

Nima Rezaei *Editor*

Pathophysiology of Cancer: An Interdisciplinary Approach

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Nima Rezaei 

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Editor

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 Springer

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Preface

The Interdisciplinary Cancer Research series publishes comprehensive volumes on different cancers. It plans to present the most updated and peer-reviewed interdisciplinary chapters on cancers. This interdisciplinary book series is of special value to researchers and practitioners working on cell biology, immunology, hematology, biochemistry, genetics, oncology and related fields. This is the main concept of Cancer Immunology Project (CIP) and Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), which are two active interest groups of the Universal Scientific Education and Research Network (USERN).

The twenty-second volume of the book entitled *Pathophysiology of Cancer: An Interdisciplinary Approach* focuses on pathophysiology of cancer. The volume starts with a chapter on risk of cancer development, followed by other chapters on strategies to reduce the burden of cancers and molecular biology of cancer disparities. The pattern of epithelial carcinogenesis, mitochondrial energy metabolism in carcinogenesis, and the role of mitochondrial DNA mutations in cancer development are discussed in the following chapters. The obesity epidemic and cancer risk, urban road dust, genotoxicity, aberrant glycosylation, melatonin, and matrix metalloproteinases as players in cancer development and progression are explained in other chapters. The subsequent chapters discuss vascular endothelium, extracellular vesicles and extracellular matrix, circulating tumor cells, cancer-associated programmed cell death, and epithelial-to-mesenchymal transition in cancer. Importance of antioxidants, decoding metabolic changes, and effects of secondary metabolites in cancer therapy are the subjects of the last chapters.

I hope that this interdisciplinary book will be comprehensible, cogent, and of special value for those who wish to have an update on pathophysiology of cancer.

Stockholm, Sweden

Nima Rezaei

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About the Editor



Nima Rezaei, MD, PhD, gained his medical degree (MD) from Tehran University of Medical Sciences and subsequently obtained an MSc in Molecular and Genetic Medicine and a PhD in Clinical Immunology and Human Genetics from the University of Sheffield, UK. He also spent a short-term fellowship of Pediatric Clinical Immunology and Bone Marrow Transplantation in the Newcastle General Hospital. Professor Rezaei is now the Full Professor of Immunology and Vice Dean of Research and Technologies, School of Medicine, Tehran University of Medical Sciences, and the co-founder and Head of the Research Center for Immunodeficiencies. He is also the Founder of Universal Scientific Education and Research Network (USERN). Prof. Rezaei has already been the Director of more than 200 research projects and has designed and participated in several international collaborative projects. Prof. Rezaei is the editor, editorial assistant, or editorial board member of more than 50 international journals. He has edited more than 100 international books, has presented more than 1000 lectures/posters in congresses/meetings, and has published more than 1500 scientific papers in international journals.



Micro(Nano)Plastics and the Risk of Cancer Development: Calling for Attention to a Macro Issue

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Abstract

Microplastics and nanoplastics are ever-present environmental contaminants with inevitable exposure to humans. Recently, research evaluating the effect of micro-

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and nanoplastics (MNPs) on human health, and more specifically their contribution to the risk of malignancies has increased. Nonetheless, current knowledge about the effect of these particles is limited. Thus, in the present work, we are providing a thorough review of microplastic shapes, routes of exposure to humans, pathogenic mechanisms, and types of cancers proven to be associated. Importantly, there are several ways MNPs can enter the body, including, ingestion, inhalation, dermal contact, iatrogenic and medical instruments, and through the placenta during the prenatal period. The exposure causes endocrine disruption, gut epithelium impairment, gut dysbiosis, and immune system impairment and, subsequently, inflammation, cell death, genotoxicity, and gene mutations. Digestive system tract malignancies, including hepatocellular carcinoma, pancreatic, gastric, and colorectal cancers are reported to be associated with MNPs exposure as ingestion is one important way of exposure. Lung, skin, renal, and hematological malignancies, including acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CML), and cancers of the reproductive system, including breast, ovarian, and prostatic are other types of malignancies. Nevertheless, knowledge of MNPs contamination effects is still limited and highly influenced by particle properties, exposure concentration, types of additives, affected tissues, and individual susceptibility. Due to the constant exposure, these particles must be considered to be an alarming issue that their effects cannot be negligible. Therefore, future research projects are encouraged to dive into the carcinogenic effects of these invisible plastic particles as the mentioned cancers are among the most prevalent types of cancers, as well.

Keywords

Cancer · Human health · Micro(nano)plastics · Microplastics · Nanoplastics · Plastic pollution · Toxicity

Abbreviations

AML	acute myeloid leukemia
BMP2 and BMP4	bone morphogenetic protein 2
BPA	bisphenol A
CDK-4	cyclin-dependent kinase-4
CML	chronic lymphocytic leukemia
CRC	colorectal cancer
EDCs	endocrine disrupting chemicals
ER	estrogen receptor
ERR γ	estrogen-related receptor gamma
GI	gastrointestinal
GnRH	gonadotropin-releasing hormone
GP α	G protein-coupled receptor
H.pylori	Helicobacter pylori

HCC	hepatocellular carcinoma
HE4	human epididymis protein 4
IARC	International Agency for Research on Cancer
ISCs	intestinal stem cells
Krt14	keratin 14
MAPK	mitogen-activated protein kinase
MNPs	micro- and nanoplastics
NETs	neutrophils extracellular traps
NF- κ B	nuclear factor kappa B
NPs	nanoplastics
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PE	polyethylene
PET	polyethylene terephthalate
PP	polypropylene
PS	polystyrene
PSA	prostate-specific antigen
PVC	polyvinyl chloride
ROS	reactive oxygen species
VCM	vinyl chloride monomer
WWTPs	wastewater treatment plants

1 Introduction

Plastic pollution is an emerging public health issue that has received more attention over the past two decades. Plastics are everywhere, from the deepest parts of the oceans to the highest peaks (Cózar et al. 2014; Kasamesiri et al. 2023). Plastic particles are a crucial part of this pollution, and a big concern has been raised about their impact on human health on a global scale (Wright and Kelly 2017). They come in different sizes, shapes, and types. Based on how big they are, these particles are categorized as macro-, micro-, and nanoplastics (Thompson et al. 2004).

Of note, micro- and nanoplastics (MNPs) captured emerging attention as they were shown to exert potential negative impacts on marine life (Cole et al. 2011). Their impact on human health, however, is not restricted to water supply as soil and air are also contaminated (Allen et al. 2022), which makes humans to be in chronic exposure to MNPs and susceptible to possible adverse effects, as previously proved about marine creatures (Naidoo and Glassom 2019).

Among the possible adverse effects, malignancies have a high risk of being developed due to the chronic nature of the disease pathophysiology and potential carcinogens in plastic make-ups (Verma et al. 2016). Most of the additives found in MNPs have potential carcinogenic effects and are able to disrupt the endocrine system (Alabi et al. 2019). Polystyrene, polycarbonate, and bisphenol A (BPA) are

well-known carcinogens abundant in plastics and thus present in MNPs (Proshad et al. 2018). Furthermore, MNPs absorb heavy metals, including As, Cr, Co, Cu, Mn, Pb, Ni, Zn, and Cd, from the environment, which are carcinogens (Chen et al. 2022a, b). Although malignancies are highly multifactorial, as genetics and lifestyle are involved, environmental pollution might contribute to developing cancer (Zandalinas et al. 2021).

It is important to note that these particles are invisible, and the possible adverse effects are not negligible. Importantly, these MNPs are always with human beings as people are continuously exposed to the sources of these particles. Given the increased rate of plastic production, population growth, and increased need for manufactured plastics (Hale et al. 2020), the increased risk of cancer development due to plastic exposure must be a public health issue that needs solutions to be sought.

This review, therefore, will discuss the sources, ways of exposure, and reported cancers resulting from MNPs exposure. We will also tackle the suggested mechanisms to provide a detailed review of the current evidence of the association.

2 Plastics Particles Spectrum

In recent years, there has been much interest in the spectrum of plastic particles as they are everywhere in the environment. These particles come from several places, such as the breakdown of more significant pieces of plastic, industrial waste, and microbeads in personal care products (Thompson et al. 2009; Andrady 2011).

The size of the plastic particles in the spectrum changes from microplastics to nanoplastics. Microplastics (MPs) are considered to be less than 5 mm, while nanoplastics (NPs) are less than 1,000 nm (Molenaar et al. 2021). These particles can be fibers, films, or small pieces, depending on where they come from and how they break down (Gigault et al. 2016). Furthermore, depending on the type of plastic and the additives used, the color of plastic particles can range from clear to opaque (Mattsson et al. 2015).

Plastic particles can have colorants, stabilizers, and flame retardants in addition to the polymer, which can change how toxic they are and how long they stay in the environment. There are many different kinds of polymers in plastic particles, such as polypropylene (PP), polyethylene (PE), and polystyrene (PS) (Hartmann et al. 2019).

3 Plastic Particles Sources and Types

3.1 Sources

Plastic particles can be made when plastic products break down in the environment, like when exposed to sunlight; this makes MPs, which can enter the environment when plastic items are used, thrown away, or recycled (Eerkes-Medrano et al. 2015;

Barboza et al. 2018). For instance, plastic bags can break down into small pieces that can build up in the environment (Danopoulos et al. 2020). In the same way, plastic bottles are important sources of these particles (Karami et al. 2018).

Wastewater treatment plants (WWTPs) are another important source. Studies have shown that although treated effluents have a relatively low MPs concentration, the daily amount of MPs discharged is still relatively high which is due to the high capacity of WWTPs in treating the water (Sun et al. 2019). MPs are found in both the incoming and outgoing water of WWTPs, and the amount of items is dependent on treatment levels (preliminary, primary, secondary, and tertiary). WWTPs treat wastewater originating from homes, factories, hospitals, etc. (Xiang et al. 2022). The MPs composition of the wastewater depends on the complexity of the treatment system and the composition of incoming water. It is estimated that the average daily discharged MPs from some WWTPs was 1.6×10^9 items per liter; however, the total discharged MPs were affected by the characteristics of the systems (Cheng et al. 2021).

Plastic particles can also get into the environment through agricultural activities. Plastic mulch films used in agriculture can break up into microplastics, which can be carried by wind or water to other ecosystems like soil, water, and air (Ren et al. 2021). The use of plastic packaging for agricultural products can also contribute to the release of plastic particles into the environment (Glenn et al. 2014).

Products like toothpaste, facial scrubs, and cosmetics contain MPs, which are released into the environment through WWTPs (Guerranti et al. 2019). For instance, facial cleansers and toothpaste emit 229,000 and 4000 MPs in domestic sewage in each use, respectively (Cheung and Fok 2017; Kokalj et al. 2018).

Tire wear is another source of MPs, which enters the aquatic environment (Kole et al. 2017; Siegfried et al. 2017). The mechanical abrasion of tires and scratches by asphalt and road makes tire wear particles (Panko et al. 2013). Tire wear particles are produced in relatively high amount compared to the general rate of MPs production (Tamis et al. 2021).

3.2 Types and Sizes

3.2.1 Macroplastics

Plastics bigger than 5 mm and visible to the naked eye are called macroplastics. Items like bottles, bags, and packaging materials are among them (Eriksen et al. 2014). Plastics like these are often found in marine environments, where they can hurt marine life (Fu et al. 2018; Barboza et al. 2019). They can also damage boats and fishing gear, which increases the costs and is important from an economic aspect (Borrelle et al. 2020; Lechthaler et al. 2020).

3.2.2 Primary and Secondary Microplastics

Microplastics are smaller than 5 mm and can only be seen with a microscope (Andrady 2017). MPs can be categorized into two major groups: primary and secondary. Primary MPs are the type of MPs that are intentionally manufactured

and are primarily in microscopic sizes. Microbeads found in personal care products, nurdles, and pellets used to make plastic are examples of primary MPs and made to be small on purpose. Primary particles can be found in salt and fresh water and are ingested by aquatic animals (Arthur et al. 2009; Hidalgo-Ruz et al. 2012; Cole et al. 2013).

Whereas secondary MPs are produced over time and are the result of plastic waste degradation by external forces or the breakdown of large particles. Plastic bags and bottles break down through photodegradation, mechanical abrasion, and chemical weathering; this makes secondary MPS (Koelmans et al. 2015). Table 1 provides more detailed description and examples of different types of MPs.

Table 1 Type of microplastic components

Type of microplastic component ^a	Description	Examples	Key references
Primary microplastics	Small plastic particles that are intentionally produced and used in a variety of consumer products	Microbeads, plastic pellets, resin pellets	Courtene-Jones et al. (2017)
Microbeads	Plastic fragments ranging from roughly 5 μm to 1 mm in size.	Abrasive scrubbers, including body washes, cosmetics, face washes, and cleaning supplies	Rochman et al. (2015a, b)
Nurdles	Small plastic pellets used in the manufacturing of plastic products as a raw material	The raw plastic material used for injection molding and other production processes	Horton and Dixon (2018)
Secondary microplastics	Formed as a result of the breakdown of larger plastic materials such as bags, bottles, and other debris	Fragmented plastic items, fibers, films	Horton and Dixon (2018)
Fibers	Small fibers that are produced from the breakdown of larger plastic materials or from the shedding of synthetic textiles such as clothing and carpeting	Acrylic, polyester, nylon, polyamide, polyester	Rebelein et al. (2021)
Fragmented microplastics	Small pieces of plastic that break off from larger items through physicochemical or abiotic processes	Bottle caps, plastic utensils, broken toys	Mateos-Cárdenas et al. (2020)
Microplastic films	Thin plane or sheets of flimsy plastic that are commonly used in packaging and wrapping	Plastic bags, agricultural mulch film	Sruthy and Ramasamy (2017) and Ren et al. (2021)

^aThis table is not exhaustive, and other types of microplastic components may not be listed here

Microfibers are small pieces of synthetic material that come off clothes when washed (Hartmann et al. 2019). These particles are a type of secondary MPs, which are especially harmful as they are familiar and easy to swallow (Cole et al. 2013). They can be found in both freshwater and saltwater environments. Recent research suggests that microfibers are one of the most common types of MPs reported in the environment and can hurt the health of marine organisms (Hartmann et al. 2019). Foams are another type of MPs, which are disposed through domestic wastewater systems and thus be discharged into natural water sources (Lebreton et al. 2017).

3.2.3 Nanoplastics

Nanoplastics (NPs) are a type of plastic particle ranging from 1 to 1000 nm in size (Molenaar et al. 2021). NPs possess some unique characteristics that are assumed to be different from both MPs and engineered nanoparticles (Gigault et al. 2021). These particles are reported to be found in many places, like freshwater, saltwater, and even land (Sangkham et al. 2022). These nano-sized particles can get into the body through eating, breathing, or skin touching and most of what consequences they contribute to are still unknown (van der Zande et al. 2014; Yee et al. 2021; Castrogiovanni et al. 2022; Ge et al. 2022).

4 The Plastic Cycle

The plastic cycle is the whole life cycle of plastic, from when it is made to when it is thrown away (Geyer et al. 2017). As suggested, this cycle comprises several exchanges between land, atmosphere, ocean, and animals, including the land-atmosphere exchange, ocean-atmosphere exchange, and land-ocean exchange (Zhu 2021). Importantly, atmospheric MPs produced in the plastic cycle have been shown to be derived from secondary re-emission sources, mostly from roads (84%) in the west areas of the United States. The ocean and agricultural soil dust have been responsible for 11% and 5% of re-emission, respectively (Brahney et al. 2021).

4.1 The Plastic Cycle and Human Health

The plastic cycle influences people's health in many ways. Chemicals in plastic products can leak into the environment and get into our bodies through our food, water, and air. Chemicals like phthalates and BPA are known to mess up the endocrine system and have been linked to cancer, problems with reproduction, and other health problems (Yang et al. 2011; Gore et al. 2015; Rochester and Bolden 2015; Schymanski et al. 2018; Ghosh and Sahu 2022). Furthermore, plastic waste can cause physical harm in addition to chemical harm. Plastic trash in the oceans and other bodies of water can hurt marine animals (Thompson et al. 2009; Koelmans et al. 2016; Trevisan et al. 2022). Plastic waste can make the effects of climate change worse. As manufacturing plastics takes much energy, and eliminating plastic waste adds to greenhouse gas emissions. Plastic waste also negatively affects the

natural beauty of our world, which can affect people's well-being and mental health (Andrady 2011; Eriksen et al. 2014; Geyer et al. 2017; Enyoh et al. 2019; Manisalidis et al. 2020).

4.2 Micro(Nano)Plastics and the Plastic Cycle

There are several ways that MPs are made within the plastic cycle. They can be used in products, such as microbeads in some products or synthetic fibers in clothing (Rochman et al. 2015a, b; Mason et al. 2018). In this cycle, MPs can also be made when more pieces of plastic in different sizes break down in the sun, heat, or other environmental conditions (Andrady 2011; Song et al. 2015). Furthermore, when the trash is thrown away, plastic waste in landfills can break down over time into smaller pieces, and when plastic waste is burned, microplastics can be released into the air (Horn et al. 2019). Plastic trash can be broken down into tiny pieces during physical and chemical processes, which can lead to MPs (de Souza Machado et al. 2019). In other words, both primary and secondary MPs are involved in the cycle.

4.3 Ways to Minimize Exposure to Humans

People and society as a whole can take some steps to reduce the adverse effects of the plastic cycle on public health. One way to reduce plastic chemicals is to use fewer plastic products, like straws, bags, and utensils, that only need to be used once. Instead, it is suggested that things like metal straws, cloth bags, and bamboo utensils that can be used more than once (Narancic and O'Connor 2019; Ricciardi et al. 2021).

Recycling and proper disposal can cut down on the amount of plastic trash that ends up in landfills and oceans, and the right way to get rid of plastic trash can keep it from getting into waterways. Using less plastic by choosing products with less packaging, buying in bulk, and fixing or reusing things instead of throwing them away (Heidbreder et al. 2020).

Regulations can also help reduce people's exposure to plastic chemicals and trash. Governments can make rules to limit certain plastic products' use or encourage recycling and proper disposal (Onyena et al. 2022). Industry can also help by making products with less plastic packaging and less plastic used in them (Babaremu et al. 2022; Huang et al. 2022; Satyro et al. 2023).

5 Microplastic Components

5.1 Bisphenol A

Bisphenol A (BPA) is a synthetic organic compound that belongs to the group of diphenylmethane derivatives (Li et al. 2010; Siracusa et al. 2018). Since the 1950s, it has been widely used to make polycarbonate plastics, epoxy resins, and other things

because of its unique physical and chemical properties (Rochester 2013; Rochester and Bolden 2015; Liu et al. 2021a, b). Nevertheless, in recent years, BPA has gotten much attention because it might harm people's health and the environment. As a result, many countries have taken steps to limit or ban its use in certain products (Vandenberg et al. 2012; Sifakis et al. 2017; Wazir and Mokbel 2019).

BPA in MPs enters the environment through industrial effluents, landfill leachate, and WWTPs discharges (Sukanya et al. 2020; Kovačič et al. 2022) and remains for a long time. Thus, it is found in many places, like water, sediment, soil, and air (Lee et al. 2018; Mustieles et al. 2020). This chemical has also been found in fish and shellfish, which shows the potential to build up in the food chain (Vandenberg et al. 2012; Lai et al. 2016).

Given its carcinogenic and adverse cardiovascular effects (Jin et al. 2020), the use of BPA in food industry has been reduced or banned (Vandenberg et al. 2007; Cantonwine et al. 2013). However, more assessment is needed to eventually understand the effects of BPA on human health and the environment, especially when it comes to long-term exposure and the possibility that it could build up in the food chain (Rochester 2013; Almeida et al. 2018). More research is also needed to find and create effective ways to stop BPA from getting into the environment and causing problems (Bhatnagar and Anastopoulos 2017; Hashemi Orimi et al. 2020).

5.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds comprising two or more bonded aromatic rings (Samanta et al. 2002). PAHs can be made when fossil fuels, wood, and tobacco are incompletely burned (Låg et al. 2020) and enter the environment in several ways industrial emissions, vehicle exhaust, and coal and oil deposits that break down over time (Gasull et al. 2013; Sheikh Fakhradini et al. 2019).

With the potential to accumulate in the tissues of aquatic and land organisms (Moorthy et al. 2015; Kim et al. 2017; Låg et al. 2020), PAHs contribute to developing cancer and mutagenesis in animals and humans (Huang et al. 2017; Li et al. 2019). Some PAHs were labeled as carcinogens to humans by the International Agency for Research on Cancer (IARC) (Moorthy et al. 2015; Francová et al. 2017; Cheng et al. 2018).

However, more research is needed to understand PAHs' effects on health and the environment, especially their long-term effects and how they might build up in the food chain. More research is also needed to find and create effective ways to stop PAHs from getting into the environment and causing problems (Kuppusamy et al. 2017; Lawal 2017; Shoaie et al. 2023).

5.3 Polystyrene

Polystyrene is a manufactured polymer used in many industries because it is lightweight, insulates well, and can be made into many different things. It is often

used to make packaging, disposable cutlery, insulation, and materials for the building industry (Yu et al. 2018; Moskalyuk et al. 2020; Lim et al. 2021a, b; Prasittisopin et al. 2022). However, the widespread use of polystyrene has enormously affected the environment as it remains for a long time (Nugnes et al. 2022; Pandey et al. 2023; Ugwu and Obele 2023). This polymer enters the environment in many ways, such as through littering, industrial waste, or an accident. Once entered, it can be moved around by air and water currents, which causes it to build up in places like water bodies, soils, and sediments (Browne 2015; Carney Almroth and Eggert 2019; Yang et al. 2021a, b), being mistaken for food by marine animals and significantly affecting ecosystems and species as it contains styrene monomers (Gola et al. 2021; Chowdhury et al. 2022; von Hellfeld et al. 2022).

Polystyrene has been shown to affect human health negatively. Studies have shown that styrene monomers, released in the process of polystyrene breaks down, can cause cancer and damage nerve cells in people (Lu et al. 2018; Sarma et al. 2022). Examples of the efforts to deal with the burden of pollution are replacing biodegradable and compostable with polystyrene in the packaging (Song et al. 2009a, b; Razza et al. 2015; Moustafa et al. 2019) and recycling. Polystyrene can be recycled into things like picture frames, rulers, and insulation, and there are efforts to increase the amount of recycled polystyrene (Maafa 2021; Welle 2023).

5.4 Polyethylene

Polyethylene is another common synthetic polymer commonly used in packaging, plastic bags, toys, and medical devices as it is a light, flexible material that is easy to make and work with (Babu et al. 2013; Farah et al. 2016). Nevertheless, the widespread use of polyethylene has affected the environment. Polyethylene does not break down, easily enters the environment, and thus remains there for hundreds of years, in many ways, such as through littering, industrial waste, and accidents (Ghosh and Chatterjee 2020; Yao et al. 2022).

Once polyethylene is in the environment, it can be moved around by air and water currents and build up in places like water bodies, soils, and sediments (Shah et al. 2008; Andradý 2017; Wang et al. 2021a, b, c). Similar to the previously discussed polymers, polyethylene pollution can affect different species (Wright et al. 2013; Prata et al. 2020; Zhou et al. 2020; Uy and Johnson 2022). Studies have shown that chemicals like BPA and phthalates released by polyethylene can harm human health. For example, they can disrupt hormones and cause congenital disabilities (Xu et al. 2019a, b; Campanale et al. 2020).

5.5 Polyvinyl Chloride

Polyvinyl chloride (PVC) is an artificial polymer used in many industries because it is flexible, cheap, and durable. It is often used to make pipes, flooring, building materials, packaging, and medical devices (Elena-Diana et al. 2020; Darabi et al.

2022; Ledniowska et al. 2022). When making PVC, toxic chemicals like vinyl chloride monomer (VCM) are used, thus making it hazardous (Bidoki and Wittlinger 2010; Akovali 2012). PVC products can also release harmful chemicals like phthalates and heavy metals, hurting aquatic and land organisms (Praveena et al. 2021; Šimunović et al. 2022).

PVC pollution can harm both aquatic and terrestrial ecosystems. Marine animals may ingest PVC particles, which can lead to choking, internal injuries, or even death. Additionally, PVC waste can cause physical entanglement, further threatening wildlife (Lusher et al. 2017; Ye et al. 2017; Cássio et al. 2022). It can also be bad for people's health. Studies have shown that chemicals like phthalates and dioxins released by PVC can harm human health, potentially disrupting hormones and causing congenital disabilities (Thompson et al. 2009; Karbalaie et al. 2018).

Similar to the efforts on other compounds, there have been attempts to cut down on the use of PVC in different industries. Biodegradable and compostable materials are being developed and pushed as possible replacements for PVC in packaging and other uses (Kale et al. 2007; Maraveas 2020). Recycling is another way to lessen PVC detrimental effects to the environment. There are efforts to increase the amount of PVC that is recycled (Sadat-Shojai and Bakhshandeh 2011; Lewandowski and Skórczewska 2022). Even though work is being done to reduce the usage and encourage alternatives, more research and action are needed to stop PVC from polluting the environment (Baitz et al. 2005; Feng et al. 2018; Elena-Diana et al. 2020; Lewandowski and Skórczewska 2022; Li et al. 2023).

6 Routes of Exposure

Micro- and nanoplastics exposure to humans could be through various ways, including the oral by ingesting contaminated food or water or use of care products, inhalation of airborn MPs, and, to a lesser extent, direct skin contact (Basri et al. 2021). Evidence has also been found of human contamination with MPs during the fetal period and through medical devices during procedures or implanting plastic-based prostheses (Ragusa et al. 2021; Field et al. 2022).

6.1 Ingestion

People are directly and consistently in risk of exposure to MNPs by ingesting contaminated water, food products, personal care products (toothbrushes, toothpaste), and settled dust. The particles may also be ingested through mucociliary clearance after inhalation. Although the diversity of these particles in chemical composition, size, and density makes it difficult to be precise in the type and amount of each particle in different food or water, ingestion is widely accepted as the main route of human exposure (Galloway and Galloway 2015), which is strongly supported by the presence of MNPs in the human stool (2019).

6.1.1 Water

Microplastic contamination has been found in almost all aquatic habitats, including oceans, seas, and all sources of natural fresh waters (Boucher and Friot 2017; Li et al. 2020a, b).

A broad range of concentrations of MNPs has been found in both tap and bottled water. MNPs in tap water could be originated from different sources, including ground, surface run-off, treated and untreated wastewater, sewer overflows, industrial effluent, degraded plastic waste, possible erosion and/or degradation of the plastic components of distribution networks, for instance, pipes and tubing and atmospheric deposition (Pivokonsky et al. 2018; Mintenig et al. 2019; Kirstein et al. 2021a, b).

Bottled water is also contaminated by plastic bottles used as packaging or through the process of manufacture (Winkler et al. 2019; Zuccarello et al. 2019; Giese et al. 2021; Makhdoumi et al. 2021). Recent studies have confirmed a higher concentration of MNPs in bottled water than in tap water, turning on a new alarm for public health due to the increased prevalence of bottled water consumption (Tosun et al. 2020; Gambino et al. 2022).

Using contaminated water to incorporate into food could be an additional pathway for human exposure (Danopoulos et al. 2020).

6.1.2 Food

All human food sources can be potentially contaminated with MNPs. Sea foods are one of the key sources of MPs transmission due to marine organism entanglement and ingestion of MNPs in the polluted sea and ocean water (Verla et al. 2019).

Meat and dairy products can also be contaminated with these particles following the feeding of animals from water and food containing microplastics (Enyoh et al. 2020). Vegetables and fruits irrigated with contaminated water and grown in contaminated soil contain high amounts of MNPs. Table salt, sugar (Honey), and alcohol are other known sources of MNPs (Cox et al. 2019).

Contents of MNPs have also been detected in cow milk samples (Kutralam-Muniasamy et al. 2020). In addition, these particles can contaminate food by transferring it through plastic packaging (Domenech and Marcos 2021). Since MNPs can travel up the food chain to higher levels and humans are at the top, they have been cumulatively exposed to MNPs through contaminated foods.

Traces of MNPs have also been detected in human breast milk, bringing a new concern regarding the exposure of infants through breastfeeding (Ragusa et al. 2022; Liu et al. 2023).

6.2 Inhalation

The surrounding atmosphere is a medium of MNPs dispersion and transportation regionally and globally and is being inhaled directly and continuously, highlighting the air as a major and constant source of pollution (Allen et al. 2019). Respiratory

exposure is related to pathologies such as bronchitis, asthma, fibrosis, pneumothorax, granulomatous lesions, and cancer (Song et al. 2009a, b; Lim et al. 2021a, b).

Inhalation of MNPs suspended in indoor and outdoor air and settled dust has recently become the dominant route of human exposure and health concern (Wang et al. 2021a, b, c). Investigations showed higher concentrations and lesser sizes of MNPs in indoor air than in outdoor air (Gaston et al. 2020). As particles with smaller sizes are more abundant, and humans spend most of their time indoors, it can be concluded that there is a higher risk of exposure to indoor air (Jenner et al. 2021).

Those particles reaching the intrathoracic cavity, entering and accumulating in the deep lungs, are classified as respirable. These particles are separated from synthetic textiles as small fibers easily tear from fiber products such as mats, curtains, mattresses, and from cloths during wearing, cleaning, and drying. Degradation of manufactured furniture, plastic goods, electrical cables, indoor paint, building materials, kitchen items (plates, cups, utensils, bowls, bottles), and waste incineration are other sources of MPs particles in around atmosphere (Webster et al. 2009; Dris et al. 2015; Dris et al. 2016; Allen et al. 2019).

PE, PP, PS, and polyethylene terephthalate (PET) are the main polymers component in atmosphere fallout, with fibers as the dominant shape (Prata 2018).

Since the beginning of the COVID-19 pandemic, wearing a face mask has become a new routine all around the world. Although wearing a mask can act as a protector against breathing microplastics in the atmosphere, the mask itself can be a source of MNPs inhalation, depending on the material it is made of (Li et al. 2021; Cao et al. 2023).

6.3 Dermal Contact

Although inhalation and ingestion are the two major ways for human exposure, MNPs particles can also cross the dermal barrier and enter the human body. Dermal exposure may occur when there is close skin contact with MNPs used in cosmetics and continuous reduction of textile microfibers, or settled dust particles, indoor and outdoor air, contaminated soil, and during the shower with polluted water (Galloway 2015; Hernandez et al. 2017).

Interestingly, studies suggest that particles smaller than 100 nm can directly penetrate the skin barrier, while particles with larger sizes could pass through hair follicles, sweat glands, or open dermal injuries (Schneider et al. 2009; Revel et al. 2018).

6.4 Iatrogenic Exposure

The vast amount of medical equipment used generally in hospitals is made from plastic or wrapped with one. Humans can be exposed to MPs originating from medical devices through ingesting and inhaling (Field et al. 2022).

Regardless of the contamination of medical environments with MPs, like all-natural environments, plastic particles separated from polyethylene-based implants have been observed in the capsule and joint space (Willert and Semlitsch 1996; Urban et al. 2000; Massin and Achour 2017). Some previous studies indicated that the release of these particles is associated with triggering a local immunoreaction and inflammatory response and the macrophages presence in the space around the implant (Devane et al. 1995; Nich and Goodman 2014; Massin and Achour 2017).

6.5 Prenatal Exposure

MPs exposure during early development in fetuses and neonates is a major health concern because it could lead to irreversible changes in vital organs of the body (Amran et al. 2022). Since the placenta is the interface between the fetus and the external environment, nanoparticle plastics must reach the placenta through the mother's bloodstream, crossing the placental barrier and distributing and accumulating in the fetus (Graffmueller et al. 2015; Barboza et al. 2018).

Prenatal exposure to MNPs could affect the central nervous system and is related to alternations in the developing brain, and therefore, increasing the risk of neurodevelopmental disorders, including autism spectrum disorder (Solleiro-Villavicencio et al. 2020; Zaheer et al. 2022).

Recent studies detected microplastic fragments in human placenta samples, in both maternal and fetal sides and amniochorial membranes (Ragusa et al. 2021). MPs also were detected in meconium, showing another proof of neonatal exposure through the placenta (Braun et al. 2021; Liu et al. 2023).

7 Suggested Mechanisms

7.1 Hormonal Mimicry

High surface area-to-volume ratios make MNPs potent for carrying various environmental pollutants, including toxic chemicals, pesticides, heavy metals, micro-organisms, and bioactive compounds (Curren and Leong 2019; Yu et al. 2019; Liu et al. 2021a, b). Some of these absorbed chemicals, such as PAHs as well as additives including BPA, phthalates, and polychlorinated biphenyls, can interfere with the endocrine activity and act as endocrine disrupting chemicals (EDCs) (Darbre 2015; Campanale et al. 2020).

EDCs could alter hormone synthesis in endocrine glands, interfere with the transport of the physiological hormone, compete for binding sites in target cells, mimic or antagonize the effects of the hormone, and finally disturb the elimination process. Thus, may affect fertility, fetal and child development, and promote some cancers (Meeker et al. 2009; Louis et al. 2014; Darbre 2015).

The disruptive activity of BPA, phthalate esters, and PAHs is associated with mimicking the effect and interfering with the activity of thyroid and steroid

hormones. Both thyroid and steroid hormones play a fundamental role in different cancer development (Ahmad and Kumar 2011; Krashin et al. 2019). Being exposed to PAHs in rats leads to a reduced rate of metabolism following the breakdown of thyroid hormones (Schrappau et al. 2015). Some PAH derivatives share similar structures with 17 β -estradiol, and some of the PAH metabolites demonstrate antiestrogenic activity by interacting with estrogen receptors (Arcaro et al. 1999). A positive association between PAH exposure and levels of serum testosterone in males and estradiol level in females exists (Yuan et al. 2022), which may be related to increased gonadotropin-releasing hormone (GnRH) mRNA expression (Wang et al. 2019a, b).

Given the fact that BPA acts as xenoestrogen (Fenichel et al. 2013), it influences estrogen-related pathways and increases aromatase expression, the key enzyme necessary for converting androgens into estrogens (Wetherill et al. 2007; Williams and Darbre 2019). There is also a link between BPA exposure and the incidence of endocrine-related cancer, including breast, ovarian, uterine, prostate, testicular, and hepatic cancers, alteration of thyroid hormones, and disruption of glucose homeostasis (insulin resistance and type 2 diabetes) (Fenichel et al. 2013; Wang et al. 2017; Hafezi and Abdel-Rahman 2019).

Exposure to phthalates, another group of EDCs, is related to breast cancer incidence. These chemicals can promote tumor growth via estrogen receptor signaling and induce cell proliferation in estrogen-independent breast cancer with antiestrogenic effects (Okubo et al. 2003; Ahern et al. 2019). The adverse effects of phthalate on the prostate gland, causing prostatic enlargement and intraepithelial neoplasia through the estrogenic pathways, have also been reported in rats (Jiang et al. 2007; Di Lorenzo et al. 2018).

7.2 Oxidative Stress, Mitochondria Dysfunction, and DNA Damage

Numerous studies have proven that the mitochondrial cycle dysfunction and oxidative stress play a critical role in the occurrence and development of many diseases, including the development of various cancers (Luo et al. 2020). Oxidative stress, disruption of mitochondrial dynamics, and genome instability are probably the critical causes of cellular toxicity and malignant transformation related to MNPs exposure, but the exact mechanism of MNPs carcinogenesis remains to be investigated (Alimba et al. 2021). The imbalance in reactive oxygen species (ROS) production is the initiating step of oxidative stress. MNPs initiate oxidative stress by triggering the intra and extracellular formation of ROS. Moreover, activation of redox-sensitive signaling pathways such as mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF- κ B) and dysregulated expression of antioxidant enzymes gene has been observed post MNPs exposure in recent studies (Jeong et al. 2017; Hu and Palić 2020; Woo et al. 2023).

The particles' size and surface charge are related to the severity of induced oxidative stress, as more severe damage has been reported after exposure to

negatively charged nanoplastics (Jeong et al. 2016; Lin et al. 2019). Following oxidative stress and increased intracellular ROS, the mitochondrial inner and outer membranes permeability is affected, and the ionic flux through the ion channels reduces the potential of the mitochondrial membrane (Hu and Palić 2020). Changes in potential membranes cause positive feedback for more ROS generation by electron transfer change leading to many more cellular and mitochondrial injuries (Zorov et al. 2006).

MNPs exposure is also involved in mitochondrial autophagy (Liu et al. 2022). In vitro testing also demonstrated autophagic cell death in macrophage and epithelial cell cultures of lungs exposed to MNPs caused by ROS over-production and endoplasmic reticulum stress (Chiu et al. 2015).

ROS and oxidative stress also damage mitochondrial and cellular DNA (Zorov et al. 2014). Mutagenesis associated with MNPs exposure happens after ROS breaks the double strands of DNA, followed by rearrangements of chromosomes (Hu et al. 2021). NPs directly interact with DNA. Due to their free passage across the cell membrane, they freely enter the cell nucleus and cause physical damage to the genetic material (Singh et al. 2009). Genotoxicity and genome instability are reported in several studies caused by DNA single-strand breaks, micronuclei formation, chromosomal aberrations, sister chromatid exchange, and glycophorin A somatic cell mutations (Barale 1991; Bigbee et al. 1996; Laffon et al. 2002; Ballesteros et al. 2020). Furthermore, MNPs significantly disturb the cell viability and cell cycle by cell cycle arrest induction at the S phase (Synthesis phase), pro-apoptotic proteins activation, apoptosis-related transcriptional genes downregulation, and increased DNA fragmentation, which could explain tumor initiation and progression (Avio et al. 2015; Xu et al. 2019a, b; Hamed et al. 2020).

7.3 Immune Function Alteration

Microplastics can alter the immune system function and trigger a cascade of inflammatory responses at different levels and with different mechanisms. The innate immune system recognizes MNPs particles as xenobiotics. MNPs exposure interferes with the cytokine release and nitric oxide production by macrophages and monocytes, which are the main part of the first line of the innate immune system response that mainly uptake microplastic particles. Phagocytic functions can also be impaired by inhibiting the chemotaxis process of macrophages and neutrophils into the target tissue (Prietl et al. 2014).

MNPs induce neutrophils extracellular traps (NETs) release and increase the neutrophils primary granules degranulation, suggesting that MNPs as phagocyte activators and potential stressors of innate immunity. Studies also reported that engulfing MNPs resulted in massive neutrophil death (Greven et al. 2016; Yang et al. 2022).

At the molecular level, IL-1 α is increased significantly in the serum, and Th17 and Treg cells are reduced after MNPs exposure (Li et al. 2020a, b). NPs exposure impairs the gene regulation of proinflammatory cytokines such as IL-6, IL-8, and

IL1 β in human gastric adenocarcinoma cells (Forte et al. 2016). Increased level of L-1 α , IL1 β , and IFN mRNA and their respective protein was reported related to MPs exposure in zebrafish (Jin et al. 2018).

MNPs have disrupting effect on the immunity system response by altering the activity of immune enzyme, decreasing cell viability, and promoting cell apoptosis. The signaling pathways involved in T cell differentiation, such as PKC θ /NF- κ B and IL-2R/STAT5, can also be inhibited by MNPs, which may cause increased risk of infection and cancer (Tang et al. 2020; Li et al. 2022). Furthermore, MNPs contribute to immunosuppression by reducing anti-inflammatory cytokines production such as IL-10 and dendritic cell activity. Exposure could also lead to impairment in Th2 activation as a result of T-effector cell production (Saravia et al. 2014). In vitro studies also showed induced innate immunity responses in bone-marrow-derived macrophages which are accompanied by increased inflammatory cytokines secretion (such as interferons, IL-6, and TNF- α) and reduced production cytokines (such as IL-10) with anti-inflammatory effects (Lebre et al. 2020). Induced inflammation was also observed in mice liver tissue after oral ingestion via the NF- κ B pathway in the non-parenchymal cells of the liver. Evaluation of non-parenchymal liver cells revealed increased infiltration of macrophages and natural killer cells and reduced B cell recruitment to the mentioned cells. In response to MNPs exposure, expression of IFN- γ , TNF- α , IL-1 β , IL-6, and IL-33 were upregulated in liver tissue; in contrast, IL-4, IL-5, IL-10, IL-18, and TGF- β 1 were downregulated (Zhao et al. 2021). Although much more extensive research is needed to determine the exact effect of microplastics on immune system function, data from previous studies clearly show that the accumulation of MPs in animal and human tissues is associated with the creation of an inflammatory microenvironment and a chronic inflammatory response that can potentially lead to neoplasia Exposure could also lead to impairment in Th2 activation as a result of T-effector cell production (Saravia et al. 2014; Lebre et al. 2020).

7.4 Gut Epithelium Impairment

The intestinal epithelium integrity plays an essential role in maintenance of the host's normal homeostasis of the digestive tract, balancing the intestinal environment, and restricting the entry of microorganisms and toxins. Its function is tightly associated with endogenous factors, including the performance of gut microbiota, mucosal layer, immune cells, and intestinal stem cells (ISCs) (Vancamelbeke and Vermeire 2017; Gieryńska et al. 2022). Since the most important way of MPs exposure is inhalation and ingestion, these particles must pass through the epithelial barrier of the intestine and lungs to reach the distant organs. Environmental pollutants, including MNPs, could disrupt the intestinal barrier with different adverse effects, which may ultimately lead to pathologic conditions such as autoimmune and inflammatory disease, irritable bowel disease, celiac disease, diabetes, obesity, and even cancer (Groschwitz and Hogan 2009; Celebi Sözen et al. 2020; Ahmad Kendong et al. 2021; Genua et al. 2021). Gut epithelium is not only affected

by ingested particles, but microplastics absorbed from the respiratory epithelium can reach systemic circulation and exert an immune effect on the digestive epithelium through the gut-lung axis path (Enaud et al. 2020).

Previously, investigations revealed post-MNPs exposure effects on gut epithelium homeostasis and cell morphology. MNPs exposure in animal studies led to deformed and disorderly arranged intestinal cells due to a significant decrease in the transcription levels of tight junction proteins in the colon and ileum (Luo et al. 2019; Wang et al. 2019a, b).

Increased vascularization, triggered villi and microvilli damage, and thinning of the intestinal wall were also reported related to MNPs (Qiao et al. 2019a, b). A recent study suggests that MNPs interfere with self-renewal and differentiation of colonic epithelial cells triggered by the over-proliferation of ISCs and loss of goblet cells, exacerbating the severity of colitis (Xie et al. 2023). MNPs impact the immune barrier of the intestine by decreasing the number of M1 macrophages, increasing T lymphocytes in the intestinal microenvironment, and changing the chemotaxis of B lymphocytes (Gu et al. 2020). Moreover, an inflammatory process begins in the exposed gut epithelium to MNPs by increased secretion of proinflammatory cytokines and reduced activity of digestive enzymes, which may cause mucosal injury and intestinal leakage (Huang et al. 2020). Excessive oxidative stress followed by the over-production of ROS and a change in the expression of genes involved in response to oxidative stress has also been observed after exposure to microplastics (Huang et al. 2021). Previous studies also demonstrated increased level of proinflammatory and pro-apoptotic proteins in mice colon tissue, while the expression of anti-inflammatory and anti-apoptotic proteins was significantly reduced. As known, oxidative stress and activation of the inflammatory pathway can also promote abnormal apoptosis in colonic cells (Liang et al. 2021; Jia et al. 2023). The literature also provided evidence for similar harmful effects and cytotoxicity caused by inhaled particles in the respiratory epithelium that may lead to pulmonary disease and cancer (Dong et al. 2020; Celis et al. 2021).

7.5 Alteration in Gut Microbiota

Human gut microbiota is a dynamic and complex community of many commensal microbes, that act as a critical player in individual's health status, not only influencing the entire digestive system; however, several other organs are also influenced. A well-known association is demonstrated between gut microbiota composition alternation and several metabolic, neurologic, and immunological diseases and cancer development (Sonnenburg and Bäckhed 2016; Siddiqui et al. 2021).

Studies have found that MPs exposure can lead to nasal and intestinal microbiota alternation causing disease in multiple systems (Zhang et al. 2022a, b, c, d). Given the heterogeneity of composition, shape, dose, and time of exposure to MNPs, as well as the great diversity of gut microbiota composition in animal models, the results of studies influencing microbiome alteration caused by microplastics are very

heterogeneous. In general, the data indicate that MNPs are capable of changing the composition and diversity of the bacterial community and triggering intestinal dysbiosis (Souza-Silva et al. 2022).

Studies also suggest that MNPs influence bacterial metabolism and increase mineral absorption by gut microbiota (Duan et al. 2021). Intestinal microflora modulates the inflammatory process in gut epithelium by its effects on proinflammatory or anti-inflammatory cytokines and maintains intestinal mucosal immune function by regulating signal transduction pathways related to intestinal defense mechanism (Kamada et al. 2013). Intestinal dysbacteriosis impairs the antimicrobial activity of the normal flora and provides an opportunity for commensal bacteria to invade the intestinal lining and cause inflammation. Chronic inflammation and excessive production of inflammatory cytokines trigger cancers, speed up tumor invasion, and may lead to metastasis (Meng et al. 2018; Li et al. 2020a, b). Moreover, several resident bacterial species are known to have anticancer properties, and some contribute to cancer development; therefore, changing the intestinal microbial composition can directly induce cancer (Arthur et al. 2012; Meng et al. 2018). Figure 1 summarizes current knowledge and findings about MNPs sources and shapes, the suggested mechanisms, and the routes of exposures.

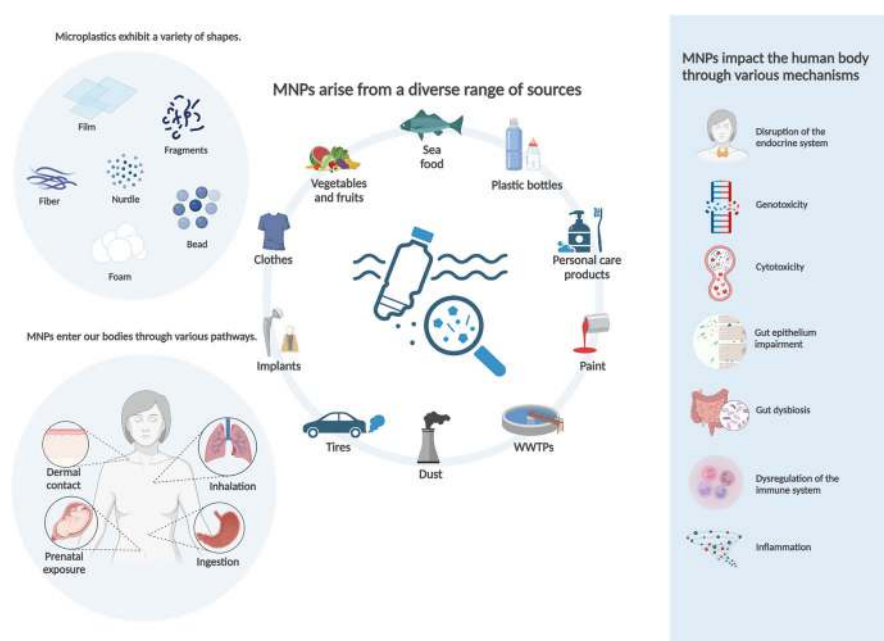


Fig. 1 Micro- and nanoplastics are either manufactured or made during the mechanical abrasion of plastic products. MPs can be in the form of film, fibers, nurdles, fragments, beads, and foams. Humans are constantly exposed to MNPs through inhalation, ingestion, skin, and even placenta during prenatal contact. The entered particles can show the effects through hormonal mimicry and disrupting the endocrine system, gut epithelium impairment, gut dysbiosis, contributing to inflammation, and disrupting the immune system, therefore, increasing the risk of mutation and genotoxicity

8 Microplastic and Cancers

8.1 Gastric Cancer

Gastric cancer is the fifth most commonly diagnosed malignancy and the third cause of death related to cancer worldwide. The annual burden is predicted to increase to about 1.8 million new cases and 1.3 million deaths by 2040 (Morgan et al. 2022a, b). Considering that the gastrointestinal (GI) tract is a major route of exposure, MNPs directly interact with GI epithelium and pose a potential hazard. Kim et al. found that MNPs can accumulate in stomach cells after digestion. Prolonged exposure to MNPs disturbed the expression of genes and distribution of RNA isoforms involved in gastric cancer promotion, leading to increased tumor growth and survival in the rat. Gastric cancer hallmarks were also reinforced in gastric cancer cell lines, and resistance to multiple therapeutic drugs was observed (Kim et al. 2022).

Several mechanisms have been suggested; exposure to MNPs leads to exacerbated immune response and accelerated proliferation, cell invasion, and migration in human gastric cancer (Hwang et al. 2020; Yan et al. 2021; Kim et al. 2022). Oxidative stress, activated apoptosis-related signaling pathways, and DNA chain break in gastric cancer cells were observed post MPs exposure (Yan et al. 2020).

A recent study demonstrated that *Helicobacter pylori* (H.pylori) which is selectively colonized on the gastric mucosal surface and is a potential carcinogen for gastric cancer itself, can attach to the surfaces of MPs to form complex biofilms. The formation facilitates H.pylori colonization in mice's stomachs and allows microplastics with a larger diameter to the intestinal tissue, synergistically promoting gastric injury and inflammation (Tong et al. 2022).

Furthermore, plastic additives such as BPA and phthalates stimulate the mechanisms involved in gastric cancer progression (Hanafi et al. 2019; Nyman and Ennis et al. 2019). A positive association has also been observed between PAH exposure and the risk of gastric cancer (Liao et al. 2014). As shown in Fig. 2 several cancers are demonstrated to be in association with MNPs exposure.

8.2 Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of death related to malignancies worldwide. CRC has the second-highest cancer treatment cost, and the incidence and mortality burden are believed to constantly increase (Morgan et al. 2022b). Importantly, investigations have found that the tumoral tissue of the colon in patients with colorectal adenocarcinoma, compared to the non-tumorous tissue of the colon in the control group, contained higher amounts of MPs, which suggests a possible relationship between the MPs exposure and the increased risk of CRC incidence (Cetin et al. 2023). Epidemiological studies of occupational risk factors for CRC also indicated that exposure to

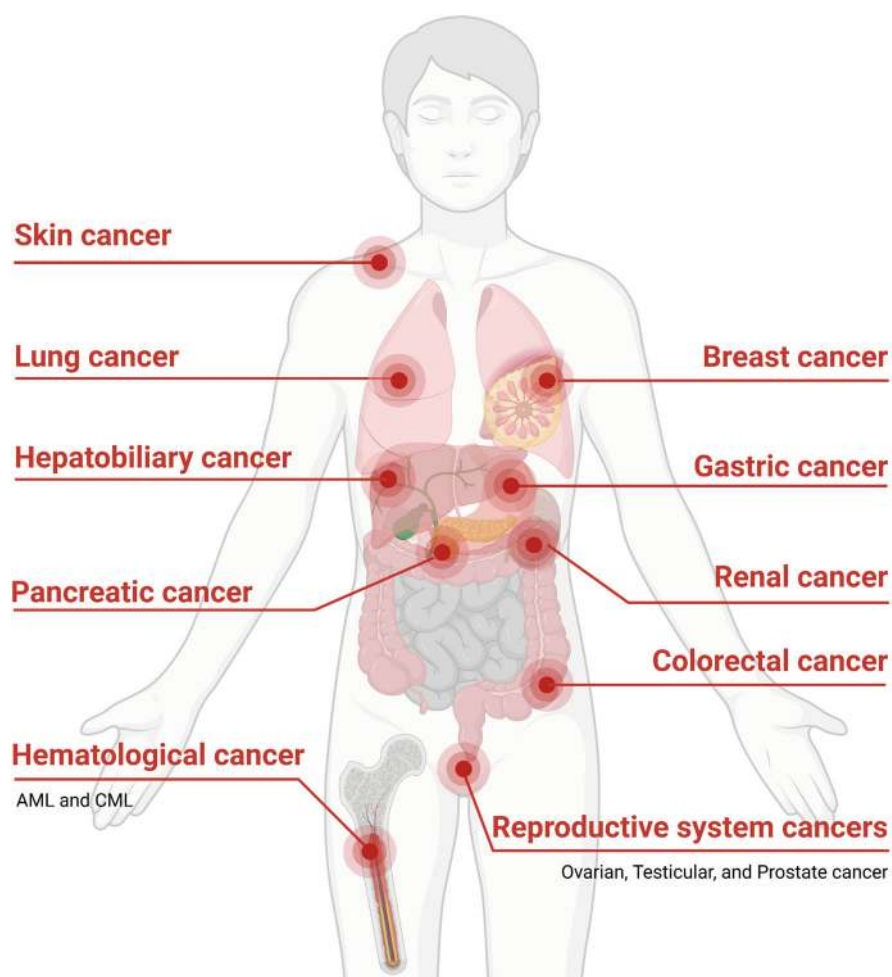


Fig. 2 Micro- and nanoplastics can contribute to the development of several types of cancer in both men and women. Hepatocellular carcinoma, pancreatic, gastric, and colorectal cancers are reported to be associated with MNPs exposure. Lung, skin, renal, and hematological malignancies, i.e., acute myeloid leukemia and chronic lymphocytic leukemia, and cancers of the reproductive system including breast, ovarian, and prostatic are other types of malignancies

plastics might be a potential cause of CRC (Acquavella et al. 1989; Oddone et al. 2014).

As previously stated, MPs could induce intestinal inflammation and dysbacteriosis. Investigating both in-vitro studies and in-vivo models found that MNPs exposure is related to increased ROS production in colorectal cells that cause oxidative damage and induce apoptosis, necrosis, genotoxicity, and possible carcinogenesis (Shi et al. 2014; Vecchiotti et al. 2021). Furthermore, both BPA and phthalate as by-products of the plastic industry are related to colon cancer

progression. Tumor metastasis and chemoresistance related to BPA and phthalate exposure is observed in colon cancer cells (Chen et al. 2018; Xia et al. 2022). Gut microflora dysbiosis is one of the interesting findings in patients with colorectal malignancies (Gagnière et al. 2016). A change in the gut microbiota occurs following exposure to microplastics. It can be involved in the development and progression of CRC, which is observed by reducing the diversity of resident bacterial species and the enrichment of the detrimental bacteria population (Sánchez-Alcoholado et al. 2020; Souza-Silva et al. 2022; Zhang et al. 2022a, b, c, d).

8.3 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the primary and most prevalent type of liver cancer globally, and develops in patients with long-term liver disease (Rumgay et al. 2022). Cirrhosis and hepatitis, which cause chronic inflammation in liver tissue, are known as the major risk factors for HCC (Janevska et al. 2015). It takes years for a cancerous hepatic lesion to develop as it grows slowly, but some toxic chemicals could enhance the process, including plastics and their additives (Erkekoglu et al. 2017).

Tissue sampling of patients with liver cirrhosis showed significantly higher MPs concentrations than individuals without liver diseases (Horvatits et al. 2022). Furthermore, Donato et al. found significantly high serum levels of Polychlorinated biphenyls (PCBs) prior to the HCC diagnosis in patients (Donato et al. 2021).

According to the current evidence, there are different mechanisms by which MNPs exposure can accelerate or induce HCC. For instance, PCB, a highly carcinogenic organic compound previously used in plastic synthesis and found in MPs, exposures induced significant lipidomic changes related to malignancy and tumor metastasis and initiated processes involved in damage to the DNA and increased oxidative stress levels in HCC (Ludewig and Robertson 2013; Menéndez-Pedriz et al. 2022). Cheng et al. also provided evidence of MPs-related hepatotoxicity in the human pluripotent stem cells-derived liver organoids arising from altered lipid metabolism, increased ROS production, excessive oxidative stress, and chronic inflammation (Cheng et al. 2022). MPs exposure can directly damage the DNA of both the nucleus and mitochondria and initiated the downstream cascade of inflammation and liver fibrosis (Shen et al. 2022). Reports also documented the hepatic tumors incidence following BPA, phthalates, and PAH exposure which are all commonly found in MP particles (Weinhouse et al. 2014; Wang and Xue 2015; Čtveráková et al. 2020).

8.4 Pancreatic Cancer

Pancreatic cancer is one of the most aggressive cancers, with increasing incidence rates and low improvement in survival rates, originating from the exocrine or endocrine tissue of the pancreas (Khalaf et al. 2021). Few studies have been

conducted to assess the impacts of MNPs on pancreatic cancers. The results of a case-control study showed an increased incidence of pancreatic cancer among plastic workers in the USA (Selenskask et al. 1995).

Another study demonstrated a higher concentration of MPs derivatives in the KRAS (an oncogenic signaling pathway involved in pancreatic ductal adenocarcinoma and exocrine pancreatic cancer) mutant compared to the non-mutant group (Baj et al. 2022). Activation of the KRAS gene was also reported in exocrine pancreatic cancer patients with a history of occupational exposure to PAH (Alguacil et al. 2003). Experimental evidence also associates BPA and phthalate exposure with carcinogenesis of endoderm glands, including the pancreas (Wang et al. 2012; Cuomo et al. 2017). However, more extensive research is needed to determine the precise link between MPs and pancreatic cancer.

8.5 Lung Cancer

Lung cancer is the leading cause of cancer mortality and morbidity globally and an emerging public health concern (Thandra et al. 2021). The first report of inhaled nonmineral fibers, plant and plastic, in human tissue back to 1998 in a study by Pauly and colleagues (Pauly et al. 1998), in which bioresistant plastic fibers were introduced as candidate agents increasing the lung cancer risk. According to the study, inhaled fibers were found in 83% (67 of 81 specimens) of non-neoplastic lung specimens. However, this rate was reported in 97% (32 of 33 specimens) of malignant lung specimens. Twenty-three years later, a recent study characterized the environmental MPs found in human lungs. Polypropylene and polyethylene were the most frequent polymers, which also were more prevalently found than fibers. All polymeric particles were smaller than 5.5 μm in size (Amato-Lourenço et al. 2021).

As humans persistently inhale MNPs particles, the epithelial surface of the respiratory system is constantly under its effects. As previously demonstrated, prolonged exposure to MPs causes disruption of lung barrier integrity by the increased inflammatory response (Donkers et al. 2022; Lu et al. 2022). Induced ROS production, redox imbalance, epithelial cell apoptosis, and overproduction of TNF- α , IL-6, TGF- β , and IL-1 β contribute to the initiation of the inflammatory process and interference with surfactant biophysical function were observed post-MP exposure in lung epithelial cells (Yang et al. 2021a, b; Lu et al. 2022). Genotoxicity and cytotoxicity may be enhanced by further intracellular accumulation of MNPs (Shi et al. 2022).

Environmental MPs exposure predisposes the lungs to ground glass nodule formation (Chen et al. 2021), which is an important clinical dilemma in oncology as lung cancer indicators (Lee 2015). Workplace exposure in plastics and rubber manufacturing is also associated with an increased risk of lung cancer development and mortality (DeBono et al. 2020). Additionally, BPA exposure may stimulate migration and invasion of the lung cancer cells by upregulating the matrix metalloproteinases signaling pathway (Zhang et al. 2014). Similarly, phthalates

promote DNA damage, cell proliferation, and inflammation via activating Akt/NF- κ B and Wnt/ β -catenin signaling pathways in lung cancer cell lines (Kim 2019; Urade et al. 2023). It is also reported that exposure to PCB and PAH could induce oxidative stress and cell proliferation and promote lung cancer (Recio-Vega et al. 2013; Stading et al. 2021).

8.6 Breast Cancer

This cancer is the most common invasive cancer diagnosed in women, and the incidence continues to increase until it overtakes lung cancer. One out of every eight women will be diagnosed with breast cancer, and 3% (1 in 39) will not survive the disease (Giaquinto et al. 2022). Epidemiological studies confirmed estrogen as a major carcinogen for breast cancer development (Russo and Russo 2006).

Although clinical studies have yet to show a link between MPs and the risk of breast cancer, such a link is probable. A causal association has also been observed between an individual's exposure to PAH and PCB and breast cancer incidence and progression (Rundle et al. 2000; Rodgers et al. 2018). Observational studies on breast cancer risk and phthalate suggest breast tumor growth promotion by phthalate through estrogen receptor (ER) signaling (Ahern et al. 2019; Wu et al. 2021). Epidemiological studies also demonstrated an increased risk of breast cancer due to occupational exposure in plastic industries among premenopausal women (Brophy et al. 2012).

As previously discussed MNPs contain a mixture of EDCs with the ability of estrogen mimicry. Shreds of evidence confirmed BPA as an important breast cancer stimulator. Carcinogenic effects were observed on breast cancer cells following low-dose BPA exposure. The compound can damage the DNA structure, cause epigenetic changes, influence stem cell differentiation, and alter the cancer microenvironment (Wang et al. 2017). Palacios-Arreola et al. observed and evaluated endocrine alterations and cytokine expression along with increased infiltration and metastasis of breast cancer cells into the lungs of murine models induced by BPA injection (Palacios-Arreola et al. 2021). In vitro studies have demonstrated that BPA exposure can alter several molecular pathways related to breast cancer. These include the G protein-coupled receptor (GPER) pathway, estrogen-related receptor gamma (ERR γ) pathway, the HOXB9 (a homeobox gene) pathway, bone morphogenetic proteins 2 and 4 (BMP2 and BMP4) pathways. Additionally, BPA exposure leads disruptions in immunoregulatory cytokines and impacts the EGFR-STAT3 pathway. There is also an increased expression of breast cancer markers, such as SOX2, which is a cancer stem cell marker (Lillo et al. 2017; Shafei et al. 2018). Interestingly, prenatal exposure to BPA leads to the development of larger breast tumors in adult mice and changes the tumor microenvironment by modulating the infiltration of immune cells (Palacios-Arreola et al. 2017). Furthermore, MNPs synergistically increase the paraben effect on estrogen-sensitive breast cancer cell proliferation (Roje et al. 2019).

8.7 Ovarian Cancer

Ovarian cancer is the third most common gynecological cancer among women after cervical and uterine cancer, and the overall burden is on the rise. Ovarian cancer is a highly aggressive malignancy, three times more lethal than breast cancer (Momenimovahed et al. 2019; Zhang et al. 2022a, b, c, d).

Experiments indicated that MPs particles could be taken by ovarian granulosa cells, accumulate in ovary tissue, increase IL-1 β and IL-18 production, and induce pyroptosis and apoptosis (Recio-Vega et al. 2013). MNPs are able to induce cellular toxicity in the mammalian ovary by causing oxidative stress and membrane damage (Stading et al. 2021). Some epidemiological studies suggest a positive association between occupational exposure in plastic industries and an increased risk of ovarian cancer (Shields et al. 2002).

It has been long recognized that estrogen and ER dysfunction have strong association with the risk of ovarian cancer development (Cunat et al. 2004). As BPA mimics the effects of estrogen, evidence is suggestive of the theory that BPA is implicated in ovarian malignancy occurrence in animal models and humans (Hui et al. 2018). Similarly, phthalates can exert estrogenic effects; therefore, stimulate cell growth in ER-positive cancer cells via upregulation of downstream target genes such as cyclin D and cyclin-dependent kinase-4 (CDK-4) (Park et al. 2012). Furthermore, biomarkers related to tumor initiation and resistance to chemotherapeutic drugs are expressed under low-dose of BPA and PCB (Guo et al. 2020). A causal association is also discovered between PAH exposure and increased risk of ovarian cancer, detected by increased human epididymis protein 4 (HE4) levels, a potential crucial biomarker for ovarian cancer (Liang et al. 2022).

8.8 Prostate Cancer

Prostate cancer is the second most frequent cancer and the fifth leading cause of death due to malignancy in reported males (Wang et al. 2022). Similar to breast and ovarian cancer, prostate cancer is an endocrine-related malignancy that can be predisposed by estrogen (Dobbs et al. 2019).

Some environmental and occupational factors may drive the risk of metastatic prostate cancer (Vigneswaran et al. 2021). Prostate carcinogenesis due to epigenome alternation and DNA damage is reported after early life exposure to BPA (Prins 2008). In addition, prostate cancer cell lines exposed to this compound have shown signs of early-onset prostate cancer and centrosome amplification (Tarapore et al. 2014).

Similar to BPA a clear relationship is demonstrated between phthalate and prostate cancer. Phthalate metabolites increase prostate-specific antigen (PSA) and activate MAPK/AP-1 pathway that potentially contributes to cell proliferation in prostate cancer (Zhu et al. 2018; Guo et al. 2023). A higher urinary concentration of phthalate metabolites is also found in obese patients with prostate cancer (Chuang et al. 2020). Findings from the observational and experimental studies found PCB as

a significant environmental risk factor for high-grade and fatal prostate cancer (Emeville et al. 2015; Ali et al. 2016). The risk of prostate cancer is also increased in individuals exposed at high levels to occupational PAH (Rybicki et al. 2006; Barul and Parent 2021).

8.9 Testicular Cancer

Testicular cancer is a relatively rare cancer, although incidence rates have raised over the last decade and the chance of development reached about one in 270 (Znaor et al. 2022). It has been documented that MNPs can pass across the blood-testis barrier and affect fertility by reducing the sperms motility and concentration in both animals and humans (Zhang et al. 2022a, b, c, d). Experimental studies on animals revealed harmful effects of NPs exposure on signaling pathways testis that facilitate biological processes, including cell growth, proliferation, differentiation, apoptosis, and survival. Other signaling pathways that orchestrate cancer progression, survival, migration, adhesion, angiogenesis, and metastasis can also be activated by NPs (Xiao et al. 2023). Consumption of specific personal care products containing amounts of EDCs such as phthalates BPA, especially in the prenatal period is reported to be associated with a higher risk of testicular cancer (Bräuner et al. 2021). Although the results of the epidemiological study are controversial, occupational exposure to plastics might be a risk factor for this cancer, especially seminoma (Hansen 1999; Ohlson and Hardell 2000; Hardell et al. 2004; McGlynn and Trabert 2012).

8.10 Skin Cancer

Skin cancer, both melanoma and non-melanoma, are now among the most prevalent cancers, especially among the white population. One in five Americans will develop skin cancer in their lifetime, and the increasing incidence is at an alarming rate for a global epidemic (Leiter et al. 2020).

Schmidt et al. showed that being exposed to MNPs for the long term promotes intracellular ROS production and modulates transcriptional patterns of apoptosis-related genes in skin cells. Oxidative stress in skin cells affects and reorganizes the actin cytoskeleton architecture, disturbs the cell shape, motility, and gene expression, and also downregulates keratin 14 (Krt14) (Schmidt et al. 2023). Krt14 participates in the maintenance of skin cell integrity, and its dysfunction is correlated with aggressive skin lesions, including malignant melanoma (Roth et al. 2012; Han et al. 2021). Studies in animal models showed potential tumorigenesis and persistent inflammation in skin tissue after BPA exposure (Ju and Zouboulis 2016; Kitagawa et al. 2023). Numerous reports confirmed the role of PAH as an oncogene, causing DNA damage in skin cells that may be the initiating step in carcinogenesis (Ju and Zouboulis 2016). Cao et al. found a positive association between PCB plasma level and the risk of malignant melanoma (Cao et al. 2019). A meta-analysis based on

epidemiological studies also confirmed the link between PCB exposure and the incidence of melanoma (Zani et al. 2017).

8.11 Renal Cancer

Kidney cancer is one of the ten most common neoplasms in both men and women (2023). Occupational exposure is believed to play a substantial role in renal cancer pathogenesis (Scelo and Larose 2018). Xiao et al. conducted an in vivo study that showed signaling pathways in cancer development, including the MAPK signaling pathway post-MNP, and involved proteins dysregulated after exposure in renal cells (Xiao et al. 2023). MPs leave nephrotoxic effects on human kidney tissue by inducing oxidative stress, inflammation, mitochondrial dysfunction, and autophagy which may initiate tumorigenesis (Wang et al. 2021a, b, c; Chen et al. 2022a, b). Studies have confirmed that long exposure to NPs jeopardizes kidney function by affecting the cell cycle and viability, leading to renal failure (Atis et al. 2005) and maybe carcinogenesis.

8.12 Hematological Cancer

Hematologic cancers are a heterogeneous subgroup group of malignancies, accounting for 6.5% of all cancers worldwide, originating from the uncontrolled division of hematopoietic stem cells of the bone marrow and lymphoid tissues. Since the hematological system mediates the transfer of MPs in the body, MPs could accumulate in the bloodstream as in the destination organs (Leslie et al. 2022).

Although a clear connection has not yet been found between MPs exposure and the incidence of hematological malignancies, studies show hematological system disturbance in contact with MPs. Hematopoietic impairment has been found in marine animals and mammals exposed to MPs caused by bone marrow damage and altered gene expression (Kim et al. 2021; Xu et al. 2021). MPs hematotoxicity can be manifested by the interference in metabolic pathways, cellular misarrangement, inhibited colony-forming, reduced self-renewal and differentiation capacity, decreased number of granulocytes, and increased lymphocytes in the systemic circulation (Sun et al. 2021; Jing et al. 2022). NPs exposure contributes to the downregulation of the T cell differentiation signaling pathway into the CD8⁺ T cells subtype in the murine spleen and multiplies the risk of cancer and infection. Mitochondrial function and cell viability are also adversely affected in the exposed splenic lymphocytes (Li et al. 2022). Sun et al. have found that MPs exposure may lead to cell cycle disturbance through the Jak/Stat pathway which is one of the known pathways with a key role in hematological malignancies (Mughal et al. 2014; Sun et al. 2021). Furthermore, BPA is able to promote acute myeloid leukemia (AML) proliferation, and evidence supports the link between PCB and B-cell chronic lymphocytic leukemia (CML) (Georgiadis et al. 2019; Zhang et al. 2020).

According to the observations made so far, further studies are needed to clarify the impact of microplastic exposure on the incidence of hematological cancers.

9 Conclusion

MNPs are an emerging public health issue due to the inevitable exposure and different mechanisms of effects leading to mutations. Using less plastic and properly recycling are suggested to prevent these particles from spreading more. MNPs have been reported to be associated with lung, skin, renal, and hematological malignancies, including AML and CML, and cancers of the reproductive system, including breast, ovarian, and prostatic cancer. The mechanisms by which MNPs contribute to cancer development are yet to be clearly described; however, inducing physical disruption of the gut epithelium, promoting pro-inflammatory pathways, and carcinogenic effects of plastics additives are among the most important suggested ways MNPs could contribute to cancer development in human bodies. Given the carcinogenic potentials and types of cancers discussed in the review, managing MNPs contamination might significantly reduce the rate of cancer incidence worldwide. Thus, more research and interdisciplinary cooperation are needed to be done in this field with being focused on cancers and these plastic particles.

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References

- (2019) Detection of various microplastics in human stool. *Ann Internal Med* 171:453–457
- (2023) Key statistics about kidney cancer. from <https://www.cancer.org/cancer/kidney-cancer/about/key-statistics.html#:~:text=How%20common%20is%20kidney%20cancer,will%20die%20from%20this%20disease>
- (3/21/2023) Is exposure to a specific carcinogen associated with a certain type of cancer? Retrieved 3/21/2023, 2023, from https://www.ccohs.ca/oshanswers/diseases/cancer/carcinogen_site.html
- Acquavella JF, Douglass TS, Vernon S, Hughes JI, Thar WE (1989) Assessment of colorectal cancer screening outcomes among workers involved in polypropylene manufacture. *Journal of occupational medicine*:785–791, 31
- Ahern TP, Broe A, Lash TL, Cronin-Fenton DP, Ulrichsen SP, Christiansen PM, Cole BF, Tamimi RM, Sørensen HT, Damkier P (2019) Phthalate exposure and breast cancer incidence: a Danish Nationwide cohort study. *J Clin Oncol* 37:1800–1809

- Ahmad Kendong SM, Raja Ali RA, Nawawi KNM, Ahmad HF, Mokhtar NM (2021) Gut dysbiosis and intestinal barrier dysfunction: potential explanation for early-onset colorectal cancer. *Front Cell Infect Microbiol* 11:1244
- Ahmad N, Kumar R (2011) Steroid hormone receptors in cancer development: a target for cancer therapeutics. *Cancer Lett* 300:1–9
- Akovali G (2012). 2 – plastic materials: polyvinyl chloride (PVC). Pacheco-Torgal F, Jalali S, Fucic A (eds), *Toxicity of building materials*. Woodhead Publishing, pp 23–53
- Alabi OA, Ologbonjaye KI, Awosolu O, Alalade OE (2019) Public and environmental health effects of plastic wastes disposal: a review. *J Toxicol Risk Assess* 5:1–13
- Alguacil J, Porta M, Kauppinen T, Malats N, Kogevinas M, Carrato A, Group fitPIS (2003) Occupational exposure to dyes, metals, polycyclic aromatic hydrocarbons and other agents and K-ras activation in human exocrine pancreatic cancer. *Int J Cancer* 107:635–641
- Ali I, Julin B, Glynn A, Högborg J, Berglund M, Johansson J-E, Andersson S-O, Andrén O, Giovannucci E, Wolk A, Stenius U, Åkesson A (2016) Exposure to polychlorinated biphenyls and prostate cancer: population-based prospective cohort and experimental studies. *Carcinogenesis* 37:1144–1151
- Alimba CG, Faggio C, Sivanesan S, Ogunkanmi AL, Krishnamurthi K (2021) Micro(nano)-plastics in the environment and risk of carcinogenesis: insight into possible mechanisms. *J Hazard Mater* 416:126143
- Allen S, Allen D, Phoenix VR, Le Roux G, Durántez Jiménez P, Simonneau A, Binet S, Galop D (2019) Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nat Geosci* 12:339–344
- Allen S, Allen D, Karbalaei S, Maselli V, Walker TR (2022) Micro (nano) plastics sources, fate, and effects: what we know after ten years of research. *J Hazard Mater Adv* 6:100057
- Almeida S, Raposo A, Almeida-González M, Carrascosa C (2018) Bisphenol a: food exposure and impact on human health. *Compr Rev Food Sci Food Saf* 17:1503–1517
- Amato-Lourenço LF, Carvalho-Oliveira R, Júnior GR, dos Santos GL, Ando RA, Mauad T (2021) Presence of airborne microplastics in human lung tissue. *J Hazard Mater* 416:126124
- Amran NH, Zaid SSM, Mokhtar MH, Manaf LA, Othman S (2022) Exposure to microplastics during early developmental stage: review of current evidence. *Toxics* 10
- Andrady AL (2011) Microplastics in the marine environment. *Mar Pollut Bull* 62:1596–1605
- Andrady AL (2017) The plastic in microplastics: a review. *Mar Pollut Bull* 119:12–22
- Arcaro KF, O'Keefe PW, Yang Y, Clayton W, Gierthy JF (1999) Antiestrogenicity of environmental polycyclic aromatic hydrocarbons in human breast cancer cells. *Toxicology* 133:115–127
- Arthur C, Baker JE, Bamford HA (2009) Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris, September 9–11, 2008. University of Washington Tacoma, Tacoma
- Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan T-J, Campbell BJ, Abujamel T, Dogan B, Rogers AB (2012) Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338:120–123
- Atis S, Tutluoglu B, Levent E, Ozturk C, Tunaci A, Sahin K, Saral A, Oktay I, Kanik A, Nemery B (2005) The respiratory effects of occupational polypropylene flock exposure. *Eur Respir J* 25: 110–117
- Avio CG, Gorbi S, Milan M, Benedetti M, Fattorini D, d'Errico G, Pauletto M, Bargelloni L, Regoli F (2015) Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environ Pollut* 198:211–222
- Babaremu KO, Okoya SA, Hughes E, Tijani B, Teidi D, Akpan A, Igwe J, Karera S, Oyinlola M, Akinlabi ET (2022) Sustainable plastic waste management in a circular economy. *Heliyon* 8: e09984
- Babu RP, O'Connor K, Seeram R (2013) Current progress on bio-based polymers and their future trends. *Prog Biomater* 2:8
- Baitz M, Kreissig J, Makishi C (2005) Life cycle assessment of PVC in product optimisation and green procurement & 8211; fact-based decisions towards sustainable solutions. *Plastics Rubber and Composites – PLAST RUBBER COMPOS* 34

- Baj J, Dring JC, Czezelewski M, Kozyra P, Forma A, Flieger J, Kowalska B, Buszewicz G, Teresiński G (2022) Derivatives of plastics as potential carcinogenic factors: the current state of knowledge. *Cancers* 14. <https://doi.org/10.3390/cancers14194637>
- Ballesteros S, Domenech J, Bargailla I, Cortés C, Marcos R, Hernández A (2020) Genotoxic and immunomodulatory effects in human white blood cells after ex vivo exposure to polystyrene nanoplastics. *Environ Sci Nano* 7:3431–3446
- Barale R (1991) The genetic toxicology of styrene and styrene oxide. *Mutat Res/Rev Genet Toxicol* 257:107–126
- Barboza LGA, Dick Vethaak A, Lavorante B, Lundebye AK, Guilhermino L (2018) Marine microplastic debris: an emerging issue for food security, food safety and human health. *Mar Pollut Bull* 133:336–348
- Barboza LGA, Cózar A, Gimenez BCG, Barros TL, Kershaw PJ, Guilhermino L (2019). Chapter 17 – acropastics pollution in the marine environment. *World seas: an environmental evaluation*, 2nd edn.. C. Sheppard, Academic, pp 305–328
- Barul C, Parent M-E (2021) Occupational exposure to polycyclic aromatic hydrocarbons and risk of prostate cancer. *Environ Health* 20:71
- Basri K S, Daud A, Astuti RDP, K B (2021) Detection of exposure to microplastics in humans: a systematic review. *Open Access Maced J Med Sci* 9:275–280
- Bhatnagar A, Anastopoulos I (2017) Adsorptive removal of bisphenol a (BPA) from aqueous solution: a review. *Chemosphere* 168:885–902
- Bidoki SM, Wittlinger R (2010) Environmental and economical acceptance of polyvinyl chloride (PVC) coating agents. *J Clean Prod* 18:219–225
- Bigbee WL, Grant SG, Langlois RG, Jensen RH, Anttila A, Pfäffli P, Pekari K, Norppa H (1996) Glycophorin a somatic cell mutation frequencies in Finnish reinforced plastics workers exposed to styrene. *Cancer Epidemiol Biomarkers Prev* 5:801–810
- Borrelle SB, Ringma J, Law KL, Monnahan CC, Lebreton L, McGivern A, Murphy E, Jambeck J, Leonard GH, Hilleary MA, Eriksen M, Possingham HP, De Frond H, Gerber LR, Polidoro B, Tahir A, Bernard M, Mallos N, Barnes M, Rochman CM (2020) Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science* 369:1515–1518
- Boucher J, Friot D (2017) Primary microplastics in the oceans: a global evaluation of sources. IUCN Gland, Switzerland
- Brahney J, Mahowald N, Prank M, Cornwell G, Klimont Z, Matsui H, Prather KA (2021) Constraining the atmospheric limb of the plastic cycle. *Proc Natl Acad Sci* 118:e2020719118
- Braun T, Ehrlich L, Henrich W, Koepfel S, Lomako I, Schwabl P, Liebmann B (2021) Detection of microplastic in human placenta and meconium in a clinical setting. *Pharmaceutics* 13
- Bräuner EV, Lim Y-H, Koch T, Uldbjerg CS, Gregersen LS, Pedersen MK, Frederiksen H, Petersen JH, Coull BA, Andersson A-M, Hickey M, Skakkebaek NE, Hauser R, Juul A (2021) Endocrine disrupting chemicals and risk of testicular cancer: a systematic review and meta-analysis. *J Clin Endocrinol Metabol* 106:e4834–e4860
- Brophy JT, Keith MM, Watterson A, Park R, Gilbertson M, Maticka-Tyndale E, Beck M, Abu-Zahra H, Schneider K, Reinhartz A, DeMatteo R, Luginaah I (2012) Breast cancer risk in relation to occupations with exposure to carcinogens and endocrine disruptors: a Canadian case-control study. *Environ Health* 11:87
- Browne MA (2015) Sources and pathways of microplastics to habitats:229–244
- Campanale C, Massarelli C, Savino I, Locaputo V, Uricchio VF (2020) A detailed review study on potential effects of microplastics and additives of concern on human health. *Int J Environ Res Public Health* 17
- Cantonwine DE, Hauser R, Meeker JD (2013) Bisphenol a and human reproductive health. *Expert rev. Obstet Gynecol* 8:329
- Cao J, Fan T, Li W, Xiao S (2019) Association study between plasma levels of polychlorinated biphenyls and risk of cutaneous malignant melanoma. *Environ Int* 126:298–301
- Cao J, Shi Y, Yan M, Zhu H, Chen S, Xu K, Wang L, Sun H (2023) Face mask: as a source or protector of human exposure to microplastics and phthalate plasticizers? *Toxics*:11

- Carney Almroth B, Eggert H (2019) Marine plastic pollution: sources, impacts, and policy issues. *Rev Environ Econ Policy* 13:317–326
- Cássio F, Batista D, Pradhan A (2022) Plastic interactions with pollutants and consequences to aquatic ecosystems: what we know and what we do not know. *Biomolecules*:12
- Castrogiovanni F, Borea L, Corpuz MVA, Buonerba A, Vigliotta G, Ballesteros F Jr, Hasan SW, Belgiorio V, Naddeo V (2022) Innovative encapsulated self-forming dynamic bio-membrane bioreactor (ESFDMBR) for efficient wastewater treatment and fouling control. *Sci Total Environ* 805:150296
- Celebi Sözen Z, Cevhertas L, Nadeau K, Akdis M, Akdis CA (2020) Environmental factors in epithelial barrier dysfunction. *J Allergy Clin Immunol* 145:1517–1528
- Celis JE, Espejo W, Paredes-Osses E, Contreras SA, Chiang G, Bahamonde P (2021) Plastic residues produced with confirmatory testing for COVID-19: classification, quantification, fate, and impacts on human health. *Sci Total Environ* 760:144167
- Cetin M, Demirkaya Miloglu F, Kilic Baygutalp N, Ceylan O, Yildirim S, Eser G, Gul Hİ (2023) Higher number of microplastics in tumoral colon tissues from patients with colorectal adenocarcinoma. *Environ Chem Lett* 21:639–646
- Chen HP, Lee YK, Huang SY, Shi PC, Hsu PC, Chang CF (2018) Phthalate exposure promotes chemotherapeutic drug resistance in colon cancer cells. *Oncotarget* 9:13167–13180
- Chen Q, Gao J, Yu H, Su H, Yang Y, Cao Y, Zhang Qu, Ren Y, Shi H, Chen C, Liu H (2021) An emerging role of microplastics in the etiology of lung ground glass nodules. *medRxiv:2021.2004.2022.21255586*
- Chen G, Fu Q, Tan X, Yang H, Luo Y, Shen M, Gu Y (2022a) Speciation and release risk of heavy metals bonded on simulated naturally-aged microplastics prepared from artificially broken macroplastics. *Environ Pollut* 295:118695
- Chen Y-C, Chen K-F, Lin K-YA, Chen J-K, Jiang X-Y, Lin C-H (2022b) The nephrotoxic potential of polystyrene microplastics at realistic environmental concentrations. *J Hazard Mater* 427:127871
- Cheng H, Li L, Zhang M, Jiang Y, Yu P, Ma F, Mao L (2018) Recent advances on in vivo analysis of ascorbic acid in brain functions. *TrAC Trends Anal Chem* 109:247–259
- Cheng YL, Kim J-G, Kim H-B, Choi JH, Tsang YF, Baek K (2021) Occurrence and removal of microplastics in wastewater treatment plants and drinking water purification facilities: a review. *Chem Eng J* 410:128381
- Cheng W, Li X, Zhou Y, Yu H, Xie Y, Guo H, Wang H, Li Y, Feng Y, Wang Y (2022) Polystyrene microplastics induce hepatotoxicity and disrupt lipid metabolism in the liver organoids. *Sci Total Environ* 806:150328
- Cheung PK, Fok L (2017) Characterisation of plastic microbeads in facial scrubs and their estimated emissions in mainland China. *Water Res* 122:53–61
- Chiu H-W, Xia T, Lee Y-H, Chen C-W, Tsai J-C, Wang Y-J (2015) Cationic polystyrene nanospheres induce autophagic cell death through the induction of endoplasmic reticulum stress. *Nanoscale* 7:736–746
- Chowdhury GW, Koldewey HJ, Niloy MNH, Sarker S (2022) The ecological impact of plastic pollution in a changing climate. *Emerging Topics in Life Sciences* 6:389–402
- Chuang S-C, Chen H-C, Sun C-W, Chen Y-A, Wang Y-H, Chiang C-J, Chen C-C, Wang S-L, Chen C-J, Hsiung CA (2020) Phthalate exposure and prostate cancer in a population-based nested case-control study. *Environ Res* 181:108902
- Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. *Mar Pollut Bull* 62:2588–2597
- Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, Galloway TS (2013) Microplastic ingestion by zooplankton. *Environ Sci Technol* 47:6646–6655
- Courtene-Jones W, Quinn B, Gary S, Mogg A, Narayanaswamy B (2017) Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall trough, North Atlantic Ocean. *Environ Pollut* 231:271–280

- Cox KD, Covernton GA, Davies HL, Dower JF, Juanes F, Dudas SE (2019) Human consumption of microplastics. *Environ Sci Technol* 53:7068–7074
- Cózar A, Echevarría F, González-Gordillo JJ, Irigoien X, Ubeda B, Hernández-León S, Palma AT, Navarro S, García-de-Lomas J, Ruiz A, Fernández-de-Puelles ML, Duarte CM (2014) Plastic debris in the open ocean. *Proc Natl Acad Sci USA* 111:10239–10244
- Čtveráčková L, Jančula D, Raška J, Babica P, Sovadinová I (2020) Structure-dependent effects of phthalates on intercellular and intracellular communication in liver oval cells. *Int J Mol Sci* 21
- Cunat S, Hoffmann P, Pujol P (2004) Estrogens and epithelial ovarian cancer. *Gynecol Oncol* 94: 25–32
- Cuomo D, Porreca I, Cobellis G, Tarallo R, Nassa G, Falco G, Nardone A, Rizzo F, Mallardo M, Ambrosino C (2017) Carcinogenic risk and Bisphenol A exposure: a focus on molecular aspects in endoderm derived glands. *Mol Cell Endocrinol* 457:20–34
- Curren E, Leong SCY (2019) Profiles of bacterial assemblages from microplastics of tropical coastal environments. *Sci Total Environ* 655:313–320
- Danopoulos E, Twiddy M, Rotchell JM (2020) Microplastic contamination of drinking water: a systematic review. *PLoS One* 15:e0236838
- Darabi H, Baradaran A, Ebrahimpour K (2022) Subacute toxic effects of polyvinyl chloride microplastics (PVC-MPs) in juvenile common carp, *Cyprinus carpio* (Pisces: Cyprinidae). *Caspian J Environ Sci* 20:233–242
- Darbre (2015) *Endocrine disruption and human health*. Elsevier, New York
- de Souza Machado AA, Lau CW, Kloas W, Bergmann J, Bachelier JB, Faltin E, Becker R, Görlich AS, Rillig MC (2019) Microplastics can change soil properties and affect plant performance. *Environ Sci Technol* 53:6044–6052
- DeBono NL, Logar-Henderson C, Warden H, Shakik S, Dakouo M, MacLeod J, Demers PA (2020) Cancer surveillance among workers in plastics and rubber manufacturing in Ontario, Canada. *Occup Environ Med* 77:847–856
- Devane PA, Bourne RB, Rorabeck CH, MacDonald S, Robinson EJ (1995) Measurement of polyethylene wear in metal-backed acetabular cups: II. Clinical application. *Clin Orthopaedics Relat Res* 319:317–326
- Di Lorenzo M, Forte M, Valiante S, Laforgia V, De Falco M (2018) Interference of dibutylphthalate on human prostate cell viability. *Ecotoxicol Environ Saf* 147:565–573
- Dobbs RW, Malhotra NR, Greenwald DT, Wang AY, Prins GS, Abem MR (2019) Estrogens and prostate cancer. *Prostate Cancer Prostatic Dis* 22:185–194
- Domenech J, Marcos R (2021) Pathways of human exposure to microplastics, and estimation of the total burden. *Curr Opin Food Sci* 39:144–151
- Donato F, Moneda M, Portolani N, Rossini A, Molfino S, Ministrini S, Contessi GB, Pesenti S, De Palma G, Gaia A (2021) Polychlorinated biphenyls and risk of hepatocellular carcinoma in the population living in a highly polluted area in Italy. *Sci Rep* 11:3064
- Dong C-D, Chen C-W, Chen Y-C, Chen H-H, Lee J-S, Lin C-H (2020) Polystyrene microplastic particles: in vitro pulmonary toxicity assessment. *J Hazard Mater* 385:121575
- Donkers JM, Höppener EM, Grigoriev I, Will L, Melgert BN, van der Zaan B, van de Steeg E, Kooter IM (2022) Advanced epithelial lung and gut barrier models demonstrate passage of microplastic particles. *Microplast Nanoplast* 2:6
- Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin B (2015) Microplastic contamination in an urban area: a case study in greater Paris. *Environ Chem* 12:592–599
- Dris R, Gasperi J, Saad M, Mirande C, Tassin B (2016) Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? *Mar Pollut Bull* 104:290–293
- Duan Y, Xiong D, Wang Y, Zhang Z, Li H, Dong H, Zhang J (2021) Toxicological effects of microplastics in *Litopenaeus vannamei* as indicated by an integrated microbiome, proteomic and metabolomic approach. *Sci Total Environ* 761:143311
- Eerkes-Medrano D, Thompson RC, Aldridge DC (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res* 75:63–82

- Elena-Diana U-C, Ghinea C, Ca M, Simion I, Petraru M, Gavrilescu M (2020) Environmental impacts of polyvinyl chloride (PVC) production process
- Emeville E, Giusti A, Coumoul X, Thomé JP, Blanchet P, Multigner L (2015) Associations of plasma concentrations of dichlorodiphenyldichloroethylene and polychlorinated biphenyls with prostate cancer: a case-control study in Guadeloupe (French West Indies). *Environ Health Perspect* 123:317–323
- Enaud R, Prevel R, Ciarlo E, Beaufile F, Wieërs G, Guery B, Delhaes L (2020) The gut-lung Axis in health and respiratory diseases: a place for inter-organ and inter-kingdom Crosstalks. *Front Cell Infect Microbiol* 10:9
- Enyoh CE, Verla AW, Verla EN, Ibe FC, Amaobi CE (2019) Airborne microplastics: a review study on method for analysis, occurrence, movement and risks. *Environ Monit Assess* 191:668
- Enyoh CE, Shafea L, Verla AW, Verla EN, Qingyue W, Chowdhury T, Paredes M (2020) Microplastics exposure routes and toxicity studies to ecosystems: An overview. *Environ Anal Health Toxicol* 35:e2020004
- Eriksen M, Lebreton LC, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, Reisser J (2014) Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS One* 9:e111913
- Erkekoglu P, Oral D, Chao M-W, Kocer-Gumusel B (2017) Hepatocellular carcinoma and possible chemical and biological causes: a review. *J Environ Pathol Toxicol Oncol* 36:171
- Farah S, Anderson DG, Langer R (2016) Physical and mechanical properties of PLA, and their functions in widespread applications - a comprehensive review. *Adv Drug Deliv Rev* 107:367–392
- Feng G, Hu L, Ma Y, Jia P, Hu Y, Zhang M, Liu C, Zhou Y (2018) An efficient bio-based plasticizer for poly (vinyl chloride) from waste cooking oil and citric acid: synthesis and evaluation in PVC films. *J Clean Prod* 189:334
- Fenichel P, Chevalier N, Brucker-Davis F (2013) Bisphenol A: an endocrine and metabolic disruptor, vol 74. Elsevier, *Annales d'endocrinologie*, p 211
- Field DT, Green JL, Bennett R, Jenner LC, Sadofsky LR, Chapman E, Loubani M, Rotchell JM (2022) Microplastics in the surgical environment. *Environ Int* 170:107630
- Forte M, Iachetta G, Tussellino M, Carotenuto R, Prisco M, De Falco M, Laforgia V, Valiante S (2016) Polystyrene nanoparticles internalization in human gastric adenocarcinoma cells. *Toxicol In Vitro* 31:126–136
- Francová A, Chrástný V, Šillerová H, Vítková M, Kocourková J, Komárek M (2017) Evaluating the suitability of different environmental samples for tracing atmospheric pollution in industrial areas. *Environ Pollut* 220:286–297
- Fu C, Travers-Trolet M, Velez L, Grüss A, Bundy A, Shannon LJ, Fulton EA, Akoglu E, Houle JE, Coll M, Verley P, Heymans JJ, John E, Shin Y-J (2018) Risky business: the combined effects of fishing and changes in primary productivity on fish communities. *Ecol Model* 368:265–276
- Gagnière J, Raisch J, Veziat J, Barnich N, Bonnet R, Buc E, Bringer M-A, Pezet D, Bonnet M (2016) Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 22:501–518
- Galloway TS (2015) Micro-and nano-plastics and human health. *Marine Anthropog Lit*:343–366
- Galloway T, Galloway T (2015). Micro-and nano-plastics and Human Health: 343–366
- Gambino I, Bagordo F, Grassi T, Panico A, De Donno A (2022) Occurrence of microplastics in tap and bottled water: current knowledge. *Int J Environ Res Public Health* 19
- Gaston E, Woo M, Steele C, Sukumaran S, Anderson S (2020) Microplastics differ between indoor and outdoor air masses: insights from multiple microscopy methodologies. *Appl Spectrosc* 74: 1079–1098
- Gasull M, Pumarega J, Rovira G, López T, Alguacil J, Porta M (2013) Relative effects of educational level and occupational social class on body concentrations of persistent organic pollutants in a representative sample of the general population of Catalonia, Spain. *Environ Int* 60:190–201
- Ge X, Ma S, Huo Y, Yang Y, Luo X, Yu Y, An T (2022) Mixed bromine/chlorine transformation products of tetrabromobisphenol a: potential specific molecular markers in e-waste dismantling areas. *J Hazard Mater* 423:127126

- Genua F, Raghunathan V, Jenab M, Gallagher WM, Hughes DJ (2021) The role of gut barrier dysfunction and microbiome Dysbiosis in colorectal cancer development. *Frontiers. Oncology* 11
- Georgiadis P, Gavril M, Rantakokko P, Ladoukakis E, Botsivali M, Kelly RS, Bergdahl IA, Kiviranta H, Vermeulen RCH, Spaeth F, Hebbels D, Kleinjans JCS, de Kok T, Palli D, Vineis P, Kyrtopoulos SA (2019) DNA methylation profiling implicates exposure to PCBs in the pathogenesis of B-cell chronic lymphocytic leukemia. *Environ Int* 126:24–36
- Geyer R, Jambeck JR, Law KL (2017) Production, use, and fate of all plastics ever made. *Sci Adv* 3: e1700782
- Ghosh S, Chatterjee K (2020) Poly(ethylene glycol) functionalized graphene oxide in tissue engineering: a review on recent advances. *Int J Nanomedicine* 15:5991–6006
- Ghosh S, Sahu M (2022) Phthalate pollution and remediation strategies: a review. *J Hazard Mater Adv* 6:100065
- Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, Jemal A, Siegel RL (2022) Breast cancer statistics, 2022. *CA Cancer J Clin* 72:524–541
- Gieryńska M, Szulc-Dąbrowska L, Struzik J, Mielcarska MB, Gregorczyk-Zboroch KP (2022) Integrity of the intestinal barrier: the involvement of epithelial cells and microbiota: a mutual relationship. *Animals (Basel)* 12
- Giese A, Kerpen J, Weber F, Prediger J (2021) A preliminary study of microplastic abrasion from the screw cap system of reusable plastic bottles by Raman microspectroscopy. *ACS ES&T Water* 1:1363–1368
- Gigault J, Pedrono B, Maxit B, Ter Halle A (2016) Marine plastic litter: the unanalyzed nano-fraction. *Environ Sci Nano* 3:346–350
- Gigault J, El Hadri H, Nguyen B, Grassl B, Rowenczyk L, Tufenkji N, Feng S, Wiesner M (2021) Nanoplastics are neither microplastics nor engineered nanoparticles. *Nat Nanotechnol* 16:501–507
- Glenn GM, Orts W, Imam S, Chiou B-S, Wood DF (2014) Starch plastic packaging and agriculture applications. Elsevier, *Starch Polymers*, pp 421–452
- Gola D, Kumar Tyagi P, Arya A, Chauhan N, Agarwal M, Singh SK, Gola S (2021) The impact of microplastics on marine environment: a review. *Environ Nanotechnol Monitor Manage* 16: 100552
- Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT (2015) EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals. *Endocr Rev* 36:E1–e150
- Grafmueller S, Manser P, Diener L, Diener P-A, Maeder-Althaus X, Maurizi L, Jochum W, Krug HF, Buerki-Thurnherr T, Von Mandach U (2015) Bidirectional transfer study of polystyrene nanoparticles across the placental barrier in an ex vivo human placental perfusion model. *Environ Health Perspect* 123:1280–1286
- Greven AC, Merk T, Karagöz F, Mohr K, Klapper M, Jovanović B, Palić D (2016) Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 35:3093–3100
- Groschwitz KR, Hogan SP (2009) Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 124:3–20
- Gu W, Liu S, Chen L, Liu Y, Gu C, Ren H-q, Wu B (2020) Single-cell RNA sequencing reveals size-dependent effects of polystyrene microplastics on immune and secretory cell populations from zebrafish intestines. *Environ Sci Technol* 54:3417–3427
- Guerranti C, Martellini T, Perra G, Scopetani C, Cincinelli A (2019) Microplastics in cosmetics: environmental issues and needs for global bans. *Environ Toxicol Pharmacol* 68:75–79
- Guo Y, Li B, Yan X, Shen X, Ma J, Liu S, Zhang D (2020) Bisphenol A and polychlorinated biphenyls enhance the cancer stem cell properties of human ovarian cancer cells by activating the WNT signaling pathway. *Chemosphere* 246:125775
- Guo T, Meng X, Liu X, Wang J, Yan S, Zhang X, Wang M, Ren S, Huang Y (2023) Associations of phthalates with prostate cancer among the US population. *Reprod Toxicol* 116:108337

- Hafezi SA, Abdel-Rahman WM (2019) The endocrine disruptor Bisphenol a (BPA) exerts a wide range of effects in carcinogenesis and response to therapy. *Curr Mol Pharmacol* 12:230–238
- Hale RC, Seeley ME, La Guardia MJ, Mai L, Zeng EY (2020) A global perspective on microplastics. *J Geophys Res Oceans* 125:e2018JC014719
- Hamed M, Soliman HA, Osman AG, Sayed AE-DH (2020) Antioxidants and molecular damage in Nile tilapia (*Oreochromis niloticus*) after exposure to microplastics. *Environ Sci Pollut Res* 27: 14581–14588
- Han W, Hu C, Fan Z-J, Shen G-L (2021) Transcript levels of keratin 1/5/6/14/15/16/17 as potential prognostic indicators in melanoma patients. *Sci Rep* 11:1023
- Hanafi NI, Kadir SHSA, Musa M, Othman MHD, Kamaludin R, Zulkifli NA, Abd Latip N, Karim ZRA (2019) Low concentration of bisphenol A induces proliferation of gastric cancer cells, HGC-27. *Jurnal Teknologi*:81
- Hansen J (1999) Risk for testicular cancer after occupational exposure to plastics. *Int J Cancer* 82: 911–912
- Hardell L, Malmqvist N, Ohlson C-G, Westberg H, Eriksson M (2004) Testicular cancer and occupational exposure to polyvinyl chloride plastics: a case-control study. *International journal of cancer. J Intl du Cancer* 109:425–429
- Hartmann NB, Huffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M (2019) Are we speaking the same language? ACS Publications, Recommendations for a definition and categorization framework for plastic debris
- Hashemi Orimi SM, Khavarpour M, Kazemi S (2020) Removal of bisphenol a from water solution using molecularly imprinted nanopolymers: isotherm and kinetic studies. *J Water Environ Nanotechnol* 5:56–67
- Heidbreder LM, Steinhorst J, Schmitt M (2020) Plastic-free July: An experimental study of limiting and promoting factors in encouraging a reduction of single-use plastic consumption. *Sustain For* 12. <https://doi.org/10.3390/su12114698>
- Hernandez LM, Yousefi N, Tufenkji N (2017) Are there nanoplastics in your personal care products? *Environ Sci Technol Lett* 4:280–285
- Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M (2012) Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ Sci Technol* 46: 3060–3075
- Horn D, Miller M, Anderson S, Steele C (2019) Microplastics are ubiquitous on California beaches and enter the coastal food web through consumption by Pacific mole crabs. *Mar Pollut Bull* 139: 231–237
- Horton AA, Dixon SJ (2018) Microplastics: an introduction to environmental transport processes. *Wiley Interdiscip Rev Water* 5:e1268
- Horvatits T, Tamminga M, Liu B, Sebode M, Carambia A, Fischer L, Püschel K, Huber S, Fischer EK (2022) Microplastics detected in cirrhotic liver tissue. *eBioMedicine*:82
- Hu M, Palić D (2020) Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. *Redox Biol* 37:101620
- Hu X, Biswas A, Sharma A, Sarkodie H, Tran I, Pal I, De S (2021) Mutational signatures associated with exposure to carcinogenic microplastic compounds bisphenol a and styrene oxide. *NAR cancer* 3:zcab004
- Huang X, Zhou C, Tian M, Kang JQ, Shen W, Verdier K, Pimenta A, MacDonald RL (2017) Overexpressing wild-type $\gamma 2$ subunits rescued the seizure phenotype in Gabrg2(+/-Q390X) Dravet syndrome mice. *Epilepsia* 58:1451–1461
- Huang J-N, Wen B, Zhu J-G, Zhang Y-S, Gao J-Z, Chen Z-Z (2020) Exposure to microplastics impairs digestive performance, stimulates immune response and induces microbiota dysbiosis in the gut of juvenile guppy (*Poecilia reticulata*). *Sci Total Environ* 733:138929
- Huang Z, Weng Y, Shen Q, Zhao Y, Jin Y (2021) Microplastic: a potential threat to human and animal health by interfering with the intestinal barrier function and changing the intestinal microenvironment. *Sci Total Environ* 785:147365

- Huang S, Wang H, Ahmad W, Ahmad A, Ivanovich Vatin N, Mohamed AM, Deifalla AF, Mehmood I (2022) Plastic waste management strategies and their environmental aspects: a Scientometric analysis and comprehensive review. *Int J Environ Res Public Health* 19. <https://doi.org/10.3390/ijerph19084556>
- Hui L, Li H, Lu G, Chen Z, Sun W, Shi Y, Fu Z, Huang B, Zhu X, Lu W, Xia D, Wu Y (2018) Low dose of Bisphenol A modulates ovarian cancer gene expression profile and promotes epithelial to mesenchymal transition via canonical Wnt pathway. *Toxicol Sci* 164:527–538
- Hwang J, Choi D, Han S, Jung SY, Choi J, Hong J (2020) Potential toxicity of polystyrene microplastic particles. *Sci Rep* 10:1–12
- Janevska D, Chaloska-Ivanova V, Janevski V (2015) Hepatocellular carcinoma: risk factors, diagnosis and treatment. *Open Access Maced J Med Sci* 3:732–736
- Jenner LC, Sadofsky LR, Danopoulos E, Rotchell JM (2021) Household indoor microplastics within the Humber region (United Kingdom): quantification and chemical characterisation of particles present. *Atmos Environ* 259:118512
- Jeong C-B, Won E-J, Kang H-M, Lee M-C, Hwang D-S, Hwang U-K, Zhou B, Souissi S, Lee S-J, Lee J-S (2016) Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (*Brachionus koreanus*). *Environ Sci Technol* 50:8849–8857
- Jeong C-B, Kang H-M, Lee M-C, Kim D-H, Han J, Hwang D-S, Souissi S, Lee S-J, Shin K-H, Park HG (2017) Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod *Paracyclopsina nana*. *Sci Rep* 7:41323
- Jia R, Han J, Liu X, Li K, Lai W, Bian L, Yan J, Xi Z (2023) Exposure to polypropylene microplastics via Oral ingestion induces colonic apoptosis and intestinal barrier damage through oxidative stress and inflammation in mice. *Toxics* 11:127
- Jiang J, Ma L, Yuan L, Wang X, Zhang W (2007) Study on developmental abnormalities in hypospadiac male rats induced by maternal exposure to di-n-butyl phthalate (DBP). *Toxicology* 232:286–293
- Jin Y, Xia J, Pan Z, Yang J, Wang W, Fu Z (2018) Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. *Environ Pollut* 235:322–329
- Jin H, Xie J, Mao L, Zhao M, Bai X, Wen J, Shen T, Wu P (2020) Bisphenol analogue concentrations in human breast milk and their associations with postnatal infant growth. *Environ Pollut* 259:113779
- Jing J, Zhang L, Han L, Wang J, Zhang W, Liu Z, Gao A (2022) Polystyrene micro-/nanoplastics induced hematopoietic damages via the crosstalk of gut microbiota, metabolites, and cytokines. *Environ Int* 161:107131
- Ju Q, Zouboulis CC (2016) Endocrine-disrupting chemicals and skin manifestations. *Rev Endocr Metabol Disord* 17:449–457
- Kale G, Kijchavengkul T, Auras R, Rubino M, Selke SE, Singh SP (2007) Compostability of bioplastic packaging materials: an overview. *Macromol Biosci* 7:255–277
- Kamada N, Seo S-U, Chen GY, Núñez G (2013) Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol* 13:321–335
- Karami A, Golieskardi A, Choo CK, Larat V, Karbalaee S, Salamatinia B (2018) Microplastic and mesoplastic contamination in canned sardines and sprats. *Sci Total Environ* 612:1380–1386
- Karbalaee S, Hanachi P, Walker TR, Cole M (2018) Occurrence, sources, human health impacts and mitigation of microplastic pollution. *Environ Sci Pollut Res Int* 25:36046–36063
- Kasamesiri P, Panchan R, Thaimuangphol W (2023) Spatial-temporal distribution and ecological risk assessment of microplastic pollution of inland fishing ground in the Ubolratana reservoir. *Thailand Water* 15:330
- Khalaf N, El-Serag HB, Abrams HR, Thrift AP (2021) Burden of pancreatic cancer: from epidemiology to practice. *Clin Gastroenterol Hepatol* 19:876–884
- Kim JH (2019) Di(2-ethylhexyl) phthalate promotes lung cancer cell line A549 progression via Wnt/ β -catenin signaling. *J Toxicol Sci* 44:237–244

- Kim K-H, Kabir E, Jahan SA (2017) Exposure to pesticides and the associated human health effects. *Sci Total Environ* 575:525–535
- Kim J-H, Yu Y-B, Choi J-H (2021) Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: a review. *J Hazard Mater* 413:125423
- Kim H, Zaheer J, Choi EJ, Kim JS (2022) Enhanced ASGR2 by microplastic exposure leads to resistance to therapy in gastric cancer. *Theranostics* 12:3217–3236
- Kirstein IV, Gomiero A, Vollertsen J (2021a) Microplastic pollution in drinking water. *Curr Opin Toxicol* 28:70–75
- Kirstein IV, Hensel F, Gomiero A, Iordachescu L, Vianello A, Wittgren HB, Vollertsen J (2021b) Drinking plastics?—quantification and qualification of microplastics in drinking water distribution systems by μ FTIR and Py-GCMS. *Water Res* 188:116519
- Kitagawa K, Shibata E, Yamamoto M, Harada H, Yoshino K, Iwashita T, Oshima M, Tsuji M (2023) Subacute exposure to bisphenol F diglycidyl-ether induces chronic dermatitis characterized by psoriasis-like skin inflammation in mice. *Genes Cells* 28:42–52
- Koelmans AA, Besseling E, Shim WJ (2015) Nanoplastics in the aquatic environment. *Critic Rev Mar Anthropogen Litt*:325–340
- Koelmans AA, Bakir A, Burton GA, Janssen CR (2016) Microplastic as a vector for Chemicals in the Aquatic Environment: critical review and model-supported reinterpretation of empirical studies. *Environ Sci Technol* 50:3315–3326
- Kokalj AJ, Horvat P, Skalar T, Kržan A (2018) Plastic bag and facial cleanser derived microplastic do not affect feeding behaviour and energy reserves of terrestrial isopods. *Sci Total Environ* 615:761–766
- Kole PJ, Löhr AJ, Van Belleghem FG AJ, Ragas AMJ (2017) Wear and tear of tyres: a stealthy source of microplastics in the environment. *Int J Environ Res Public Health* 14:1265
- Kovačič A, Modic M, Hojnik N, Vehar A, Kosjek T, Heath D, Walsh JL, Cvelbar U, Heath E (2022) Degradation of bisphenol a and S in wastewater during cold atmospheric pressure plasma treatment. *Sci Total Environ* 837:155707
- Krashin E, Piekietko-Witkowska A, Ellis M, Ashur-Fabian O (2019) Thyroid hormones and cancer: a comprehensive review of preclinical and clinical studies. *Front Endocrinol (Lausanne)* 10:59
- Kuppusamy S, Thavamani P, Venkateswarlu K, Lee YB, Naidu R, Megharaj M (2017) Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: technological constraints, emerging trends and future directions. *Chemosphere* 168:944–968
- Kutralam-Muniasamy G, Pérez-Guevara F, Elizalde-Martínez I, Shruti V (2020) Branded milks—are they immune from microplastics contamination? *Sci Total Environ* 714:136823
- Laffon B, Páraso E, Méndez J (2002) Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene. *Toxicology* 171:175–186
- Låg M, Øvreivik J, Refsnes M, Holme JA (2020) Potential role of polycyclic aromatic hydrocarbons in air pollution-induced non-malignant respiratory diseases. *Respir Res* 21:299
- Lai KP, Chung YT, Li R, Wan HT, Wong CK (2016) Bisphenol A alters gut microbiome: comparative metagenomics analysis. *Environ Pollut* 218:923–930
- Lawal AT (2017) Polycyclic aromatic hydrocarbons: a review. *Cogent Environm Sci* 3:1339841
- Lebre F, Boland JB, Gouveia P, Gorman AL, Lundahl ML, Lynch RI, O'Brien FJ, Coleman J, Lavelle EC (2020) Pristine graphene induces innate immune training. *Nanoscale* 12:11192–11200
- Lebreton LCM, Van Der Zwet J, Damsteeg J-W, Slat B, Andrady A, Reisser J (2017) River plastic emissions to the world's oceans. *Nat Commun* 8:15611
- Lechthaler S, Waldschl ger K, Stauch G, Sch tttrumpf H (2020) The way of macroplastic through the environment. *Environments* 7:73
- Ledniewska K, Nosal-Kovalenko H, Janik W, Krasuska A, Sta ńczyk D, Sabura E, Bartoszewicz M, Rybak A (2022) Effective, environmentally friendly PVC plasticizers based on succinic acid. *Polymers* 14. <https://doi.org/10.3390/polym14071295>

- Lee CT (2015) What do we know about ground-glass opacity nodules in the lung? *Transl Lung Cancer Res* 4:656–659
- Lee J, Choi K, Park J, Moon HB, Choi G, Lee JJ, Suh E, Kim HJ, Eun SH, Kim GH, Cho GJ, Kim SK, Kim S, Kim SY, Kim S, Eom S, Choi S, Kim YD, Kim S (2018) Bisphenol a distribution in serum, urine, placenta, breast milk, and umbilical cord serum in a birth panel of mother-neonate pairs. *Sci Total Environ* 626:1494–1501
- Leiter U, Keim U, Garbe C (2020) Epidemiology of skin cancer: update 2019. In Reichrath J (ed) *Sunlight, Vitamin D and skin cancer*. Springer, Cham, pp 123–139
- Leslie HA, Van Velzen MJ, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH (2022) Discovery and quantification of plastic particle pollution in human blood. *Environ Int* 163: 107199
- Lewandowski K, Skórczewska K (2022) A brief review of poly (vinyl chloride)(PVC) recycling. *Polymers* 14:3035. <https://doi.org/10.3390/polym14153035>
- Li DK, Zhou Z, Miao M, He Y, Qing D, Wu T, Wang J, Weng X, Ferber J, Herrinton LJ, Zhu Q, Gao E, Yuan W (2010) Relationship between urine bisphenol-A level and declining male sexual function. *J Androl* 31:500–506
- Li Z, Feng Y, Yuan X, Wang H, Zhao Y, Wang J (2019) Photo-triggered reversible phase transfer of Azobenzene-based ionic liquid surfactants between oil and water. *Int J Mol Sci* 20
- Li B, Ding Y, Cheng X, Sheng D, Xu Z, Rong Q, Wu Y, Zhao H, Ji X, Zhang Y (2020a) Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. *Chemosphere* 244:125492
- Li C, Busquets R, Campos LC (2020b) Assessment of microplastics in freshwater systems: a review. *Sci Total Environ* 707:135578
- Li L, Zhao X, Li Z, Song K (2021) COVID-19: performance study of microplastic inhalation risk posed by wearing masks. *J Hazard Mater* 411:124955
- Li Y, Xu M, Zhang Z, Halimu G, Li Y, Li Y, Gu W, Zhang B, Wang X (2022) In vitro study on the toxicity of nanoplastics with different charges to murine splenic lymphocytes. *J Hazard Mater* 424:127508
- Li J, Yu Y, Chen X, Yu S, Cui M, Wang S, Song F (2023) Effects of biochar on the phytotoxicity of polyvinyl chloride microplastics. *Plant Physiol Biochem* 195:228–237
- Liang B, Zhong Y, Huang Y, Lin X, Liu J, Lin L, Hu M, Jiang J, Dai M, Wang B, Zhang B, Meng H, Lelaka JJJ, Sui H, Yang X, Huang Z (2021) Underestimated health risks: polystyrene micro- and nanoplastics jointly induce intestinal barrier dysfunction by ROS-mediated epithelial cell apoptosis. *Part Fibre Toxicol* 18:20
- Liang HI, Cheng YW, Chang HS, Su KM, Chen WL (2022) Exposure to polycyclic aromatic hydrocarbons and its relationship with increased expression of human epididymal secretory protein 4. *Cancer* 13:14
- Liao LM, Hofmann JN, Kamangar F, Strickland PT, Ji BT, Yang G, Li HL, Rothman N, Zheng W, Chow WH, Gao YT, Shu XO (2014) Polycyclic aromatic hydrocarbons and risk of gastric cancer in the Shanghai Women's health study. *Int J Mol Epidemiol Genet* 5:140–144
- Lillo MA, Nichols C, Seagroves TN, Miranda-Carboni GA, Krum SA (2017) Bisphenol A induces Sox2 in ER+ breast cancer stem-like cells. *Hormones and Cancer* 8:90–99
- Lim D, Jeong J, Song KS, Sung JH, Oh SM, Choi J (2021a) Inhalation toxicity of polystyrene micro (nano)plastics using modified OECD TG 412. *Chemosphere* 262:128330
- Lim J-V, Bee S-T, Tin Sin L, Ratnam CT, Abdul Hamid ZA (2021b) A review on the synthesis, properties, and Utilities of Functionalized carbon nanoparticles for polymer nanocomposites. *Polymers* 13. <https://doi.org/10.3390/polym13203547>
- Lin W, Jiang R, Hu S, Xiao X, Wu J, Wei S, Xiong Y, Ouyang G (2019) Investigating the toxicities of different functionalized polystyrene nanoplastics on *Daphnia magna*. *Ecotoxicol Environ Saf* 180:509–516
- Liu J, Zhang L, Lu G, Jiang R, Yan Z, Li Y (2021a) Occurrence, toxicity and ecological risk of Bisphenol a analogues in aquatic environment - a review. *Ecotoxicol Environ Saf* 208:111481

- Liu S, Shi J, Wang J, Dai Y, Li H, Li J, Liu X, Chen X, Wang Z, Zhang P (2021b) Interactions between microplastics and heavy metals in aquatic environments: a review. *Front Microbiol* 12: 652520
- Liu T, Hou B, Wang Z, Yang Y (2022) Polystyrene microplastics induce mitochondrial damage in mouse GC-2 cells. *Ecotoxicol Environ Saf* 237:113520
- Liu S, Guo J, Liu X, Yang R, Wang H, Sun Y, Chen B, Dong R (2023) Detection of various microplastics in placentas, meconium, infant feces, breastmilk and infant formula: a pilot prospective study. *Sci Total Environ* 854:158699
- Louis GMB, Sundaram R, Sweeney AM, Schisterman EF, Maisog J, Kannan K (2014) Urinary bisphenol a, phthalates, and couple fecundity: the longitudinal investigation of fertility and the environment (LIFE) study. *Fertil Steril* 101:1359–1366
- Lu L, Wan Z, Luo T, Fu Z, Jin Y (2018) Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. *Sci Total Environ* 631–632:449–458
- Lu K, Zhan D, Fang Y, Li L, Chen G, Chen S, Wang L (2022) Microplastics, potential threat to patients with lung diseases. *Front Toxicol* 4:958414
- Ludewig G, Robertson LW (2013) Polychlorinated biphenyls (PCBs) as initiating agents in hepatocellular carcinoma. *Cancer Lett* 334:46–55
- Luo T, Wang C, Pan Z, Jin C, Fu Z, Jin Y (2019) Maternal polystyrene microplastic exposure during gestation and lactation altered metabolic homeostasis in the dams and their F1 and F2 offspring. *Environ Sci Technol* 53:10978–10992
- Luo Y, Ma J, Lu W (2020) The significance of mitochondrial dysfunction in cancer. *Int J Mol Sci* 21
- Lusher A, Hollman P, Mendoza J (2017) Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. *Food and Agriculture of the United Nations, Rome*, p 41. ISSN: 2070-7010
- Maafa IM (2021) Pyrolysis of polystyrene waste: a review. *Polymers* 13. <https://doi.org/10.3390/polym13020225>
- Makhdoumi P, Amin AA, Karimi H, Pirsaeheb M, Kim H, Hossini H (2021) Occurrence of microplastic particles in the most popular Iranian bottled mineral water brands and an assessment of human exposure. *J Water Process Eng* 39:101708
- Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E (2020) Environmental and health impacts of air pollution: a review *Front Pub Health*:8
- Maraveas C (2020) Production of sustainable and biodegradable polymers from agricultural waste. *Polymers* 12. <https://doi.org/10.3390/polym12051127>
- Mason SA, Welch VG, Neratko J (2018) Synthetic polymer contamination in bottled water. *Front Chem* 6:407
- Massin P, Achour S (2017) Wear products of total hip arthroplasty: the case of polyethylene. *Morphologie* 101:1–8
- Mateos-Cárdenas A, O'Halloran J, van Pelt FNAM, Jansen MAK (2020) Rapid fragmentation of microplastics by the freshwater amphipod *Gammarus duebeni* (Lillj.). *Sci Rep* 10:12799
- Mattsson K, Hansson LA, Cedervall T (2015) Nano-plastics in the aquatic environment. *Environ Sci Process Impacts* 17:1712–1721
- McGlynn KA, Trabert B (2012) Adolescent and adult risk factors for testicular cancer. *Nat Rev Urol* 9:339–349
- Meeker JD, Sathyanarayana S, Swan SH (2009) Phthalates and other additives in plastics: human exposure and associated health outcomes. *Philos Trans Royal Soc B Biol Sci* 364:2097–2113
- Menéndez-Pedriza A, Jaumot J, Bedia C (2022) Lipidomic analysis of single and combined effects of polyethylene microplastics and polychlorinated biphenyls on human hepatoma cells. *J Hazard Mater* 421:126777
- Meng C, Bai C, Brown TD, Hood LE, Tian Q (2018) Human gut microbiota and gastrointestinal cancer. *Genomics Proteomics Bioinformatics* 16:33–49
- Mintenig SM, Löder MGJ, Primpke S, Gerdts G (2019) Low numbers of microplastics detected in drinking water from ground water sources. *Sci Total Environ* 648:631–635

- Molenaar R, Chatterjee S, Kamphuis B, Segers-Nolten IMJ, Claessens MMAE, Blum C (2021) Nanoplastic sizes and numbers: quantification by single particle tracking. *Environ Sci Nano* 8: 723–730
- Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H (2019) Ovarian cancer in the world: epidemiology and risk factors. *Int J Women's Health* 11:287–299
- Moorthy B, Chu C, Carlin DJ (2015) Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. *Toxicol Sci* 145:5–15
- Morgan E, Arnold M Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, et al. (2022a) Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Retrieved 3/19/2023, 2023, from <https://www.iarc.who.int/news-events/global-burden-of-colorectal-cancer-in-2020-and-2040-incidence-and-mortality-estimates-from-globocan/>
- Morgan E, Arnold M, Camargo MC, Gini A, Kunzmann AT, Matsuda T, Meheus F, Verhoeven RHA, Vignat J, Laversanne M, Ferlay J, Soerjomataram I (2022b) The current and future incidence and mortality of gastric cancer in 185 countries, 2020–40: a population-based modelling study. *eClinicalMedicine*:47
- Moskalyuk OA, Belashov AV, Beltukov YM, Ivan'kova EM, Popova EN, Semenova IV, Yelokhovskiy VY, Yudin VE (2020) Polystyrene-based nanocomposites with different fillers: fabrication and mechanical properties. *Polymers* 12. <https://doi.org/10.3390/polym12112457>
- Moustafa H, Youssef AM, Darwish NA, Abou-Kandil AI (2019) Eco-friendly polymer composites for green packaging: future vision and challenges. *Compos Part B* 172:16–25
- Mughal TI, Girmius S, Rosen ST, Kumar S, Wiestner A, Abdel-Wahab O, Kiladjian J-J, Wilson WH, Van Etten RA (2014) Emerging therapeutic paradigms to target the dysregulated Janus kinase/signal transducer and activator of transcription pathway in hematological malignancies. *Leuk Lymphoma* 55:1968–1979
- Mustieles V, d'Cruz SC, Couderq S, Rodríguez-Carrillo A, Fini J-B, Hofer T, Steffensen I-L, Dirven H, Barouki R, Olea N, Fernandez-Olalla M, David A (2020) Bisphenol A and its analogues: a comprehensive review to identify and prioritize effect biomarkers for human biomonitoring. *Environ Int* 144:105811
- Naidoo T, Glassom D (2019) Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. *Mar Pollut Bull* 145:254–259
- Narancic T, O'Connor KE (2019) Plastic waste as a global challenge: are biodegradable plastics the answer to the plastic waste problem? *Microbiology (Reading)* 165:129–137
- Nich C, Goodman SB (2014) Role of macrophages in the biological reaction to wear debris from joint replacements. *J Long-Term Eff Med Implants* 24:259
- Nugnes R, Lavorgna M, Orlo E, Russo C, Isidori M (2022) Toxic impact of polystyrene microplastic particles in freshwater organisms. *Chemosphere* 299:134373
- Nymand Ennis Z, Arnspar Pedersen S, Rix Hansen M, Pottgård A, Patrick Ahern T, Hallas J, Damkier P (2019) Use of phthalate-containing prescription drugs and the risk of gastric cancer: a Danish nationwide case-control study. *Acta Oncol* 58:852–858
- Oddone E, Modonesi C, Gatta G (2014) Occupational exposures and colorectal cancers: a quantitative overview of epidemiological evidence. *World J Gastroenterol: WJG* 20:12431
- Ohlson C-G, Hardell L (2000) Testicular cancer and occupational exposures with a focus on xenoestrogens in polyvinyl chloride plastics. *Chemosphere* 40:1277–1282
- Okubo T, Suzuki T, Yokoyama Y, Kano K, Kano I (2003) Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. *Biol Pharm Bull* 26:1219–1224
- Onyena AP, Aniche DC, Ogbolu BO, Rakib MR, Uddin J, Walker TR (2022) Governance strategies for mitigating microplastic pollution in the marine environment: a review. *Microplastics* 1:15–46. <https://doi.org/10.3390/microplastics1010003>

- Palacios-Arreola MI, Nava-Castro KE, Río-Araiza VHD, Pérez-Sánchez NY, Morales-Montor J (2017) A single neonatal administration of Bisphenol A induces higher tumour weight associated to changes in tumour microenvironment in the adulthood. *Sci Rep* 7:10573
- Palacios-Arreola MI, Nava-Castro KE, Mendoza-Moreno NA, Segovia-Mendoza M, Perez-Torres-A, Morales-Montor J (2021) Environmental pollution and breast cancer: the microplastic component BPA regulates the intratumoral immune microenvironment and increases lung metastasis. <https://www.preprints.org/manuscript/202109.0017/v2>
- Pandey B, Pathak J, Singh P, Kumar R, Kumar A, Kaushik S, Thakur TK (2023) Microplastics in the ecosystem: an overview on detection, removal, toxicity assessment, and control release. *Water* 15. <https://doi.org/10.3390/w15010051>
- Panko JM, Kreider ML, McAtee BL, Marwood C (2013) Chronic toxicity of tire and road wear particles to water-and sediment-dwelling organisms. *Ecotoxicology* 22:13–21
- Park MA, Hwang KA, Lee HR, Yi BR, Jeung EB, Choi KC (2012) Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. *Mol Med Rep* 5:761–766
- Pauly JL, Stegmeier SJ, Allaart HA, Cheney RT, Zhang PJ, Mayer AG, Streck RJ (1998) Inhaled cellulosic and plastic fibers found in human lung tissue. *Cancer Epidemiol Biomarkers Prev* 7: 419–428
- Pivokonsky M, Cermakova L, Novotna K, Peer P, Cajthaml T, Janda V (2018) Occurrence of microplastics in raw and treated drinking water. *Sci Total Environ* 643:1644–1651
- Prasittisopin L, Termkhajornkit P, Kim YH (2022) Review of concrete with expanded polystyrene (EPS): performance and environmental aspects. *J Clean Prod* 366:132919
- Prata JC (2018) Airborne microplastics: consequences to human health? *Environ Pollut* 234:115–126
- Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T (2020) Environmental exposure to microplastics: an overview on possible human health effects. *Sci Total Environ* 702:134455
- Praveena SM, Siok Fong C, Amaruddin AF (2021) Phthalates in children toys available in Malaysian market: quantification and potential human health risk. *J Steroid Biochem Mol Biol* 213:105955
- Prietl B, Meindl C, Roblegg E, Pieber T, Lanzer G, Fröhlich E (2014) Nano-sized and micro-sized polystyrene particles affect phagocyte function. *Cell Biol Toxicol* 30:1–16
- Prins GS (2008) Endocrine disruptors and prostate cancer risk. *Endocr Relat Cancer* 15:649–656
- Proshad R, Kormoker T, Islam MS, Haque MA, Rahman MM, Mithu MMR (2018) Toxic effects of plastic on human health and environment: a consequences of health risk assessment in Bangladesh. *Intl J Health* 6:1–5
- Qiao R, Deng Y, Zhang S, Wolosker MB, Zhu Q, Ren H, Zhang Y (2019a) Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere* 236:124334
- Qiao R, Sheng C, Lu Y, Zhang Y, Ren H, Lemos B (2019b) Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. *Sci Total Environ* 662:246–253
- Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, Papa F, Rongioletti MCA, Baiocco F, Draghi S, D'Amore E, Rinaldo D, Matta M, Giorgini E (2021) Plasticenta: first evidence of microplastics in human placenta. *Environ Int* 146:106274
- Ragusa A, Notarstefano V, Svelato A, Belloni A, Gioacchini G, Blondeel C, Zucchelli E, De Luca C, D'Avino S, Gulotta A (2022) Raman microspectroscopy detection and characterisation of microplastics in human breastmilk. *Polymers* 14:2700
- Razza F, Degli Innocenti F, Dobon A, Aliaga C, Sanchez C, Hortal M (2015) Environmental profile of a bio-based and biodegradable foamed packaging prototype in comparison with the current benchmark. *J Clean Prod* 102:493–500
- Rebelein A, Int-Veen I, Kammann U, Scharsack JP (2021) Microplastic fibers—underestimated threat to aquatic organisms? *Sci Total Environ* 777:146045

- Recio-Vega R, Mendez-Henandez A, Gabriel AP, Jacobo-Avila A, Portales-Castanedo A, Hernandez-Gonzalez S, Gallegos-Arreola MP, Ocampo-Gomez G (2013) Potentially estrogenic polychlorinated biphenyls congeners serum levels and its relation with lung cancer. *J Appl Toxicol* 33:906–914
- Ren S-Y, Kong S-F, Ni H-G (2021) Contribution of mulch film to microplastics in agricultural soil and surface water in China. *Environ Pollut* 291:118227
- Revel M, Châtel A, Mouneyrac C (2018) Micro(nano)plastics: a threat to human health? *Curr Opin Environ Sci Health* 1:17–23
- Ricciardi M, Pironti C, Motta O, Miele Y, Proto A, Montano L (2021) Microplastics in the aquatic environment: occurrence, persistence, analysis, and human exposure. *Water* 13. <https://doi.org/10.3390/w13070973>
- Rochester JR (2013) Bisphenol a and human health: a review of the literature. *Reprod Toxicol* 42: 132–155
- Rochester JR, Bolden AL (2015) Bisphenol S and F: a systematic review and comparison of the hormonal activity of Bisphenol a substitutes. *Environ Health Perspect* 123:643–650
- Rochman CM, Kross SM, Armstrong JB, Bogan MT, Darling ES, Green SJ, Smyth AR, Verissimo D (2015a) Scientific evidence supports a ban on microbeads, vol 49. ACS Publications, p 10759
- Rochman CM, Tahir A, Williams SL, Baxa DV, Lam R, Miller JT, Teh F-C, Werorilangi S, Teh SJ (2015b) Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci Rep* 5:14340
- Rodgers KM, Udesky JO, Rudel RA, Brody JG (2018) Environmental chemicals and breast cancer: An updated review of epidemiological literature informed by biological mechanisms. *Environ Res* 160:152–182
- Roje Ž, Ilić K, Galić E, Pavičić I, Turčić P, Stanec Z, Vrček IV (2019) Synergistic effects of parabens and plastic nanoparticles on proliferation of human breast cancer cells. *Arh Hig Rada Toksikol* 70:310–314
- Roth W, Kumar V, Beer H-D, Richter M, Wohlenberg C, Reuter U, Thiering S, Staratschek-Jox A, Hofmann A, Kreusch F (2012) Keratin 1 maintains skin integrity and participates in an inflammatory network in skin through interleukin-18. *J Cell Sci* 125:5269–5279
- Rumgay H, Arnold M, Ferlay J, Lesi O, Cabaasag CJ, Vignat J, Laversanne M, McGlynn KA, Soerjomataram I (2022) Global burden of primary liver cancer in 2020 and predictions to 2040. *J Hepatol* 77:1598–1606
- Rundle A, Tang D, Hibshoosh H, Estabrook A, Schnabel F, Cao W, Grumet S, Perera FP (2000) The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. *Carcinogenesis* 21:1281–1289
- Russo J, Russo IH (2006) The role of estrogen in the initiation of breast cancer. *J Steroid Biochem Mol Biol* 102:89–96
- Rybicki BA, Neslund-Dudas C, Nock NL, Schultz LR, Eklund L, Rosbalt J, Bock CH, Monaghan KG (2006) Prostate cancer risk from occupational exposure to polycyclic aromatic hydrocarbons interacting with the GSTP1 Ile105Val polymorphism. *Cancer Detect Prev* 30: 412–422
- Sadat-Shojai M, Bakhshandeh G-R (2011) Recycling of PVC wastes. *Polym Degrad Stab* 96:404–415
- Samanta SK, Singh OV, Jain RK (2002) Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. *Trends Biotechnol* 20:243–248
- Sánchez-Alcoholado L, Ramos-Molina B, Otero A, Laborda-Illanes A, Ordóñez R, Medina JA, Gómez-Millán J, Queipo-Ortuño MI (2020) The role of the gut microbiome in colorectal cancer development and therapy response. *Cancers (Basel)* 12
- Sangham S, Faikhaw O, Munkong N, Sakunkoo P, Arunlertaree C, Chavali M, Mousazadeh M, Tiwari A (2022) A review on microplastics and nanoplastics in the environment: their occurrence, exposure routes, toxic studies, and potential effects on human health. *Mar Pollut Bull* 181:113832

- Saravia J, You D, Thevenot P, Lee GI, Shrestha B, Lomnicki S, Cormier SA (2014) Early-life exposure to combustion-derived particulate matter causes pulmonary immunosuppression. *Mucosal Immunol* 7:694–704
- Sarma DK, Dubey R, Samarth RM, Shubham S, Chowdhury P, Kumawat M, Verma V, Tiwari RR, Kumar M (2022) The biological effects of polystyrene Nanoplastics on human peripheral blood lymphocytes. *Nano* 12. <https://doi.org/10.3390/nano12101632>
- Satyro WC, Contador JC, Monken SF, Lima AF, Soares Junior GG, Gomes JA, Neves JV, do Nascimento JR, de Araújo JL, Correa ED, Silva LS (2023) Industry 4.0 implementation projects: the cleaner production strategy – a literature review. *Sustain For* 15. <https://doi.org/10.3390/su15032161>
- Scelo G, Larose TL (2018) Epidemiology and risk factors for kidney cancer. *J Clin Oncol* 36:3574
- Schmidt A, da Silva Brito WA, Singer D, Mühl M, Berner J, Saadati F, Wolff C, Miebach L, Wende K, Bekeschus S (2023) Short- and long-term polystyrene nano- and microplastic exposure promotes oxidative stress and divergently affects skin cell architecture and Wnt/beta-catenin signaling. *Part Fibre Toxicol* 20:3
- Schneider M, Stracke F, Hansen S, Schaefer UF (2009) Nanoparticles and their interactions with the dermal barrier. *Dermatoendocrinol* 1:197–206
- Schraplau A, Schewe B, Neuschäfer-Rube F, Ringel S, Neuber C, Kleuser B, Püschel GP (2015) Enhanced thyroid hormone breakdown in hepatocytes by mutual induction of the constitutive androstane receptor (CAR, NR1I3) and arylhydrocarbon receptor by benzo[a]pyrene and phenobarbital. *Toxicology* 328:21–28
- Schymanski D, Goldbeck C, Humpf HU, Fürst P (2018) Analysis of microplastics in water by micro-Raman spectroscopy: release of plastic particles from different packaging into mineral water. *Water Res* 129:154–162
- Selenskas S, Teta MJ, Vitale JN (1995) Pancreatic cancer among workers processing synthetic resins. *Am J Ind Med* 28:385–398
- Shafei A, Matboli M, Mostafa E, Sannat S, Mahmoud A, Lewis B, Muhammad B, Mohamed S, Mostafa R (2018) Stop eating plastic, molecular signaling of bisphenol a in breast cancer. *Environ Sci Pollut Res* 25:23624
- Shah AA, Hasan F, Hameed A, Ahmed S (2008) Biological degradation of plastics: a comprehensive review. *Biotechnol Adv* 26:246–265
- Sheikh Fakhradini S, Moore F, Keshavarzi B, Lahijanzadeh A (2019) Polycyclic aromatic hydrocarbons (PAHs) in water and sediment of Hoor Al-Azim wetland, Iran: a focus on source apportionment, environmental risk assessment, and sediment-water partitioning. *Environ Monit Assess* 191:233
- Shen R, Yang K, Cheng X, Guo C, Xing X, Sun H, Liu D, Liu X, Wang D (2022) Accumulation of polystyrene microplastics induces liver fibrosis by activating cGAS/STING pathway. *Environ Pollut* 300:118986
- Shi J, Sun X, Lin Y, Zou X, Li Z, Liao Y, Du M, Zhang H (2014) Endothelial cell injury and dysfunction induced by silver nanoparticles through oxidative stress via IKK/NF-κB pathways. *Biomaterials* 35:6657–6666
- Shi X, Wang X, Huang R, Tang C, Hu C, Ning P, Wang F (2022) Cytotoxicity and Genotoxicity of polystyrene micro- and Nanoplastics with different size and surface modification in A549 cells. *Int J Nanomedicine* 17:4509–4523
- Shields T, Gridley G, Moradi T, Adami J, Plato N, Dosemeci M (2002) Occupational exposures and the risk of ovarian cancer in Sweden. *Am J Ind Med* 42:200–213
- Shoaei F, Talebi-Ghane E, Amirsadeghi S, Mehri F (2023) The investigation of polycyclic aromatic hydrocarbons (PAHs) in milk and its products: a global systematic review, meta-analysis and health risk assessment. *Intl Dairy J* 142:105645
- Siddiqui R, Qaisar R, Goswami N, Khan NA, Elmoselhi A (2021) Effect of microgravity environment on gut microbiome and angiogenesis. *Life* 11:1008
- Siegfried M, Koelmans AA, Besseling E, Kroeze C (2017) Export of microplastics from land to sea. A modelling approach. *Water Res* 127:249–257

- Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA (2017) Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. *Environ Toxicol Pharmacol* 51:56–70
- Šimunović A, Tomić S, Kranjčec K (2022) Medical devices as a source of phthalate exposure: a review of current knowledge and alternative solutions. *Arh Hig Rada Toksikol* 73:179–190
- Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, Maffei TG, Wright CJ, Doak SH (2009) NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials* 30:3891–3914
- Siracusa JS, Yin L, Measel E, Liang S, Yu X (2018) Effects of bisphenol A and its analogs on reproductive health: a mini review. *Reprod Toxicol* 79:96–123
- Solheiro-Villavicencio H, Gomez-De León CT, Del Río-Araiza VH, Morales-Montor J (2020) The detrimental effect of microplastics on critical periods of development in the neuroendocrine system. *Birth Defects Res* 112:1326–1340
- Song JH, Murphy RJ, Narayan R, Davies GB (2009a) Biodegradable and compostable alternatives to conventional plastics. *Philos Trans R Soc Lond Ser B Biol Sci* 364:2127–2139
- Song Y, Li X, Du X (2009b) Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. *Eur Respir J* 34:559
- Song YK, Hong SH, Jang M, Han GM, Shim WJ (2015) Occurrence and distribution of microplastics in the sea surface microlayer in Jinhae Bay, South Korea. *Arch Environ Contam Toxicol* 69:279–287
- Sonnenburg JL, Bäckhed F (2016) Diet–microbiota interactions as moderators of human metabolism. *Nature* 535:56–64
- Souza-Silva TG, Oliveira IA, Silva GG, Giusti FCV, Novaes RD, Paula HAA (2022) Impact of microplastics on the intestinal microbiota: a systematic review of preclinical evidence. *Life Sci* 294:120366
- Struthy S, Ramasamy EV (2017) Microplastic pollution in Vembanad Lake, Kerala, India: the first report of microplastics in lake and estuarine sediments in India. *Environ Pollut* 222:315–322
- Stading R, Gastelum G, Chu C, Jiang W, Moorthy B (2021) Molecular mechanisms of pulmonary carcinogenesis by polycyclic aromatic hydrocarbons (PAHs): implications for human lung cancer. *Semin Cancer Biol* 76:3–16
- Sukanya M, Khushboo S, Geetika S, Mandeep S, Pooja C (2020) Sources, fate, and impact of microplastics in aquatic environment. emerging contaminants. N. Aurel. Rijeka, IntechOpen: Ch. 13
- Sun J, Dai X, Wang Q, van Loosdrecht MCM, Ni B-J (2019) Microplastics in wastewater treatment plants: detection, occurrence and removal. *Water Res* 152:21–37
- Sun R, Xu K, Yu L, Pu Y, Xiong F, He Y, Huang Q, Tang M, Chen M, Yin L, Zhang J, Pu Y (2021) Preliminary study on impacts of polystyrene microplastics on the hematological system and gene expression in bone marrow cells of mice. *Ecotoxicol Environ Saf* 218:112296
- Tamis JE, Koelmans AA, Dröge R, Kaag NHBM, Keur MC, Tromp PC, Jongbloed RH (2021) Environmental risks of car tire microplastic particles and other road runoff pollutants. *Microplast Nanoplast* 1:1–17
- Tang Y, Rong J, Guan X, Zha S, Shi W, Han Y, Du X, Wu F, Huang W, Liu G (2020) Immunotoxicity of microplastics and two persistent organic pollutants alone or in combination to a bivalve species. *Environ Pollut* 258:113845
- Tarapore P, Ying J, Ouyang B, Burke B, Bracken B, Ho SM (2014) Exposure to bisphenol A correlates with early-onset prostate cancer and promotes centrosome amplification and anchorage-independent growth in vitro. *PLoS One* 9:e90332
- Thandra KC, Barsouk A, Saginala K, Aluru JS, Barsouk A (2021) Epidemiology of lung cancer. *Contemp Oncol (Pozn)* 25:45–52
- Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AW, McGonigle D, Russell AE (2004) Lost at sea: where is all the plastic? *Science* 304:838
- Thompson RC, Moore CJ, Vom Saal FS, Swan SH (2009) Plastics, the environment and human health: current consensus and future trends. *Philos Trans Royal Soc B Biol Sci* 364:2153–2166

- Tong X, Li B, Li J, Li L, Zhang R, Du Y, Zhang Y (2022) Polyethylene microplastics cooperate with helicobacter pylori to promote gastric injury and inflammation in mice. *Chemosphere* 288: 132579
- Tosun J, Scherer U, Schaub S, Horn H (2020) Making Europe go from bottles to the tap: political and societal attempts to induce behavioral change. *Wiley Interdiscip Rev Water* 7:e1435
- Trevisan R, Ranasinghe P, Jayasundara N, Di Giulio RT (2022) Nanoplastics in aquatic environments: impacts on aquatic species and interactions with environmental factors and pollutants. *Toxics* 10. <https://doi.org/10.3390/toxics10060326>
- Ugwu S, Obele C (2023) A mini-review on expanded polystyrene waste recycling and its applications. *World J Adv Eng Technol Sci* 8:315–329
- Urade R, Chou CK, Chou HL, Chen BH, Wang TN, Tsai EM, Hung CT, Wu SJ, Chiu CC (2023) Phthalate derivative DEHP disturbs the antiproliferative effect of camptothecin in human lung cancer cells by attenuating DNA damage and activating Akt/NF- κ B signaling pathway. *Environ Toxicol* 38:332–342
- Urban RM, Jacobs JJ, Tomlinson MJ, Gavrilovic J, Black J, Peoc'h M (2000) Dissemination of wear particles to the liver, spleen, and abdominal lymph nodes of patients with hip or knee replacement. *JBJS* 82:457
- Uy CA, Johnson DW (2022) Effects of microplastics on the feeding rates of larvae of a coastal fish: direct consumption, trophic transfer, and effects on growth and survival. *Mar Biol* 169:27
- van der Zande M, Vandebriel RJ, Groot MJ, Kramer E, Herrera Rivera ZE, Rasmussen K, Ossenkoppele JS, Tromp P, Gremmer ER, Peters RJB, Hendriksen PJ, Marvin HJP, Hoogenboom RLAP, Peijnenburg AACM, Bouwmeester H (2014) Sub-chronic toxicity study in rats orally exposed to nanostructured silica. *Part Fibre Toxicol* 11:8
- Vancamelbeke M, Vermeire S (2017) The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol* 11:821–834
- Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007) Human exposure to bisphenol a (BPA). *Reprod Toxicol* 24:139–177
- Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP (2012) Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev* 33:378–455
- Vecchiotti G, Colafarina S, Aloisi M, Zarivi O, Di Carlo P, Poma A (2021) Genotoxicity and oxidative stress induction by polystyrene nanoparticles in the colorectal cancer cell line HCT116. *PLoS One* 16:e0255120
- Verla AW, Enyoh CE, Verla EN, Nwamnorh KO (2019) Microplastic–toxic chemical interaction: a review study on quantified levels, mechanism and implication. *SN Appl Sci* 1:1400
- Verma R, Vinoda KS, Papireddy M, Gowda ANS (2016) Toxic pollutants from plastic waste—a review. *Procedia Environ Sci* 35:701–708
- Vigneswaran HT, Jagai JS, Greenwald DT, Patel AP, Kumar M, Dobbs RW, Moreira DM, Abern MR (2021) Association between environmental quality and prostate cancer stage at diagnosis. *Prostate Cancer Prostatic Dis* 24:1129–1136
- von Hellfeld R, Zarzuelo M, Zaldibar B, Cajaraville MP, Orbea A (2022) Accumulation, depuration, and biological effects of polystyrene microplastic spheres and adsorbed cadmium and benzo(a)pyrene on the mussel *Mytilus galloprovincialis*. *Toxics* 10
- Wang Q, Xue Y (2015) Characterization of solid tumors induced by polycyclic aromatic hydrocarbons in mice. *Med Sci Monit Basic Res* 21:81–85
- Wang Y-C, Chen H-S, Long C-Y, Tsai C-F, Hsieh T-H, Hsu C-Y, Tsai E-M (2012) Possible mechanism of phthalates-induced tumorigenesis. *Kaohsiung J Med Sci* 28:S22–S27
- Wang Z, Liu H, Liu S (2017) Low-dose bisphenol a exposure: a seemingly instigating carcinogenic effect on breast cancer. *Adv Sci* 4:1600248
- Wang W, Chen J, Fang Y, Wang B, Zou Q, Wang L, Zhang W, Huang X, Lv H, Zhang C (2019a) Identification of gnrh2 and gnrh3 and their expression during brood pouch growth and short-term benzo (a) pyrene exposure in lined seahorse (*Hippocampus erectus*). *Comparat Biochem Physiol Part C Toxicol Pharmacol* 225:108579

- Wang Y, Mao Z, Zhang M, Ding G, Sun J, Du M, Liu Q, Cong Y, Jin F, Zhang W (2019b) The uptake and elimination of polystyrene microplastics by the brine shrimp, *Artemia parthenogenetica*, and its impact on its feeding behavior and intestinal histology. *Chemosphere* 234:123–131
- Wang C, Zhao J, Xing B (2021a) Environmental source, fate, and toxicity of microplastics. *J Hazard Mater* 407:124357
- Wang Y, Huang J, Zhu F, Zhou S (2021b) Airborne microplastics: a review on the occurrence, migration and risks to humans. *Bull Environ Contam Toxicol* 107:657–664
- Wang YL, Lee YH, Hsu YH, Chiu IJ, Huang CC, Huang CC, Chia ZC, Lee CP, Lin YF, Chiu HW (2021c) The kidney-related effects of polystyrene microplastics on human kidney proximal tubular epithelial cells HK-2 and male C57BL/6 mice. *Environ Health Perspect* 129:57003
- Wang L, Lu B, He M, Wang Y, Wang Z, Du L (2022) Prostate cancer incidence and mortality: global status and temporal trends in 89 countries from 2000 to 2019. *Frontiers. Public Health* 10
- Wazir U, Mokbel K (2019) Bisphenol a: a concise review of literature and a discussion of health and regulatory implications. *In Vivo* 33:1421–1423
- Webster TF, Harrad S, Millette JR, Holbrook RD, Davis JM, Stapleton HM, Allen JG, McClean MD, Ibarra C, Abdallah MA, Covaci A (2009) Identifying transfer mechanisms and sources of decabromodiphenyl ether (BDE 209) in indoor environments using environmental forensic microscopy. *Environ Sci Technol* 43:3067–3072
- Weinhouse C, Anderson OS, Bergin IL, Vandenbergh DJ, Gyekis JP, Dingman MA, Yang J, Dolinoy DC (2014) Dose-dependent incidence of hepatic tumors in adult mice following perinatal exposure to bisphenol a. *Environ Health Perspect* 122:485–491
- Welle F (2023) Recycling of post-consumer polystyrene packaging waste into new food packaging applications – part 1: direct food contact. *Recycling* 8. <https://doi.org/10.3390/recycling8010026>
- Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM (2007) In vitro molecular mechanisms of bisphenol a action. *Reprod Toxicol* 24:178–198
- Willert HG, Semlitsch M (1996) Tissue reactions to plastic and metallic wear products of joint endoprostheses. *Clin Orthop Relat Res*:4–14
- Williams GP, Darbre PD (2019) Low-dose environmental endocrine disruptors, increase aromatase activity, estradiol biosynthesis and cell proliferation in human breast cells. *Mol Cell Endocrinol* 486:55–64
- Winkler A, Santo N, Ortenzi MA, Bolzoni E, Bacchetta R, Tremolada P (2019) Does mechanical stress cause microplastic release from plastic water bottles? *Water Res* 166:115082
- Woo J-H, Seo HJ, Lee J-Y, Lee I, Jeon K, Kim B, Lee K (2023) Polypropylene nanoplastic exposure leads to lung inflammation through p38-mediated NF- κ B pathway due to mitochondrial damage. *Part Fibre Toxicol* 20:2
- Wright SL, Kelly FJ (2017) Plastic and human health: a micro issue? *Environ Sci Technol* 51:6634–6647
- Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine organisms: a review. *Environ Pollut* 178:483–492
- Wu AH, Franke AA, Wilkens LR, Tseng C, Conroy SM, Li Y, Polfus LM, De Rouen M, Caberto C, Haiman C, Stram DO, Le Marchand L, Cheng I (2021) Urinary phthalate exposures and risk of breast cancer: the multiethnic cohort study. *Breast Cancer Res* 23:44
- Xia T, Guo J, Zhang B, Song C, Zhao Q, Cui B, Liu Y (2022) Bisphenol a promotes the progression of colon cancer through dual-targeting of NADPH oxidase and mitochondrial electron-transport chain to produce ROS and Activating HIF-1 α /VEGF/PI3K/AKT Axis. *Front Endocrinol (Lausanne)* 13:933051
- Xiang S, Xie Y, Sun X, Du H, Wang J (2022) Identification and quantification of microplastics in aquaculture environment. *Frontiers in marine. Science* 8

- Xiao M, Li X, Zhang X, Duan X, Lin H, Liu S, Sui G (2023) Assessment of cancer-related signaling pathways in responses to polystyrene nanoplastics via a kidney-testis microfluidic platform (KTP). *Sci Total Environ* 857:159306
- Xie S, Zhang R, Li Z, Liu C, Chen Y, Yu Q (2023) Microplastics perturb colonic epithelial homeostasis associated with intestinal overproliferation, exacerbating the severity of colitis. *Environ Res* 217:114861
- Xu M, Halimu G, Zhang Q, Song Y, Fu X, Li Y, Li Y, Zhang H (2019a) Internalization and toxicity: a preliminary study of effects of nanoplastic particles on human lung epithelial cell. *Sci Total Environ* 694:133794
- Xu X, Zhou G, Lei K, LeBlanc GA, An L (2019b) Phthalate esters and their potential risk in PET bottled water stored under common conditions. *Int J Environ Res Public Health* 17
- Xu D, Ma Y, Han X, Chen Y (2021) Systematic toxicity evaluation of polystyrene nanoplastics on mice and molecular mechanism investigation about their internalization into Caco-2 cells. *J Hazard Mater* 417:126092
- Yan X, Zhang Y, Lu Y, He L, Qu J, Zhou C, Hong P, Sun S, Zhao H, Liang Y, Ren L, Zhang Y, Chen J, Li C (2020) The complex toxicity of tetracycline with polystyrene spheres on gastric cancer cells. *Int J Environ Res Public Health* 17. <https://doi.org/10.3390/ijerph17082808>
- Yan Z, Liu Y, Zhang T, Zhang F, Ren H, Zhang Y (2021) Analysis of microplastics in human feces reveals a correlation between fecal microplastics and inflammatory bowel disease status. *Environ Sci Technol* 56:414–421
- Yang CZ, Yaniger SI, Jordan VC, Klein DJ, Bittner GD (2011) Most plastic products release estrogenic chemicals: a potential health problem that can be solved. *Environ Health Perspect* 119:989–996
- Yang H, Chen G, Wang J (2021a) Microplastics in the marine environment: sources, fates, impacts and microbial degradation. *Toxics* 9. <https://doi.org/10.3390/toxics9020041>
- Yang S, Cheng Y, Chen Z, Liu T, Yin L, Pu Y, Liang G (2021b) In vitro evaluation of nanoplastics using human lung epithelial cells, microarray analysis and co-culture model. *Ecotoxicol Environ Saf* 226:112837
- Yang W, Jannatun N, Zeng Y, Liu T, Zhang G, Chen C, Li Y (2022) Impacts of microplastics on immunity *Front Toxicol*:4
- Yao Z, Seong HJ, Jang Y-S (2022) Environmental toxicity and decomposition of polyethylene. *Ecotoxicol Environ Saf* 242:113933
- Ye L, Qi C, Hong J, Ma X (2017) Life cycle assessment of polyvinyl chloride production and its recyclability in China. *J Clean Prod* 142:2965–2972
- Yee MS, Hii LW, Looi CK, Lim WM, Wong SF, Kok YY, Tan BK, Wong CY, Leong CO (2021) Impact of Microplastics and nanoplastics on human health. *Nanomaterials* (Basel):11
- Yu C, Ke Y, Deng Q, Lu S, Ji J, Hu X, Zhao Y (2018) Synthesis and characterization of polystyrene-montmorillonite nanocomposite particles using an anionic-surfactant-modified clay and their friction performance. *Appl Sci* 8. <https://doi.org/10.3390/app8060964>
- Yu F, Yang C, Zhu Z, Bai X, Ma J (2019) Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. *Sci Total Environ* 694:133643
- Yuan Q, Jin K, Zhou X, Qiu Z, Li J, Jin D, Zhang Z, Zhang C, Yang L, Zhan Y, Qiu S, Wei Q (2022) Urinary polycyclic aromatic hydrocarbon metabolites are positively related to serum testosterone levels of males and serum estradiol levels of females among U.S. adults. *Front Endocrinol (Lausanne)* 13:1037098
- Zaheer J, Kim H, Ko IO, Jo E-K, Choi E-J, Lee H-J, Shim I, Woo H-j, Choi J, Kim G-H (2022) Pre/post-natal exposure to microplastic as a potential risk factor for autism spectrum disorder. *Environ Int* 161:107121
- Zandalinas SI, Fritsch FB, Mittler R (2021) Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. *Trends Plant Sci* 26:588–599
- Zani C, Ceretti E, Covolo L, Donato F (2017) Do polychlorinated biphenyls cause cancer? A systematic review and meta-analysis of epidemiological studies on risk of cutaneous melanoma and non-Hodgkin lymphoma. *Chemosphere* 183:97–106

- Zhang K-S, Chen H-Q, Chen Y-S, Qiu K-F, Zheng X-B, Li G-C, Yang H-D, Wen C-J (2014) Bisphenol A stimulates human lung cancer cell migration via upregulation of matrix metalloproteinases by GPER/EGFR/ERK1/2 signal pathway. *Biomed Pharmacother* 68: 1037–1043
- Zhang S, Li J, Fan J, Wu X (2020) Bisphenol A triggers the malignancy of acute myeloid leukemia cells via regulation of IL-4 and IL-6. *J Biochem Mol Toxicol* 34:e22412
- Zhang C, Chen J, Ma S, Sun Z, Wang Z (2022a) Microplastics may be a significant cause of male infertility. *Am J Mens Health* 16:15579883221096549
- Zhang S, Cheng C, Lin Z, Xiao L, Su X, Zheng L, Mu Y, Liao M, Ouyang R, Li W, Ma J, Cai J, Liu L, Wang D, Zeng F, Liu J (2022b) The global burden and associated factors of ovarian cancer in 1990–2019: findings from the global burden of disease study 2019. *BMC Public Health* 22:1455
- Zhang X, He Y, Xie Z, Peng S, Xie C, Wang H, Liu L, Kang J, Yuan H, Liu Y (2022c) Effect of microplastics on nasal and gut microbiota of high-exposure population: protocol for an observational cross-sectional study. *Medicine* 101
- Zhang X, Wang H, Peng S, Kang J, Xie Z, Tang R, Xing Y, He Y, Yuan H, Xie C, Liu Y (2022d) Effect of microplastics on nasal and intestinal microbiota of the high-exposure population. *Front Pub Health*:10
- Zhao L, Shi W, Hu F, Song X, Cheng Z, Zhou J (2021) Prolonged oral ingestion of microplastics induced inflammation in the liver tissues of C57BL/6J mice through polarization of macrophages and increased infiltration of natural killer cells. *Ecotoxicol Environ Saf* 227: 112882
- Zhou Y, Wang J, Zou M, Jia Z, Zhou S, Li Y (2020) Microplastics in soils: a review of methods, occurrence, fate, transport, ecological and environmental risks. *Sci Total Environ* 748:141368
- Zhu X (2021) The plastic cycle—an unknown branch of the carbon cycle. *Front Marine Sci*:1227
- Zhu M, Huang C, Ma X, Wu R, Zhu W, Li X, Liang Z, Deng F, Wu J, Geng S, Xie C, Zhong C (2018) Phthalates promote prostate cancer cell proliferation through activation of ERK5 and p38. *Environ Toxicol Pharmacol* 63:29–33
- Znaor A, Skakkebaek NE, Rajpert-De Meyts E, Kuliš T, Laversanne M, Gurney J, Sarfati D, McGlynn KA, Bray F (2022) Global patterns in testicular cancer incidence and mortality in 2020. *Int J Cancer* 151:692–698
- Zorov DB, Juhaszova M, Sollott SJ (2006) Mitochondrial ROS-induced ROS release: an update and review. *Biochimica et Biophysica Acta (BBA). Bioenergetics* 1757:509–517
- Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 94:909–950
- Zuccarello P, Ferrante M, Cristaldi A, Copat C, Grasso A, Sangregorio D, Fiore M, Conti GO (2019) Exposure to microplastics (<10 µm) associated to plastic bottles mineral water consumption: the first quantitative study. *Water Res* 157:365–371



Cancer: Insight and Strategies to Reduce the Burden

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Abstract

Cancer is associated with abnormal cell growth and becomes deadly when it metastasizes to other areas of the body. Risk of cancer can be reduced by adopting early screening, testing, healthy lifestyle, and preventive interventions like avoiding tobacco, protection against sun, getting vaccinated, and lowering the exposure of environmental toxins. Policy and legislation are essential in addressing the global burden of cancer, including prevention, screening, assessment, therapy, and care. The National Cancer Institute (NCI) has developed public health strategies to lower cancer risk, including reducing tobacco use, addressing obesity, and investigating emerging risk factors. Although cancer awareness in public not only increases their knowledge about the disease but also proves to be conducive to minimize social stigma about the disease. The “World Health Organization” (WHO) is an agency of the United Nations that aims to promote health globally and to address major health issues, including cancer. There are many international policies and initiatives aimed at increasing awareness about cancer and reducing the burden of the disease globally. These include World Cancer Day, “World Health Organization” (WHO) action plan for control and prevention of Non-communicable Diseases (NCDs), “United Nations (UN) Sustainable Development Goals (SDGs)”.

Keywords

Cancer public health research · Cancer survivorship · Risk factors · SDGs and cancer · Sustainable development and quality of life · Union for International Cancer Control (UICC)

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1 Introduction

The word “cancer” encompasses a broad range of diseases which can develop from various tissues and organs within the body. It happens when abnormal cells proliferate and uncontrollably multiply, invading neighboring tissues and potentially spreads to various surrounding areas. This phenomenon, called metastasis, is a significant factor in causing deaths related to cancer (National Cancer Institute 2022c). Cancer is also referred to as a neoplasm or malignant tumor. The failure of normal cellular regulation mechanisms can lead to the growth and reproduction of damaged or abnormal cells, resulting in the formation of tumors, which can be benign or malignant (Stanford Health Care 2022).

Malignant tumors, commonly referred to as cancerous tumors, have the ability to infiltrate surrounding tissues and spread to other parts of the body, resulting in the development of new tumors through a process called metastasis (Canadian Cancer Society 2023).

- Cancer and other non-communicable illnesses are often seen as major dangers to human health and development (Organization, W. H 2022b).
- COVID-19 has had an influence on healthcare systems’ capacity to recognize and treat cancer patients (Richards et al. 2020).
- The use of technology can be highly beneficial in the fight against cancer (National Cancer Institute 2022b).

2 Causes of Cancer

Cancer is primarily caused by mutations or changes in the DNA of cells. These genetic variations can be inherited or arise as a result of environmental factors (Cancer.Net 2018). External causes of these mutations include physical agents such as radiation and UV light, chemical agents such as cigarette smoke, asbestos, alcohol, pollution, and hazardous food and water, and biological agents such as viruses, microbes, and parasites (Parsa 2012). There are additional factors that can contribute to the onset of cancer, such as:

- **A sedentary lifestyle.** Foods with high fat or high-sugar products can raise your chance of developing a variety of cancers. You are also more susceptible to sickness if you do not exercise regularly (American Cancer Society 2020a).
- **A hazardous environment.** Toxins in the climate, like asbestos, insecticides, and radon, ultimately cause cancer (Theodore and Theodore 2021).
- **Exposure to radiation.** The sun’s ultraviolet radiation dramatically raises your chance of developing skin cancer. Overexposure to radiation therapy might potentially pose a concern (Watson et al. 2016).
- **Hormone replacement treatment.** Women who use hormone replacement treatment are at a greater risk of breast and endometrial cancer (Cancer Research UK 2022a).

3 Cancer Types

Cancer is diversified into five forms which are:

- **Carcinoma:** A malignant neoplasm that arises from epithelial tissue and affects vital organs and glands like the lungs, breasts, pancreas, and skin, and is the most prevalent type of cancer.
- **Sarcoma:** A malignant neoplasm that originates from mesenchymal tissues such as muscle, fat, bone, cartilage, or blood vessels, and spreads to other areas of the body, which can be life-threatening.
- **Melanoma:** A type of cancer that affects the melanocytes, which are the cells that provide color to the skin, and can spread to other parts of the body if not detected early.
- **Lymphoma:** A type of malignancy that affects the lymphocytes, a type of white blood cell that plays a crucial role in the immune system, and can occur in the lymph nodes, spleen, bone marrow, or other organs.
- **Leukemia:** A cancer of the blood and bone marrow, where abnormal white blood cells are produced and accumulate, leading to weakened immunity and other complications (Cleveland Clinic 2022).

4 Hallmarks of Cancer

The eight hallmarks of cancer include the ability to sustain proliferative signaling, evade growth suppressors, resist cell death, enable replicative immortality, induce access vasculature, activate invasion and metastasis, reprogram cellular metabolism, and avoid immune destruction. Recently, “deregulating cellular metabolism” and “avoiding immune destruction” have been recognized as additional hallmarks, but now, 11 years later, it is clear that these two hallmarks are also core features of cancer and are included in the current depiction (Fig. 1; Hanahan 2022).

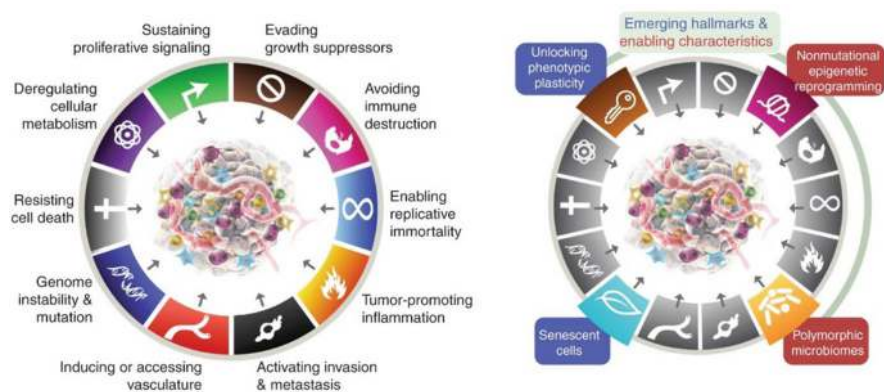


Fig. 1 Hallmarks of cancer. (Hanahan 2022)

5 Risk Factors

Different risk factors might raise the chances of getting cancer. According to analysis, these risk factors can include: cigarette usage, heavy alcohol use, an unhealthy diet and processed meat, sugary beverages and salty snacks, starchy meals, and refined carbs including sugars and processed grains, lack of physical exercise, exposure to external pollution, exposure to radiation, uncontrolled exposure to UV light, like sunlight and infection with viruses such as helicobacter pylori, hepatitis C, HIV, and the Epstein-Barr virus, which promotes infectious mononucleosis (Healthline Media [2022](#)).

The chance of having cancer rises with age as well. According to the National Cancer Institute (NCI), chance to acquire cancer appears to develop until the age of 70–80 and then decreases (National Cancer Institute [2022a](#)).

5.1 Incidence of Cancer

The incidence of cancer varies worldwide and by type of cancer. The World Health Organization (WHO) reported that in 2020, approximately 38.8 million new cases of cancer emerged globally, with the prevalent types being lung, breast, and colorectal cancer. Factors that can affect the incidence of cancer include genetics, lifestyle, and environmental factors.

In 2020, there were around 19.3 million new cases of cancer and ten million deaths globally. With 2.3 million cases, breast cancer has become the most commonly diagnosed cancer, followed by lung, colorectal, prostate, and stomach cancers. However, lung cancer remains the primary cause of cancer-related deaths, with 1.8 million deaths followed by colorectal, liver, stomach, and breast. In transitioned countries, the incidence of cancer is 2–3 times higher compared to transitioning countries, but death rates vary less. In transitioning countries, death rates for breast and cervical cancers are higher compared to transitioned countries. It is estimated that the burden of cancer will rise to 28.4 million cases by the year 2040, with a greater increase in transitioning countries. To control global cancer, it is crucial to establish a sustainable infrastructure for cancer prevention and treatment in transitioning countries (Sung et al. [2021](#)).

6 Reducing Cancer Burdens

Avoiding risk factors and implementing evidence-based preventive measures can now prevent 30–50% of cancer cases. Timely detection, proper care, and treatment can also reduce the burden of cancer. If diagnosed early and treated effectively, many types of cancer can be cured (World Health Organization (WHO) [2023](#)).

7 Cancer-Related Policies

If you are a public health or cancer medical expert, or you're just wondering about why cancer would be a public health concern, cancer affects practically everyone on the planet. Whether you've had cancer, a loved one has suffered from cancer, or you've lived with the anxiety of awaiting cancer testing results, getting diagnosed with cancer throws up a world of unsettling unknowns.

While cancer therapies are growing more improved by the year, there is currently no cure. Cancer and global health will continue to just go hand in hand as long as cancer claims the lives of individuals all over the world (The Mesothelioma Center [2023](#)).

7.1 Policies and Legislation Dealing with Cancer

Policy and law are critical in addressing the global and local burden of cancer. Effective utilization of the legislation to accomplish population health goals demands cross-sector coordination.

In 2011, the UN General Assembly had a historic high-level conference that ended in a decision to address non-communicable diseases (NCDs) as a key development concern. The World Health Organization Global Initiative on NCDs was endorsed by the World Health Assembly in 2013, stressing whole-of-society approaches to reducing the key causes of avoidable NCDs. The UN Sustainable Development Goals included a goal to minimize premature death from NCDs by one-third in 2015.

The proper choice of the legislation is crucial in the fight against cancer as well as other NCDs. This applies to the entire spectrum of cancer and non-communicable diseases (NCDs), including prevention (reducing exposure to risk factors such as tobacco, alcohol, unhealthy diet, air pollution, and occupational hazards), as well as the gathering and management of personal health information (protection of privacy rights while permitting for the conduct and distribution of vital medical and public health research), screening, assessment, therapy, and care (access, cost, and quality).

Collaboration among sectors is required for the successful application of the law: government, civilized society, academics, health professionals, localities, persons afflicted by cancer and NCDs, their caregivers and families, and, when appropriate, the private sector. Collaboration across government is also required, as few issues can be resolved by health ministry's functioning alone.

The fight against cancer and non-communicable diseases (NCDs) through law requires participation in various legal and governance frameworks at domestic, regional, and global levels. This includes laws governing health, individual rights, global trade, intellectual property, financing, environment, and occupational safety and health. It also involves the ability to resist legal action or threats of litigation by corporate interests in the tobacco, alcohol, and food sectors, which is becoming more prevalent. Legal competence is a crucial aspect of cancer and NCD treatment (The Cancer Atlas [2022](#)).

8 Cancer-Related Health Policies

8.1 The Importance of Cancer Public Health Research

The impact of cancer and its associated complications on public health is substantial, affecting individuals in various ways such as premature death, reduced productivity, and long-term effects on the quality of life for cancer survivors. As the population continues to age, it is anticipated that the impact of cancer on public health will increase. Although cancer can impact people and their families differently, analyzing its effect on large groups can yield valuable information for improving practices, policies, and initiatives that benefit millions of individuals annually. Through NCI-funded research, there have been significant advancements in public health and cancer control, resulting in improvements in several areas. For example:

- Policies based on scientific research have contributed to a reduction in tobacco-related deaths and illnesses worldwide. The prevalence of cigarette smoking among adults has significantly decreased from 42% in 1964 to 14% in 2017. Tobacco use has been linked to at least 15 different types of cancer, and if smoking were eradicated, it could prevent approximately one-third of cancer-related deaths, in addition to reducing the incidence of other tobacco-related diseases, such as cardiovascular disorders.
- Since 1973, the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program has been gathering and publishing official statistics on cancer prevalence in the United States, including incidence, mortality, and survival trends for various cancer types. This knowledge has helped guide research and public policy.
- Additionally, NCI-supported epidemiology research has led to the identification of hereditary cancer syndromes like Lynch syndrome and Li-Fraumeni syndrome, which has improved clinical treatment and the ability to identify individuals at high risk.

8.2 The National Cancer Institute's Plan for Public Health and Cancer Control Research

The NCI creates an Annual Plan and Budget Plan for Congress and the President, which outlines the Institute's scientific objectives and promising opportunities across the research spectrum. The plan encompasses a strategic aim and approach for enhancing research on cancer control and healthcare system, among other things. Cancer research breakthroughs will benefit all populations equally.

8.3 Implement Public Health Strategies to Lower Cancer Risk

It is believed that minimizing susceptibility to cigarette smoke as well as other atmospheric toxins, encouraging individuals to maintain a healthy weight and

undergo recommended cancer screenings and vaccinations could potentially prevent 30–50% of the cancer cases diagnosed currently. The development of innovative strategies aimed at facilitating behavioral changes and the adoption of healthy habits could result in a considerable effect. Its primary aim includes:

- The reduction of tobacco use across all age groups, as well as a deeper understanding of the health implications associated with electronic cigarettes and other alternative tobacco delivery methods, is crucial.
- The creation of behavioral and educational treatments to decrease obesity and the danger of obesity-related cancers is important.
- Researching the relationship between physical activity and cancer incidence, as well as patient outcomes, and translating that knowledge into effective interventions for individuals who are at risk, receiving treatment, or have completed treatment is essential.
- Additionally, developing interventions to increase the adoption of established and effective cancer preventive measures, such as HPV vaccination, and diagnostic techniques, such as colorectal and lung cancer screening, is crucial.

8.4 Encouraging Cancer Research to Decrease Inequities

Some malignancies affect certain populations disproportionately, particularly specific racial/ethnic groupings and rural communities. There are several explanations for these discrepancies, including biological variables and ineffective cancer treatment and prevention initiatives in healthcare setting. Our primary goals are as follows:

- Developing new, affordable, and portable technology for cancer screening and diagnosis that can be used in various healthcare settings, such as clinics or doctor's offices, is crucial.
- Developing smoking cessation programs that are specifically tailored for disadvantaged groups and can be implemented on a large scale to have a broad population impact is important.
- Evaluating the effectiveness of a single-dose HPV vaccine and alternative methods that do not require refrigeration to increase HPV vaccination rates, particularly in underserved populations, is necessary.
- Increasing the participation of ethnic minorities and underserved populations in clinical trials sponsored by the NCI is important.

8.5 Ensure that Cancer Victims Enjoy the Best Quality of Life Possible

- Adult survivors of cancer and childhood cancer survivors alike often experience health problems resulting from the tumor or treatment, which may require

ongoing care. However, compared to adult survivors, those who had cancer as children may face these consequences for a much longer period of time. The primary goals are as follows:

- Evaluating the immediate negative impacts of cancer as well as creating strategies to mitigate their impact upon patients.
- Determining risk variables, particularly genetic factors, which contribute to disparities in incidence, such as the likelihood of recurring malignancies.
- By assessing the impact of comorbidities on treatment-induced side effects, we can improve our ability to provide comprehensive care for patients.
- Developing interventions that promote healthy behaviors in cancer survivors can lead to improved clinical outcomes and a better quality of life (Cancer.Net [2020](#)).

9 National Cancer Control Program with Early Diagnosis of Cancer

Early cancer identification not only enhances survival, minimizing treatment intensity, but also improves cancer patients' life expectancy. The Diagnosis of Symptomatic Cancer Plan 2022–2025 of the National Cancer Control Programme (NCCP) aims to establish a comprehensive, all-encompassing approach to raise the proportion of symptomatic malignancies discovered at a preliminary phase (stages I and II) in Ireland (Our Health Service [2023](#)).

10 ESMO (European Society for Medical Oncology)

10.1 The ESMO Pledge

ESMO presented a public announcement during the Forum as part of its “special consultative standing” with ECOSOC. The ESMO statement raised concerns as the WHO 2020 update on Cancer showed just 12 countries can join the cancer target of 3.4 by 2030. ESMO mentioned its different resources that might help governments reduce early cancer mortality, like

- The ESMO Clinical Practice Guidelines, which consist of more than 80 evidence-based recommendations, have become the benchmark for cancer care.
- The ESMO COVID-19 with Cancer Portal is a comprehensive resource that includes guidelines for cancer treatment, as well as advice for patients and caregivers, and a call to action for prioritizing COVID-19 vaccination for those with cancer.
- The European Society of Medical Oncology (ESMO) has issued recommendations for avoiding and addressing shortages of affordable essential cancer medications that are used in the treatment of many different types of cancer.
- The ESMO-Magnitude of Clinical Impact Scale is a tool used to prioritize expensive new cancer therapies, in order to optimize the use of limited public and private (European Society for Medical Oncology [2021](#)).

11 American Society of Clinical Oncology (ASCO)

ASCO, the largest professional organization for cancer care specialists in the United States, is dedicated to advocating for policies that ensure all cancer patients have access to high-quality and high-value treatment.

Advocating for policymaking that ensures the access with provision of cancer care, working to develop tools to assist patients and physicians address the increased prices of precision medicine, promoting initiatives to minimize disparities in disease treatment and outcomes, continuing work to establish a robust oncology workers to fulfill patient needs are all examples of efforts focused on improving the availability and provision of high-quality cancer care.

11.1 Clinical Trials

ASCO believes that it is critical to promote greater involvement toward clinical studies to improve research system, focusing on the incorporation of best science and guaranteeing appropriate payment for expenditures of patients engaged in clinical trials.

11.2 Shortage of Drugs

Many vital medicines are in limited supply in the cancer community, and the situation is deteriorating. This is a complicated issue with various causes involving regulatory and production difficulties. Many groups and activists are collaborating with legislators to highlight the important shortages with propose solutions to avoid them in future.

12 Federal Cancer Research Funding

The funding allocated by the federal government for cancer research has remained stagnant for over a hundred years and has decreased in real terms due to inflation. In order to advance cancer care in the future, there is a need to increase and revitalize the funding for key organizations such as the National Institutes of Health, the National Cancer Institute, and the U.S. Food and Drug Administration. Addressing the challenge of high cancer care costs is a key priority for organizations like ASCO, which are actively pursuing various initiatives to promote reforms and tools that can empower patients and physicians to collaborate on this front. By joining forces and leveraging their expertise, these organizations hope to make a meaningful impact on the quality and affordability of cancer care.

12.1 Quality Cancer Care

One of the most significant parts of a good treatment outcome is the quality care that the patient acquires. There are several methods to advocate for patients to guarantee they receive the most effective treatment.

12.2 Cancer Care Value

Developing value in patient management is essential for reducing the financial pressures that patients confront as a consequence of increased healthcare costs. Advocating for policy prioritizes values (Cancer.Net 2020).

12.3 Diagnosis of Cancer

Early detection of cancer often offers the highest chance of a cure. Detection of few cancers in the early stage can save life. Screening tests for other cancers are only advised for people who are at high risk. A doctor may employ one or maybe more methods to diagnose cancer that include:

- **Taking a medical history:** A doctor will ask about the patient's symptoms, medical history, and family history of cancer.
- **Physical examination:** During a physical examination, a doctor may feel for lumps or other changes in the body that could indicate cancer.
- **Imaging tests:** Imaging tests, such as X-rays, CT scans, MRI scans, and PET scans, can help to identify changes in the body that could be indicative of cancer.
- **Biopsy:** A biopsy is a procedure in which a small sample of tissue is removed from the body and analyzed in a laboratory to determine if it is cancerous. Biopsies can be performed using a needle, a surgical procedure, or other methods.
- **Blood tests:** Blood tests can measure certain substances in the blood that may indicate the presence of cancer. For example, elevated levels of certain proteins or abnormal cells can indicate the presence of cancer.
- **Endoscopic procedures:** Endoscopic procedures, such as colonoscopies and bronchoscopies, allow a doctor to examine the inside of organs and tissues using a special camera attached to a flexible tube.
- **Tumor markers:** Tumor markers are substances produced by cancer cells that can be detected in the blood, urine, or other bodily fluids. Measuring these markers can help to diagnose certain types of cancer.
- **Staging:** Once a cancer diagnosis is confirmed, the next step is to determine the stage of the cancer, which is a measure of how advanced the cancer is. This information is important for determining the most appropriate treatment options.

The most appropriate diagnostic strategy for a particular patient will depend on several factors, including the type and stage of the cancer, the patient's symptoms,

and the patient's overall health. In many cases, a combination of diagnostic strategies may be used to confirm or rule out a cancer diagnosis (National Institute of Cancer 2023).

13 Prevention of Cancer

Prevention is defined as “the protection of one's health through individual and community efforts (Miller et al. 2008).” This is accomplished by describing the cancer burden, identifying its causative factors, and assessing cancer prevention interventions (Nagai and Kim 2017). Previously, cancer prevention research has primarily focused on lowering cancer incidence and mortality. Early cancer prevention efforts focused on both synthetic and natural chemicals (e.g., tamoxifen and retinoids) and natural compounds (e.g., omega-3 fish oil and carotene) (Lippman 2009). Efforts have recently expanded to include ‘pre-disease’ interventions or those focused on delaying carcinogenesis (Foucar 2005). Organizations at the national and global levels, such as the World Health Organization (WHO), National Cancer Institute (NCI), and the International Agency for Research on Cancer (IARC), have the capabilities and resources to accurately reflect the cancer burden at the population level. However, prevention initiatives should have a significant impact on the individual level before they can have an impact on the population level, implying that population health is the primary health experience of individuals. A combination of genetic and environmental factors, such as behavioral, lifestyle, and environmental exposures, influence the risk of developing cancer (Fig. 2; Loomans-Kropp and Umar 2019).



Fig. 2 Prevention of cancer

Prevention of carcinogenic infections and initiatives to improve lifestyle are crucial for lowering the incidence of various cancers. In low-income nations, cancers linked to infections predominate. Contrarily breast, colorectal, and lung cancer have high incidence rates in high-income countries and are strongly linked to risk factors related to lifestyle, such as obesity, alcoholism, and cigarette smoking. Early cancer screening and identification of patients with high risk of cancer especially those with family history are crucial for improving survival (Loud and Murphy 2017). Prevention against cancer can be divided into primary prevention and secondary prevention:

13.1 Primary Prevention Against Cancer

Prevention of cancer means to stop cancer before it starts developing. Cancer is a preventable disease. Primary prevention encourages people to engage in healthy behaviors that reduce their risk of cancer development.

Cancer can be prevented by:

- **Avoiding tobacco:** Using tobacco products is the leading cause of preventable deaths worldwide, including deaths from cancer. Quitting smoking and avoiding exposure to secondhand smoke can significantly reduce the risk of cancer.
- **Maintaining a healthy diet:** A diet that includes whole grains, fruits, vegetables, and red meats can reduce the risk of cancer.
- **Staying physically active:** Regular physical activity can help to prevent cancer by maintaining a healthy weight, reducing inflammation, and improving immune function.
- **Protecting against the sun:** Ultraviolet (UV) radiation from the sun can increase the risk of skin cancer. Wearing sunscreen, protective clothing, and sunglasses, and seeking shade when the sun is strongest can help to reduce the risk of skin cancer.
- **Getting vaccinated:** Some vaccines, such as the HPV vaccine, can help to prevent cancer.
- **Lowering exposure of environmental toxins:** Exposure to certain chemicals, such as those found in certain pesticides and industrial products can increase the risk of cancer. Limiting exposure to these substances can help to reduce the risk of cancer.
- **Getting screened:** Early diagnosis of cancer can improve the chances of successful treatment. Regular screenings, such as mammograms and colonoscopies, can help to identify cancer at an early stage (Centers for Disease Control and Prevention 2021).

13.2 Secondary Prevention

Secondary prevention involves identifying diseases in their early stages, even among asymptomatic individuals. Early detection enables timely treatment, leading to improved outcomes, reduced mortality rates, and more effective management of

the disease. Secondary prevention in cancer refers to measures that are taken to prevent cancer recurrence or to detect cancer at an early stage in individuals who have already been diagnosed with cancer. This can include surveillance through regular cancer screenings, such as mammograms or colonoscopies, as well as adaptation of healthy lifestyle, including maintaining a healthy body weight or quitting smoking, which can help reduce the risk of cancer recurrence.

Some specific strategies for secondary prevention in cancer may include:

- **Regular cancer screenings:** These can help in the early diagnosis of cancer when it is more likely to be treatable.
- **Adopting a healthy lifestyle:** This can include maintaining a healthy weight, getting regular exercise, and having a healthy diet, which may help reduce the risk of cancer recurrence.
- **Taking medications as prescribed:** If you are taking medication to treat cancer or to prevent cancer relapse, it is important to take it as prescribed by your healthcare provider.
- **Managing stress:** Research has shown that stress can affect the immune system and may potentially increase the risk of cancer recurrence. Managing stress through techniques such as relaxation, meditation, or exercise may help reduce this risk (Santaballa et al. [2020](#)).

14 Cancer Awareness Through Social Media

In public health intervention, social media is widely used to educate large community on cancer screening and early diagnosis. Social media can be a powerful tool for raising cancer awareness and promoting cancer prevention. Through social media message can be targeted to specific demographic group and geographical regions that have poor knowledge about prevention, symptoms, and screening of disease (Cavallo et al. [2014](#); Dunn et al. [2018](#)). As a result, social media intervention strategies may be more effective than traditional interventions in addressing health disparities (Chou et al. [2009](#); Knoll [2016](#)). There are many ways in which cancer awareness campaigns can be implemented through social media, including:

- **Sharing information using social media:** Social media platforms can be used to share information about early detection and prevention of cancer, such as the importance of regular cancer screenings and the benefits of adopting a healthy lifestyle (Pagoto et al. [2019](#)).
- **Promoting cancer awareness events:** Social media can be used to promote cancer awareness events, such as fundraisers or community walks, and to encourage people to participate in these events (Schliemann et al. [2019](#)).
- **Sharing personal stories:** Personal stories and experiences with cancer can be powerful tools for raising awareness and providing support to others. Sharing these stories through social media can help raise awareness and encourage others to take steps to prevent cancer or detect it early.

- **Partnering with influencers:** Collaborating with influencers or celebrities who have a large social media following can help raise awareness about cancer and its prevention.

15 Food Labeling Initiatives to Prevent Cancer

Food labeling initiatives that can help prevent cancer by providing nutritional content information to consumers and potential health impacts of the foods they are purchasing include:

- **Nutrient labeling:** Nutrient labeling, such as the Nutrition Facts panel found on most packaged foods, provides information about the various nutrients, calories, and serving size (e.g., protein, fat, and carbohydrates) in a food product. Obesity and overweight have been linked to an increased risk of 13 cancers, according to the Centers for Disease Control (CDC). This information can help consumers make informed choices about the foods they eat and assist in preventing cancer by allowing them to choose foods that are lower in unhealthy nutrients (e.g., saturated, and trans fats) and higher in healthy nutrients (e.g., fiber). Obesity and overweight have been linked to an increased risk of 13 cancers, according to the Centers for Disease Control (CDC) (Thompson Cancer Survival Center 2021).
- **Front-of-pack labeling:** Front-of-pack labeling is a system that uses symbols or icons to indicate the nutritional quality of a food product briefly. This type of labeling can help consumers quickly identify healthier options and assist in preventing cancer by allowing them to make healthier choices more easily.
- **Health claims:** Health claims are statements made on food labels that link a food or a dietary component to a reduced risk of a specific health condition or disease. For example, a food label that states “low in saturated fat” may include a health claim linking the low saturated fat content to a reduced risk of heart disease. Health claims can help consumers make informed choices about the foods they eat and assist in preventing cancer by allowing them to choose foods that may have a lower risk of certain health conditions. Dietary fibers play important role in prevention of cancer and cardiovascular diseases. According to the American Institute of Cancer Research, when diet contains 30 g of dietary fiber per day then the risk of colorectal cancer is reduced by 7%. Fruits, vegetables, whole grain breads, peas, and beans are rich sources of dietary fiber.
- **Sugars added are now listed on the label:** This is crucial for cancer prevention because you will now be aware of the amount of sugar your food contains such as sweetened fruit drinks, yogurt, and flavored milk. According to AICR research, eating foods and drinks with high sugar content can lead to obesity which is a risk factor for 11 different types of cancers. AICR and other health organizations urged the FDA to implement this change (Research, A. I. o. C 2016).

16 Public Awareness on Cancer

On February 4th, World Cancer Day is celebrated globally to raise cancer awareness among the public. Cancer awareness is crucial in the twenty-first century due to the increase in mortality rate. Despite major advances in cancer diagnosis and treatment still number of new cancer cases increases each year around the globe. The World Health Organization (WHO) estimates that about 40% of cancer-related deaths can be avoided. The main objectives of celebrating Cancer Day include:

- Increasing cancer awareness and reducing the stigma and fear associated with it.
- Assist patients in early diagnosis of cancer so that they can seek treatment sooner.
- Educating people about the major risk factors, as changing one's lifestyle, or avoiding the risk factors could prevent more than 30% of cancer cases.
- To raise awareness about the importance of regular screenings and check-ups (Sparsh Hospital [2022](#)).

The main aim of Cancer Day is to make people aware of diseases and to minimize the fear connected with it. Cancer is one of the largest causes of death worldwide. Lung, breast, cervical, and colorectal cancers are the most prevalent types of cancers. World Cancer Day aims at making the world healthier and better place free of deadly diseases.

16.1 Cancer Days Calendar

The aim of World Cancer Day is to raise cancer awareness and encourage cancer prevention, detection, and treatment. The theme of World Cancer Day changes each year, but the overall goal remains the same: to draw attention to the worldwide cancer burden and to encourage actions to address it (World Cancer Day [2022](#)).

Cancer is a major cause of death worldwide, due to cancer about ten million people died in the year 2020. World Cancer Day aims to reduce this number by promoting the importance of cancer prevention, early detection, and access to treatment. The day also serves as a platform for advocacy and the promotion of cancer research, causes, prevention, and treatment (Organization, W. H [2022a](#)).

World Cancer Day provides an opportunity for people, organizations, and governments all over the world to gather in the fight against cancer. It is a time to raise awareness about the impact of cancer on individuals and communities, and to take action to reduce the cancer burden on a global scale.

16.2 International Organizations Work for Cancer Awareness

There are many international organizations that work to raise cancer awareness and to promote prevention, early detection, and treatment of cancer (Fig. 3). Some examples include:



Fig. 3 International organization works for cancer awareness

- **“World Health Organization (WHO)”**: The WHO is an agency of the United Nations that aims to promote health globally and to address major health issues, including cancer. The WHO provides information and resources on cancer prevention, screening, treatment, and research, and it also works to improve cancer care access in middle- and low-income countries (Organization, W. H [2022a](#)).
- **“Union for International Cancer Control (UICC)”**: The UICC is an organization that fights against cancer on the global level. It does this through advocacy, education, and research, and by promoting the importance of prevention of cancer, early detection, and access to treatment. The UICC also organizes “World Cancer Day”, which takes place on February 4th each year (UICC [2022](#)).
- **“American Cancer Society (ACS)”**: The American Cancer Society is a non-profit organization devoted to eradicating cancer as a major health risk. It does this through advocacy, education, and research, and by providing support and resources to cancer patients and their families (American Cancer Society [2022c](#)).
- **“Cancer Research UK”**: “Cancer Research UK” is a charitable organization that funds and conducts research into cancer causes, prevention, and treatment. It also provides information and support to cancer patients and their families (Cancer Research UK [2022b](#)).

17 SDGs and Cancer

SDGs that are relevant to cancer include:

- **“Goal 1” (No Poverty)**: Poverty is a major barrier to cancer prevention, detection, and treatment. Target 1.3 of this goal aims to “implement nationally appropriate

social protection systems and measures for all, and by 2030 achieve substantial coverage of the poor and the vulnerable.”

- “Goal 5” (Gender Equality): Cancer affects women and men differently, and gender-based barriers can affect access to cancer prevention, detection, and treatment.
- “Goal 11” (Sustainable Cities and Communities): Urbanization and population growth can contribute to the risk of cancer, and urban areas often face challenges in providing adequate cancer prevention, detection, and treatment services. The main aim of this goal is “reduce the adverse per capita environmental impact of cities, including by paying special attention to air quality and municipal and other waste management “(UICC 2019).

18 Psychological Support for Cancer Patients

Cancer and its treatment can be emotionally and physically challenging for patients and their families. It is important for cancer patients to have access to psychological support to help them cope with the emotional and psychological impact of the disease.

Psychological support for cancer patients can take many forms, including:

- **Counseling:** With the help of counseling, cancer patients and their families can deal with the emotional and psychological challenges of cancer. This may include individual counseling, group counseling, or couples counseling (American Cancer Society 2020b).
- **Support groups:** Support groups provide a space for cancer patients and their families to connect with others who are facing similar challenges. These groups can be a valuable source of emotional support and can help people to feel less isolated.
- **Psychotherapy:** Psychotherapy, or “talk therapy,” can help cancer patients to explore their emotions, cope with stress, and develop coping strategies. This may include cognitive-behavioral therapy (CBT) that helps people to change their negative thoughts, or other types of therapy, including interpersonal therapy or acceptance and commitment therapy (ACT) (UC San Diego Health 2022).
- **Mind-body practices:** Some cancer patients find that practices such as meditation, yoga, or deep breathing can help to reduce stress and improve their overall well-being (Heeter and Lehto 2018).

19 International Policies on Cancer

Policy and legislation are required to address the global and local burden of cancer. There are many international policies and initiatives aimed at increasing awareness about cancer and reducing the burden of the disease globally. Some examples include:

- **World Cancer Day:** This annual event, organized by the “Union for International Cancer Control” (UICC), held on February 4th, to raise cancer awareness and to encourage prevention, detection, and treatment (UICC).
- **“World Health Organization” (WHO) action plans for control and prevention of Non-communicable Diseases (NCDs):** The prime objective of this plan is to lower premature deaths from NCDs, including cancer, by one-third by 2030. It includes several strategies for increasing awareness about cancer, such as promoting healthy lifestyles, strengthening health systems, and increasing access to cancer prevention, screening, and treatment services.
- **“United Nations (UN) Sustainable Development Goals (SDGs)”:** The United Nation’s SDGs include a specific goal to bring down the number of deaths caused by NCDs, including cancer, by one-third by 2030. This goal is being pursued through a variety of initiatives, including efforts to increase cancer awareness and to improve access to cancer prevention, screening, and treatment services (United Nations 2022).
- **Cancer Moonshot:** In 2016, the U.S. government launched an initiative that aims to advance the cancer research and treatment and to make significant progress in the fight against cancer within the next decade. One of the main purposes of the Cancer Moonshot is to raise awareness about cancer and to encourage more people to get screened for the disease (National Cancer Institute 2022d).

20 International Cancer Registries

International cancer registries are organizations that collect and maintain data on cancer cases and cancer-related deaths in a specific geographic area. These registries are typically maintained by government agencies or non-profit organizations, and they may be organized at the national, regional, or local level.

The main purpose of international cancer registries is to monitor and track the prevalence and distribution of cancer in a population, as well as to gather information on risk factors, treatment patterns, and survival outcomes. This information is used to inform cancer research, policy, and public health efforts, and to help identify trends and patterns in cancer incidence and mortality.

There are numerous international cancer registries around the world, and they vary in their scope and coverage. Some registries cover only a specific type of cancer or a specific population, while others cover all types of cancer in a broader population. Many international cancer registries also collaborate with each other and with other organizations, such as the World Health Organization (WHO), to share data and resources.

Overall, international cancer registries play a significant role in understanding and addressing the burden of cancer globally. They provide valuable insights into the epidemiology and outcomes of cancer, and they help to inform cancer prevention and control efforts at the local, national, and international levels (International Cancer Control Partnership 2022).

21 Conclusion

Cancer is a complex and widespread disease induced by genetic mutations, and environmental and lifestyle factors. There are various types of cancer, each with distinct characteristics and treatment options. The hallmarks of cancer include several features that allow cancer cells to grow and spread. Cancer incidence varies worldwide and by type, and the burden of the disease is predicted to increase in the future. However, advances in technology and research provide hope for improving prevention, diagnosis, and treatment of cancer.

Cancer prevention is crucial in reducing cancer incidence and mortality rates. Efforts in cancer prevention research have evolved from focusing on lowering cancer incidence and mortality rates to include pre-disease interventions and delaying carcinogenesis. Prevention against cancer can be divided into primary and secondary prevention. Strategies for cancer prevention include lifestyle changes, vaccination, and regular screenings. Social media is an effective medium for raising cancer awareness with promoting cancer prevention.

Patients and their families affected by cancer necessitate psychological support, international policies, initiatives for cancer control, prevention, and international cancer registries for monitoring and tracking the prevalence and distribution of cancer cases. Psychological support can take various forms, such as counseling and support groups. International policies and legislation dealing with cancer aim to increase awareness, promote healthy lifestyles, strengthen health systems, and improve access to cancer prevention, screening, and treatment services. International cancer registries play a significant role in understanding and addressing the global burden of cancer.

The cancer-related health policy has a substantial influence on public health, with the potential to affect individuals and their families in various ways. Major advancements in public health and cancer control have been made by the contributions of the Cancer Institutes through scientific research, policies, and initiatives. Early cancer identification is the first step in enhancing survival, minimizing treatment intensity, and improving cancer patients' quality of life, and several initiatives have been launched to improve early diagnosis of symptomatic cancer. The goal is to achieve cancer goals at the UN High-Level Political Forum on Sustainable Development, ensuring that cancer research breakthroughs benefit all populations equally.

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References

- American Cancer Society (2020a) American Cancer Society guideline for diet and physical activity. Retrieved from <https://www.cancer.org/healthy/eat-healthy-get-active/acs-guidelines-nutrition-physical-activity-cancer-prevention/guidelines.html>
- American Cancer Society (2020b) Psychosocial support options for people with cancer. Retrieved from <https://www.cancer.org/treatment/survivorship-during-and-after-treatment/coping/understanding-psychosocial-support-services.html>

- American Cancer Society (2022c) Every cancer every life. Retrieved from <https://www.cancer.org/>
- Canadian Cancer Society (2023) What is metastatic cancer? Retrieved from <https://cancer.ca/en/cancer-information/cancer-types/metastatic/what-is-metastatic-cancer>
- Cancer Research UK (2022a) Does hormone replacement therapy (HRT) increase cancer risk? Retrieved from <https://www.cancerresearchuk.org/about-cancer/causes-of-cancer/hormones-and-cancer/does-hormone-replacement-therapy-increase-cancer-risk#:~:text=Most%20types%20of%20HRT%20increase,slightly%20increases%20breast%20cancer%20risk>
- Cancer Research UK (2022b) Together we will beat cancer. Retrieved from <https://www.cancerresearchuk.org/>
- Cancer.Net (2018) The genetics of cancer
- Cancer.Net (2020) Public policy advocacy. Retrieved from <https://www.cancer.net/research-and-advocacy/public-policy-advocacy>
- Cavallo DN, Chou W-YS, McQueen A, Ramirez A, Riley WT (2014) Cancer prevention and control interventions using social media: user-generated approaches cancer prevention and control interventions using social Media. *Cancer Epidemiol Biomarkers Prev* 23(9): 1953–1956. <https://doi.org/10.1158/1055-9965.EPI-14-0593>
- Centers for Disease Control and Prevention. (2021). Emphasizing primary prevention of cancer. Retrieved from <https://www.cdc.gov/cancer/ncccp/priorities/primary-prevention.htm>
- Chou W-YS, Hunt YM, Beckjord EB, Moser RP, Hesse BW (2009) Social media use in the United States: implications for health communication. *J Med Internet Res* 11(4):e1249. <https://doi.org/10.2196/jmir.1249>
- Cleveland Clinic (2022) Types of cancer. Retrieved from <https://my.clevelandclinic.org/health/diseases/23180-carcinoma>
- Dunn AG, Mandl KD, Coiera E (2018) Social media interventions for precision public health: promises and risks. *NPJ Dig Med* 1(1):47. <https://doi.org/10.1038/s41746-018-0054-0>
- European Society for Medical Oncology (2021) ESMO statement on achieving cancer goals at UN high-level political forum on sustainable development. Retrieved from <https://www.esmo.org/policy/policy-news/esmo-statement-on-achieving-cancer-goals-at-un-high-level-political-forum-on-sustainable-development>
- Foucar E (2005) Diagnostic precision and accuracy in interpretation of specimens from cancer screening programs. *Semin Diagn Pathol* 22(2):147–155. <https://doi.org/10.1053/j.semdp.2006.02.002>
- Hanahan D (2022) Hallmarks of cancer: new dimensions. *Cancer Discov* 12(1):31–46. <https://doi.org/10.1158/2159-8290.CD-21-1059>
- Healthline Media (2022) Cancer: types, causes, prevention, and more. Retrieved from <https://www.healthline.com/health/cancer#What-is-cancer?>
- Heeter C, Lehto R (2018) Benefits of yoga and meditation for patients with cancer
- International Cancer Control Partnership (2022) Cancer registries. Retrieved from <https://www.iccp-portal.org/cancer-registries>
- Knoll J (2016) Advertising in social media: a review of empirical evidence. *Int J Advert* 35(2): 266–300. <https://doi.org/10.1080/02650487.2015.1021898>
- Lippman SM (2009) Cancer prevention research: back to the future. *Cancer Prev Res* 2(6):503–513. <https://doi.org/10.1158/1940-6207.CAPR-09-0092>
- Loomans-Kropp HA, Umar A (2019) Cancer prevention and screening: the next step in the era of precision medicine. *NPJ Precis Oncol* 3(1):3. <https://doi.org/10.1038/s41698-018-0075-9>
- Loud JT, Murphy J (2017) Cancer screening and early detection in the 21st century. Paper presented at the *Semin Oncol Nurs*. <https://doi.org/10.1016/j.soncn.2017.02.002>
- Miller SM, Bowen DJ, Lyle J, Clark M, Mohr D, Wardle J et al (2008) Primary prevention, aging, and cancer: overview and future perspectives. *Cancer* 113(S12):3484–3492. <https://doi.org/10.1002/cncr.23945>
- Nagai H, Kim YH (2017) Cancer prevention from the perspective of global cancer burden patterns. *J Thorac Dis* 9(3):448. <https://doi.org/10.21037/jtd.2017.02.75>

- National Cancer Institute (2022a) Age and cancer risk. Retrieved from <https://www.cancer.gov/about-cancer/causes-prevention/risk/age>
- National Cancer Institute (2022b) The tech revolutionizing cancer research and care. Retrieved from <https://www.cancer.gov/news-events/nca50/stories/technologies-and-innovations>
- National Cancer Institute (2022c) What is cancer? Retrieved from <https://www.cancer.gov/about-cancer/understanding/what-is-cancer>
- National Cancer Institute (2022d) Cancer Moonshot. Retrieved from <https://www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative>
- National Institute of Cancer (2023) How cancer is diagnosed. Retrieved from <https://www.cancer.gov/about-website>
- Organization, W. H (2022a) Cancer. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/cancer>
- Organization, W. H (2022b) Noncommunicable diseases. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases>
- Our Health Service (2023) National cancer control programme early diagnosis of symptomatic cancer plan 2022–2025. Retrieved from [https://www.hse.ie/eng/services/list/5/cancer/news/launch-of-nccp-early-diagnosis-of-symptomatic-cancer-plan-2022-2025.html#:~:text=The%20HSE's%20National%20Cancer%20Control,I%20and%20II\)%%20in%20Ireland](https://www.hse.ie/eng/services/list/5/cancer/news/launch-of-nccp-early-diagnosis-of-symptomatic-cancer-plan-2022-2025.html#:~:text=The%20HSE's%20National%20Cancer%20Control,I%20and%20II)%%20in%20Ireland)
- Pagoto S, Waring ME, Xu R (2019) A call for a public health agenda for social media research. *J Med Internet Res* 21(12):e16661. <https://doi.org/10.2196/16661>
- Parsa N (2012) Environmental factors inducing human cancers. *Iran J Public Health* 41(11):1–9
- Research, A. I. o. C (2016) Nutrition label finalized—good for cancer prevention. Retrieved from <https://www.aicr.org/resources/blog/nutrition-label-finalized-good-for-cancer-prevention/>
- Richards M, Anderson M, Carter P, Ebert BL, Mossialos E (2020) The impact of the COVID-19 pandemic on cancer care. *Nat Cancer* 1(6):565–567. <https://doi.org/10.1038/s43018-020-0074-y>
- Santaballa A, Pinto Á, Balanya RP, Ramírez Merino N, Martín IR, Grau SS et al (2020) SEOM clinical guideline for secondary prevention (2019). *Clin Transl Oncol* 22(2):187–192. <https://doi.org/10.1007/s12094-020-02302-0>
- Schliemann D, Su TT, Paramasivam D, Treanor C, Dahlui M, Loh SY, Donnelly M (2019) Effectiveness of mass and small media campaigns to improve cancer awareness and screening rates in Asia: a systematic review. *J Glob Oncol* 5:1–20. <https://doi.org/10.1200/JGO.19.00011>
- Sparsh Hospital (2022) What is world cancer day and its importance. Retrieved from <https://www.sparshhospital.com/blog/what-is-world-cancer-day-and-its-importance/>
- Stanford Health Care (2022) What is cancer? Retrieved from <https://stanfordhealthcare.org/medical-conditions/cancer/cancer.html>
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71(3):209–249. <https://doi.org/10.3322/caac.21660>
- The Cancer Atlas (2022) Policies & legislation. Retrieved from <https://canceratlas.cancer.org/taking-action/policies-and-legislation/>
- The Mesothelioma Center (2023) The future of cancer treatment: using technology to find a cure. Retrieved from <https://www.asbestos.com/featured-stories/future-cancer-treatment/>
- Theodore MK, Theodore L (2021) Effects of pollutants. In: *Introduction to environmental management*. CRC Press, Boca Raton, pp 61–70
- Thompson Cancer Survival Center (2021) The nutrition facts label: breaking it down. Retrieved from <https://www.thompsoncancer.com/nutrition-facts-label/>
- UC San Diego Health (2022) Counseling and emotional support for cancer patients and families. Retrieved from <https://health.ucsd.edu/specialties/cancer/resources/support/pages/default.aspx>
- UICC (2019) Cancer and SDGs. Retrieved from <https://www.uicc.org/what-we-do/advocacy/global-commitment/cancer-and-sdgs>
- UICC (2022) Leading the global fight against cancer. Retrieved from <https://www.uicc.org/>

- United Nations (2022) The 17 goals. Retrieved from <https://sdgs.un.org/goals>
- Watson M, Holman DM, Maguire-Eisen M (2016) Ultraviolet radiation exposure and its impact on skin cancer risk. *Semin Oncol Nurs* 32(3):241–254. <https://doi.org/10.1016/j.soncn.2016.05.005>
- World Cancer Day (2022) Close the care gap. Retrieved from <https://www.worldcancerday.org/about-us>
- World Health Organization (WHO) (2023) World cancer day. Retrieved from <https://www.emro.who.int/noncommunicable-diseases/campaigns/world-cancer-day-2023.html>



The Molecular Biology of Cancer Disparities

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Abstract

Cancer health disparities are characterized by adverse differences in cancer burden and outcomes, especially affecting populations of different ancestries. This is caused by a complex interplay between biological factors, including key molecular processes of the cell and social determinants of health. Current technology facilitates the classification of individuals using genetic ancestry markers, avoiding sociopolitical issues associated with self-reported ethnicity data. Such categorization has proved valuable in identifying ancestry-related molecular alterations that contribute to these disparities. This review examines the influence of molecular changes, including DNA methylation, genomic variations, and transcriptomic/proteomic patterns, on cancer progression, survival rates, treatment outcomes, and frequency of various cancer forms across ancestries. Finally, we address the historical overrepresentation of Caucasian populations in all aspects of cancer research, emphasizing the need for inclusive studies that reflect the genetic diversity of global populations.

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1 Introduction

Cancer health disparities refer to the adverse differences in cancer burden and outcomes among specific groups, characterized by race/ethnicity, socioeconomic status, age, or sexual orientation. These disparities encompass differences in cancer incidence, prevalence, mortality, treatment quality, survival rates, along with other related factors. Cancer disparities predominantly affect populations of diverse ancestral backgrounds due to a complex interplay between (1) medical and biological factors, representing different molecular processes in the cell and (2) the social determinants of health, described as the conditions in which people are born, grow, live, work, and age (Freedman et al. 2021; Hacker et al. 2022; Symioti et al. 2023).

Significant advancements have been achieved in the last 10 years in identifying cancer health disparities across different racial and ethnic groups (Ellis et al. 2018; McLeod et al. 2022; Williams et al. 2022; Yedjou et al. 2019; Zavala et al. 2020). Recently, The AACR Cancer Disparities Progress Report 2022 shed light on significant cancer health disparities among different ethnicities in the United States. It revealed that black individuals face higher incidence and mortality rates for certain cancers such as stomach, prostate, and multiple myeloma, with their death rates more than doubling those of white individuals. Similarly, American Indian or Alaska Native populations showed elevated incidence rates in cancers like lung, colorectal, and kidney, along with liver, stomach, and cervical cancers linked to infectious agents, compared to their white counterparts. The Asian population is also more affected by cancers caused by infectious agents. In contrast, Hispanic individuals generally have lower incidences of breast, colorectal, lung, and prostate cancers than non-Hispanic white individuals. However, they are at a higher risk for cancers of the gallbladder, liver, stomach, and cervix (Williams et al. 2022).

In breast adenocarcinoma (BRAC), the prevalence and mortality rates show significant disparities across racial and ethnic populations. White women were most likely to develop breast cancer, but black women faced a higher risk of death from the disease. These higher mortality rates are mainly due to advanced disease stages at diagnosis, prevalent comorbid conditions like obesity and diabetes, income disparities, limited access to cancer screening and care, and treatment delays. Additionally, there is evidence that these disparities might impact the biology of triple-negative breast cancer (TNBC) in this group. TNBC is a subtype of breast cancer that is more aggressive and has fewer treatment options (Dietze et al. 2015; Yedjou et al. 2019). Similarly, in colorectal cancer (COREAD), black individuals have the highest incidence and mortality, followed by American Indian/Alaskan Native, white, Hispanic/Latino, and Asian/Pacific Islander groups. These disparities are attributed to differences in social determinants of health, which include economic

stability, education access, healthcare quality, and living environment (McLeod et al. 2022).

Despite these efforts, all these rates, calculated from self-reported ethnicity data, may be subject to sociopolitical influences and might not accurately represent individuals' genetic ancestry (Williams et al. 2022). Indeed, race and ethnicity are more accurately understood as social constructs rather than reliable indicators of genetic variation (Mersha and Beck 2020). In that respect, current technology enables the categorization of individuals based on genetic ancestry markers, thereby avoiding sociopolitical controversies. Such categorization proves valuable in identifying ancestry-related molecular differences that contribute to cancer health disparities, allowing the distinction between biological factors from social determinants (Arora et al. 2022; Carrot-Zhang et al. 2020; Jiagge et al. 2023).

Over the last 5 years, extensive research has shown that these molecular differences are present in all major aspects of gene expression. These studies have corroborated previous findings and identified novel alterations that impact clinical outcomes among ancestral groups (Arora et al. 2022; Carrot-Zhang et al. 2020; Jiagge et al. 2023; Lee et al. 2022; Ma et al. 2010). The aim of this chapter is to underscore the significance of these studies and thoroughly explore all molecular aspects associated with genetic expression that contribute to cancer disparities. Our chapter takes an initial focus on human genetic diversity, setting the foundation for a comprehensive exploration of epigenetic, genomic, transcriptomic, and proteomic alterations that affect cancer burden and outcomes across different ancestries.

2 Human Genetic Diversity

To comprehend ethnic disparities in cancer, it is crucial to first understand the genetic diversity that exists among human populations. In that respect, significant large-scale initiatives have established a robust basis for exploring human genetic variation, which may have implications for understanding the disparities in cancer outcomes observed among various racial and ethnic groups (Auton et al. 2015; Fu et al. 2013; Lek et al. 2016; Liao et al. 2023; Sudmant et al. 2015).

The 1000 Genomes Project Consortium has been instrumental in providing a comprehensive landscape of this diversity by sequencing the genomes of individuals from various global populations. In their landmark study, the consortium analyzed the genomes of 2504 individuals from 26 different populations and included populations from Africa, East Asia, Europe, South Asia, and the Americas. This was accomplished through a combination of low-coverage whole-genome sequencing, deep exome sequencing, and high-density microarray genotyping (Auton et al. 2015).

Through this massive effort, they identified a remarkable genetic variation across human populations: more than 88 million variants, including 84.7 million single nucleotide polymorphisms (SNPs), 3.6 million short insertions/deletions (indels), and 60,000 structural variants. All these genetic modifications were carefully phased onto high-quality haplotypes, providing an extensive reference for genetic variation

that encompasses over 99% of SNP variants with a frequency of more than 1% across various ancestries. Interestingly, while common genetic variants are generally shared among populations, rarer variants tend to be limited to populations that are closely related (Auton et al. 2015).

This study also revealed that a typical human genome exhibits between 4.1 and 5.0 million variations when compared to the reference genome. While more than 99.9% of genetic variations consist of SNPs and short indels, 2100–2500 structural variants have also been observed in a typical genome. This large-scale genotyping effort has contributed substantially to the public dbSNP database (<https://www.ncbi.nlm.nih.gov/snp/>), adding around 40 million new SNPs and indels, with a significant proportion of novel variants coming from South Asian and African populations, which contributed to 24% and 28% of the novel variants, respectively (Auton et al. 2015).

Similarly, a profound exploration into this diversity has been conducted through the Exome Aggregation Consortium (ExAC), which has analyzed a high-quality exome sequencing dataset including 60,706 individuals of European, African, South Asian, East Asian, and admixed American ancestry. ExAC identified 7.4 million high-quality variants, of which 317,381 were insertions or deletions. This corresponds to an average of one variant at every eight base pairs of the exome. These variants are predominantly of very low frequency, not present in earlier, smaller datasets: 99% have a prevalence of less than 1%, over half are singletons (variants seen only once in the data set), and nearly three-quarters were not reported in prior databases, such as 1000 Genomes Project (Auton et al. 2015) or NHLBI Exome Sequencing Project (Fu et al. 2013).

A comprehensive understanding of ethnic disparities in cancer outcomes requires a deep appreciation of the genetic diversity inherent in human populations. These variants are crucial elements that can influence how individuals respond to cancer and its treatments, highlighting the need for tailored approaches to study cancer among human populations.

3 Ancestral Diversity in Cancer Epigenomics

Epigenetic modification of the genome via methylation significantly impacts the control of different cellular activities. Alterations in DNA methylation patterns are linked to complex diseases, including different types of cancer such as breast (Batra et al. 2021; Garcia-Recio et al. 2023), colorectal (Batra et al. 2021), and prostate cancers (Li et al. 2020; Zhao et al. 2020). These changes have been shown to vary across ancestries and are influenced by both genetic and environmental factors (Song et al. 2021; Wattacheril et al. 2023).

Carrot-Zhang et al. (2020) conducted a pan-cancer analysis to uncover differentially DNA-methylated regions among ancestries ($n = 6264$ tumor samples). They analyzed 482,421 CpG sites from the HM450 array and identified significant differences in methylation between ancestries in 374 CpG sites. Forty-one genes have multiple ancestry-differential methylation sites, with ten of these genes

supported by at least four probes (Carrot-Zhang et al. 2020). These include known methylation quantitative trait loci (meQTLs) such as *SPATC1L* and *PM20D1*, genes like *HOOK2* with previously recognized variable methylation (Kraus et al. 2015), and others such as *FLJ26850*, *PACS2*, and *FAAP20* where methylation variation is newly described. In nine of these ten genes, the associated probes showed consistent ancestry effects. For example, *FAAP20* methylation was more frequent in Africans, compared with Europeans, Asians, and Native/Latin Americans. Conversely, *HOOK2* exhibited lower methylation levels in individuals of African descent while showing higher frequencies in those of European and Native/Latin American ancestries. Additionally, all seven *PM20D1* sites were more frequently methylated in Europeans and South Asians relative to other ethnic groups. Interestingly, *SPATC1L* exhibited opposite methylation patterns when comparing its promoter region with its transcription termination site (TTS). Africans showed higher methylation levels in the promoter region and lower at the transcription termination site (TTS) compared to other ethnic groups. Methylation at the promoter region was negatively correlated with *SPATC1L* mRNA levels across ethnicities, with Africans showing the strongest correlation (Carrot-Zhang et al. 2020).

As previously described, Lee et al. (2022) determined differential gene expression signatures associated with genetic ancestry (GA). In breast adenocarcinoma (BRCA), five protein-coding differential expressed genes (*PAQR6*, *LIME1*, *MXD3*, *SAP25*, and *CCER2*) displayed significant gene-by-genetic ancestry ($G \times GA$) interactions. All were overexpressed in the African ancestry group compared to the European group. Notably, increased expression of these genes was associated with a higher mortality risk in African patients with BRCA. For head and neck squamous cell carcinoma (HNSCC), one protein-coding gene, *CTSW*, and one long noncoding RNA transcript, AC005330.1, showed significant $G \times GA$ interactions for patients of African ancestry. In skin cutaneous carcinoma (SKCM), the gene *RFLNA*, overexpressed in the admixed American ancestry group compared with the Europeans, was also associated with an increased relative risk of mortality. Lee et al. (2022) then performed a methylation analysis of CpG sites to explore regulatory differences contributing to the differential expression of these genes. Significant correlations were found between DNA methylation levels and gene expression levels for *MXD3*, *LIME1*, and *PAQR6* (Fig. 1). In their final survival model, the inclusion of differential methylation data markedly accentuated the observed survival disparities between ancestries. This indicates that differential methylation plays a regulatory role in gene expression and greatly contributes to cancer survival disparities (CSD) (Lee et al. 2022).

In prostate cancer, Rai et al. (2019) aimed to identify epigenetic variations between 43 African American and 162 Caucasian men with prostate adenocarcinoma (PRAD) and explore how these differences relate to clinical outcomes. The study found differential activation of canonical and non-canonical Wnt signaling pathways between African American and Caucasian patients. For instance, non-canonical Wnt/ Ca^{+2} signaling was upregulated in Caucasian men but downregulated in African American men. The study further highlighted, in a small cohort, distinct methylation patterns that were uniquely characteristic of each ethnic group. Specifically, it was

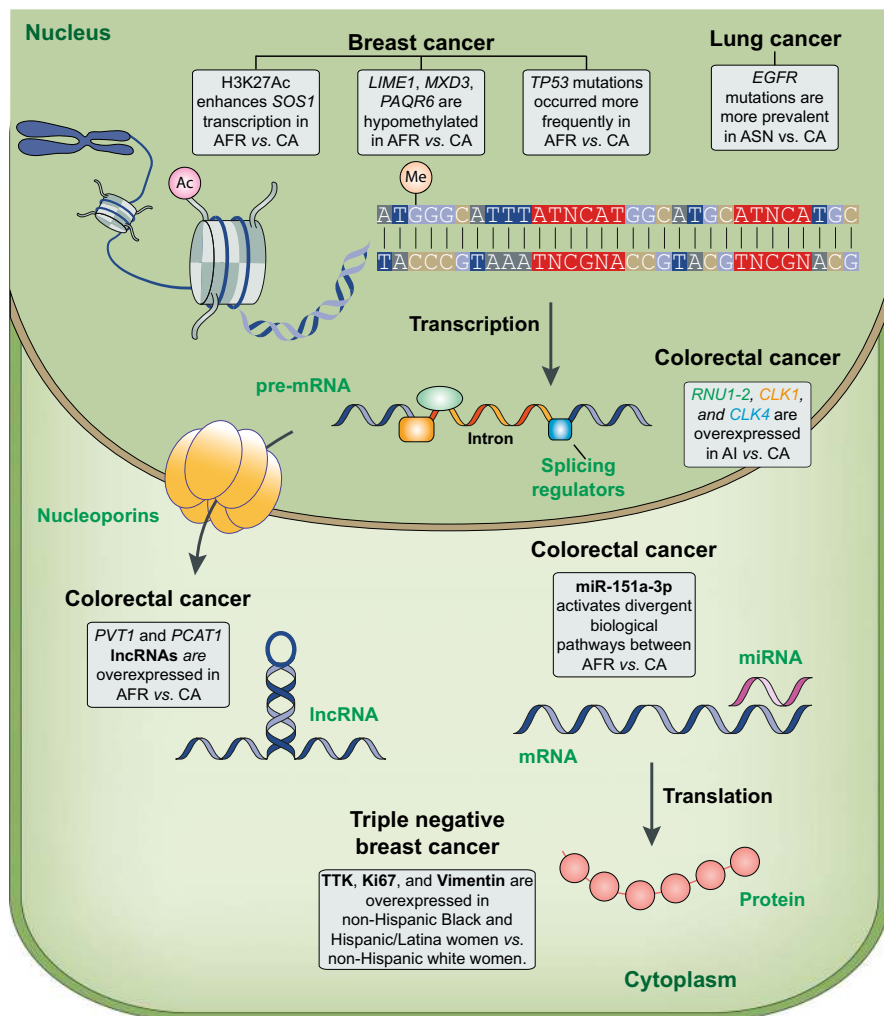


Fig. 1 Molecular basis of cancer disparities. The diagram provides a visual overview of all molecular aspects that contribute to cancer disparities. Examples of key epigenomic, genomic, transcriptomic, and proteomic alterations are highlighted across different cancer types and ancestries. *AFR* African, *CA* Caucasian, *AI* American Indian, *ASN* Asian, *lncRNA* long noncoding RNA, *miRNA* microRNA, *Ac* acetylation, *Me* methylation

observed that 32 genes were hypomethylated in Caucasian patients, while 19 genes showed hypomethylation in African American patients. Interestingly, the distinct epigenetic patterns in African American and Caucasian men were correlated with differences in survival and recurrence outcomes. Notably, the *PDIA3* gene was found to be hypomethylated in African American patients and hypermethylated in Caucasian patients. This gene has been linked to malignant PRAD and could potentially serve as

a biomarker (Diaz Cruz et al. 2021; Pressinotti et al. 2009). Additionally, genes such as *IDH3 β* and *PIKFYVE* were hypermethylated in African American patients and have been associated with cancer (Huang et al. 2021; Wu et al. 2019b).

In COREAD, racial disparities have been observed in both age at diagnosis and its location within the colon (Devall et al. 2021; Rutter et al. 2023). For further understanding, Devall et al. (2021) conducted a DNA methylation analysis on matched right and left colon biopsies from 128 individuals, comprising 88 African Americans and 40 European Americans. The study revealed race-specific differences in epigenetic aging of normal colon tissue. In African Americans, the right colon exhibited accelerated epigenetic aging compared to the left, with an average increase of 1.51 years. Conversely, European Americans showed a notable age deceleration in the right colon by 1.93 years. They identified 456 and 558 race-associated differentially methylated positions in the right and left colon, respectively. A significant proportion of positions in the right colon of African Americans were hypermethylated (69.5%), suggesting a unique pattern of epigenetic modification. The hypermethylated differentially methylated positions in the African American right colon were found to be enriched for cytosines associated with ancestry and aging but also to COREAD development (Devall et al. 2021).

Similar to the patterns of epigenetic variation seen in colorectal cancer, the research conducted by Xing et al. (2021), sheds light on the critical role of histone modifications in the regulation of genes associated with BRCA among African American women vs. Caucasian women. The study pinpointed a marked increase in the acetylation of histone H3 at lysine 27 (H3K27Ac) around a specific enhancer region near the *SOS1* gene, a modification unique to cell lines derived from African American individuals (Fig. 1). This particular enhancer, spanning roughly 1.3 kb, was amplifying the expression of *SOS1*. Such elevation in gene expression, driven by histone acetylation, was distinct to the African American cohort (Xing et al. 2021).

Overall, these findings demonstrate the profound impact of epigenetic modifications, particularly DNA methylation, on cancer outcomes across different ancestries. The evidence shows that methylation patterns vary significantly between ethnic groups, influencing gene expression and consequently, disease progression and prognosis in various cancer types.

4 Ancestry-Based Genomic Fingerprints in Cancer

In recent years, the analysis of genomic differences across ethnic groups has advanced significantly. This progress has been achieved by studying large cohorts of patients, with their genetic ancestry determined through sequencing data analysis (Arora et al. 2022; Carrot-Zhang et al. 2020; Jiage et al. 2023; Lee et al. 2022). These studies have corroborated well-known reports and found new cancer genetic markers among ancestries.

In that respect, Arora et al. (2022) examined the link between ancestry and somatic driver alterations in cancer. In a cohort of 45,157 patients, who underwent

prospective sequencing, they found 18 gene-ancestry associations between Europeans vs. other populations. In lung adenocarcinoma (LUAD), a higher frequency of *EGFR* mutations was detected in both East and South Asian groups compared with Europeans, consistent with other reports (Gibson et al. 2019; Izumi et al. 2020; Ma et al. 2010; Fig. 1). Among never-smokers, a higher frequency of *EGFR* mutations in Africans vs. Europeans was also detected. Similarly, a higher frequency of *TP53* mutations in African ancestry women vs. Europeans with breast invasive ductal carcinoma was also reported (Fig. 1). This trend aligns with earlier research and is partially attributed to the higher rates of TNBC in African ancestry patients (32% vs. 17% in Europeans) (Dietze et al. 2015; Keenan et al. 2015).

Additionally, this study also revealed a higher alteration frequency of *FOXA1* in East Asian patients with prostate adenocarcinoma (PRAD), *MYC* in African ancestry patients with PRAD, and *KRAS* in African patients with colon adenocarcinoma. Also, *RBI* mutations were more prevalent in East Asian patients with hepatocellular carcinoma (HCC). The researchers also identified a lower occurrence of *TERT* mutations, particularly in the promoter region, in East Asian patients with both glioblastoma multiforme and HCC, compared to European patients (Arora et al. 2022). Notably, these findings align with and reinforce previous research in the field (Kang et al. 2013; Keenan et al. 2015; Koga et al. 2020; Li et al. 2020; Stopsack et al. 2022; Yao et al. 2016). Ultimately, Arora et al. (2022) determined the rate of clinically actionable alterations across ancestries. Although these rates were similar across various populations, the proportion of actionable mutations was lower in African, Native American, and South Asian populations, each at 30%, in contrast to the slightly higher rates in East Asian at 34% and European populations at 33% (Arora et al. 2022).

Similarly, Lee et al. (2022) examined the relationship between GA and CSD across 33 cancers in 9818 patients. The study found that GA is a significant factor in CSD, particularly in BRCA, HNSCC, kidney renal clear cell carcinoma (KIRC), and SKCM. Their study identified 15 significantly differentially mutated genes (DMGs) with somatic variants in BRCA (African vs. European ancestry), 20 in HNSCC (African vs. European ancestry), and 2 in KIRC (admixed American vs. European ancestry). They also identified 85 DMGs when comparing admixed American vs. European ancestry, and 27 when comparing East Asian vs. European ancestry in SKCM (Lee et al. 2022).

Among these DMGs, *TP53* stands out for its role in preventing the proliferation of cells with damaged DNA, with mutations leading to various aggressive cancers (Aubrey et al. 2016). Similarly, *PIK3CA* mutations are linked to several cancers and are targets for therapy due to their role in cell growth pathways (Samuels and Waldman 2010). *CDH1*, essential for cell adhesion, is another gene where mutations can contribute to the progression and metastasis of stomach and breast cancers (Corso et al. 2020). *NOTCH3*, involved in cell differentiation, is implicated in leukemias and other cancers when its pathway is disrupted (Aburjania et al. 2018). Lastly, *KIT* gene, often mutated in gastrointestinal stromal tumors, has become a focal point for targeted cancer treatments, underscoring its significance in oncology (Xu et al. 2014).

In line with these findings, Carrot-Zhang et al. (2020) have studied the frequencies of somatic mutations (including single nucleotide variants/indels) and copy-number alterations (CNAs) among 10,678 patients (33 cancer types) from The Cancer Genome Atlas (TCGA). At a pan-cancer level, they found that African patients showed enriched mutations in the *TP53* and *FBXW7* genes, while Europeans exhibited an increased frequency of *PIK3CA* mutations. Within cancer types, they identified differential mutation rates in four specific genes. Notably, in KIRC, African samples showed a lack of mutations in two genes: *VHL* and *PBRM1*, compared with Europeans. Additionally, East Asian samples of bladder and esophageal cancers revealed enriched mutations in *HRAS* and *NFE2L2* genes (Carrot-Zhang et al. 2020). These results also corroborate previous findings (Deng et al. 2017; Krishnan et al. 2016; Nassar et al. 2019; Wu et al. 2019a; Yuan et al. 2018).

Recently, Jiagge et al. (2023) identified genomic alterations in relation to African ancestry across six common cancer types. By interrogating a large dataset of 333,908 tumor samples for the initial discovery and 64,173 paired tumor-normal samples for validation, the study identified significant differences in genomic drivers and biomarkers. Key findings include the enrichment of *MYC* amplifications in lung, breast, and prostate cancers and depletion of *BRAF* alterations in colorectal and pancreatic cancers among patients with African ancestry. The study also notes differences in *KRAS* and *TP53* mutations based on smoking status, suggesting an ancestry-environment interaction (Jiagge et al. 2023).

This study also found significant enrichment of certain US Food and Drug Administration (FDA)-approved (<https://www.oncokb.org/>), actionable genetic alterations in patients with African ancestry. This included *ROS1* fusion in non-small cell lung cancer patients without a smoking history, as well as *CDK12* alteration and *NTRK3* fusion in prostate cancer. Additionally, a higher frequency of specific *KRAS* mutations (G12D, G13D, G12V, and Q61H), which are known to confer resistance to standard treatments, was observed in colorectal cancer patients with African ancestry. Conversely, *KRAS* G12C mutations, targetable by recently approved inhibitors, were less common in both smokers and nonsmokers of African ancestry. Interestingly, while most actionable mutations in the *EGFR* gene were more common in African ancestry, the *EGFR* L858R mutation, crucial for predicting response to standard *EGFR* tyrosine kinase inhibitors in NSCLC, was found to be less frequent in this population (Jiagge et al. 2023).

These comprehensive studies have uncovered distinct patterns of somatic mutations and driver alterations across various ancestries. These findings reveal that specific gene-ancestry associations are crucial factors in cancer development and treatment response. The research collectively indicates that genetic ancestry not only contributes to variations in cancer incidence and survival rates but also influences the effectiveness of targeted therapies. This underscores the necessity of integrating ancestry-based genomic data into cancer research and clinical practice for effective personalized medicine.

5 Differences in Gene Expression Across Ancestries

In the field of oncology, understanding the mechanisms of cancer development and progression is essential (Freedman et al. 2021; Lee et al. 2022). Central to this is gene expression, the cellular process responsible for generating functional RNA, regulated by a myriad of genetic and epigenetic elements such as DNA mutations, DNA methylation, chromatin remodeling, transcription factor activity, microRNA (miRNA)-gene interactions, and long noncoding RNA (lncRNA) (Distefano et al. 2022; Freedman et al. 2021; Ramanto et al. 2021; Yap 2023). This process plays a key role not only in the initiation and progression of cancer but also in how cancer responds to therapies and develops resistance (Carrot-Zhang et al. 2020; Distefano et al. 2022). Recent studies have revealed significant ancestry-related differences in gene expression across various cancer types, such as breast, colon, lung, and prostate cancers (Carrot-Zhang et al. 2020).

In BRCA, several studies examining protein levels in tumors from a racially diverse cohort have shown that specific gene regulators and networks can predict BRCA survival differently across races (García-Cárdenas et al. 2022; Lee et al. 2022; Ramanto et al. 2021; Xing et al. 2021). For instance, the work of Xing et al. (2021) highlights this concept. They discovered that *SOS1*, a crucial regulator of the Ras pathway, is more highly expressed in African American breast cancer patients compared to Caucasian-American counterparts. This variation in expression can be attributed to factors like the higher obesity rates in African American women, influencing the activation of the c-Met pathway signaling through the hepatocyte growth factor from adipocytes. Additionally, elevated *SOS1* mRNA levels in African American patients are associated with increased cancer cell stemness and macrophage polarization, driven by changes in gene expression and epigenetic modulation by a super enhancer. Furthermore, the posttranscriptional regulation of *SOS1* mRNA via miR-483, which is less expressed in African American patients due to specific histone modifications, underscores the complexity of cancer regulation and opens potential avenues for targeted therapy, such as the use of Taxifolin to inhibit SOS1 signaling. These findings not only add to our understanding of the molecular underpinnings of cancer but also emphasize the importance of considering racial differences in cancer research and treatment strategies (Xing et al. 2021).

Another study explored the potential of miRNAs as biomarkers for BRCA, focusing on how their regulation varies across different cancer stages and between non-Hispanic white and non-Hispanic black ethnic groups. They identified 160 abnormal miRNAs, noting that upregulated miRNAs were more common in the white population, while fewer stage-specific aberrant miRNAs were seen in the black population. They found 26 unique aberrant miRNAs in the non-Hispanic white group and 19 in the non-Hispanic black group, showing significant variation in miRNA expression across cancer stages within each ethnicity. The study also revealed significant differences in miRNA expression, with some miRNAs in the white population showing very high expression changes. Certain miRNAs, including two that were downregulated and seven that were upregulated, had been previously validated as potential BRCA biomarkers (Ramanto et al. 2021).

Likewise, Byun et al. (2020) analyzed data from 262 African American and 293 European American breast cancer patients, focusing on hormone receptor-positive subtypes. They used immunohistochemistry to examine biomarkers like *ESR1*, *FOXA1*, and *GATA3*, and profiled gene expression through RNA sequencing. The study found significant racial differences in the prognostic value of these biomarkers for survival. African American women exhibited less favorable or nonsignificant hazard ratios compared to European American women, despite having similar biomarker levels. This suggests that these biomarkers impact BRCA biology differently in African American and European American patients. Furthermore, the study identified *FOXA1* as a particularly strong independent predictor of overall BRCA survival, surpassing the predictive value of both the estrogen receptor and *GATA3*. This held true even after adjusting for demographic factors like age, race, and cancer stage. An analysis of genes related to *ESR1*, *FOXA1*, and *GATA3* found specific genes that could optimally predict race and 3-year survival. These findings emphasize substantial racial disparities in BRCA outcomes, with African American women experiencing less favorable outcomes compared to European American women, even when their biomarker profiles were similar (Byun et al. 2020). Additionally, using the BRCA data from TCGA, Mani et al. (2023) focused on understanding why *RAD51* is upregulated in TNBC, especially in African American women. miRNA sequencing revealed a significant decrease in *RAD51*-targeting miRNAs, specifically miR-214-5P and miR-142-3P, in TNBC cell lines from African American women compared to European American women. Further validation confirmed the miRNA-mediated regulation of *RAD51*. The study identified *RAD51* as a poor prognostic marker for TNBCs and noted a substantial reduction of miR-214-5P and miR-142-3P in TNBC cell lines from African American women. Using miR-214-5P mimics, they managed to reduce *RAD51* expression and induce homologous recombination deficiency. Based on these results, Mani et al. (2023) suggested a novel treatment combining miR-214-5P and olaparib for HR-proficient African American TNBC cell lines. This combination was more effective than individual treatments, showing potential as a new therapeutic approach for treating HR-proficient TNBCs and addressing racial disparities in treatment outcomes (Mani et al. 2023).

Lastly, Ramos et al. (2020) examined the role of *NRF1* in breast cancer, focusing on its impact on the disease's aggressiveness and molecular signature across different races and ethnicities, including black, white, Asian, and Hispanic women. The study found increased *NRF1* activity in all stages of BRCA, particularly in luminal A and B, HER2+, and TNBC subtypes among various racial and ethnic backgrounds. Notably, *NRF1* activity was significantly higher in non-Hispanic white patients compared to race-matched noncancer controls, as well as in Asian, black, and Hispanic white BRCA patients compared to noncancer non-Hispanic white controls. The research identified unique *NRF1* motif-enriched gene signatures for each BRCA molecular subtype in non-Hispanic white women. Additionally, it revealed distinct *NRF1* motif-enriched gene signatures in luminal A breast cancer that varied by race and ethnicity, indicating unique genetic profiles for different groups. For instance, Asian women's cancers were associated with specific genes like *CACNG4* and *ENDOG*, black women's cancers with genes like *DVL3* and *HSPA2*, and

Hispanic white women's cancers with genes such as *LAMTOR4* and *MAPKAPK5* (Ramos et al. 2020).

We have explored the role of genetic expression and its impact on breast cancer across different ethnicities. Similar patterns of ethnic disparities and gene expression are evident in other cancers as well. For instance, prostate cancer, another prevalent form of cancer globally, exhibits significant ethnic variations, particularly affecting men of African descent and those leading a Western lifestyle (Yuan et al. 2020). PRAD is the second most diagnosed non-skin cancer among men worldwide, with men of primarily African ancestry facing a higher incidence and lower survival rates compared to men of predominantly European ancestry (Morgan et al. 2021; Yuan et al. 2020). Yuan et al. (2020) investigated the differences in PRAD incidence and survival rates between men of African ancestry and European ancestry. Analyzing genomic profiles of 470 patients from the TCGA PRAD cohort, the study found significant disparities in gene expression between African ancestry and European ancestry patients. Specifically, 3787 of 60,483 genes were differentially expressed, a number that rose to 5964 after adjusting for age and Gleason score. Notably, the *SPINK1* gene, linked to rapid prostate cancer progression, was upregulated in African ancestry patients, indicative of wider differences in gene expression across these groups. A key finding was the pronounced upregulation of immune-related signaling pathways in African ancestry patients, suggesting substantial differences in immune response and inflammation in prostate tumors based on genetic ancestry. Additionally, the study identified 1868 out of 15,900 long noncoding RNAs (lncRNAs) that were differentially expressed between African ancestry and European ancestry patients. Among these, *PVT1* and *PCAT1*, located near the *MYC* oncogene and implicated in cancer risk, were notably overexpressed in African patients (Fig. 1). *PVT1* is associated with *MYC* protein stabilization and miRNA production that promotes tumorigenesis, while *PCAT1* is known to encourage PRAD growth through its interactions with androgen receptor and LSD1 (Yuan et al. 2020).

In COREAD, given the need for more precise biomarkers (García-Cárdenas et al. 2023), ethnically inclusive studies have been performed (Wu et al. 2020; Yamada et al. 2023). Wu et al. (2020) compared COREAD in Taiwanese patients with data primarily from European ancestry populations in TCGA. They discovered significant molecular differences between the two groups. While Taiwanese COREAD tumors showed gene expression changes similar to those in the TCGA dataset, they exhibited unique enrichments in metastasis-related pathways, including cell cycle, DNA replication, and Wnt signaling pathways for upregulated genes, and oxidative phosphorylation and metabolism-related pathways for downregulated genes. This suggests distinct molecular alterations in the Taiwanese cohort. The study also identified novel gene fusion events in Taiwanese COREAD, which may be less common in European ancestry populations, indicating different molecular alterations of COREAD across ethnicities. Additionally, variations in miRNA profiles in the Taiwanese group pointed to unique regulatory mechanisms at the miRNA level. Furthermore, Wu et al. (2020) developed a new stratification method based on miRNA-mRNA-associated subtyping, which revealed that suppressed WNT

signaling activity correlated with poorer prognosis in Taiwanese COREAD, a trend less evident in European ancestry data. This finding demonstrated that survival outcomes based on molecular subtypes differed between the Taiwanese cohort and the TCGA dataset, highlighting the importance of considering ethnic-specific factors in COREAD pathogenesis (S. M. Wu et al. 2020).

Similarly, Yamada et al. (2023) focused on the disparities in COREAD among different racial groups in the United States, particularly examining Alabama African Americans, Oklahoma American Indians, and white Americans. The findings indicated significant transcriptomic changes among the groups, suggesting that standard COREAD treatment strategies might not be equally effective for all racial and ethnic populations. In Alabama African Americans, this cancer showed increased expression of immune-related markers and cytokines (such as *CD69*, *CD48*, *CD22*, *CCL13*, and *CCL11*) and vesicle trafficking molecules (like *AP1S2*, *HERC1*, and *RFTN1*), but a decreased expression of genes in the ID1/BMP/SMAD axis, *IL22RA1*, *APOBEC3*, and Mucins. These results suggest a distinct immune response and potential mechanisms of immune evasion in African Americans with COREAD, as well as differences in the usage of oncogenic pathways that might influence treatment responses. For Oklahoma American Indians, the study found higher expression of *PTGS2/COX2* and splicing regulators (including *RNU1-2*, *CLK1*, and *CLK4*), but lower expression of tumor suppressor genes (like *TOB2*, *PCGF2*, and *BAP1*) and genes involved in genome stability (such as *CHTF8* and *EPHB2*). This indicates a significant role of *COX2*-mediated pro-oncogenic inflammation and splicing misregulation in American Indians with COREAD, coupled with reduced activities of tumor suppressor genes. Comparing COREAD patients from African Americans to American Indians, the study highlighted differences in pro-oncogenic inflammatory signaling and the reliance on varying pathways such as Notch signaling and chromatin remodeling. These findings underscore the molecular disparities in COREAD between these ancestral groups (Yamada et al. 2023).

Concerning LUAD, Distefano et al. (2022) explored miRNA isoform dysregulation focusing on racial disparities between African American and Caucasian American patients. Specifically, the study highlighted the edited miRNA, miR-151a-3p, with an A-to-I editing event at position 3. This miRNA exhibited differential biological effects in African American versus Caucasian American LUAD patients, suggesting its potential role in activating divergent biological pathways between these groups (Fig. 1). Furthermore, the analysis identified distinct race-specific miRNA isoforms that correlated with prognosis in LUAD patients, suggesting the potential of miRNA isoforms as clinically meaningful biomarkers in LUAD (Distefano et al. 2022).

To gain a broader understanding of the impact of ethnicity on cancer, Lee et al. (2022) performed a pan-cancer analysis involving 9818 patients across 33 cancer types, focusing on the links between CSD, genetic ancestry, and tumor molecular signatures. The study found significant connections between GA and CSD in four specific cancer types: BRCA, HNSCC, KIRC, and SKCM. One key finding was the variation in gene expression and methylation among different ancestry groups, which correlates with CSD. In their analysis of differential gene expression, they

discovered distinct patterns between ancestries. For example, in BRCA, many genes (*PAQR6*, *LIME1*, *MXD3*, *SAP25*, and *CCER2*) were more highly expressed in African ancestry groups compared to European groups, whereas in HNSCC, KIRC, and SKCM, more genes were upregulated in European groups. This indicated significant disparities in gene expression levels between these groups. The study also performed gene set enrichment analysis, revealing that different ancestral groups have unique gene expression patterns related to specific cancer pathways. Additionally, the study found that the expression levels of certain genes were significantly linked to GA, influencing cancer survival outcomes (Lee et al. 2022). Finally, Lee et al. (2022) reveal that the long noncoding RNA transcript AC005330.1 is overexpressed in HNSCC among African ancestry patients, leading to a lower relative risk of mortality compared to European ancestry (Lee et al. 2022).

Similarly, Carrot-Zhang et al. (2020) focus on a comprehensive study involving 10,678 patients across 33 cancer types from TCGA, focusing on the impact of ancestry on mutation rates, DNA methylation, and both mRNA and miRNA expression. The pan-cancer analysis revealed significant differences in mRNA expression across ancestries, with 327 genes differing between African ancestry and European samples, and 654 genes between East Asian and European samples. Of these, 85 genes were common to both comparisons, with varying levels of expression in Europeans compared to African ancestry and East Asian groups. The study also aligned with previous GTEx consortium findings, identifying 221 protein-coding genes associated with African ancestry, 44 of which overlapped with similar effect sizes in this study. Notably, fewer significant associations were observed in specific cancer types, the most being 61 genes in the East Asian-European comparison for HCC. Key genes identified across various cancer types included *CRYBB2*, *NOTCH2NL*, *LOC90784*, and *PPIL3*, which are associated with African ancestry. Additionally, Carrot-Zhang et al. (2020) explored the relationship between genetic ancestry and miRNA expression in cancer. They found that ancestry-related differences in miRNA expression were generally specific to individual cancer types, mirroring patterns seen in DNA methylation and mRNA expression. The study identified 71 miRNAs with significant differences in expression related to ancestry (Carrot-Zhang et al. 2020).

6 Ancestry-Specific Proteomic Patterns in Cancer

As previously emphasized, precision oncology has relied mainly on genomics and transcriptomics to identify and tailor specific therapeutic drugs for individual cancer cases. However, average RNA expression data has shown to be an ineffective predictor of protein expression (Nusinow et al. 2020). Moreover, recent research has shown that the protein-coding potential of the human genome has been significantly underestimated, with a growing discovery of novel functional microproteins translated from previously considered noncoding regions (Hassel et al. 2023; Sandmann et al. 2023). Importantly, these microproteins have been identified as either oncogenic drivers or tumor suppressors in cancer (Chen et al. 2020; Hassel et al. 2023).

In recent years, significant efforts have been made to ultimately define the cancer proteome. Consequently, extensive analysis of proteomic datasets has led to the discovery of new cancer biomarkers, which could potentially enhance diagnostic and therapeutic options for medical practitioners and patients (Álvarez et al. 2023; Gonçalves et al. 2022; Jarnuczak et al. 2021; Lv et al. 2013). For example, Bueno-Álvarez et al. (2023) characterized the plasma proteome of 1477 patients including 12 cancer types. This study identified cancer-specific proteome profiles through differential expression analyses, comparing each cancer type in contrast to the rest. Significant upregulated proteins included GFAP in glioma and FLT3 in acute myeloid leukemia. Additionally, FKBP prolyl isomerase 1B was upregulated in colorectal cancer, and PAEP was significantly upregulated in ovarian cancer (Álvarez et al. 2023). Despite these efforts, no large-scale proteomic studies analyzing differences among ancestries have been conducted. However, significant differences in key cancer biomarkers have been identified among ethnicities (Libes et al. 2014; Liu et al. 2011), particularly in breast (Preat et al. 2014; Rivera-Rivera et al. 2022; Srivastava et al. 2019) and prostate (Minas et al. 2022; Ye et al. 2022).

Rivera-Rivera et al. (2022) focused on racial/ethnic differences in breast cancer aggressiveness and survival, based on key diagnostic biomarkers. They analyzed formalin-fixed paraffin-embedded tissue microarrays of 147 non-Hispanic black, 168 Hispanic/Latina, and 112 non-Hispanic white women. Through immunohistochemical staining, the study evaluated levels of TTK, p-TBK1, E-cadherin (an epithelial marker), Vimentin (a mesenchymal marker), and Ki67 (a proliferation marker). TTK, Ki67, and Vimentin were significantly more expressed in TNBC tumors, which are more prevalent in non-Hispanic black and Hispanic/Latina women (Fig. 1). These proteins are associated with aggressive cancer characteristics like high proliferation rates and mesenchymal states. In contrast, E-cadherin, an epithelial marker indicative of less aggressive cancers, was less expressed in these groups. Furthermore, analysis of TCGA data indicated that mRNA levels of various mitotic kinases and related transcription factors were elevated in non-Hispanic black women compared to non-Hispanic whites, and these were associated with higher rates of aneuploidy (Rivera-Rivera et al. 2022).

Similarly, Srivastava et al. (2019) identified noninvasive protein biomarkers that might help in tailoring interventions for reducing recurrence and metastasis risk in breast cancer, particularly in African American and Caucasian American women. The study involved 240 subjects, including controls and various immunohistochemistry-based subtypes of breast cancer cases, subdivided by menopausal status and race. Thus, they discovered several proteins that were present in higher concentrations in cancer patient serum compared to controls, including Pyk2, SAPK/JNK, and PTEN. Notably, it found significant differences in the expression of certain proteins between African American and Caucasian American cancer patients. The proteins c-kit and Rb were significantly overrepresented in African American cancer serum compared to Caucasian American cancer serum. Additionally, VEGFR2, linked to metastasis and poor prognosis, was significantly overrepresented in African American cancer serum relative to African American controls, a pattern not observed in Caucasian American patients. This suggests that African American breast cancer patients may be at a higher

risk for aggressive cancer due to increased VEGFR2 expression (Srivastava et al. 2019).

Concerning prostate cancer, Ye et al. (2022) examined the differential expression of proteins in African American vs. Caucasian American patients to identify biomarkers associated with racial disparities in prostate cancer. The study analyzed 90 fresh frozen prostate samples obtained from radical prostatectomy specimens. Using protein pathway array analysis (PPAA), a total of 286 proteins and phosphoproteins were assessed. The PPAA analysis revealed that 33 proteins were significantly differentially expressed in tumor tissue compared to benign tissue. Among these, three proteins—Aurora, cyclin D1, and HNF-3a—were found to be differentially expressed between African American and Caucasian American tumor groups. Aurora was significantly increased in African American tumors, while cyclin D1 and HNF-3a were downregulated in African American tumors. These findings suggest that these proteins may be involved in different pathways leading to aggressive prostate cancer behavior in African American patients, potentially serving as biomarkers for prostate cancer racial disparity. Similarly, Zewdu-Minas et al. (2022) measured 82 circulating proteins in nearly 3000 men of Ghanaian, African American, and European American descent. The study found distinctive protein signatures in men of African ancestry, particularly elevated levels of proteins suppressing tumor immunity and influencing chemotaxis. They identified two markers, pleiotrophin and TNFRSF9, that predict poor disease survival, particularly among African American men. The findings suggest that immune-oncology marker profiles vary between men of African and European descent, potentially contributing to the higher incidence of lethal prostate cancer in men of African ancestry (Minas et al. 2022).

7 Addressing Racial/Ethnic Disparities in Cancer Research

Despite the significant influence of genetic ancestry on cancer outcomes and its critical implications for healthcare quality, there has been a historical overrepresentation of white populations in all aspects of cancer research. This imbalance spans from fundamental aspects, such as patient-derived oncological models, biobanks, genomics, and epigenomics, to applied cancer research including clinical trials (Breeze et al. 2022; Cheung et al. 2023; Gao et al. 2023; García-Cárdenas et al. 2024; Guerrero et al. 2018; Herring et al. 2022; Raghavan 2022). Several factors have been identified to promote underrepresentation of ethnic minorities, such as institutional exclusion, mistrust in research, socioeconomic disadvantages, and linguistic and cultural barriers. To address this critical challenge, future approaches may involve expanding clinical trials to a global scale, employing molecular methods to determine ancestry, and increasing diversity among researchers to build trust in underrepresented groups. Researchers and practitioners need to implement targeted strategies to increase ethnic minority participation in health studies, ensuring equitable access to cancer research advancements (Bustamante et al. 2011;

George et al. 2014; Giuliano et al. 2000; Guerrero et al. 2018; Hu et al. 2013; Mattei et al. 2022; Riaz et al. 2023; Webb et al. 2018).

8 Conclusion

Cancer health disparities among ancestries arise from a complex multifactorial interplay of molecular factors and the social determinants of health (Freedman et al. 2021; Hacker et al. 2022; Syrnioti et al. 2023). Molecular changes at the epigenomic, genomic, transcriptomic, and proteomic levels are caused by (1) social determinants, such as exposure to mutagens, lifestyle choices like diet and exercise, or socioeconomic status and (2) natural variations linked to ancestry-based genetic predispositions and differences in gene expression caused at different levels (Freedman et al. 2021; Hacker et al. 2022; Syrnioti et al. 2023). For instance, communities lacking clean water or air may expose residents to cancer-causing substances, or neighborhoods without affordable healthy foods or safe exercise areas can lead to lifestyles with increasing molecular alterations that could lead to cancer (Naudin et al. 2020; Turner et al. 2020).

In this chapter, we focused on the molecular factors affecting cancer health disparities and showed that ancestry-based molecular alterations are found in all levels of gene expression. Such modifications can profoundly impact survival rates, treatment responses, and occurrence of specific cancer forms in different ancestries. For instance, East and South Asian groups show a higher frequency of *EGFR* mutations in LUAD compared to Europeans, a trend also observed among African never-smokers. This can significantly affect treatment response in this type of cancer. African ancestry women exhibit an increased frequency of *TP53* mutations in breast invasive ductal carcinoma, correlating with higher rates of triple-negative breast cancer. In prostate adenocarcinoma, East Asian patients have a higher alteration frequency of *FOXAI*, while African ancestry patients show increased *MYC* mutations. *KRAS* mutations are more prevalent in African patients with colon adenocarcinoma, and *RBI* mutations are notably more common in East Asian patients with HCC. East Asians also exhibit a lower occurrence of *TERT* mutations in glioblastoma and HCC compared to Europeans. Across different ancestries, the rate of clinically actionable mutations varies, with lower proportions in African, Native American, and South Asian populations compared to East Asian and European groups. Furthermore, genetic ancestry significantly influences cancer survival disparities in various cancers, including breast invasive carcinoma, head and neck squamous cell carcinoma, kidney renal clear cell carcinoma, and skin cutaneous carcinoma. Understanding these differences is crucial for developing more effective, personalized cancer therapies that consider both molecular factors and health challenges faced by individuals of diverse ancestries.

References

- Aburjania Z, Jang S, Whitt J, Jaskula-Stzul R, Chen H, Rose JB (2018) The role of Notch3 in cancer. *Oncologist* 23(8):900–911. <https://doi.org/10.1634/THEONCOLOGIST.2017-0677>
- Álvez MB, Edfors F, von Feilitzen K, Zwahlen M, Mardinoglu A, Edqvist PH, Sjöblom T, Lundin E, Rameika N, Enblad G, Lindman H, Höglund M, Hesselager G, Ståhlberg K, Enblad M, Simonson OE, Häggman M, Axelsson T, Åberg M et al (2023) Next generation pan-cancer blood proteome profiling using proximity extension assay. *Nat Commun* 14(1): 1–13. <https://doi.org/10.1038/s41467-023-39765-y>
- Arora K, Tran TN, Kemel Y, Mehine M, Liu YL, Nandakumar S, Smith SA, Brannon AR, Ostrovskaya I, Stopsack KH, Razavi P, Safonov A, Rizvi HA, Hellmann MD, Vijai J, Reynolds TC, Fagin JA, Carrot-Zhang J, Offit K et al (2022) Genetic ancestry correlates with somatic differences in a real-world clinical cancer sequencing cohort. *Cancer Discov* 12(11):2552. <https://doi.org/10.1158/2159-8290.CD-22-0312>
- Aubrey BJ, Strasser A, Kelly GL (2016) Tumor-suppressor functions of the TP53 pathway. *Cold Spring Harb Perspect Med* 6(5). <https://doi.org/10.1101/CSHPERSPECT.A026062>
- Auton A, Abecasis GR, Altshuler DM, Durbin RM, Bentley DR, Chakravarti A, Clark AG, Donnelly P, Eichler EE, Flicek P, Gabriel SB, Gibbs RA, Green ED, Hurles ME, Knoppers BM, Korbel JO, Lander ES, Lee C, Leach H et al (2015) A global reference for human genetic variation. *Nature* 526(7571):68–74. <https://doi.org/10.1038/NATURE15393>
- Batra RN, Lifshitz A, Vidakovic AT, Chin SF, Sati-Batra A, Sammut SJ, Provenzano E, Ali HR, Dariush A, Bruna A, Murphy L, Purushotham A, Ellis I, Green A, Garrett-Bakelman FE, Mason C, Melnick A, Aparicio SAJR, Rueda OM et al (2021) DNA methylation landscapes of 1538 breast cancers reveal a replication-linked clock, epigenomic instability and cis-regulation. *Nat Commun* 12(1):1–13. <https://doi.org/10.1038/s41467-021-25661-w>
- Breeze CE, Beck S, Berndt SI, Franceschini N (2022) The missing diversity in human epigenomic studies. *Nat Genet* 54(6):737–739. <https://doi.org/10.1038/s41588-022-01081-4>
- Bustamante CD, De La Vega FM, Burchard EG (2011) Genomics for the world. *Nature* 475(7355): 163–165. <https://doi.org/10.1038/475163a>
- Byun JS, Singhal SK, Park S, Yi DI, Yan T, Caban A, Jones A, Mukhopadhyay P, Gil SM, Hewitt SM, Newman L, Davis MB, Jenkins BD, Sepulveda JL, De Siervi A, Nápoles AM, Vohra NA, Gardner K (2020) Racial differences in the association between luminal master regulator gene expression levels and breast cancer survival. *Clin Cancer Res* 26(8):1905–1914. <https://doi.org/10.1158/1078-0432.CCR-19-0875>
- Carrot-Zhang J, Chambwe N, Damrauer JS, Knijnenburg TA, Robertson AG, Yau C, Zhou W, Berger AC, Huang KL, Newberg JY, Mashl RJ, Romanel A, Sayaman RW, Demichelis F, Felau I, Frampton GM, Han S, Hoadley KA, Kemal A et al (2020) Comprehensive analysis of genetic ancestry and its molecular correlates in cancer. *Cancer Cell* 37(5):639. <https://doi.org/10.1016/J.CCELL.2020.04.012>
- Chen J, Brunner A-D, Cogan JZ, Nuñez JK, Fields AP, Adamson B, Itzhak DN, Li JY, Mann M, Leonetti MD, Weissman JS (2020) Pervasive functional translation of noncanonical human open reading frames. *Science* 367(6482):1140–1146. <https://doi.org/10.1126/science.aay0262>
- Cheung ATM, Palapattu EL, Pompa IR, Aldrighetti CM, Niemierko A, Willers H, Huang F, Vapiwala N, Van Allen E, Kamran SC (2023) Racial and ethnic disparities in a real-world precision oncology data registry. *NPJ Precis Oncol* 7(1). <https://doi.org/10.1038/S41698-023-00351-6>
- Corso G, Montagna G, Figueiredo J, La Vecchia C, Romario UF, Fernandes MS, Seixas S, Roviello F, Trovato C, Guerini-Rocco E, Fusco N, Pravettoni G, Petrocchi S, Rotili A, Massari G, Magnoni F, De Lorenzi F, Bottoni M, Galimberti V et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: A multidisciplinary clinical review. *Cancers* 12(6):1–25. <https://doi.org/10.3390/CANCERS12061598>

- Deng J, Chen H, Zhou D, Zhang J, Chen Y, Liu Q, Ai D, Zhu H, Chu L, Ren W, Zhang X, Xia Y, Sun M, Zhang H, Li J, Peng X, Li L, Han L, Lin H et al (2017) Comparative genomic analysis of esophageal squamous cell carcinoma between Asian and Caucasian patient populations. *Nat Commun* 8(1). <https://doi.org/10.1038/S41467-017-01730-X>
- Devall M, Sun X, Yuan F, Cooper GS, Willis J, Weisenberger DJ, Casey G, Li L (2021) Racial disparities in epigenetic aging of the right vs left colon. *J Natl Cancer Inst* 113(12):1779–1782. <https://doi.org/10.1093/JNCI/DJAA206>
- Diaz Cruz MA, Karlsson S, Szekeres F, Faresjö M, Lund D, Larsson D (2021) Differential expression of protein disulfide-isomerase A3 isoforms, PDIA3 and PDIA3N, in human prostate cancer cell lines representing different stages of prostate cancer. *Mol Biol Rep* 48(3): 2429–2436. <https://doi.org/10.1007/S11033-021-06277-1>
- Dietze EC, Sistrunk C, Miranda-Carboni G, O'Regan R, Seewaldt VL (2015) Triple-negative breast cancer in African-American women: disparities versus biology. *Nat Rev Cancer* 15(4):248–254. <https://doi.org/10.1038/NRC3896>
- Distefano R, Nigita G, Le P, Romano G, Acunzo M, Nana-Sinkam P (2022) Disparities in lung cancer: miRNA isoform characterization in lung adenocarcinoma. *Cancers* 14(3). <https://doi.org/10.3390/CANCERS14030773>
- Ellis L, Canchola AJ, Spiegel D, Ladabaum U, Haile R, Gomez SL (2018) Racial and ethnic disparities in cancer survival: the contribution of tumor, sociodemographic, institutional, and neighborhood characteristics. *J Clin Oncol* 36(1):25–33. <https://doi.org/10.1200/JCO.2017.74.2049>
- Freedman JA, Al Abo M, Allen TA, Piwarski SA, Wegermann K, Patierno SR (2021) Biological aspects of cancer health disparities. 72:229–241. <https://doi.org/10.1146/ANNUREV-MED-070119-120305>
- Fu W, O'Connor TD, Jun G, Kang HM, Abecasis G, Leal SM, Gabriel S, Altshuler D, Shendure J, Nickerson DA, Bamshad MJ, Akey JM (2013) Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. *Nature* 493(7431):216–220. <https://doi.org/10.1038/NATURE11690>
- Gao Y, Sharma T, Cui Y (2023) Addressing the challenge of biomedical data inequality: an artificial intelligence perspective. *Annu Rev Biomed Data Sci* 6:153–171. <https://doi.org/10.1146/ANNUREV-BIODATASCI-020722-020704>
- García-Cárdenas JM, Armendáriz-Castillo I, Pérez-Villa A, Indacochea A, Jácome-Alvarado A, López-Cortés A, Guerrero S (2022) Integrated in silico analyses identify PUF60 and SF3A3 as new spliceosome-related breast cancer RNA-binding proteins. *Biology* 11(4):481. <https://doi.org/10.3390/BIOLOGY11040481>
- García-Cárdenas JM, Armendáriz-Castillo I, García-Cárdenas N, Pesantez-Coronel D, López-Cortés A, Indacochea A, Guerrero S (2023) Data mining identifies novel RNA-binding proteins involved in colon and rectal carcinomas. *Front Cell Dev Biol* 11:1088057. <https://doi.org/10.3389/FCELL.2023.1088057>
- García-Cárdenas JM, Indacochea A, Pesantez-Coronel D, Terán-Navas M, Aleaga A, Armendáriz-Castillo I, Zúñiga LP, Morán-Erazo C, Chávez-Vélez E, Pérez-Villa A, López-Cortés A, Guerrero S (2024) Toward equitable precision oncology: monitoring racial and ethnic inclusion in genomics and clinical trials. <https://doi.org/10.1200/PO.23.00398>
- García-Recio S, Hinoue T, Wheeler GL, Kelly BJ, Garrido-Castro AC, Pascual T, De Cubas AA, Xia Y, Felsheim BM, McClure MB, Rajkovic A, Karaesmen E, Smith MA, Fan C, Ericsson PIG, Sanders ME, Creighton CJ, Bowen J, Leraas K et al (2023) Multiomics in primary and metastatic breast tumors from the AURORA US network finds microenvironment and epigenetic drivers of metastasis. *Nat Cancer* 4(1):128–147. <https://doi.org/10.1038/S43018-022-00491-X>
- George S, Duran N, Norris K (2014) A systematic review of barriers and facilitators to minority research participation among African Americans, Latinos, Asian Americans, and Pacific islanders. *Am J Public Health* 104(2). <https://doi.org/10.2105/AJPH.2013.301706>

- Gibson AJW, D'Silva A, Elegbede AA, Tudor RA, Dean ML, Bebb DG, Hao D (2019) Impact of Asian ethnicity on outcome in metastatic EGFR-mutant non-small cell lung cancer. *Asia Pac J Clin Oncol* 15(6):343–352. <https://doi.org/10.1111/AJCO.13234>
- Giuliano AR, Mokuau N, Hughes C, Tortolero-Luna G, Risendal B, Ho RCS, Prewitt TE, McCaskill-Stevens WJ (2000) Participation of minorities in cancer research: the influence of structural, cultural, and linguistic factors. *Ann Epidemiol* 10(8 Suppl):S22–S34. <http://www.ncbi.nlm.nih.gov/pubmed/11189089>
- Gonçalves E, Poulos RC, Cai Z, Barthorpe S, Manda SS, Lucas N, Beck A, Bucio-Noble D, Dausmann M, Hall C, Hecker M, Koh J, Lightfoot H, Mahboob S, Mali I, Morris J, Richardson L, Seneviratne AJ, Shepherd R et al (2022) Pan-cancer proteomic map of 949 human cell lines. *Cancer Cell* 40(8):835–849.e8. <https://doi.org/10.1016/J.CCELL.2022.06.010>
- Guerrero S, López-Cortés A, Indacochea A, García-Cárdenas JM, Zambrano AK, Cabrera-Andrade A, Guevara-Ramírez P, González DA, Leone PE, Paz-y-Miño C (2018) Analysis of racial/ethnic representation in select basic and applied cancer research studies. *Sci Rep* 8(1):13978. <https://doi.org/10.1038/s41598-018-32264-x>
- Hacker K, Auerbach J, Ikeda R, Philip C, Houry D (2022) Social determinants of health – an approach taken at CDC. *J Public Health Manag Pract* 28(6):589–594. <https://doi.org/10.1097/PHH.0000000000001626>
- Hassel KR, Brito-Estrada O, Makarewich CA (2023) Microproteins: overlooked regulators of physiology and disease. *IScience* 26(6):106781. <https://doi.org/10.1016/J.ISCI.2023.106781>
- Herring BR, Bonner A, Guenter RE, Vickers S, Yates C, Lee G, Dhall D, Chen H, Rose JB (2022) Under-representation of racial groups in genomics studies of gastroenteropancreatic neuroendocrine neoplasms. *Cancer Res Commun* 2(10):1162–1173. <https://doi.org/10.1158/2767-9764.CRC-22-0093>
- Hu Y, Willer C, Zhan X, Kang HM, Abecasis GR (2013) Accurate local-ancestry inference in exome-sequenced admixed individuals via off-target sequence reads. *Am J Hum Genet* 93(5):891–899. <https://doi.org/10.1016/j.ajhg.2013.10.008>
- Huang PT, Einav S, Asquith CRM (2021) PIKfyve: a lipid kinase target for COVID-19, cancer and neurodegenerative disorders. *Nat Rev Drug Discov* 20(10):730. <https://doi.org/10.1038/D41573-021-00158-9>
- Izumi M, Suzumura T, Ogawa K, Matsumoto Y, Sawa K, Yoshimoto N, Tani Y, Watanabe T, Kaneda H, Mitsuoka S, Asai K, Kawaguchi T (2020) Differences in molecular epidemiology of lung cancer among ethnicities (Asian vs. Caucasian). *J Thorac Dis* 12(7):3776. <https://doi.org/10.21037/JTD.2019.08.61>
- Jarnuczak AF, Najgebauer H, Barzine M, Kundu DJ, Ghavidel F, Perez-Riverol Y, Papatheodorou I, Brazma A, Vizcaíno JA (2021) An integrated landscape of protein expression in human cancer. *Sci Data* 8(1):1–14. <https://doi.org/10.1038/s41597-021-00890-2>
- Jiagge E, Jin DX, Newberg JY, Perea-Chamblee T, Pekala KR, Fong C, Waters M, Ma D, Dei-Adomakoh Y, Erb G, Arora KS, Maund SL, Njiraini N, Ntekim A, Kim S, Bai X, Thomas M, van Eeden R, Hegde P et al (2023) Tumor sequencing of African ancestry reveals differences in clinically relevant alterations across common cancers. *Cancer Cell* 41(11):1963–1971.e3. <https://doi.org/10.1016/J.CCELL.2023.10.003>
- Kang M, Shen XJ, Kim S, Araujo-Perez F, Galanko JA, Martin CF, Sandler RS, Keku TO (2013) Somatic gene mutations in African Americans may predict worse outcomes in colorectal cancer. *Cancer Biomark* 13(5):359–366. <https://doi.org/10.3233/CBM-130366>
- Keenan T, Moy B, Mroz EA, Ross K, Niemierko A, Rocco JW, Isakoff S, Ellisen LW, Bardia A (2015) Comparison of the genomic landscape between primary breast cancer in African American versus white women and the Association of Racial Differences with Tumor Recurrence. *J Clin Oncol* 33(31):3621–3627. <https://doi.org/10.1200/JCO.2015.62.2126>
- Koga Y, Song H, Chalmers ZR, Newberg J, Kim E, Carrot-Zhang J, Piou D, Polak P, Abdulkadir SA, Ziv E, Meyerson M, Frampton GM, Campbell JD, Huang FW (2020) Genomic profiling of prostate cancers from men with African and European ancestry. *Clin Cancer Res* 26(17):4651–4660. <https://doi.org/10.1158/1078-0432.CCR-19-4112>

- Kraus WE, Muoio DM, Stevens R, Craig D, Bain JR, Grass E, Haynes C, Kwee L, Qin X, Slentz DH, Krupp D, Muehlbauer M, Hauser ER, Gregory SG, Newgard CB, Shah SH (2015) Metabolomic quantitative trait loci (mQTL) mapping implicates the ubiquitin proteasome system in cardiovascular disease pathogenesis. *PLoS Genet* 11(11):e1005553. <https://doi.org/10.1371/JOURNAL.PGEN.1005553>
- Krishnan B, Rose TL, Kardos J, Milowsky MI, Kim WY (2016) Intrinsic genomic differences between African American and white patients with clear cell renal cell carcinoma. *JAMA Oncol* 2(5):664–667. <https://doi.org/10.1001/JAMAONCOL.2016.0005>
- Lee KK, Rishishwar L, Ban D, Nagar SD, Mariño-Ramírez L, McDonald JF, Jordan IK (2022) Association of genetic ancestry and molecular signatures with cancer survival disparities: a pan-cancer analysis. *Cancer Res* 82(7):1222–1233. <https://doi.org/10.1158/0008-5472.CAN-21-2105>
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J et al (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536(7616):285–291. <https://doi.org/10.1038/nature19057>
- Li J, Xu C, Lee HJ, Ren S, Zi X, Zhang Z, Wang H, Yu Y, Yang C, Gao X, Hou J, Wang L, Yang B, Yang Q, Ye H, Zhou T, Lu X, Wang Y, Qu M et al (2020) A genomic and epigenomic atlas of prostate cancer in Asian populations. *Nature* 580(7801):93–99. <https://doi.org/10.1038/S41586-020-2135-X>
- Liao WW, Asri M, Ebler J, Doerr D, Haukness M, Hickey G, Lu S, Lucas JK, Monlong J, Abel HJ, Buonaiuto S, Chang XH, Cheng H, Chu J, Colonna V, Eizenga JM, Feng X, Fischer C, Fulton RS et al (2023) A draft human pangenome reference. *Nature* 617(7960):312–324. <https://doi.org/10.1038/s41586-023-05896-x>
- Libes JM, Seeley EH, Li M, Axt JR, Pierce J, Correa H, Newton M, Hansen E, Judd A, McDonald H, Caprioli RM, Naranjo A, Huff V, O'Neill JA, Lovvorn HN (2014) Race disparities in peptide profiles of north American and Kenyan Wilms tumor specimens. *J Am Coll Surg* 218(4):707–720. <https://doi.org/10.1016/J.JAMCOLLSURG.2013.12.044>
- Liu Z, Feng JG, Tuersun A, Liu T, Liu H, Liu Q, Zheng ST, Huang CG, Lv GD, Sheyhidin I, Lu XM (2011) Proteomic identification of differentially-expressed proteins in esophageal cancer in three ethnic groups in Xinjiang. *Mol Biol Rep* 38(5):3261–3269. <https://doi.org/10.1007/S11033-010-0586-0>
- Lv D, Li D, Ang Cai YY, Guo J, Chu S, Yu J, Liu K, Jiang T, Ding N, Jin X, Li Y, Xu J (2013) CancerProteome: a resource to functionally decipher the proteome landscape in cancer. *Nucleic Acids Res* 41(12):56879:13–14. <https://doi.org/10.1093/NAR/GKAD824>
- Ma BB, Hui EP, Mok TS (2010) Population-based differences in treatment outcome following anticancer drug therapies. *Lancet Oncol* 11(1):75–84. [https://doi.org/10.1016/S1470-2045\(09\)70160-3](https://doi.org/10.1016/S1470-2045(09)70160-3)
- Mani C, Acharya G, Saamrathy K, Ochola D, Mereddy S, Pruitt K, Manne U, Palte K (2023) Racial differences in RAD51 expression are regulated by miRNA-214-5P and its inhibition synergizes with olaparib in triple-negative breast cancer. *Breast Cancer Res* 25(1). <https://doi.org/10.1186/S13058-023-01615-6>
- Mattei LH, Robb L, Banning K, Polan RM, Cote ML (2022) Enrollment of individuals from racial and ethnic minority groups in gynecologic cancer precision oncology trials. *Obstet Gynecol* 140(4):654–661. <https://doi.org/10.1097/AOG.0000000000004917>
- McLeod MR, Galoosian A, May FP (2022) Racial and ethnic disparities in colorectal cancer screening and outcomes. *Hematol Oncol Clin North Am* 36(3):415–428. <https://doi.org/10.1016/J.HOC.2022.02.003>
- Mersha TB, Beck AF (2020) The social, economic, political, and genetic value of race and ethnicity in 2020. *Hum Genomics* 14(1):1–5. <https://doi.org/10.1186/S40246-020-00284-2/FIGURES/2>
- Minas TZ, Candia J, Dorsey TH, Baker F, Tang W, Kiely M, Smith CJ, Zhang AL, Jordan SV, Obadi OM, Ajao A, Tetey Y, Biritwum RB, Adjei AA, Mensah JE, Hoover RN, Jenkins FJ, Kittles R, Hsing AW et al (2022) Serum proteomics links suppression of tumor immunity to

- ancestry and lethal prostate cancer. *Nat Commun* 13(1):1–14. <https://doi.org/10.1038/s41467-022-29235-2>
- Morgan R, da Silveira WA, Kelly RC, Overton I, Allott EH, Hardiman G (2021) Long non-coding RNAs and their potential impact on diagnosis, prognosis, and therapy in prostate cancer: racial, ethnic, and geographical considerations. *Expert Rev Mol Diagn* 21(12):1257–1271. <https://doi.org/10.1080/14737159.2021.1996227>
- Nassar AH, Umeton R, Kim J, Lundgren K, Harshman L, Van Allen EM, Preston M, Dong F, Bellmunt J, Mouw KW, Choueiri TK, Sonpavde G, Kwiatkowski DJ (2019) Mutational analysis of 472 urothelial carcinoma across grades and anatomic sites. *Clin Cancer Res* 25(8):2458–2470. <https://doi.org/10.1158/1078-0432.CCR-18-3147>
- Naudin S, Solans Margalef M, Saberi Hosnijeh F, Nieters A, Kyrø C, Tjønneland A, Dahm CC, Overvad K, Mahamat-Saleh Y, Besson C, Boutron-Ruault MC, Kühn T, Canzian F, Schulze MB, Peppas E, Karakatsani A, Trichopoulou A, Sieri S, Masala G et al (2020) Healthy lifestyle and the risk of lymphoma in the European prospective investigation into cancer and nutrition study. *Int J Cancer* 147(6):1649–1656. <https://doi.org/10.1002/IJC.32977>
- Nusinow DP, Szpyt J, Ghandi M, Rose CM, McDonald ER, Kalocsay M, Jané-Valbuena J, Gelfand E, Schweppe DK, Jedrychowski M, Golji J, Porter DA, Rejtar T, Wang YK, Kryukov GV, Stegmeier F, Erickson BK, Garraway LA, Sellers WR, Gygi SP (2020) Quantitative proteomics of the cancer cell line encyclopedia. *Cell* 180(2):387–402.e16. <https://doi.org/10.1016/J.CELL.2019.12.023>
- Preat F, Simon P, Noel JC (2014) Differences in breast carcinoma immunohistochemical subtypes between immigrant Arab and European women. *Diagn Pathol* 9(1). <https://doi.org/10.1186/1746-1596-9-26>
- Pressinotti NC, Klocker H, Schäfer G, Luu VD, Ruschhaupt M, Kuner R, Steiner E, Poustka A, Bartsch G, Sültmann H (2009) Differential expression of apoptotic genes PDIA3 and MAP 3K5 distinguishes between low- and high-risk prostate cancer. *Mol Cancer* 8(1):1–12. <https://doi.org/10.1186/1476-4598-8-130/FIGURES/4>
- Raghavan S (2022) How inclusive are cell lines in preclinical engineered cancer models? *Dis Model Mech* 15(5). <https://doi.org/10.1242/DMM.049520>
- Rai R, Yadav SS, Pan H, Khan I, O'Connor J, Alshalalfa M, Davicioni E, Taioli E, Elemento O, Tewari AK, Yadav KK (2019) Epigenetic analysis identifies factors driving racial disparity in prostate cancer. *Cancer Rep* 2(2). <https://doi.org/10.1002/CNR2.1153>
- Ramanto KN, Widiarto KJ, Wibowo SSH, Agustriawan D (2021) The regulation of microRNA in each of cancer stage from two different ethnicities as potential biomarker for breast cancer. *Comput Biol Chem* 93. <https://doi.org/10.1016/J.COMPBIOLCHEM.2021.107497>
- Ramos J, Yoo C, Felty Q, Gong Z, Liuzzi JP, Poppiti R, Thakur IS, Goel R, Vaid AK, Komotar RJ, Ehteshami NZ, Hasnain SE, Roy D (2020) Sensitivity to differential NRF1 gene signatures contributes to breast cancer disparities. *J Cancer Res Clin Oncol* 146(11):2777–2815. <https://doi.org/10.1007/S00432-020-03320-9>
- Riaz IB, Islam M, Ikram W, Naqvi SAA, Maqsood H, Saleem Y, Riaz A, Ravi P, Wang Z, Hussain SA, Warner JL, Odedina FT, Duma N, Singh P, Kehl KL, Kamran SC, Murad MH, Landman A, Van Allen E, Bryce AH (2023) Disparities in the inclusion of racial and ethnic minority groups and older adults in prostate cancer clinical trials: a meta-analysis. *JAMA Oncol* 9(2):180–187. <https://doi.org/10.1001/JAMAONCOL.2022.5511>
- Rivera-Rivera Y, Vargas G, Jaiswal N, Núñez-Marrero A, Li J, Chen DT, Eschrich S, Rosa M, Johnson JO, Dutil J, Chellappan SP, Saavedra HI (2022) Ethnic and racial-specific differences in levels of centrosome-associated mitotic kinases, proliferative and epithelial-to-mesenchymal markers in breast cancers. *Cell Div* 17(1). <https://doi.org/10.1186/S13008-022-00082-3>
- Rutter CM, Nascimento de Lima P, Maerzluft CE, May FP, Murphy CC (2023) Black-White disparities in colorectal cancer outcomes: a simulation study of screening benefit. *JNCI Monographs* 2023(62):196–203. <https://doi.org/10.1093/JNCIMONOGRAPHS/LGAD019>
- Samuels Y, Waldman T (2010) Oncogenic mutations of PIK3CA in human cancers. *Curr Top Microbiol Immunol* 347:21. https://doi.org/10.1007/82_2010_68

- Sandmann CL, Schulz JF, Ruiz-Orera J, Kirchner M, Ziehm M, Adami E, Marczenke M, Christ A, Liebe N, Greiner J, Schoenenberger A, Muecke MB, Liang N, Moritz RL, Sun Z, Deutsch EW, Gotthardt M, Mudge JM, Prensner JR et al (2023) Evolutionary origins and interactomes of human, young microproteins and small peptides translated from short open reading frames. *Mol Cell* 83(6):994–1011.e18. <https://doi.org/10.1016/J.MOLCEL.2023.01.023>
- Song MA, Seffernick AE, Archer KJ, Mori KM, Park SY, Chang L, Ernst T, Tiirikainen M, Peplowska K, Wilkens LR, Le Marchand L, Lim U (2021) Race/ethnicity-associated blood DNA methylation differences between Japanese and European American women: an exploratory study. *Clin Epigenet* 13(1). <https://doi.org/10.1186/S13148-021-01171-W>
- Srivastava M, Eidelman O, Craig J, Starr J, Kvecher L, Liu J, Hueman M, Pollard HB, Hu H, Shriver CD (2019) Serum biomarkers for racial disparities in breast cancer progression. *Mil Med* 184(Suppl 1):652–657. <https://doi.org/10.1093/MILMED/USY417>
- Stopsack KH, Nandakumar S, Arora K, Nguyen B, Vasselmann SE, Nweji B, McBride SM, Morris MJ, Rathkopf DE, Slovin SF, Danila DC, Autio KA, Scher HI, Mucci LA, Solit DB, Gönen M, Chen Y, Berger MF, Schultz N et al (2022) Differences in prostate cancer genomes by self-reported race: contributions of genetic ancestry, modifiable cancer risk factors, and clinical factors. *Clin Cancer Res* 28(2):318–326. <https://doi.org/10.1158/1078-0432.CCR-21-2577>
- Sudmant PH, Rausch T, Gardner EJ, Handsaker RE, Abyzov A, Huddleston J, Zhang Y, Ye K, Jun G, Fritz MHY, Konkel MK, Malhotra A, Stütz AM, Shi X, Casale FP, Chen J, Hormozdiari F, Dayama G, Chen K et al (2015) An integrated map of structural variation in 2,504 human genomes. *Nature* 526(7571):75–81. <https://doi.org/10.1038/nature15394>
- Syrnioti G, Eden CM, Johnson JA, Alston C, Syrnioti A, Newman LA (2023) Social determinants of cancer disparities. *Ann Surg Oncol* 30(13). <https://doi.org/10.1245/S10434-023-14200-0>
- Turner MC, Andersen ZJ, Baccarelli A, Diver WR, Gapstur SM, Pope CA, Prada D, Samet J, Thurston G, Cohen A (2020) Outdoor air pollution and cancer: an overview of the current evidence and public health recommendations. *CA Cancer J Clin* 70(6):460–479. <https://doi.org/10.3322/CAAC.21632>
- Wattacheril JJ, Raj S, Knowles DA, Grealley JM (2023) Using epigenomics to understand cellular responses to environmental influences in diseases. *PLoS Genet* 19(1):e1010567. <https://doi.org/10.1371/JOURNAL.PGEN.1010567>
- Webb FJ, Khubchandani J, Striley CW, Cottler LB (2018) Black–White differences in willingness to participate and perceptions about health research: results from the population-based Healthstreet study. *J Immigr Minor Health* 1–7. <https://doi.org/10.1007/s10903-018-0729-2>
- Williams PA, Zaidi SK, Sengupta R (2022) AACR cancer disparities progress report 2022. *Cancer Epidemiol Biomark Prev* 31(7):1249–1250. <https://doi.org/10.1158/1055-9965.EPI-22-0542/699401/P/AACR-CANCER-DISPARITIES-PROGRESS-REPORT-2022>
- Wu S, Ou T, Xing N, Lu J, Wan S, Wang C, Zhang X, Yang F, Huang Y, Cai Z (2019a) Whole-genome sequencing identifies ADGRG6 enhancer mutations and FRS2 duplications as angiogenesis-related drivers in bladder cancer. *Nat Commun* 10(1). <https://doi.org/10.1038/S41467-019-08576-5>
- Wu Q, Zhang W, Xue L, Wang Y, Fu M, Ma L, Song Y, Zhan QM (2019b) APC/C-CDH1-regulated IDH3 β coordinates with the cell cycle to promote cell proliferation. *Cancer Res* 79(13):3281–3293. <https://doi.org/10.1158/0008-5472.CAN-18-2341>
- Wu SM, Tsai WS, Chiang SF, Lai YH, Ma CP, Wang JH, Lin J, Lu PS, Yang CY, Tan BCM, Liu H (2020) Comprehensive transcriptome profiling of Taiwanese colorectal cancer implicates an ethnic basis for pathogenesis. *Sci Rep* 10(1):1–18. <https://doi.org/10.1038/s41598-020-61273-y>
- Xing F, Zhao D, Wu SY, Tyagi A, Wu K, Sharma S, Liu Y, Deshpande R, Wang Y, Cleary J, Miller LD, Chittiboyina AG, Yalamanchili C, Mo YY, Watabe K (2021) Epigenetic and posttranscriptional modulation of SOS1 can promote breast cancer metastasis through obesity-activated c-met signaling in African-American women. *Cancer Res* 81(11):3008–3021. <https://doi.org/10.1158/0008-5472.CAN-19-4031>

- Xu Z, Huo X, Tang C, Ye H, Nandakumar V, Lou F, Zhang D, Jiang S, Sun H, Dong H, Zhang G, Liu Z, Dong Z, Guo B, Yan H, Yan C, Wang L, Su Z, Li Y et al (2014) Frequent KIT mutations in human gastrointestinal stromal tumors. *Sci Rep* 4. <https://doi.org/10.1038/SREP05907>
- Yamada HY, Xu C, Jones KL, O'Neill PH, Venkateshwar M, Chiliveru S, Kim HG, Doescher M, Morris KT, Manne U, Rao CV (2023) Molecular disparities in colorectal cancers of white Americans, Alabama African Americans, and Oklahoma American Indians. *NPJ Precis Oncol* 7(1):1–7. <https://doi.org/10.1038/s41698-023-00433-5>
- Yao S, Johnson C, Hu Q, Yan L, Liu B, Ambrosone CB, Wang J, Liu S (2016) Differences in somatic mutation landscape of hepatocellular carcinoma in Asian American and European American populations. *Oncotarget* 7(26):40491–40499. <https://doi.org/10.18632/ONCOTARGET.9636>
- Yap YS (2023) Outcomes in breast cancer—does ethnicity matter? *ESMO Open* 8(3). <https://doi.org/10.1016/J.ESMOOP.2023.101564>
- Ye F, Han X, Shao Y, Lo J, Zhang F, Wang J, Melamed J, Deng F-M, Sfanos KS, De Marzo A, Ren G, Wang D, Zhang D, Lee P (2022) Identification of novel biomarkers differentially expressed between African-American and Caucasian-American prostate cancer patients. *Am J Cancer Res* 12(4):1660. [/pmc/articles/PMC9077070/](https://pubmed.ncbi.nlm.nih.gov/39077070/)
- Yedjou CG, Sims JN, Miele L, Noubissi F, Lowe L, Fonseca DD, Alo RA, Payton M, Tchounwou PB (2019) Health and racial disparity in breast cancer. *Adv Exp Med Biol* 1152:31–49. https://doi.org/10.1007/978-3-030-20301-6_3
- Yuan J, Hu Z, Mahal BA, Zhao SD, Kensler KH, Pi J, Hu X, Zhang Y, Wang Y, Jiang J, Li C, Zhong X, Montone KT, Guan G, Tanyi JL, Fan Y, Xu X, Morgan MA, Long M et al (2018) Integrated analysis of genetic ancestry and genomic alterations across cancers. *Cancer Cell* 34(4):549–560.e9. <https://doi.org/10.1016/J.CCELL.2018.08.019>
- Yuan J, Kensler KH, Hu Z, Zhang Y, Zhang T, Jiang J, Xu M, Pan Y, Long M, Montone KT, Tanyi JL, Fan Y, Zhang R, Hu X, Rebbeck TR, Zhang L (2020) Integrative comparison of the genomic and transcriptomic landscape between prostate cancer patients of predominantly African or European genetic ancestry. *PLoS Genet* 16(2). <https://doi.org/10.1371/JOURNAL.PGEN.1008641>
- Zavala VA, Bracci PM, Carethers JM, Carvajal-Carmona L, Coggins NB, Cruz-Correa MR, Davis M, de Smith AJ, Dutil J, Figueiredo JC, Fox R, Graves KD, Gomez SL, Llera A, Neuhausen SL, Newman L, Nguyen T, Palmer JR, Palmer NR et al (2020) Cancer health disparities in racial/ethnic minorities in the United States. *Br J Cancer* 124(2):315–332. <https://doi.org/10.1038/s41416-020-01038-6>
- Zhao SG, Chen WS, Li H, Foye A, Zhang M, Sjöström M, Aggarwal R, Playdle D, Liao A, Alumkal JJ, Das R, Chou J, Hua JT, Barnard TJ, Bailey AM, Chow ED, Perry MD, Dang HX, Yang R et al (2020) The DNA methylation landscape of advanced prostate cancer. *Nat Genet* 52(8):778–789. <https://doi.org/10.1038/s41588-020-0648-8>



The Pattern of Epithelial Carcinogenesis

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Abstract

Epithelial tissues constitute the outer surface of the body, line most internal organ cavities, and form a significant portion of glandular structures. Malignant neoplasms originating from epithelia typically begin as hyperplasias progressing to carcinomas in situ, which serve as non-obligate precursors to invasive lesions and advanced metastatic disease. This chapter compares the fundamental characteristics of the epithelial tissue organization and the changes occurring during the initiation and progression of carcinomas. It reviews similarities across nine types of epithelial neoplasms, focusing on their association with risk factors and shared molecular, cellular, and histological processes underlying precursor lesions. Additionally, the chapter explores events driving progression to high-grade carcinomas, emphasizing recent studies on the role of cellular senescence and epithelial to mesenchymal transition in dynamic alterations from early stages to advanced poorly differentiated disease. The discussion highlights discoveries enhancing our understanding of epithelial degeneration, malignant transformation, and the development of resistance to anticancer therapies. The convergence of these findings suggests a pattern, that may facilitate the identification of general principles crucial for advancing comprehension, prevention, and treatment strategies for the disease.

Keywords

Carcinogenesis · Carcinomas · Epithelial neoplasms

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1 Introduction

Most malignant neoplasms are generated from well-located anatomical regions, so their broadest classification is based on their site of origin. In this way, carcinomas are the type of cancer that develop from epithelial tissues (Avery et al. 2022). In them, reiterative changes are observed during the process of malignant transformation at the molecular, cellular, and histological levels that have relevant implications for the establishment of their prognosis and treatment (Méndez-López 2022). This recurrent pattern in the progression is perceptible even in the way in which its metastases migrate and does not seem to be influenced by the latest advances in medicine (Budczies et al. 2015). Despite the above, for a long time it has prevailed in cancer research the notion that tumors are highly heterogeneous entities governed largely by random mutagenic events and Darwinian selection (Vogelstein and Kinzler 1993; Greenman et al. 2006). However, from the first implementations of genomic analysis to carcinomas it was revealed that the supposed complexity could be reduced extensively to only a few well-defined entities (Malhotra et al. 2010) that were less diverse than expected even by conventional histopathological and immunohistochemical classification (Lakhani et al. 2012). It is worth mentioning that similarities have been established between neoplasms that originate from various types of tissues (Ben-Porath et al. 2008). For example, contrary to what was expected, results from new-generation technologies of gene-sequencing demonstrated a great similarity between the various types of carcinomas and surprisingly also with leukemias, melanomas, and gliomas (Heim et al. 2014). Recent research suggests that the malignant transformation of various types of carcinomas could be the result of the same generic event by which epithelial cells recover the ability to behave like embryonic phenotypes capable of generating neoplasia (Willet et al. 2018). In this regard, similar conclusions were reached by other authors through theoretical approaches and computational analyses that include a potential mechanism involved in the loss of differentiation in epithelial cells in the context of the histological progression of carcinomas (Méndez-López et al. 2017a; Méndez-López 2022). Although cancer can arise from practically any cell type, the correspondences of tumor progression among different histological types of neoplasms, particularly in carcinomas, led to the postulation that this disease could be a single entity (Clark 1991). This chapter emphasizes those events occurring in epithelial carcinogenesis that suggest a universal behavior. In addition, we reviewed the results from lineage tracing and single-cell transcriptomics regarding the origin of carcinomas in their histological, cellular, and molecular contexts. Although we do not intend to discuss the reasons underlying such similarities, we would like to highlight that this convergence suggests a pattern, which could be useful for the identification of generic principles that allow a significant progress in the understanding, prevention, and treatment of disease.

2 The Hallmarks of Carcinoma

Carcinomas comprise approximately 90% of all neoplasms diagnosed in the adult population (Siegel et al. 2023). These originate in the epithelia, which are the type of tissue that lines the surface and all the cavities of the body and whose organization allows them to act as a barrier and carry out the transport of molecules between body compartments (Tyler 2003). In addition to the skin, the epithelia most susceptible to carcinogenesis are found forming part of glands or covering the mucous membranes of the gastrointestinal, genitourinary, and respiratory tract (DePinho 2000). Hence, most of the mortality related to cancer in humans comes from lesions generated in the breast, endometrium, cervix, prostate, colon, liver, pancreas, bladder, and lung (Siegel et al. 2023). Another peculiarity of carcinomas is that they have a strong association with lifestyle factors (Byrne et al. 2023), advanced age (DePinho 2000), inflammatory processes (Murata 2018), and exhibit a set of gradual histological changes during their gestation and progression (Dupont et al. 1993). All of the aforementioned carcinomas arise from structures formed by the interaction of the epithelium with an associated mesenchyme (Tyler 2003). Epithelia are tissues made up of aligned cells that form the covering, structure and that give specific functions to the organ, that is, its parenchyma (Tyler 2003). Epithelial cells are usually flat, cuboidal, or columnar and adhere to the basement membrane which establishes an axis of polarity (Tyler 2003). They also join together through intercellular cohesion proteins such as cadherins, claudins, and occludins (Kalluri 2009). In fact, epithelia and their neoplasms express intensely E-cadherin, which makes it one of the most used markers for the characterization of this tissue (Behrens et al. 1991). The basal surface of epithelium is associated with the extracellular matrix, which is synthesized by fibroblasts (Peinado et al. 2004). Although these mesenchymal cells are highly heterogeneous, they are characterized by a flattened, elongated, and fusiform morphology (Kalluri 2009). Unlike epithelial cells, fibroblasts do not exhibit some particular alignment within the extracellular matrix, and even though they present several binding proteins, the most abundant is N-cadherin. Furthermore, these cells can migrate and constitutively express vimentin, a component of the intermediate filaments necessary for motility (Peinado et al. 2004). Therefore, to identify these mesenchymal cells, their fusiform morphology is used together with the expression of N-cadherin, and vimentin (Peinado et al. 2004). The extracellular matrix composed mainly of collagen, elastin, and glycoproteins together with fibroblasts is considered the mesenchyme of the organs, that is, the supporting tissue also called stroma (Tyler 2003).

The histological criteria most used to estimate the degree of structural transformation of epithelial tissue during carcinogenesis are related to cellularity, nuclear size, the presence of nucleoli, and the basement membrane (Dupont et al. 1993). According to the current consensus, carcinomas are preceded by focal hyperplastic lesions, in other words, subpopulations of cells with dense cellularity and atypical cytology, which are considered the precursors of the *in situ* carcinomas (Dupont et al. 1993). In turn, epithelial neoplasms tend to invade and lose differentiation during disease progression (Cserni 2002). At the histological level, simple

hyperplasias are benign abnormal proliferations of epithelial cells that lack nuclear atypia and although display structural thickening in general resemble normal tissues. In them, the glandular compartment seems dilated, but the stroma may also be involved. It should be noted that the vast majority of epithelial neoplasms originate from the glandular tissue, which is why they are classified as adenocarcinomas (Silverberg 2000). At a molecular level, hyperplastic lesions are characterized by the positivity of biomarkers of senescence (Radisky et al. 2011). This cellular state is distinguished by the loss of the ability to proliferate, the adoption of an enlarged and flattened shape, an increased granularity, and by expression of β -galactosidase. The nucleus presents elongation and invaginations with large and fragmented nucleoli. Additionally, telomeres exhibit shortening and the formation of DNA repair foci (Kuilman et al. 2010). The genome damage response is activated and depends on the protein kinase ATM (Ataxia telangiectasia mutated), p53, and Chk2 (Checkpoint kinase 2) (Rodier et al. 2009). Furthermore, the epigenetic silencing is lost in the Ink4b/ARF/Ink4a locus of the kinase inhibitors p15 and p16 (Agherbi et al. 2009). Cellular senescence entails a marked activity of polycomb complexes and enzymes that modify histones leading to significant changes in the configuration of chromatin and therefore gene expression (Agherbi et al. 2009) that include the adoption of a secretory phenotype characterized by cytokines, proteases, and growth factors (Rodier et al. 2009).

The malignant transformation of hyperplasias is related to the acquisition of a greater degree of proliferation, a loss of the normal orientation of epithelial cells with displacement of the stroma, and the gain of atypia, that is, the appearance of cells with large, pleomorphic and hyperchromatic nuclei, enlarged nucleoli and cytoplasm with eosinophilia (Silverberg 2000). A recurrent observation during the progression of hyperplastic lesions toward carcinomas is that atypia increases along with a diminution of senescence markers (Umbricht et al. 1999; Meeker et al. 2004a, b; Pare et al. 2019) and the infiltration of lymphocytes (DeNardo and Coussens 2007). Relatedly, although hyperplasias share some of the architectural and cytological characteristics with carcinomas in situ, the former are morphologically and phenotypically more heterogeneous while the latter present greater homogeneity (Pinder 2010). Carcinomas in situ are characterized by confined epithelial malignant cells, without evidence of invasion through the basement membrane, and unlike hyperplasias, these present greater nuclear atypia in proliferating large cells within the parenchymal structures (Pinder and Ellis 2003). On the other hand, once invasive carcinomas develop, they present a tendency to progress characterized by a loss of differentiation (Van Dooijeweert et al. 2022; Giordano et al. 2023). In essence, well-differentiated grade 1 tumors are composed by 90% of structures that resemble normal tissue whereas those categorized as grade 3 or poorly differentiated display have lost more than 50% of the original epithelial architecture (Van Dooijeweert et al. 2022). The classification also considers the degree of variation in the size and shape of the nucleus, the pattern of nucleoli and chromatin as well as the number of mitoses generally observed in the most proliferative solid area in the periphery of the tumor (Van Dooijeweert et al. 2022). Thus, a high-grade carcinoma is characterized by a solid growth pattern, with necrotic areas, neoplastic cells that do not stick

together, uniform appearance, oval nuclei with prominent nucleoli, and frequent mitotic figures (Giordano et al. 2023). High-grade tumors have also been described as small-cell carcinomas or stromal sarcomas (Giordano et al. 2023). The percentage of tumor epithelial cells tends to decrease as the tumors progress toward high histological grades where the stroma associated with the tumor increases (Sullivan et al. 2022). This excessive proliferation of fibroblasts with an increase in the production of the extracellular matrix is also called desmoplasia (Wernicke et al. 2003). At the molecular level, the loss of differentiation of carcinomas is associated with the epithelial-to-mesenchymal transition (EMT), where the activation of the transcription factors Snail, Slug, Twist, and Zeb leads to the repression of the epithelial phenotype and the formation of tumors with higher histological grades (Ye et al. 2015). In addition, it is known that mesenchymal transcription factors inhibit those responsible for the epithelial phenotype and vice versa (Chakrabarti et al. 2012). The group of proteins that regulate gene expression, morphogenesis, and development of epithelial tissues includes ESE (ETS specific of epithelium) proteins such as ESE-1, ESE-2, ESE-3, and PDEF (prostate-derived ETS factor) (Hollenhorst et al. 2004). Interestingly, the silencing of ESE transcription factors is sufficient for the loss of differentiation and the generation of carcinomas (Donnison et al. 2005). Another notable fact is that fibroblasts are the most abundant cell type in high-grade carcinomas, however, they present increased plasticity and multipotency that is accompanied by the expression of embryonic markers (Selman et al. 2008; Koch et al. 2011; Xie et al. 2016; Lai et al. 2017). Therefore, these tumors can be considered as neoplastic entities that have lost differentiation, adopting a phenotype similar to that observed during early embryonic development or during regenerative processes (Giordano et al. 2023). This increase in cellular plasticity, stromal content, and mesenchymal markers is associated with greater invasiveness, metastasis, drug resistance, and worse prognosis (Ugnat et al. 2004; Finak et al. 2008; Sarrió et al. 2008; Chen et al. 2015). Furthermore, the loss of differentiation during tumor progression has been described in the vast majority of carcinomas and can occur *de novo* or present as a recurrence of a previously well-differentiated neoplasia (Giordano et al. 2023).

The importance of histological changes in epithelia in relation to the course of the disease and prognosis has been recognized for more than a century and has not been replaced by the most modern molecular diagnostic methods (Giordano et al. 2023). However, the precise delimitation between each of the histological entities referred here continues to represent a challenge even for experts on the subject. Moreover, different diagnostic criteria are usually used for each type of carcinoma and even among its benign and malignant precursor lesions (Silverberg 2000; Cserni 2002). On the other hand, histological and molecular studies demonstrate that in many cases the different types of hyperplastic lesions coexist within tumors together with carcinomas of several histological grades (Silverberg 2000; Pinder 2010; Lehmann et al. 2011). Therefore, although the description regarding the histological criteria for the classification of carcinomas in their various stages of progression represents a very exaggerated simplification, we consider it essential for the purposes of the chapter. In addition, it will serve as a guide for the unfamiliar reader when pointing

out the repetitive events in the tissue and cellular context in the different carcinomas reviewed, as well as to understand the new findings about their most plausible origin.

3 Breast Cancer

Malignant neoplasms of the breast are the most commonly diagnosed type of cancer worldwide with 2,261,419 million new cases and 685,000 deaths in 2020 (Arnold et al. 2022). Epidemiological studies indicate that the most relevant risk factors for the development of the disease include advanced age, obesity, metabolic syndrome, use of hormone therapy, physical inactivity, unhealthy diet, smoking, and alcohol consumption (Friedenreich et al. 2000; Anand et al. 2008; Djuric et al. 2009; Siegel et al. 2016). According to some reports, up to 20% of breast cancer cases can be attributed to obesity, another 20% to alcohol consumption, 16% to a sedentary lifestyle, and 11% to the use of hormones (Sprague et al. 2008). It is well established that chronic exposure to high circulating levels of estrogens, insulin, IGF-1, IL-8, IL-6, and leptin produces carcinogenic effects in the epithelium of the mammary gland (Calle and Kaaks 2004; Howell et al. 2014). Breast tissue is particularly sensitive to the endocrine environment since its proliferation rate increases in relation to the production and activity of growth factors, cytokines, and hormonal or nutritional stimuli (Bendrik and Dabrosin 2009; Scaling et al. 2014). In fact, benign proliferative diseases of the breast and its hyperplasias are associated with inflammatory processes or with hormonal disorders (Donnelly et al. 1975; Dupont et al. 1993; Lambe et al. 2009). Carcinomas are detected more frequently after the age of 50 and in 80% of cases these are associated with the presence of one or several risk factors (Benz 2008). It is worth mentioning that obesity is associated with an appearance of breast cancer up to 5 years earlier, estimated at 47 years (Azrad et al. 2019), but in general, its diagnosis increases exponentially after menopause, and its highest point is reached on average at 79 years of age (Benz 2008). An interesting fact is that low-grade tumors are detected in patients under 50 years of age while high-grade tumors prevail in those over 70 (Anderson et al. 2006). As in other carcinomas, breast carcinogenesis presents as a progressive process in which atypical hyperplasias are considered the precursor lesions of carcinomas in situ. Actually, their diagnosis increases five times the risk of developing invasive carcinomas (Dupont et al. 1993). A common finding in breast hyperplasias is the expression of cellular senescence markers, that is, short telomeres and the p16 protein can be detected (Radisky et al. 2011). Among the events associated with its progression is the infiltration of immune system cells (DeNardo and Coussens 2007; Pare et al. 2019). In the case of invasive carcinomas, a recovery of the telomeric regions, telomerase activity (Umbricht et al. 1999), and decreased expression of p16 are common findings (Meeker et al. 2004a, b). More than 80% of breast carcinomas correspond to the ductal subtype, that is, they originate in the luminal epithelial cells of the ducts (Malhotra et al. 2010). The acquisition of a high histological grade is accompanied by a loss of glandular architecture and biomarkers of the luminal epithelium of the breast such as the estrogen receptor, the progesterone receptor,

and E-cadherin (Mendez-Lopez et al. 2010; Younis et al. 2010). During breast tumor progression, the loss of hormonal dependence and other growth factors is observed. This phenotype is called triple negative, since it does not express the proteins normally present in the epithelium of the mammary gland, including the receptors for estrogen, progesterone, and epidermal growth factors. Dedifferentiated tumors eventually gain expression of mesenchymal markers associated with EMT (Chen et al. 2008) and with breast cancer stem cells such as the expression of the CD44 marker (Dongre et al. 2017). Triple-negative breast carcinomas present high-grade histological features, an increased number of metastasis, and resistance to chemotherapy, radiotherapy, and immunotherapy (Lakhtakia et al. 2017). In general, metastases are usually found in bones, lungs, brain, and liver (Minn et al. 2005). The prognosis of patients with localized disease and low-grade carcinomas is good, since around 95% survive their cancer for 5 years after diagnosis. In contrast, in patients with metastatic disease the prognosis is adverse with an average survival of 1 year (Kassam et al. 2009); however, it has been observed that the presence of liver and brain metastases can reduce it to only 3 months (Patanaphan et al. 1988).

4 Endometrial Cancer

Endometrial cancer is the sixth most common cancer in women worldwide, with 417,000 new cases and 97,370 deaths reported in 2020 (Huang et al. 2023). Among the risk factors most associated with its development are advanced age, obesity, the use of estrogens, polycystic ovary syndrome, consumption of an unhealthy diet, and smoking (Beavis et al. 2016). Like the epithelium of the breast, the endometrial is very sensitive to hormonal changes related to metabolic disorders associated with obesity such as diabetes, hypertension, and dyslipidemias. Approximately 57% of endometrial cancer cases can be attributed to overweight and obesity (Onstad et al. 2017). The endocrine profile of patients at risk of developing endometrial carcinoma is characterized by the presence of elevated levels of insulin, estrogen, EGF, IL-18, IL-6, IGF-1, and leptin (Rutanen 1998; Punyadeera et al. 2004; Ramos-Nino 2013). This hormonal environment promotes inflammatory and proliferative conditions in the endometrial epithelium such as hyperplasia (Cusack et al. 1996; Villavicencio et al. 2010). Therefore, it is considered that chronic exposure to this hormonal environment promotes the risk of malignant transformation of the endometrium, especially in obese and postmenopausal patients. The average age for the diagnosis of this carcinoma is 61 years but the maximum incidence is reached at 79 years. It stands out that among patients with obesity, the average age for diagnosis is 50 years, highlighting the carcinogenic role of adipose tissue on the endometrium (Patsner 2000; Nevadunsky et al. 2014). Atypical hyperplasias of the endometrium are considered the precursor lesions of endometrial carcinomas. These histological entities present positive reactivity for cellular senescence markers such as the p16 protein (Hisamatsu et al. 2017). Furthermore, the infiltration of immune system cells and the loss of expression of senescence markers are associated with their progression to carcinoma (Mingels et al. 2014; Hu et al. 2015). Incipient malignant lesions

of the endometrium are also called intraepithelial neoplasias and their diagnosis is related to the discovery of invasive carcinomas in the following year (Jarboe and Mutter 2010). Unlike their hyperplasias, endometrial carcinomas express the enzyme telomerase and can recover telomere extension (Saito et al. 1997; Maida et al. 2002; Danescu et al. 2013). Up to 80% of sporadic endometrial carcinomas are adenocarcinomas since arise in the glandular epithelium and resemble normal features (Jarboe and Mutter 2010). However, their progression is accompanied by loss of differentiation, the silencing of hormone and leptin receptors, and E-cadherin (Bartosch et al. 2015; Méndez-López et al. 2017b). On the other hand, the content of mesenchymal lineage markers increases, including EMT and vimentin, but also display embryonic transcription factors and the marker CD133 (Nakamura et al. 2014; Nesina et al. 2018). In endometrial carcinoma, EMT is associated with all aspects related to progression, including metastasis, invasion, and resistance to treatment (Nakamura et al. 2014). The 5-year survival of localized endometrial tumors is greater than 95%; however, for women diagnosed with metastatic disease, this percentage is less than 17%. The response rate to treatment in these stages is unfavorable with an average survival of 4 to 9 months. Metastases occur most frequently in the pelvic lymph nodes, vagina, peritoneum, and lungs. Less frequently they can be found in the liver, bones, and brain (Thigpen et al. 2004; Kurra et al. 2013; Nassir et al. 2014).

5 Prostate Cancer

Prostate cancer is the second most commonly diagnosed malignancy and the fifth cause of cancer death among men worldwide, with an estimated 1,414,000 new cases and 375,304 deaths in 2020 (Wang et al. 2022). Factors associated with the risk of developing it include advanced age, obesity, androgen levels, unhealthy diet, sexually transmitted diseases, and smoking (Gann 2002; Wilson and Mucci 2019; Wang et al. 2022). However, aging is by far the most important risk factor, with 80% of diagnoses occurring in men over 60 (Pathirana et al. 2022). The risk factors associated with this cancer are characterized by chronic exposure to high circulating levels of sex hormones, insulin, IGF-1, leptin, and IL-6 (Gann 2002; Barnard 2007), which in turn are well known for their proliferative and secretory effects on the prostatic epithelium (Giri et al. 2001; Arnold and Isaacs 2002; Roberts 2004). Relatedly, patients with metabolic syndrome or those who suffer from urinary tract infections develop prostatitis, which is also associated with an increased risk of developing prostate carcinoma (Gann 2002; Puhr et al. 2016). While 80% of young patients present either normal prostate histology, infections, or benign inflammatory conditions, 34% of patients over 70 years would display hyperplastic and neoplastic lesions, illustrating again the relevance of aging for the carcinogenesis of this gland. The average age for diagnosis of prostate carcinoma is 68 years and it is estimated that more than half of men will suffer from it by the age of 80 (Harbitz and Haugen 1972; Crawford 2003). Like other carcinomas, the average age for diagnosis tends to decrease among patients with overweight and obesity (Popovici et al. 2023). Its

precursor lesion is prostatic hyperplasia, whose histological diagnosis is characterized by an increase in the proliferation of the glandular epithelium. Its progression to the entity known as intraepithelial neoplasia of the prostate (Harvey 1995) increases the diagnosis of carcinoma by up to 15 times (Bostwick 1996). At a molecular level, the presence of short telomeres can be detected (Castro et al. 2003; Vukovic et al. 2003) along with the expression of the enzyme β -galactosidase, and the p16 protein (Zhang et al. 2006). Its malignant transformation is associated with the infiltration of leukocytes (Perletti et al. 2010), the accumulation of senescent cells (Meeker et al. 2002), and the reactivation of telomerase activity (Koenen et al. 1998; Henshall et al. 2001). In 95% of the cases, these lesions advance to carcinomas with luminal phenotype (Shen and Abate-Shen 2010); however, these neoplasms do not remain unchanged, they also tend to progress accompanied by the loss of epithelial architecture and its biomarkers (Arnold and Isaacs 2002). The acquisition of high tumor grades in prostate neoplasms has also been related to the EMT process, the increase in expression of mesenchymal transcription factors, the expression of N-cadherin, the loss of glandular architecture, and the presence of metastasis (Kwok et al. 2005; Kolijn et al. 2018). In patients with prostate cancer undergoing androgen deprivation treatment, EMT is also associated with the adoption of a hormone-independent phenotype, resistance to chemotherapy, radiotherapy, and the gain of cancer stem cells. In prostate tissue, the CD44 marker has been useful to characterize cells with stem behavior (Gravdal et al. 2007). Tumors with higher expression of CD44 present a greater recurrence, number of metastases, and overall unfavorable prognosis (Liu et al. 2011; Shang et al. 2015). The survival rate at 5 years for localized tumors is practically 100%; however, patients on deprivation therapy may present progression 1 year after the drug regimen. In the case of those diagnosed with metastatic disease, the average survival is reduced to just 14 months. This carcinoma tends to generate metastases mainly to the bone, viscera, and lymph nodes, and the most unfavorable prognosis is for those with bone metastases (Gandaglia et al. 2015).

6 Colorectal Cancer

Worldwide, in 2020, 1,880,725 malignant neoplasms arising in the colon or rectum were diagnosed and 930,000 deaths were attributed to them (Morgan et al. 2023). Their development is strongly associated with lifestyle factors, particularly an unhealthy diet, consumption of meat, alcoholic beverages, smoking, and obesity (Chan and Giovannucci 2010). It is estimated that up to 28% of colorectal cancer cases can be attributed to low fiber intake, 13% to meat consumption, and 11% to obesity (Brown et al. 2018). These habits, as well as the metabolic syndrome, are associated with elevated blood levels of leptin, insulin, IGF-1, glucose, and proinflammatory cytokines, as well as with an increased risk of developing colorectal cancer (Frezza et al. 2006). It has been documented that exposure to IGF-1, IL-6, leptin, heterocyclic amines, and saturated fats promotes the proliferation of colon epithelial cells, which is why they are considered carcinogens (Chen et al. 1999;

Becker et al. 2004; Rouet-Benzineb et al. 2004; Chan and Giovannucci 2010). Relatedly, unhealthy eating habits have a direct role in the development of inflammatory bowel diseases (Fleming et al. 2012), which are preceded by many years of carcinoma diagnosis (Lakatos and Lakatos 2008). On average, colorectal cancer is diagnosed at 72 years of age, but the majority are registered at 88 and only 10% of cases are detected before the age of 55 (Kolligs 2016). However, patients with poor eating habits usually develop this type of carcinoma before the age of 50 (Sardo Molmenti et al. 2020), while the finding before the age of 40 is associated with a more aggressive tumor biology (Cusack et al. 1996). More than 50% of patients diagnosed with hyperplasia will develop colon adenocarcinoma within the same year (Hyman et al. 2004; Fleming et al. 2012). The gain of atypia in hyperplasia can be accompanied by markers of cellular senescence, such as positivity for the p16 protein and shortening of the telomeres (Hardikar et al. 2018), as well as lymphocytic infiltration (Hisamatsu et al. 2017). On the other hand, in carcinomas, the activity of the enzyme telomerase is observed (Tang et al. 1998). Up to 90% of colon carcinomas are classified as adenocarcinomas by histological diagnosis (Fleming et al. 2012). Like other cancers originating from the epithelia, these progress by losing their glandular architecture along with the expression of E-cadherin and other characteristics associated with the differentiation state (Dorudi et al. 1993). On the other hand, high histological grade carcinomas overexpress the transcription factors Snail, Twist, and Zeb, contain markers of cancer stem cells, and generally present a higher number of metastases and poor prognosis (Francí et al. 2009; Gomez et al. 2011; Skog et al. 2011; Zhang et al. 2013; Calon et al. 2015). Colon tumor-derived cancer cells induced by EMT adopt a stem phenotype with the expression of CD133 and CD144. These cells are highly tumorigenic, metastatic, and resistant to treatment (Brabletz et al. 2005; Zhou et al. 2016). Interestingly, the genetic programs of cells that emerge from EMT with those isolated as cancer stem cells overlaps (Leng et al. 2018). The prognosis also correlates with tumor progression since around 90% of patients with localized disease survive more than 5 years. In contrast, in metastatic disease, survival reduces to 14% and most lesions occur in the liver, followed by the lungs, bone, and brain. Those that confer a worse prognosis are the latter reducing the survival to only 5 months (Patanaphan and Salazar 1993; Kabbinar et al. 2005).

7 Lung Cancer

Globally, in 2020 there were 2,206,771 new cases of lung cancer and 1,796,144 people died from this cause (Li et al. 2023). Up to 85% of diagnoses are attributed to smoking and for patients who do not smoke, the main risk factors are occupational exposure to some chemical and infectious agents, alcohol consumption, obesity, and unhealthy eating habits (Khan et al. 2010). Studies performed in vitro demonstrated that some chemical agents present in tobacco smoke can induce malignant transformation of epithelial cells, giving them tumorigenic and invasive properties (Hecht et al. 1994; Masubuchi et al. 1998; Yang et al. 2011; Zhao et al. 2012). Another

finding with these cell models was that cigarette smoke stimulates an inflammatory process in lung cells with the production of IL-8 and MCP-1 (Masubuchi et al. 1998; Yang et al. 2011; Zhao et al. 2012). Exposure to tobacco smoke is associated with inflammatory processes in the lung epithelium and the development of chronic obstructive pulmonary disease, emphysema, and fibrosis, which in turn increase the risk of developing lung cancer (Petty 1997; Sevenoaks and Stockley 2006; Smith et al. 2006). Advanced age is another factor associated with the development of lung cancer since 60% of diagnoses occur in patients over 65 years of age and the maximum incidence is reached in octogenarians (Owonikoko et al. 2007). Among smokers, lung adenocarcinomas are detected at a younger age and in advanced stage (Dragani et al. 2023). An interesting fact is that young patients are usually diagnosed with adenocarcinomas, while older patients frequently present with squamous cell carcinomas (Kreuzer et al. 1999). As in other carcinomas, atypical hyperplasias are considered the precursors of lung cancer and coexist with them in 36% of cases (Chapman and Kerr 2000; Kerr 2001). Among the molecular markers present in these lesions are those of cellular senescence such as the p16 protein (Sevenoaks and Stockley 2006) and telomere shortening (Yashima et al. 1997). The early events of their malignant transformation are associated with greater accumulation of senescent cells and with the infiltration of immune cells (Mori et al. 2001; Kawai et al. 2007). Eighty-five percent of lung carcinomas correspond to non-small cell histology (Siegel et al. 2016). However, progression is accompanied by the loss of E-cadherin expression (Kase et al. 2000) and the gain of EMT-related proteins such as Twist and Snail, the adoption of mesenchymal histology, the production of cytokines, and a more unfavorable prognosis (Chae et al. 2018). The induction of EMT in lung cancer cells confers them expression of markers associated with cancer stem cells, such as Oct, Nanog, CD44, and CD133 (Chiou et al. 2010), the ability to initiate tumors, and metastatic activity (Chen et al. 2008; Bertolini et al. 2009). Unlike other carcinomas, histological grade does not usually represent a great difference in survival. For patients with localized disease, it is approximately 1 year while for metastatic patients it is around 5 months. The most common sites for metastases are the brain, liver, and bones, where those who suffer from hepatic lesions tend to have a worse prognosis with a survival of only 3 months (Riihimäki et al. 2014).

8 Pancreatic Cancer

A total of 495,773 new cases and 466,003 deaths from pancreatic cancer were reported worldwide in 2020 (Ilic and Ilic 2022). Among the main risk factors for developing this neoplasia are advanced age, obesity, diabetes, smoking, and occupational exposure to some chemical agents (Lo et al. 2007). Up to 25% of all cases of pancreatic cancer are related to smoking and much of what is known about this causality is attributed to carcinogens present in cigarette smoke (Lochan et al. 2011). This chronic exposure generates a deterioration and persistent renewal in the epithelium of the pancreas that is accompanied by the overexpression of proinflammatory

mediators such as TNF- α , IL-1, and IL-6 (Lochan et al. 2011). This proliferation and constant inflammation contribute to the development of histological alterations, including pancreatitis associated with an increased risk of cancer. It is worth mentioning that this condition is frequently diagnosed among smokers and heavy drinkers (Momi et al. 2012; Yadav and Lowenfels 2013). The average age for the diagnosis of pancreatic cancer is 71 years; however, among smokers, it drops to 56. Up to 80% occur after the age of 50, but most cases are registered at the age of 91. It has been seen that patients under 58 years usually suffer from endocrine tumors, while those over 67 are diagnosed with adenocarcinomas and mucinous tumors (Fesinmeyer et al. 2005; Koloddecik et al. 2014). The diagnosis of pancreatic intraductal neoplasia usually precedes the development of adenocarcinoma by at least 2 years and is considered its hyperplastic precursor lesion (Brat et al. 1998). Frequent findings in these tumors include the expression of the p16 protein, shortening of the telomeric region, infiltration of immune components, and stromal changes associated with inflammation (Brat et al. 1998; Van Heek et al. 2002; Zińczuk et al. 2018). On the other hand, in pancreatic adenomas, the expression of the enzyme telomerase and the decrease in senescent cells are common (Hashimoto et al. 2008). Over 95% of patients are diagnosed with pancreatic cancer corresponding with the histological classification of ductal adenocarcinoma (Brat et al. 1998). Its progression is accompanied by the gradual loss of epithelial differentiation and the expression of E-cadherin (Hong et al. 2011). In contrast, the adoption of high tumor grades is associated with the presence of mesenchymal markers and with an unfavorable prognosis (Lawlor et al. 2019). The induction of transcription factors Snail1 and Zeb1 in pancreatic cancer cells increases the populations of malignant stem cells that express the markers CD24 and CD44 (Li et al. 2007). In murine models of pancreatitis, cells with EMT markers exhibit properties of cancer stem cells (Rhim et al. 2012). In this regard, it is considered that mesenchymal transcription factors are essential for the acquisition of the embryonic phenotype and the formation of metastases (Krebs et al. 2017; Poruk et al. 2017). In similitude to lung cancer, the overall prognosis of pancreatic cancer is always adverse with survival at 5 years lower than 30% even when the disease is localized. Patients who are diagnosed with metastases have an average survival of less than 3 months and when treated with chemotherapy quickly develop resistance (Peixoto et al. 2015). The most common sites of metastases are the peritoneum, liver, lung, and lymph nodes (Allendorf et al. 2008).

9 Bladder Cancer

A total of 573,278 new cases and 212,536 deaths from bladder cancer were reported worldwide in 2020 (Wu et al. 2023). Risk factors associated with this neoplasia include smoking, diabetes, occupational exposure, obesity, and schistosomiasis (Wu et al. 2023). Around 50% of the cases of bladder cancer are attributed to smoking, while in endemic areas of *Schistosoma haematobium*, infections play a relevant role (Tanaka et al. 2011; Saginala et al. 2020). Therefore, a large part of the

mechanisms described for the carcinogenesis of this epithelium come from studies with smokers or parasitosis. In smokers, it is common to detect the presence of toxic substances derived from tobacco and genetic damage in the bladder. In cell cultures the exposure to these substances generates alterations in DNA and inflammation (Letašiová et al. 2012; Jin et al. 2017). Schistosomiasis also produces carcinogenic metabolites that can be detected in the urine of infected patients (Mostafa et al. 1999). Chronic exposure to these nitrosamines and activation of the immune system provides an environment for the malignant transformation of the bladder epithelium (Mostafa et al. 1999). In both situations histological alterations occur in the epithelium and stroma characterized by proliferation, inflammation, and the production of TNF- α , IL-6, and IL-1 α (Rosin et al. 1994; Letašiová et al. 2012). This leads to the development of cystitis, which is a condition that increases the risk of developing bladder carcinoma regardless of the etiological agent that generated it (Michaud 2007). Transitional cell cancer is the most common type of bladder cancer in developed countries while in endemic regions of the parasite is the squamous cell variety. On average, this type of cancer is diagnosed at 62 years of age, and the majority of them at 89 (Aliramaji et al. 2015). However, among patients with active infections, the onset of cancer is early than 40, it generally presents with an advanced clinical stage and responds poorly to therapy (Efared et al. 2022). When it is not associated with infection, patients diagnosed younger than 40 usually have small, low-grade tumors (Telli et al. 2014). The finding of urothelial hyperplasia can precede the development of invasive carcinoma by up to 2 years. Among the abnormalities of this lesion is the presence of atypia and markers of cellular senescence such as the p16 protein, short telomere length, and lymphocytic infiltration (Edgecombe et al. 2012; Sanfrancesco et al. 2013; Williamson et al. 2013). On the other hand, carcinomas in situ reactivate the expression of the enzyme telomerase (Morii et al. 2010). Around 90% of bladder carcinomas correspond to the urothelial type, expressing E-cadherin and other epithelial markers (Edgecombe et al. 2012). However, they tend to dedifferentiate increasing the mesenchymal proteins. The expression of vimentin, Twist, and the presence of a greater number of metastases is a hallmark of high-grade tumors (Bryan and Tselepis 2010; Paliwal et al. 2012). Likewise, EMT markers correlate with the phenotype of cancer stem cells and with metastases (Choi et al. 2014). In the bladder, the proteins CD44 and CD133 have been used as markers for the identification of cancer stem cells (Kuncova et al. 2007; Hedegaard et al. 2016; Li et al. 2017). The prognosis when the disease is localized tends to be favorable with 95% of patients surviving more than 5 years; however, it is reduced to only 7 months in the case of disseminated neoplasms. The tissues most affected by metastases are the bones, brain, liver, and lungs (Ecke et al. 2009; Hedegaard et al. 2016).

10 Liver Cancer

Globally, it is estimated that 905,677 people were diagnosed with liver cancer and 830,200 died from this cause in 2020 (Rumgay et al. 2022). Risk factors for liver cancer include advanced age, infections, excessive alcohol consumption, metabolic

syndrome, diabetes, obesity, and fatty liver disease (Srivatanakul et al. 2004; El-Serag and Rudolph 2007). Approximately, 80% of cases are attributed to viral diseases while the remaining to smoking and alcohol consumption (Rumgay et al. 2022). The most important infectious agents to increase the risk of liver cancer are the hepatitis virus, B and C, and the parasites of the genera *Clonorchis*, *Opisthorchis*, and *Fasciola* (Srivatanakul et al. 2004; El-Serag and Rudolph 2007). These conditions have been related to increased oxidative stress, inflammation, and proliferation in the liver as response to damage. The above augment cytokine production such as IL-6, TNF- α , and inflammatory signaling pathways mediated by NF- κ B responsible for mechanisms implicating in survival, renewal, and immunity (Karin and Dhar 2016). As expected, chronic alcohol consumption generates hyperproliferation in the liver, but also acetaldehyde, its metabolic product is toxic and carcinogenic (Testino 2011). Frequent exposure to such risk factors induces histological changes associated with hepatitis, fatty liver disease, and cirrhosis, which in turn precede the development of liver carcinoma (Villanueva and Luedde 2016). In up to 95% of cases, hepatocarcinoma is diagnosed after 45 years, with an average of 63, but the maximum peak is reached at 83 years. It stands out that, in the case of viral coinfections, the age of diagnosis can be as early as 32 years (de Mattos et al. 2021). In 80% of cases, histological subtype corresponds to hepatocellular carcinoma while the rest to other less common varieties (Petrick et al. 2016). The exposition of liver to chemicals or pathogens increase the proliferation rate leading to the eventual appearance of senescence markers such as telomere shortening (Nishimoto et al. 1998), activation of the INK4a locus, formation of heterochromatin foci, and expression of β -galactosidase (Pacheco-Rivera et al. 2016). These changes frequently occur in high-grade dysplastic nodules, which present an epithelium with nuclear and cell atypia. In 50% of cases, these lesions progress to hepatocellular carcinoma in the same year in which they were diagnosed (Leong and Leong 2005). The malignant transformation of high-grade dysplastic nodules is associated with leukocyte infiltration and cytokine overexpression (Venturi et al. 2007). At this stage of carcinogenesis, the reactivation of telomerase (Nault et al. 2013) and the epigenetic silencing of p16 are common findings (Hui et al. 1996). Most carcinomas that arise in the liver correspond to the hepatocellular variety, which is characterized by having good differentiation (Zhai et al. 2008). Progression to higher histological grades is accompanied by the loss of normal morphology, including the expression of E-cadherin. In contrast, tumors increase the EMT transcription factors, such as Snail, Slug, and Twist (Yang et al. 2009; Yamada et al. 2014) along with the presence of metastasis and display an overall aggressive behavior (Li et al. 2013; Huaman et al. 2018). It has been documented that macrophages from the tumor can induce EMT in liver epithelial cancer cells through the production of IL-6, giving them cancer stem cell properties and the expression of CD44 and CD133. Those cells are highly resistant to therapy and have been implicated in recurrences and worse survival (Wan et al. 2014; Fernando et al. 2015). The 5-year survival rate in patients with localized disease is 30% whereas in those with metastatic disease

decreases to only 3%. Most metastases are found in the bone, lungs, and brain, the latter being those associated with the most adverse prognosis since its survival is only 5 months (Cai et al. 2018).

11 Cervical Cancer

Malignant neoplasia of the cervix is the fourth most common cancer in women globally with around 604,127 new cases and 341,831 deaths in 2020 (Rumgay et al. 2022). Factors associated with the risk of developing it include smoking, human papillomavirus (HPV) infections, herpesviruses, and hormone therapy. HPV is considered the main etiological agent of cervical cancer and more than 70% of cases are attributed to it (Rock et al. 2000). Exposure of the epithelial cells of the cervix to the virus induces their proliferation, the activation of cyclooxygenases, the production of prostaglandins, and cytokines such as IL-1 α and IL-6 (Castrilli et al. 1997). HPV infections are persistent and generate low-grade squamous intraepithelial lesions that can eventually progress to invasive cervical carcinoma. This can also be induced by other viruses, bacteria, protozoans, trauma, or chemicals, which can generate pelvic inflammatory diseases that may also progress to cervical carcinomas (Deivendran et al. 2014). The average age at the time of diagnosis of cervical cancer is usually 50 years, but 80% of cases are detected between 35 and 54. The most frequent histological variety occurring corresponds to squamous cell carcinoma, with 80% and the rest to adenomasquamous carcinomas (Yost and Hoekstra 2018). The precursor lesion of this type of cancer is the cervical intraepithelial neoplasia (Rozario et al. 2019). It is estimated that the process of malignant transformation takes an average of 20 years, but when intraepithelial neoplasia is associated with HPV infection, progression reduces to half (Vink et al. 2013). Intraepithelial neoplasms overexpress the p16 protein (Liu et al. 2017), and present telomere shortening and lymphocytic infiltrations related to the increase in the histological grade (Maskey et al. 2019). In carcinomas, the markers of senescence diminish while telomeric regions increase (Cheah et al. 2002; Meeker et al. 2004b). The epithelial phenotype of cervical tumors gradually loses its characteristics, while gaining mesenchymal markers. Remarkably, the more invasive the cervical cancer sample, the higher the expression of vimentin (Lee et al. 2008). High-grade tumors usually present a greater number of metastases and the expression of mesenchymal proteins such as Snail, Slug, Twist, and those related to cancer stem cells including CD133, Oct4, Sox3, and Nanog (Ye et al. 2008; Lin et al. 2015; Tyagi et al. 2016). The prognosis is favorable when the disease is localized with a 5-year survival rate greater than 90%; however, when it is disseminated, this is reduced to only 11 months despite the administration of treatment. The predominant sites of metastasis in cervical cancer include the bone, lung, liver, and lymph nodes, with hepatic metastases significantly compromising prognosis, often reducing survival to only 6 months (Yin et al. 2019).

12 Insights on the Origin of Carcinoma

The molecular and cellular mechanisms underlying the initiation and progression of the different histological stages of carcinomas are not yet fully understood (Méndez-López 2022). Furthermore, although the increase in epithelium is the pathological manifestation of carcinomas, other cell types also undergo dynamic changes from normal epithelium to hyperplasias and metastatic carcinomas (Ye et al. 2015; Ren et al. 2022). In other words, tumoral masses are not only composed of epithelial cells but are complex microenvironments made up of cells with different phenotypes, genotypes, and epigenetic states (Ren et al. 2022). Some of the most frequent cells in the tumor microenvironment are fibroblasts, leukocytes, endothelial cells, pericytes, and stem cells, which also play an important role in tumorigenesis and the metastatic process (Place et al. 2011). Therefore, it is also debated what cell gives rise to carcinomas and from where they arise in the context of their multistage histological progression (Méndez-López 2022). This has led to speculation of different plausible origins of the cancer cell in the epithelia since there is evidence of the capacity of various cell lineages to become malignant and cause metastatic tumors (Baker 2020; Vinuesa-Pitarch et al. 2021). The above includes a potential origin in the stroma, either by fibroblasts or mesenchymal stem cells (Kato et al. 2010) or in the parenchyma from epithelial stem cells (Yu et al. 2012) or by dedifferentiation of terminally differentiated epithelial cells (Schwitalla et al. 2013; Méndez-López 2022) and even by tissue-infiltrating cells such as pericytes (Baker 2020). Studies analyzing the events involved in epithelial carcinogenesis have long been hampered by the low feasibility of obtaining tissue samples from all stages of progression from the same patient (Ren et al. 2022). However, the use of various histological stages from different patients, as well as the development of murine models that recapitulate them (Behbod et al. 2018) in conjunction with single-cell transcriptomic technologies (Ren et al. 2022) and lineage tracing studies (Vinuesa-Pitarch et al. 2021) have made it possible to analyze the dynamical processes that epithelial tissue undergoes during its malignant transformation and the most plausible origin of the cell that gives rise to carcinomas and from the first stage in which they arise (Ye et al. 2015; Ren et al. 2022). In this sense, as a first approximation, it is considered that the models that best simulate the progression of human carcinomas are those that faithfully reproduce the development of various histological stages (Behbod et al. 2018). That is, in the first instance, benign proliferative lesions are generated that give rise to atypical hyperplasias, from which well-differentiated localized and then invasive carcinomas emerge which in turn progress toward the high-grade and metastatic neoplasias (Behbod et al. 2018). With this type of model, it has been possible to confirm that chemical carcinogens, hormones, oncogenes, and radiation are capable of reproducing the gestation and progression of multiple stages of carcinomas (Behbod et al. 2018). In all cases, hyperplasias are the earliest lesions and retain the epithelial phenotype, dependence on ligands, and an architecture similar to the original tissue (Silverberg 2000). However, they are growths of cells with morphological alterations that already show signs of decline of differentiation, although they remain forming relatively well-organized structures (Silverberg 2000).

An interesting fact about the cells that make up hyperplasias is that they show immortality, chromosomal alterations, and can mimic the histological and molecular characteristics of carcinomas in situ (Behbod et al. 2018). It is worth mentioning that, the most recent studies are consistent in that the epithelial cells of hyperplasias and carcinomas present chromosomal alterations in common, but that they differ greatly from normal epithelial cells (Ren et al. 2022). Independent observations have also pointed out that the majority of genetic alterations important for tumor progression occur from atypical hyperplasias and persist until metastatic disease (Ma et al. 2003). This suggests that hyperplasias already possess cancerous features and is consistent with findings derived from studies of transplantation in mice where it was found that, unlike normal epithelial cells, which suffer senescence after 6 to 7 generations, those derived from hyperplastic lesions are immortal (Daniel et al. 1975). On the other hand, although the transplantation of normal epithelial cells can give rise to hyperplastic lesions, the use of cells derived from hyperplastic lesions increases the probability of generating carcinomas that can metastasize (DeOme et al. 1959). In this sense, it was observed that all incipient carcinomas in vivo show markers of senescence, which is why it has been proposed as a sensitive indicator of neoplastic transformation (Burd et al. 2013). Similarly, according to their hypomethylation pattern, senescent cells had already been considered to be susceptible to cancerous behavior (Cruickshanks et al. 2013). Consistent with previous observations that hyperplasias already present malignant potential, recent research supports that the origin of cancer cells in carcinomas comes from well-differentiated glandular epithelial cells that undergo oncogenic changes which are evident for the first time in this histological stage (Ren et al. 2022). Immunostaining analysis confirmed that these cells appear for the first time in the hyperplastic tissues, and show features of senescence with epithelial morphology that are maintained in low-grade carcinomas but are absent in normal epithelial tissues (Ren et al. 2022). The results of another study are not only consistent with the notion that differentiated epithelial cells are the origin of the first transformed entities with the capacity to initiate invasive and metastatic tumors, but also found that these clones appear for the first time in atypical hyperplastic lesions (Ye et al. 2015). Although EMT was initially related to the acquisition of metastatic abilities and therefore with late events in the progression of carcinomas (Kalluri 2009) this new research reveals that it could be a process used from the earliest stages of epithelial carcinogenesis (Ye et al. 2015). Specifically, they reported that luminal cells in hyperplasia expressing Snail were capable of producing complex heterogeneous tumors when implanted into another mice (Ye et al. 2015). Furthermore, although at the beginning they exhibited epithelial characteristics, when they progressed gave rise to high-grade carcinomas and produced metastasis which in turn resembled glandular structures (Ye et al. 2015). Another interesting result of this research is that the compartment of normal epithelial stem cells remained with few changes during tumor progression (Ye et al. 2015). On the other hand, the cells that originate from hyperplasia expressing Snail protein became cancerous and although they manifest epithelial features, they eventually separate from the tumor mass and gain a fibroblastic morphology and loss E-cadherin expression (Ye et al. 2015). Together these data support that EMT is

related with the generation of cancer stem cells *in vivo* from hyperplasia. Specifically, these cells derive from terminally differentiated epithelial cells rather than from the normal stem compartment. Moreover, they give rise to well-differentiated tumors that subsequently evolve and acquire the characteristics of high-grade metastatic disease (Ye et al. 2015). Remarkably, they found the same results in other models of epithelial carcinogenesis suggesting that it could be a highly conserved characteristic in the generation of neoplasms induced by a wide variety of stimuli (Ye et al. 2015).

Among the most evident histological changes associated with the transition to the invasive stage are the disappearance of the basement membrane and a more aberrant organization of the tissue with the invasion of cancer cells into the surrounding stroma (Behbod et al. 2018). The above has been recurrently associated with the upregulation of EMT genes (Behbod et al. 2018). Invading tumor cells migrate to an abnormal environment that has been profoundly remodeled by the developing carcinoma (Hinck and Näthke 2014). In carcinomas *in situ*, these extracellular changes increase and include infiltration of leukocytes, fibroblasts, and endothelial cells (Ren et al. 2022). These accumulate in the stroma surrounding the epithelial mass and promote it by remodeling the extracellular matrix and with the supply of cytokines, growth factors, and signals for the formation of blood vessels (Hinck and Näthke 2014). Again many of these events are related to the mesenchymal lineage, since, on the one hand, it is known that E-cadherin levels decrease in invasive tumors while vimentin, extracellular matrix metalloproteases, and the expression of EMT transcription factors increase (McConkey et al. 2010). On the other hand, tumor-associated fibroblasts promote the proliferation of cancer cells and activate the expression of embryonic genes (Farhood et al. 2019). Other proteins induced are metalloproteases, which contribute to the capacity of cancer cells at the margin tumor to remodel its environment and facilitate its spread (McConkey et al. 2010).

The new findings are also consistent in that the proportion of cells with epithelial markers increases in hyperplasias and expands further in well-differentiated carcinomas. The study also demonstrates a reduction in the proportion of stromal cells as the progression advances from normal epithelium to hyperplasias, further diminishing in low-grade carcinomas (Ren et al. 2022). In the case of lymphocytes and macrophages, these increase significantly in hyperplasias in comparison to that of normal epithelium but do not appear to be different from what is observed in early carcinomas (Ren et al. 2022). Unlike what happens in the early events of epithelial carcinogenesis, during its progression, practically all carcinomas gradually lose the epithelial phenotype, and some can become mesenchymal tumors similar to sarcomas. This phenomenon is called sarcomatoid transformation and was described more than a century ago (McConkey et al. 2010). Interestingly, the undifferentiated component with sarcoma features corresponds to spindle cells that have greater instability that is manifested with their susceptibility to differentiation toward osteoblastic, and chondroblastic lineages (McConkey et al. 2010). Sarcomatoid carcinomas exhibit complex morphologies with epithelial and mesenchymal elements and are usually lethal as they are resistant to currently known treatment protocols (McConkey et al. 2010). These tumors present a greater number of

chromosomal abnormalities and overexpress EMT and embryonic markers. Therefore, the high histological grades of carcinomas, their poorly differentiated and sarcomatoid varieties, are largely attributed to the EMT process (McConkey et al. 2010), as it confers mesenchymal, stem features and increases their potential to generate metastasis, resistance, and relapses (Wilson et al. 2020). Recent studies indicate that parenchymal epithelial cells undergo EMT in vivo and represent most fibroblastic cells in desmoplasia associated with carcinomas. It has been observed that the majority of cancer-associated fibroblasts arise from the EMT of epithelial cells and some acquire the gene expression profile of cancer stem cells (Bartoschek et al. 2018). Cancer cells that exhibit hybrid characteristics between the epithelial and mesenchymal stem cells are usually more aggressive than those with more compromised differentiation (Lu and Kang 2019). Interestingly, normal epithelial stem cells also show intermediate expression levels of epithelial and mesenchymal markers (Ye et al. 2015). Furthermore, tumor cells do not necessarily undergo complete EMT to disperse (Hinck and Näthke 2014). Instead, pathologists typically observe clusters of epithelial cells moving through tissue and the bloodstream as a group of physically and functionally connected cells in a process called collective cell migration (Hinck and Näthke 2014). These groups exhibit qualities of EMT, including alterations in polarity and invasiveness, and although they remain in aggregates they are not completely epithelial (Hinck and Näthke 2014). Similarities between collective migration and EMT suggest that the states of epithelial and mesenchymal cells may not always be uniquely represented (Hinck and Näthke 2014). Instead, cells can express a variety of epithelial and mesenchymal characteristics (Lu and Kang 2019). This has led to the suggestion that partial EMT within a tumor tissue could provide cells with the flexibility necessary to respond to different environments and for their dissociation (Hinck and Näthke 2014). Another recurrent observation is that at the invasive margins of the tumor, a more complete EMT event seems to occur (Hinck and Näthke 2014). Finally, it is important to mention that neither cytotoxic agents nor radiotherapy provide a significant advantage in the survival of patients with high-grade and disseminated disease regardless of the epithelium of origin (Carr et al. 1993; Ecke et al. 2009; Crissien and Frenette 2014; Peixoto et al. 2015; Hedegaard et al. 2016; Yin et al. 2019). The new findings on the appearance of markers of senescence or EMT from terminally differentiated cells in hyperplasias with the capacity to generate complex carcinomas suggest a fundamental role of both processes of dedifferentiation within the histological changes that occur during epithelial carcinogenesis. As we will see later, the above suggests a paradigmatic change in the prevention, detection, and treatment of carcinomas.

13 Conclusion

Epithelia is constantly renewed and therefore it has been assumed that carcinomas originate predominantly from cells that undergo more cell divisions since proliferation occurs in accordance with their functional demand (DePinho 2000). Given the

above, it is understandable that the tumorigenesis of epithelial tissues increases as we age and is associated with inflammatory processes and lifestyle risk factors (Méndez-López 2022). Furthermore, this is also consistent with the observation that senescent cells progressively accumulate with age (Krishnamurthy et al. 2004) and correlate with a diminution in the replicative capacity of epithelial tissues (Krishnamurthy et al. 2006). Histologically, this exhaustion manifests with the appearance of hyperplasias and the positive expression of the biomarkers of cellular senescence (Kawai et al. 2007). A very interesting finding is that the progression of these precursor lesions is frequently accompanied by the infiltration of cells of the immune system (Perletti et al. 2010). This causal relationship between carcinogenesis and inflammation is only comparable to the one existing with the aging process (Ahmad et al. 2009). Furthermore, the link was reinforced when the secretory phenotype of senescent cells was discovered (Rodier et al. 2009) since many proinflammatory cytokines secreted by aged cells are inducers of EMT (Xie et al. 2012) that in turn suppress cellular senescence and confers most cancer hallmarks (Ansieau et al. 2008). Taken together, the findings reviewed suggest a close mechanistic relationship between cellular senescence, inflammation, and EMT in the emergence of cancer cells in epithelia (Méndez-López 2022). A growing number of investigations link both events not only with the gain of cellular plasticity, stemness, and aggressive behavior in carcinomas (Wilson et al. 2020; Benítez et al. 2021) but also with the molecular responses associated with tumor resistance in patients who do not respond to pharmacological or radiological treatment (Gerashchenko et al. 2016; Wei et al. 2020). Despite cellular senescence and EMT are processes that seem to be essential in each of the histological stages of epithelial carcinogenesis *in vivo*, their temporal sequence, interrelationships, involved lineages, and true contribution to sporadic carcinoma generation in humans remain incompletely deciphered (Méndez-López 2022). Furthermore, although aging eventually leads to the development of benign epithelial lesions (Krishnamurthy et al. 2004), it is well known that up to 70% of them will not progress to carcinomas (Hartmann et al. 2014). Therefore, one of the most important questions that remains to be solved is the factor that determines which hyperplasias will progress. The potential role of inflammatory status and stromal architecture in this discrepancy was discussed in another contribution since in this chapter we predominantly focused on changes in the epithelial and mesenchymal compartments. However, it is well established that many of the processes underlying the histological progression of carcinomas are influenced at all times by the tumor microenvironment (Méndez-López 2022). Modern evidence continues to indicate that the genomic instability conferred by cellular senescence seems to play a fundamental role in the susceptibility of epithelia for the generation of neoplasias (Méndez-López 2022). However, inflammation could be the key to the progression of premalignant lesions through the induction of EMT both in incipient carcinomas and in high-grade carcinomas that do not respond to current treatment (Méndez-López 2022). Taken together, reviewed discoveries support that cellular senescence and EMT contribute significantly to the dedifferentiation and malignant transformation of epithelia, giving rise to the characteristic pattern of multistage histological changes, but also to the mechanisms involved in tumor resistance and

relapse. Research in our laboratory and others continues to explore whether cellular aging and EMT are sufficient and necessary to explain most molecular, cellular, and histological phenomena involved in the gestation and progression of carcinomas. One of the most important implications of this research would be the need to develop new strategies against cancer since conventional therapeutic management is one of the most important inducers of both processes, not only in tumor but also in healthy tissues. Hopefully, the study of the generic events that determine the progression of early lesions toward invasive and metastatic carcinomas, as well as their resistance to therapy, lead to a more effective screening methods and broad-spectrum anticancer drugs with a lesser toxicity against neoplasms that arise from epithelial tissues.

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References

- Agherbi H, Gaussmann-Wenger A, Verthuy C, Chasson L, Serrano M, Djabali M (2009) Polycomb mediated epigenetic silencing and replication timing at the INK4a/ARF locus during senescence. *PLoS One* 4(5):e5622
- Ahmad A, Banerjee S, Wang Z, Kong D, Majumdar AP, Sarkar FH (2009) Aging and inflammation: etiological culprits of cancer. *Curr Aging Sci* 2(3):174–186
- Aliramaji A, Kaseean A, Pasha YRY, Shafi H, Kamali S, Safari M, Moudi EJ (2015) Age distribution types of bladder cancers and their relationship with opium consumption and smoking. *Caspian J Intern Med* 6(2):82
- Allendorf JD, Lauerma M, Bill A, DiGiorgi M, Goetz N, Vakiani E, Remotti H, Schrope B, Sherman W, Hall M et al (2008) Neoadjuvant chemotherapy and radiation for patients with locally unresectable pancreatic adenocarcinoma: feasibility, efficacy, and survival. *J Gastrointest Surg* 12(1):91–100
- Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (2008) Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 25(9):2097–2116
- Anderson WF, Pfeiffer RM, Dores GM, Sherman ME (2006) Comparison of age distribution patterns for different histopathologic types of breast carcinoma. *Cancer Epidemiol Biomarkers Prev* 15(10):1899–1905
- Ansieau S, Bastid J, Doreau A, Morel A-P, Bouchet BP, Thomas C, Fauvet F, Puisieux I, Doglioni C, Piccinin S (2008) Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. *Cancer Cell* 14(1):79–89
- Arnold J, Isaacs J (2002) Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault. *Endocr Relat Cancer* 9(1):61–73
- Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S (2022) Current and future burden of breast cancer: global statistics for 2020 and 2040. *Breast* 66:15–23
- Avery A, Alden J, Kramish C, Caballero C, Wright-Void C, Bruner ET (2022) The pathologic diagnosis of carcinoma in various tissues. *Adv Cancer Res* 154:1–14

- Azrad M, Blair CK, Rock CL, Sedjo RL, Wolin KY, Demark-Wahnefried W (2019) Adult weight gain accelerates the onset of breast cancer. *Breast Cancer Res Treat* 176:649–656
- Baker SG (2020) Rethinking carcinogenesis: the detached pericyte hypothesis. *Med Hypotheses* 144:110056
- Barnard RJ (2007) Prostate cancer prevention by nutritional means to alleviate metabolic syndrome. *Am J Clin Nutr* 86(3):889S–893S
- Bartosch C, Monteiro-Reis S, Vieira R, Pereira A, Rodrigues M, Jerónimo C, Lopes JM (2015) Endometrial endometrioid carcinoma metastases show decreased ER- α and PR-A expression compared to matched primary tumors. *PLoS One* 10(8):e0134969
- Bartoschek M, Oskolkov N, Bocci M, Lötvot J, Larsson C, Sommarin M, Madsen CD, Lindgren D, Pekar G, Karlsson G et al (2018) Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. *Nat Commun* 9(1):5150
- Beavis AL, Smith AJB, Fader AN (2016) Lifestyle changes and the risk of developing endometrial and ovarian cancers: opportunities for prevention and management. *Int J Women's Health* 8:151
- Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A, Burg J, Strand S, Kiesslich R, Huber S (2004) TGF- β 3 suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity* 21(4):491–501
- Behbod F, Gomes AM, Machado HL (2018) Modeling human ductal carcinoma in situ in the mouse. *J Mammary Gland Biol Neoplasia* 23:269–278
- Behrens J, Lowrick O, Klein-Hitpass L, Birchmeier W (1991) The E-cadherin promoter: functional analysis of a G.C-rich region and an epithelial cell-specific palindromic regulatory element. *Proc Natl Acad Sci USA* 88(24):11495–11499
- Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, Weinberg RA (2008) An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet* 40(5):499–507
- Bendrik C, Dabrosin C (2009) Estradiol increases IL-8 secretion of normal human breast tissue and breast cancer in vivo. *J Immunol* 182(1):371–378
- Benítez S, Cordero A, Santamaría PG, Redondo-Pedraza J, Rocha AS, Collado-Solé A, Jimenez M, Sanz-Moreno A, Yoldi G, Santos JC (2021) RANK links senescence to stemness in the mammary epithelia, delaying tumor onset but increasing tumor aggressiveness. *Dev Cell* 56(12):1727–1741.e7
- Benz C (2008) Impact of aging on the biology of breast cancer. *Crit Rev Oncol Hematol* 66(1):65–74
- Bertolini G, Roz L, Perego P, Tortoreto M, Fontanella E, Gatti L, Pratesi G, Fabbri A, Andriani F, Tinelli S et al (2009) Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. *Proc Natl Acad Sci USA* 106(38):16281–16286
- Bostwick DG (1996) Prospective origins of prostate carcinoma: prostatic intraepithelial neoplasia and atypical adenomatous hyperplasia. *Cancer* 78(2):330–336
- Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T (2005) Migrating cancer stem cells—an integrated concept of malignant tumour progression. *Nat Rev Cancer* 5(9):744–749
- Brat DJ, Lillemoe KD, Yeo CJ, Warfield PB, Hruban RH (1998) Progression of pancreatic intraductal neoplasias to infiltrating adenocarcinoma of the pancreas. *Am J Surg Pathol* 22(2):163–169
- Brown KF, Rumgay H, Dunlop C, Ryan M, Quartly F, Cox A, Deas A, Elliss-Brookes L, Gavin A, Hounsborne L (2018) The fraction of cancer attributable to modifiable risk factors in England, Wales, Scotland, Northern Ireland, and the United Kingdom in 2015. *Br J Cancer* 118(8):1130–1141
- Bryan RT, Tselepis C (2010) Cadherin switching and bladder cancer. *J Urol* 184(2):423–431
- Budczies J, von Winterfeld M, Klauschen F, Bockmayr M, Lennerz JK, Denkert C, Wolf T, Warth A, Dietel M, Anagnostopoulos I (2015) The landscape of metastatic progression patterns across major human cancers. *Oncotarget* 6(1):570

- Burd CE, Sorrentino JA, Clark KS, Darr DB, Krishnamurthy J, Deal AM, Bardeesy N, Castrillon DH, Beach DH, Sharpless NE (2013) Monitoring tumorigenesis and senescence in vivo with a p16INK4a-luciferase model. *Cell* 152(1–2):340–351
- Byrne S, Boyle T, Ahmed M, Lee SH, Benyamin B, Hyppönen E (2023) Lifestyle, genetic risk and incidence of cancer: a prospective cohort study of 13 cancer types. *Int J Epidemiol* 52(3): 817–826
- Cai H, Wang H, Li Z, Lin J, Yu J (2018) The prognostic analysis of different metastatic patterns in extensive-stage small-cell lung cancer patients: a large population-based study. *Future Oncol* 14(14):1397–1407
- Calle EE, Kaaks R (2004) Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 4(8):579–591
- Calon A, Lonardo E, Berenguer-Llergo A, Espinet E, Hernando-Momblona X, Iglesias M, Sevillano M, Palomo-Ponce S, Tauriello DV, Byrom DJ et al (2015) Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. *Nat Genet* 47(4):320
- Carr BI, Iwatsuki S, Starzl TE, Selby R, Madariaga J (1993) Regional cancer chemotherapy for advanced stage hepatocellular carcinoma. *J Surg Oncol Supp* 3:100
- Castrilli G, Tatone D, Diodoro M, Rosini S, Piantelli M, Musiani P (1997) Interleukin 1alpha and interleukin 6 promote the in vitro growth of both normal and neoplastic human cervical epithelial cells. *Br J Cancer* 75(6):855
- Castro P, Giri D, Lamb D, Ittmann M (2003) Cellular senescence in the pathogenesis of benign prostatic hyperplasia. *Prostate* 55(1):30–38
- Chae YK, Chang S, Ko T, Anker J, Agte S, Iams W, Choi WM, Lee K, Cruz M (2018) Epithelial-mesenchymal transition (EMT) signature is inversely associated with T-cell infiltration in non-small cell lung cancer (NSCLC). *Sci Rep* 8(1):1–8
- Chakrabarti R, Hwang J, Blanco MA, Wei Y, Lukačičin M, Romano R-A, Smalley K, Liu S, Yang Q, Ibrahim T (2012) Elf5 inhibits the epithelial–mesenchymal transition in mammary gland development and breast cancer metastasis by transcriptionally repressing Snail2. *Nat Cell Biol* 14(11):1212–1222
- Chan AT, Giovannucci EL (2010) Primary prevention of colorectal cancer. *Gastroenterology* 138(6):2029–2043.e10
- Chapman A, Kerr K (2000) The association between atypical adenomatous hyperplasia and primary lung cancer. *Br J Cancer* 83(5):632
- Cheah P, Looi L, Ng MH, Sivanesaratnam V (2002) Telomerase activation and human papillomavirus infection in invasive uterine cervical carcinoma in a set of Malaysian patients. *J Clin Pathol* 55(1):22–26
- Chen K, Nezu R, Wasa M, Sando K, Kamata S, Takagi Y, Okada A (1999) Insulin-like growth factor-1 modulation of intestinal epithelial cell restitution. *J Parenter Enter Nutr* 23(5 suppl): S89–S92
- Chen Y-C, Hsu H-S, Chen Y-W, Tsai T-H, How C-K, Wang C-Y, Hung S-C, Chang Y-L, Tsai M-L, Lee Y-Y et al (2008) Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. *PLoS One* 3(7):e2637
- Chen Y, Zhang L, Liu W, Liu X (2015) Prognostic significance of the tumor-stroma ratio in epithelial ovarian cancer. *Biomed Res Int* 2015:589301
- Chiou S-H, Wang M-L, Chou Y-T, Chen C-J, Hong C-F, Hsieh W-J, Chang H-T, Chen Y-S, Lin T-W, Hsu H-S, Wu CW (2010) Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial–mesenchymal transdifferentiation. *Cancer Res* 70(24):10433–10444
- Choi W, Porten S, Kim S, Willis D, Plimack ER, Hoffman-Censits J, Roth B, Cheng T, Tran M, Lee I-L et al (2014) Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. *Cancer Cell* 25(2):152–165
- Clark W (1991) Tumour progression and the nature of cancer. *Br J Cancer* 64(4):631–644
- Crawford ED (2003) Epidemiology of prostate cancer. *Urology* 62(6):3–12

- Crissien AM, Frenette C (2014) Current management of hepatocellular carcinoma. *Gastroenterol Hepatol (N Y)* 10(3):153
- Cruikshanks HA, McBryan T, Nelson DM, VanderKraats ND, Shah PP, van Tuyn J, Rai TS, Brock C, Donahue G, Dunican DS (2013) Senescent cells harbour features of the cancer epigenome. *Nat Cell Biol* 15:1495–1506
- Cserni G (2002) Tumour histological grade may progress between primary and recurrent invasive mammary carcinoma. *J Clin Pathol* 55(4):293–297
- Cusack JC, Giacco GG, Cleary K, Davidson BS, Izzo F, Skibber J, Yen J, Curley SA (1996) Survival factors in 186 patients younger than 40 years old with colorectal adenocarcinoma. *J Am Coll Surg* 183(2):105–112
- Danescu A, Herrero Gonzalez S, Di Cristofano A, Mai S, Hombach-Klonisch SJG (2013) Three-dimensional nuclear telomere architecture changes during endometrial carcinoma development. *Genes Chromosomes Cancer* 52(8):716–732
- Daniel C, Aidells B, Medina D, Faulkin L Jr (1975) Unlimited division potential of precancerous mouse mammary cells after spontaneous or carcinogen-induced transformation. In: *Biology of aging and development*. FASEB, Bethesda, pp 123–130
- de Mattos ÂZ, Debes JD, Boonstra A, Yang J-D, Balderramo DC, Sartori GD, de Mattos AA (2021) Current impact of viral hepatitis on liver cancer development: the challenge remains. *World J Gastroenterol* 27(24):3556
- Deivendran S, Marzook KH, Pillai MR (2014) The role of inflammation in cervical cancer. In: *Inflammation and cancer*. Springer, Basel, pp 377–399
- DeNardo DG, Coussens LM (2007) Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. *Breast Cancer Res* 9(4):1
- DeOme K, Faulkin L Jr, Bern HA, Blair PB (1959) Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. *Cancer Res* 19(5):515
- DePinho RA (2000) The age of cancer. *Nature* 408(6809):248–254
- Djuric Z, Edwards A, Madan S, Darga L, Ren J, Blake C, Koletsky M, Heilbrun LK (2009) Obesity is associated with atypia in breast ductal lavage of women with proliferative breast disease. *Cancer Epidemiol* 33(3–4):242–248
- Dongre A, Rashidian M, Reinhardt F, Bagnato A, Keckesova Z, Ploegh HL, Weinberg RA (2017) Epithelial-to-mesenchymal transition contributes to immunosuppression in breast carcinomas. *Cancer Res* 77(15):3982–3989
- Donnelly P, Baker K, Carney J, O’Fallon W (1975) Benign breast lesions and subsequent breast carcinoma in Rochester, Minnesota. *Mayo Clinic proceedings*
- Donnison M, Beaton A, Davey HW, Broadhurst R, L’Huillier P, Pfeffer PL (2005) Loss of the extraembryonic ectoderm in *Elf5* mutants leads to defects in embryonic patterning. *Development* 132(10):2299–2308
- Dorudi S, Sheffield J, Poulson R, Northover J, Hart I (1993) E-cadherin expression in colorectal cancer. An immunocytochemical and in situ hybridization study. *Am J Pathol* 142(4):981
- Dragani TA, Muley T, Schneider MA, Kobinger S, Eichhorn M, Winter H, Hoffmann H, Kriegsmann M, Noci S, Incarbone M (2023) Lung adenocarcinoma diagnosed at a younger age is associated with advanced stage, female sex, and ever-smoker status, in patients treated with lung resection. *Cancers* 15(8):2395
- Dupont WD, Parl FF, Hartmann WH, Brinton LA, Winfield AC, Worrell JA, Schuyler PA, Plummer WD (1993) Breast cancer risk associated with proliferative breast disease and atypical hyperplasia. *Cancer* 71(4):1258–1265
- Ecke T, Gerullis H, Bartel P, Koch S (2009) Palliative chemotherapy with gemcitabine, paclitaxel, and cisplatin as first-line treatment following gemcitabine monotherapy for patients with transitional cell carcinoma of the urothelium. *Minerva Urol Nefrol* 61(1):1–8

- Edgecombe A, Nguyen BN, Djordjevic B, Belanger EC, Mai KT (2012) Utility of cytokeratin 5/6, cytokeratin 20, and p16 in the diagnosis of reactive urothelial atypia and noninvasive component of urothelial neoplasia. *Appl Immunohistochem Mol Morphol* 20(3):264–271
- Efared B, Bako ABA, Idriss B, Alhousseini D, Boureima HS, Sodé HC, Nouhou H (2022) Urinary bladder *Schistosoma haematobium*-related squamous cell carcinoma: a report of two fatal cases and literature review. *Trop Dis Travel Med Vaccines* 8(1):3
- El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 132(7):2557–2576
- Farhood B, Najafi M, Mortezaee K (2019) Cancer-associated fibroblasts: secretions, interactions, and therapy. *J Cell Biochem* 120(3):2791–2800
- Fernando J, Malfettone A, Cepeda EB, Vilarrasa-Blasi R, Bertran E, Raimondi G, Fabra À, Alvarez-Barrientos A, Fernández-Salguero P, Fernández-Rodríguez CM et al (2015) A mesenchymal-like phenotype and expression of CD44 predict lack of apoptotic response to sorafenib in liver tumor cells. *Int J Cancer* 136(4):E161–E172
- Fesinmeyer MD, Austin MA, Li CI, De Roos AJ, Bowen DJ (2005) Differences in survival by histologic type of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 14(7):1766–1773
- Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, Chen H, Omeroglu G, Meterissian S, Omeroglu A (2008) Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med* 14(5):518–527
- Fleming M, Ravula S, Tatishchev SF, Wang HL (2012) Colorectal carcinoma: pathologic aspects. *J Gastrointest Oncol* 3(3):153–173
- Francí C, Gallen M, Alameda F, Baro T, Iglesias M, Virtanen I, de Herreros AG (2009) Snail1 protein in the stroma as a new putative prognosis marker for colon tumours. *PLoS One* 4(5):e5595
- Frezza EE, Wachtel MS, Chiriva-Internati M (2006) Influence of obesity on the risk of developing colon cancer. *Gut* 55(2):285–291
- Friedenreich C, Bryant H, Alexander F, Hugh J, Danyluk J, Page D (2000) Risk factors for benign proliferative breast disease. *Int J Epidemiol* 29(4):637–644
- Gandaglia G, Karakiewicz PI, Briganti A, Passoni NM, Schiffmann J, Trudeau V, Graefen M, Montorsi F, Sun M (2015) Impact of the site of metastases on survival in patients with metastatic prostate cancer. *Eur Urol* 68(2):325–334
- Gann PH (2002) Risk factors for prostate cancer. *Rev Urol* 4(Suppl 5):S3
- Gerashchenko B, Salmira K, Eglitis J, Huna A, Grjunberga V, Erenpreisa J (2016) Disentangling the aneuploidy and senescence paradoxes: a study of triploid breast cancers non-responsive to neoadjuvant therapy. *Histochem Cell Biol* 145(4):497–508
- Giordano G, Ferioli E, Guareschi D, Tafuni A (2023) Dedifferentiated endometrial carcinoma: a rare aggressive neoplasm-clinical, morphological and immunohistochemical features. *Cancers* 15(21):5155
- Giri D, Ozen M, Ittmann M (2001) Interleukin-6 is an autocrine growth factor in human prostate cancer. *Am J Pathol* 159(6):2159–2165
- Gomez I, Peña C, Herrera M, Muñoz C, Larriba MJ, Garcia V, Dominguez G, Silva J, Rodriguez R, de Herreros AG et al (2011) TWIST1 is expressed in colorectal carcinomas and predicts patient survival. *PLoS One* 6(3):e18023
- Gravdal K, Halvorsen OJ, Haukaas SA, Akslen LA (2007) A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clin Cancer Res* 13(23):7003–7011
- Greenman C, Wooster R, Futreal PA, Stratton MR, Easton DF (2006) Statistical analysis of pathogenicity of somatic mutations in cancer. *Genetics* 173(4):2187–2198
- Harbitz TB, Haugen OA (1972) Histology of the prostate in elderly men: a study in an autopsy series. *Acta Pathol Microbiol Scand A* 80(6):756–768
- Hardikar S, Burnett-Hartman AN, Phipps AI, Upton MP, Zhu L-C, Newcomb PA (2018) Telomere length differences between colorectal polyp subtypes: a colonoscopy-based case-control study. *BMC Cancer* 18(1):513

- Hartmann LC, Radisky DC, Frost MH, Santen RJ, Vierkant RA, Benetti LL, Tarabishy Y, Ghosh K, Visscher DW, Degnim AC (2014) Understanding the premalignant potential of atypical hyperplasia through its natural history: a longitudinal cohort study. *Cancer Prev Res* 7(2):211–217
- Harvey H (1995) A unifying hypothesis that links benign prostatic hyperplasia and prostatic intraepithelial neoplasia with prostate cancer: invited comments. *Pathol Res Pract* 191(9): 924–934
- Hashimoto Y, Murakami Y, Uemura K, Hayashidani Y, Sudo T, Ohge H, Fukuda E, Shimamoto F, Sueda T, Hiyama E (2008) Telomere shortening and telomerase expression during multistage carcinogenesis of intraductal papillary mucinous neoplasms of the pancreas. *J Gastrointest Surg* 12(1):17–28; discussion 28–19
- Hecht SS, Isaacs S, Trushin N (1994) Lung tumor induction in A/J mice by the tobacco smoke carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and benzo [a] pyrene: a potentially useful model for evaluation of chemopreventive agents. *Carcinogenesis* 15(12):2721–2725
- Hedegaard J, Lamy P, Nordentoft I, Algaba F, Høyer S, Ulhøi BP, Vang S, Reinert T, Hermann GG, Mogensen K et al (2016) Comprehensive transcriptional analysis of early-stage urothelial carcinoma. *Cancer Cell* 30(1):27–42
- Heim D, Budczies J, Stenzinger A, Treue D, Hufnagl P, Denkert C, Dietel M, Klauschen F (2014) Cancer beyond organ and tissue specificity: next-generation-sequencing gene mutation data reveal complex genetic similarities across major cancers. *Int J Cancer* 135(10):2362–2369
- Henshall SM, Quinn DI, Lee CS, Head DR, Golovsky D, Brenner PC, Delprado W, Stricker PD, Grygiel JJ, Sutherland RL (2001) Overexpression of the cell cycle inhibitor p16INK4A in high-grade prostatic intraepithelial neoplasia predicts early relapse in prostate cancer patients. *Clin Cancer Res* 7(3):544–550
- Hinck L, Näthke I (2014) Changes in cell and tissue organization in cancer of the breast and colon. *Curr Opin Cell Biol* 26:87–95
- Hisamatsu K, Noguchi K, Tomita H, Muto A, Yamada N, Kobayashi K, Hirata A, Kanayama T, Niwa A, Ishida K et al (2017) Distinctive crypt shape in a sessile serrated adenoma/polyp: distribution of Ki67-, p16INK4a-, WNT5A-positive cells and intraepithelial lymphocytes. *Oncol Rep* 38(2):775–784
- Hollenhorst PC, Jones DA, Graves BJ (2004) Expression profiles frame the promoter specificity dilemma of the ETS family of transcription factors. *Nucleic Acids Res* 32(18):5693–5702
- Hong S-M, Li A, Olin K, Wolfgang CL, Herman JM, Schulick RD, Iacobuzio-Donahue C, Hruban RH, Goggins M (2011) Loss of E-cadherin expression and outcome among patients with resectable pancreatic adenocarcinomas. *Mod Pathol* 24(9):1237–1247
- Howell A, Anderson AS, Clarke RB, Duffy SW, Evans DG, Garcia-Closas M, Gescher AJ, Key TJ, Saxton JM, Harvie MN (2014) Risk determination and prevention of breast cancer. *Breast Cancer Res* 16(5):1
- Hu H-L, Bai H-S, Pan H-X (2015) Correlation between TAMs and proliferation and invasion of type I endometrial carcinoma. *Asian Pac J Trop Med* 8(8):643–650
- Huaman J, Bach C, Ilboudo A, Ogunwobi OO (2018) Epithelial-to-mesenchymal transition in hepatocellular carcinoma. In: *Precision molecular pathology of liver cancer*. Springer, Cham, pp 131–152
- Huang J, Chan WC, Ngai CH, Lok V, Zhang L, Lucero-Prisno DE III, Xu W, Zheng ZJ, Elcarte E, Withers M, Wong MCS (2023) Global incidence and mortality trends of corpus uteri cancer and associations with gross domestic product, human development index, lifestyle, and metabolic risk factors. *Int J Gynecol Obstet* 162(3):998–1009
- Hui A, Sakamoto M, Kanai Y, Ino Y, Gotoh M, Yokota J, Hirohashi S (1996) Inactivation of p16INK4 in hepatocellular carcinoma. *Hepatology* 24(3):575–579
- Hyman NH, Anderson P, Blasyk H (2004) Hyperplastic polyposis and the risk of colorectal cancer. *Dis Colon Rectum* 47(12):2101–2104
- Ilic I, Ilic M (2022) International patterns in incidence and mortality trends of pancreatic cancer in the last three decades: a joinpoint regression analysis. *World J Gastroenterol* 28(32):4698

- Jarboe EA, Mutter GL (2010) Endometrial intraepithelial neoplasia. *Semin Diagn Pathol* 27(4): 215–225
- Jin F, Thaiparambil J, Donepudi SR, Vantaku V, Piyaathna DWB, Maity S, Krishnapuram R, Putluri V, Gu F, Purwaha P et al (2017) Tobacco-specific carcinogens induce hypermethylation, DNA adducts, and DNA damage in bladder cancer. *Cancer Prev Res (Phila)* 10(10):588–597
- Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E, Sarkar S (2005) Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. *J Clin Oncol* 23(16):3706–3712
- Kalluri R (2009) EMT: when epithelial cells decide to become mesenchymal-like cells. *J Clin Invest* 119(6):1417–1419
- Karin M, Dhar D (2016) Liver carcinogenesis: from naughty chemicals to soothing fat and the surprising role of NRF2. *Carcinogenesis* 37(6):541–546
- Kase S, Sugio K, Yamazaki K, Okamoto T, Yano T, Sugimachi K (2000) Expression of E-cadherin and β -catenin in human non-small cell lung cancer and the clinical significance. *Clin Cancer Res* 6(12):4789–4796
- Kassam F, Enright K, Dent R, Dranitsaris G, Myers J, Flynn C, Fralick M, Kumar R, Clemons M (2009) Survival outcomes for patients with metastatic triple-negative breast cancer: implications for clinical practice and trial design. *Clin Breast Cancer* 9(1):29–33
- Kato K, Takao T, Kuboyama A, Tanaka Y, Ohgami T, Yamaguchi S, Adachi S, Yoneda T, Ueoka Y, Kato K (2010) Endometrial cancer side-population cells show prominent migration and have a potential to differentiate into the mesenchymal cell lineage. *Am J Pathol* 176(1): 381–392
- Kawai T, Hiroi S, Nakanishi K, Meeker AK (2007) Telomere length and telomerase expression in atypical adenomatous hyperplasia and small bronchioloalveolar carcinoma of the lung. *Am J Clin Pathol* 127(2):254–262
- Kerr K (2001) Pulmonary preinvasive neoplasia. *J Clin Pathol* 54(4):257–271
- Khan N, Afaq F, Mukhtar H (2010) Lifestyle as risk factor for cancer: evidence from human studies. *Cancer Lett* 293(2):133–143
- Koch CM, Suschek CV, Lin Q, Bork S, Goergens M, Joussen S, Pallua N, Ho AD, Zenke M, Wagner W (2011) Specific age-associated DNA methylation changes in human dermal fibroblasts. *PLoS One* 6(2):e16679
- Koenenman KS, Pan C-X, Jin J-K, Pyle JM, Flanigan RC, Shankey TV, Diaz MO (1998) Telomerase activity, telomere length, and DNA ploidy in prostatic intraepithelial neoplasia (PIN). *J Urol* 160(4):1533–1539
- Kolijn K, Verhoef EI, Smid M, Böttcher R, Jenster GW, Debets R, van Leenders GJ (2018) Epithelial–mesenchymal transition in human prostate cancer demonstrates enhanced immune evasion marked by IDO1 expression. *Cancer Res* 78(16):4671–4679
- Kolligs FT (2016) Diagnostics and epidemiology of colorectal cancer. *Visc Med* 32(3):158–164
- Kolodczek T, Shugrue C, Ashat M, Thrower EC (2014) Risk factors for pancreatic cancer: underlying mechanisms and potential targets. *Front Physiol* 4:415
- Krebs AM, Mitschke J, Losada ML, Schmalhofer O, Boerries M, Busch H, Boettcher M, Mougiakakos D, Reichardt W, Bronsert P et al (2017) The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. *Nat Cell Biol* 19(5):518–529
- Kreuzer M, Kreienbrock L, Müller KM, Gerken M, Wichmann E (1999) Histologic types of lung carcinoma and age at onset. *Cancer* 85(9):1958–1965
- Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, Su L, Sharpless NE (2004) Ink4a/Arf expression is a biomarker of aging. *J Clin Invest* 114(9):1299–1307
- Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, Bonner-Weir S, Sharpless NE (2006) p16INK4a induces an age-dependent decline in islet regenerative potential. *Nature* 443(7110): 453–457
- Kuilman T, Michaloglou C, Mooi WJ, Peeper DS (2010) The essence of senescence. *Genes Dev* 24(22):2463–2479

- Kuncova J, Urban M, Mandys V (2007) Expression of CD44s and CD44v6 in transitional cell carcinomas of the urinary bladder: comparison with tumour grade, proliferative activity and p53 immunoreactivity of tumour cells. *APMIS* 115(11):1194–1205
- Kurra V, Krajewski KM, Jagannathan J, Giardino A, Berlin S, Ramaiya N (2013) Typical and atypical metastatic sites of recurrent endometrial carcinoma. *Cancer Imaging* 13(1):113
- Kwok WK, Ling M-T, Lee T-W, Lau TC, Zhou C, Zhang X, Chua CW, Chan KW, Chan FL, Glackin C et al (2005) Up-regulation of TWIST in prostate cancer and its implication as a therapeutic target. *Cancer Res* 65(12):5153–5162
- Lai P-L, Lin H, Chen S-F, Yang S-C, Hung K-H, Chang C-F, Chang H-Y, Lu FL, Lee Y-H, Liu Y-C et al (2017) Efficient generation of chemically induced mesenchymal stem cells from human dermal fibroblasts. *Sci Rep* 7(1):1–13
- Lakatos PL, Lakatos L (2008) Risk for colorectal cancer in ulcerative colitis: changes, causes and management strategies. *World J Gastroenterol* 14(25):3937
- Lakhani SR, Ellis IO, Schnitt S, Tan PH, van de Vijver M (2012) WHO classification of tumours of the breast. International Agency for Research on Cancer, Lyon
- Lakhtakia R, Aljarrah A, Furrukh M, Ganguly SS (2017) Epithelial mesenchymal transition (EMT) in metastatic breast cancer in Omani women. *Cancer Microenviron* 10(1–3):25–37
- Lambe M, Johansson AL, Altman D, Eloranta S (2009) Mastitis and the risk of breast cancer. *Epidemiology* 20(5):747–751
- Lawlor RT, Veronese N, Nottegar A, Malleo G, Smith L, Demurtas J, Cheng L, Wood LD, Silvestri N, Salvia R et al (2019) Prognostic role of high-grade tumor budding in pancreatic ductal adenocarcinoma: a systematic review and meta-analysis with a focus on epithelial to mesenchymal transition. *Cancers (Basel)* 11(1):113
- Lee M-Y, Chou C-Y, Tang M-J, Shen M-R (2008) Epithelial-mesenchymal transition in cervical cancer: correlation with tumor progression, epidermal growth factor receptor overexpression, and snail up-regulation. *Clin Cancer Res* 14(15):4743–4750
- Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 121(7):2750–2767
- Leng Z, Xia Q, Chen J, Li Y, Xu J, Zhao E, Zheng H, Ai W, Dong J (2018) Lgr5+ CD44+ EpCAM+ strictly defines cancer stem cells in human colorectal cancer. *Cell Physiol Biochem* 46(2):860–872
- Leong TY-M, Leong AS-Y (2005) Epidemiology and carcinogenesis of hepatocellular carcinoma. *HPB (Oxford)* 7(1):5–15
- Letašiová S, Medvedová A, Šovčíková A, Dušinská M, Volkovová K, Mosoiu C, Bartonová A (2012) Bladder cancer, a review of the environmental risk factors. *Environ Health* 11(1):1
- Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM (2007) Identification of pancreatic cancer stem cells. *Cancer Res* 67(3):1030–1037
- Li Y, Xu S, Li J, Han K, Pi H, Zheng L, Zuo G, Huang X, Li H, Zhao H et al (2013) Epithelial–mesenchymal transition markers expressed in circulating tumor cells in hepatocellular carcinoma patients with different stages of disease. *Cell Death Dis* 4(10):e831
- Li Y, Lin K, Yang Z, Han N, Quan X, Guo X, Li C (2017) Bladder cancer stem cells: clonal origin and therapeutic perspectives. *Oncotarget* 8(39):66668
- Li C, Lei S, Ding L, Xu Y, Wu X, Wang H, Zhang Z, Gao T, Zhang Y, Li L (2023) Global burden and trends of lung cancer incidence and mortality. *Chin Med J* 136(13):1583–1590
- Lin J, Liu X, Ding D (2015) Evidence for epithelial-mesenchymal transition in cancer stem-like cells derived from carcinoma cell lines of the cervix uteri. *Int J Clin Exp Pathol* 8(1):847
- Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, Patrawala L, Yan H, Jeter C, Honorio S et al (2011) The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med* 17(2):211
- Liu Y, Alqatari M, Sultan K, Ye F, Gao D, Sigel K, Zhang D, Kalir T (2017) Using p16 immunohistochemistry to classify morphologic cervical intraepithelial neoplasia 2: correlation of ambiguous staining patterns with HPV subtypes and clinical outcome. *Hum Pathol* 66:144–151

- Lo A-C, Soliman AS, El-Ghawalby N, Abdel-Wahab M, Fathy O, Khaled HM, Omar S, Hamilton SR, Greenson JK, Abbruzzese JL (2007) Lifestyle, occupational, and reproductive factors in relation to pancreatic cancer risk. *Pancreas* 35(2):120–129
- Lochan R, Reeves HL, Daly AK, Charnley RM (2011) The role of tobacco-derived carcinogens in pancreas cancer. *ISRN Oncol* 2011:249235
- Lu W, Kang Y (2019) Epithelial-mesenchymal plasticity in cancer progression and metastasis. *Dev Cell* 49(3):361–374
- Ma X-J, Salunga R, Tuggle JT, Gaudet J, Enright E, McQuary P, Payette T, Pistone M, Stecker K, Zhang BM (2003) Gene expression profiles of human breast cancer progression. *Proc Natl Acad Sci* 100(10):5974–5979
- Maida Y, Kyo S, Kanaya T, Wang Z, Tanaka M, Yatabe N, Nakamura M, Inoue M (2002) Is the telomerase assay useful for screening of endometrial lesions? *Int J Cancer* 100(6):714–718
- Malhotra GK, Zhao X, Band H, Band V (2010) Histological, molecular and functional subtypes of breast cancers. *Cancer Biol Ther* 10(10):955–960
- Maskey N, Thapa N, Maharjan M, Shrestha G, Maharjan N, Cai H, Liu S (2019) Infiltrating CD4 and CD8 lymphocytes in HPV infected uterine cervical milieu. *Cancer Manag Res* 11:7647
- Masubuchi T, Koyama S, Sato E, Takamizawa A, Kubo K, Sekiguchi M, Nagai S, Izumi T (1998) Smoke extract stimulates lung epithelial cells to release neutrophil and monocyte chemotactic activity. *Am J Pathol* 153(6):1903–1912
- McConkey DJ, Lee S, Choi W, Tran M, Majewski T, Lee S, Siefker-Radtke A, Dinney C, Czerniak B (2010) Molecular genetics of bladder cancer: emerging mechanisms of tumor initiation and progression. *Urol Oncol* 28(4):429–440
- Meeker AK, Hicks JL, Platz EA, March GE, Bennett CJ, Delannoy MJ, De Marzo AM (2002) Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. *Cancer Res* 62(22):6405–6409
- Meeker AK, Hicks JL, Gabrielson E, Strauss WM, De Marzo AM, Argani P (2004a) Telomere shortening occurs in subsets of normal breast epithelium as well as in situ and invasive carcinoma. *Am J Pathol* 164(3):925–935
- Meeker AK, Hicks JL, Iacobuzio-Donahue CA, Montgomery EA, Westra WH, Chan TY, Ronnett BM, De Marzo AM (2004b) Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. *Clin Cancer Res* 10(10):3317–3326
- Méndez-López LF (2022) Revisiting epithelial carcinogenesis. *Int J Mol Sci* 23(13):7437
- Mendez-Lopez LF, Zapata-Benavides P, Zavala-Pompa A, Aguado-Barrera ME, Pacheco-Calleros-J, Rodriguez-Padilla C, Cerda-Flores RM, Cortes-Gutierrez EI, Davila-Rodriguez MI (2010) Immunohistochemical analysis of prostate apoptosis response-4 (Par-4) in Mexican women with breast cancer: a preliminary study. *Arch Med Res* 41(4):261–268
- Méndez-López LF, Davila-Velderrain J, Domínguez-Hüttinger E, Enríquez-Olguín C, Martínez-García JC, Alvarez-Buylla ER (2017a) Gene regulatory network underlying the immortalization of epithelial cells. *BMC Syst Biol* 11(1):24
- Méndez-López LF, Zavala-Pompa A, Cortés-Gutiérrez EI, Cerda-Flores RM, Davila-Rodriguez MI (2017b) Leptin receptor expression during the progression of endometrial carcinoma is correlated with estrogen and progesterone receptors. *Arch Med Sci* 13(1):228
- Michaud DS (2007) Chronic inflammation and bladder cancer. *Urol Oncol* 25(3):260–268
- Mingels MJ, Masadah R, Geels YP, Otte-Höller I, de Kievit IM, van der Laak JA, van Ham MA, Bulten J, Massuger LFA (2014) High prevalence of atypical hyperplasia in the endometrium of patients with epithelial ovarian cancer. *Am J Clin Pathol* 142(2):213–221
- Minn AJ, Kang Y, Serganova I, Gupta GP, Giri DD, Doubrovin M, Ponomarev V, Gerald WL, Blasberg R, Massagué J (2005) Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *J Clin Invest* 115(1):44–55
- Momi N, Kaur S, Krishn S, Batra SK (2012) Discovering the route from inflammation to pancreatic cancer. *Minerva Gastroenterol Dietol* 58(4):283

- Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag C, Laversanne M, Vignat J, Ferlay J, Murphy N, Bray F (2023) Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. *Gut* 72(2):338–344
- Mori M, Rao SK, Popper HH, Cagle PT, Fraire AE (2001) Atypical adenomatous hyperplasia of the lung: a probable forerunner in the development of adenocarcinoma of the lung. *Mod Pathol* 14(2):72–84
- Morii A, Komiya A, Okumura A, Fuse H (2010) Telomerase activity in bladder cancer tissue. *Exp Ther Med* 1(1):85–88
- Mostafa MH, Sheweita S, O'Connor PJ (1999) Relationship between schistosomiasis and bladder cancer. *Clin Microbiol Rev* 12(1):97–111
- Murata M (2018) Inflammation and cancer. *Environ Health Prev Med* 23(1):1–8
- Nakamura M, Zhang X, Mizumoto Y, Maida Y, Bono Y, Takakura M, Kyo S (2014) Molecular characterization of CD133+ cancer stem-like cells in endometrial cancer. *Int J Oncol* 44(3):669–677
- Nassir M, Roth A, Gasimli K, Braicu EI, Fotopoulou C, Mawrin C, Badakhshi H, Warnke J-P, Sehoul J (2014) Is endometrial cancer really a neurophobic tumor? A case report and review of the literature. *Anticancer Res* 34(1):249–257
- Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, Laurent A, Cherqui D, Balabaud C, Zucman-Rossi J (2013) High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* 4:2218
- Nesina I, Iurchenko N, Buchynska L (2018) Markers of the epithelial-mesenchymal transition in cells of endometrial carcinoma. *Exp Oncol* 40(3):218–222
- Nevadunsky NS, Van Arsdale A, Strickler HD, Moadel A, Kaur G, Levitt J, Girda E, Goldfinger M, Goldberg GL, Einstein MH (2014) Obesity and age at diagnosis of endometrial cancer. *Obstet Gynecol* 124(2 PART 1):300–306
- Nishimoto A, Miura N, Oshimura M (1998) Clinical significance of telomerase activity in precancerous lesion of the liver (adenomatous hyperplasia). *Nihon Rinsho* 56(5):1244–1247
- Onstad MA, Schmandt RE, Lu KH (2017) Addressing the role of obesity in endometrial cancer risk, prevention, and treatment. *Obstet Gynecol Surv* 72(3):169–170
- Owonikoko TK, Ragin CC, Belani CP, Oton AB, Gooding WE, Taioli E, Ramalingam S (2007) Lung cancer in elderly patients: an analysis of the surveillance, epidemiology, and end results database. *J Clin Oncol* 25(35):5570–5577
- Pacheco-Rivera R, Fattel-Fazenda S, Arellanes-Robledo J, Silva-Olivares A, Alemán-Lazarini L, Rodríguez-Segura M, Pérez-Carreón J, Villa-Treviño S, Shibayama M, Serrano-Luna J (2016) Double staining of β -galactosidase with fibrosis and cancer markers reveals the chronological appearance of senescence in liver carcinogenesis induced by diethylnitrosamine. *Toxicol Lett* 241:19–31
- Paliwal P, Arora D, Mishra AK (2012) Epithelial mesenchymal transition in urothelial carcinoma: twist in the tale. *Indian J Pathol Microbiol* 55(4):443
- Pare R, Soon PS, Shah A, Lee CS (2019) Differential expression of senescence tumour markers and its implications on survival outcomes of breast cancer patients. *PLoS One* 14(4):e0214604
- Patanaphan V, Salazar O (1993) Colorectal cancer: metastatic patterns and prognosis. *South Med J* 86(1):38–41
- Patanaphan V, Salazar OM, Risco R (1988) Breast cancer: metastatic patterns and their prognosis. *South Med J* 81(9):1109–1112
- Pathirana T, Sequeira R, Del Mar C, Dickinson JA, Armstrong BK, Bell KJ, Glasziou P (2022) Trends in prostate specific antigen (PSA) testing and prostate cancer incidence and mortality in Australia: a critical analysis. *Cancer Epidemiol* 77:102093
- Patsner B (2000) Endometrial cancer in women 45 years of age or younger. *Eur J Gynaecol Oncol* 21(3):249–250
- Peinado H, Portillo F, Cano A (2004) Transcriptional regulation of cadherins during development and carcinogenesis. *Int J Dev Biol* 48(5–6):365–375

- Peixoto RDA, Speers C, McGahan CE, Renouf DJ, Schaeffer DF, Kennecke HF (2015) Prognostic factors and sites of metastasis in unresectable locally advanced pancreatic cancer. *Cancer Med* 4(8):1171–1177
- Perletti G, Montanari E, Vral A, Gazzano G, Marras E, Mione S, Magri V (2010) Inflammation, prostatitis, proliferative inflammatory atrophy: ‘fertile ground’ for prostate cancer development? *Mol Med Rep* 3(1):3–12
- Petrack JL, Braunlin M, Laversanne M, Valery PC, Bray F, McGlynn KA (2016) International trends in liver cancer incidence, overall and by histologic subtype, 1978–2007. *Int J Cancer* 139(7):1534–1545
- Petty TL (1997) Lung cancer and chronic obstructive pulmonary disease. *Hematol Oncol Clin North Am* 11(3):531–541
- Pinder SE (2010) Ductal carcinoma in situ (DCIS): pathological features, differential diagnosis, prognostic factors and specimen evaluation. *Mod Pathol* 23:S8–S13
- Pinder SE, Ellis IO (2003) The diagnosis and management of pre-invasive breast disease: ductal carcinoma in situ (DCIS) and atypical ductal hyperplasia (ADH)—current definitions and classification. *Breast Cancer Res* 5:1–4
- Place AE, Jin Huh S, Polyak K (2011) The microenvironment in breast cancer progression: biology and implications for treatment. *Breast Cancer Res* 13(6):227
- Popovici D, Stanisav C, Pricop M, Dragomir R, Saftescu S, Ciurescu D (2023) Associations between body mass index and prostate cancer: the impact on progression-free survival. *Medicina* 59(2):289
- Poruk KE, Blackford AL, Weiss MJ, Cameron JL, He J, Goggins M, Rasheed ZA, Wolfgang CL, Wood LD (2017) Circulating tumor cells expressing markers of tumor-initiating cells predict poor survival and cancer recurrence in patients with pancreatic ductal adenocarcinoma. *Clin Cancer Res* 23(11):2681–2690
- Puhr M, De Marzo A, Isaacs W, Lucia MS, Sfanos K, Yegnasubramanian S, Culig Z (2016) Inflammation, microbiota, and prostate cancer. *Eur Urol Focus* 2(4):374–382
- Punyadeera C, Dunselman G, Marbaix E, Kamps R, Galant C, Nap A, de Goeij A, Ederveen A, Groothuis P (2004) Triphasic pattern in the ex vivo response of human proliferative phase endometrium to oestrogens. *J Steroid Biochem Mol Biol* 92(3):175–185
- Radisky DC, Santisteban M, Berman HK, Gauthier ML, Frost MH, Reynolds CA, Vierkant RA, Pankratz VS, Visscher DW, Tlsty T, Hartmann LC (2011) p16INK4a expression and breast cancer risk in women with atypical hyperplasia. *Cancer Prev Res (Phila)* 4(12):1953–1960
- Ramos-Nino ME (2013) The role of chronic inflammation in obesity-associated cancers. *ISRN Oncol* 2013:697521
- Ren X, Liang J, Zhang Y, Jiang N, Xu Y, Qiu M, Wang Y, Zhao B, Chen X (2022) Single-cell transcriptomic analysis highlights origin and pathological process of human endometrioid endometrial carcinoma. *Nat Commun* 13(1):6300
- Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH (2012) EMT and dissemination precede pancreatic tumor formation. *Cell* 148(1–2):349–361
- Riihimäki M, Hemminki A, Fallah M, Thomsen H, Sundquist K, Sundquist J, Hemminki K (2014) Metastatic sites and survival in lung cancer. *Lung Cancer* 86(1):78–84
- Roberts CT Jr (2004) IGF-1 and prostate cancer. In: *Biology of IGF-1: its interaction with insulin in health and malignant states*, vol 193. Wiley, Chichester
- Rock CL, Michael CW, Reynolds RK, Ruffin MT (2000) Prevention of cervix cancer. *Crit Rev Oncol Hematol* 33(3):169–185
- Rodier F, Coppe JP, Patil CK, Hoeijmakers WA, Munoz DP, Raza SR, Freund A, Campeau E, Davalos AR, Campisi J (2009) Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol* 11(8):973–979
- Rosin MP, Anwar WA, Ward AJ (1994) Inflammation, chromosomal instability, and cancer: the schistosomiasis model. *Cancer Res* 54(7 Supplement):1929s–1933s

- Rouet-Benzineb P, Aparicio T, Guilmeau S, Pouzet C, Descatoire V, Buyse M, Bado A (2004) Leptin counteracts sodium butyrate-induced apoptosis in human colon cancer HT-29 cells via NF- κ B signaling. *J Biol Chem* 279(16):16495–16502
- Rozario S d, da Silva IF, Koifman RJ, Silva IF (2019) Characterization of women with cervical cancer assisted at Inca by histological type. *Rev Saude Publica* 53:88
- Rumgay H, Arnold M, Ferlay J, Lesi O, Cabaasag CJ, Vignat J, Laversanne M, McGlynn KA, Soerjomataram I (2022) Global burden of primary liver cancer in 2020 and predictions to 2040. *J Hepatol* 77(6):1598–1606
- Rutanen E-M (1998) Insulin-like growth factors in endometrial function. *Gynecol Endocrinol* 12(6):399–406
- Saginala K, Barsouk A, Aluru JS, Rawla P, Padala SA, Barsouk A (2020) Epidemiology of bladder cancer. *Med Sci (Basel)* 8(1):15
- Saito T, Schneider A, Martel N, Mizumoto H, Bulgay-Moerschel M, Kudo R, Nakazawa HJB (1997) Proliferation-associated regulation of telomerase activity in human endometrium and its potential implication in early cancer diagnosis. *Biochem Biophys Res Commun* 231(3):610–614
- Sanfrancesco J, Jones JS, Hansel DE (2013) Diagnostically challenging cases: what are atypia and dysplasia? *Urol Clin North Am* 40(2):281
- Sardo Molmenti CL, Hibler EA, Yang J, Kolb JM, Yang J, Hussain M, Andersen SW, Shivappa N, Hébert JR, Lance P (2020) Characteristics and risk factors for early-onset metachronous colorectal adenoma. *Colorectal Cancer* 9(3):CRC2033
- Sanrió D, Rodríguez-Pinilla SM, Hardisson D, Cano A, Moreno-Bueno G, Palacios J (2008) Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. *Cancer Res* 68(4):989–997
- Scaling AL, Prossnitz ER, Hathaway HJ (2014) GPER mediates estrogen-induced signaling and proliferation in human breast epithelial cells and normal and malignant breast. *Horm Cancer* 5(3):146–160
- Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Göktuna SI, Ziegler PK, Canli O, Heijmans J, Huels DJ, Moreaux G (2013) Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell* 152(1–2):25–38
- Selman M, Pardo A, Kaminski N (2008) Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? *PLoS Med* 5(3):e62
- Sevenoaks MJ, Stockley RA (2006) Chronic obstructive pulmonary disease, inflammation and co-morbidity – a common inflammatory phenotype? *Respir Res* 7(1):70
- Shang Z, Cai Q, Zhang M, Zhu S, Ma Y, Sun L, Jiang N, Tian J, Niu X, Chen J et al (2015) A switch from CD44+ cell to EMT cell drives the metastasis of prostate cancer. *Oncotarget* 6(2):1202
- Shen MM, Abate-Shen C (2010) Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev* 24(18):1967–2000
- Siegel RL, Miller KD, Jemal A (2016) Cancer statistics, 2016. *CA Cancer J Clin* 66(1):7–30
- Siegel RL, Miller KD, Wagle NS, Jemal A (2023) Cancer statistics, 2023. *CA Cancer J Clin* 73(1):17–48
- Silverberg SG (2000) Problems in the differential diagnosis of endometrial hyperplasia and carcinoma. *Mod Pathol* 13(3):309–327
- Skog M, Bono P, Lundin M, Lundin J, Louhimo J, Linder N, Petrova TV, Andersson LC, Joensuu H, Alitalo K, Haglund CH (2011) Expression and prognostic value of transcription factor PROX1 in colorectal cancer. *Br J Cancer* 105(9):1346–1351
- Smith CJ, Perfetti TA, King JA (2006) Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. *Inhal Toxicol* 18(9):667–677
- Sprague BL, Trentham-Dietz A, Egan KM, Titus-Ernstoff L, Hampton JM, Newcomb PA (2008) Proportion of invasive breast cancer attributable to risk factors modifiable after menopause. *Am J Epidemiol* 168(4):404–411
- Srivatanakul P, Sriplung H, Deerasamee S (2004) Epidemiology of liver cancer: an overview. *Asian Pac J Cancer Prev* 5(2):118–125

- Sullivan L, Pacheco RR, Kmeid M, Chen A, Lee H (2022) Tumor stroma ratio and its significance in locally advanced colorectal cancer. *Curr Oncol* 29(5):3232–3241
- Tanaka T, Miyazawa K, Tsukamoto T, Kuno T, Suzuki K (2011) Pathobiology and chemoprevention of bladder cancer. *J Oncol* 2011:528353
- Tang R, Cheng A-J, Wang J-Y, Wang T-C (1998) Close correlation between telomerase expression and adenomatous polyp progression in multistep colorectal carcinogenesis. *Cancer Res* 58(18):4052–4054
- Telli O, Sarici H, Ozgur BC, Doluoglu OG, Sunay MM, Bozkurt S, Eroglu M (2014) Urothelial cancer of bladder in young versus older adults: clinical and pathological characteristics and outcomes. *Kaohsiung J Med Sci* 30(9):466–470
- Testino G (2011) The burden of cancer attributable to alcohol consumption. *Maedica (Bucur)* 6(4):313
- Thigpen JT, Brady MF, Homesley HD, Malfetano J, DuBeshter B, Burger RA, Liao S (2004) Phase III trial of doxorubicin with or without cisplatin in advanced endometrial carcinoma: a gynecologic oncology group study. *J Clin Oncol* 22(19):3902–3908
- Tyagi A, Vishnoi K, Mahata S, Verma G, Srivastava Y, Masaldan S, Roy BG, Bharti AC, Das BC (2016) Cervical cancer stem cells selectively overexpress HPV oncoprotein E6 that controls stemness and self-renewal through upregulation of HES1. *Clin Cancer Res* 22(16):4170–4184
- Tyler S (2003) Epithelium—the primary building block for metazoan complexity. *Integr Comp Biol* 43(1):55–63
- Ugnat A, Xie L, Morriss J, Semenciw R, Mao Y (2004) Survival of women with breast cancer in Ottawa, Canada: variation with age, stage, histology, grade and treatment. *Br J Cancer* 90(6):1138
- Umbrecht CB, Sherman ME, Dome J, Carey L, Marks J, Kim N, Sukumar S (1999) Telomerase activity in ductal carcinoma in situ and invasive breast cancer. *Oncogene* 18(22):3407–3414
- Van Dooijeweert C, Van Diest P, Ellis I (2022) Grading of invasive breast carcinoma: the way forward. *Virchows Arch* 480(1):33–43
- Van Heek NT, Meeker AK, Kern SE, Yeo CJ, Lillemoe KD, Cameron JL, Offerhaus GJA, Hicks JL, Wilentz RE, Goggins MG et al (2002) Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am J Pathol* 161(5):1541–1547
- Venturi A, Piscaglia F, Vidili G, Flori S, Righini R, Golfieri R, Bolondi L (2007) Diagnosis and management of hepatic focal nodular hyperplasia. *J Ultrasound* 10(3):116–127
- Villanueva A, Luedde T (2016) The transition from inflammation to cancer in the liver. *Clin Liver Dis (Hoboken)* 8(4):89–93
- Villavicencio A, Aguilar G, Argüello G, Dünner C, Gabler F, Soto E, Gaete F, Peñaloza P, Celis M, Rojas C (2010) The effect of overweight and obesity on proliferation and activation of AKT and ERK in human endometria. *Gynecol Oncol* 117(1):96–102
- Vink MA, Bogaards JA, van Kemenade FJ, de Melker HE, Meijer CJ, Berkhof J (2013) Clinical progression of high-grade cervical intraepithelial neoplasia: estimating the time to preclinical cervical cancer from doubly censored national registry data. *Am J Epidemiol* 178(7):1161–1169
- Vinuesa-Pitarch E, Ortega-Álvarez D, Rodilla V (2021) How lineage tracing studies can unveil tumor heterogeneity in breast cancer. *Biomedicines* 10(1):3
- Vogelstein B, Kinzler KW (1993) The multistep nature of cancer. *Trends Genet* 9(4):138–141
- Vukovic B, Park PC, Al-Maghrabi J, Beheshti B, Sweet J, Evans A, Trachtenberg J, Squire J (2003) Evidence of multifocality of telomere erosion in high-grade prostatic intraepithelial neoplasia (HPIN) and concurrent carcinoma. *Oncogene* 22(13):1978–1987
- Wan S, Zhao E, Kryczek I, Vatan L, Sadovskaya A, Ludema G, Simeone DM, Zou W, Welling TH (2014) Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. *Gastroenterology* 147(6):1393–1404
- Wang L, Lu B, He M, Wang Y, Wang Z, Du L (2022) Prostate cancer incidence and mortality: global status and temporal trends in 89 countries from 2000 to 2019. *Front Public Health* 10:176

- Wei LY, Zhang XJ, Wang L, Hu LN, Zhang XD, Li L, Gao JN (2020) A six-epithelial–mesenchymal transition gene signature may predict metastasis of triple-negative breast cancer. *Onco Targets Ther* 13:6497–6509
- Wernicke M, Piñeiro LC, Caramutti D, Dorn VG, Raffo MML, Guixa HG, Telenta M, Morandi AA (2003) Breast cancer stromal myxoid changes are associated with tumor invasion and metastasis: a central role for hyaluronan. *Mod Pathol* 16(2):99–107
- Willet SG, Lewis MA, Miao ZF, Liu D, Radyk MD, Cunningham RL, Burclaff J, Sibbel G, Lo HYG, Blanc V (2018) Regenerative proliferation of differentiated cells by mTORC 1-dependent paligenosis. *EMBO J* 37(7):e98311
- Williamson SR, Zhang S, Lopez-Beltran A, Montironi R, Wang M, Cheng L (2013) Telomere shortening distinguishes inverted urothelial neoplasms. *Histopathology* 62(4):595–601
- Wilson KM, Mucci LA (2019) Diet and lifestyle in prostate cancer. In: *Prostate cancer*. Springer, Cham, pp 1–27
- Wilson MM, Weinberg RA, Lees JA, Guen VJJ (2020) Emerging mechanisms by which EMT programs control stemness. *Trends Cancer* 6(9):775–780
- Wu Y, Deng Y, Dai Z, Ma Y, Lyu L, Lei C, Zheng Y, Li Y, Wang Z, Gao J (2023) Estimates of bladder cancer burden attributable to high fasting plasma glucose: Findings of the Global Burden of Disease Study 2019. *Cancer Med* 12(15):16469–16481
- Xie G, Yao Q, Liu Y, Du S, Liu A, Guo Z, Sun A, Ruan J, Chen L, Ye C, Yuan Y (2012) IL-6-induced epithelial-mesenchymal transition promotes the generation of breast cancer stem-like cells analogous to mammosphere cultures. *Int J Oncol* 40(4):1171–1179
- Xie T, Liang J, Liu N, Huan C, Zhang Y, Liu W, Kumar M, Xiao R, D’Armiento J, Metzger D et al (2016) Transcription factor TBX4 regulates myofibroblast accumulation and lung fibrosis. *J Clin Invest* 126(8):3063–3079
- Yadav D, Lowenfels AB (2013) The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 144(6):1252–1261
- Yamada S, Okumura N, Wei L, Fuchs BC, Fujii T, Sugimoto H, Nomoto S, Takeda S, Tanabe KK, Kodera Y (2014) Epithelial to mesenchymal transition is associated with shorter disease-free survival in hepatocellular carcinoma. *Ann Surg Oncol* 21(12):3882–3890
- Yang MH, Chen CL, Chau GY, Chiou SH, Su CW, Chou TY, Peng WL, Wu JC (2009) Comprehensive analysis of the independent effect of twist and snail in promoting metastasis of hepatocellular carcinoma. *Hepatology* 50(5):1464–1474
- Yang Y, Yu C, Zhou M, Pang N, Li N, Nie H, Liao J, Bai Y, Liu H (2011) Metabolic study of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to the enantiomers of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in vitro in human bronchial epithelial cells using chiral capillary electrophoresis. *J Chromatogr A* 1218(37):6505–6510
- Yashima K, Litzky LA, Kaiser L, Rogers T, Lam S, Wistuba II, Milchgrub S, Srivastava S, Piatyszek MA, Shay JW, Gazdar AF (1997) Telomerase expression in respiratory epithelium during the multistage pathogenesis of lung carcinomas. *Cancer Res* 57(12):2373–2377
- Ye F, Zhou C, Cheng Q, Shen J, Chen H (2008) Stem-cell-abundant proteins Nanog, Nucleostemin and Musashi1 are highly expressed in malignant cervical epithelial cells. *BMC Cancer* 8(1):108
- Ye X, Tam WL, Shibue T, Kaygusuz Y, Reinhardt F, Ng Eaton E, Weinberg RA (2015) Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. *Nature* 525(7568):256–260
- Yin Z, Tang H, Li L, Ni J, Yuan S, Lou H, Chen M (2019) Impact of sites versus number of metastases on survival of patients with organ metastasis from newly diagnosed cervical cancer. *Cancer Manag Res* 11:7759
- Yost S, Hoekstra A (2018) Cervical cancer in women over 65: an analysis of screening. *Gynecol Oncol Rep* 25:48–51
- Younis LK, El Sakka H, Haque I (2010) The prognostic value of E-cadherin expression in breast cancer. *J Health Sci* 1(1):43–51
- Yu Z, Pestell TG, Lisanti MP, Pestell RG (2012) Cancer stem cells. *Int J Biochem Cell Biol* 44(12):2144–2151

- Zhai B, Yan H-X, Liu S-Q, Chen L, Wu M-C, Wang H-Y (2008) Reduced expression of E-cadherin/catenin complex in hepatocellular carcinomas. *World J Gastroenterol* 14(37):5665
- Zhang Z, Rosen DG, Yao JL, Huang J, Liu J (2006) Expression of p14ARF, p15INK4b, p16INK4a, and DCR2 increases during prostate cancer progression. *Mod Pathol* 19(10):1339–1343
- Zhang G-J, Zhou T, Tian H-P, Liu Z-L, Xia S-S (2013) High expression of ZEB1 correlates with liver metastasis and poor prognosis in colorectal cancer. *Oncol Lett* 5(2):564–568
- Zhao P, Fu J, Yao B, Song Y, Mi L, Li Z, Shang L, Hao W, Zhou Z (2012) In vitro malignant transformation of human bronchial epithelial cells induced by benzo(a)pyrene. *Toxicol In Vitro* 26(2):362–368
- Zhou J-Y, Chen M, Ma L, Wang X, Chen Y-G, Liu S-L (2016) Role of CD44^{high}/CD133^{high} HCT-116 cells in the tumorigenesis of colon cancer. *Oncotarget* 7(7):7657
- Zińczuk J, Zaręba K, Guzińska-Ustymowicz K, Kędra B, Kemon A, Pryczynicz A (2018) p16, p21, and p53 proteins play an important role in development of pancreatic intraepithelial neoplastic. *Ir J Med Sci* 187(3):629–637

Mitochondria as a Key Player in Cancer

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Abstract

Cancer is regarded as a serious issue affecting the health of entire societies. It is defined as a disease in which certain body cells begin to uncontrollably divide, grow, and spread to other body parts. This process necessitates an increased supply of energy in the form of ATP, along with an elevated amount of cofactors such as NADH and NADPH, as well as the building blocks of molecules. Mitochondria are widely recognized as the cell's powerhouse; they serve as biosynthetic as well as signaling organelles that are part of stress perception and enable cellular adaptation to the environment. Moreover, metabolic reprogramming plays a fundamental role in the initiation and maintenance of tumors and has thus been proposed as one of the hallmarks of cancer. Recent research highlights mitochondria as a crucial target for cancer therapy, underscoring the importance of understanding and targeting mitochondrial function in developing new cancer treatments.

The function of mitochondria plays a crucial role in the development and pathology of various cancers. Mitochondrial dysfunction leads to physiological imbalances and metabolic alteration in the cell, contributing to cancer progression. Understanding the mechanisms of mitochondrial function in cancer is

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essential for further developing strategies to treat this disease and to create new anticancer drugs.

Focusing on mitochondrial metabolism, understanding the importance and function of the electron transport chain, as well as redox signaling pathways and, of course, ROS homeostasis, has become the primary focus of researchers.

Keywords

Cancer · Mitochondria · Mitochondria as a target · Mitochondria metabolism · Reactive oxygen species

1 Introduction

Human malignancies generally represent a complex set of diseases characterized by an interplay of pathophysiology, genetics, environmental factors, and immunology. Cancer encompasses a wide variety of conditions with a variable clinical course, ranging from extremely indolent to highly aggressive (Hill 2019). Cancer cells have an interesting property that they change their metabolism in order to maintain their rapid proliferation and survival. Key features of cancer cells include unlimited replicative capacity, evasion of apoptosis, resistance to growth suppressors, induction of angiogenesis, tissue invasion and metastasis, and reprogramming of cell metabolism and bioenergetics (Ghanem et al. 2021; Schiliro and Firestein 2021). In order to acquire and maintain these energy-demanding characteristics, tumor cells must reprogram their metabolic pathways. Increased growth and proliferation in cancer cells due to cell division necessitate an elevated supply of energy in the form of ATP, increased cofactors such as NADH, NADPH, and building block molecules like carbon skeletons. The heightened consumption of energy and building molecules in tumor cells leads to changes in key metabolic pathways, including alterations in the metabolism of proteins (relation to glutamine and branched-chain amino acids metabolism), lipids (involving lipid acquisition and fatty acid oxidation), carbohydrates (in glycolysis), nucleic acids, and the tricarboxylic acid (TCA) cycle (Ghanem et al. 2021; Schiliro and Firestein 2021).

There is substantial evidence indicating that mitochondria, as owing to their bioenergetic and biosynthetic functions, serve as the metabolic hub for tumor cell proliferation, survival, and metastasis (Liu and Shi 2020; Wang et al. 2023). Their functions vary between cells, depending on specific requirements for energy distribution, metabolite biosynthesis, and cellular signaling (Glancy et al. 2020). Mitochondria are crucial in controlling redox homeostasis, oncogenic signaling, innate immunity, and apoptosis. Recognized as the cell's powerhouse, mitochondria are bioenergetic and biosynthetic organelles responsible for energy production. Numerous metabolic pathways are housed in mitochondria. These include the electron transport chain (ETC), oxidative phosphorylation (OXPHOS), fatty acid oxidation, the TCA cycle, as well as the synthesis of amino acids, heme biosynthesis, lipid synthesis, and nucleotide synthesis (Zong et al. 2016). Mitochondria are

associated with various physiological functions, including calcium homeostasis, iron-sulfur cluster synthesis, apoptosis, participation in cellular aging, and involvement in developmental processes (Protasoni and Zeviani 2021; Shen et al. 2022; Scheffler 2008).

Proteins that are part of the ETC and OXPHOS have been identified as cancer targets. Due to this discovery, many inhibitors have been developed recently. Additionally, several intermediates in the TCA cycle, localized in mitochondria, are crucial for cancer cell metabolism. Mitochondria are critical organelles in processes such as tumor proliferation, survival, metastasis, and drug resistance (Liu and Shi 2020; Wang et al. 2023). Mutations in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) associated with the TCA cycle are frequently observed in cancer cells; these mutations are involved in cancer metabolism remodeling (Liu and Shi 2020). Mitochondria are considered promising targets for cancer therapy, providing new insights into cancer treatment opportunities. It is important to focus specifically on the mechanisms for mitochondrial reprogramming in every different cancer medium, because it is precisely this altered mitochondrial metabolism that may ultimately be an essential strategy for developing anticancer drugs (Liu and Shi 2020).

2 Cancer

The National Cancer Institute (NCI) defines cancer as a disease in which certain body cells begin to uncontrollably divide, grow, and spread to other parts of the body (NCI: <https://www.cancer.gov/>). Cancer can occur in any place in the human body, and cancer cells can form tumors, which are classified into two groups: malignant or benign. Malignant tumors spread to or invade nearby tissues and can travel to distant sites in the body to form metastases. Benign tumors do not spread and do not invade nearby tissues. After surgical removal, benign tumors usually do not grow. However, if benign tumors reach significant sizes, they can be life-threatening, as is the case with benign tumors in the brain (NCI: <https://www.cancer.gov/>; WHO: <https://www.who.int/>). According to the World Health Organization (WHO), cancer is the second leading cause of death worldwide, accounting for an estimated 9.6 million deaths in 2018 (or 1 in 6 deaths). In men, the most common types of cancer include prostate, lung, bronchus, colorectal, stomach, liver, and urinary bladder cancers. In women, the most prevalent types are breast, lung, colorectal, cervical, and thyroid cancers (Siegel et al. 2016). The types of cancer most commonly found in children differ from those in adults. According to the American Cancer Society (ACS), the most common cancer types in children are leukemia, brain and spinal cord tumors, neuroblastoma, Wilms tumor, lymphoma, rhabdomyosarcoma, retinoblastoma, and bone cancer (ACS: <https://www.cancer.org/>).

Cancer is considered a serious problem that affects the health of individuals and entire societies. The WHO states that 30% to 50% of cancer deaths could be prevented by modifying or eliminating key risk factors and implementing existing evidence-based prevention strategies (WHO: <https://www.who.int/>). A great

Table 1 The IARC classification of carcinogenic agents (adjusted according to IARC: <https://monographs.iarc.who.int/agents-classified-by-the-iarc/>)

Group	Meaning	Number of agents
Group 1	Carcinogenic to humans	127 agents
Group 2A	Probably carcinogenic to humans	95 agents
Group 2B	Possibly carcinogenic to humans	323 agents
Group 3	Not classifiable as to its carcinogenicity to humans	500 agents

challenge is early diagnosis followed by effective treatment. However, the most cost-effective long-term cancer control strategy is cancer prevention (WHO: <https://www.who.int/>; Hassanpour and Dehghani 2017).

Compounds capable of causing and increasing the risk of cancer are generally called carcinogens. The International Agency for Research on Cancer (IARC) has developed a system for the classification of carcinogenic agents in humans (Table 1).

Carcinogenesis can result from the action of one or more carcinogens, which may be chemical, physical, biological, or genetic. Common and frequent carcinogens include chemical compounds; for instance, smoking itself involves carcinogenic chemicals that can lead to lung cancer. Environmental chemicals exhibiting carcinogenic properties can directly or indirectly affect the cytoplasm and nucleus of normal cells, potentially causing genetic disorders and gene mutations. Other significant factors contributing to carcinogenesis include viruses, bacteria, and radiation rays (Hassanpour and Dehghani 2017).

Carcinogenesis is considered a multistage process involving various molecular and cellular changes. The process consists of initiation, promotion, malignant conversion, and progression stages (Weston and Harris 2003; Siddiqui et al. 2015). In initiation, the first stage results from irreversible genetic damage caused by one or more gene mutations, which can be spontaneous or caused by some carcinogenic agent. These genetic changes can disturb the regulation of important biochemical signaling pathways linked to cell proliferation, survival, and differentiation. Factors influencing these changes include the rate and type of carcinogen metabolism and the response of DNA repair function (Siddiqui et al. 2015; Pitot 1993).

The second phase, promotion, is a reversible process where actively proliferating preneoplastic cells expand, with the rate of mutation accumulation proportional to the rate of cell division. This stage involves changes in genome expression mediated by promoter-receptor interactions (Siddiqui et al. 2015, Pitot 1993). Tumor promoters, generally non-mutagenic, can affect the carcinogenesis process by reducing the latency period for tumor formation or increasing the number of tumors formed in a tissue (Weston and Harris 2003). Chemopreventive agents can modify this stage and influence the rate of tumor growth (Siddiqui et al. 2015). Malignant conversion, the next stage, requires further genetic changes, transforming preneoplastic cells into those expressing a malignant phenotype. The total dose of the tumor promoter is less significant at this stage, but frequent and repeated administration is crucial. Disruption of the tumor promoter before malignant

conversion may lead to regression of benign and premalignant lesions (Loeb and Cheng 1990). The final stage is tumor progression, characterized by genetic and phenotypic changes, karyotype instability, and typical cell proliferation. Rapid increase in tumor size is observed, along with proto-oncogenes activation and functional loss of tumor suppressor genes. Tumor progression involves the expression of a malignant phenotype, making malignant cells more susceptible to gaining aggressive characteristics. Metastasis, the ability of tumor cells to secrete proteases, allows them to attack tissues immediately surrounding the primary tumor (Siddiqui et al. 2015; Pitot 1993; Weston and Harris 2003).

2.1 Cancer Metabolism

Cancer has long been perceived in society as a genetic disease, driven by numerous mutations that propel cancer progression. However, recent years have seen an accumulation of new knowledge pointing to the uncontrolled metabolism of tumor cells as more than just a characteristic but potentially the fundamental cause of tumors. Consequently, cancer is increasingly considered a metabolic disease (Gyamfi et al. 2022).

The metabolism of tumor cells is primarily geared toward the production and proliferation of biomass. This results in the depletion of nutrients in the local tissue and the accumulation of metabolic waste. Metabolism alteration is a typical sign of cancer cells, adapted to sustain their growth. Selected metabolites serve as substrates for energy and biomass production, while also acting as regulators of gene and protein expression, influencing the behavior of normal cells surrounding the tumor. As tumors grow and metastasize, they can affect nutrient distribution in the body, and, conversely, they can be influenced by it (Pavlova et al. 2022).

Cancer cells often take advantage of established cellular processes, such as the Warburg effect, and adapt them to their advantage. A fundamental characteristic of many cancer cell types is altered metabolism, involving increased glucose uptake and glycolysis alongside reduced oxidative metabolism. Tumor cells have a fascinating property: their ability to reprogram their cellular metabolism to obtain as much energy as possible for their growth, which is a typical feature of oncogenic transformation. This modification is necessary for tumor cells to meet the anabolic requirements essential for the growth and division of cells. In multicellular organisms, nutrient intake is strictly regulated by multiple control systems to avoid abnormal cellular proliferation. However, tumor cells overcome these metabolic limitations due to mutations in essential genes, including tumor suppressors and oncogenes (Valle-Mendiola and Soto-Cruz 2020). There is mounting evidence suggesting that a major function of active oncogenes and inactive tumor suppressors is the reprogramming of cellular metabolism (Kroemer and Pouyssegur 2008). Cancer cells are generally assumed to be more dependent on glycolysis than on OXPHOS for energy production. In normal tissues, more than 90% of the cellular energy is produced by the aerobic respiratory chain located in the mitochondria, compared to approximately 10% of the cellular energy obtained by glycolysis. In

contrast, in cancer cells, approximately 50% of cellular energy is produced through glycolysis, with the remaining energy derived from mitochondrial oxidative phosphorylation (Dasu et al. 2003). This shift is permanent even when O_2 is sufficient to maintain mitochondrial function (anaerobic glycolysis) (Zeng et al. 2015).

Recently, new oncometabolites have been discovered. An oncometabolite is considered a substance, an endogenous cellular metabolite whose concentration increases in cancers, promoting cancer growth and metastasis. For example, 2-hydroxyglutarate, which is found at elevated levels in gliomas, is considered an oncometabolite. This metabolite is known to contribute to the functional modification of the histone methylation patterns, and its activity has also been observed in altering the expression of various genes and thus contributes to carcinogenesis (Chowdhury et al. 2011). Oncometabolites that have been recently characterized include fumarate (renal cell carcinoma), sarcosine (prostate cancer), glycine (breast cancer), asparagine (leukemia), choline (prostate, brain, and breast cancer), and also glucose, lactate, glutamine, and serine in several cancers. The increased levels and accumulation of oncometabolites in tumors are associated with the necessity to maintain processes essential for the tumor environment (e.g., one-carbon metabolism, glutaminolysis, aerobic glycolysis) (Yang et al. 2012; Yang et al. 2013).

2.2 Energy Metabolism in Cancer Cell

All cells require energy for their basic functions, obtained through the degradation of macromolecules. Glycolysis, the TCA cycle, and the respiratory chain play central roles in energy metabolism. Cancer cells can reprogram their energy metabolism to meet the increased demands necessary for their uncontrolled and rapid growth.

Glycolysis is a metabolic pathway present in all cells in the living body. It is a principal pathway for ATP synthesis in tissues without mitochondria, such as erythrocytes, cornea, and lens. The brain is also dependent on glucose for energy. The glycolysis pathway produces many intermediate products essential for other metabolic pathways in the body, such as amino acid and lipid synthesis. In tumor cells, the rate of glycolysis is about 200 times higher compared to normal cells, a phenomenon described by Otto Warburg in 1930, hence referred to as the Warburg effect. According to Warburg, dysfunctional mitochondrial metabolism, not uncontrolled cell growth, is the primary cause of cancer. Cancer cells prioritize and reprogram their metabolism, focusing on glycolysis even in the presence of oxygen, resulting in aerobic glycolysis, unlike normal cells that convert glucose through glycolysis into pyruvate, metabolized in the mitochondria into carbon dioxide, ATP, and water (Akram 2013; Ghanem et al. 2021). Glycolysis consists of 10 sequential enzymatic reactions converting glucose into two molecules of pyruvate, linked to other metabolic pathways. In glycolysis, two high-energy ATP molecules and two NADH molecules are formed. ATP is the primary energy source for metabolic work, and NADH serves various functions. NADH acts as a source of reducing power in anabolic reactions or can be oxidized in the respiratory chain (in the presence of oxygen). Pyruvate produced in glycolysis enters the Krebs cycle, and anaerobically,

lactic acid is produced, accumulating in muscles, causing fatigue and pain. Many glycolytic enzymes also play crucial roles in non-glycolytic processes, such as transcriptional regulation, contributing to cancer cells meeting diverse cellular requirements. All enzymes in this pathway are deregulated in cancer (Valle-Mendiola and Soto-Cruz 2020).

Cancer cells exhibit increased glucose uptake and glycolysis. Rapidly growing tumors often have hypoxic blood vessels. Cancer cells adapt to hypoxic glycolysis through the involvement of the hypoxia-inducible transcription factor (HIF), which increases the synthesis of glucose transporters and glycolytic enzymes (Lu et al. 2002). This leads to predominantly anaerobic glycolysis in tumor cells, producing lactic acid for energy. Lipids and proteins can also serve as alternative energy sources, but due to hypoxia and mitochondrial damage, the TCA cycle does not function correctly in oxidizing proteins and lipids. Consequently, cancer cells, especially those with impaired mitochondrial respiration, depend on ATP production from the glycolysis pathway. Inhibition of glycolysis seriously depletes ATP production, resulting in dephosphorylation of the proapoptotic BAD (BCL2-associated agonist of cell death) molecule, integrating glycolysis and apoptosis, relocalization of regulatory BAX (BCL2-associated X protein) to mitochondria, and massive cell death (Akram 2013).

The pentose phosphate pathway (PPP) also plays a crucial role in the metabolism of tumor cells. It is a metabolic pathway parallel to glycolysis, directing glucose to its oxidative branch and leading to the production of intermediates such as NADPH and ribose-5-phosphate, essential for nucleotide synthesis. The PPP is located in the cytosol and consists of an irreversible oxidation stage and a non-oxidative phase. In the oxidative stage of the PPP, glucose 6-phosphate is converted into ribulose-5-phosphate, CO₂, and NADPH. In the non-oxidative stage, glycolytic intermediates, namely fructose 6-phosphate and glyceraldehyde 3-phosphate, and sedoheptulose sugars, are produced, resulting in the generation of ribose-5-phosphate. Ribose-5-phosphate is an important precursor for amino acid synthesis and is an essential molecule for nucleic acid synthesis. Another very important metabolite is NADPH. NADPH is crucial for the synthesis of fatty acids, and also in the process of cell survival, especially during stress, which is associated with an increased concentration of intracellular ROS. In cancer cells, most PPP enzymes are deregulated (Ramos-Martinez 2017; Patra and Hay 2014; Jin and Zhou 2019; Valle-Mendiola and Soto-Cruz 2020; Ghanem et al. 2021).

The PPP is vital for regulating tumor cell growth, involving many enzymes. Tumor cells, dividing rapidly, have developed mechanisms by which PPP is regulated to meet increased demands for the synthesis of nucleic acid, synthesis of fatty acids, generation of NADPH, and cell survival. Signaling pathways such as PI3K/Akt, Ras, and Src are known pro-oncogenic pathways, and may hyperactivate G6PD (glucose-6-phosphate dehydrogenase) by post-translational mechanisms (Patra and Hay 2014). The tumor suppressor p53 can stimulate flux through glycolysis while regulating apoptosis. TIGAR (TP53-inducible glycolysis and apoptosis regulator) works as fructose-2,6-bisphosphatase, diverting glycolytic intermediates into PPP. Moreover, p53 has the ability to repress the transcription of the glucose transporters GLUT1 and GLUT4. However, another tumor suppressor, p21, via

p21-activated kinase 4, supports intake of glucose, production of NADPH, and biosynthesis of lipids. Activation of the mammalian target of rapamycin complex 1 in tumor cells is associated with upregulation in the oxidative stage of PPP, mainly G6PD, through increased activity of the transcription factor sterol regulatory element-binding protein (Ghanem et al. 2021). Targeting PPP for cancer treatment may be attractive due to its role in distinguishing cancer cells from normal cells (Patra and Hay 2014).

The TCA cycle is a metabolic pathway located in the matrix of mitochondria. It consists of a series of enzymatic reactions, and is considered a central metabolic pathway for cellular energy production, providing precursors (e.g., α -ketoglutarate and oxaloacetate) for biosynthetic pathways and redox balance. This is extremely important in highly proliferating tumor cells (Eniafe and Jiang 2021; Anderson et al. 2018; Valle-Mendiola and Soto-Cruz 2020). Several enzymes catalyzing TCA cycle reactions are deregulated in cancer (Valle-Mendiola and Soto-Cruz 2020), and certain cancer types contain mutations affecting its integrity, predominantly at the levels of isocitrate dehydrogenase, succinate dehydrogenase, and fumarate hydratase. These mutations lead to dysregulation of TCA cycle metabolite production, likely contributing to cancer initiation, development, and progression (Eniafe and Jiang 2021). Despite the dogma that cancer cells primarily use aerobic glycolysis as an energy source, recent evidence suggests that some cancer cells use the TCA cycle for the production of energy and for the synthesis of macromolecules (Anderson et al. 2018).

The ETC, located in the mitochondria, generates energy in the form of ATP, oxidizing NADH and FADH₂ and reducing oxygen. The ETC consists of protein complexes I to IV, ubiquinone, and cytochrome c. Protein complexes connect redox reactions and form the electrochemical gradient needed for the synthesis of ATP by the protein ATP-synthase in the process called oxidative phosphorylation (Zhao et al. 2019; Ahmad et al. 2023). Electrons from various metabolic processes are directed to the ETC to facilitate oxidative phosphorylation. Electrons escaping from the ETC may react with oxygen to form reactive oxygen species (ROS), activating signaling pathways near the mitochondrial system to control cell proliferation and survival. Increased ROS production is a metabolic feature of tumor cells (Raimondi et al. 2020; Neagu et al. 2019). If ROS accumulate in large quantities, cell apoptosis occurs, prompting tumor cells to generate high amounts of NADPH in the mitochondria and cytosol to limit ROS accumulation. Glucose-dependent metabolism and mitochondrial metabolism are integral to tumor cell proliferation (Neagu et al. 2019).

3 Mitochondria

For many years, mitochondria have been considered crucial organelles, often referred to as the powerhouse of the cell, as they house processes essential for cellular life. The initial discovery of mitochondria is attributed to Swiss physiologist Albert von Kölliker, who, in 1857, first observed intracellular structures that were

most likely mitochondria (Kölliker 1857). In 1890, German pathologist and histologist Richard Altmann named them ‘bioblasts’ (life germs) and characterized these organelles as fundamental living units with metabolic and genetic autonomy (Altmann 1890). Eight years later, in 1898, microbiologist Carl Benda renamed them, introducing the term ‘mitochondria.’ The name originates from Greek, meaning ‘*mitos*,’ or ‘thread,’ and ‘*chondrion*,’ or ‘granule’ (Benda 1898). The understanding of mitochondrial function unfolded gradually. An important milestone was the discovery of the Krebs cycle by Sir Hans Adolf Krebs, elucidating the cycle of reactions and identifying the role of the citric acid cycle in the intermediary metabolism within the mitochondria of eukaryotic cells (Krebs and Johnson 1980). Krebs received the Nobel Prize for this groundbreaking discovery. Another Nobel Prize was awarded to British biochemist Peter Mitchell for his chemiosmotic theory, describing the connection between oxidation and phosphorylation (ATP synthesis) as proton and voltage gradients across biological membranes (Mitchell 1961).

3.1 Origin of Mitochondria

The evidence supporting the origin of life on Earth—similar to ongoing efforts in the search for life on the Moon or other planets—is primarily derived from radiometric fossil research and genetic evidence such as gene mapping. The oldest known fossils trace the origin of prokaryotes, the first organisms on Earth, back to approximately 3.5 billion years ago, about 1 billion years after the formation of the Earth’s crust. These early prokaryotic organisms adapted to the challenging conditions prevailing on Earth at that time. Shielded from intense solar radiation, frequent volcanic activity, and high temperatures, the first organisms thrived in the deep ocean or deep below the Earth’s surface. To synthesize organic structures for building their own cells, they utilized energy from sunlight, with carbon sourced primarily from the abundant carbon dioxide and electrons likely derived from water, sulfate, or hydrogen (Sim et al. 2019).

Cyanobacteria, descendants of the initial phototrophs, initiated the oxygenation of the atmosphere by producing oxygen as a byproduct of their fermentation. Over the next approximately half to 1 billion years, the Earth gradually became saturated with oxygen. Additionally, a portion of molecular oxygen was transformed into ozone, effectively absorbing the hazardous ultraviolet light from solar radiation. Prokaryotic organisms had to adapt to these new aerobic conditions, but on the other hand, they could now colonize previously uninhabitable territories. While some species perished, others migrated to environments where anaerobic conditions persisted, and others developed mechanisms to utilize oxygen to their advantage (Cooper 2000a, b).

Ultimately, the evolution of aerobic prokaryotes marked a crucial step toward the emergence of the first eukaryotes. The theory proposing the evolution of eukaryotic organisms through the serial symbiotic coexistence of prokaryotes gains significant support from research on mitochondria and chloroplasts. Mitochondria are believed to have evolved from bacteria that lived within larger cells. This is supported not

only by their similar type of division through binary fission but, more importantly, by research on the mitochondrial genome, confirming the bacterial ancestry of mitochondria (Gray 2012).

Theories about the phylogenetic origin of mitochondria vary, although most are grounded in genetic mapping. Many studies support an alphaproteobacterial origin, with an ancestor related to Rickettsiales (Wang and Wu 2015; Williams et al. 2007; Fitzpatrick et al. 2006; Sassera et al. 2011; Andersson et al. 1998; Rodríguez-Ezpeleta and Embley 2012; Ferla et al. 2013). Others propose that mitochondria evolved from free-living strains within alphaproteobacteria (Thrash et al. 2011; Brindefalk et al. 2011; Viklund et al. 2013). According to Martijn et al., mitochondria evolved from a proteobacterial lineage that branched off before the divergence of previously studied alphaproteobacteria (Martijn et al. 2018). In a more in-depth examination of endosymbioses, Zachar and Boza (2020) support the idea that mitochondria originated from an exploitative—parasitic or phagotrophic—interaction rather than a syntrophic one between a mitochondrial ancestor and its host. Both genomic and cell biology research have revealed that all known existing eukaryotes descended from a mitochondria-containing ancestor, the last eukaryote common ancestor (LECA), possessing most of the genetic and cellular characteristics of modern eukaryotes.

3.2 Structure and Function of Mitochondria

Mitochondria are cell organelles considered as markers of cellular health. Their function varies between cells, depending on their particular requirements for energy distribution, biosynthesis of metabolites, and cellular signaling (Glancy et al. 2020). Mitochondria are well-known as the powerhouse of the cell due to their role in the production of energy in the form of ATP through oxidative phosphorylation. Numerous metabolic pathways, such as β -oxidation, heme biosynthesis, certain phospholipids, and other metabolites, are located in mitochondria. They are considered signaling organelles associated with various physiological functions, including calcium homeostasis, iron-sulfur cluster synthesis, apoptosis (programmed cell death), participation in cellular aging, and developmental processes (Protasoni and Zeviani 2021; Shen et al. 2022; Scheffler 2008). For all cell functions to be fulfilled, mitochondrial function is optimized through the structural properties (Glancy et al. 2020). The term ‘mitochondrial dynamics’ encompasses the synchronized processes of fission and fusion, which ensure that mitochondria maintain their size, shape, and distribution. Transient and fast morphological customization occur due to key metabolic processes in the cells such as the cell cycle, immunity, apoptosis, and also the quality control of mitochondria itself (Tilokani et al. 2018).

Mitochondria are considered semi-autonomous organelles (Fig. 1) bounded by a double membrane system—the inner mitochondrial membrane (IMM) and the outer mitochondrial membrane (OMM). The IMM forms folds called cristae, increasing the surface area for chemical reactions. The IMM and OMM divide the entire organelle into compartments: the mitochondrial matrix and the intermembrane space, each with its specific role and protein components (Protasoni and Zeviani

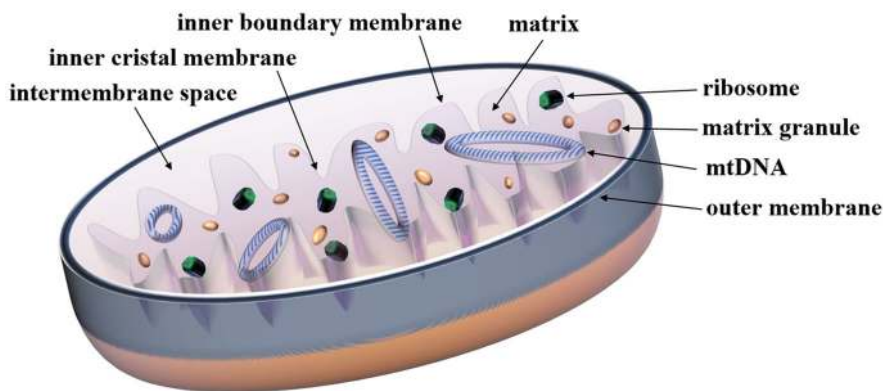


Fig. 1 Structure of mitochondria

2021; Glancy et al. 2020). The complex architecture of the double-bonded mitochondrial membrane was elucidated through advancements in the field of electron microscopy in the 1950s (Palade 1953). Each membrane has a different structure and function, differing significantly in their permeability. The OMM is porous, allowing ions and small molecules to pass through pore-forming membrane proteins called porins, like the voltage-dependent anion channel (VDAC). Due to its porosity, there is no membrane potential across the outer membrane (Protasoni and Zeviani 2021; Kühlbrandt 2015; Cooper 2000a, b). The OMM contains enzymes involved in various metabolic pathways, such as fatty acid elongation and enzymes involved in epinephrine oxidation and tryptophan degradation (specifically, fatty acid-CoA ligase, monoamine oxidase, kynurenine hydroxylase, and rotenone-insensitive NADH-cytochrome c-reductase) (Chipuk et al. 2006). The physical connection between the OMM and endoplasmic reticulum (ER) membrane, known as mitochondria-associated ER-membranes (MAMs), plays a vital role in lipid metabolism, autophagy, and Ca^{2+} transport between the ER and mitochondria (Yang et al. 2020).

The IMM is a functional barrier that allows specific ions and molecules to cross only through selective transporters, forming an electrochemical gradient used for ATP formation and the regulation of other ions' concentrations, such as calcium (important for cell signaling) (Protasoni and Zeviani 2021, Kühlbrandt 2015, Cooper 2000a, b). The inner mitochondrial membrane (IMM) is subdivided into two main regions. The first is the inner boundary membrane, which is smooth and parallel to the OMM. The second is the cristae membranes, forming deep folds that penetrate into the mitochondrial matrix. The inner boundary membrane houses membrane fusion machineries and protein translocation, playing a crucial role in communication with the cytosol and the transport of metabolites, such as pyruvate and fatty acids, between the mitochondria and cytosol. In contrast, the cristae membranes are specialized for oxidative phosphorylation, harboring the majority of electron transport chain complexes, ATP synthase dimers that consume and generate the proton-

motive force across the membrane to produce ATP, and proteins involved in iron-sulfur cluster assembly (Colina-Tenorio et al. 2020; Klecker and Westermann 2021; Cooper 2000a, 2000b; Glancy et al. 2020).

The mitochondrial matrix contains mitochondrial DNA (mtDNA), ribosomes, mitochondrial matrix granules, enzymes, small organic molecules, and inorganic ions. It is the site of organellar DNA replication, transcription, protein biosynthesis, and numerous enzymatic reactions. Mitochondrial DNA is a circular, double-stranded molecule of 16.6 kb, structurally more similar to prokaryotic genomes than nuclear DNA. mtDNA represents about 1% of total cellular DNA in humans. The number of copies per mitochondrion ranges from 5 to 10 (Habbane et al. 2021). mtDNA encodes 37 coding genes, including genes for 2 rRNA, 22 tRNA, and 13 polypeptides responsible for subunits of the electron transport chain and ATP synthase. Other mitochondrial peptides are encoded by nuclear genes, synthesized on ribosomes in the cytosol, and imported into mitochondria. Coordinated regulation between mitochondrial and nuclear genomes and protein import into mitochondria are crucial for mitochondrial biogenesis and maintenance (Sherratt 1991; Taanman 1999; van den Heuvel and Smeitink 2001; Lin et al. 2023). The presence of mitochondrial matrix granules was initially documented by Palade (1953) and subsequently confirmed by Sjostrand and Rhodin (1953) in mitochondria isolated from liver and kidney. Despite limited information on the composition of these granules, available data suggest that mitochondrial matrix granules contain phospholipids (e.g., cardiolipin), glycoproteins or lipids, calcium-precipitable lipoprotein, and cytochrome c oxidase. Regarding their function, two hypotheses exist. The first posits that the granules serve as a storage site for cations while simultaneously regulating their concentrations. The second hypothesis suggests that these granules may act as precursors to the inner mitochondrial membrane during mitochondrial fusion (Jacob et al. 1994).

Mitochondrial ribosomes (mitoribosomes), attached to membranes, synthesize proteins inside mitochondria. During evolution, mitoribosomes specialized for synthesizing mitochondrial membrane proteins, resulting in major changes in their structure and composition. In human cells, they produce hydrophobic membrane protein subunits that integrate directly into the inner mitochondrial membrane after translation (Greber and Ban 2016; Kühlbrandt 2015). Enzymes in the mitochondrial matrix play a key role in the crucial reactions of oxidative metabolism. Processes within the matrix encompass the citric acid cycle (TCA or Krebs cycle) and the enzymatic reactions initiated by the conversion of pyruvate and fatty acids into acetyl coenzyme A. Pyruvate and fatty acids are transported into the mitochondria from the cytoplasm via membrane-bound permeases. Acetyl-CoA is subsequently oxidized through the citric acid cycle, resulting in the production of carbon dioxide and the reduced coenzymes NADH (nicotinamide adenine dinucleotide) and FADH₂ (flavin adenine dinucleotide). NADH and FADH₂ serve as electron sources for the electron transport chain within the inner mitochondrial membrane, constituting a metabolic pathway that culminates in ATP formation (Cooper 2000a, b; Kühlbrandt 2015; Gray 2013).

3.3 Mitochondrial Dynamics

Mitochondrial dynamics is an essential feature of mitochondria that plays a crucial role in monitoring the quality of mitochondria, ensuring proper mitochondrial function, and responding to cellular needs and the cell’s metabolic state. It involves two genetically regulated processes, fission, and fusion, which are usually balanced (Fig. 2). Any imbalance in the expression levels of these processes can result in morphological changes in mitochondria. Recent research suggests that the molecular mechanisms underlying fission and fusion are coordinated at the contact points between mitochondria and the endoplasmic reticulum (Annesley and Fisher 2019; Glancy et al. 2020; Wang et al. 2022).

Mitochondrial fission involves splitting a single mitochondrion into two daughter mitochondria, contributing to organelle inheritance during the process of cell division; it is crucial for the proper distribution of mitochondria. This process is also important in the release of cytochrome c during apoptosis. Fission also facilitates the removal of damaged organelles through mitophagy (Adebayo et al. 2021). On the other hand, mitochondrial fusion is defined as the merging of two mitochondria into one, resulting in a mixing of their contents. It is worth mentioning that fusion can occur within one mitochondrion when a circular morphology occurs (Chan 2020). Dysregulation of these processes leads to either a fragmented network, characterized by numerous small, round-shaped mitochondria, or a hyperfused network with

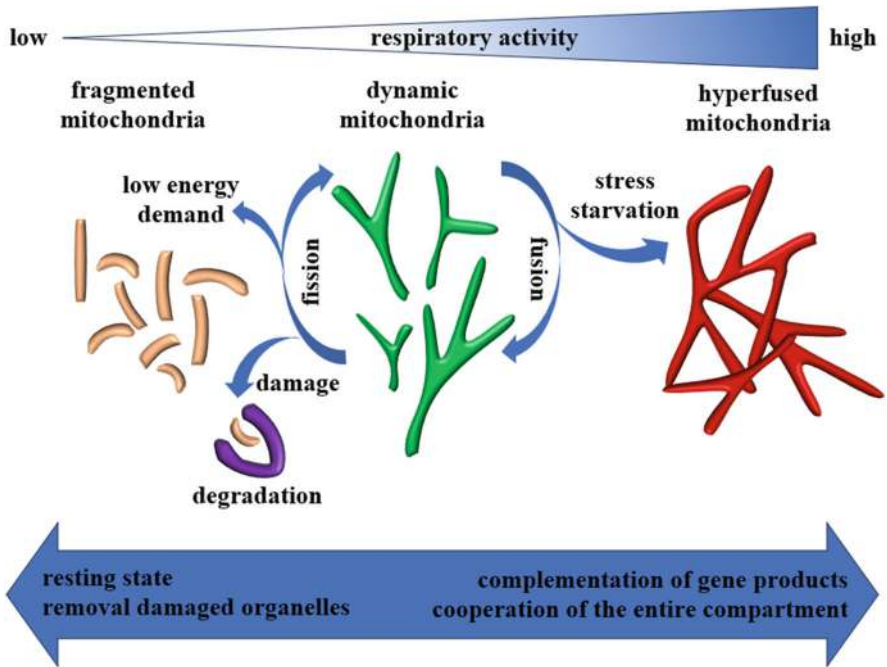


Fig. 2 Mitochondrial dynamics. (Adjusted according to Klecker and Westerman 2021)

elongated, highly interconnected mitochondria. Various morphological conditions are associated with physiological and pathophysiological states. For instance, mitochondrial fragmentation is linked to dysfunction, observed during elevated stress levels and cell death (Tilokani et al. 2018). The fusion-fission cycle is regulated by a conserved mechanism involving a set of large GTPase proteins from the Dynamin family located on IMM and OMM. Mitofusins 1/2 (MFN1/2) and optic atrophy 1 (OPA1) drive the fusion process, while dynamin-related protein 1 (DRP1) and dynamin-2 (DNM2) mediate fission (Wang et al. 2022; Ferguson and De Camilli 2012).

Mitochondrial fusion comprises two sequential processes: OMM fusion and subsequently IMM fusion. MFNs are inserted into the OMM through two transmembrane domains that are separated by a short loop. This exposes an N-terminal region containing the GTPase and coil-coil heptad repeat 1 (HR1) domain and a C-terminal region containing the HR2 domain, both directed toward the cytosol (Tilokani et al. 2018). OMM fusion is followed by IMM fusion, which is regulated by OPA1, potentially cooperating with MFN to orchestrate the fusion of both mitochondrial membranes. OMM fusion has been demonstrated even in OPA1-deficient cells; however, the loss of OPA1 leading to abortive IMM fusion results in the fragmentation of the mitochondrial network (Ge et al. 2020; Adebayo et al. 2021). IMM fusion is controlled by OPA1 and a specific IMM lipid component, such as cardiolipin. ATP-dependent zinc metalloprotease (YME1L1) and ATP-independent metalloendopeptidase (OMA1) are two IMM-bound proteases accountable for OPA1 proteolysis. Following proteolysis, the long form of OPA1 (L-OPA1) interacts with cardiolipin on opposite sides of the mitochondrial membranes, serving to bind the IMM (Ban et al. 2017). DRP1 plays a crucial role in mitochondrial fission. Upon recruitment from the cytosol to the OMM, DRP1 forms a ring structure around mitochondria, initiating membrane narrowing and subsequent GTP-dependent constriction. The assembly of DRP1 in the OMM is facilitated by a central domain, giving rise to DRP1-oligomeric helices from two distinct membrane points (Tilokani et al. 2018). Endoplasmic reticulum (ER) tubules have a pivotal role during fission, contacting mitochondria to constrict their diameter from approximately 300–500 nm to around 150 nm. This facilitates DRP1 recruitment and the formation of DRP1 oligomeric rings. ER-bound inverted-formin 2 (INF2) at ER-mitochondria contact sites, in collaboration with the mitochondria-anchored formin-binding Spire1C, regulates actin assembly crucial for mitochondrial constriction before DRP1 recruitment and oligomerization (Adebayo et al. 2021). DRP1 is necessary but not sufficient for mitochondrial fission because receptors must recruit the enzyme to the OMM. Three other membrane proteins have an important role in the recruitment of DRP1 to mitochondria, namely mitochondrial fission factor (MFF) and mitochondrial dynamic proteins MID49 and MID51 (Kalia et al. 2018). Subsequently, DRP1 undergoes oligomerization, creating a helical structure. IMM constriction is hypothesized as a spontaneous and recurring event, serving as the priming mechanism for fission, independent of the OMM (Wang et al. 2022).

Mitochondrial dynamics are pivotal in various physiological conditions, during cell development, and aging. Distorted mitochondrial dynamics, altered morphology, and dysfunction are observed in various pathological conditions. For instance,

cancer cells exhibit more fragmented mitochondria with impaired mitochondrial capacity. Irregular mitochondrial shapes during pathological conditions are recognized as key factors and valuable targets for pharmaceutical treatment (Glancy et al. 2020).

3.4 Mitochondrial Networking

Mitochondria are characterized by highly dynamic properties, including the two important processes mentioned above—fusion and fission, resulting in various mitochondrial morphologies ranging from fragmented states to forming a continuous mitochondrial network. Consequently, mitochondria can manifest as discrete, isolated organelles or part of an extensive, continuous reticulum. The cellular benefits of these fused mitochondrial networks remain unclear (Hoitzing et al. 2015). Current knowledge suggests that the mitochondrial network forms through the fusion of individual mitochondria, playing a crucial role in maintaining mitochondrial DNA integrity and facilitating the exchange of mitochondrial materials (Wang and Wu 2015). Disturbances in the mitochondrial network can impact essential mitochondrial functions, including ATP production, metabolic integration, calcium homeostasis, apoptosis, and, last but not least, the response to cellular stress. All these disturbances can eventually lead to abnormal development as well as the occurrence of various human diseases (Du et al. 2021; Wang et al. 2015).

4 Mitochondria in Cancer

Mitochondria play a crucial role in cancer metabolism, serving as the focal point for the proliferation, survival, and metastasis of tumor cells due to their bioenergetic and biosynthetic functions. The ETC, a series of complexes on the IMM, is responsible for ATP generation through OXPHOS. Proteins and complexes within the ETC and OXPHOS have been identified as targets for cancer treatment, leading to the development of several inhibitors in recent years. Mitochondria serve as the primary cellular source of NADH and are integral to pyrimidine and lipid biosynthetic pathways, contributing to various aspects of cancer cell metabolism. Additionally, numerous intermediates in the TCA cycle play critical roles in cancer cell metabolism. Overall, mitochondria emerge as key organelles influencing tumor proliferation, survival, metastasis, and drug resistance (Liu et al. 2023).

Mutations in mtDNA and nuclear genes related to the TCA cycle are frequently observed in cancer cells, contributing to the remodeling of cancer metabolism. These mutations, found in oncogenes and tumor suppressor genes such as phosphatidylinositol 3-phosphate kinase (PI3K)-AKT, MYC, RAS, and hypoxia-inducible factor 1 (HIF-1), lead to the production of oncogenic metabolites crucial for tumor initiation. Additionally, these mutations impact mitochondrial function, causing alterations in mitochondrial metabolic pathways such as OXPHOS, fatty acid metabolism, and glutamine utilization, facilitating tumor transformation. Metabolic reprogramming stands out as a pivotal factor

in both tumor initiation and maintenance, being recognized as one of the hallmarks of cancer. Recent research underscores the significance of mitochondria as a promising target for cancer therapy (Liu et al. 2023).

4.1 Mitochondrial Dynamics Alteration in Cancer

The structure and function of mitochondria vary significantly in different types of cells and tissues. An imbalance in mitochondrial dynamics is accompanied by various human diseases, which are characterized by impaired mitochondrial function and, at the same time, increased cell death. Notably, significant changes in mitochondrial dynamics occur during the cellular stress response. Cancer is one disease where alterations in both the shape and function of mitochondria have been observed. Mitochondrial fragmentation is linked to metabolic changes typical of tumor cells in many cancer cells. This fragmentation facilitates rapid growth and survival. However, promoting a more interconnected mitochondrial network could potentially slow growth and sensitize these cells to programmed cell death (Whitley et al. 2019).

Mitochondrial fusion increases connections among mitochondria, simultaneously enhancing the OXPHOS capacity to meet the energy needs of cells. When fusion occurs between healthy and damaged mitochondria, it reduces metabolic stress, minimizes mitochondrial fragmentation, and preserves mitochondrial function. This implies that mitochondrial dynamics can contribute to reducing cellular stress induced by mtDNA mutation. Conversely, a high level of mitochondrial fission may result from metastatic potential mediated by mtDNA depletion. Mitochondrial fission contributes to the poor prognosis of various cancer types, promoting increased proliferation, metastasis, and chemoresistance, ultimately advancing cancer progression to malignancy (Wang et al. 2023). Therefore, mitochondrial dynamics presents a potential target in cancer treatment. Over the last decades, numerous studies have been conducted to uncover the molecular changes and functioning of the mitochondrial fusion/fission mechanism in tumors. Mitochondrial dynamics plays a crucial role in cancer progression, where mitochondria often exhibit a fragmented pattern (fission) within different types of tumor cells, associated with upregulation of DRP1. Increased mitochondrial dynamics, specifically mitochondrial fission observed in tumor cells, is linked to metabolic reprogramming, cell cycle progression, and enhanced migration, invasiveness, and metastatic capacities. Consequently, inhibiting DRP1 is anticipated to reduce tumor proliferation (Rodrigues and Ferraz 2020). DRP1 is considered a critical factor in tumor initiation and progression, and it is highly expressed in various human cancers, including breast, lung, pancreatic, colon, cutaneous squamous cell carcinoma, and glioblastoma. Activation of DRP1 correlates with a poor prognosis in glioblastoma. In extramammary Paget's disease, a positive correlation between DRP1 and clinical stages suggests DRP1 as a potential therapeutic target (Huang et al. 2022).

4.2 mtDNA Alteration in Cancer

Mitochondrial DNA encodes proteins and RNA essential for mitochondrial function, as well as cellular homeostasis. Modifications in mtDNA are associated with various diseases, and mtDNA mutations contribute to the potential of a cell to become cancerous. Therefore, mtDNA mutations are considered biomarkers for certain types of tumors. Abnormalities in mtDNA, including deletions, inversions, point mutations, and changes in copy number, are linked to a wide variety of cancer types. These changes in mtDNA influence the proliferation, progression, and metastasis of cancer cells, as well as their interactions with the immune system and the tumor microenvironment (TME). MtDNA plays a crucial role in cancer development and appears to be a promising target for cancer treatment. Mutations in mtDNA may decrease the efficiency of the ETC and lead to higher production of ROS. Studies have demonstrated that the OXPHOS system and OXPHOS-related superoxide significantly influence tumor progression (Lin et al. [2023](#); van Gisbergen et al. [2015](#)).

Even in certain types of tumors, variations in the mtDNA sequence are quite common. According to the study, two types of cancer variants of mtDNA can be characterized: firstly, *de novo* mutations, which can be considered initiators of carcinogenesis, and secondly, functional variants, which act as adaptors, enabling cancer cells to thrive in different environments.

These two mentioned mtDNA variants may have different origins; they can belong to the group of inherited variants that occur in families or be somatic mutations arising in individual cells. Alternatively, they may be groups of variants associated with ancient mtDNA lineages, enabling adaptation to changing tissue or geographic environments. The number of mtDNA copies and possibly nuclear transfer of mtDNA sequences also contribute to certain cancers. Alterations in mitochondrial genes encoded by nuclear DNA have confirmed the importance of mitochondrial metabolism in cancer. Therefore, subtle changes in mitochondrial genotype can have profound effects on the nucleus, as well as on carcinogenesis and cancer progression (Kopinski et al. [2021](#)). Mutations in nuclear genes encoding mitochondrial components that are associated with an increased risk of cancer include, for example, succinate dehydrogenase (SDHB, SDHC, and SDHD genes) and isocitrate dehydrogenase (IDH1 and IDH2 genes) (van Gisbergen et al. [2015](#)).

Lin et al. ([2023](#)) provided a comprehensive review of recent progress in mtDNA-targeted cancer treatment strategies, categorizing them into two types based on their mechanisms of action: indirect and direct targeting strategies. Indirect strategies aim to induce mtDNA damage and dysfunction by modulating pathways involved in mtDNA stability and integrity. On the other hand, direct strategies involve the use of molecules that can selectively bind or cleave mtDNA to achieve therapeutic efficacy. In the realm of indirect strategies, it is possible to target mtDNA by stimulating ROS, inhibiting POL γ and TFAM (mitochondrial transcription factor A), and activating the cGAS-STING pathway. Pathways responsible for mtDNA stability and integrity are affected, resulting in mtDNA damage and dysfunction.

Lin et al. (2023) categorized indirect mtDNA-targeted cancer therapy into three groups: the first group includes increasing ROS, the second one includes influencing mtDNA genetic functions, and the third one includes activating immunity. Disrupting these aspects can further damage mtDNA, leading to tumor suppression through mitochondrial dysfunction. With direct targeting of mtDNA, it is possible to avoid the effect on nuclear genes and to face the challenge of preventing the effect on nuclear genes, which may be an advantage of this strategy. Recently, mtDNA-targeted therapy has been applied in clinical settings using mitochondrial localization molecules to modulate mtDNA function directly. Following Lin et al.'s (2023) categories for indirect cancer therapy targeting mtDNA, the stimulation of ROS can induce mtDNA defects and mitochondrial dysfunction, leading to apoptosis. This property is utilized in various studies investigating cancer therapy. Substances that induce mtDNA target inhibition and mitochondrion-mediated apoptosis include the Ir(III)-2H-indazole complex with an isopropyl substituent, which induces toxicity in cancer cell lines by contributing to the effects of ROS-induced mitochondrial disruption along with chromosomal and mitochondrial DNA target inhibition (Panchangam et al. 2021), then VB12-sericin-PBLG-IR780 nanomicelles, which showed brilliant antitumor effect and could activate DC maturation, initiate T-cell recruitment, and enhance adaptive antitumor efficiency (Guo et al. 2022), and others is the antiviral drug Acyclovir which causes damage of mitochondrial DNA, reduces mitochondrial membrane potential, and alters mitochondrial shape and size (Benedetti et al. 2022). Among compounds influencing mtDNA genetic functions is an inhibitor of Pol γ - 3,3'-[(1,1'-Biphenyl)-4',4'-(diyl)bis(azo)]bis[4-amino-1-naphthalenesulfonic acid]—as a high-affinity binder to the Pol γ protein and potent inhibitor of the Pol γ strand displacement and one-nucleotide incorporation DNA synthesis activities in vitro and in vivo (Somuncu et al. 2022), and also the antiviral drug zalcitabine causing TFAM degradation (Li et al. 2021). The effect of the combination of cisplatin and epoxomicin, which inhibits TFAM, is also described (Franco et al. 2018; Kong et al. 2023). Additionally, the combination of violacein and silver nanoparticles showed anticancer activity by inhibiting TFAM (Pandey and Verma 2020).

In the activation of immunity, one antitumor treatment method involves using nanocarrier systems that can activate innate immune response pathways while inducing damage and release of mtDNA. The carrier system helps solve the problem of preventing nuclear genes from being affected when targeting mtDNA. Nanocarrier systems are used to target mtDNA specifically, and common carriers include molecules like cyanine and triphenylphosphonium (TPP) (Wang et al. 2020; Chen et al. 2020). Metal complexes containing elements such as Fe, Ir, Ru, and Zn play a significant role in nanosystems targeting mtDNA formation. Phototherapy, also known as light therapy, is a standard treatment that uses light waves to treat certain skin conditions. In cancer treatment, it uses light for drugs/materials activation, which then has the ability to destroy tumor cells due to targeting mtDNA, which is crucial for the survival and proliferation of tumors. Promising cancer phototherapies include photodynamic therapy (PDT), photothermal therapy (PTT), and photochemotherapy (PCT) (Lin et al. 2023).

4.3 Electron Transport Chain and OXPHOS Alteration in Cancer

In human cells, three metabolic pathways are involved in energy production: OXPHOS, glycolysis, and fatty acid oxidation. Deregulation of cellular energetics has been recognized as a prominent characteristic of cancer. The OXPHOS metabolic pathway, located in the mitochondria, serves as a center for both catabolic and anabolic metabolism. It diverts carbon from glucose for macromolecular synthesis while simultaneously providing bioenergetic requirements in the form of ATP. In the OXPHOS process, enzymes of the TCA cycle and transmembrane protein complexes in the ETC play crucial roles. In the TCA cycle, the electron donors NADH and FADH₂ are generated for the ETC complexes (Greene et al. 2022).

The electron transport chain located in the inner mitochondrial membrane represents a series of four protein complexes, which transfer electrons from their donors to acceptors in redox reactions (Fig. 3). ETC complexes are among the largest protein-membrane complexes in the cell.

Complex I (NADH—ubiquinone oxidoreductase) represents the entrance gate of electrons to the respiratory chain. Complex I contains 44 subunits, seven of which are encoded by the mitochondrial genome and the others by the nuclear genome (Giachin et al. 2016). In complex I, two electrons from NADH reduce ubiquinone to ubiquinol, with the simultaneous transport of four protons to the IMS. Complex II (succinate dehydrogenase) plays a dual role in the ETC. It catalyzes the oxidation reaction of succinate to fumarate in the Krebs cycle and simultaneously transfers

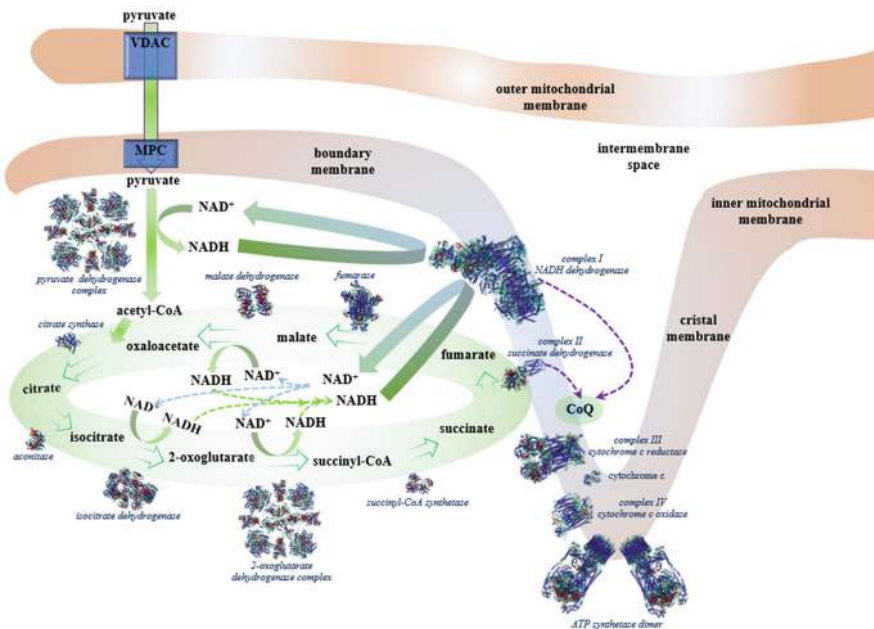


Fig. 3 Electron transport chain

electrons from succinate to ubiquinone in the ETC, and therefore, is considered as the second entrance gate of electrons (Bandara et al. 2021). It is the only respiratory chain complex that does not transfer protons through the IMM. Coenzyme Q (ubiquinone, CoQ) is a mobile electron carrier in the respiratory chain. It transfers electrons from complexes I and II to complex III of the respiratory chain (Ahmad et al. 2023). Complex III (ubiquinol—cytochrome c reductase) connects the transfer of electrons from ubiquinol (QH₂, reduced CoQ) to cytochrome c; simultaneously, four protons are translocated into the IMS. The process of electron transfer by complex III is carried out by the Q-cycle (Sun and Trumpower 2003; Chandel 2010).

Complex IV (cytochrome c oxidase) is the terminal oxidase of the respiratory chain; it transfers electrons from cytochrome c to the final acceptor—oxygen, which is reduced to H₂O, and at the same time transfers two protons from the mitochondrial matrix to the IMS. Protons that are transferred into the IMS (through complexes I, III, and IV) create an electrochemical gradient. This proton gradient drives ATP formation through ATP synthase, known as complex V of the electron transport chain (Ahmad et al. 2023).

Cancer development is linked to defects in energy production within the OXPHOS metabolic pathway in mitochondria. OXPHOS is responsible for generating the majority of energy required for normal cellular function. Any deficiencies in mitochondrial function, mitochondrial structure, or mitochondria number result in altered cell metabolism from an energetic point of view. Over time, this leads to the utilization of energy production by fermentation, which is why aerobic fermentation to lactic acid, known as the Warburg effect, is often observed in cancer cells. Another potential compensatory mechanism is substrate-level mitochondrial phosphorylation. Flawed OXPHOS, coupled with compensatory fermentation, generates ROS, which are associated with mutagenic and carcinogenic properties (Gyamfi et al. 2022).

ROS are primarily generated as byproducts of OXPHOS, accounting for approximately 90% of ROS production. Traditionally, complex I was considered the primary source of ROS. However, in many recent publications is mentioned that complex III is also a significant contributor to ROS generation. The literature now define the mechanism of this process, indicating that ROS are formed through both direct and reverse electron transfer. Although complex II was traditionally not viewed as a major ROS producer, recent evidence suggests that under specific physiological conditions, particularly in the presence of excess succinate, complex II can indeed produce ROS (Kausar et al. 2018).

A growing body of evidence indicates that certain cancers heavily rely on OXPHOS, and numerous studies have demonstrated the effectiveness of OXPHOS inhibition in targeting these cancer subtypes (Ashton et al. 2018). Since the majority of ATP in tumor cells is generated in the mitochondria, targeting this energy metabolic pathway holds promise for cancer therapy. Cells at the core of solid tumors survive in a nutrient-poor environment with reduced glucose and oxygen levels. Blocking mitochondrial ATP production through targeted drugs can induce apoptosis in these cells. For tumors highly dependent on oxidative phosphorylation, such as skin melanoma, drugs inhibiting mitochondrial ATP production can be

employed, leading to apoptosis in the targeted tumor cells. Another approach in metabolically directed therapy involves inhibitors of the intracellular PI3K signaling pathway, which synergize with glycolysis-reducing therapies. In such therapeutic scenarios, tumor cells selectively take up inhibitors of mitochondrial ATP production (Neagu et al. 2019).

To date, numerous compounds with therapeutic potential are being studied as OXPHOS inhibitors (Table 2), either in vivo, in the clinic, or in vitro (Ashton et al. 2018). One of the most promising inhibitors of OXPHOS is metformin, known as an antidiabetic drug and an inhibitor of complex I, effectively reducing OXPHOS and subsequently tumor growth in various cancer models. An alternative compound to metformin is phenformin, another biguanide easily transported into cancer cells. Phenformin exhibits higher affinity to the mitochondrial membrane compared to metformin (Greene et al. 2022; Kim and Cheong 2020). The group of complex I inhibitors also includes molecules such as BAY 87-2243, Carboxyamidotriazole (CAI), ME-344, OPB-111,077, mIBG, and IACS-010,759. BAY 87-2243 is a potent and selective small molecule with antitumor activity in vivo and in melanoma cell lines. CAI, a cytostatic inhibitor, acts as an agonist of non-voltage-gated calcium channels, inhibiting angiogenesis, tumor growth, and metastatic potential. ME-344, a synthetic small molecule, is a demethylated metabolite of an isoflavone derivative. OPB-111,077 inhibits STAT3, leading to mitochondrial STAT3-mediated inhibition of complex I and complex II. mIBG, a tumor-targeted radiopharmaceutical, inhibits both complexes I and III. IACS-010,759 is a potent oral selective inhibitor of mitochondrial complex I (Greene et al. 2022; Ashton et al. 2018).

Several drugs used for lipid metabolism, including statins, fibrates, and thiazolidinediones, have been shown to inhibit OXPHOS. Fenofibrate, in particular, targets complex I of the ETC (Greene et al. 2022; Ashton et al. 2018). Mitochondrial poisons of complex I include rotenone, piericidin A, and MPTP (Kim and Cheong 2020; Ashton et al. 2018; Liu and Shi 2020).

For complex II inhibitors, relatively few well-characterized ones exist, including ionidamine, the vitamin E analog α -tocopheryl succinate (α -TOS), and malonate (Ashton et al. 2018; Kim and Cheong 2020).

Complex III inhibitors include atovaquone, mIBG, antimycin A, myxothiazol, and stigmatellin. Atovaquone, a hydroxy-1,4-naphthoquinone analog of ubiquinone, is among them. Antimycin A, myxothiazol, and stigmatellin are classical mitochondrial poisons (Ashton et al. 2018; Greene et al. 2022; Kim and Cheong 2020).

Complex IV inhibitors include arsenic trioxide, NO, hydrocortisone, cyanide, azide, and CO (Ashton et al. 2018; Kim and Cheong 2020).

Targeting ATP synthase (complex V) appears to be another suitable therapeutic option. Oligomycin is a well-known inhibitor of this complex (Greene et al. 2022; Kim and Cheong 2020; Ashton et al. 2018; Liu and Shi 2020).

A recent glioblastoma study identified a new OXPHOS inhibitor, gboxin, which selectively inhibits the oxidative phosphorylation in cancer cells (Shi et al. 2019). There is also evidence that bedaquiline inhibits yeast and human mitochondrial ATP synthase (Luo et al. 2020).

Table 2 Inhibitors of OXPHOS

Complex of ETC	Inhibitor	References
Complex I	Metformin	Greene et al. (2022), Kim and Cheong (2020), and Ghosh et al. (2020)
	Phenformin	Greene et al. (2022), Kim and Cheong (2020)
	BAY 87–2,243	Greene et al. (2022) and Ashton et al. (2018)
	Carboxyamidotriazole (CAI)	Greene et al. (2022) and Ashton et al. (2018)
	ME-344	Greene et al. (2022) and Ashton et al. (2018)
	OPB-111,077	Greene et al. (2022) and Ashton et al. (2018)
	mIBG (meta-iodobenzylguanidine)	Greene et al. (2022) and Ashton et al. (2018)
	IACS-010,759	Greene et al. (2022) and Ashton et al. (2018)
	Rotenon	Kim and Cheong (2020), Ashton et al. (2018), and Liu and Shi (2020)
	Piericidin A	Kim and Cheong (2020), Ashton et al. (2018), and Liu and Shi (2020)
	MPTP	Kim and Cheong (2020), Ashton et al. (2018), and Liu and Shi (2020)
Complex II	OPB-111,077	Greene et al. (2022) and Ashton et al. (2018)
	Lonidamine	Ashton et al. (2018) and Kim and Cheong (2020)
	α -TOS (α -tocopheryl succinate)	Ashton et al. (2018) and Kim and Cheong (2020)
	Malonate	Ashton et al. (2018) and Kim and Cheong (2020)
Complex III	Atovaquone	Ashton et al. (2018), Greene et al. (2022), and Kim and Cheong (2020)
	mIBG (meta-iodobenzylguanidine)	Ashton et al. (2018), Greene et al. (2022), and Kim and Cheong (2020)
	Antimycin A	Ashton et al. (2018), Greene et al. (2022), and Kim and Cheong (2020)
	Myxothiazol	Ashton et al. (2018), Greene et al. (2022), and Kim and Cheong (2020)
	Stigmatellin	Ashton et al. (2018), Greene et al. (2022), and Kim and Cheong (2020)
Complex IV	Arsenic trioxide	Ashton et al. (2018) and Kim and Cheong (2020)
	NO	Ashton et al. (2018) and Kim and Cheong (2020)
	Hydrocortisone	Ashton et al. (2018) and Kim and Cheong (2020)
	Cyanide	Ashton et al. (2018), and Kim and Cheong (2020)
	Azide	Ashton et al. (2018), and Kim and Cheong (2020)
Complex V	CO	Ashton et al. (2018), and Kim and Cheong (2020)
	Oligomycin	Greene et al. (2022), Kim and Cheong (2020), Ashton et al. (2018), and Liu and Shi (2020)
	Gboxin	Shi et al. (2019)
	Bedaquiline	Luo et al. (2020)

4.4 TCA Cycle Alteration in Cancer

The TCA cycle (Fig. 3) is considered the bioenergetic center of cellular metabolism, biosynthesis, and redox balance. It serves as a metabolic pathway for the final oxidation of carbohydrates, proteins, and fats (Ghosh et al. 2020). Acetyl-CoA, the molecule entering this cycle, can originate from various sources—either from pyruvate in glycolysis, beta-oxidation of fatty acids, or the metabolism of ketogenic amino acids and ketones. In a series of reactions, the TCA cycle generates reduced coenzymes (NADH and FADH₂), which are subsequently oxidized by the electron transport chain to produce adenosine triphosphate (ATP) (Bender 2003). Additionally, the cycle yields molecules crucial for other metabolic pathways, including gluconeogenesis, lipid synthesis, nucleotide synthesis, and protein synthesis (Grasso et al. 2020). Anaplerotic reactions, such as glutaminolysis (producing α -ketoglutarate from glutamine) and pyruvate carboxylation (producing oxaloacetate from glucose-derived pyruvate), are required to replenish the molecules and sustain the TCA cycle. Maintaining a balance between energy production and biosynthetic pathways is essential to ensure a steady supply of metabolic intermediates during periods of low or high energy consumption (Sainero-Alcolado et al. 2022).

The TCA cycle holds a special position in the metabolism of the human body, making the inhibition of various aspects of altered bioenergetic metabolism an attractive therapeutic strategy for cancer treatment (Ghosh et al. 2020). There is increasing evidence suggesting that mutations in genes encoding enzymes of the TCA cycle and abnormal accumulation of TCA cycle intermediates may promote carcinogenesis (Grasso et al. 2020). Recent findings indicate that mutations in TCA coding genes, particularly isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and fumarate hydratase (FH), may contribute to carcinogenesis (Ghosh et al. 2020; Sainero-Alcolado et al. 2022; Marquez et al. 2019; Grasso et al. 2020). These mutations result in the simultaneous abnormal accumulation of various metabolites (D-2-hydroxyglutarate, succinate, and fumarate), collectively referred to as oncometabolites. This accumulation leads to the deregulation of signaling and, concurrently, the promotion of cancer progression (Todisco et al. 2019; Liu and Shi 2020).

The IDH family consists of three isoforms: IDH1, IDH2, and IDH3, catalyzing the oxidative decarboxylation of isocitrate to α -KG (Sainero-Alcolado et al. 2022; Todisco et al. 2019). Mutations in the isocitrate dehydrogenase 1 and 2 genes (IDH1 and IDH2) have been identified in various cancers, making them two of the most often mutated metabolic genes in cancer (Ghosh et al. 2020; Liu and Shi 2020). IDH1 and IDH2 point mutations result in a gain-of-function, converting α -KG to the oncometabolite 2-hydroxyglutarate (2-HG), leading to the accumulation of this oncometabolite (Liu and Shi 2020; Sainero-Alcolado et al. 2022).

Succinate dehydrogenase (SDH) is a hetero-tetrameric protein, consisting of four subunits (SDHA, SDHB, SDHC, and SDHD). Deficiency in this enzyme activates tumor formation through the dysregulation of HIF activity (Marquez et al. 2019; Sainero-Alcolado et al. 2022). SDH is the only complex involved in both the TCA

cycle and ETC. It constitutes complex II of the ETC and catalyzes the oxidation of succinate to fumarate in the TCA cycle, simultaneously reducing FAD to FADH₂, followed by the transfer of electrons to ubiquinone (Anderson et al. 2018). Mutations in genes encoding different SDH subunits are associated with the accumulation of succinate and are the cause of hereditary paraganglioma and pheochromocytoma (Baysal et al. 2000).

Fumarate hydratase (FH) is a homotetrameric enzyme encoded by the FH gene, present in both cytosolic and mitochondrial forms. In mitochondria, as part of the TCA cycle, FH catalyzes the hydration and dehydration of fumarate to malate. In the cytoplasm, FH is involved in the urea cycle, as well as nucleotide and amino acid metabolism (Marquez et al. 2019; Anderson et al. 2018; Todisco et al. 2019).

The increased accumulation of fumarate in mitochondria inhibits prolyl hydroxylases, responsible for regulating HIF-1 α degradation (Marquez et al. 2019). Accumulated fumarate also acts as an oncometabolite, inhibiting α -KG-dependent dioxygenases involved in DNA and histone demethylation (Grasso et al. 2020). While all intermediates of the TCA cycle are present throughout the body, high levels of succinate and fumarate are detected in some tumors due to mutations in *SDH* and *FH* (Todisco et al. 2019; Liu and Shi 2020). Both *SDH* and *FH* are considered tumor suppressor genes (Sainero-Alcolado et al. 2022).

TCA cycle inhibitors have shown potential in cancer treatment, and therapeutic targeting of TCA cycle enzymes seems to be a promising strategy. Among the inhibitors of IDH, which is responsible for 2-HG accumulation, are Enasidenib (IDH1-mutant inhibitor) and Ivosidenib (IDH2-mutant inhibitor). Both reduce the level of 2-HG and have been approved by the FDA as first-in-class drugs for IDH-mutated acute myeloid leukemia (Golub et al. 2019; Stein et al. 2017; Dang and Su 2017; Anderson et al. 2018).

5 Conclusion

Mitochondria are crucial organelles in eukaryotic cells, serving as the cell's energy center. They play a pivotal role in key cellular processes, including the generation of ATP through oxidative phosphorylation, cell signaling, and, notably, apoptosis—cell death. Simultaneously, mitochondria are recognized as the primary producers of reactive oxygen species (ROS), by-products of the respiratory chain. While low concentrations of ROS are beneficial for the organism, excessive production can damage vital biological macromolecules (such as nucleic acids, lipids, and proteins), disrupting their function, causing cell damage, and ultimately leading to apoptosis. Mitochondrial dysfunction contributes to the pathology of various diseases, namely cardiovascular diseases, neurodegenerative diseases, metabolic diseases, and, last but not least, cancer. Consequently, mitochondria represent crucial targets for numerous drugs. Researchers are increasingly focusing on mitochondrial metabolism, encompassing electron transport chain function, redox signaling pathways, and ROS homeostasis. Understanding the underlying mechanisms of mitochondrial

disorders remains a significant challenge, posing obstacles to proper diagnosis and treatment.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

References

- Adebayo M, Singh S, Singh AP, Dasgupta S (2021) Mitochondrial fusion and fission: the fine-tune balance for cellular homeostasis. *FASEB J* 35(6):e21620. <https://doi.org/10.1096/fj.202100067R>
- Ahmad M, Wolberg A, Kahwaji CI (2023) Biochemistry, electron transport chain. [Updated 2022 Sep 5]. In StatPearls [internet]. StatPearls Publishing, Treasure Island. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK526105/>
- Akram M (2013) Mini-review on glycolysis and cancer. *J Cancer Educ* 28(3):454–457. <https://doi.org/10.1007/s13187-013-0486-9>
- Altmann R (1890) Die Elementarorganismen und ihre Beziehungen zu den Zellen [Elementary Organisms and Their Relations to Cells] (in German). Veit & Co, Leipzig, p 125
- American Cancer Society (ACS). <https://www.cancer.org/cancer/types/cancer-in-children/types-of-childhood-cancers.html>. Available o 12 Oct 2023
- Anderson NM, Mucka P, Kern JG, Feng H (2018) The emerging role and targetability of the TCA cycle in cancer metabolism. *Protein Cell* 9(2):216–237. <https://doi.org/10.1007/s13238-017-0451-1>
- Andersson SG, Zomorodipour A, Andersson JO, Sicheritz-Pontén T, Alsmark UC, Podowski RM, Näslund AK, Eriksson AS, Winkler HH, Kurland CG (1998) The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* 396(6707):133–140. <https://doi.org/10.1038/24094>
- Annesley SJ, Fisher PR (2019) Mitochondria in health and disease. *Cells* 8(7):680. <https://doi.org/10.3390/cells8070680>
- Ashton TM, McKenna WG, Kunz-Schughart LA, Higgins GS (2018) Oxidative phosphorylation as an emerging target in cancer therapy. *Clin Cancer Res* 24(11):2482–2490. <https://doi.org/10.1158/1078-0432.CCR-17-3070>
- Ban T, Ishihara T, Kohno H, Saita S, Ichimura A, Maenaka K, Oka T, Mihara K, Ishihara N (2017) Molecular basis of selective mitochondrial fusion by heterotypic action between OPA1 and cardiolipin. *Nat Cell Biol* 19(7):856–863. <https://doi.org/10.1038/ncb3560>
- Bandara AB, Drake JC, Brown DA (2021) Complex II subunit SDHD is critical for cell growth and metabolism, which can be partially restored with a synthetic ubiquinone analog. *BMC Mol and Cell Biol* 22(35). <https://doi.org/10.1186/s12860-021-00370-w>
- Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, Richard CW 3rd, Cornelisse CJ, Devilee P, Devlin B (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 287(5454):848–851. <https://doi.org/10.1126/science.287.5454.848>
- Benda C (1898) Mitochondria. *Arch F Anat U Physiol Abt* 73:397
- Bender DA (2003) Tricarboxylic acid cycle. In: Caballero B (ed) *Encyclopedia of food sciences and nutrition*, 2nd edn. Academic, pp 5851–5856. ISBN 9780122270550. <https://doi.org/10.1016/B0-12-227055-X/01363-8>
- Benedetti S, Catalani S, Canonico B, Nasoni MG, Luchetti F, Papa S, Potenza L, Palma F (2022) The effects of acyclovir administration to NCI-H1975 non-small cell lung cancer cells. *Toxicol In Vitro* 79:105301. <https://doi.org/10.1016/j.tiv.2021.105301>

- Brindefalk B, Ettema TJ, Viklund J, Thollesson M, Andersson SG (2011) A phylometagenomic exploration of oceanic alphaproteobacteria reveals mitochondrial relatives unrelated to the SAR11 clade. *PLoS One* 6(9):e24457. <https://doi.org/10.1371/journal.pone.0024457>
- Chan DC (2020) Mitochondrial dynamics and its involvement in disease. *Annu Rev Pathol Mech Dis* 15:235–259. <https://doi.org/10.1146/annurev-pathmechdis-012419-032711>
- Chandel NS (2010) Mitochondrial complex III: an essential component of universal oxygen sensing machinery? *Respir Physiol Neurobiol* 174(3):175–181. <https://doi.org/10.1016/j.resp.2010.08.004>
- Chen W, Hu S, Mao S, Xu Y, Guo H, Li H, Paulsen MT, Chen X, Ljungman M, Neamati N (2020) Discovery of mitochondrial transcription inhibitors active in pancreatic cancer cells. *ChemMedChem* 15(21):2029–2039. <https://doi.org/10.1002/cmdc.202000494>
- Chipuk JE, Bouchier-Hayes L, Green DR (2006) Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. *Cell Death Differ* 13(8):1396–1402. <https://doi.org/10.1038/sj.cdd.4401963>
- Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagge EA, Rose NR, Leung IK, Li XS, Woon EC, Yang M, McDonough MA, King ON, Clifton IJ, Klose RJ, Claridge TD, Ratcliffe PJ, Schofield CJ, Kawamura A (2011) The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep* 12(5):463–469. <https://doi.org/10.1038/embor.2011.43>
- Colina-Tenorio L, Horten P, Pfanner N, Rampelt H (2020) Shaping the mitochondrial inner membrane in health and disease. *J Intern Med* 287(6):645–664. <https://doi.org/10.1111/joim.13031>
- Cooper GM (2000a) The cell: a molecular approach, 2nd edn. Sinauer Associates, The Origin and Evolution of Cells, Sunderland (MA). Available from: <https://www.ncbi.nlm.nih.gov/books/NBK9841>
- Cooper GM (2000b) The cell: a molecular approach, 2nd edn. Sinauer Associates; Mitochondria, Sunderland (MA). Available from: <https://www.ncbi.nlm.nih.gov/books/NBK9896>
- Dang L, Su SM (2017) Isocitrate dehydrogenase mutation and (R)-2-Hydroxyglutarate: from basic discovery to therapeutics development. *Annu Rev Biochem* 86:305–331. <https://doi.org/10.1146/annurev-biochem-061516-044732>
- Dasu A, Toma-Dașu I, Karlsson M (2003) Theoretical simulation of tumour oxygenation and results from acute and chronic hypoxia. *Phys Med Biol* 48(17):2829–2842. <https://doi.org/10.1088/0031-9155/48/17/307>
- Du W, Di X, Su QP (2021) Studying mitochondrial network formation by in vivo and in vitro reconstitution assay. *Methods Mol Biol* 2276:333–341. https://doi.org/10.1007/978-1-0716-1266-8_25
- Eniafe J, Jiang S (2021) The functional roles of TCA cycle metabolites in cancer. *Oncogene* 40(19):3351–3363. <https://doi.org/10.1038/s41388-020-01639-8>
- Ferguson SM, De Camilli P (2012) Dynamin, a membrane-remodelling GTPase. *Nat Rev Mol Cell Biol* 13(2):75–88. <https://doi.org/10.1038/nrm3266>
- Ferla MP, Thrash JC, Giovannoni SJ, Patrick WM (2013) New rRNA gene-based phylogenies of the Alphaproteobacteria provide perspective on major groups, mitochondrial ancestry and phylogenetic instability. *PLoS One* 8(12):e83383. <https://doi.org/10.1371/journal.pone.0083383>
- Fitzpatrick DA, Creevey CJ, McInerney JO (2006) Genome phylogenies indicate a meaningful alpha-proteobacterial phylogeny and support a grouping of the mitochondria with the Rickettsiales. *Mol Biol Evol* 23(1):74–85. <https://doi.org/10.1093/molbev/msj009>
- Franco DG, Moretti IF, Marie SKN (2018) Mitochondria transcription factor a: a putative target for the effect of melatonin on U87MG malignant glioma cell line. *Molecules* 23(5):1129. <https://doi.org/10.3390/molecules23051129>
- Ge Y, Shi X, Boopathy S, McDonald J, Smith AW, Chao LH (2020) Two forms of Opa1 cooperate to complete fusion of the mitochondrial inner-membrane. *elife*. <https://doi.org/10.7554/eLife.50973>

- Ghanem N, El-Baba C, Araji K, El-Khoury R, Usta J, Darwiche N (2021) The pentose phosphate pathway in cancer: regulation and therapeutic opportunities. *Chemotherapy* 66(5–6):179–191. <https://doi.org/10.1159/000519784>
- Ghosh P, Vidal C, Dey S, Zhang L (2020) Mitochondria targeting as an effective strategy for cancer therapy. *Int J Mol Sci* 21(9):3363. <https://doi.org/10.3390/ijms21093363>
- Giachin G, Bouverot R, Acajjaoui S, Pantalone S, Soler-López M (2016) Dynamics of human mitochondrial complex I assembly: implications for neurodegenerative diseases. *Front Mol Biosci* 3(43). <https://doi.org/10.3389/fmolb.2016.00043>
- Glancy B, Kim Y, Katti P, Willingham TB (2020) The functional impact of mitochondrial structure across subcellular scales. *Front Physiol* 11:541040. <https://doi.org/10.3389/fphys.2020.541040>
- Golub D, Iyengar N, Dogra S, Wong T, Bready D, Tang K, Modrek AS, Placantonakis DG (2019) Mutant Isocitrate dehydrogenase inhibitors as targeted cancer therapeutics. *Front Oncol* 9:417. <https://doi.org/10.3389/fonc.2019.00417>
- Grasso D, Zampieri LX, Capelôa T, Van de Velde JA, Sonveaux P (2020) Mitochondria in cancer. *Cell. Stress* 4(6):114–146. <https://doi.org/10.15698/cst2020.06.221>
- Gray MW (2012) Mitochondrial evolution. *Cold Spring Harb Perspect Biol* 4(9):a011403. <https://doi.org/10.1101/cshperspect.a011403>
- Gray MW (2013) Mitochondria. In: Maloy S, Hughes K (eds) *Brenner's encyclopedia of genetics*, 2nd edn. Academic, pp 430–432, ISBN 9780080961569. <https://doi.org/10.1016/B978-0-12-374984-0.00957-8>
- Greber BJ, Ban N (2016) Structure and function of the mitochondrial ribosome, annual review of biochemistry. *Annu Rev Biochem* 85(1):103–132. <https://doi.org/10.1146/annurev-biochem-060815-014343>
- Greene J, Segaran A, Lord S (2022) Targeting OXPHOS and the electron transport chain in cancer: molecular and therapeutic implications. *Semin Cancer Biol* 86(Pt2):851–859. <https://doi.org/10.1016/j.semcancer.2022.02.002>
- Guo W, Li Z, Huang H, Xu Z, Chen Z, Shen G, Li Z, Ren Y, Li G, Hu Y (2022) VB12-Sericin-PBLG-IR780 Nanomicelles for programming cell Pyroptosis via Photothermal (PTT)/photodynamic (PDT) effect-induced mitochondrial DNA (mitoDNA) oxidative damage. *ACS Appl Mater Interfaces* 14(15):17008–17021. <https://doi.org/10.1021/acsami.1c22804>
- Gyamfi J, Kim J, Choi J (2022) Cancer as a metabolic disorder. *Int J Mol Sci* 23(3):1155. <https://doi.org/10.3390/ijms23031155>
- Habbane M, Montoya J, Rhouda T, Sbaoui Y, Radallah D, Emperador S (2021) Human mitochondrial DNA: particularities and diseases. *Biomedicines* 9(10):1364. <https://doi.org/10.3390/biomedicines9101364>
- Hassanpour SH, Dehghani M (2017) Review of cancer from perspective of molecular. *J Cancer Res Pract* 4(4):127–129. <https://doi.org/10.1016/j.jcrpr.2017.07.001>
- Hill BT (2019) Etiology of cancer. In: Singh A, Damato B (eds) *Clinical ophthalmic oncology*. Springer, Cham. https://doi.org/10.1007/978-3-030-04489-3_2
- Hoitzing H, Johnston IG, Jones NS (2015) What is the function of mitochondrial networks? A theoretical assessment of hypotheses and proposal for future research. *BioEssays* 37(6):687–700. <https://doi.org/10.1002/bies.201400188>
- Huang TL, Chang CR, Chien CY, Huang GK, Chen YF, Su LJ, Tsai HT, Lin YS, Fang FM, Chen CH (2022) DRP1 contributes to head and neck cancer progression and induces glycolysis through modulated FOXM1/MMP12 axis. *Mol Oncol* 16(13):2585–2606. <https://doi.org/10.1002/1878-0261.13212>
- Jacob WA, Bakker A, Hertsens RC, Biermans W (1994) Mitochondrial matrix granules: their behavior during changing metabolic situations and their relationship to contact sites between inner and outer mitochondrial membranes. *Microsc Res Tech* 27(4):307–318. <https://doi.org/10.1002/jemt.1070270406>
- Jin L, Zhou Y (2019) Crucial role of the pentose phosphate pathway in malignant tumors. *Oncol Lett* 17(5):4213–4221. <https://doi.org/10.3892/ol.2019.10112>

- Kalia R, Wang RY, Yusuf A, Thomas PV, Agard DA, Shaw JM, Frost A (2018) Structural basis of mitochondrial receptor binding and constriction by DRP1. *Nature* 558(7710):401–405. <https://doi.org/10.1038/s41586-018-0211-2>
- Kausar S, Wang F, Cui H (2018) The role of mitochondria in reactive oxygen species generation and its implications for neurodegenerative diseases. *Cells* 7(12):274. <https://doi.org/10.3390/cells7120274>
- Kim J, Cheong JH (2020) Role of mitochondria-cytoskeleton interactions in the regulation of mitochondrial structure and function in cancer stem cells. *Cells* 9(7):1691. <https://doi.org/10.3390/cells9071691>
- Klecker T, Westermann B (2021) Pathways shaping the mitochondrial inner membrane. *Open Biol* 11(12):210238. <https://doi.org/10.1098/rsob.210238>
- Kölliker A (1857) Einige bemerkungen uber die endigungen der hautnerven und den bau der muskein. *Zwiss Zool* 8:311–325
- Kong Q, Yan X, Cheng M, Jiang X, Xu L, Shen L, Yu H, Sun L (2023) p62 promotes the mitochondrial localization of p53 through its UBA domain and participates in regulating the sensitivity of ovarian cancer cells to cisplatin. *Int J Mol Sci* 23(6):3290. <https://doi.org/10.3390/ijms23063290>
- Kopinski PK, Singh LN, Zhang S, Lott MT, Wallace DC (2021) Mitochondrial DNA variation and cancer. *Nat Rev Cancer* 21(7):431–445. <https://doi.org/10.1038/s41568-021-00358-w>
- Krebs HA, Johnson WA (1980) The role of citric acid in intermediate metabolism in animal tissues. *FEBS Lett* 117(Suppl):K1–K10. <https://doi.org/10.4159/harvard.9780674366701.c143>
- Kroemer G, Pouyssegur J (2008) Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* 13(6):472–482. <https://doi.org/10.1016/j.ccr.2008.05.005>
- Kühlbrandt W (2015) Structure and function of mitochondrial membrane protein complexes. *BMC Biol* 13:89. <https://doi.org/10.1186/s12915-015-0201-x>
- Li C, Zhang Y, Liu J, Kang R, Klionsky DJ, Tang D (2021) Mitochondrial DNA stress triggers autophagy-dependent ferroptotic death. *Autophagy* 17(4):948–960. <https://doi.org/10.1080/15548627.2020.1739447>
- Lin Y, Yang B, Huang Y, Zhang Y, Jiang Y, Ma L, Shen YQ (2023) Mitochondrial DNA-targeted therapy: a novel approach to combat cancer. *Cell Insight* 2(4):100113. <https://doi.org/10.1016/j.cellin.2023.100113>
- Liu Y, Shi Y (2020) Mitochondria as a target in cancer treatment. *MedComm* 1(2):129–139. <https://doi.org/10.1002/mco2.16>
- Liu Y, Sun Y, Guo Y, Shi X, Chen X, Feng W, Wu LL, Zhang J, Yu S, Wang Y, Shi Y (2023) An overview: the diversified role of mitochondria in cancer metabolism. *Int J Biol Sci* 19(3):897–915. <https://doi.org/10.7150/ijbs.81609>
- Loeb LA, Cheng KC (1990) Errors in DNA synthesis: a source of spontaneous mutations. *Mutat Res* 238(3):297–304. [https://doi.org/10.1016/0165-1110\(90\)90021-3](https://doi.org/10.1016/0165-1110(90)90021-3)
- Lu H, Forbes RA, Verma A (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J Biol Chem* 277(26):23111–23115. <https://doi.org/10.1074/jbc.M202487200>
- Luo M, Zhou W, Patel H et al (2020) Bedaquiline inhibits the yeast and human mitochondrial ATP synthases. *Commun Biol* 3:452. <https://doi.org/10.1038/s42003-020-01173-z>
- Marquez J, Flores J, Kim AH, Nyamaa B, Nguyen ATT, Park N, Han J (2019) Rescue of TCA cycle dysfunction for cancer therapy. *J Clin Med* 8(12):2161. <https://doi.org/10.3390/jcm8122161>
- Martijn J, Vosseberg J, Guy L et al (2018) Deep mitochondrial origin outside the sampled alphaproteobacteria. *Nature* 557:101–105. <https://doi.org/10.1038/s41586-018-0059-5>
- Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 191:144–148. <https://doi.org/10.1038/191144a0>
- National Cancer Institute (NCI). <https://www.cancer.gov/about-cancer/understanding/what-is-cancer>. Available on 9 Oct 2023

- Neagu M, Constantin C, Popescu ID, Zipeto D, Tzanakakis G, Nikitovic D, Fenga C, Stratakis CA, Spandidos DA, Tsatsakis AM (2019) Inflammation and metabolism in cancer cell-mitochondria key player. *Front Oncol* 9:348. <https://doi.org/10.3389/fonc.2019.00348>
- Palade GE (1953) An electron microscope study of the mitochondrial structure. *J Histochem Cytochem* 1:188–211
- Panchangam RL, Rao RN, Balamurali MM, Hingamire TB, Shanmugam D, Manickam V, Chanda K (2021) Antitumor effects of Ir(III)-2H-Indazole complexes for triple negative breast cancer. *Inorg Chem* 60(23):17593–17607. <https://doi.org/10.1021/acs.inorgchem.1c02193>
- Pandey AK, Verma S (2020) Combination drug therapy for multimodal treatment of cancer by targeting mitochondrial transcriptional pathway: an in-silico approach. *Med Hypotheses* 143: 110075. <https://doi.org/10.1016/j.mehy.2020.110075>
- Patra KC, Hay N (2014) The pentose phosphate pathway and cancer. *Trends Biochem Sci* 39:347–354. <https://doi.org/10.1016/j.tibs.2014.06.005>
- Pavlova NN, Zhu J, Thompson CB (2022) The hallmarks of cancer metabolism: still emerging. *Cell Metab* 34(3):355–377. <https://doi.org/10.1016/j.cmet.2022.01.007>
- Pitot HC (1993) The molecular biology of carcinogenesis. *Cancer* 72(3 Suppl):962–970. [https://doi.org/10.1002/1097-0142\(19930801\)72:3+<962::aid-cnrcr2820721303>3.0.co;2-h](https://doi.org/10.1002/1097-0142(19930801)72:3+<962::aid-cnrcr2820721303>3.0.co;2-h)
- Protasoni M, Zeviani M (2021) Mitochondrial structure and bioenergetics in normal and disease conditions. *Int J Mol Sci* 22(2):586. <https://doi.org/10.3390/ijms22020586>
- Raimondi V, Ciccarese F, Ciminale V (2020) Oncogenic pathways and the electron transport chain: a dangerROS liaison. *Br J Cancer* 122:168–181. <https://doi.org/10.1038/s41416-019-0651-y>
- Ramos-Martinez JI (2017) The regulation of the pentose phosphate pathway: remember Krebs. *Arch Biochem Biophys* 614:50–52. <https://doi.org/10.1016/j.abb.2016.12.012>
- Rodrigues T, Ferraz LS (2020) Therapeutic potential of targeting mitochondrial dynamics in cancer. *Biochem Pharmacol* 182:114282. <https://doi.org/10.1016/j.bcp.2020.114282>
- Rodríguez-Ezpeleta N, Embley TM (2012) The SAR11 group of alpha-proteobacteria is not related to the origin of mitochondria. *PLoS One* 7(1):e30520. <https://doi.org/10.1371/journal.pone.0030520>
- Sainero-Alcolado L, Liaño-Pons J, Ruiz-Pérez MV, Arsenian-Henriksson M (2022) Targeting mitochondrial metabolism for precision medicine in cancer. *Cell Death Differ* 29(7): 1304–1317. <https://doi.org/10.1038/s41418-022-01022-y>
- Sassera D, Lo N, Epis S, D'Auria G, Montagna M, Comandatore F, Horner D, Peretó J, Luciano AM, Franciosi F, Ferri E, Crotti E, Bazzocchi C, Daffonchio D, Sacchi L, Moya A, Latorre A, Bandi C (2011) Phylogenomic evidence for the presence of a flagellum and cbb(3) oxidase in the free-living mitochondrial ancestor. *Mol Biol Evol* 28(12):3285–3296. <https://doi.org/10.1093/molbev/msr159>
- Scheffler IE (2008) *Mitochondria*, 2nd edn. Wiley, Hoboken. ISBN:978-0-470-04073-7
- Schiliro C, Firestein BL (2021) Mechanisms of metabolic reprogramming in cancer cells supporting enhanced growth and proliferation. *Cells* 10(5):1056. <https://doi.org/10.3390/cells10051056>
- Shen K, Pender CL, Bar-Ziv R, Zhang H, Wickham K, Willey E, Durieux J, Ahmad Q, Dillin A (2022) Mitochondria as cellular and organismal signaling hubs. *Annu Rev Cell Dev Biol* 38: 179–218. <https://doi.org/10.1146/annurev-cellbio-120420-015303>
- Sherratt HS (1991) Mitochondria: structure and function. *Rev Neurol (Paris)* 147(6–7):417–430
- Shi Y, Lim SK, Liang Q, Iyer SV, Wang HY, Wang Z, Xie X, Sun D, Chen YJ, Tabar V, Gutin P, Williams N, De Brabander JK, Parada LF (2019) Gboxin is an oxidative phosphorylation inhibitor that targets glioblastoma. *Nature* 567(7748):341–346. <https://doi.org/10.1038/s41586-019-0993-x>
- Siddiqui IA, Sanna V, Ahmad N, Sechi M, Mukhtar H (2015) Resveratrol nanoformulation for cancer prevention and therapy. *Ann N Y Acad Sci* 1348(1):20–31. <https://doi.org/10.1111/nyas.12811>

- Siegel RL, Miller KD, Jemal A (2016) Cancer statistics. *CA Cancer J Clin* 66(1):7–30. <https://doi.org/10.3322/caac.21332>
- Sim MS, Ogata H, Lubitz W et al (2019) Role of APS reductase in biogeochemical sulfur isotope fractionation. *Nat Commun* 10:44. <https://doi.org/10.1038/s41467-018-07878-4>
- Sjostrand FS, Rhodin J (1953) The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy. *Exp Cell Res*:4426–4456
- Somuncu B, Ekmekcioglu A, Antmen FM, Ertuzun T, Deniz E, Keskin N, Park J, Yazici IE, Simsek B, Erman B, Yin W, Erman B, Muftuoglu M (2022) Targeting mitochondrial DNA polymerase gamma for selective inhibition of MLH1 deficient colon cancer growth. *PLoS One* 17(6):e0268391. <https://doi.org/10.1371/journal.pone.0268391>
- Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, Stone RM, DeAngelo DJ, Levine RL, Flinn IW, Kantarjian HM, Collins R, Patel MR, Frankel AE, Stein A, Sekeres MA, Swords RT, Medeiros BC, Willekens C, Vyas P, Tosolini A, Xu Q, Knight RD, Yen KE, Agresta S, de Botton S, Tallman MS (2017) Enasidenib in mutant *IDH2* relapsed or refractory acute myeloid leukemia. *Blood* 130(6):722–731. <https://doi.org/10.1182/blood-2017-04-779405>
- Sun J, Trumpower BL (2003) Superoxide anion generation by the cytochrome bc1 complex. *Arch Biochem Biophys* 419(2):198–206. <https://doi.org/10.1016/j.abb.2003.08.028>
- Taanman JW (1999) The mitochondrial genome: structure, transcription, translation and replication. *Biochim Biophys Acta* 1410(2):103–123. [https://doi.org/10.1016/s0005-2728\(98\)00161-3](https://doi.org/10.1016/s0005-2728(98)00161-3)
- The International Agency for Research on Cancer (IARC). <https://monographs.iarc.who.int/agents-classified-by-the-iarc/>. Available on: 13 Oct 2023
- Thrash JC, Boyd A, Huggett MJ, Grote J, Carini P, Yoder RJ, Robbertse B, Spatafora JW, Rappé MS, Giovannoni SJ (2011) Phylogenomic evidence for a common ancestor of mitochondria and the SAR11 clade. *Sci Rep* 1:13. <https://doi.org/10.1038/srep00013>
- Tilokani L, Nagashima S, Paupe V, Prudent J (2018) Mitochondrial dynamics: overview of molecular mechanisms. *Essays Biochem* 62(3):341–360. <https://doi.org/10.1042/EBC20170104>
- Todisco S, Convertini P, Iacobazzi V, Infantino V (2019) TCA cycle rewiring as emerging metabolic signature of hepatocellular carcinoma. *Cancers (Basel)* 12(1):68. <https://doi.org/10.3390/cancers12010068>
- Valle-Mendiola A, Soto-Cruz I (2020) Energy metabolism in cancer: the roles of STAT3 and STAT5 in the regulation of metabolism-related genes. *Cancers (Basel)* 12(1):124. <https://doi.org/10.3390/cancers12010124>
- van den Heuvel L, Smeitink J (2001) The oxidative phosphorylation (OXPHOS) system: nuclear genes and human genetic diseases. *BioEssays* 23(6):518–525. <https://doi.org/10.1002/bies.1071>
- van Gisbergen MW, Voets AM, Starmans MH, de Coo IF, Yadak R, Hoffmann RF, Boutros PC, Smeets HJ, Dubois L, Lambin P (2015) How do changes in the mtDNA and mitochondrial dysfunction influence cancer and cancer therapy? Challenges, opportunities and models. *Mutat Res Rev Mutat Res* 764:16–30. <https://doi.org/10.1016/j.mrrev.2015.01.001>
- Viklund J, Martijn J, Ettema TJ, Andersson SG (2013) Comparative and phylogenomic evidence that the alphaproteobacterium HIMB59 is not a member of the oceanic SAR11 clade. *PLoS One* 8(11):e78858. <https://doi.org/10.1371/journal.pone.0078858>
- Wang Z, Wu M (2015) An integrated phylogenomic approach toward pinpointing the origin of mitochondria. *Sci Rep* 5:7949. <https://doi.org/10.1038/srep07949>
- Wang C, Du W, Su QP, Zhu M, Feng P, Li Y, Zhou Y, Mi N, Zhu Y, Jiang D, Zhang S, Zhang Z, Sun Y, Yu L (2015) Dynamic tubulation of mitochondria drives mitochondrial network formation. *Cell Res* 25(10):1108–1120. <https://doi.org/10.1038/cr.2015.89>
- Wang K, Xiang Y, Pan W, Wang H, Li N, Tang B (2020) Dual-targeted photothermal agents for enhanced cancer therapy. *Chem Sci* 11(31):8055–8072. <https://doi.org/10.1039/d0sc03173a>
- Wang S, Tan J, Miao Y, Zhang Q (2022) Mitochondrial dynamics, mitophagy, and mitochondria-endoplasmic reticulum contact sites crosstalk under hypoxia. *Front Cell Dev Biol* 10:848214. <https://doi.org/10.3389/fcell.2022.848214>
- Wang SF, Tseng LM, Lee HC (2023) Role of mitochondrial alterations in human cancer progression and cancer immunity. *J Biomed Sci* 30(1):61. <https://doi.org/10.1186/s12929-023-00956-w>

- Weston A, Harris CC (2003) Multistage carcinogenesis. In: Kufe DW, Pollock RE, Weichselbaum RR et al (eds) *Holland-Frei cancer medicine*, 6th edn. BC Decker, Hamilton. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK13982>
- Whitley BN, Engelhart EA, Hoppins S (2019) Mitochondrial dynamics and their potential as a therapeutic target. *Mitochondrion* 49:269–283. <https://doi.org/10.1016/j.mito.2019.06.002>
- Williams KP, Sobral BW, Dickerman AW (2007) A robust species tree for the alphaproteobacteria. *J Bacteriol* 189(13):4578–4586. <https://doi.org/10.1128/JB.00269-07>
- World Health Organisation (WHO). https://www.who.int/health-topics/cancer#tab=tab_1. Available on 12 Oct 2023
- Yang M, Soga T, Pollard PJ, Adam J (2012) The emerging role of fumarate as an oncometabolite. *Front Oncol* 2:85. <https://doi.org/10.3389/fonc.2012.00085>
- Yang M, Soga T, Pollard PJ (2013) Oncometabolites: linking altered metabolism with cancer. *J Clin Invest* 123(9):3652–3658. <https://doi.org/10.1172/JCI67228>
- Yang M, Li C, Yang S, Xiao Y, Xiong X, Chen W, Zhao H, Zhang Q, Han Y, Sun L (2020) Mitochondria-associated ER membranes—the origin site of autophagy. *Front Cell Dev Biol* 8:595. <https://doi.org/10.3389/fcell.2020.00595>
- Zachar I, Boza G (2020) Endosymbiosis before eukaryotes: mitochondrial establishment in protoeukaryotes. *Cell Mol Life Sci* 77(18):3503–3523. <https://doi.org/10.1007/s00018-020-03462-6>
- Zeng W, Liu P, Pan W, Singh SR, Wei Y (2015) Hypoxia and hypoxia inducible factors in tumor metabolism. *Cancer Lett* 356(2 Pt A):263–267. <https://doi.org/10.1016/j.canlet.2014.01.032>
- Zhao RZ, Jiang S, Zhang L, Yu ZB (2019) Mitochondrial electron transport chain, ROS generation and uncoupling (review). *Int J Mol Med* 44(1):3–15. <https://doi.org/10.3892/ijmm.2019.4188>
- Zong WX, Rabinowitz JD, White E (2016) Mitochondria and cancer. *Mol Cell* 61(5):667–676. <https://doi.org/10.1016/j.molcel.2016.02.011>



Mitochondrial Energy Metabolism in Carcinogenesis

Natalia Kurhaluk and Halina Tkaczenko

Abstract

The chapter examines and analyses scientific publications devoted to the role of mitochondria in the support of the life of transformed cells and to the study of the characteristics and structural and functional dysfunctions of mitochondria. Issues of the mitochondrial control of cellular redox homeostasis and sites of mitochondrial reactive oxygen species (ROS) production in the inner mitochondrial membrane are considered. The features of the structural and functional reorganisation of mitochondria in malignant tumour cells and the mechanisms of reprogramming energy metabolism, increasing ROS production, and adaptation to the conditions of hypoxia and metabolic stress are analysed. Mitochondrial dysfunction, activation of oncogenes, inactivation of anti-oncogenes, and the tumour cell microenvironment have a profound effect on the metabolism of cancer cells and determine the heterogeneity of metabolic profiles between different tumour types. The importance of identifying specific metabolic changes in each malignant tumour is clear given the need to effectively influence its growth. Understanding the role of mitochondrial dysfunction is very important for studying the pathophysiology of cancer and many other common diseases. This gives impetus to the development of mitochondrial pharmacology using mitochondria as targets for different types of effects, which can serve as the biochemical basis for the development of new antitumour agents. Perhaps the combined use of mitochondrial metabolism modulators and antitumour agents will contribute to the emergence of a new direction in antitumour therapy that will significantly increase the effectiveness of treatment for cancer patients.

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Keywords

Carcinogenesis · Mitochondrial dysfunction · Reactive oxygen species · Tumour metabolism · Warburg effect

1 Introduction

One hundred years ago, Otto Warburg, twice winner of the Nobel Prize for Physiology and Medicine, discovered that healthy cells produce energy through the oxidative breakdown of organic acids in the mitochondria, whereas tumour and cancer cells obtain energy through the non-oxidative breakdown of glucose (Pascale et al. 2020). According to Warburg, the transition to an oxygen-free energy mode leads to an autonomous uncontrolled existence of the cell, which begins to behave as an independent organism that wants to reproduce. Based on this discovery, scientists proposed that cancer could be considered a mitochondrial disease. Otto Warburg's hypothesis and his biochemical theory of cancer caused controversy in the scientific world, but somewhat later, researchers discovered that malignant changes in cells and uncontrolled cell growth are caused by genetic mutations (Courtney et al. 2015). In addition to explaining the Warburg effect, data from various researchers are changing the conventional view of biochemists that metabolism is secondary to enzyme signalling; therefore, regulation of cellular metabolism may be an effective way to prevent cancer growth (Xu et al. 2021; Cortellino and Longo 2023).

The fundamental questions about the origin, structure, and function of the mitochondrion and its genome determine the role of mitochondria in the pathogenesis of malignant neoplasms (Tulah and Birch-Machin 2013). The involvement of the mitochondrial genome in cancer development as a polyetioloical process has not been fully elucidated, and the debate among scientists about the primary role of an oncogene or the mitochondrion in cancer initiation continues (Kaplia et al. 2015). Tumour transformation of cells is a multistep pathological process during which transformed cells acquire new phenotypic properties: (1) growth autonomy and insensitivity to anti-growth signals; (2) unlimited proliferative potential; (3) capacity for invasive growth and metastasis; (4) ability to stimulate angiogenesis; (5) genomic instability; (6) reduction of apoptotic death and ability to evade immune attack; and (7) metabolic reprogramming. Tumour transformation is the longest and most hidden stage of carcinogenesis. Various genetic and epigenetic perturbations in cells that occur during this stage lead to the activation of cellular proto-oncogenes within them, the inactivation of suppressor genes and genes controlling apoptosis and DNA repair, the disruption of the functioning of cell cycle “checkpoints” and, ultimately, the formation of a clone of cells with an “unstable” genome, and the ability to proliferate in an unregulated manner (Alitalo and Schwab 1986). Metabolic reprogramming, primarily characterised by a shift in energy supply from mitochondrial oxidative phosphorylation to aerobic glycolysis, is the major hallmark that cells acquire during

the process of tumour transformation (Hanahan and Weinberg 2011). Although this sign is the most obvious change in the metabolism of cancer cells, it is only part of the picture of their metabolic reorganisation. In order to proliferate rapidly, tumour cells must increase the rate of metabolic reactions in order to provide themselves not only with sufficient energy in the form of ATP molecules but also with the building blocks (amino acids, lipids, and nucleotides) necessary for the formation of new tumour cells (Hanahan and Weinberg 2011). Mitochondrial dysfunction and mitochondrial genome mutations may play an important role in the pathogenesis of malignancies. Despite many years of research, this issue is still the subject of scientific debate. The aim of this review is to analyse studies on mitochondrial dysfunction in the development of malignant neoplasms. The main features of metabolism in tumour cells, in particular the Warburg effect, and the biological significance of such alterations are also analysed. Special attention is given to the discussion of the mechanisms of alterations in the major metabolic reactions in tumour cells.

2 Mitochondria: Structure, Origin, and Function

Mitochondria are double-membrane organelles found in most eukaryotic cells (Ng et al. 2021). The main function of mitochondria is the synthesis of ATP molecules. ATP is a universal source of energy for living organisms. Mitochondrial electron transport chain (ETC) complexes play a leading role in the synthesis of ATP molecules (Vercellino and Sazanov 2022). Mitochondria accumulate an electrochemical proton gradient at the inner membrane, and these processes are associated with the absorption of oxygen and the formation of water molecules. The generated proton potential is then used for the synthesis of ATP, which is carried out by the enzyme ATPase, or is dissipated as heat.

According to the endosymbiotic theory, mitochondria arose as a result of the symbiosis of α -proteobacteria with a cell (Andrieux et al. 2021). As a result, pre-eukaryotic cells began to receive significantly more energy. During evolution, the genetic material from proteobacteria was transferred to nuclear DNA, and eukaryotes also acquired additional genes for the full functioning of mitochondria. Mitochondrial DNA in mammalian cells encodes 22 transfer RNAs, 2 ribosomal RNAs, and 13 proteins that are present in the mitochondrial electron transport chain (ETC) complexes: seven in complex 1, one in complex 3, and three in complex 4. In addition, two proteins are found in ETC complex 5 (ATPase) (Schäfer et al. 2006). More than a thousand proteins encoded by the nuclear DNA are transported into the mitochondria by special transport proteins. Of the 850 proteins found in mitochondria, only 15% are directly involved in energy production. Other proteins are used for DNA synthesis, transcription, transport, and protein folding (Chaban et al. 2014; Yan et al. 2019). Mitochondria also contain proteins essential for organelle division and the induction of apoptosis (Green 2022). There are also proteins involved in the biosynthesis of iron-sulphur proteins (Dudkina et al. 2011).

Mitochondria have their own genome, the mitochondrial DNA (mtDNA). In humans, mtDNA is present in multiple copies (from 100 to 10,000 copies per cell), depending on the intensity of ATP production (Miller et al. 2003). For example, the heart contains two times more copies of mtDNA than skeletal muscle (Miller et al. 2003). The mitochondrial genome is responsible for the expression of 13 subunits of the respiratory chain and ATP synthase enzyme complexes located in the inner mitochondrial membrane (Andrews et al. 1999; Nicholls and Minczuk 2014; Rath et al. 2021). Proteins encoded by mtDNA account for only about 1% of the mitochondrial proteome. According to the latest revised list in MitoCarta 3.0, mitochondria contain proteins and RNA encoded by 1136 genes (Rath et al. 2021), of which only 37 genes are localised in the mitochondrial genome (Anderson et al. 1981). As a result of evolution, most of the mitochondrial proteome is encoded by the nuclear genome.

3 Mitochondria and Oxidative Stress

Reactive oxygen species (ROS) are generated as superoxide anion radicals in non-specific reactions with oxygen molecules. At the same time, ROS can act as second messengers in cellular processes (Kasai et al. 2020). ROS production has been studied in all ETC complexes, submitochondrial particles, and mitochondria themselves from a variety of biological specimens. Substrate-specific features of ROS production have been identified (Zhang et al. 2022).

In mitochondria, the superoxide anion, which has a short half-life (10^{-6} s at +37 °C), is generated with the highest intensity, and transition metal ions, such as Fe^{2+} , are converted into $\text{OH}^\bullet$ and $\text{HOO}^\bullet$ radicals (Mailloux 2021). O_2^- and $\text{OH}^\bullet$ (the most aggressive radical) are potent oxidants of biopolymers and lipids. These ROS are able to inactivate enzymes, damage nucleic acids, modify signalling proteins, depolymerise polysaccharides, and induce lipid peroxidation processes (LPO) in biomembranes (Mailloux 2018).

The major sources of ROS in cells are complexes I and III of the mitochondrial ETC (Yin and O'Neill 2021; Chen et al. 2022). ROS can be generated by ETC complex I in two different states. In the first case, generation occurs during direct electron transport. ROS production is greatly increased when rotenone, which binds to the Q site, is added to mitochondria (Morgan-Hughes et al. 1988; Pitkanen and Robinson 1996). In the second case, ROS generation occurs during reverse electron transfer from ETC complex II (Lanciano et al. 2013). This process requires a high membrane potential. The production of ROS by ETC complex I has been studied in an isolated enzyme (NADH-ubiquinone oxidoreductase), intact mitochondria, and submitochondrial particles (Chenna et al. 2022). When ROS are generated by ETC complex I, reactive oxygen species enter the mitochondrial matrix. Peroxiredoxin 3 and peroxiredoxin 5, as well as glutathione peroxidase and reduced glutathione, are involved in protecting the mitochondrial matrix from the negative effects of ROS (Byun et al. 2018).

The important role of flavin mononucleotide (FMN) in the generation of ROS for ETC I during direct electron transfer has been determined, as shown in the paper by Curtabbi and Enríquez (2022). The flavin radical and iron-sulphur clusters can be donors for one-electron oxygen reduction. There are two points of view regarding the role of ETC complex I and the membrane potential ($\Delta\psi$) in direct electron transfer through ETC complex I, from insignificant stimulation to a highly stimulatory effect.

The mechanism of ROS generation in mitochondrial complex III, as presented in a number of papers, is as follows: the inhibition of ubiquinone at the P-site of the ubiquinone cycle by antimycin or high membrane potential causes electrons to be directed in the opposite direction to the high-potential cytochrome bn, thereby increasing the lifetime and accumulation of ubesemiquinone, which is capable of reacting directly with oxygen molecules, leading to one-electron reduction and the formation of a superoxide anion radical (Chen et al. 2022).

Cytochrome c is also currently being considered as a potential ROS generator in mitochondria, particularly in pathophysiological conditions (Ramzan et al. 2020). ATP synthetase may be involved in ROS production by raising the membrane potential, which stimulates production at both complex I and complex III of the ETC (Yin and O'Neill 2021; Chen et al. 2022). The literature also considers the possibility of ROS production by complex II of the mitochondrial ETC (Dröse 2013), which is the only complex of the electron transport chain involved in the Krebs cycle. It has been concluded that the ubiquinone pool plays the most important role in ROS generation, as the effect of an inhibitor on ETC complex II increases ROS production at the Qo site (Lanciano et al. 2013).

The manifestations of mitochondrial oxidative stress are related to the features of non-enzymatic oxidation in this closed compartment of cells (Fig. 1).

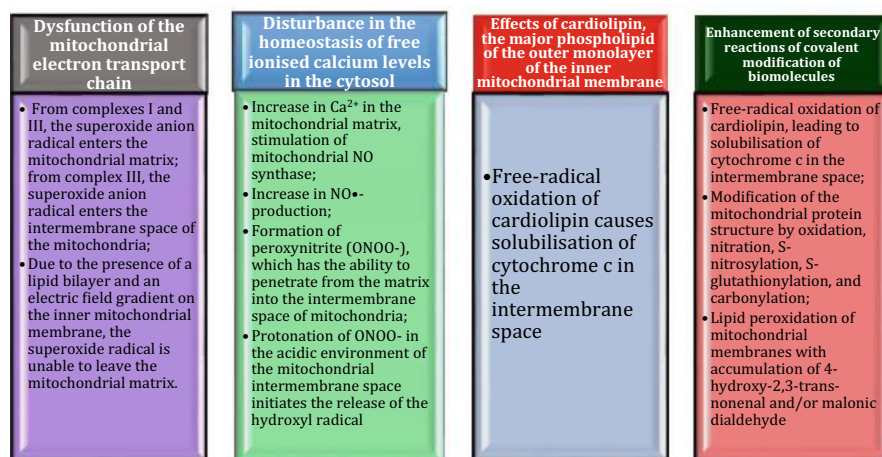


Fig. 1 Aspects of mitochondrial oxidative stress

Mitochondrial Dysfunction: Role in the Development of Malignant Neoplasms

Carcinogenesis is a multistep process in which the accumulation of mutations and other genetic alterations leads to perturbations in the regulation of the cell cycle, apoptosis, differentiation, cell movement, and morphogenetic responses as well as the control of genome integrity. All this, in turn, ensures that the cell and its progeny acquire a number of properties, such as self-sufficiency in proliferative signals, increased migratory ability, insensitivity to growth-inhibiting effects, absence of replicative ageing, increased viability in unfavourable environmental conditions or intracellular damage, genetic instability, and others, which determine neoplastic transformation and further tumour progression. Dysfunction of proto-oncogenes and tumour suppressors plays a key role in the emergence of these characteristics of a neoplastic cell (Hanahan and Weinberg 2011).

Tumour cell transformation as a multi-stage pathological process is shown in Fig. 2.

Mitochondrial dysfunction is a major cause of tumourigenesis. Retrograde signalling initiated by dysfunctional mitochondria can cause global changes in gene expression that affect cell morphology and function as well as tumour transformation. This is usually associated with the disruption of key mitochondrial functions, leading to metabolic remodelling of the tumour-transformed cell.

Changes in mitochondrial dynamics contribute to the restructuring of the bioenergetic and biosynthetic profile of the cancer cell through the transmission of signals from “defective” mitochondria to the nucleus, leading to changes in

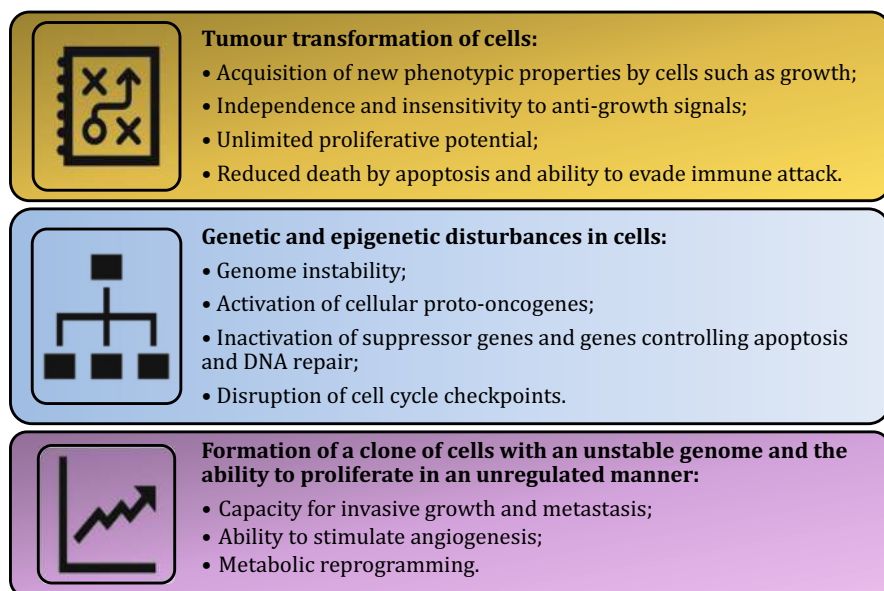


Fig. 2 Tumour cell transformation as a multi-stage pathological process

transcription and/or gene activity. Intratumoural heterogeneity suggests the existence of different metabolic patterns among cancer cells within the same tumour mass, and the restructuring of their metabolic profile is largely determined by the degree of mitochondrial dysfunction. In addition to these factors, mitochondrial dynamics may play an important role in stress signalling. Normal mitochondria are highly dynamic organelles whose size, shape, and network are controlled by cellular physiology. Aberrant mitochondrial dynamics and dysfunction of these organelles have been reported in many cancers. Recent studies indicate that increased mitochondrial fission is referred to as a protumourigenic phenotype. Mitochondrial dysfunction is a hallmark of cancer and is associated with its progression to a metastatic and drug-resistant phenotype (Srinivasan et al. 2017).

Mitochondria are now emerging as key players in tumour transformation and progression. This is not surprising since mitochondria are actively involved in bioenergetics and act as metabolic and signalling centres linking cellular metabolism and signalling. In this context, mitochondria play a key role not only in tumourigenesis but also in cancer metastasis. Transformed malignant cells require functionally active mitochondria for tumour growth, proliferation, and metastasis. Alterations in mitochondrial activity are known to promote prometastatic features, including increased production of mitochondrial reactive oxygen species (mtROS), increased resistance to apoptosis, and increased or de novo production of metabolic intermediates. The TCA cycle functions as a producer of oncometabolites, such as succinate, fumarate, and D-2-hydroxyglutarate, which control energy production, redox processes, etc. The metabolism of lipids and amino acids, such as glutamine, glutamate, and proline, is also becoming a central control point for cancer metastasis (Porporato et al. 2016).

For example, mutations in fumarate hydratase (FH), an enzyme in the tricarboxylic acid cycle, cause hereditary leiomyomatosis and renal cell carcinoma. Renal cancer with fumarate hydratase deficiency is very aggressive and metastasises at an early stage. Fumarate, a low-molecular-weight metabolite that accumulates in fumarate hydratase deficient cells plays a key role in cell transformation, making it a target for studying the role of this metabolite in maintaining and promoting cell proliferation. Fumarate has been shown to inhibit α -ketoglutarate-dependent dioxygenases involved in DNA and histone demethylation. However, the relationship between fumarate accumulation, epigenetic changes, and tumourigenesis is unclear (Sciacovelli et al. 2016).

Loss of fumarate hydratase and subsequent accumulation of fumarate in mouse and human cells causes a phenotypic switch associated with cancer initiation, invasion, and metastasis. Fumarate inhibits Tet-mediated demethylation of the regulatory region of the antimetastatic cluster miRNA mir-200ba429, resulting in the expression of epithelial-mesenchymal transition (EMT)-related transcription factors (TFs) and improved migratory properties. These epigenetic and phenotypic changes are recapitulated by incubating fumarate hydratase-positive cells with cell-permeable fumarate. Loss of fumarate hydratase is associated with downregulation of miR-200 and the epithelial-mesenchymal transition signature in renal cancer and is associated with poor clinical outcomes. These findings suggest that loss of

fumarate hydratase and accumulation of fumarate contribute to the development of aggressive tumour characteristics, such as early metastasis and rapid expansion of the primary lesion (Girolimetti et al. 2017).

In this context, mitochondrial metabolic changes may stimulate epithelial-mesenchymal transition, or the activation of epithelial-mesenchymal transition may regulate the metabolism of cancer cells by influencing the expression of metabolic genes. The precise mechanisms underlying mitochondrial dysfunction are diverse and may include deregulated autophagic processes, imbalances in reactive oxygen species (ROS) homeostasis, and mutations in oxidative phosphorylation complexes (OXPHOS), electron transport chains (ETCs), or Krebs cycle enzymes (TCA). Despite the heterogeneity of mechanisms, the induction of epithelial-mesenchymal transition has been described as one of the terminal phenotypes in many epithelial tumour cells affected by mitochondrial dysfunction (Guerra et al. 2017).

The mechanisms contributing to mitochondrial dysfunction can be diverse and occur at the level of mitochondrial proteins encoded by the mtDNA or at the level of the nucleus. Mutations in the TCA cycle enzymes, i.e. fumarate hydratase (FH), isocitrate dehydrogenase (IDH), and succinate dehydrogenase (SDH), have long been recognised as oncogenic, but only recently have they been linked to the activation of epithelial-mesenchymal transition (EMT). Mutations in fumarate hydratase inhibit the conversion of fumarate to malate and cause hereditary leiomyomatosis and highly aggressive renal cell carcinoma that can metastasise at an early stage, even when the primary tumour is very small (Schmidt and Linehan 2014). The accumulation of fumarate in FH cells (mutated in the fumarate dehydrogenase gene) stimulates epithelial-mesenchymal transition through an epigenetic mechanism: fumarate suppresses the antimetastatic cluster of miRNA mir-200ba429, inhibiting demethylation of the regulatory region, which leads to the expression of EMT-TFs (Sciacovelli and Frezza 2016). This mechanism explains the aggressive nature of FH mutant tumours.

Isocitrate dehydrogenase promotes the oxidative decarboxylation of isocitrate to α -ketoglutarate. Mutations in IDH1/2 isoforms are frequently found in oligodendrogliomas, astrocytomas, leukaemia, melanoma, and prostate, colon, and lung cancers (Cairns and Mak 2013). Mutant IDHs are neomorphic and catalyse the conversion of α -ketoglutarate to 2-hydroxyglutarate, an oncometabolite that induces EMT and is associated with the presence of distant metastases in colorectal cancer (Colvin et al. 2016). Succinate dehydrogenase is another TCA cycle enzyme involved in EMT. It catalyses the conversion of succinate to fumarate, and loss-of-function SDH mutations predispose to hereditary pheochromocytoma, paraganglioma, gastrointestinal stromal tumour, and renal cell carcinoma (Dalla Pozza et al. 2020; Bardella et al. 2011). In metastatic pheochromocytoma and paraganglioma, mutations in the *SDHB* gene subunit are associated with the activation of the transcription factors SNAIL and SLUG as a result of epigenetic remodelling due to the hypermethylation of promoter CpG islands, contributing to physiological changes leading to epithelial-mesenchymal transition (EMT) (Letouzé et al. 2013; Lorient et al. 2015). Focal deletions of *SDHB* have also been identified in

serous ovarian (Aspuria et al. 2014) and colorectal (Wang et al. 2017) cancers and have been shown to stimulate EMT through an epigenetic mechanism.

4 Warburg's Hypothesis and the Energetics of Cancer Cells

At the beginning of the twentieth century, German scientist Otto Warburg formulated a hypothesis about the origin of cancer (Warburg 1956). According to this hypothesis, cancer arises as a result of a long process in which there is a selection of cells that switch to anaerobic respiration ("fermentation") due to a violation of oxidative phosphorylation, i.e. impaired ATP production or mitochondrial dysfunction. A number of researchers note that mitochondrial dysfunction can be defined as a violation of ATP production due to enzymatic, transport, structural, or regulatory failure (Stamerra et al. 2022). However, scientists are still debating whether mitochondrial dysfunction is a cause or a consequence of the transformation of normal cells into cancer cells (Senyilmaz and Teleman 2015). As suggested by D. Senyilmaz and Teleman (2015), both Otto Warburg's supporters and his opponents may be right in some cases. This view fits in with the idea of the polyetiology of cancer.

The key provisions of Warburg's hypothesis about the cause of cancerous tumour formation are statements according to which a violation of cellular respiration initiates the process of tumour formation, and the energy obtained through glycolysis compensates for the insufficient energy generation during the respiration of tumour cells. The following statement postulates the conversion of glucose to lactate in the presence of oxygen in cancer cells with irreversible impairment of cellular respiration (Liberti and Locasale 2016).

Otto Warburg, a physician, noticed that cancer cells obtain energy from glucose sugar in a strangely inefficient way: instead of burning it with oxygen, cancer cells do what yeast does: they ferment glucose. The anaerobic process is not only very fast but also very inefficient: most of the glucose energy is wasted. Otto Warburg suggested that anaerobic nutrition was the cause of cancer. Subsequent studies showed that cancer cells can have any type of energy supply, including the characteristics of normal cells, and the effect discovered by Warburg began to be considered a consequence rather than a cause of cancer (Pascale et al. 2020).

In addition to explaining the Warburg effect, data from other researchers have suggested that metabolism is secondary to enzyme signalling pathways. Therefore, the regulation of cellular metabolism may be an effective way to prevent cancer growth. The question of whether it is the metabolic changes that trigger carcinogenesis or the tumourigenic cell transformation that triggers the metabolic changes is still controversial (Vaupel and Multhoff 2021). However, many studies show that the increased dependence on glycolysis is characteristic of most malignant tumours and that glycolysis provides tumour cells with ATP molecules and metabolic intermediates for their growth and proliferation (Vaupel et al. 2019).

Several mechanisms by which mitochondria may be involved in carcinogenesis have been discussed in the literature. The first involves mitochondrial dysfunction as

the primary cause of tumour development and aerobic glycolysis. The second mechanism involves metabolic rearrangement of the tumour cell as a consequence of tumour transformation through mitochondrial dysfunction. However, this can be seen as a second step in the metabolic transformation of tumour cells (Lin et al. 2023).

Studies have shown examples of tumour cell lines in which reduced mitochondrial function was found to be caused by mutations in mitochondrial DNA or nuclear DNA encoding some mitochondrial proteins. In contrast, in other tumour cell lines, low levels of oxidative phosphorylation may be a consequence of increased glycolysis caused by hypoxia or genetic alterations in oncogenes and oncosuppressor genes. In this case, the dilemma of what is primary and what is secondary in mitochondrial dysfunction in carcinogenesis has unfortunately not yet been resolved, but the existence of a close relationship between oncogenes and the Warburg effect is no longer in doubt.

It is now accepted that aerobic glycolysis is present in tumour cells at very early stages of carcinogenesis, even before cancer cells become hypoxic (Vander Heiden et al. 2009). The presence of the Warburg effect in tumour cells does not mean that tissue respiration and oxidative phosphorylation are completely switched off in these cells. In fact, in most cancer cells, the proportion of energy produced by aerobic glycolysis does not exceed 50% of the total, with the remainder coming from mitochondrial oxidative phosphorylation (Pedersen 2007). Although the Warburg effect is not universal to all types of cancer tumours (Funes et al. 2007), increased glucose uptake by tumour cells is a fairly common phenomenon. Its discovery served as the basis for the development of principles for visualising cancer tumours and their metastases in clinical practice using a fluorinated analogue of glucose, 2-(18F)-2-deoxy-D-glucose, with positron emission tomography (Plathow and Weber 2008). In addition, an increase in the expression of genes encoding glycolytic enzymes has been found in at least 70% of all human cancers (Altenberg and Greulich 2004).

Increased glucose uptake and high rates of aerobic glycolysis may provide cancer cells with certain survival advantages over non-tumour cells. First, active glycolysis allows cells to survive in conditions that will sooner or later develop into a malignant tumour (Warburg 1956; Pouyssegur et al. 2006). Second, cancer cells use glycolytic pathway intermediates for anabolic reactions and to enhance their antioxidant defences (DeBerardinis et al. 2008; Berardi and Fantin 2011). For example, glucose 6-phosphate, a product of the first reaction of glycolysis, can be sent to the pentose phosphate pathway (PPP) to form ribose 5-phosphate and NADPH, which are necessary for nucleotide synthesis and maintenance of cellular redox potential. In addition, NADPH and three-carbon glycolytic intermediates are also important for lipid biosynthesis. Third, cancer cells produce lactate as the end product of aerobic glycolysis, which is removed from the cell by monocarboxylate transporter-4 (MCT-4). The development of extracellular acidosis contributes not only to the invasion and metastasis of cancer cells but also to their evasion from immune attack (Fischer et al. 2007; Goetze et al. 2011). In addition, lactate produced in tumour cells

suffering from hypoxia can serve as a carbon source for cells located in aerobic areas of the tumour (Sonveaux et al. 2008; Semenza 2008).

The molecular mechanisms that trigger aerobic glycolysis in tumour cells are quite complex and can work in concert or independently. The switch to a glycolytic phenotype in cancer cells is explained by several mechanisms (Kim and Dang 2006; Kroemer and Pouyssegur 2008; Fogg et al. 2011):

1. Activation of the transcription factor HIF-1 and its effect on glucose metabolism: In tumour cells in hypoxic conditions, the rate of HIF-1 degradation decreases and its level increases. In cancer cells that receive sufficient oxygen, the level and activity of this transcription factor can increase due to increased expression of the *HIF-1* gene following the activation of oncogenes (ras, src, and myc), inactivation of suppressor genes (p53, PTEN, and VHL), and activation of intracellular signalling pathways (PI3K/Akt).
2. Hypoxia-inducible factor (HIF)-independent modification of the expression pattern of genes whose products are involved in energy metabolism reactions as a result of the activation of oncogenes (ras, c-myc, and akt) and inactivation of suppressor genes (p53).
3. Changes in metabolic and bioenergetic reactions in the mitochondria due to mutations in genes in the nuclear and/or mitochondrial DNA.

It should be noted that the switch to a glycolytic phenotype with the formation of lactate, together with the activation of the biosynthesis of lipids and other macromolecules, is also observed in rapidly proliferating non-tumour cells, such as mitogen-activated T lymphocytes, thymocytes, and some haematopoietic cell lines (Bauer et al. 2004; Maciver et al. 2008).

5 Hypoxia and HIF-1 α in the Pathogenesis of Tumour Growth

Hypoxia is considered to play an inducing role in the pathogenesis of tumour growth (Jing et al. 2019). Furthermore, hypoxia is one of the most characteristic features of a tumour and has, therefore, become the subject of many scientific studies aimed at increasing the efficacy of antitumour therapy. Hypoxia is an important (limiting) factor in the development of cancerous tumours. Reduced diffusion of oxygen from surrounding tissues limits tumour growth. However, over time, hypoxic conditions stimulate vascularisation, thereby increasing tumour growth and metastasis (Infantino et al. 2021). It is well known that hypoxia leads to functional and then structural changes in organs and tissues as a result of a decrease in intracellular oxygen tension. This also applies to hypoxia of tumour cells (intratumoural hypoxia). Many cancers have areas of hypoxia. Intratumoural hypoxia significantly worsens the prognosis of the disease due to the very intense angiogenesis in tumour tissue. It is thought to be one of the causes of the rapid growth of malignant tumours. In addition, increased angiogenesis in the tumour promotes metastasis of its cells,

which ultimately leads to increased mortality in such patients (Poole and Macleod 2021).

Under the influence of hypoxia in tumour cells, there is selection for a genotype that supports cell survival in hypoxic conditions (e.g. p53 mutation), changes in the expression of genes that suppress apoptosis and support autophagy, predominance of anabolic processes, decreased DNA repair, an increased tyrosine kinase-mediated reaction cascade, epithelial-mesenchymal transition, invasiveness, metastasis, and suppression of immune responses (O'Malley et al. 2020). Increased levels of p53, which induces cell cycle arrest and apoptosis, may explain the delayed development of recurrence, presence of dormant micrometastases, and growth arrest in larger tumours (Vaupel and Multhoff 2021). However, the increased formation of free radicals and, consequently, the increase in the number of mutations lead to a violation of this mechanism. In tissue hypoxia, the hypoxia-inducible factor (HIF) is activated in cells, stimulating the expression of hundreds of genes (Fedotova et al. 2023). One of the most important effects is the switch from oxidative to glycolytic metabolism as well as the stimulation of metastasis and radiation resistance (Vaupel and Multhoff 2020) (Fig. 3).

Hypoxia produces radicals [reactive oxygen species and nitric oxide (NO)] that react with cellular molecules, altering their biochemical and physical properties and leading to cell death (Xing et al. 2023). Nitric oxide is formed with the involvement of nitric oxide synthase (NOS), and all three isoforms of this enzyme have been

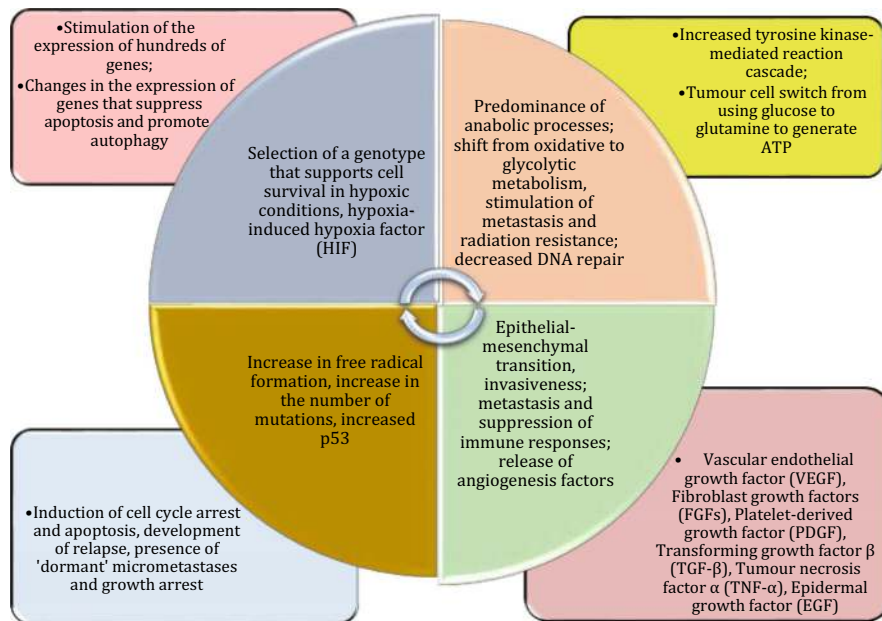


Fig. 3 Effects of hypoxia in tumour cells

identified in tumours: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS). Under chronic hypoxia, endothelial and tumour cells increase iNOS expression. NO produced by endothelial cells has at least two important functions: maintenance of adequate blood flow through vasodilation and antithrombotic endothelial activity. The end product of the interaction between NO and $O_2^{\cdot-}$ radicals—nitrotyrosine—is a marker of the level of oxidative stress (O'Malley et al. 2020).

It is clear that hypoxia causes the death of normal cells via apoptosis or necrosis but promotes the survival of tumour cells by activating glycolysis and angiogenesis pathways, thereby stimulating tumour progression (Vaupel 2008). Malignant neoplasms are an example of cellular adaptation to hypoxic conditions. In fact, the imbalance between high rates of proliferation and insufficient rates of vascularisation, leading to a shortage of oxygen and energy substrates, ensures the evolutionary selection of tumour clones with adaptations favourable for maintaining viability in these conditions. In turn, hypoxia-induced genetic changes ensure tumour progression as a result of clonal selection (Chen et al. 2009; Lozy and Karantza 2012). A model has been proposed according to which hypoxia during tumour progression mediates the selection of cells with reduced apoptotic potential, deficiency of the p53 transcription factor (tumour suppressor protein) or expression of its mutant variant, and overexpression of the anti-apoptotic protein Bcl-2 and proangiogenic factors (in particular the vascular endothelial growth factor—VEGF), which may contribute to the formation of a more aggressive tumour phenotype with greater resistance to the action of antitumour agents (Graeber et al. 1996; Zhou et al. 2006).

In addition to the peculiarities of cell cycle regulation, implementation of apoptotic signals, autophagy, and functioning of signal transduction pathways in tumour cells, the modification of metabolic processes and ATP synthesis pathways, which ensure the growth and viability of malignant tumours under metabolic stress, is also a decisive factor (Vaupel and Harrison 2004).

Oxygen deprivation activates the hypoxia-inducible factor (HIF) signalling pathway in cells (Luo et al. 2022). Its different isoforms, such as HIF-1, HIF-2, and HIF-3, regulate the expression of several thousand genes (Kozal and Krześlak 2022). During tumour development and progression, HIF activation stimulates angiogenesis and promotes changes in cell metabolism, adhesion, invasion, and metastasis. HIF isoforms may play opposing roles in the development of inflammatory and tumour processes. HIF-1 and hypoxia-regulated target genes are involved in key processes, i.e. erythropoiesis, angiogenesis, glycolysis, glucose transport, metastasis, and cell survival (Agani and Jiang 2013) and metabolic pathways, such as the phosphatidylinositol 3-kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR), which is closely related to the mitogen-activated protein kinase (MAPK) signalling pathway and is becoming an intensively described intracellular signalling pathway in tumorigenesis, as demonstrated by data (Polivka Jr and Janku 2014; Yeh et al. 2018). As shown in a convincing number of studies (Courtney et al. 2015), cancer PI3K signalling pathways play a role in many cellular processes, e.g. inflammation, cell metabolism and motility, and ultimately cancer progression (Wang et al. 2016) (Fig. 4).

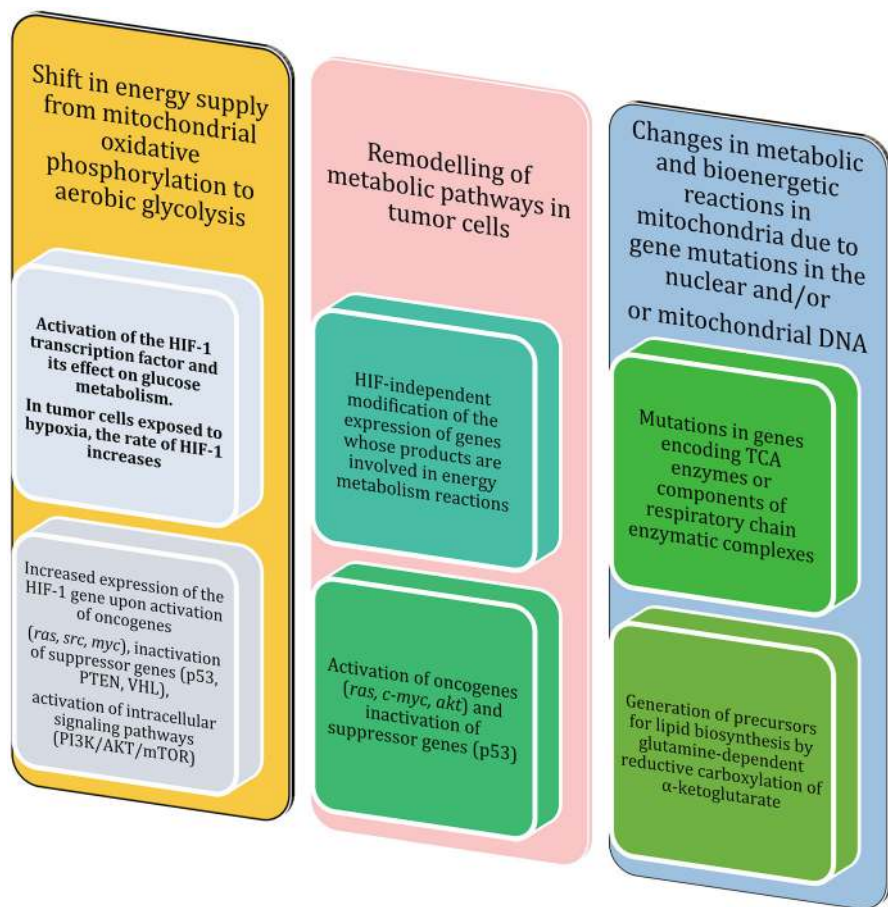


Fig. 4 Metabolic reprogramming or alteration of major metabolic pathways in cancer cells (Courtney et al. 2015; Xie et al. 2019). HIF-1—hypoxia-inducible factor 1, the basic domain transcription factor; p53—transformation-related protein 53; TCA—tricarboxylic acid cycle; PTEN—human tumour suppressor gene on chromosome 10 (its protein: phosphatase and tensin homologue), MMAC1—mutated in multiple advanced cancers 1; VHL—von Hippel-Lindau disease tumour suppressor gene

Paradoxically, in hypoxic conditions, the production of mitochondrial ROS increases, the main source of which is complex III of the ETC, which are physiological regulators of HIF1 (Finkel 2012). The stability and function of HIF1 are highly dependent on the level of oxygen in the cellular environment and have a regulatory effect on the state of energy metabolism. Under normoxia, the labile α -subunit of HIF1 is hydroxylated by prolyl hydroxylases. This is a signal for the onset of its ubiquitylation under the action of VHL (von Hippel-Lindau) E3 ligase and further proteasomal degradation (Brahimi-Horn et al. 2007; Vaupel and Mayer 2007). ROS may contribute to the stabilisation of HIF1 by inhibiting prolyl hydroxylases

(Pugh and Ratcliffe 2003). In conditions where glycolysis predominates and mitochondrial function is impaired, the level of ketoacids (pyruvate and oxaloacetate) increases, which can also have an inhibitory effect on prolyl hydroxylases (Kim et al. 2007). As a result, the degree of the association of labile HIF1 α and constitutive HIF1 β subunits with the formation of the active transcription factor HIF1 is increased (Mole et al. 2001; Vaupel and Mayer 2007; Kaplia et al. 2015).

The important role of HIF1 in suppressing oxidative phosphorylation reactions in the mitochondria of tumour cells is determined by its ability to act as a negative regulator of the synthesis of ETC enzymes and to stimulate the expression of glycolytic enzymes, regulatory enzymes, signalling molecules, etc. (Gatenby and Gillies 2004). The activation of HIF1 mediates the activation of the expression of pyruvate dehydrogenase kinase (KPDH)—an inhibitor of the pyruvate dehydrogenase complex of mitochondria in tumour cells through phosphorylation of pyruvate dehydrogenase (PDH) (Iyer et al. 1998), glycolytic enzymes—hexokinase II and the hypoxia-inducible isoform of the M-subunit of lactate dehydrogenase adapted to anaerobic conditions, and VEGF (Kim et al. 2006; Papandreou et al. 2006). PDH converts pyruvate to acetyl-CoA, which enters the Krebs cycle as part of the general pool of acetyl-CoA, whose reactions produce NADH and FADH₂ supplying electrons to complexes I and II of the respiratory chain. Mitochondrial PDH is the target of the regulatory control of glucose metabolism, determining its completion at the stage of glycolysis in the cytosol with the formation of pyruvate or lactate or its continuation during the Krebs cycle and oxidative phosphorylation (Roche and Hiromasa 2007). KPDH regulates the activity of the multi-enzyme complex PDH, thereby controlling the intensity of oxidation of energy substrates in mitochondria and providing a mechanism for switching metabolism and cellular adaptation to hypoxic conditions (Kim et al. 2006). PDH substrates: pyruvate, NAD⁺, and CoA are inhibitors of PDH activity and products of the pyruvate dehydrogenase reaction: NADH and acetyl-CoA are its activators. In physiological conditions, the balance of NAD⁺/NADH, CoA/acetyl-CoA, and pyruvate in the mitochondria determines the activity of KPD, depending on the metabolic needs of the cell. It is known that, under metabolic stress in tumour cells, HIF1-induced activation of KPD expression and corresponding inhibition of PDH, restriction of pyruvate entry into mitochondria, reduction in the intensity of tricarboxylic acid cycle reactions, and hyperproduction of lactate occur (Gogvadze et al. 2010). One of the causes of the decrease in pyruvate levels in tumour cells may also be the change in the expression of aldehyde dehydrogenase isoforms, leading to an increase in the level of acetaldehyde, which interacts in the active site of PDH with decarboxylated pyruvate to form a competitive PDH inhibitor, acetoin, an abnormal metabolite of tumour cells (Baggetto and Lehninger 1987; Kaplia et al. 2015).

In tumour cells, hexokinase II acquires the ability to bind to the outer mitochondrial membrane through porin-like proteins—voltage-dependent anion channels (VDACs). A correlation has been found between the activity of hexokinase II associated with mitochondria and a high rate of tumour growth (Bustamante et al. 1981). On the one hand, the mitochondrial localisation of this enzyme maintains a high intensity of glycolysis in many malignant tumours; on the other hand, it

mediates the mechanism of inhibition of apoptosis as a “gatekeeper”, thus ensuring the immortality of cancer cells (Mathupala et al. 2009). HIF1 is also a modulator of gene expression and cytochrome oxidase activity; excess HIF1 leads to changes in the subunit composition of the enzyme through proteolysis, activating transcription of the mitochondrial protease *LON* gene (Boutin and Johnson 2007). Reduced expression of the proapoptotic protein p53 in tumour cells leads to the disruption of the cytochrome oxidase assembly as a result of the insufficient expression of the assembly factors SCO1 and SCO2 (Gogvadze et al. 2010). The lack of functional cytochrome oxidase leads to a shift in oxidative metabolism in tumour cells towards glycolysis (Kaplia et al. 2015).

Bioenergetic Profile of Tumour Cells The question of primary metabolic changes that trigger carcinogenesis or metabolic changes that are a consequence of tumour cell transformation is controversial. However, studies show that the increased reliance on glycolysis is characteristic of many cancers, and glycolysis provides cancer cells with ATP molecules and metabolic intermediates for their growth and proliferation. The breakdown of glucose to lactate is a less efficient but a faster process for energy production. In addition, the high rate of glycolysis promotes tumour cell growth by increasing the formation of precursors for lipid, nucleotide, and amino acid synthesis, while the metabolites of glycolysis (lactate and protons) cause acidification of the extracellular space. Acidification of the environment favours not only invasion and metastasis of cancer cells but also their evasion of the attack by immune system cells (Vaupel et al. 2019; Vaupel and Multhoff 2020, 2021).

Changes in metabolic pathways may vary from one tumour to another (Ferns et al. 2021). It is known that, even within the same tumour, there can be cells with different metabolic phenotypes. Therefore, the determination of the “bioenergetic profile” of a cancerous tumour, from glycolytic to oxidative, in order to identify the main metabolic pathway, depends on the type of tumour, the microenvironment of tumour cells, the degree of differentiation, and the stage of tumour cell growth (Martínez-Reyes and Chandel 2021). Changes in metabolic pathways suggest the following possible types of cancer therapy (Biswas 2015; Ferns et al. 2021). The typical “glycolytic” phenotype of cancer cells is characterised by increased glycolysis and low efficiency of mitochondrial oxidation, whereas the “oxidative” phenotype is dominated by mitochondrial oxidation of glucose and/or glutamine. Accordingly, for cancer cells with a “glycolytic” phenotype, a therapeutic strategy targeted at the inhibition of glycolysis seems logical. The effects on glutamine metabolism may have a significant impact on cancer cells whose survival and proliferation are dependent on glutamine. The metabolic adaptation of cancer cells is also important where targeting different pathways of energy metabolism would be appropriate (Pavlova et al. 2022).

Two therapeutic strategies for cancer cells have been considered in the literature. One involves direct inhibition of glycolysis by affecting the activity of the first enzyme in the reaction cascade, hexokinase-2, which in cancer cells is mostly bound

to the outer mitochondrial membrane via the voltage-dependent anion channel (VDAC) and plays an important role in preventing apoptosis (Mazure 2017). The second strategy involves the indirect inhibition of glycolysis through effects on the signalling pathways that regulate this process (inhibition of HIF1- α and phosphatidylinositol 3-kinase/Akt/m-TOR signalling pathway and activation of adenosine monophosphate kinase (AMPK)), as shown in publications (Narayanankutty 2020; Xiang et al. 2022).

A characteristic feature of cells from most types of malignant tumours is the ability to meet energy requirements for ATP synthesis, even in aerobic conditions, mainly through the glycolytic pathway (Lu et al. 2015a, b). This phenomenon was identified by O. Warburg in 1926, who showed that tumour samples produced significantly more lactic acid than normal cells, regardless of the presence of oxygen. He hypothesised that the inhibition of energy metabolism in the mitochondria of tumour cells was the cause of the increased intensity of glycolysis and a prerequisite for the development of a tumour phenotype (Warburg 1956). For example, the level of ATP generation in the mitochondria of tumour cells, especially those with high rates of proliferative activity, reaches only 50% of the total level, whereas it is 90% in normal cells (Capuano et al. 1996). This suppression of energy metabolism reduces the oxygen demand of tumour cells, as up to 70–90% of the oxygen consumed by normal cells is used for mitochondrial function (Frezza and Gottlieb 2009), and switching the ATP synthesis mechanism to the oxygen-independent glycolytic pathway is considered an effective mechanism for tumour cell adaptation to metabolic stress under oxygen deprivation (Höckel and Vaupel 2001). Despite its low energy efficiency, aerobic glycolysis in tumour cells is also considered to be a mechanism for protecting cells from excess ROS that would be generated in the conditions of intense substrate degradation in mitochondria (Chen et al. 2007) and for providing appropriate conditions for tumour cell invasion into surrounding tissues (Smallbone et al. 2007).

It has been shown that the use of glycolysis inhibitors does not lead to a compensatory increase in the functional efficiency of the ETC of tumour cells (Koseki et al. 2019). In addition, the majority of malignant tumours do not exhibit the Pasteur effect—inhibition of glycolysis by respiration (Vaupel 2008), suggesting that impaired mitochondrial function in tumour cells plays a primary role in shaping their ability to use glycolysis as a key pathway for ATP synthesis. However, the significance of mitochondrial dysfunction for the biology of tumour cells is currently not clearly understood (Kaplia et al. 2015).

A direct correlation has been established between the intensity of ATP synthesis in aerobic glycolysis reactions in tumour cells and their proliferative activity and invasive potential (Simonnet et al. 2002; Zhang et al. 2021). Increased glycolysis in malignant tumour cells leads to peritumoural acidification due to the diffusion of excess hydrogen ions into the surrounding tissue, which induces the death of normal cells via necrosis or caspase-mediated activation of p53-dependent apoptosis. In turn, extracellular acidosis promotes angiogenesis, degradation of the extracellular matrix, and inhibition of the body's immune response to tumour antigens (Gatenby et al. 2006). Excessive production of lactate also leads to its excretion into the

extracellular environment, and a defect in blood flow leads to its retention in tumour tissue and changes in tumour pH (Fang et al. 2008).

It is known that lactate, as a paracrine regulator of tumour growth, affects the proliferative potential and energy metabolism of tumour cells and promotes the expression of the hypoxia-inducible factor (HIF1) in these cells (Nagel et al. 2016). Lactate also induces the synthesis of proteins required for cellular adaptation to hypoxia, in particular, glycolytic enzymes and anti-apoptotic proteins (Zong et al. 2016), and inhibits the expression of mitochondrial ETC components (Shehade et al. 2014). The ability of the end products of glycolysis, primarily lactate, to degrade the extracellular matrix and increase the migratory activity of tumour cells is well known and appears to increase the metastatic potential of a malignant tumour (Gillies and Gatenby 2015). In primary human carcinoma cells, a correlation has been shown between excess lactate levels and high lactate dehydrogenase (LDH) activity in tumours with a greater degree of invasiveness and aggressiveness (Hirschhaeuser et al. 2011).

Malignant cells, in contrast to normal cells, are characterised by the hyperpolarisation of the IMM with a shift towards more negative values (from -150 to -170 mV) (Davis et al. 1985). This may be caused by disturbances in the activity of the enzymes of the ETC complexes, electron transport by these complexes, transmembrane proton transfer, insufficient utilisation of $\Delta\mu\text{H}^+$, and modification of the conductivity of the mitochondrial membrane (Fogg et al. 2011). It has been shown that isolated mitochondria from hepatocellular carcinoma are characterised by lower levels of ATP hydrolysis, ADP phosphorylation, and electron transport compared to normal cells (Formentini et al. 2010). A modification of the ATP synthase F_0F_1 is also often observed in tumour cell mitochondria, characterised by a decrease in V_{max} , lower content of the β -subunit in the F_1 component, and/or the associated overexpressed protein inhibitor (IF1) (Capuano et al. 1996), which explains the lack of utilisation of the proton gradient in tumour cell mitochondria. The utilisation of acetoin with the formation of citrate enhances the biosynthesis of cholesterol in cancer cells, and as a result, the additional incorporation of cholesterol into the IMM causes a significant decrease in passive proton permeability and voltage-dependent metabolism in tumour cells (Baggetto and Testa-Parussini 1990). Thus, the hyperpolarisation of the IMM of tumour cells, the reduced proton permeability of the membrane with an increase in its cholesterol content, and the inhibition of ATP synthase activity contribute to the transition of electron carriers to the reduced state (Andreyev et al. 2005). In this case, ubiquinone molecules bound to the CoQ sites of complexes I and III are converted to the ubisemiquinone radical, which undergoes alternative reactions with molecular oxygen to form the toxic superoxide anion by-product (Mazat et al. 2020).

Despite their ability to survive and proliferate under oxygen deprivation in their microenvironment, tumour cells retain residual aerobic respiratory activity (Fogg et al. 2011). They are highly sensitive to inhibitors of mitochondrial respiration. The use of rotenone, a specific inhibitor of electron transport in complex I, leads to the hypergeneration of ROS and partial death of tumour cells (Carew and Huang 2002). The disruption of mitochondrial respiration in tumour cells also leads to an increase

in the NADH pool, which inactivates the PTEN phosphatase (phosphatase and tensin homologue), an antagonist of phosphatidylinositol 3-kinase (PI₃K) and its target PKB/Akt (Antico Arciuch et al. 2012). The activation of these signalling enzymes causes additional stimulation of glycolysis and a decrease in energy production in the mitochondria, which promotes the survival of tumour cells under metabolic stress and the development of their resistance to a number of antitumour drugs (Lu et al. 2015c).

Activation of oncogenes (*MYC*, *AKT*, and *RAS*) and inactivation of tumour suppressors also play an important role in maintaining the state of aerobic glycolysis in tumour cells (Marbaniang and Kma 2018). Increased expression of *AKT* and *MYC* leads to increased glycolysis and glutaminolysis, which is manifested by decreased expression of the tumour suppressors *P53* and *VHL* (Dang 2013). Loss of the functional p53 protein has been shown to cause a metabolic shift in aerobic respiration towards ATP production via the glycolytic pathway (Puzio-Kuter 2011). There are studies (Matoba et al. 2006) that support the notion that an increase in the intensity of aerobic glycolysis in tumour cells occurs as a result of mutations in genes involved in the regulation of oxidative phosphorylation (in particular, the *P53* gene). The ROS-mediated activation of transcription factors plays an important role in the development of the tumour phenotype in hypoxic conditions: NF- κ B (nuclear factor κ B), AP-1 (activator protein 1), and GRP78 (glucose-regulated protein 78) (Morgan and Liu 2011).

6 ROS Production During Tumour Growth

The inhibition of the activity of the respiratory chain of the inner mitochondrial membrane (IMM) observed in malignant tumour cells, for example, as a result of disturbances in the structural stability of the electron transport chain complexes, synthesis of mutant versions of its components, inhibition of respiration by glycolysis, loss of functionally active cytochrome c, or with an increase in the intensity of reverse electron transport (RET), is accompanied by an increase in the NADH/NAD⁺ ratio (Hirst et al. 2008)—a factor that enhances the formation of O₂^{•−} by both complex I and other enzymes that interact with NADH (e.g. α -ketoglutarate dehydrogenase, etc.) (Starkov et al. 2004; Kaplia et al. 2015).

A correlation has been established between a decrease in the ability of NADH-CoQ oxidoreductase to oxidise NAD⁺-dependent substrates (pyruvate, glutamate, and malate) as a result of structural defects or oxidative modification of functionally active groups and an increase in the level of ROS generation by mitochondrial complex I (Jezek and Hlavatá 2005), whose intensity increases at high transmembrane potential values on the IMM (Votyakova and Reynolds 2001), typical of malignant tumours (Davis et al. 1985; Bonnet et al. 2007; Kaplia et al. 2015).

The formation of ROS during RET is significantly suppressed during the utilisation of the proton gradient, for example, during ATP synthesis or during other energy-dependent processes, such as Ca²⁺ uptake by mitochondria, under the influence of mitochondrial energy dissipation systems—proteins that uncouple

respiration and oxidative phosphorylation (Ledesma et al. 2002). It should be emphasised that, in the hypoxic conditions typical of tumour cells, intense ROS synthesis in complex I occurs precisely during RET (Jezek and Hlavatá 2005; Murphy 2009).

The slowing down of electron transport along the ETC of the IMM in tumour cell mitochondria is accompanied by an increase in the lifetime of the ubisemiquinone radical with the generation of a significant amount of ROS in complexes I and III (Penta et al. 2001; Carew and Huang 2002). It has also been shown that endogenous NO regulates O_2^- production by modifying the redox state of cytochrome c oxidase under O_2 deficiency (Palacios-Callender et al. 2004). An increase in the level of reduction of cytochromes a + a3 and c + c1 under the influence of endogenous NO contributes to the mechanism of maintaining the rate of O_2 consumption at low concentrations (Palacios-Callender et al. 2004). An increase in NO synthase activity, observed in some malignant tumours, leads to the inhibition of cytochrome c oxidoreductase (Xu et al. 2002). This inhibition of complex IV leads to increased generation of the superoxide anion as a factor in promoting mtDNA mutations, synthesis of dysfunctional proteins, and modification of mitochondrial proteins and lipids in tumour cells (Kaplia et al. 2015).

In hypoxia or oxidative damage to the structure of the succinate dehydrogenase enzyme in complex II during the oxidation of the flavin semiquinone radical and in the absence of substrates for immediate electron transfer, the state of the enzyme can switch from catalysing its own succinate dehydrogenase reaction to fumarate reductase activity, leading to an increase in the intensity of RET (Andreyev et al. 2005; Jezek and Hlavatá 2005). At low levels of oxygen uptake by cells, the necessary reduction of energy consumption (ATP) requires inhibition of the activity of energy-consuming enzymes, such as Na^+, K^+ -ATPase. Studies have shown that its activity decreases with the degree of dedifferentiation of colorectal adenocarcinoma cells, which determines the increase in tumour malignancy (Kaplia et al. 2007, 2015). Downregulation of the expression of this transport ATPase has been found in some carcinomas (Kaplia and Morozova 2010). In addition, it has been shown that mitochondrial ROS also contribute to the inhibition of Na^+, K^+ -ATPase activity by stimulating its endocytosis (Dada et al. 2003).

In tumour cells, the formation of ROS in mitochondria and an increase in the NADH/NAD⁺ ratio cause activation of the protein kinase PKB/Akt, which phosphorylates transcription factors of the FOXO (forkhead box O) group, leading to a decrease in the expression of Mn-SOD and mitochondrial catalase (Robey and Hay 2009; Fan et al. 2010). Depletion of NAD⁺ leads to the inhibition of the NAD-dependent deacetylase sirtuin 3, a product of a tumour suppressor gene, which deacetylates and inactivates FOXO (He et al. 2012). In tumour cells, mitochondrial ROS also contribute to the inhibition of phosphatases, in particular, the PTEN phosphatase, a PKB/Akt antagonist, and MAP kinase phosphatases. In the conditions of suppression of Mn-SOD activity, it is important to note the role of mitochondrial ROS in stimulating the activity of the transcription factor NF- κ B and inactivating JNK phosphatases, resulting in the formation of resistance of tumour cells to apoptosis-inducing signals from the tumour necrosis factor TNF α (Meng

et al. 2002). For example, in breast cancer cells, increased generation of ROS in the mitochondria against a background of inhibition of antioxidant enzyme activity and decreased expression of tumour suppressor proteins was found to correlate with an increase in the number of mtDNA mutations, high metastatic potential, and resistance to proapoptotic stimuli (Imanishi et al. 2011).

Also, the loss of the mitochondrial membrane potential is a critical event in the cell and its entry into apoptosis due to the formation of ROS (Murphy 2009). A correlation has been shown between the transmembrane potential of mitochondria isolated from human peripheral blood lymphocytes and the production of ROS and the occurrence of nitrosative stress (Doherty and Perl 2017). A significant and prolonged increase in the mitochondrial transmembrane potential, accompanied by an increase in ROS production, can lead to damage to the mitochondrial membrane, loss of its functions, and, as a result, cell death (Brand 2016).

Thus, the intensity of $O_2^{\cdot -}$ and H_2O_2 generation in normal cells is balanced by the functioning of antioxidant defence enzymes localised directly in the mitochondria (Andreyev et al. 2005), whereas tumour cells are characterised by a spatio-temporal imbalance between the functional activity of antioxidant enzymes and ROS-generating systems of the mitochondria (Chatterjee et al. 2011). The result may be an increase in the destabilising and damaging effects of ROS in relation to the ETC of the IMM (Kaplia et al. 2015).

7 Cancer Cell Heterogeneity and Metabolic Antitumour Therapy

Two therapeutic strategies are currently being used to target the Warburg effect in cancer cells. One is based on direct inhibition of glycolysis by targeting the activity of glycolytic enzymes and the other involves indirect inhibition of glycolysis by targeting signalling pathways that regulate this process. The most attractive target for glycolysis inhibition is the second isoform of the first enzyme in the glycolysis cascade—hexokinase-2. In contrast to normal cells, the activity and amount of hexokinase-2 are significantly increased in cancer cells. This enzyme, which in cancer cells is mostly associated with the outer mitochondrial membrane through the voltage-dependent anion channel (VDAC), plays an important role in preventing apoptosis (Ho et al. 2016).

The metabolically inactive analogue of glucose, 2-deoxyglucose, was found to inhibit hexokinase-2 and inhibit tumour growth *in vitro*. In this case, deoxyglucose selectively kills cancer cells with defects in mitochondrial respiration or those exposed to hypoxic conditions (Dwarakanath 2009). However, due to the low antitumour efficacy of 2-deoxyglucose *in vivo*, the efficacy of its use in combination with radiotherapy and traditional chemotherapeutic drugs is currently being investigated (Sheng and Tang 2016; Sobhakumari et al. 2016).

Another hexokinase-2 inhibitor, 3-bromopyruvate, induces apoptosis in cancer cells by disrupting the enzyme's association with the mitochondrial voltage-gated anion channel. An inhibitory effect of bromopyruvate on other glycolytic enzymes,

in particular 3-phosphoglycerate dehydrogenase and lactate dehydrogenase, has also been found. In addition, in tumour tissue, bromopyruvate inhibits oxidative phosphorylation and suppresses angiogenesis and ATP-binding cassette transporters that remove many drugs from the cell, thereby promoting the development of chemoresistance (Baghdadi 2017; Fan et al. 2019). In the presence of bromopyruvate, tumour cells die quickly while normal cells remain unharmed, as normal cells, unlike tumour cells, do not have significant amounts of monocarboxylic acid transporters (MCT-1) on their surface. The antitumour activity of bromopyruvate has been confirmed in animal studies and by other scientific laboratories. In addition, the inhibition of glycolysis by bromopyruvate not only enhances the cytotoxic effect of the antitumour antibiotics daunorubicin and doxorubicin *in vitro* but also significantly inhibits tumour growth when used in combination with doxorubicin in animal experiments *in vivo* (Korga et al. 2019). Bromopyruvate has been shown to be effective in humans, but data are still limited (Ho et al. 2016).

Lonidamine, an indazole-3-carboxylic acid derivative, also slows tumour growth by inhibiting hexokinase-2, reducing oxygen consumption and lactate formation (Hammoudi et al. 2011). In addition, lonidamine inhibits membrane monocarboxylic acid transporters, preventing the export of lactate from the cell and leading to a decrease in intracellular pH. Lonidamine has also been shown to inhibit mitochondrial respiratory complex II (Nath et al. 2016). The efficacy of its combination with doxorubicin or cisplatin has already been evaluated in clinical trials in patients with breast, ovarian, lung, and brain tumours (Huang et al. 2020). New methods of delivering lonidamine to tumour cells using nanoparticles are being developed (Milane et al. 2011).

A logical approach to targeted inhibition of glycolysis is to inhibit the terminal enzyme of anaerobic glycolysis, lactate dehydrogenase (LDH), which is elevated in cancer cells. Oxamate, a structural analogue of pyruvate and one of the first LDH inhibitors, greatly reduces the rate of glycolysis and suppresses tumour cell growth. Unfortunately, oxamate is poorly absorbed by cells and only inhibits cancer cell proliferation at very high concentrations. However, the combination of oxamate with the widely used chemotherapeutic agent paclitaxel (Taxol) has been shown to have a synergistic inhibitory effect on Taxol-resistant cells (Zhao et al. 2013). The use of a synthetic LDH inhibitor, galloflavin, has been shown to have potent antitumour activity in cultured breast tumour cells (Farabegoli et al. 2012). A derivative of cinnamic acid, α -cyano-4-hydroxycinnamate, also inhibits glycolysis and proliferation of glioblastoma cells by inhibiting the transmembrane transport of lactate (Draoui et al. 2013).

The attempt to neutralise the low pH of the tumour microenvironment by using bicarbonate to reduce the invasiveness of tumours and minimise the possibility of metastasis also seems interesting and promising (Robey et al. 2009). This approach will make it possible to control the proliferation of malignant tumour cells via the alteration of the pH of the tumour microenvironment by affecting the activity and/or number of membrane bicarbonate transporters and membrane carbonic anhydrases (Kulikov and Belyaeva 2016).

Another way of inhibiting glycolysis consists in acting on the pyruvate dehydrogenase (PDH) enzyme complex, the activity of which determines the fate of pyruvate (either its conversion to acetyl-CoA or its reduction to lactate). The KPDH is phosphorylated, thereby inhibiting the action of this enzyme complex. The KPDH inhibitor, dichloroacetate, has been used to treat lactic acidosis by increasing the formation of acetyl-CoA from pyruvate, which enters the Krebs cycle, thereby switching energy metabolism from glycolysis to oxidative phosphorylation. Dichloroacetate has been shown to inhibit the proliferation of cancer cells and increase their death via apoptosis. The mechanism of action of dichloroacetate is based on (1) reversal of the glycolytic phenotype of cells to an oxidative one; (2) increased generation of reactive oxygen species and activation of the p53 protein with subsequent triggering of apoptosis; (3) inhibition of the transcription factor HIF-1. The therapeutic activity of dichloroacetate has been demonstrated in animal studies and in some clinical trials (Saunier et al. 2016; Stacpoole 2017). However, there are scientific publications on the lack of antitumour activity of dichloroacetate both in vitro and in vivo (Shen et al. 2015).

Since glycolysis depends on the transmembrane transport of glucose into cells, inhibition of membrane glucose transporters (GLUTs) is another alternative method of inhibiting glycolysis in cancer cells (Adekola et al. 2012). Phloretin, a GLUT-1 inhibitor, induces apoptosis of tumour cells and helps to overcome drug resistance to chemotherapy in hypoxic conditions (Tilekar et al. 2020). Attempts are being made to inhibit GLUT using monoclonal antibodies and antisense oligonucleotides against *SLC2A* genes that encode GLUT (Pliszka and Szablewski 2021).

Phosphofructokinase-1 (PFK-1) is the main rate-limiting enzyme in glycolysis. Its activity is tightly controlled, making it possible to modulate the rate of glycolysis according to the energy status of the cell or to direct glycolysis intermediates into the pentose phosphate cycle. An indirect inhibitor of this enzyme, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one, reduces glucose uptake and inhibits the growth of some cancer cell lines (Lea et al. 2015). A new and more potent derivative of this inhibitor, PFK15 (1-(4-pyridinyl)-3-(2-quinolinyl)-2-propen-1-one), induces cell cycle arrest and apoptosis and inhibits invasion in gastric cancer (Zhu et al. 2016).

Phosphoglycerate mutase-1 (PGM1) catalyses another important step in glycolysis, i.e. the conversion of 3-phosphoglycerate to 2-phosphoglycerate. This enzyme regulates the flux of glycolytic intermediates by influencing the concentration of its substrate 3-phosphoglycerate, the accumulation of which can inhibit 6-phosphogluconate dehydrogenase, an enzyme in the oxidative branch of the pentose phosphate pathway (PPP). Loss of p53 protein function in tumour cells due to mutations in its gene leads to increased levels of phosphoglycerate mutase-1. The rapid metabolism of 3-phosphoglycerate promotes the occurrence of PPP reactions, creating the conditions for rapidly dividing tumour cells to be supplied with nucleotides. Inhibition of PGM1 in cancer cells not only reduces the rate of glycolysis and PPP but also inhibits tumour cell growth (Jin et al. 2018; Li and Liu 2020).

Another important glycolytic enzyme, pyruvate kinase (PK), catalyses the irreversible transfer of a phosphate group from phosphoenolpyruvate to ADP to form

pyruvate and ATP. Mammalian pyruvate kinase has four isoforms (M1, M2, L, and R) that are present in different cell types. In tumour cells, the M2 isoform predominates in a low-active dimeric form sometimes called tumour PKM2. The dimeric form of PK has a lower affinity for phosphoenolpyruvate than its active tetrameric form. This leads to the accumulation of glycolytic intermediates in tumour cells, which contributes to their targeting of biosynthetic pathways (Israelien and Vander Heiden 2015). PKM2 activators TEPP-40, DASA-58, and ML-265 promote increased PKM2 activity, which is associated with a decrease in the rate of tumour cell proliferation under hypoxia and inhibition of tumour growth in mice due to a slowdown of cellular biosynthetic processes (Anastasiou et al. 2012).

The transcription factor HIF-1 α not only causes the activation of almost all glycolytic enzymes but also directly activates the gene encoding PDH kinase-1. The HIF-1 α inhibitor PX-478 reduces the levels of this transcription factor by decreasing the amount of mRNA and increasing its ubiquitination (Welsh et al. 2004). PX-478 has been shown to have antitumour activity against malignant tumours derived from xenograft colon, lung, breast, and pancreatic tumours in mice (Zhao et al. 2015). Another HIF-1 α inhibitor, acriflavine, binds directly to HIF-1 α and inhibits tumour growth in mice with prostate and mammary tumours (Lee et al. 2009).

The phosphatidylinositol 3-kinase/Akt/m-TOR signalling pathway plays a key role in energy metabolism and regulates cell growth and proliferation. Animal studies of the first phosphatidylinositol 3-kinase inhibitors, wortmannin and LY294002, showed high toxicity, and further studies were discontinued (Zhao et al. 2012). However, a new potent and selective phosphatidylinositol 3-kinase inhibitor, pictilisib (GDC-0941), is in early clinical trials (Schöffski et al. 2018). An Akt enzyme inhibitor, perifosine, blocks the movement of Akt to the cell membrane, thereby inhibiting the growth of a number of cancer cells (lung, breast, prostate, colon, and melanoma). Perifosine treatment shows a complex molecular response, including the inhibition of Akt or induction of apoptosis via clustering of death receptors in the lipid raft (Fensterle et al. 2014). m-TOR inhibitors are derivatives of rapamycin, a classical inhibitor of the m-TORC1 complex. They include temsirolime, everolime, and deforolime. These inhibitors have moderate antitumour activity against a range of tumours. A new generation of m-TOR inhibitors, e.g. BEZ235 and XL765, simultaneously inhibit m-TOR and phosphatidylinositol 3-kinase and are in first-line clinical trials (Xie et al. 2016).

Metformin is the first-line treatment for type 2 diabetes mellitus and plays an effective role in the treatment of several diseases, including cardiovascular disease, neurodegenerative disease, cancer, and ageing (Kurhaluk et al. 2018a, b; Kurhaluk and Tkachenko 2022). Epidemiological studies have shown that the use of metformin is associated with a reduced risk of developing malignant tumours in various sites. In tumour cells, metformin activates the adenosine monophosphate kinase (AMPK) signalling pathway and inhibits their growth. Metformin has been shown to have antitumour activity against pancreatic, breast, ovarian, and prostate cancer cells (Aljofan and Riethmacher 2019). The combination of metformin and doxorubicin kills cancer stem cells in cell culture (Hirsch et al. 2009). It is important to note that

AMP kinase is a double-edged sword that can act either as a tumour suppressor or as a pro-survival factor for tumour cells, depending on the amount of energy available in the cell. When tumour cells are in the proliferative phase and receive sufficient nutrients, AMPK may have a tumour-suppressing effect. Conversely, if tumour cells are energy starved, AMPK may promote tumour cell survival by shifting energy pathways towards mitochondrial oxidation. Therefore, drugs that activate AMPK should be used with caution, taking into account the bioenergetic status of tumour cells (Vincent et al. 2015).

Although inhibition of glycolysis is an attractive target for cancer treatment, some studies suggest that the use of glycolysis inhibitors alone in certain tumours may not be sufficient to completely block energy metabolism. For example, some malignant tumours of the lung, breast, cervix, ovaries, and skin actively use mitochondrial oxidative phosphorylation to produce ATP in addition to glycolysis (Zhang and Yang 2013). This suggests that the antitumour therapeutic strategy of inhibiting glycolysis cannot be considered universal, but may be appropriate for cancer cells with mitochondrial defects or those in hypoxic conditions (Pelicano et al. 2006).

Lactate produced by tumour cells located at a significant distance (more than 70–100 μm) from blood vessels, i.e. in hypoxic conditions, can be used as a “metabolic fuel” by its oxidative metabolism in tumour cells located near blood vessels (Sonveaux et al. 2008). Furthermore, when the metabolic profile of neoplastic epithelial cells and associated fibroblasts (tumour stromal cells) was studied, it was found that epithelial cancer cells secrete hydrogen peroxide at a concentration that can cause oxidative stress in neighbouring tumour stromal cells. This in turn induces glycolysis and autophagy in these cells, which may contribute to the survival of tumour cells under energy deprivation. Lactate released from tumour stromal cells can be taken up by tumour epithelial cells, where it is oxidised to CO_2 and H_2O via mitochondrial oxidation (Li et al. 2022). Thus, therapeutic targeting of the stromal-epithelial metabolic pathway and mitochondrial pathways represent important avenues for the development of new anticancer drugs. For example, chloroquine inhibits autophagy in tumour stromal cells and prevents the supply of energy substrates from the stroma to epithelial tumour cells (Varisli et al. 2020). An inhibitor of respiratory complex I (rotenone) induces the development of apoptosis in several cancer cell lines (Deng et al. 2010). The respiratory complex II inhibitor α -tocopheryl succinate (a vitamin E analogue) causes electron leakage with subsequent generation of cytotoxic reactive oxygen species (Dong et al. 2008). α -tocopheryl succinate competes with ubiquinone for binding to the second respiratory complex, which “breaks” the flow of electrons and promotes the formation of the superoxide anion. An inhibitor of respiratory complex III (benzyl isothiocyanate) promotes the formation of reactive oxygen species and induces the development of apoptosis in breast, liver, and lung cancer cells (Han et al. 2019). A respiratory complex V inhibitor (oligomycin) has antitumour activity in the treatment of liver cancer (Li et al. 2004). Resveratrol, a natural component of green tea, can specifically target the mitochondria of tumour cells and has antitumour activity. Resveratrol inhibits the first and third respiratory complexes of mitochondria. Today, resveratrol,

whose antitumour mechanisms are much broader than those described, is in the early stages of clinical trials (Kursvietiene et al. 2023).

Glucose and glutamine are two essential ingredients for cell growth. In cancer cells, glucose and glutamine metabolism are coupled and actively coordinated (Wang et al. 2018). The level of glutamine in cells can influence the pattern of glucose uptake. Thus, an increase in glutamine levels in the cell will ultimately result in increased glucose uptake. When tumour cells are cultured in a glucose-deficient medium, the activity of the enzyme glutamate dehydrogenase, which catalyses the conversion of glutamate to α -ketoglutarate used in TCA cycle reactions, is greatly increased. This promotes the survival of tumour cells even when glycolysis is severely impaired. On the contrary, the inhibition of glutamine metabolism in tumour cells after experimental “silencing” of glutaminase is compensated for by the activation of pyruvate carboxylase in these cells, which uses the pyruvate formed during glycolysis to replenish the TCA cycle with oxaloacetate (Cheng et al. 2011). Recently, another mechanism for the survival of at least some tumour cell lines under severe glucose starvation has been identified. This mechanism is the pathway of phosphoenolpyruvate formation from glutamine via increased activity of the mitochondrial isoform of phosphoenolpyruvate carboxykinase. Phosphoenolpyruvate is also used for metabolic biosynthetic pathways normally supported by glucose metabolism, such as the synthesis of serine and purines (Yu et al. 2021). It should be noted that the function of the mitochondrial isoform of phosphoenolpyruvate carboxykinase was long unknown. Only recently has it been shown that this enzyme is part of the survival programme of tumour cells in stress conditions, e.g. glucose or glutamine deprivation or endoplasmic reticulum stress (Méndez-Lucas et al. 2014).

In addition to the Warburg effect and glutaminolysis, a characteristic metabolic property of tumour cells is the activation of the synthesis of higher fatty acids (Currie et al. 2013). More than half a century ago, it was shown that tumour cells synthesise fatty acids de novo from glucose, and subsequent studies have demonstrated that the inhibition of fatty acid synthesis in these cells can be used as a chemotherapeutic strategy. Tumour cells are characterised by high levels of expression of genes encoding lipogenic enzymes, including citrate lyase, acetyl-CoA carboxylase, and higher fatty acid synthase (HFAS) (Montesdeoca et al. 2020). Inhibition of these enzymes both in vitro and in vivo slows down the proliferation of malignant cells and inhibits tumour growth. Recent data suggest that increased expression of HFAS not only is important for tumour growth but also determines the development of resistance to chemotherapeutic drugs (Zaytseva et al. 2015). Some fatty acid synthase inhibitors, such as cerulenin (Cerulenin, an antifungal antibiotic isolated from a culture filtrate of *Cephalosporium caerulens*) and orlistat (N-formyl-L-leucine dodecyl ester), have antitumour activity and increase the efficacy of in vitro antitumour therapy in combination with anti-mitotic drugs, such as docetaxel and doxorubicin (Kridel et al. 2004; Shiragami et al. 2013).

Thus, inhibition of glycolysis and interference with glutamine metabolism and fatty acid synthesis are three possible approaches to cancer therapy. Mitochondrial dysfunction, activation of oncogenes, inactivation of anti-oncogenes, and microenvironment of tumour cells have a pronounced effect on the metabolism of cancer

cells and determine the heterogeneity of metabolic profiles between different tumour types. The importance of identifying specific metabolic alterations for each malignant tumour is clear given the need to effectively target its growth. In addition, the combination of traditional chemotherapeutic drugs and metabolic modulators may improve the efficacy of cancer therapy.

8 Conclusion

In addition to the key energy function, mitochondria control the maintenance of redox and calcium homeostasis, which are critical for ensuring the viability of transformed cells, oncogenic signalling, regulation of receptor-independent apoptosis pathways, implementation of redox and Ca^{2+} -dependent signalling mechanisms, and control of nuclear function. In malignant neoplasms, mitochondrial rearrangement, in part due to mutations in the mitochondrial genome, is associated with reprogramming of energy metabolism. At the same time, some cancers have mutations in mitochondrial Krebs cycle enzymes that produce oncogenic metabolites. The removal of mitochondrial DNA (mtDNA) limits tumourigenesis, and rare human tumours with mutant mitochondrial genomes transform from benign to malignant forms. Thus, mitochondria play an important and multifunctional role in the progression of malignant tumours, and targeting mitochondria will contribute to the emergence of new therapeutic options.

Aerobic glycolysis, first described more than 95 years ago by renowned biochemist Otto Warburg, is the most striking metabolic feature of cancer cells. Other important features of metabolic changes in cancer cells are the active use of glutamine and the synthesis of higher fatty acids. Although these metabolic differences between normal and cancer cells are not absolute, they may provide a biochemical basis for the development of new cancer drugs. Inhibition of glycolysis and interference with glutamine metabolism and fatty acid synthesis are three possible approaches to cancer therapy. One of the most promising areas of antitumour therapy is the search for agents that selectively affect mitochondria, taking into account the specific restructuring of both the mitochondria themselves and the mitochondrial metabolism of the transformed cell and/or the activation of energy metabolism by reprogrammed mitochondria. This gives impetus to the development of mitochondrial pharmacology to exploit the special properties of the mitochondria of transformed cells as targets for antitumour effects.

References

- Adekola K, Rosen ST, Shanmugam M (2012) Glucose transporters in cancer metabolism. *Curr Opin Oncol* 24(6):650–654. <https://doi.org/10.1097/CCO.0b013e328356da72>
- Agani F, Jiang BH (2013) Oxygen-independent regulation of HIF-1: novel involvement of PI3K/AKT/mTOR pathway in cancer. *Curr Cancer Drug Targets* 13(3):245–251. <https://doi.org/10.2174/1568009611313030003>

- Alitalo K, Schwab M (1986) Oncogene amplification in tumor cells. *Adv Cancer Res* 47:235–281. [https://doi.org/10.1016/s0065-230x\(08\)60201-8](https://doi.org/10.1016/s0065-230x(08)60201-8)
- Aljofan M, Riethmacher D (2019) Anticancer activity of metformin: a systematic review of the literature. *Future Sci OA* 5(8):FSO410. <https://doi.org/10.2144/fsoa-2019-0053>
- Altenberg B, Greulich KO (2004) Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. *Genomics* 84(6):1014–1020. <https://doi.org/10.1016/j.ygeno.2004.08.010>
- Anastasiou D, Yu Y, Israelsen WJ et al (2012) Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. *Nat Chem Biol* 8(10):839–847. <https://doi.org/10.1038/nchembio.1060>
- Anderson S, Bankier AT, Barrell BG et al (1981) Sequence and organization of the human mitochondrial genome. *Nature* 290(5806):457–465. <https://doi.org/10.1038/290457a0>
- Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* 23(2):147. <https://doi.org/10.1038/13779>
- Andreyev AY, Kushnareva YE, Starkov AA (2005) Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Mosc)* 70(2):200–214. <https://doi.org/10.1007/s10541-005-0102-7>
- Andrieux P, Chevillard C, Cunha-Neto E, Nunes JPS (2021) Mitochondria as a cellular hub in infection and inflammation. *Int J Mol Sci* 22(21):11338. <https://doi.org/10.3390/ijms222111338>
- Antico Arciuch VG, Elguero ME, Poderoso JJ, Carreras MC (2012) Mitochondrial regulation of cell cycle and proliferation. *Antioxid Redox Signal* 16(10):1150–1180. <https://doi.org/10.1089/ars.2011.4085>
- Aspuria PP, Lunt SY, Våremo L et al (2014) Succinate dehydrogenase inhibition leads to epithelial-mesenchymal transition and reprogrammed carbon metabolism. *Cancer Metab* 2:21. <https://doi.org/10.1186/2049-3002-2-21>
- Baggetto LG, Lehninger AL (1987) Isolated tumoral pyruvate dehydrogenase can synthesize acetoin which inhibits pyruvate oxidation as well as other aldehydes. *Biochem Biophys Res Commun* 145(1):153–159. [https://doi.org/10.1016/0006-291x\(87\)91300-3](https://doi.org/10.1016/0006-291x(87)91300-3)
- Baggetto LG, Testa-Parussini R (1990) Role of acetoin on the regulation of intermediate metabolism of Ehrlich ascites tumor mitochondria: its contribution to membrane cholesterol enrichment modifying passive proton permeability. *Arch Biochem Biophys* 283(2):241–248. [https://doi.org/10.1016/0003-9861\(90\)90638-f](https://doi.org/10.1016/0003-9861(90)90638-f)
- Baghdadi HH (2017) Targeting cancer cells using 3-bromopyruvate for selective cancer treatment. *Saudi J Med Med Sci* 5(1):9–19. <https://doi.org/10.4103/1658-631X.194253>
- Bardella C, Pollard PJ, Tomlinson I (2011) SDH mutations in cancer. *Biochim Biophys Acta* 1807(11):1432–1443. <https://doi.org/10.1016/j.bbabi.2011.07.003>
- Bauer DE, Harris MH, Plas DR et al (2004) Cytokine stimulation of aerobic glycolysis in hematopoietic cells exceeds proliferative demand. *FASEB J* 18(11):1303–1305. <https://doi.org/10.1096/fj.03-1001fje>
- Berardi MJ, Fantin VR (2011) Survival of the fittest: metabolic adaptations in cancer. *Curr Opin Genet Dev* 21(1):59–66. <https://doi.org/10.1016/j.gde.2010.10.001>
- Biswas SK (2015) Metabolic reprogramming of immune cells in cancer progression. *Immunity* 43(3):435–449. <https://doi.org/10.1016/j.immuni.2015.09.001>
- Bonnet S, Archer SL, Allalunis-Turner J et al (2007) A mitochondria-K⁺ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* 11(1):37–51. <https://doi.org/10.1016/j.ccr.2006.10.020>
- Boutin AT, Johnson RS (2007) Waiting to inhale: HIF-1 modulates aerobic respiration. *Cell* 129(1):29–30. <https://doi.org/10.1016/j.cell.2007.03.031>
- Brahimi-Horn MC, Chiche J, Pouyssegur J (2007) Hypoxia signalling controls metabolic demand. *Curr Opin Cell Biol* 19(2):223–229. <https://doi.org/10.1016/j.ceb.2007.02.003>
- Brand MD (2016) Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radic Biol Med* 100:14–31. <https://doi.org/10.1016/j.freeradbiomed.2016.04.001>

- Bustamante E, Morris HP, Pedersen PL (1981) Energy metabolism of tumor cells. Requirement for a form of hexokinase with a propensity for mitochondrial binding. *J Biol Chem* 256(16): 8699–8704
- Byun JM, Kim SS, Kim KT et al (2018) Overexpression of peroxiredoxin-3 and -5 is a potential biomarker for prognosis in endometrial cancer. *Oncol Lett* 15(4):5111–5118. <https://doi.org/10.3892/ol.2018.7909>
- Cairns RA, Mak TW (2013) Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. *Cancer Discov* 3(7):730–741. <https://doi.org/10.1158/2159-8290.CD-13-0083>
- Capuano F, Varone D, D'Eri N et al (1996) Oxidative phosphorylation and F₀F₁ ATP synthase activity of human hepatocellular carcinoma. *Biochem Mol Biol Int* 38(5):1013–1022
- Carew JS, Huang P (2002) Mitochondrial defects in cancer. *Mol Cancer* 1:9. <https://doi.org/10.1186/1476-4598-1-9>
- Chaban Y, Boekema EJ, Dudkina NV (2014) Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilisation. *Biochim Biophys Acta* 1837(4): 418–426. <https://doi.org/10.1016/j.bbabi.2013.10.004>
- Chatterjee A, Dasgupta S, Sidransky D (2011) Mitochondrial subversion in cancer. *Cancer Prev Res (Phila)* 4(5):638–654. <https://doi.org/10.1158/1940-6207.CAPR-10-0326>
- Chen Z, Lu W, Garcia-Prieto C, Huang P (2007) The Warburg effect and its cancer therapeutic implications. *J Bioenerg Biomembr* 39(3):267–274. <https://doi.org/10.1007/s10863-007-9086-x>
- Chen Y, Cairns R, Papandreou I, Koong A, Denko NC (2009) Oxygen consumption can regulate the growth of tumors, a new perspective on the Warburg effect. *PLoS One* 4(9):e7033. <https://doi.org/10.1371/journal.pone.0007033>
- Chen CL, Zhang L, Jin Z, Kasumov T, Chen YR (2022) Mitochondrial redox regulation and myocardial ischemia-reperfusion injury. *Am J Physiol Cell Physiol* 322(1):C12–C23. <https://doi.org/10.1152/ajpcell.00131.2021>
- Cheng T, Sudderth J, Yang C et al (2011) Pyruvate carboxylase is required for glutamine-independent growth of tumor cells. *Proc Natl Acad Sci U S A* 108(21):8674–8679. <https://doi.org/10.1073/pnas.1016627108>
- Chenna S, Koopman WJH, Prehn JHM, Connolly NMC (2022) Mechanisms and mathematical modeling of ROS production by the mitochondrial electron transport chain. *Am J Physiol Cell Physiol* 323(1):C69–C83. <https://doi.org/10.1152/ajpcell.00455.2021>
- Colvin H, Nishida N, Konno M et al (2016) Oncometabolite D-2-hydroxyglutarate directly induces epithelial-mesenchymal transition and is associated with distant metastasis in colorectal cancer. *Sci Rep* 6:36289. <https://doi.org/10.1038/srep36289>
- Cortellino S, Longo VD (2023) Metabolites and immune response in tumor microenvironments. *Cancers (Basel)* 15(15):3898. <https://doi.org/10.3390/cancers15153898>
- Courtney R, Ngo DC, Malik N, Ververis K, Tortorella SM, Karagiannis TC (2015) Cancer metabolism and the Warburg effect: the role of HIF-1 and PI3K. *Mol Biol Rep* 42(4): 841–851. <https://doi.org/10.1007/s11033-015-3858-x>
- Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr (2013) Cellular fatty acid metabolism and cancer. *Cell Metab* 18(2):153–161. <https://doi.org/10.1016/j.cmet.2013.05.017>
- Curtabbi A, Enriquez JA (2022) The ins and outs of the flavin mononucleotide cofactor of respiratory complex I. *IUBMB Life* 74(7):629–644. <https://doi.org/10.1002/iub.2600>
- Dada LA, Chandel NS, Ridge KM, Pedemonte C, Bertorello AM, Sznajder JI (2003) Hypoxia-induced endocytosis of Na,K-ATPase in alveolar epithelial cells is mediated by mitochondrial reactive oxygen species and PKC- ζ . *J Clin Invest* 111(7):1057–1064. <https://doi.org/10.1172/JCI16826>
- Dalla Pozza E, Dando I, Pacchiana R et al (2020) Regulation of succinate dehydrogenase and role of succinate in cancer. *Semin Cell Dev Biol* 98:4–14. <https://doi.org/10.1016/j.semcdb.2019.04.013>

- Dang CV (2013) MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med* 3(8):a014217. <https://doi.org/10.1101/cshperspect.a014217>
- Davis S, Weiss MJ, Wong JR, Lampidis TJ, Chen LB (1985) Mitochondrial and plasma membrane potentials cause unusual accumulation and retention of rhodamine 123 by human breast adenocarcinoma-derived MCF-7 cells. *J Biol Chem* 260(25):13844–13850
- DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB (2008) The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 7(1):11–20. <https://doi.org/10.1016/j.cmet.2007.10.002>
- Deng YT, Huang HC, Lin JK (2010) Rotenone induces apoptosis in MCF-7 human breast cancer cell-mediated ROS through JNK and p38 signaling. *Mol Carcinog* 49(2):141–151. <https://doi.org/10.1002/mc.20583>
- Doherty E, Perl A (2017) Measurement of mitochondrial mass by flow cytometry during oxidative stress. *React Oxyg Species (Apex)* 4(10):275–283. <https://doi.org/10.20455/ros.2017.839>
- Dong LF, Low P, Dyason JC et al (2008) Alpha-tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. *Oncogene* 27(31):4324–4335. <https://doi.org/10.1038/onc.2008.69>
- Draoui N, Schicke O, Fernandes A et al (2013) Synthesis and pharmacological evaluation of carboxycoumarins as a new antitumor treatment targeting lactate transport in cancer cells. *Bioorg Med Chem* 21(22):7107–7117. <https://doi.org/10.1016/j.bmc.2013.09.010>
- Dröse S (2013) Differential effects of complex II on mitochondrial ROS production and their relation to cardioprotective pre- and postconditioning. *Biochim Biophys Acta* 1827(5):578–587. <https://doi.org/10.1016/j.bbabi.2013.01.004>
- Dudkina NV, Kudryashev M, Stahlberg H, Boekema EJ (2011) Interaction of complexes I, III, and IV within the bovine respirasome by single particle cryoelectron tomography. *Proc Natl Acad Sci U S A* 108(37):15196–15200. <https://doi.org/10.1073/pnas.1107819108>
- Dwarakanath BS (2009) Cytotoxicity, radiosensitization, and chemosensitization of tumor cells by 2-deoxy-D-glucose *in vitro*. *J Cancer Res Ther* 5(Suppl. 1):S27–S31. <https://doi.org/10.4103/0973-1482.55137>
- Fan Y, Dickman KG, Zong WX (2010) Akt and c-Myc differentially activate cellular metabolic programs and prime cells to bioenergetic inhibition. *J Biol Chem* 285(10):7324–7333. <https://doi.org/10.1074/jbc.M109.035584>
- Fan T, Sun G, Sun X, Zhao L, Zhong R, Peng Y (2019) Tumor energy metabolism and potential of 3-bromopyruvate as an inhibitor of aerobic glycolysis: implications in tumor treatment. *Cancers (Basel)* 11(3):317. <https://doi.org/10.3390/cancers11030317>
- Fang JS, Gillies RD, Gatenby RA (2008) Adaptation to hypoxia and acidosis in carcinogenesis and tumor progression. *Semin Cancer Biol* 18(5):330–337. <https://doi.org/10.1016/j.semcancer.2008.03.011>
- Farabegoli F, Vettraino M, Manerba M, Fiume L, Roberti M, Di Stefano G (2012) Galloflavin, a new lactate dehydrogenase inhibitor, induces the death of human breast cancer cells with different glycolytic attitude by affecting distinct signaling pathways. *Eur J Pharm Sci* 47(4):729–738. <https://doi.org/10.1016/j.ejps.2012.08.012>
- Fedotova M, Barysheva E, Bushueva O (2023) Pathways of hypoxia-inducible factor (HIF) in the orchestration of uterine fibroids development. *Life (Basel)* 13(8):1740. <https://doi.org/10.3390/life13081740>
- Fensterle J, Aicher B, Seipelt I, Teifel M, Engel J (2014) Current view on the mechanism of action of perifosine in cancer. *Anticancer Agents Med Chem* 14(4):629–635. <https://doi.org/10.2174/1871520614666140309225912>
- Ferns GA, Shahini Shams Abadi M, Raeisi A, Arjmand MH (2021) The potential role of changes in the glucose and lipid metabolic pathways in gastrointestinal cancer progression: strategy in cancer therapy. *Gastrointest Tumors* 8(4):169–176. <https://doi.org/10.1159/000517771>
- Finkel T (2012) Signal transduction by mitochondrial oxidants. *J Biol Chem* 287(7):4434–4440. <https://doi.org/10.1074/jbc.R111.271999>

- Fischer K, Hoffmann P, Voelkl S et al (2007) Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* 109(9):3812–3819. <https://doi.org/10.1182/blood-2006-07-035972>
- Fogg VC, Lanning NJ, Mackeigan JP (2011) Mitochondria in cancer: at the crossroads of life and death. *Chin J Cancer* 30(8):526–539. <https://doi.org/10.5732/cjc.011.10018>
- Formentini L, Martínez-Reyes I, Cuezva JM (2010) The mitochondrial bioenergetic capacity of carcinomas. *IUBMB Life* 62(7):554–560. <https://doi.org/10.1002/iub.352>
- Frezza C, Gottlieb E (2009) Mitochondria in cancer: not just innocent bystanders. *Semin Cancer Biol* 19(1):4–11. <https://doi.org/10.1016/j.semcancer.2008.11.008>
- Funes JM, Quintero M, Henderson S et al (2007) Transformation of human mesenchymal stem cells increases their dependency on oxidative phosphorylation for energy production. *Proc Natl Acad Sci U S A* 104(15):6223–6228. <https://doi.org/10.1073/pnas.0700690104>
- Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* 4(11):891–899. <https://doi.org/10.1038/nrc1478>
- Gatenby RA, Gawlinski ET, Gmitro AF, Kaylor B, Gillies RJ (2006) Acid-mediated tumor invasion: a multidisciplinary study. *Cancer Res* 66(10):5216–5223. <https://doi.org/10.1158/0008-5472.CAN-05-4193>
- Gillies RJ, Gatenby RA (2015) Metabolism and its sequelae in cancer evolution and therapy. *Cancer J* 21(2):88–96. <https://doi.org/10.1097/PPO.0000000000000102>
- Girolimetti G, Guerra F, Iommarini L et al (2017) Platinum-induced mitochondrial DNA mutations confer lower sensitivity to paclitaxel by impairing tubulin cytoskeletal organization. *Hum Mol Genet* 26(15):2961–2974. <https://doi.org/10.1093/hmg/ddx186>
- Goetze K, Walenta S, Ksiazkiewicz M, Kunz-Schughart LA, Mueller-Klieser W (2011) Lactate enhances motility of tumor cells and inhibits monocyte migration and cytokine release. *Int J Oncol* 39(2):453–463. <https://doi.org/10.3892/ijo.2011.1055>
- Gogvadze V, Zhivotovsky B, Orrenius S (2010) The Warburg effect and mitochondrial stability in cancer cells. *Mol Aspects Med* 31(1):60–74. <https://doi.org/10.1016/j.mam.2009.12.004>
- Graeber TG, Osmanian C, Jacks T et al (1996) Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 379(6560):88–91. <https://doi.org/10.1038/379088a0>
- Green DR (2022) The mitochondrial pathway of apoptosis: part I: MOMP and beyond. *Cold Spring Harb Perspect Biol* 14(5):a041038. <https://doi.org/10.1101/cshperspect.a041038>
- Guerra F, Guaragnella N, Arbini AA, Bucci C, Giannattasio S, Moro L (2017) Mitochondrial dysfunction: a novel potential driver of epithelial-to-mesenchymal transition in cancer. *Front Oncol* 7:295. <https://doi.org/10.3389/fonc.2017.00295>
- Hammoudi N, Ahmed KB, Garcia-Prieto C, Huang P (2011) Metabolic alterations in cancer cells and therapeutic implications. *Chin J Cancer* 30(8):508–525. <https://doi.org/10.5732/cjc.011.10267>
- Han KWW, Po WW, Sohn UD, Kim HJ (2019) Benzyl isothiocyanate induces apoptosis via reactive oxygen species-initiated mitochondrial dysfunction and DR4 and DR5 death receptor activation in gastric adenocarcinoma cells. *Biomolecules* 9(12):839. <https://doi.org/10.3390/biom9120839>
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- He X, Nie H, Hong Y, Sheng C, Xia W, Ying W (2012) SIRT2 activity is required for the survival of C6 glioma cells. *Biochem Biophys Res Commun* 417(1):468–472. <https://doi.org/10.1016/j.bbrc.2011.11.141>
- Hirsch HA, Iliopoulos D, Tschlis PN, Struhl K (2009) Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res* 69(19):7507–7511. <https://doi.org/10.1158/0008-5472.CAN-09-2994>
- Hirschhaeuser F, Sattler UG, Mueller-Klieser W (2011) Lactate: a metabolic key player in cancer. *Cancer Res* 71(22):6921–6925. <https://doi.org/10.1158/0008-5472.CAN-11-1457>
- Hirst J, King MS, Pryde KR (2008) The production of reactive oxygen species by complex I. *Biochem Soc Trans* 36(Pt 5):976–980. <https://doi.org/10.1042/BST0360976>

- Ho N, Morrison J, Silva A, Coomber BL (2016) The effect of 3-bromopyruvate on human colorectal cancer cells is dependent on glucose concentration but not hexokinase II expression. *Biosci Rep* 36(1):e00299. <https://doi.org/10.1042/BSR20150267>
- Höckel M, Vaupel P (2001) Biological consequences of tumor hypoxia. *Semin Oncol* 28(2 Suppl 8):36–41
- Huang Y, Sun G, Sun X et al (2020) The potential of lonidamine in combination with chemotherapy and physical therapy in cancer treatment. *Cancers (Basel)* 12(11):3332. <https://doi.org/10.3390/cancers12113332>
- Imanishi H, Hattori K, Wada R et al (2011) Mitochondrial DNA mutations regulate metastasis of human breast cancer cells. *PLoS One* 6(8):e23401. <https://doi.org/10.1371/journal.pone.0023401>
- Infantino V, Santarsiero A, Convertini P, Todisco S, Iacobazzi V (2021) Cancer cell metabolism in hypoxia: role of HIF-1 as key regulator and therapeutic target. *Int J Mol Sci* 22(11):5703. <https://doi.org/10.3390/ijms22115703>
- Israelsen WJ, Vander Heiden MG (2015) Pyruvate kinase: function, regulation and role in cancer. *Semin Cell Dev Biol* 43:43–51. <https://doi.org/10.1016/j.semcdb.2015.08.004>
- Iyer NV, Kotch LE, Agani F et al (1998) Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 12(2):149–162. <https://doi.org/10.1101/gad.12.2.149>
- Jezek P, Hlavatá L (2005) Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. *Int J Biochem Cell Biol* 37(12):2478–2503. <https://doi.org/10.1016/j.biocel.2005.05.013>
- Jin GZ, Zhang Y, Cong WM et al (2018) Phosphoglucosmutase 1 inhibits hepatocellular carcinoma progression by regulating glucose trafficking. *PLoS Biol* 16(10):e2006483. <https://doi.org/10.1371/journal.pbio.2006483>
- Jing X, Yang F, Shao C et al (2019) Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Mol Cancer* 18(1):157. <https://doi.org/10.1186/s12943-019-1089-9>
- Kaplia AA, Morozova VS (2010) [Na⁺,K⁺]-ATPase activity in polarized cells]. *Ukr Biokhim Zh* (1999) 82(1):5–20
- Kaplia AA, Kudriavtseva AG, Kizhniak SV, Osinskii DS, Demin EN (2007) [Na⁺,K⁺-ATPase activity characteristics in human colon adenocarcinoma]. *Ukr Biokhim Zh* (1999) 79(4):90–96
- Kaplia AA, Sorokina LV, Khyzhnyak SV (2015) [Reprogramming of mitochondrial energy metabolism in malignant neoplasms]. *Ukr Biochem J* 87(6):19–35. <https://doi.org/10.15407/ubj87.06.019>
- Kasai S, Shimizu S, Tataru Y, Mimura J, Itoh K (2020) Regulation of Nrf2 by mitochondrial reactive oxygen species in physiology and pathology. *Biomolecules* 10(2):320. <https://doi.org/10.3390/biom10020320>
- Kim JW, Dang CV (2006) Cancer's molecular sweet tooth and the Warburg effect. *Cancer Res* 66(18):8927–8930. <https://doi.org/10.1158/0008-5472.CAN-06-1501>
- Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 3(3):177–185. <https://doi.org/10.1016/j.cmet.2006.02.002>
- Kim JW, Gao P, Dang CV (2007) Effects of hypoxia on tumor metabolism. *Cancer Metastasis Rev* 26(2):291–298. <https://doi.org/10.1007/s10555-007-9060-4>
- Korga A, Ostrowska M, Iwan M, Herbet M, Dudka J (2019) Inhibition of glycolysis disrupts cellular antioxidant defense and sensitizes HepG2 cells to doxorubicin treatment. *FEBS Open Bio* 9(5):959–972. <https://doi.org/10.1002/2211-5463.12628>
- Koseki J, Konno M, Ishii H (2019) Computational analyses for cancer biology based on exhaustive experimental backgrounds. *Cancer Drug Resist* 2(3):419–427. <https://doi.org/10.20517/cdr.2019.33>
- Kozal K, Krześlak A (2022) The role of hypoxia-inducible factor isoforms in breast cancer and perspectives on their inhibition in therapy. *Cancers (Basel)* 14(18):4518. <https://doi.org/10.3390/cancers14184518>

- Kridel SJ, Axelrod F, Rozenkrantz N, Smith JW (2004) Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity. *Cancer Res* 64(6):2070–2075. <https://doi.org/10.1158/0008-5472.can-03-3645>
- Kroemer G, Pouyssegur J (2008) Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* 13(6):472–482. <https://doi.org/10.1016/j.ccr.2008.05.005>
- Kulikov VA, Belyaeva LE (2016) Cancer cell metabolism as a therapeutic target. *Vestnik Vitebskogo Gosudarstvennogo Meditsinskogo Universiteta* 15(6):7–20. <https://doi.org/10.22263/2312-4156.2016.6.7>
- Kurhaluk N, Tkachenko H (2022) Effects of melatonin and metformin in preventing lysosome-induced autophagy and oxidative stress in rat models of carcinogenesis and the impact of high-fat diet. *Sci Rep* 12(1):4998. <https://doi.org/10.1038/s41598-022-08778-w>
- Kurhaluk N, Bojkova B, Radkowski M et al (2018a) Melatonin and metformin diminish oxidative stress in heart tissue in a rat model of high fat diet and mammary carcinogenesis. *Adv Exp Med Biol* 1047:7–19. https://doi.org/10.1007/5584_2017_128
- Kurhaluk N, Bojková B, Winklewski PJ (2018b) Liver antioxidant and aerobic status improves after metformin and melatonin administration in a rat model of high-fat diet and mammary carcinogenesis. *Can J Physiol Pharmacol* 96(8):790–797. <https://doi.org/10.1139/cjpp-2018-0001>
- Kursvietiene L, Kopustinskiene DM, Staneviciene I et al (2023) Anti-cancer properties of resveratrol: a focus on its impact on mitochondrial functions. *Antioxidants (Basel)* 12(12):2056. <https://doi.org/10.3390/antiox12122056>
- Lanciano P, Khalfaoui-Hassani B, Selamoglu N, Ghelli A, Rugolo M, Daldal F (2013) Molecular mechanisms of superoxide production by complex III: a bacterial versus human mitochondrial comparative case study. *Biochim Biophys Acta* 1827(11–12):1332–1339. <https://doi.org/10.1016/j.bbabi.2013.03.009>
- Lea MA, Altayyar M, desBordes C (2015) Inhibition of growth of bladder cancer cells by 3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one in combination with other compounds affecting glucose metabolism. *Anticancer Res* 35(11):5889–5899
- Ledesma A, de Lacoba MG, Rial E (2002) The mitochondrial uncoupling proteins. *Genome Biol* 3(12):REVIEWS3015. <https://doi.org/10.1186/gb-2002-3-12-reviews3015>
- Lee K, Zhang H, Qian DZ, Rey S, Liu JO, Semenza GL (2009) Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. *Proc Natl Acad Sci U S A* 106(42):17910–17915. <https://doi.org/10.1073/pnas.0909353106>
- Letouzé E, Martinelli C, Lorient C et al (2013) SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell* 23(6):739–752. <https://doi.org/10.1016/j.ccr.2013.04.018>
- Li N, Liu X (2020) Phosphoglycerate mutase 1: its glycolytic and non-glycolytic roles in tumor malignant behaviors and potential therapeutic significance. *Onco Targets Ther* 13:1787–1795. <https://doi.org/10.2147/OTT.S238920>
- Li YC, Fung KP, Kwok TT, Lee CY, Suen YK, Kong SK (2004) Mitochondria-targeting drug oligomycin blocked P-glycoprotein activity and triggered apoptosis in doxorubicin-resistant HepG2 cells. *Chemotherapy* 50(2):55–62. <https://doi.org/10.1159/000077803>
- Li X, Yang Y, Zhang B et al (2022) Lactate metabolism in human health and disease. *Signal Transduct Target Ther* 7(1):305. <https://doi.org/10.1038/s41392-022-01151-3>
- Liberti MV, Locasale JW (2016) The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci* 41(3):211–218. <https://doi.org/10.1016/j.tibs.2015.12.001>
- Lin Y, Yang B, Huang Y et al (2023) Mitochondrial DNA-targeted therapy: a novel approach to combat cancer. *Cell Insight* 2(4):100113. <https://doi.org/10.1016/j.cellin.2023.100113>
- Lorient C, Domingues M, Berger A et al (2015) Deciphering the molecular basis of invasiveness in Sdhb-deficient cells. *Oncotarget* 6(32):32955–32965. <https://doi.org/10.18632/oncotarget.5106>
- Lozy F, Karantza V (2012) Autophagy and cancer cell metabolism. *Semin Cell Dev Biol* 23(4):395–401. <https://doi.org/10.1016/j.semcdb.2012.01.005>
- Lu CL, Qin L, Liu HC, Candas D, Fan M, Li JJ (2015a) Tumor cells switch to mitochondrial oxidative phosphorylation under radiation via mTOR-mediated hexokinase II inhibition – a Warburg-reversing effect. *PLoS One* 10(3):e0121046. <https://doi.org/10.1371/journal.pone.0121046>

- Lu J, Tan M, Cai Q (2015b) The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett* 356(2 Pt A):156–164. <https://doi.org/10.1016/j.canlet.2014.04.001>
- Lu JF, Pokharel D, Bebawy M (2015c) MRP1 and its role in anticancer drug resistance. *Drug Metab Rev* 47(4):406–419. <https://doi.org/10.3109/03602532.2015.1105253>
- Luo Z, Tian M, Yang G et al (2022) Hypoxia signaling in human health and diseases: implications and prospects for therapeutics. *Signal Transduct Target Ther* 7(1):218. <https://doi.org/10.1038/s41392-022-01080-1>
- Maciver NJ, Jacobs SR, Wieman HL, Wofford JA, Colloff JL, Rathmell JC (2008) Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol* 84(4):949–957. <https://doi.org/10.1189/jlb.0108024>
- Mailloux RJ (2018) Mitochondrial antioxidants and the maintenance of cellular hydrogen peroxide levels. *Oxid Med Cell Longev* 2018:7857251. <https://doi.org/10.1155/2018/7857251>
- Mailloux RJ (2021) An update on methods and approaches for interrogating mitochondrial reactive oxygen species production. *Redox Biol* 45:102044. <https://doi.org/10.1016/j.redox.2021.102044>
- Marbaniang C, Kma L (2018) Dysregulation of glucose metabolism by oncogenes and tumor suppressors in cancer cells. *Asian Pac J Cancer Prev* 19(9):2377–2390. <https://doi.org/10.22034/APJCP.2018.19.9.2377>
- Martínez-Reyes I, Chandel NS (2021) Cancer metabolism: looking forward. *Nat Rev Cancer* 21(10):669–680. <https://doi.org/10.1038/s41568-021-00378-6>
- Mathupala SP, Ko YH, Pedersen PL (2009) Hexokinase-2 bound to mitochondria: cancer's stygian link to the “Warburg Effect” and a pivotal target for effective therapy. *Semin Cancer Biol* 19(1):17–24. <https://doi.org/10.1016/j.semcancer.2008.11.006>
- Matoba S, Kang JG, Patino WD et al (2006) p53 regulates mitochondrial respiration. *Science* 312(5780):1650–1653. <https://doi.org/10.1126/science.1126863>
- Mazat JP, Devin A, Ransac S (2020) Modelling mitochondrial ROS production by the respiratory chain. *Cell Mol Life Sci* 77(3):455–465. <https://doi.org/10.1007/s00018-019-03381-1>
- Mazure NM (2017) VDAC in cancer. *Biochim Biophys Acta Bioenerg* 1858(8):665–673. <https://doi.org/10.1016/j.bbabi.2017.03.002>
- Méndez-Lucas A, Hyroššová P, Novellasdemunt L, Viñals F, Perales JC (2014) Mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M) is a pro-survival, endoplasmic reticulum (ER) stress response gene involved in tumor cell adaptation to nutrient availability. *J Biol Chem* 289(32):22090–22102. <https://doi.org/10.1074/jbc.M114.566927>
- Meng TC, Fukada T, Tonks NK (2002) Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol Cell* 9(2):387–399. [https://doi.org/10.1016/s1097-2765\(02\)00445-8](https://doi.org/10.1016/s1097-2765(02)00445-8)
- Milane L, Duan Z, Amiji M (2011) Development of EGFR-targeted polymer blend nanocarriers for combination paclitaxel/Ionidamine delivery to treat multi-drug resistance in human breast and ovarian tumor cells. *Mol Pharm* 8(1):185–203. <https://doi.org/10.1021/mp1002653>
- Miller FJ, Rosenfeldt FL, Zhang C, Linnane AW, Nagley P (2003) Precise determination of mitochondrial DNA copy number in human skeletal and cardiac muscle by a PCR-based assay: lack of change of copy number with age. *Nucleic Acids Res* 31(11):e61. <https://doi.org/10.1093/nar/gng060>
- Mole DR, Maxwell PH, Pugh CW, Ratcliffe PJ (2001) Regulation of HIF by the von Hippel-Lindau tumour suppressor: implications for cellular oxygen sensing. *IUBMB Life* 52(1–2):43–47. <https://doi.org/10.1080/15216540252774757>
- Montesdeoca N, López M, Ariza X, Herrero L, Makowski K (2020) Inhibitors of lipogenic enzymes as a potential therapy against cancer. *FASEB J* 34(9):11355–11381. <https://doi.org/10.1096/fj.202000705R>
- Morgan MJ, Liu ZG (2011) Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Res* 21(1):103–115. <https://doi.org/10.1038/cr.2010.178>

- Morgan-Hughes JA, Schapira AH, Cooper JM, Clark JB (1988) Molecular defects of NADH-ubiquinone oxidoreductase (complex I) in mitochondrial diseases. *J Bioenerg Biomembr* 20(3): 365–382. <https://doi.org/10.1007/BF00769638>
- Murphy MP (2009) How mitochondria produce reactive oxygen species. *Biochem J* 417(1):1–13. <https://doi.org/10.1042/BJ20081386>
- Nagel R, Semenova EA, Berns A (2016) Drugging the addict: non-oncogene addiction as a target for cancer therapy. *EMBO Rep* 17(11):1516–1531. <https://doi.org/10.15252/embr.201643030>
- Narayanankutty A (2020) Phytochemicals as PI3K/ Akt/ mTOR inhibitors and their role in breast cancer treatment. *Recent Pat Anticancer Drug Discov* 15(3):188–199. <https://doi.org/10.2174/1574892815666200910164641>
- Nath K, Guo L, Nancolas B et al (2016) Mechanism of antineoplastic activity of lonidamine. *Biochim Biophys Acta* 1866(2):151–162. <https://doi.org/10.1016/j.bbcan.2016.08.001>
- Ng MYW, Wai T, Simonsen A (2021) Quality control of the mitochondrion. *Dev Cell* 56(7): 881–905. <https://doi.org/10.1016/j.devcel.2021.02.009>
- Nicholls TJ, Minczuk M (2014) In D-loop: 40 years of mitochondrial 7S DNA. *Exp Gerontol* 56: 175–181. <https://doi.org/10.1016/j.exger.2014.03.027>
- O'Malley J, Kumar R, Inigo J, Yadava N, Chandra D (2020) Mitochondrial stress response and cancer. *Trends Cancer* 6(8):688–701. <https://doi.org/10.1016/j.trecan.2020.04.009>
- Palacios-Callender M, Quintero M, Hollis VS, Springett RJ, Moncada S (2004) Endogenous NO regulates superoxide production at low oxygen concentrations by modifying the redox state of cytochrome c oxidase. *Proc Natl Acad Sci U S A* 101(20):7630–7635. <https://doi.org/10.1073/pnas.0401723101>
- Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 3(3): 187–197. <https://doi.org/10.1016/j.cmet.2006.01.012>
- Pascale RM, Calvisi DF, Simile MM, Feo CF, Feo F (2020) The Warburg effect 97 years after its discovery. *Cancers (Basel)* 12(10):2819. <https://doi.org/10.3390/cancers12102819>
- Pavlova NN, Zhu J, Thompson CB (2022) The hallmarks of cancer metabolism: still emerging. *Cell Metab* 34(3):355–377. <https://doi.org/10.1016/j.cmet.2022.01.007>
- Pedersen PL (2007) Warburg, me and Hexokinase 2: multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the “Warburg Effect”, i.e., elevated glycolysis in the presence of oxygen. *J Bioenerg Biomembr* 39(3):211–222. <https://doi.org/10.1007/s10863-007-9094-x>
- Pelicano H, Xu RH, Du M et al (2006) Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. *J Cell Biol* 175(6): 913–923. <https://doi.org/10.1083/jcb.200512100>
- Penta JS, Johnson FM, Wachsman JT, Copeland WC (2001) Mitochondrial DNA in human malignancy. *Mutat Res* 488(2):119–133. [https://doi.org/10.1016/s1383-5742\(01\)00053-9](https://doi.org/10.1016/s1383-5742(01)00053-9)
- Pitkanen S, Robinson BH (1996) Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. *J Clin Invest* 98(2):345–351. <https://doi.org/10.1172/JCI118798>
- Plathow C, Weber WA (2008) Tumor cell metabolism imaging. *J Nucl Med* 49(Suppl 2):43S–63S. <https://doi.org/10.2967/jnumed.107.045930>
- Pliszka M, Szablewski L (2021) Glucose transporters as a target for anticancer therapy. *Cancers (Basel)* 13(16):4184. <https://doi.org/10.3390/cancers13164184>
- Polivka J Jr, Janku F (2014) Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. *Pharmacol Ther* 142(2):164–175. <https://doi.org/10.1016/j.pharmthera.2013.12.004>
- Poole LP, Macleod KF (2021) Mitophagy in tumorigenesis and metastasis. *Cell Mol Life Sci* 78(8): 3817–3851. <https://doi.org/10.1007/s00018-021-03774-1>
- Porporato PE, Payen VL, Baselet B, Sonveaux P (2016) Metabolic changes associated with tumor metastasis, part 2: mitochondria, lipid and amino acid metabolism. *Cell Mol Life Sci* 73(7): 1349–1363. <https://doi.org/10.1007/s00018-015-2100-2>

- Pouysselgur J, Dayan F, Mazure NM (2006) Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 441(7092):437–443. <https://doi.org/10.1038/nature04871>
- Pugh CW, Ratcliffe PJ (2003) Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 9(6):677–684. <https://doi.org/10.1038/nm0603-677>
- Puzio-Kuter AM (2011) The role of p53 in metabolic regulation. *Genes Cancer* 2(4):385–391. <https://doi.org/10.1177/1947601911409738>
- Ramzan R, Vogt S, Kadenbach B (2020) Stress-mediated generation of deleterious ROS in healthy individuals – role of cytochrome c oxidase. *J Mol Med (Berl)* 98(5):651–657. <https://doi.org/10.1007/s00109-020-01905-y>
- Rath S, Sharma R, Gupta R et al (2021) MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. *Nucleic Acids Res* 49(D1):D1541–D1547. <https://doi.org/10.1093/nar/gkaa1011>
- Robey RB, Hay N (2009) Is Akt the “Warburg kinase”? – Akt-energy metabolism interactions and oncogenesis. *Semin Cancer Biol* 19(1):25–31. <https://doi.org/10.1016/j.semcancer.2008.11.010>
- Robey IF, Baggett BK, Kirkpatrick ND et al (2009) Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer Res* 69(6):2260–2268. <https://doi.org/10.1158/0008-5472.CAN-07-5575>
- Roche TE, Hiromasa Y (2007) Pyruvate dehydrogenase kinase regulatory mechanisms and inhibition in treating diabetes, heart ischemia, and cancer. *Cell Mol Life Sci* 64(7–8):830–849. <https://doi.org/10.1007/s00018-007-6380-z>
- Saunier E, Benelli C, Bortoli S (2016) The pyruvate dehydrogenase complex in cancer: an old metabolic gatekeeper regulated by new pathways and pharmacological agents. *Int J Cancer* 138(4):809–817. <https://doi.org/10.1002/ijc.29564>
- Schäfer E, Seelert H, Reifschneider NH, Krause F, Dencher NA, Vonck J (2006) Architecture of active mammalian respiratory chain supercomplexes. *J Biol Chem* 281(22):15370–15375. <https://doi.org/10.1074/jbc.M513525200>
- Schmidt LS, Linehan WM (2014) Hereditary leiomyomatosis and renal cell carcinoma. *Int J Nephrol Renovasc Dis* 7:253–260. <https://doi.org/10.2147/IJNRD.S42097>
- Schöffski P, Cresta S, Mayer IA et al (2018) A phase Ib study of pictilisib (GDC-0941) in combination with paclitaxel, with and without bevacizumab or trastuzumab, and with letrozole in advanced breast cancer. *Breast Cancer Res* 20(1):109. <https://doi.org/10.1186/s13058-018-1015-x>
- Sciacovelli M, Frezza C (2016) Oncometabolites: unconventional triggers of oncogenic signalling cascades. *Free Radic Biol Med* 100:175–181. <https://doi.org/10.1016/j.freeradbiomed.2016.04.025>
- Sciacovelli M, Gonçalves E, Johnson TI et al (2016) Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. *Nature* 537(7621):544–547. <https://doi.org/10.1038/nature19353>
- Semenza GL (2008) Tumor metabolism: cancer cells give and take lactate. *J Clin Invest* 118(12):3835–3837. <https://doi.org/10.1172/JCI37373>
- Senyilmaz D, Teleman AA (2015) Chicken or the egg: Warburg effect and mitochondrial dysfunction. *F1000Prime Rep* 7:41. <https://doi.org/10.12703/P7-41>
- Shehade H, Oldenhove G, Moser M (2014) Hypoxia in the intestine or solid tumors: a beneficial or deleterious alarm signal? *Eur J Immunol* 44(9):2550–2557. <https://doi.org/10.1002/eji.201444719>
- Shen H, Decollogne S, Dilda PJ et al (2015) Dual-targeting of aberrant glucose metabolism in glioblastoma. *J Exp Clin Cancer Res* 34(1):14. <https://doi.org/10.1186/s13046-015-0130-0>
- Sheng H, Tang W (2016) Glycolysis inhibitors for anticancer therapy: a review of recent patents. *Recent Pat Anticancer Drug Discov* 11(3):297–308. <https://doi.org/10.2174/1574892811666160415160104>
- Shiragami R, Murata S, Kosugi C et al (2013) Enhanced antitumor activity of cerulenin combined with oxaliplatin in human colon cancer cells. *Int J Oncol* 43(2):431–438. <https://doi.org/10.3892/ijo.2013.1978>

- Simonnet H, Alazard N, Pfeiffer K et al (2002) Low mitochondrial respiratory chain content correlates with tumor aggressiveness in renal cell carcinoma. *Carcinogenesis* 23(5):759–768. <https://doi.org/10.1093/carcin/23.5.759>
- Smallbone K, Gatenby RA, Gillies RJ, Maini PK, Gavaghan DJ (2007) Metabolic changes during carcinogenesis: potential impact on invasiveness. *J Theor Biol* 244(4):703–713. <https://doi.org/10.1016/j.jtbi.2006.09.010>
- Sobhakumari A, Orcutt KP, Love-Homan L et al (2016) 2-Deoxy-d-glucose suppresses the *in vivo* antitumor efficacy of erlotinib in head and neck squamous cell carcinoma cells. *Oncol Res* 24(1):55–64. <https://doi.org/10.3727/096504016X14586627440192>
- Sonveaux P, Végran F, Schroeder T et al (2008) Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J Clin Invest* 118(12):3930–3942. <https://doi.org/10.1172/JCI36843>
- Srinivasan S, Guha M, Kashina A, Avadhani NG (2017) Mitochondrial dysfunction and mitochondrial dynamics – the cancer connection. *Biochim Biophys Acta Bioenerg* 1858(8):602–614. <https://doi.org/10.1016/j.bbabi.2017.01.004>
- Stacpoole PW (2017) Therapeutic targeting of the pyruvate dehydrogenase complex/pyruvate dehydrogenase kinase (PDC/PDK) axis in cancer. *J Natl Cancer Inst* 109(11). <https://doi.org/10.1093/jnci/djx071>
- Stamerra CA, Di Giosia P, Giorgini P, Ferri C, Sukhorukov VN, Sahebkar A (2022) Mitochondrial dysfunction and cardiovascular disease: pathophysiology and emerging therapies. *Oxid Med Cell Longev* 2022:9530007. <https://doi.org/10.1155/2022/9530007>
- Starkov AA, Fiskum G, Chinopoulos C et al (2004) Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. *J Neurosci* 24(36):7779–7788. <https://doi.org/10.1523/JNEUROSCI.1899-04.2004>
- Tilekar K, Upadhyay N, Iancu CV, Pokrovsky V, Choe JY, Ramaa CS (2020) Power of two: combination of therapeutic approaches involving glucose transporter (GLUT) inhibitors to combat cancer. *Biochim Biophys Acta Rev Cancer* 1874(2):188457. <https://doi.org/10.1016/j.bbcan.2020.188457>
- Tulah AS, Birch-Machin MA (2013) Stressed out mitochondria: the role of mitochondria in ageing and cancer focussing on strategies and opportunities in human skin. *Mitochondrion* 13(5):444–453. <https://doi.org/10.1016/j.mito.2012.11.007>
- Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324(5930):1029–1033. <https://doi.org/10.1126/science.1160809>
- Varisli L, Cen O, Vlahopoulos S (2020) Dissecting pharmacological effects of chloroquine in cancer treatment: interference with inflammatory signaling pathways. *Immunology* 159(3):257–278. <https://doi.org/10.1111/imm.13160>
- Vaupel P (2008) Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis. *Oncologist* 13(Suppl 3):21–26
- Vaupel P, Harrison L (2004) Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. *Oncologist* 9(Suppl 5):4–9. <https://doi.org/10.1634/theoncologist.9-90005-4>
- Vaupel P, Mayer A (2007) Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* 26(2):225–239. <https://doi.org/10.1007/s10555-007-9055-1>
- Vaupel P, Multhoff G (2020) Fatal alliance of hypoxia-/HIF-1 α -driven microenvironmental traits promoting cancer progression. *Adv Exp Med Biol* 1232:169–176. https://doi.org/10.1007/978-3-030-34461-0_21
- Vaupel P, Multhoff G (2021) Revisiting the Warburg effect: historical dogma versus current understanding. *J Physiol* 599(6):1745–1757. <https://doi.org/10.1113/JP278810>
- Vaupel P, Schmidberger H, Mayer A (2019) The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression. *Int J Radiat Biol* 95(7):912–919. <https://doi.org/10.1080/09553002.2019.1589653>
- Vercellino I, Sazanov LA (2022) The assembly, regulation and function of the mitochondrial respiratory chain. *Nat Rev Mol Cell Biol* 23(2):141–161. <https://doi.org/10.1038/s41580-021-00415-0>

- Vincent EE, Coelho PP, Blagih J, Griss T, Viollet B, Jones RG (2015) Differential effects of AMPK agonists on cell growth and metabolism. *Oncogene* 34(28):3627–3639. <https://doi.org/10.1038/onc.2014.301>
- Votyakova TV, Reynolds IJ (2001) DeltaPsi(m)-dependent and -independent production of reactive oxygen species by rat brain mitochondria. *J Neurochem* 79(2):266–277. <https://doi.org/10.1046/j.1471-4159.2001.00548.x>
- Wang B, Shi L, Sun X, Wang L, Wang X, Chen C (2016) Production of CCL20 from lung cancer cells induces the cell migration and proliferation through PI3K pathway. *J Cell Mol Med* 20(5):920–929. <https://doi.org/10.1111/jcmm.12781>
- Wang Z, Guo W, Kuang X, Hou S, Liu H (2017) Nanopreparations for mitochondria targeting drug delivery system: current strategies and future prospective. *Asian J Pharm Sci* 12(6):498–508. <https://doi.org/10.1016/j.ajps.2017.05.006>
- Wang L, Li JJ, Guo LY et al (2018) Molecular link between glucose and glutamine consumption in cancer cells mediated by CtBP and SIRT4. *Oncogenesis* 7(3):26. <https://doi.org/10.1038/s41389-018-0036-8>
- Warburg O (1956) On the origin of cancer cells. *Science* 123(3191):309–314. <https://doi.org/10.1126/science.123.3191.309>
- Welsh S, Williams R, Kirkpatrick L, Paine-Murrieta G, Powis G (2004) Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1alpha. *Mol Cancer Ther* 3(3):233–244
- Xiang LW, Xue H, Ha MW, Yu DY, Xiao LJ, Zheng HC (2022) The effects of REG4 expression on chemoresistance of ovarian cancer. *J Obstet Gynaecol* 42(7):3149–3157. <https://doi.org/10.1080/01443615.2022.2106834>
- Xie J, Wang X, Proud CG (2016) mTOR inhibitors in cancer therapy. *F1000Res* 5:F1000 Faculty Rev-2078. <https://doi.org/10.12688/f1000research.9207.1>
- Xie B, Wang S, Jiang N, Li JJ (2019) Cyclin B1/CDK1-regulated mitochondrial bioenergetics in cell cycle progression and tumor resistance. *Cancer Lett* 443:56–66. <https://doi.org/10.1016/j.canlet.2018.11.019>
- Xing D, Benavides GA, Johnson MS, Tian R, Barnes S, Darley-Usmar VM (2023) Glutamine-dependent effects of nitric oxide on cancer cells subjected to hypoxia-reoxygenation. *Nitric Oxide* 130:22–35. <https://doi.org/10.1016/j.niox.2022.11.003>
- Xu W, Liu LZ, Loizidou M, Ahmed M, Charles IG (2002) The role of nitric oxide in cancer. *Cell Res* 12(5–6):311–320. <https://doi.org/10.1038/sj.cr.7290133>
- Xu K, Yin N, Peng M et al (2021) Glycolysis fuels phosphoinositide 3-kinase signaling to bolster T cell immunity. *Science* 371(6527):405–410. <https://doi.org/10.1126/science.abb2683>
- Yan C, Duanmu X, Zeng L, Liu B, Song Z (2019) Mitochondrial DNA: distribution, mutations, and elimination. *Cells* 8(4):379. <https://doi.org/10.3390/cells8040379>
- Yeh YH, Hsiao HF, Yeh YC, Chen TW, Li TK (2018) Inflammatory interferon activates HIF-1- α -mediated epithelial-to-mesenchymal transition via PI3K/AKT/mTOR pathway. *J Exp Clin Cancer Res* 37(1):70. <https://doi.org/10.1186/s13046-018-0730-6>
- Yin M, O'Neill LAJ (2021) The role of the electron transport chain in immunity. *FASEB J* 35(12):e21974. <https://doi.org/10.1096/fj.202101161R>
- Yu S, Meng S, Xiang M, Ma H (2021) Phosphoenolpyruvate carboxykinase in cell metabolism: roles and mechanisms beyond gluconeogenesis. *Mol Metab* 53:101257. <https://doi.org/10.1016/j.molmet.2021.101257>
- Zaytseva YY, Harris JW, Mitov MI et al (2015) Increased expression of fatty acid synthase provides a survival advantage to colorectal cancer cells via upregulation of cellular respiration. *Oncotarget* 6(22):18891–18904. <https://doi.org/10.18632/oncotarget.3783>
- Zhang Y, Yang JM (2013) Altered energy metabolism in cancer: a unique opportunity for therapeutic intervention. *Cancer Biol Ther* 14(2):81–89. <https://doi.org/10.4161/cbt.22958>
- Zhang P, Cheng S, Sheng X, Dai H, He K, Du Y (2021) The role of autophagy in regulating metabolism in the tumor microenvironment. *Genes Dis* 10(2):447–456. <https://doi.org/10.1016/j.gendis.2021.10.010>

- Zhang B, Pan C, Feng C et al (2022) Role of mitochondrial reactive oxygen species in homeostasis regulation. *Redox Rep* 27(1):45–52. <https://doi.org/10.1080/13510002.2022.2046423>
- Zhao W, Qi J, Wang L, Zhang M, Wang P, Gao C (2012) LY294002 inhibits TLR3/4-mediated IFN- β production via inhibition of IRF3 activation with a PI3K-independent mechanism. *FEBS Lett* 586(6):705–710. <https://doi.org/10.1016/j.febslet.2012.01.016>
- Zhao Y, Butler EB, Tan M (2013) Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis* 4(3):e532. <https://doi.org/10.1038/cddis.2013.60>
- Zhao T, Ren H, Jia L, Chen J, Xin W, Yan F, Li J, Wang X, Gao S, Qian D, Huang C, Hao J (2015) Inhibition of HIF-1 α by PX-478 enhances the anti-tumor effect of gemcitabine by inducing immunogenic cell death in pancreatic ductal adenocarcinoma. *Oncotarget* 6(4):2250–2262. <https://doi.org/10.18632/oncotarget.2948>
- Zhou J, Schmid T, Schnitzer S, Brüne B (2006) Tumor hypoxia and cancer progression. *Cancer Lett* 237(1):10–21. <https://doi.org/10.1016/j.canlet.2005.05.028>
- Zhu W, Ye L, Zhang J, Yu P, Wang H, Ye Z, Tian J (2016) PFK15, a small molecule inhibitor of PFKFB3, induces cell cycle arrest, apoptosis and inhibits invasion in gastric cancer. *PLoS One* 11(9):e0163768. <https://doi.org/10.1371/journal.pone.0163768>
- Zong WX, Rabinowitz JD, White E (2016) Mitochondria and cancer. *Mol Cell* 61(5):667–676. <https://doi.org/10.1016/j.molcel.2016.02.011>

Exploring the Role of Mitochondrial DNA Mutations in Cancer Development and Diagnosis

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Abstract

Cancer is an intricate web of diseases marked by the uncontrolled proliferation of abnormal cells. At its core lies the complex interplay of genes, where inherited mutations or environmental factors lead to genetic aberrations that initiate and drive cancer progression. Each cancer type, from the silent progression of pancreatic cancer to systemic impacts of leukemia, presents unique challenges, necessitating tailored approaches for diagnosis and treatment. A key aspect of cancer biology is the role of mitochondria, the cell's powerhouses. These organelles are central to various cellular functions, especially energy production through oxidative phosphorylation. Mitochondrial dysfunction, caused by disrupted calcium regulation or altered metabolic pathways, plays a significant role in cancer development and progression. For instance, mitochondrial calcium dysregulation contributes to tumor cell survival and growth. The mitochondrial genome (mtDNA) is highly susceptible to mutations due to increased ROS production and limited repair mechanisms. These mutations significantly impact cancer development and progression. The review provides an in-depth analysis of mutations in mitochondrial protein-coding genes (PCGs) across the different mitochondrial complexes, highlighting the variability in mutation rates and impacts among various cancer types. It also discusses the roles of mitochondrial tRNA and rRNA gene mutations in cancer development.

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These mutations, while not as drastic as those in PCGs, still contribute significantly to the oncogenic process. Furthermore, the review touches upon the potential of anticancer agents known as mitocans, which target mitochondrial metabolic and apoptotic pathways. These agents not only offer novel therapeutic strategies, but may also become cellular targets for future cancer therapies. The medical community eagerly anticipates the results of clinical trials involving these agents. Here, we emphasize the critical role of mtDNA mutations in cancer. This comprehensive understanding could lead to new therapeutic targets and strategies, advancing the fight against cancer.

Keywords

Cancer · D-loop mutation · Genetic mutations · Mitochondrial DNA · Mitochondrial genome · Oncology · Reactive oxygen species

1 Introduction

In the ever-evolving narrative of global health, cancer stands as an imposing adversary, with an estimated 20 million new cases and 10 million deaths reverberating across continents (AACR 2022). As these numbers paint a stark reality, a foreboding prophecy looms, the global cancer burden is poised to surge by approximately 60% in the next two decades. The crystal ball of epidemiology unveils a daunting future: a staggering 30 million new cancer cases are projected globally by 2040 (World Health Organization 2023). The global five-year prevalence of cancer, estimated at 43,841,302 cases, exhibits a predominant incidence of breast cancer, constituting 12.5% of all new diagnoses. This is followed by lung cancer at 12.2% and colorectal cancer at 10.7%. Contrary to the initial assertion, prostate cancer ranks as the fourth most prevalent, accounting for 7.8% of new cases. Notably, mesothelioma manifests as the least prevalent cancer type. These statistics, however, diverge from the latest data available, underscoring a substantial rise in global cancer incidence, which profoundly impacts health systems, communities, and individuals worldwide, indicating an impending era marked by an intensified burden of cancer (World Cancer Research Fund International 2020; World Health Organization 2023).

Cancer is a formidable adversary, an intricate web of diseases characterized by the uncontrolled proliferation of abnormal cells. To comprehend this complex foe, one must first delve into the cellular realm, where cancer's genesis occurs. Subject to genetic mutations, normal cells undergo a transformative journey that propels them into relentless growth and evasion of regulatory mechanisms (Hanahan 2022). Within the landscape of cancer, tumors emerge as key players. Benign tumors, although non-cancerous, exhibit abnormal cell growth, often encapsulated and posing minimal threat. In contrast, malignant tumors infiltrate neighboring tissues and embark on journeys through the bloodstream or lymphatic system, initiating the ominous process of metastasis (Talmadge and Fidler 2010).

At the heart of cancer lies the intricate dance of genes. Inherited mutations or exposures to environmental culprits instigate genetic aberrations that set the stage for cancerous transformations. Understanding the genetic tapestry that orchestrates these events is crucial for unraveling the mysteries of cancer initiation and progression (Jackson 2023). Breast, lung, colorectal, and prostate demand tailored approaches to diagnosis and treatment (Kotwal and Schonberg 2017). From the silent progression of pancreatic cancer to the systemic impact of leukemia, each malignancy presents a distinct challenge for patients and healthcare professionals alike (Liu et al. 2021a). A proactive defense against cancer involves an awareness of risk factors, age, genetics, lifestyle choices, and environmental exposures (Sebens 2023). With this knowledge, individuals can make informed decisions and adopt preventive measures. Vaccinations, lifestyle modifications, and early detection initiatives constitute essential components of the arsenal against cancer (Cuzzubbo et al. 2021). The path to a cancer diagnosis often involves a diagnostic odyssey, imaging tests, biopsies, and a battery of laboratory investigations. Precision is paramount, guiding clinicians in pinpointing the malignancy's type, stage, and specific genetic features (Pulumati et al. 2023). Cancer treatment is a multidimensional endeavor, embracing surgery, chemotherapy, radiation therapy, immunotherapy, and targeted therapies.

2 The Powerhouses of the Cell: Mitochondria

2.1 The Role of Mitochondria in Cancer

Mitochondria, often described as the cell's powerhouses, are crucial for various cellular functions, particularly in energy production. These double-membraned organelles are central to oxidative phosphorylation, transforming nutrients into ATP, the cell's primary energy currency. This transformation occurs through a series of reactions in the electron transport chain and chemiosmosis on the inner mitochondrial membrane (Miranda et al. 2022). Aside from energy production, mitochondria are vital in regulating intracellular calcium levels, essential for numerous cellular processes like muscle contraction, nerve impulse transmission, and enzyme activity regulation. Mitochondria help modulate cellular calcium concentrations, impacting cell signaling and overall health (Garbincius and Elrod 2022; Bagheri-Mohammadi et al. 2023).

The concept of mitochondrial homeostasis, or "mitostasis," is critical for cell function over time (Misgeld and Schwarz 2017; Vasileiou et al. 2019). This equilibrium involves the creation of new mitochondria and the removal of damaged ones through mitophagy (Misgeld and Schwarz 2017; Praharaj et al. 2021). Disruption in mitostasis leads to cellular aging through mechanisms like increased ROS production, impaired electron transport chain function, altered bioenergetics, and disrupted calcium (Ca^{2+}) homeostasis (Makena et al. 2022; Matuz-Mares et al. 2022; Bagheri-Mohammadi et al. 2023). Dysregulation in this system is associated with neurological disorders. Additionally, cardiovascular toxicity can arise from enhanced

proteome instability and impaired mitostasis (Papanagnou et al. 2022). However, these adverse effects may be mitigated by promoting autophagy, a cellular process that helps in maintaining cellular health and function.

In cancer, mitochondrial calcium regulation is often altered. A moderate Ca^{2+} signal is vital for cell proliferation, while an excessive influx or overload can trigger cell death. Cancer cells adapt by modulating mitochondrial Ca^{2+} influx and efflux, supporting their metabolic demands and survival. Dysregulated mitochondrial calcium contributes to the emergence of mitoflashes in tumor cells, influencing processes like epithelial to mesenchymal transition (Badr et al. 2022). The expression and activity of Ca^{2+} pumps, channels, and exchangers, such as *ucp2* (Madreiter-Sokolowski et al. 2021), *rab32* (Bui et al. 2010), *mcu* (Marchi et al. 2019; Liu et al. 2020), *micu1* (Zhou et al. 2015), *micu2* (Deliernieux et al. 2020), and *nclx* (De Marchi et al. 2014), are often modified in cancers. For instance, alterations in *mcu* and *micu1* expression have been observed in pancreatic cancer (Wang et al. 2022).

Mitochondria also involve several metabolic pathways, including the citric acid cycle, fatty acid oxidation, and amino acid metabolism. Alterations in mitochondrial metabolism are common in tumors, often resulting from mutations in oncogenes, tumor suppressor genes, and metabolic enzymes (Spinelli and Haigis 2018). These changes can lead to metabolic reprogramming, supporting rapid cell proliferation and increasing ROS production. Alterations in the enzymatic components of the TCA cycle, specifically in succinate dehydrogenase (SDH), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH), have been implicated in the progression of cancer. These mutations lead to the aberrant accumulation of metabolites such as fumarate, succinate, and 2-hydroxyglutarate, collectively referred to as oncometabolites. The presence of these oncometabolites is associated with oncogenic processes, indicating a significant link between metabolic dysregulation in the TCA cycle and tumorigenesis. This relationship underscores the crucial role of metabolic pathways in the development and progression of cancer (Sciacovelli and Frezza 2016).

Mitochondrial ROS production is a byproduct of energy generation, playing roles in cell signaling and pathogenic defense. However, excessive ROS production can cause cellular damage and is linked to various diseases, including cancer and neurodegenerative disorders. Increased ROS can result from enhanced metabolic activities and abnormal signaling pathways in cancer. Recent research in cancer treatment has focused on ROS-dependent therapies. For instance, lutein-induced ROS production in gastric cancer mediates NF- κ B activation and apoptosis (Eom et al. 2023). The breakdown of endothelial glycocalyx hyaluronan by ROS can compromise vascular integrity and initiate disease progression (Berdiaki et al. 2023). Drug combinations that increase intracellular ROS and reduce mitochondrial membrane potential have effectively induced apoptosis in certain cancers, like mantle cell lymphoma and B-cell lymphoma cells (Liu et al. 2021a; Santos et al. 2022).

Mitochondria have a key role in apoptosis, inhibiting oncogenesis at multiple stages, from transformation to metastasis. For cancer to develop and progress, apoptosis must be inhibited, and cell death is a major effector function of many anticancer therapies (Bui et al. 2010; Zhou et al. 2015; Liu et al. 2021b; Santos et al. 2022). Apoptotic cell death, particularly mitochondrial apoptosis, is the most

commonly deregulated form of cell death in cancer. BCL-2 family proteins regulate this process and can be blocked by cancer cells in various ways. For instance, cancer cells may upregulate anti-apoptotic BCL-2 proteins to prevent mitochondrial outer membrane permeabilization (MOMP), the defining event of apoptosis. In addition, mechanisms exist in cancer cells that inhibit caspase function following MOMP, further hindering apoptosis. They release apoptotic factors like cytochrome c into the cytoplasm, which triggers the caspase cascade, leading to cell death (Bock and Riley 2023). Various drugs have been developed to engage mitochondrial apoptosis directly, primarily by targeting the BCL-2 protein family (Czabotar and Garcia-Saez 2023). These include BH3 mimetics, which mimic pro-apoptotic BH3-only proteins and inhibit anti-apoptotic BCL-2 function, directly triggering apoptosis or sensitizing cells to other pro-apoptotic therapies. However, challenges such as resistance to these drugs remain. For example, mutations in *bax* or *bcl-2* can lead to resistance to BH3 mimetics (Montero and Haq 2022).

Additionally, the tumor microenvironment and the extent to which cancer cells are “primed” for death can affect the efficacy of these therapies. Mitochondria are points of convergence for various cell death-inducing pathways, including apoptotic and nonapoptotic programmed cell death (PCD) (Shoshan-Barmatz et al. 2023). Dysfunctional cellular pathways leading to these forms of PCD contribute to various diseases, including melanoma, leukemia, breast, lung, colorectal, and pancreatic cancer. For instance, the high-temperature requirement protein A 2 (HtrA2) is released from the intermembrane space (IMS) to the cytosol during apoptosis, aiding in the process by removing inhibitor of apoptosis proteins (IAPs) and facilitating both caspase-dependent and -independent apoptosis. Beyond its role in apoptosis, HtrA2/Omi also influences the cell cycle by cleaving LATS1, a tumor suppressor kinase, thereby affecting the G1–S transition. Furthermore, inactivation of HtrA2/Omi leads to mitochondrial DNA (mtDNA) deletions and other mitochondrial dysfunctions, highlighting its critical role in mitochondrial homeostasis (Verhagen et al. 2002; Vande Walle et al. 2008).

Mitochondria maintains energy homeostasis in metabolic tissues, including adipose tissues. Adipose tissue, categorized into white adipose tissue (WAT) and brown adipose tissue (BAT), exhibits distinct physiological roles concerning mitochondrial function and energy regulation (Zhu et al. 2022). Notably, mitochondria in white adipocytes are involved in coordinating energy synchronization. This is achieved through intercellular mitochondrial transfer and generating extracellular vesicles (EVs) in WAT. Cold exposure activates WAT, particularly the subcutaneous variant (sWAT), leading to a browning effect. These mitochondria are crucial in mediating interactions between cells and organs, and their therapeutic potential is an area of ongoing research. In the realm of heat production, mitochondria are especially pivotal in the process of thermogenesis, primarily seen in BAT. This type of adipose tissue, abundant in newborns and hibernating mammals, specializes in energy expenditure and heat generation (Matta et al. 2022; Aquilano et al. 2023). Unlike energy-storing WAT, BAT is mitochondria-rich, which accounts for its brown hue. Both activated BAT and WAT undergoing browning engage in non-shivering thermogenesis (NST), driven by uncoupling protein 1 (UCP1) in the mitochondria's

inner membrane. UCP1 facilitates this process by disrupting the mitochondrial proton gradient, typically used for ATP production, leading to releasing energy as heat instead (Boström et al. 2012; Ringholm et al. 2013). Emerging research is exploring how BAT thermogenesis might intersect with cancer metabolism. Certain studies indicate that cancer cells might exploit mechanisms akin to those in BAT thermogenesis, potentially playing a role in their survival and growth in specific contexts (Boström et al. 2012). Experimental evidence suggests that exposing tumor-bearing mice to cold conditions significantly hampers the growth of various solid tumors, including difficult-to-treat cancers like pancreatic cancer (Lemecha et al. 2022). Additionally, research has highlighted the role of the BAT-enriched mitochondrial matrix protein LETMD1 in BAT mitochondrial functionality and thermogenesis, with implications for human cervical cancer (Park et al. 2023).

Mitochondrial dysfunction is closely associated with aging and the onset of age-related conditions, including various forms of cancer. Studies have shown that as both experimental animals and humans age, there is an accumulation of mtDNA mutations across different tissues and organs like the heart and muscles (Frahm et al. 2005). This accumulation may contribute to the emergence of aging-related physical changes (Ju et al. 2014). For instance, experiments with a specific genetically modified mouse mitochondrial mutator mice (PolgAmut/mut) displayed early signs of aging. They exhibited a high frequency of mtDNA mutations, including point mutations and deletions, although there was no notable change in the number of mtDNA copies. Smith et al. show that age-associated oxidative phosphorylation (OXPHOS) deficiency causes metabolic remodeling that can cause accelerated intestinal cancer development (Smith et al. 2020). Another study showed that a reduction in complex 1 protein levels is associated with an increased rate of stem cell cycle re-entry. These changes in stem cell homeostasis could have significant implications for age-associated intestinal pathogenesis. In addition, mitochondrial dysfunction impairs osteogenesis, increases osteoclast activity, and accelerates age-related bone loss (Dobson et al. 2020). Interestingly, mitochondria have been implicated in extending lifespan observed in various treatments to prolong life. However, the exact nature of this relationship remains unclear. Changes in hormone levels also characterize mitochondrial aging. For example, as individuals age, there is a noted decrease in the levels of several hormones, including pituitary, aldosterone, calcitonin, growth hormone, and IGF-I (Colella et al. 2021; van der Reest et al. 2021; Alberico and Woods 2022). Similarly, humanin (HN) levels decline with age in both humans and rodents, particularly in areas such as the hypothalamus and skeletal muscles in older rats (Zárate et al. 2019). These hormonal changes coincide with an increased incidence of age-related diseases like Alzheimer's disease (AD), multiple sclerosis (MS), and type 2 diabetes mellitus (T2DM) (de Barcelos et al. 2019; Weidling and Swerdlow 2019). The observed reduction in the levels of small human-like peptide 2 (SHLP2) in the bloodstream, combined with its known roles in diminishing oxidative stress and safeguarding neural cells, indicates a substantial involvement of *shlp2* in regulating aging processes and the progression of diseases associated with aging (Kim et al. 2023).

In summary, mitochondria play multifaceted roles in cellular functions, particularly in energy production, calcium regulation, and metabolic pathways. Their dysfunction can contribute to various diseases, including cancer, where their altered calcium signaling and metabolism are critical in disease progression and potential therapeutic targets.

2.2 Mitochondrial Bioenergetics and Cancer

Mitochondrial dynamics, characterized by a continuous process of fusion and fission, play a pivotal role in maintaining cellular health and adapting to metabolic demands. These dynamics are essential in the context of cancer metabolism, influencing the development and progression of malignant cells. Mitochondria, crucial organelles within eukaryotic cells, are integral in regulating various metabolic pathways, significantly impacting the oncogenesis and progression of cancer by modulating energy production, redox balance, biosynthetic processes, and apoptotic pathways (Delierneux et al. 2020; Makena et al. 2022; Matta et al. 2022; Kim et al. 2023; Shoshan-Barmatz et al. 2023).

These organelles play a dual role in cancer biology. They not only meet the metabolic requirements of cancer cells by modulating ATP and NADPH production, essential for bioenergetics, but also convert various nutrients into building blocks necessary for cell growth and biosynthesis. This interaction with other cellular components is critical in sustaining the metabolic needs of cancer cells (Teoh and Lunt 2018). Beyond their primary role in energy generation, mitochondria contribute to tumor anabolism, regulate redox and calcium homeostasis, influence transcriptional regulation, and dictate the course of cell death (Delierneux et al. 2020; Wang et al. 2022; Bagheri-Mohammadi et al. 2023). Given their involvement in numerous aspects of cancer development and interaction with cancer cells, mitochondria present as promising targets for novel anticancer therapeutics.

Mitochondrial bioenergetics, a core aspect of cell biology, is pivotal in understanding energy generation and utilization within cells. Mitochondria are often described as cellular “powerhouses,” primarily responsible for synthesizing ATP through OXPHOS. This process, occurring within the inner mitochondrial membrane, involves a complex array of protein complexes known as the electron transport chain (ETC). Here, electrons are transferred through these complexes, facilitating the translocation of protons across the mitochondrial membrane, thereby establishing a proton gradient. This gradient generates an electrochemical potential, harnessed by ATP synthase to convert adenosine diphosphate (ADP) and inorganic phosphate into ATP. Oxygen’s role as the final electron acceptor in the ETC is crucial for effective ATP production (Onishi et al. 1967; Bohnensack et al. 1982; Rigoulet et al. 1988). Nutrients like glucose and fatty acids undergo metabolic pathways, including glycolysis, the citric acid cycle, and beta-oxidation, to produce electron carriers (NADH and FADH₂), which are integral to the ETC (Houten and Wanders 2010). The regulation of mitochondrial bioenergetics is closely aligned with cellular energy demands, affecting the activity of both the ETC and ATP

synthase, as well as substrate and oxygen availability (Martínez et al. 2019). Dysfunctions in this system can lead to various pathologies, including neurodegenerative disorders, metabolic syndromes, and age-related diseases, attributed to the central role of mitochondria in energy production, cellular signaling, and programmed cell death (Frahm et al. 2005; Weidling and Swerdlow 2019; Kim et al. 2023).

Mitochondrial OXPHOS, which includes respiratory complexes 1 to 4 in the electron transport chain (ETC) and complex 5, known as ATP synthase, plays a pivotal role in cellular energy metabolism. This mechanism is essential for ATP synthesis, fueling a range of cellular activities and thermoregulation. During OXPHOS, electrons derived from carbohydrates and fats are relayed through electron carriers. One such carrier is the reduced form of nicotinamide adenine dinucleotide (NADH + H⁺), which delivers electrons to complex 1 or NADH dehydrogenase. Electrons can also be transferred from succinate to complex 2, also known as succinate dehydrogenase. These complexes aid in reducing ubiquinone to ubiquinol, which then carries electrons to complex 3, the ubiquinone:cytochrome c oxidoreductase. From there, electrons pass through cytochrome c to complex 4, also referred to as cytochrome c oxidase, where they reduce oxygen to form water. The energy released during this electron transit is used by complexes 1, 3, and 4 to pump protons across the mitochondrial inner membrane. This creates an electrochemical gradient marked by a differential in charge and pH across the membrane. The protons then flow back through ATP synthase, driving the production of ATP from ADP and inorganic phosphate. The ATP generated within the mitochondria is then exchanged with cytosolic ADP through the action of adenine nucleotide translocases (ANTs). This process completes the cycle of mitochondrial energy production, as elucidated in various studies including those by Duszyński et al. (1984) and Dzbek and Korzeniewski (2008).

mtDNA mutations are prevalent in cancer cells and contribute to the rewiring of bioenergetics and biosynthesis pathways. Metabolites, particularly oncometabolites within mitochondria, play a significant role in tumor metabolism and progression (Sanchez-Contreras et al. 2023). Mutations in enzymes of the tricarboxylic acid (TCA) cycle can lead to the accumulation of specific metabolites and oncometabolites, influencing cancer development (Sciacovelli and Frezza 2016). Cancer cells often exhibit metabolic reprogramming, closely linked with mitochondrial function.

3 Warburg Effect

The Warburg effect, a phenomenon characterized by elevated glycolysis in the presence of oxygen, diverts energy production from oxidative phosphorylation, a hallmark of cancer metabolism. Mutations in mtDNA are implicated in various cancers, affecting the function of the electron transport chain and altering the cellular energy landscape, potentially facilitating carcinogenesis (Croteau and Bohr 1997; De Paepe 2012). Otto Warburg, a German physiologist and biochemist, made significant contributions to understanding cellular metabolism, particularly in the

context of cancer. His observations, often referred to as “Warburg’s rules” or the “Warburg effect,” have profoundly impacted cancer research. Warburg observed that cancer cells often exhibit a unique metabolic phenotype recognized as aerobic glycolysis or the Warburg effect (Warburg 1924). In normal cells, the preferred pathway for energy production is oxidative phosphorylation in the mitochondria. However, Warburg noted that cancer cells rely more on glycolysis, even in the presence of oxygen. Cancer cells exhibit a higher glucose uptake rate than normal cells, a hallmark known as the Warburg effect. Cancer cells exhibit a heightened rate of glucose uptake compared to their normal counterparts, a phenomenon known as the Warburg effect. In this process, cancer cells favor the conversion of glucose into lactate through glycolysis, a preference that persists even under conditions of sufficient oxygen availability (Warburg 1928).

In contrast, normal cells metabolize glucose completely to carbon dioxide and water within the mitochondria under the same oxygen-rich conditions. Warburg’s theory posits that this inclination of cancer cells towards glycolysis is linked to impaired mitochondrial function. While normal cells rely on oxidative phosphorylation for efficient energy production, cancer cells may have defective mitochondria and, as a result, favor glycolysis. According to Warburg, cancer cells utilize glycolysis for energy production and synthesizing cellular building blocks (biomass) required for rapid cell proliferation (Warburg et al. 1927; Warburg 1956). It is important to note that while Warburg’s observations have greatly contributed to our understanding of cancer metabolism, the field has evolved, and additional complexities have been uncovered.

In 1931, Otto Warburg’s seminal research highlighted the “aerobic glycolysis” phenomenon in solid tumor cells, emphasizing the pivotal role of mitochondria in cancer (Warburg 1924, 1956). He subsequently demonstrated that mitochondria are central to cellular bioenergetics, predominantly generating adenosine triphosphate (ATP) through the OXPHOS. As mitochondrial significance in cancer became evident, it garnered substantial attention. However, interest waned over time as it became apparent that OXPHOS defects were not universally characteristic of all tumors (Warburg 1956; Koppenol et al. 2011). Subsequent years saw the identification of mtDNA within cells. The mitochondrial genome, comprising hundreds to thousands of copies per cell, supplemented the approximately 1000–2000 nuclear DNA (nDNA)-encoded mitochondrial genes. Mammalian mtDNA was revealed to encode transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) integral to OXPHOS (Wallace and Chalkia 2013).

Moreover, somatic mutations, ancient adaptive polymorphisms, and germline mutations in mtDNA were associated with the multifaceted OXPHOS function, reflecting genetic factors (maternal or Mendelian inheritance) and environmental influences in diverse diseases (Wallace 2018). By the 2000s, it was established that mtDNA variants encompassed severe mutations contributing to cancer genesis, alongside milder mutations playing a crucial role in the adaptive responses of cancer cells. This dichotomy underscores the intricate interplay between mitochondrial genetics and cancer pathogenesis, with implications extending beyond cancer to encompass various disorders, including Alzheimer’s and Parkinson’s diseases, as

well as neuropsychiatric conditions like schizophrenia (Andreu et al. 2000; Elliott et al. 2008; Wallace and Fan 2010; Chalkia et al. 2017). The recognition of the dual impact of mtDNA mutations on cancer initiation and adaptation has provided a nuanced understanding of the multifaceted role of mitochondria in the complex landscape of cancer.

4 Mitogenome (mtDNA) and Cancer

The mitochondrial genome originated from ancestral alpha-proteobacteria through the process of endosymbiosis (Gray 1989). As mitochondria evolved to become energy-producing organelles within eukaryotic cells, their genome underwent a process of reductive evolution. This led to the transfer of many essential mitochondrial genes to the nuclear chromosomes. Consequently, in the nuclear genome, the orthologues of these mitochondrial genes became functional, while the mitochondrial genomic remnants of these genes ceased to function, a development outlined by Gray and colleagues in 2001 (Gray et al. 2001).

The mitogenome of bilateral animals is usually a covalently closed, circular and highly conserved molecule encoding 37 genes: 13 OXPHOS subunits (*nd1*, *nd2*, *nd3*, *nd4*, *nd4L*, *nd5*, *nd6*, *cox1*, *cox2*, *cox3*, *cytB*, *atp6*, and *atp8*), two rRNA and 22 tRNA genes (Osigus et al. 2013). In addition to 37 “formal” genes, mtDNA is known to encode at least two more peptides, at least in humans: Humanin (Lee et al. 2013) and MOTS-c (Lee et al. 2015, 2016). In addition to gene content, the mitochondrial genome contains non-coding regions. These region(s) are functional as the binding site of proteins for replication and/or transcription (Reyes et al. 1998). Mitochondrial functional proteins are encoded from the nuclear genome and taken into the mitochondria post-transcriptionally, in order to maintain mitochondrial replication/transcription and translation systems. This unique mitochondrial translation system is needed to encode 13 mitochondrial mRNAs that use a different genetic code from nuclear genes (Barrell et al. 1979).

Human mitochondrial gene content is not equally distributed across mitogenome heavy (H-) and light (L-) chains; only the *nd6* gene and a few tRNA genes are encoded from the L-chain; all other genes are encoded from the H-chain (Anderson et al. 1981). However, 99.9% of the H- chain and 97.6% of the L- chain are transcribed (Mercer et al. 2011). The transcription of almost all regions for both chains indicates that mitochondrial gene content and/or mitochondrial genome function is richer than assumed. Consistent with this, it is known that the mitogenome harbors many small RNAs, lncRNAs, miRNAs, and alternative spliced mRNA molecules (Rackham et al. 2011; Mercer et al. 2011). Therefore, mitochondria have essential functions for eukaryotic cells beyond the known.

mtDNA is susceptible to the accumulation of mutations because of the densified ROS production and the lack of repair mechanisms (Croteau and Bohr 1997; De Paepe 2012). mtDNA mutations are approximately 10-fold more common than nDNA mutations in cancer cells (Verma et al. 2007). Many changes in mtDNA that can be detected in tumor cells also potentially alter mitochondrial function.

mtDNA mutations have been found to have significant effects on the development of different types of tumors in both germ-line and somatic cells, ultimately causing tissue invasion and metastasis (Kulawiec et al. 2008; Zimmermann et al. 2009; Pereira et al. 2012).

Cancer and tumorigenesis processes appear to affect or are influenced by the mitogenome at several stages: (1) mtDNA copy number changes, (2) numtogenesis (the transfer of mtDNA sequences to the nDNA), and (3) mitochondrial mutations (detailed in the following sections).

4.1 The Relationship Between mtDNA Copy Number and Cancer

The most well-known link between cancer cells and mitochondria is related to mtDNA copy number (Beroukhim et al. 2010). The first scenario is that cancer cells induce the production of energy required for uncontrolled proliferation by having increased numbers of mitogenomes, most likely through the process of mitochondrial fission. In this mechanism, cancer cells and mitochondrial fission use different molecular mechanisms to avoid ROS accumulation and apoptosis (Grasso et al. 2020).

Another possible scenario may be that the increased mtDNA copy number raises the effect of heteroplasmic mutations, providing adaptive strength to cancer cells (Yuan et al. 2020; Kopinski et al. 2021). Cancer cells can produce numerous low heteroplasmic and phenotypically masked mutations. A small portion of these variants, which have the advantage of adapting to cancer cells' new environment, may reach high levels of heteroplasmy in cellular mtDNA (Wallace 2015, 2018). Supporting findings for the role of mtDNA in tumorigenesis include the increased frequency of mtDNA variants found in tumor tissues but not in stromal tissues. The enrichment of heteroplasmic mtDNA mutations in cancer cells potentially indicates positive selection of mtDNA mutations during tumorigenesis. In tumor cells, there exists a phenomenon of positive selection favoring disruptive variants in mtDNA, which are typically present at minimal frequencies in normal cells (Grandhi et al. 2017). A germline heteroplasmic mutation may be enriched in the homoplasmic state in tumor cells, potentially indicating selection for mutant mtDNA (Gasparre et al. 2009). mtDNA copy number variations play a significant role in ovarian cancer, a field of study that has garnered increasing interest in recent years. In ovarian cancer, alterations in mtDNA copy number have been observed, suggesting a potential link to the pathogenesis and progression of the disease. These variations in mtDNA content within tumor cells can influence the metabolic and energetic profiles of the cancer, contributing to its aggressiveness and responsiveness to treatment. Elevated mtDNA copy numbers in ovarian cancer cells may be a compensatory response to the increased energy demands of rapidly dividing cells or a result of mitochondrial dysfunction. Conversely, reduced mtDNA content might reflect a shift in energy production mechanisms, which could affect the tumor's growth and survival. The mtDNA copy number in ovarian cancer not only offers insights into the underlying mechanisms of tumor biology but also presents potential as a biomarker for

diagnosis, prognosis, and therapeutic response monitoring (Wang et al. 2006; Yuan et al. 2020).

mtDNA copy number variations, a crucial aspect in ovarian cancer, are also significant in other types of cancer, such as breast cancer. In breast cancer, similar to ovarian cancer, changes in mtDNA copy number are observed and are thought to play a role in the cancer's development and progression. These variations are indicative of the cancer cells' metabolic and energetic needs. An increased mtDNA copy number in breast cancer cells may be a response to the heightened energy requirements for cell proliferation and survival (Rai et al. 2021). On the other hand, a decrease in mtDNA content could signify a shift in the cancer cells' metabolic strategy, potentially impacting tumor growth and resistance to therapies. A notable decrease in mtDNA content has been observed in prostate cancer, particularly as the disease progresses to a more malignant phenotype. This trend, identified in various prostate cancer cell lines, suggests a significant link between reduced mtDNA levels and the aggressive nature of the cancer (Cook et al. 2012; Higuchi et al. 2006; Moro et al. 2008). Understanding the role of mtDNA copy number variations in different cancer types, including breast cancer, could lead to novel approaches in cancer diagnosis, prognosis, and treatment, enhancing the efficacy of personalized medicine strategies.

4.2 Numtogenesis (NUMTs) and Cancer

Numtogenesis (NUMTs), the process by which mtDNA sequences are transferred and integrated into the nuclear genome, is an area of increasing interest in cancer research. This phenomenon has been observed in various types of cancers and is gaining attention for its potential role in tumorigenesis. In cancer cells, numtogenesis can lead to genomic instability, a hallmark of cancer, by introducing mtDNA sequences into the nuclear DNA. These inserted mtDNA sequences, known as nuclear mitochondrial DNA sequences (NUMTs), can disrupt normal gene function and expression by various mechanisms. They may insert into gene-coding regions, regulatory elements, or even within oncogenes or tumor suppressor genes, potentially altering their function. Moreover, the process of numtogenesis may influence the cellular metabolic profile. Since mtDNA encodes components essential for oxidative phosphorylation, its integration into nuclear DNA could affect cellular energy metabolism, a key element in cancer cell survival and proliferation (Singh et al. 2017; Srinivasainagendra et al. 2017). In a study analyzing mtDNA and nDNA from tumor and control tissues across 587 tumors, researchers discovered 25 instances of mtDNA integration in 12 different tumor types, representing 2% of the cases, where these integrations were absent in the corresponding control samples. Specifically in colorectal cancer, the occurrence and quantity of nuclear mitochondrial DNA sequences (NUMTs) varied by gender, with NUMTs being 3.1 times more prevalent in male patients and 4.5 times more in female patients in tumor tissues than in healthy control tissues. This suggests that numtogenesis may contribute to colorectal cancer development and seems to be influenced by gender.

Additionally, an increase in numtogenesis was associated with higher mortality rates (Srinivasainagendra et al. 2017). It has been proposed that NUMTs could integrate into tumor suppressor genes through an unidentified mechanism, potentially altering gene structure or activating genes associated with cancer (Shay et al. 1991; Ju et al. 2015; Srinivasainagendra et al. 2017). Furthermore, the somatic transfer of mtDNA to the nucleus, a process known as somatic numtogenesis, was found to occur in more than 5% of lung, skin, breast, and uterine tumors, reaching 16% in breast cancers and 14.6% in squamous cell lung cancer (Yuan et al. 2020).

Research into NUMTs and its implications in cancer is still evolving. Understanding how NUMTs contributes to cancer development and progression could reveal novel targets for cancer therapy and offer insights into the complex genetic landscape of tumors. This knowledge may ultimately lead to more effective and personalized approaches in cancer treatment and management.

4.3 Nuclear DNA (nDNA)-Encoded Mitochondrial Genes and Cancer

The initial research into the connection between mitochondria and cancer focused on the limited gene content of the mitochondrial genome (mitogenome), particularly mutations in mitochondrial subunits encoded by the nuclear genome. It has been established that mutations in nuclear DNA (nDNA)-encoded mitochondrial components can elevate cancer risk. Notable examples include mutations in the succinate dehydrogenase (sdhB, sdhC, and sdhD) and isocitrate dehydrogenase (idh1 and idh2) genes. More recent studies highlight the significant impact of mtDNA-encoded mitochondrial component mutations on the development of various cancer types in both germline and somatic cells, which can lead to tissue invasion and metastasis (Kulawiec et al. 2008; Zimmermann et al. 2009; Pereira et al. 2012; van Gisbergen et al. 2015).

Further research has emphasized the importance of alterations in nDNA-encoded mitochondrial genes in cancer. These alterations disrupt mitochondrial metabolism, influencing mitochondrial reactive oxygen species production, redox state, and the generation of mitochondrial intermediates that serve as substrates for chromatin-modifying enzymes (Badr et al. 2022; Gopal et al. 2018). This underscores the critical role of mitochondrial function in cancer progression. Additionally, there is a hypothesis that changes in mtDNA and nDNA-encoded mitochondrial genes could alter mitochondrial metabolites, thereby impacting the epigenome and nDNA gene expression. Evidence supporting this hypothesis emerged with discoveries that mutations in nDNA-coded mitochondrial TCS cycle enzyme genes could induce cancers by altering the epigenome (Ju et al. 2015). It was also found that mtDNA mutations could lead to epigenome modifications in osteosarcoma cells (Kopinski et al. 2019). An analysis of 587 tumor-normal tissue pairs from various cancer patients revealed that 12 tumor samples (2%) exhibited a total of 25 mtDNA integration events into the nDNA, which were absent in the corresponding control samples (Ju et al. 2015).

5 mtDNA-Encoded Mitochondrial Genes and Cancer

5.1 Mutation Profiles of Mitogenome

There are three main classes of clinically significant mtDNA variants. The first class is maternally inherited variations that occur in the female germ line. This variant form is a common mtDNA variant in the cell and causes heteroplasmy of the variant and reference mtDNAs in the cytoplasm. Severely deleterious mtDNA mutations cause significant physiological changes in heteroplasmic states (for examples see, Wallace et al. 1988; Holt et al. 1990; Shoffner et al. 1990; Goto et al. 1990; Behar et al. 2012). The second class of clinically significant mtDNA variants occurs in the oocyte, during development, or in somatic tissues of the body. These mutations include base substitutions and deletions and accumulate with age. Deletions have an effect depending on their distribution in the tissue and the level of heteroplasmy (Wallace 2018). The third clinically important class of mtDNA variants consists of ancient variants that emerged due to human migration from Africa. These variants have become homoplastic due to their ancient form. These functional variants are beneficial and have been selected as humans move into new environments, giving rise to regional groups specific to ancestral haplotypes, population-specific polymorphism called haplogroups (Wallace et al. 2003; Mishmar et al. 2004; Ruiz-Pesini et al. 2004). As a result of meta-analyses of cancer mtDNA sequence variants, a hypothesis has been developed that there may be two classes of cancer mutations: severe mutations that cause cancer induction (inducer mutations) and milder mutations that may affect the adaptations of cancer cells (adaptor mutations) (Brandon et al. 2006; Kopinski et al. 2021).

There is at least one single nucleotide variation (SNV) in most of the 37 genes encoded from the mitogenome for each type of cancer. However, the non-coding D-loop region contains the most variation for all cancer types (Gorelick et al. 2021; Kopinski et al. 2021). Consistently, 190 tumor-specific somatic mtDNA mutations were reported, and of these 52% of the tumor somatic mRNA missense, 83% of the tRNA, 38% of the rRNA, and 85% of the control region mutations (Brandon et al. 2006).

Variants in mtDNA seem strand-specific, with C>T mutations in the H-strand and T>C mutations in the L-strand. 789 tumor samples obtained from PCAWG (Pan-Cancer Analysis of Whole Genome) were examined from whole genome sequencing data in TCGA (The Cancer Genome Atlas), and consistent results were obtained from both DNA and RNA data. Heteroplasmy is observed in both DNA and RNA sequencing data, and they are highly correlated (Yuan et al. 2020).

In total, 4381 mtDNA mutations were identified in 10,132 tumor samples. 57% of sampling containing 3264 matched tumors/healthy tissues shared at least one mitochondrial variant (Ju et al. 2014). Consistent with nuclear and mitochondrial genomes being associated with independent replication, transcriptional and mutagenic processes, no correlation was detected between nuclear and mitochondrial mutations. Somatic variants in mtDNA are generally (73.2%) non-truncating, non-synonymous mutations, and these mutations are generally called VUS (variants

of unknown significance) and effect tRNA/rRNA genes, missense mutations, in-frame indels, translation start site mutations and nonstop mutations (Gorelick et al. 2021).

The mutation rate in mtDNA coding regions is roughly 67.8 mutations per Mb, approximately sixfold higher than the 468 genes in the MSK-IMPACT panel, which contained 11.3 mutations per Mb (Cheng et al. 2015). Only TP53 and KRAS genes show higher rates than the most mutated mitochondrial-genes (TP53 > KRAS > mt-trnV > PTEN > mt-cytB > mt-rnrL > mt-trnL1 > mt-trnC > mt-trnP > mt-trnT > mt-rnrS > mt-nd5 > mt-trnF > mt-cox3 > PIK3 CA > mt-nd4 > mt-nd1 > mt-nd3 > mt-nd6 > mt-nd4L > mt-trnH > mt-trnL2 > mt-cox2 > mt-cox1 > mt-trnI > mt-trnA > mt-trnR > mt-nd2 > mt-trnM > CDKN2A > NRAS > mt-atp6 > mt-trnY > B2M > mt-trnG/W > > > mt-trnQ > > mt-atp8 > > mt-trnS1/D/K > > BRAF > > mt-trnS2 > > mt-trnN > > mt-trnE). Moreover, truncating variants of 13 mitochondrial PCGs appear to be 4.2-fold more common than truncating variants on 185 known TSGs (tumor-suppressor genes) in the MSK-IMPACT panel. It is 6.7 times more common than non-truncating, nonsynonymous mutations on the 168 oncogenes in the panel. Remarkably, when examining variants in genes exhibiting over 80% heteroplasmy, the truncating mutation rates for *nd4* and *nd5* genes are approximately five mutations per Mb. This is a significantly higher rate than most TSGs (Gorelick et al. 2021).

In total, 11.9% of tumors across all cancers contain truncating mtDNA variants that are not found in matched-normal tissue. In contrast, in another study, approximately 200,000 mitogenome sequences were analyzed, and only 0.15% of normal blood cells contained truncated variants (Bolze et al. 2019). The rate of truncating mutations in mtDNA genes in tumors appears to be 80-fold increased than the truncating mutations in the normal human genome. Of the 619 observed truncating mutations, 196 contained more than 80% heteroplasmy. This indicates that systematic loss of mitochondrial function is a common feature of tumors. Moreover, truncating variants exhibiting high heteroplasmy are more common than silent mutations exhibiting high heteroplasmy, in contrast, silent mutations are expected to drive by neutral selection and become widespread (Gorelick et al. 2021).

5.2 Mutations in Mitochondrial PCGs

Although most studies have focused on complexes 1 and 5, it is known that mutations in all complexes and all subunits of complexes caused significant effects on tumor formation and various types of cancer development. For silent mutations, there are no significant differences between complexes. However, the levels of influence/artifice and the type of cancer appear to vary considerably (De Paepe 2012).

In some types of cancer, complex 1 activity falls within 50% to 65% effectiveness (Simonnet et al. 2003; Mayr et al. 2008). Complex 1 subunits are highly enriched in pathogenic mutations in specific cancer tissues (Ju et al. 2014). Complex 1 genes harbored various mutations such as enriched, germ-line, heteroplasmic, and

homoplastic mutations (Gasparre et al. 2007; Horváth et al. 2008; Alston et al. 2010). Truncating variants were detected at a higher rate of 2 or more in complex 1 than other complexes. In contrast to variants in other complexes, truncating variants in complex 1 exhibit higher heteroplasmy than silent ones, suggesting a specific positive selection pressure on truncating variants in complex 1. Additionally, six highly recurrent mtDNA mutations occur at a specific A or C homopolymeric locus in complex 1 (*nd1*, *nd4*, *nd5*). These homopolymeric repeat loci account for 40% of all truncated variants and 57% of frameshift indels. It is known that complex 1 homopolymers generally occur in liver, colorectal, and thyroid cancers. Rare, recurrent mutations affect both the structure of the protein-coding gene and the RNA elements (Yuan et al. 2020).

All subunits of complex 2 (succinate: ubiquinone oxidoreductase) were encoded from nDNA. It has been reported that complex 2 activity is 6 times less than in healthy control tissues (Meierhofer et al. 2004; Owens et al. 2011). Mutations in three complex 2 genes (*sdhB*, *sdhC*, *sdhD*) result in increased mitochondrial ROS production by retarding the electron flow and causing cancer (Baysal et al. 2000; Vanharanta et al. 2004). On the other hand, mutations in *sdhA* gene would block the entry of electrons into complex and cause another multisystem disease (Bourgeron et al. 1995).

Complex 3 contains increased rates of missense mutations but does not include truncating mutations (Ju et al. 2014). In contrast to truncating variants, protein-coding VUSs were most frequently detected in complex 3 subunits (Gorelick et al. 2021). Despite its decreased activity, the UQCRFS1 and UQCRH subunits of complex 3 and the *cox1*, *cox2*, and *cox4* subunit genes of complex 4 are overexpressed in different tumor types (Luciaková and Kužela 1992; Ohashi et al. 2004; Owens et al. 2011). Two homopolymers specifically enriched for *cox3* occur in several characteristic cancer types (such as prostate and non-small-cell lung cancers).

The relationship between complex 5 (ATP synthase) function and cancer is quite complex. Complex 5 subunits appear to be swapped of all non-synonymous mutations in tumors (Ju et al. 2014). Proteomic studies performed in breast cancer cells have shown that the ATP synthase f subunit is twice as abundant (Shi et al. 2023). The complex 5 genes (*atp6-atp8*) contain significantly lower rates of truncating but not synonymous mutations. Like truncating variant patterns, VUS patterns are rare in complex 5 subunits (Gorelick et al. 2021).

Several studies have been conducted on mitochondrial mutations in various tumor types. Analysis of mtDNA in 45 oncocyomas showed that 26% contained high heteroplasmy mtDNA mutations in the *nd1*, *nd2*, *nd4*, *nd4L*, *nd5*, *nd6*, *cytB*, and *atp6* genes, with one tumor exhibiting mutations in *atp6* and *nd5* (Gasparre et al. 2007). A separate study on 9 patients with oncocyomas identified multiple mtDNA mutations, including highly heteroplasmic ones in 7 complex 1 genes (*nd1*, *nd4*, *nd5*, and *nd6*), with *cox1* mutations also found frequently in one tumor (Gasparre et al. 2008). These patients all presented complex 1 defects affecting oxidative metabolite formation. The majority of these mtDNA mutations in oncocyomas appear to be somatic; however, one case showed maternally inherited complex

1 mutations. This particular tumor was homoplasmic for *nd5* frameshift mutations, although the same mutation was present at lower heteroplasmic levels in normal tissues of the patient and her two sisters, suggesting selective enrichment in tumor cells (Gasparre et al. 2009).

In further research, whole genome sequencing (WGS) of 12 renal oncocyomas revealed mtDNA mutations in all samples, with mutant allele frequencies exceeding 75% in 7 out of 11 oncocyomas. Additionally, an analysis of off-target mtDNA reads in whole exome sequencing (WES) of 19 renal oncocyomas found protein-truncating mutations in 16 tumors, while the remaining three had conserved missense mutations in complex 1 genes. They also discovered 16 renal oncocyomas that 69% had high heteroplasmy mutations in the *nd1*, *nd4*, and *nd5* genes (Gopal et al. 2018).

In kidney, thyroid, and colorectal cancers, approximately 20% to 30% of the samples exhibit truncating variants, a rate comparable to the prevalence of tumor suppressors typically observed in these conditions (Gorelick et al. 2021). This trend suggests a line-specific positive selection for variants that lead to a loss of function in complex 1, alongside a selective pressure against disruptive changes in complex 5. Such selection for complex 5 is likely due to the potential for drastic, irreversible effects on ATP production, crista structure, and the maintenance of membrane potential (Gorelick et al. 2021). These observations underscore the crucial role of the functional impacts of mtDNA variations in shaping the patterns of somatic mtDNA mutations. Intriguingly, truncating mutations often appear at the same genomic locations, resembling hotspot mutations that counter selective pressures and accumulate in genes driving cancer (Chang et al. 2016; Chang et al. 2018). These recurring mutations, typically insertions or deletions, are characterized by their homopolymer sequence content.

5.3 Mitochondrial tRNA Mutations

When mitochondrial tRNAs were established statistically, it was determined that the 31st position in the anticodon region after folding was the recurrent mutation site for all tRNAs. Mutations at the 31st position are predicted to be more pathogenic than other tRNA positions. Interestingly, tRNA regions appear to be mutated 8 times more frequently in the light chain than in the heavy chain. Mitochondrial tRNAs are commonly mutated in germline mitochondrial diseases. In particular, *trnL1* genes are highly mutated within mitochondrial tRNAs and this form of *trnL1* cause multisystem disease (El-Hattab et al. 2015; Gorman et al. 2015). Interestingly, the somatic hotspot m.3243 A>G in *trnL1* is also the causal variant of approximately 30% of all mtDNA diseases. There are also mutations observed at adjacent positions (3242 and 3244), meaning that recurrent mutations in *trnL1* may affect the formation of the correct secondary structure, thus causing differences in tRNA processing or regulation of transcription by mTERF1 binding (Terzioglu et al. 2013).

The alteration from T to C in the third position of the *trnA* gene is believed to be associated with mitochondrial disease, specifically chronic progressive external

ophthalmoplegia. Accordingly, the specific secondary structure of mt-tRNAs can prevent recurrent mutations and ensure the continuity of mitochondrial function (Gorelick et al. 2021). When the IQM gene cluster is examined, the interesting pattern of *trnQ* attracts attention. Mutations acting on *trnI* or *trnM* cause the accumulation of polyA⁺- mediators on *trnQ*. This indicates that this antisense tRNA is not authenticated by enzymes and only acts as a simple RNA spacer. In this region, mutations acting on *trnI* were also found to be ineffective for *trnM*. Interestingly, the opposite is not true; mutations on *trnM* partially affect *trnI*, especially the 3' region. Other regions should also be examined to test the existence of this condition in similar mtDNA regions (Stewart et al. 2015). In addition, since tRNAs are known to be a potential template for sRNA molecules and are transported at high rates in cytoplasmic traffic, they may have functions other than direct mutational effects (Mercer et al. 2011).

5.4 Mitochondrial rRNA Mutations

Mutations of rRNA genes have also been detected in various types of cancer. However, since rRNAs function not only as genomic compartments but also as ribosome components, there are no dramatic variations. Mutations on the *rrnL* gene have been detected in breast cancer and other solid tumors (Fliss et al. 2000; Tan et al. 2002). Mutations in both *rrnS* and *rrnL* genes have been detected in colorectal and pancreatic cancers (Polyak et al. 1998; Jones et al. 2001). In ovarian cancer, it has been assumed that rRNA genes accumulate intense mutations, like those determined in D-loop and *cytB*, and may be mutational hotspots for ovarian cancer (Liu et al. 2001). In gastric carcinoma studies, mutational variations in conserved 12S rRNA gene regions, specifically np652 G insertion and np716 T-G transversion, were found (Han et al. 2005). More studies are needed on these genes of the mitogenome and cancer.

5.5 Mutations in the Mitochondrial D-loop

The mitochondrial D-loop (displacement loop) is a critical region for the control of mitochondrial DNA (mtDNA) replication and transcription. Mutations in this region are increasingly recognized for their potential role in cancer development and progression. The D-loop region, which is non-coding, exhibits the highest degree of variation across various cancer types (De Paepe 2012). Studies focusing on ovarian cancer have revealed the presence of at least one mutation in the D-loop region in 20% of cancerous tissues, a stark contrast to their healthy counterparts (Gisbergen et al. 2015; Liu et al. 2001). Studies have reported a significant presence of D-loop mutations in breast cancer tissues. These mutations are often associated with changes in mitochondrial function and can influence the aggressiveness and metastatic potential of the tumor. The mitochondrial D-loop region, known for its high mutation rate, is extensively researched in the context of mtDNA variants due

to its association with advanced stages and poor prognosis in breast cancer. Among the detected mutations, a total of 65 somatic mutations were identified, including 30 that were novel. Researchers found that the majority of somatic mutations, constituting 59%, were located in the coding region, while a smaller portion, approximately 11%, occurred in the D-loop region in Malaysian female breast cancer patients (Omasanggar et al. 2020). In a different study, distinct mutation profiles were observed between biopsy samples and their corresponding whole blood samples. When compared to the mtDNA from whole blood samples, the mtDNA extracted from biopsies exhibited a significantly higher frequency of heteroplasmic mutations, particularly in the D-loop region, as well as in *mr2*, *cox1*, and *cytB*. Additionally, it was noted that germline mtDNA mutations displayed a greater level of heteroplasmy compared to both lost and newly arising mutations in breast cancer patients (Li et al. 2023). The potential of D-loop mutations as predictive markers in therapeutic contexts has been proposed. Notably, the survival rate over three years for stage III colorectal cancer patients without D-loop mutations stands at 70%, compared to only 47% for those with these mutations. This survival disparity becomes more marked when fluorouracil is included in adjuvant chemotherapy, jumping from 45% to 78% (Lièvre et al. 2005). Furthermore, in an extensive study encompassing 38 types of tumors within a sample of 2658 cancer cases and corresponding control samples, the D-loop region had a notably high mutation rate (Yuan et al. 2020). As cancer cells often undergo metabolic reprogramming to support rapid growth and proliferation, mitochondrial dysfunction caused by D-loop mutations can contribute to this metabolic shift, commonly known as the Warburg effect. These observations suggest that mitochondrial D-loop mutations can play a role in the carcinogenesis and progression of various cancers. Research in this area is ongoing to better understand the exact mechanisms by which these mutations contribute to different cancer types and to explore their potential as targets for cancer therapy.

6 Conclusion

The review comprehensively explores the intricate relationship between mtDNA mutations and cancer. It delves into the complexities of mitochondrial genetics, highlighting the unique aspects of the mitochondrial genome, such as its circular structure, gene content, and the distinction between heavy (H-) and light (L-) chains. The susceptibility of mtDNA to mutations due to high reactive oxygen species (ROS) production and limited repair mechanisms is noted, emphasizing the higher mutation rate in mtDNA than in nuclear DNA. A significant portion of the review is dedicated to discussing how mtDNA mutations contribute to cancer development. This includes examining mtDNA copy number changes in cancer cells, numtogenesis (transfer of mtDNA sequences to nuclear DNA), and specific mutations in mitochondrial genes. The review highlights various mechanisms by which these mutations can influence cancer development, such as affecting mitochondrial function, altering energy production, and contributing to tumor cells'

adaptive capabilities. The text also provides detailed insights into specific mitochondrial gene mutations and their implications in different cancer types. It discusses mutations in protein-coding genes (PCGs) of the mitochondrial complexes, noting the variability in mutation rates and impacts across different complexes and cancer types. Additionally, it touches upon mutations in mitochondrial tRNA and rRNA genes and their potential roles in cancer development. Anticancer agents known as mitocans, which target mitochondrial metabolic and apoptotic pathways, not only offer novel therapeutic strategies but may also become cellular targets for future cancer therapy. The medical community is keenly awaiting the outcomes of clinical trials involving these agents. The review concludes by emphasizing the significant role of mtDNA mutations in cancer, underlining the need for further research to understand these complex interactions fully. This understanding could potentially lead to new therapeutic targets and strategies for cancer treatment and prevention.

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References

- AACR (American Association for Cancer Research) (2022) Cancer progress report 2022. Available from: https://cancerprogressreport.aacr.org/wp-content/uploads/sites/2/2022/09/AACR_CPR_2022.pdf. Accessed 5 July 2023
- Alberico HC, Woods DC (2022) Role of granulosa cells in the aging ovarian landscape: a focus on mitochondrial and metabolic function. *Front Physiol* 12. <https://doi.org/10.3389/fphys.2021.800739>
- Alston CL, Morak M, Reid C et al (2010) A novel mitochondrial MTND5 frameshift mutation causing isolated complex I deficiency, renal failure and myopathy. *Neuromuscul Disord* 20:131. <https://doi.org/10.1016/j.nmd.2009.10.010>
- Anderson S, Bankier AT, Barrell BG et al (1981) Sequence and organization of the human mitochondrial genome. *Nature* 290:457–465. <https://doi.org/10.1038/290457a0>
- Andreu AL, Checcarelli N, Iwata S et al (2000) A missense mutation in the mitochondrial cytochrome β gene in a revisited case with histiocytoid cardiomyopathy. *Pediatr Res* 48:311. <https://doi.org/10.1203/00006450-200009000-00008>
- Aquilano K, Zhou B, Brestoff JR, Lettieri-Barbato D (2023) Multifaceted mitochondrial quality control in brown adipose tissue. *Trends Cell Biol* 33:517–529. <https://doi.org/10.1016/j.tcb.2022.09.008>
- Badr H, Blutrich R, Chan K et al (2022) Proteomic characterization of a candidate polygenic driver of metabolism in non-small cell lung cancer. *J Mol Biol* 434:167636. <https://doi.org/10.1016/j.jmb.2022.167636>
- Bagheri-Mohammadi S, Farjami M, Suha AJ et al (2023) The mitochondrial calcium signaling, regulation, and cellular functions: a novel target for therapeutic medicine in neurological disorders. *J Cell Biochem* 124:635

- Barrell BG, Bankier AT, Drouin J (1979) A different genetic code in human mitochondria. *Nature* 282:189–194. <https://doi.org/10.1038/282189a0>
- Baysal BE, Ferrell RE, Willett-Brozick JE et al (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* (80-) 287:848–851. <https://doi.org/10.1126/science.287.5454.848>
- Behar DM, Van Oven M, Rosset S et al (2012) A “copernican” reassessment of the human mitochondrial DNA tree from its root. *Am J Hum Genet* 90:675. <https://doi.org/10.1016/j.ajhg.2012.03.002>
- Berdiaki A, Neagu M, Spyridaki I et al (2023) Hyaluronan and reactive oxygen species signaling – novel cues from the matrix? *Antioxidants* 12:824. <https://doi.org/10.3390/antiox12040824>
- Beroukheim R, Mermel CH, Porter D et al (2010) The landscape of somatic copy-number alteration across human cancers. *Nature* 463:899. <https://doi.org/10.1038/nature08822>
- Bock FJ, Riley JS (2023) When cell death goes wrong: inflammatory outcomes of failed apoptosis and mitotic cell death. *Cell Death Differ* 30:293–303. <https://doi.org/10.1038/s41418-022-01082-0>
- Bohnensack R, Küster U, Letko G (1982) Rate-controlling steps of oxidative phosphorylation in rat liver mitochondria. A synoptic approach of model and experiment. *Biochim Biophys Acta Bioenerg* 680:271–280. [https://doi.org/10.1016/0005-2728\(82\)90139-6](https://doi.org/10.1016/0005-2728(82)90139-6)
- Bolze A, Mendez F, White S et al (2019) Selective constraints and pathogenicity of mitochondrial DNA variants inferred from a novel database of 196,554 unrelated individuals. *bioRxiv*, <https://doi.org/10.1101/798264>
- Boström P, Wu J, Jedrychowski MP et al (2012) A PGC1- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481:463–468. <https://doi.org/10.1038/nature10777>
- Bourgeron T, Rustin P, Chretien D et al (1995) Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nat Genet* 11:144. <https://doi.org/10.1038/ng1095-144>
- Brandon M, Baldi P, Wallace DC (2006) Mitochondrial mutations in cancer. *Oncogene* 25:4647
- Bui M, Gilady SY, Fitzsimmons REB et al (2010) Rab32 modulates apoptosis onset and mitochondria-associated membrane (MAM) properties. *J Biol Chem* 285:31590–31602. <https://doi.org/10.1074/jbc.M110.101584>
- Chalkia D, Singh LN, Leipzig J et al (2017) Association between mitochondrial DNA haplogroup variation and autism spectrum disorders. *JAMA Psychiatry* 74:1161. <https://doi.org/10.1001/jamapsychiatry.2017.2604>
- Chang MT, Asthana S, Gao SP et al (2016) Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol* 34:155–163. <https://doi.org/10.1038/nbt.3391>
- Chang MT, Bhattarai TS, Schram AM et al (2018) Accelerating discovery of functional mutant alleles in cancer. *Cancer Discov* 8:174–183. <https://doi.org/10.1158/2159-8290.CD-17-0321>
- Cheng DT, Mitchell TN, Zehir A et al (2015) Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn* 17:251. <https://doi.org/10.1016/j.jmoldx.2014.12.006>
- Colella M, Cuomo D, Peluso T et al (2021) Ovarian aging: role of pituitary-ovarian axis hormones and ncRNAs in regulating ovarian mitochondrial activity. *Front Endocrinol (Lausanne)* 12:791071
- Cook CC, Kim A, Terao S, Gotoh A, Higuchi M (2012) Consumption of oxygen: a mitochondrial-generated progression signal of advanced cancer. *Cell Death Dis* 3(1):e258–e258
- Croteau DL, Bohr VA (1997) Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. *J Biol Chem* 272:25409
- Cuzzubbo S, Mangsbo S, Nagarajan D et al (2021) Cancer vaccines: adjuvant potency, importance of age, lifestyle, and treatments. *Front Immunol* 11. <https://doi.org/10.3389/fimmu.2020.615240>

- Czabotar PE, Garcia-Saez AJ (2023) Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. *Nat Rev Mol Cell Biol* 24:732
- de Barcelos IP, Troxell RM, Graves JS (2019) Mitochondrial dysfunction and multiple sclerosis. *Biology (Basel)* 8:37. <https://doi.org/10.3390/biology8020037>
- De Marchi U, Santo-Domingo J, Castelbou C et al (2014) NCLX protein, but not LETM1, mediates mitochondrial Ca^{2+} extrusion, thereby limiting Ca^{2+} -induced NAD(P)H production and modulating matrix redox state. *J Biol Chem* 289:20377. <https://doi.org/10.1074/jbc.M113.540898>
- De Paepe B (2012) Mitochondrial markers for cancer: relevance to diagnosis, therapy, and prognosis and general understanding of malignant disease mechanisms. *ISRN Pathol* 2012:1–15. <https://doi.org/10.5402/2012/217162>
- Delierneux C, Kouba S, Shanmughapriya S et al (2020) Mitochondrial calcium regulation of redox signaling in cancer. *Cells* 9(2):432
- Dobson PF, Dennis EP, Higgs D et al (2020) Mitochondrial dysfunction impairs osteogenesis, increases osteoclast activity, and accelerates age related bone loss. *Sci Rep* 10:11643. <https://doi.org/10.1038/s41598-020-68566-2>
- Duszyński J, Bogucka K, Wojtczak L (1984) Homeostasis of the protonmotive force in phosphorylating mitochondria. *BBA-Bioenergetics* 767:540. [https://doi.org/10.1016/0005-2728\(84\)90053-7](https://doi.org/10.1016/0005-2728(84)90053-7)
- Dzбек J, Korzeniewski B (2008) Control over the contribution of the mitochondrial membrane potential ($\Delta\Psi$) and proton gradient (ΔpH) to the protonmotive force ($\Delta\mu$): in silico studies. *J Biol Chem* 283:33232. <https://doi.org/10.1074/jbc.M802404200>
- El-Hattab AW, Adesina AM, Jones J, Scaglia F (2015) MELAS syndrome: clinical manifestations, pathogenesis, and treatment options. *Mol Genet Metab* 116:4
- Elliott HR, Samuels DC, Eden JA et al (2008) Pathogenic mitochondrial DNA mutations are common in the general population. *Am J Hum Genet* 83:254. <https://doi.org/10.1016/j.ajhg.2008.07.004>
- Eom JW, Lim JW, Kim H (2023) Lutein induces reactive oxygen species-mediated apoptosis in gastric cancer AGS cells via NADPH oxidase activation. *Molecules* 28. <https://doi.org/10.3390/molecules28031178>
- Fliss MS, Usadel H, Caballero OL et al (2000) Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. *Science* (80-) 287:2017–2019. <https://doi.org/10.1126/science.287.5460.2017>
- Frahm T, Mohamed SA, Bruse P et al (2005) Lack of age-related increase of mitochondrial DNA amount in brain, skeletal muscle and human heart. *Mech Ageing Dev* 126:1192–1200. <https://doi.org/10.1016/j.mad.2005.06.008>
- Garbincius JF, Elrod JW (2022) Mitochondrial calcium exchange in physiology and disease. *Physiol Rev* 102:893–992. <https://doi.org/10.1152/physrev.00041.2020>
- Gasparre G, Porcelli AM, Bonora E et al (2007) Disruptive mitochondrial DNA mutations in complex I subunits are markers of oncocyctic phenotype in thyroid tumors. *Proc Natl Acad Sci* 104:9001–9006. <https://doi.org/10.1073/pnas.0703056104>
- Gasparre G, Hervouet E, de Laplanche E et al (2008) Clonal expansion of mutated mitochondrial DNA is associated with tumor formation and complex I deficiency in the benign renal oncocytoma. *Hum Mol Genet* 17:986–995. <https://doi.org/10.1093/hmg/ddm371>
- Gasparre G, Iommarini L, Porcelli AM et al (2009) An inherited mitochondrial DNA disruptive mutation shifts to homoplasmy in oncocyctic tumor cells. *Hum Mutat* 30:391. <https://doi.org/10.1002/humu.20870>
- Gopal RK, Calvo SE, Shih AR et al (2018) Early loss of mitochondrial complex I and rewiring of glutathione metabolism in renal oncocytoma. *Proc Natl Acad Sci USA* 115:E6283. <https://doi.org/10.1073/pnas.1711888115>
- Gorelick AN, Kim M, Chatila WK et al (2021) Respiratory complex and tissue lineage drive recurrent mutations in tumour mtDNA. *Nat Metab* 3:558. <https://doi.org/10.1038/s42255-021-00378-8>

- Gorman GS, Schaefer AM, Ng Y et al (2015) Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Ann Neurol* 77:753. <https://doi.org/10.1002/ana.24362>
- Goto Y, Nonaka I, Horai S (1990) A mutation in the tRNA^{Leu}(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 348:651–653. <https://doi.org/10.1038/348651a0>
- Grandhi S, Bosworth C, Maddox W et al (2017) Heteroplasmic shifts in tumor mitochondrial genomes reveal tissue-specific signals of relaxed and positive selection. *Hum Mol Genet* 26:2912–2922. <https://doi.org/10.1093/hmg/ddx172>
- Grasso D, Zampieri LX, Capelôa T et al (2020) Mitochondria in cancer. *Cell Stress* 4:114–146. <https://doi.org/10.15698/cst2020.06.221>
- Gray MW (1989) Origin and evolution of mitochondrial DNA. *Annu Rev Cell Biol* 5:25–50
- Gray MW, Burger G, Lang B (2001) Minireview The origin and early evolution of mitochondria. *Genome Biol* 2:1–5
- Han CB, Ma JM, Xin Y et al (2005) Mutations of mitochondrial 12S rRNA in gastric carcinoma and their significance. *World J Gastroenterol* 11:31. <https://doi.org/10.3748/wjg.v11.i1.31>
- Hanahan D (2022) Hallmarks of cancer: new dimensions. *Cancer Discov* 12:31–46. <https://doi.org/10.1158/2159-8290.CD-21-1059>
- Higuchi M, Kudo T, Suzuki S, Evans TT, Sasaki R, Wada Y, . . . Gotoh A (2006) Mitochondrial DNA determines androgen dependence in prostate cancer cell lines. *Oncogene* 25(10):1437–1445
- Holt IJ, Harding AE, Petty RKH, Morgan-Hughes JA (1990) A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 46:428
- Horváth R, Reilmann R, Holinski-Feder E et al (2008) The role of complex I genes in MELAS: a novel heteroplasmic mutation 3380G>A in ND1 of mtDNA. *Neuromuscul Disord* 18:553. <https://doi.org/10.1016/j.nmd.2008.05.002>
- Houten SM, Wanders RJA (2010) A general introduction to the biochemistry of mitochondrial fatty acid β -oxidation. *J Inherit Metab Dis* 33:469
- Jackson RC (2023) Evolutionary dynamics of malignancy. Springer, Cham
- Jones JB, Song JJ, Hempen PM et al (2001) Detection of mitochondrial DNA mutations in pancreatic cancer offers a “mass”-ive advantage over detection of nuclear DNA mutations. *Cancer Res* 61:5688
- Ju YS, Alexandrov LB, Gerstung M et al (2014) Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer. *elife* 3. <https://doi.org/10.7554/eLife.02935>
- Ju YS, Tubio JMC, Mifsud W et al (2015) Frequent somatic transfer of mitochondrial DNA into the nuclear genome of human cancer cells. *Genome Res* 25:814. <https://doi.org/10.1101/gr.190470.115>
- Kim SK, Tran LT, NamKoong C et al (2023) Mitochondria-derived peptide SHLP2 regulates energy homeostasis through the activation of hypothalamic neurons. *Nat Commun* 14:4321. <https://doi.org/10.1038/s41467-023-40082-7>
- Kopinski PK et al (2019) Regulation of nuclear epigenome by mitochondrial DNA heteroplasmy. *Proc Natl Acad Sci USA* 116:16028–16035. This study is the first description of an mtDNA mutation causing changes in the nuclear epigenome
- Kopinski PK, Singh LN, Zhang S et al (2021) Mitochondrial DNA variation and cancer. *Nat Rev Cancer* 21:431–445. <https://doi.org/10.1038/s41568-021-00358-w>
- Koppenol WH, Bounds PL, Dang CV (2011) Otto Warburg’s contributions to current concepts of cancer metabolism. *Nat Rev Cancer* 11(5):325
- Kotwal AA, Schonberg MA (2017) Cancer screening in the elderly: a review of breast, colorectal, lung, and prostate cancer screening. *Cancer J (United States)* 23:246
- Kulawiec M, Safina AF, Desouki MM et al (2008) Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. *Cancer Biol Ther* 7:1732–1743. <https://doi.org/10.4161/cbt.7.11.6729>

- Lee C, Yen K, Cohen P (2013) Humanin: a harbinger of mitochondrial-derived peptides? *Trends Endocrinol Metab* 24:222–228. <https://doi.org/10.1016/j.tem.2013.01.005>
- Lee C, Zeng J, Drew BG et al (2015) The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. *Cell Metab* 21:443–454. <https://doi.org/10.1016/j.cmet.2015.02.009>
- Lee C, Kim KH, Cohen P (2016) MOTS-c: a novel mitochondrial-derived peptide regulating muscle and fat metabolism. *Free Radic Biol Med* 100:182–187. <https://doi.org/10.1016/j.freeradbiomed.2016.05.015>
- Lemecha M, Chalise JP, Takamuku Y et al (2022) Lcn2 mediates adipocyte-muscle-tumor communication and hypothermia in pancreatic cancer cachexia. *Mol Metab* 66:101612. <https://doi.org/10.1016/j.molmet.2022.101612>
- Li Y, Sundquist K, Vats S, Hong MG, Wang X, Chen Y, ... Memon AA (2023) Mitochondrial heteroplasmic shifts reveal a positive selection of breast cancer. *J Transl Med* 21(1):696
- Lièvre A, Chapusot C, Bouvier AM et al (2005) Clinical value of mitochondrial mutations in colorectal cancer. *J Clin Oncol* 23:3517. <https://doi.org/10.1200/JCO.2005.07.044>
- Liu VWS, Hui Shi H, Cheung ANY et al (2001) High incidence of somatic mitochondrial DNA mutations in human ovarian carcinomas. *Cancer Res* 61:5998–6001
- Liu Y, Jin M, Wang Y et al (2020) MCU-induced mitochondrial calcium uptake promotes mitochondrial biogenesis and colorectal cancer growth. *Signal Transduct Target Ther* 5:59. <https://doi.org/10.1038/s41392-020-0155-5>
- Liu S, Gandler HI, Tosic I et al (2021a) Mutant KRAS downregulates the receptor for leukemia inhibitory factor (LIF) to enhance a signature of glycolysis in pancreatic cancer and lung cancer. *Mol Cancer Res* 19:1283. <https://doi.org/10.1158/1541-7786.MCR-20-0633>
- Liu T, Lam V, Thieme E et al (2021b) Pharmacologic targeting of Mcl-1 induces mitochondrial dysfunction and apoptosis in B-cell lymphoma cells in a TP53- and BAX-dependent manner. *Clin Cancer Res* 27:4910. <https://doi.org/10.1158/1078-0432.CCR-21-0464>
- Luciaková K, Kužela Š (1992) Increased steady-state levels of several mitochondrial and nuclear gene transcripts in rat hepatoma with a low content of mitochondria. *Eur J Biochem* 205:1187. <https://doi.org/10.1111/j.1432-1033.1992.tb16889.x>
- Madreiter-Sokolowski CT, Gottschalk B, Sokolowski AA et al (2021) Dynamic control of mitochondrial Ca^{2+} levels as a survival strategy of cancer cells. *Front Cell Dev Biol* 9. <https://doi.org/10.3389/fcell.2021.614668>
- Makena MR, Ko M, Mekile AX et al (2022) Secretory pathway Ca^{2+} -ATPase SPCA2 regulates mitochondrial respiration and DNA damage response through store-independent calcium entry. *Redox Biol* 50:102240. <https://doi.org/10.1016/j.redox.2022.102240>
- Marchi S, Corricelli M, Branchini A et al (2019) Akt-mediated phosphorylation of MICU 1 regulates mitochondrial Ca^{2+} levels and tumor growth. *EMBO J* 38. <https://doi.org/10.15252/embj.201899435>
- Martínez J, Tarallo D, Martínez-Palma L et al (2019) Mitofusins modulate the increase in mitochondrial length, bioenergetics and secretory phenotype in therapy-induced senescent melanoma cells. *Biochem J* 476:2463–2486. <https://doi.org/10.1042/BCJ20190405>
- Matta L, de Faria CC, De Oliveira DF et al (2022) Exercise improves redox homeostasis and mitochondrial function in white adipose tissue. *Antioxidants* 11:1689. <https://doi.org/10.3390/antiox11091689>
- Matuz-Mares D, González-Andrade M, Araiza-Villanueva MG et al (2022) Mitochondrial calcium: effects of its imbalance in disease. *Antioxidants* 11:801. <https://doi.org/10.3390/antiox11050801>
- Mayr JA, Meierhofer D, Zimmermann F et al (2008) Loss of complex I due to mitochondrial DNA mutations in renal oncocytoma. *Clin Cancer Res* 14:2270–2275. <https://doi.org/10.1158/1078-0432.CCR-07-4131>
- Meierhofer D, Mayr JA, Foetschl U et al (2004) Decrease of mitochondrial DNA content and energy metabolism in renal cell carcinoma. *Carcinogenesis* 25:1005. <https://doi.org/10.1093/carcin/bgh104>

- Mercer TR, Neph S, Dinger ME et al (2011) The human mitochondrial transcriptome. *Cell* 146: 645–658. <https://doi.org/10.1016/j.cell.2011.06.051>
- Miranda M, Bonekamp NA, Kühl I (2022) Starting the engine of the powerhouse: mitochondrial transcription and beyond. *Biol Chem* 403:779
- Misgeld T, Schwarz TL (2017) Mitostasis in neurons: maintaining mitochondria in an extended cellular architecture. *Neuron* 96(3):651–666
- Mishmar D, Ruiz-Pesini E, Brandon M, Wallace DC (2004) Mitochondrial DNA-like sequences in the nucleus (NUMTs): insights into our African origins and the mechanism of foreign DNA integration. *Hum Mutat* 23:125. <https://doi.org/10.1002/humu.10304>
- Montero J, Haq R (2022) Adapted to survive: targeting cancer cells with BH3 Mimetics. *Cancer Discov* 12:1217–1232. <https://doi.org/10.1158/2159-8290.CD-21-1334>
- Moro L, Arbini AA, Marra E, Greco M (2008) Mitochondrial DNA depletion reduces PARP-1 levels and promotes progression of the neoplastic phenotype in prostate carcinoma. *Anal Cell Pathol* 30(4):307–322
- Ohashi Y, Kaneko SJ, Cupples TE, Young SR (2004) Ubiquinol cytochrome c reductase (UQCRCF1) gene amplification in primary breast cancer core biopsy samples. *Gynecol Oncol* 93:54–58. <https://doi.org/10.1016/j.ygyno.2004.01.019>
- Omasanggar R, Yu CY, Ang GY, Emran NA, Kitan N, Baghawi A, . . . Maniam S (2020) Mitochondrial DNA mutations in Malaysian female breast cancer patients. *PLoS One* 15(5): e0233461
- Onishi T, Kröger A, Heldt HW et al (1967) The response of the respiratory chain and adenine nucleotide system to oxidative phosphorylation in yeast mitochondria. *Eur J Biochem*. <https://doi.org/10.1111/j.1432-1033.1967.tb00074.x>
- Osigus HJ, Eitel M, Bernt M et al (2013) Mitogenomics at the base of Metazoa. *Mol Phylogenet Evol* 69:339–351. <https://doi.org/10.1016/j.ympev.2013.07.016>
- Owens KM, Kulawiec M, Desouki MM et al (2011) Impaired OXPHOS complex III in breast cancer. *PLoS One* 6:e23846. <https://doi.org/10.1371/journal.pone.0023846>
- Papanagnou E, Gumeni S, Sklirou AD et al (2022) Autophagy activation can partially rescue proteasome dysfunction-mediated cardiac toxicity. *Aging Cell* 21:e13715. <https://doi.org/10.1111/ace1.13715>
- Park A, Kim K, Park I et al (2023) Mitochondrial matrix protein LETMD1 maintains thermogenic capacity of brown adipose tissue in male mice. *Nat Commun* 14:3746. <https://doi.org/10.1038/s41467-023-39106-z>
- Pereira L, Soares P, Máximo V, Samuels DC (2012) Somatic mitochondrial DNA mutations in cancer escape purifying selection and high pathogenicity mutations lead to the oncocyctic phenotype: pathogenicity analysis of reported somatic mtDNA mutations in tumors. *BMC Cancer* 12. <https://doi.org/10.1186/1471-2407-12-53>
- Polyak K, Li Y, Zhu H et al (1998) Somatic mutations of the mitochondrial genome in human colorectal tumours. *Nat Genet* 20:291–293. <https://doi.org/10.1038/3108>
- Praharaj PP, Panigrahi DP, Bhol CS et al (2021) Mitochondrial rewiring through mitophagy and mitochondrial biogenesis in cancer stem cells: a potential target for anti-CSC cancer therapy. *Cancer Lett* 498:217–228. <https://doi.org/10.1016/j.canlet.2020.10.036>
- Pulumati A, Pulumati A, Dwarakanath BS et al (2023) Technological advancements in cancer diagnostics: improvements and limitations. *Cancer Rep* 6(2):e1764
- Rackham O, Shearwood A-MJAMJ, Mercer TR et al (2011) Long noncoding RNAs are generated from the mitochondrial genome and regulated by nuclear-encoded proteins. *RNA* 17:2085–2093. <https://doi.org/10.1261/rna.029405.111>
- Rai NK, Panjwani G, Ghosh AK, Haque R, Sharma LK (2021) Analysis of mitochondrial DNA copy number variation in blood and tissue samples of metastatic breast cancer patients (A pilot study). *Biochem Biophys Res* 26:100931. <https://doi.org/10.1016/j.bbrep.2021.100931>
- Reyes A, Gissi C, Pesole G, Saccone C (1998) Asymmetrical directional mutation pressure in the mitochondrial genome of mammals. *Mol Biol Evol* 15:957–966. <https://doi.org/10.1093/oxfordjournals.molbev.a026011>

- Rigoulet M, Averet N, Mazat JP et al (1988) Redistribution of the flux-control coefficients in mitochondrial oxidative phosphorylations in the course of brain edema. *BBA-Bioenergetics* 932:116. [https://doi.org/10.1016/0005-2728\(88\)90145-4](https://doi.org/10.1016/0005-2728(88)90145-4)
- Ringholm S, Grunnet Knudsen J, Leick L et al (2013) PGC-1 α is required for exercise- and exercise training-induced UCP1 up-regulation in mouse white adipose tissue. *PLoS One* 8:e64123. <https://doi.org/10.1371/journal.pone.0064123>
- Ruiz-Pesini E, Mishmar D, Brandon M et al (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* (80-). <https://doi.org/10.1126/science.1088434>
- Sanchez-Contreras M, Sweetwyne MT, Tsantilas KA et al (2023) The multi-tissue landscape of somatic mtDNA mutations indicates tissue-specific accumulation and removal in aging. *elife* 12. <https://doi.org/10.7554/eLife.83395>
- Santos JC, Profitós-Pelejà N, Ribeiro ML, Roué G (2022) Antitumor activity of simvastatin in preclinical models of mantle cell lymphoma. *Cancers (Basel)* 14. <https://doi.org/10.3390/cancers14225601>
- Sciacovelli M, Frezza C (2016) Oncometabolites: unconventional triggers of oncogenic signalling cascades. *Free Radic Biol Med* 100:175–181. <https://doi.org/10.1016/j.freeradbiomed.2016.04.025>
- Sebens N (2023) Carcinogenesis and genetic susceptibility: unravelling the risk factors. *J Carcinog* 22(1):43–52. https://doi.org/10.4103/jcar.jcar_22_01_06
- Shay JW, Baba T, Zhan Q et al (1991) HeLaTG cells have mitochondrial DNA inserted into the c-myc oncogene. *Oncogene* 6:1869
- Shi Y, Wu L, Ji X et al (2023) Relationship between breast cancer risk and polymorphisms in CLOCK gene: a systematic review and meta-analysis. *Biochem Genet* 61:2348–2362. <https://doi.org/10.1007/s10528-023-10372-2>
- Shoffner JM, Lott MT, Lezza AMS et al (1990) Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA^{Lys} mutation. *Cell* 61:931. [https://doi.org/10.1016/0092-8674\(90\)90059-N](https://doi.org/10.1016/0092-8674(90)90059-N)
- Shoshan-Barmatz V, Arif T, Shteinfer-Kuzmine A (2023) Apoptotic proteins with non-apoptotic activity: expression and function in cancer. *Apoptosis* 28:730–753. <https://doi.org/10.1007/s10495-023-01835-3>
- Simonnet H, Demont J, Pfeiffer K et al (2003) Mitochondrial complex I is deficient in renal oncocytomas. *Carcinogenesis* 24:1461–1466. <https://doi.org/10.1093/carcin/bgg109>
- Singh KK, Choudhury AR, Tiwari HK (2017) Numtogenesis as a mechanism for development of cancer. *Semin Cancer Biol* 47:101
- Smith ALM, Whitehall JC, Bradshaw C et al (2020) Age-associated mitochondrial DNA mutations cause metabolic remodeling that contributes to accelerated intestinal tumorigenesis. *Nat Cancer* 1:976–989. <https://doi.org/10.1038/s43018-020-00112-5>
- Spinelli JB, Haigis MC (2018) The multifaceted contributions of mitochondria to cellular metabolism. *Nat Cell Biol* 20:745
- Srinivasainagendra V, Sandel MW, Singh B et al (2017) Migration of mitochondrial DNA in the nuclear genome of colorectal adenocarcinoma. *Genome Med* 9:31. <https://doi.org/10.1186/s13073-017-0420-6>
- Stewart JB, Alaei-Mahabadi B, Sabarinathan R et al (2015) Simultaneous DNA and RNA mapping of somatic mitochondrial mutations across diverse human cancers. *PLoS Genet* 11:e1005333. <https://doi.org/10.1371/journal.pgen.1005333>
- Talmadge JE, Fidler IJ (2010) AACR centennial series: the biology of cancer metastasis: historical perspective. *Cancer Res* 70:5649
- Tan DJ, Bai RK, Wong LJC (2002) Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. *Cancer Res* 62:1083
- Teoh ST, Lunt SY (2018) Metabolism in cancer metastasis: bioenergetics, biosynthesis, and beyond. *WIREs Syst Biol Med* 10. <https://doi.org/10.1002/wsbm.1406>

- Terzioglu M, Ruzzenente B, Harmel J et al (2013) MTERF1 binds mtDNA to prevent transcriptional interference at the light-strand promoter but is dispensable for rRNA gene transcription regulation. *Cell Metab* 17:618–626. <https://doi.org/10.1016/j.cmet.2013.03.006>
- van der Reest J, Nardini Cecchino G, Haigis MC, Kordowitzki P (2021) Mitochondria: their relevance during oocyte ageing. *Ageing Res Rev* 70:101378. <https://doi.org/10.1016/j.arr.2021.101378>
- van Gisbergen MW, Voets AM, Starmans MHW et al (2015) How do changes in the mtDNA and mitochondrial dysfunction influence cancer and cancer therapy? Challenges, opportunities and models. *Mutat Res Rev Mutat Res* 764:16–30
- Vande Walle L, Lamkanfi M, Vandenabeele P (2008) The mitochondrial serine protease HtrA2/Omi: an overview. *Cell Death Differ* 15:453
- Vanharanta S, Buchta M, McWhinney SR et al (2004) Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable Paraganglioma. *Am J Hum Genet* 74:153. <https://doi.org/10.1086/381054>
- Vasileiou P, Evangelou K, Vlasik K et al (2019) Mitochondrial homeostasis and cellular senescence. *Cells* 8:686. <https://doi.org/10.3390/cells8070686>
- Verhagen AM, Silke J, Ekert PG et al (2002) HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins. *J Biol Chem* 277:445. <https://doi.org/10.1074/jbc.M109891200>
- Verma M, Naviaux RK, Tanaka M et al (2007) Meeting report: mitochondrial DNA and cancer epidemiology. *Cancer Res* 67(2):437–439
- Wallace DC (2015) Mitochondrial DNA variation in human radiation and disease. *Cell* 163:33–38. <https://doi.org/10.1016/j.cell.2015.08.067>
- Wallace DC (2018) Mitochondrial genetic medicine. *Nat Genet* 50:1642
- Wallace DC, Chalkia D (2013) Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. *Cold Spring Harb Perspect Biol* 5:a021220–a021220. <https://doi.org/10.1101/cshperspect.a021220>
- Wallace DC, Fan W (2010) Energetics, epigenetics, mitochondrial genetics. *Mitochondrion* 10:12
- Wallace DC, Singh G, Lott MT et al (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* (80-). <https://doi.org/10.1126/science.3201231>
- Wallace DC, Ruiz-Pesini E, Mishmar D (2003) mtDNA variation, climatic adaptation, degenerative diseases, and longevity. *Cold Spring Harb Symp Quant Biol* 68:471. <https://doi.org/10.1101/sqb.2003.68.471>
- Wang Y, Liu VWS, Xue WC et al (2006) Association of decreased mitochondrial DNA content with ovarian cancer progression. *Br J Cancer* 95:1087–1091. <https://doi.org/10.1038/sj.bjc.6603377>
- Wang X, Li Y, Li Z et al (2022) Mitochondrial calcium uniporter drives metastasis and confers a targetable cystine dependency in pancreatic cancer. *Cancer Res* 82:2254. <https://doi.org/10.1158/0008-5472.CAN-21-3230>
- Warburg O (1924) Über den Stoffwechsel der Carcinomzelle. *Naturwissenschaften* 12:1131. <https://doi.org/10.1007/BF01504608>
- Warburg O (1928) The chemical constitution of respiration ferment. *Science* (80-) 68:437–443. <https://doi.org/10.1126/science.68.1767.437>
- Warburg O (1956) On the origin of cancer cells. *Science* (80-). <https://doi.org/10.1126/science.123.3191.309>
- Warburg O, Wind F, Negelein E (1927) The metabolism of tumors in the body. *J Gen Physiol* 8: 519. <https://doi.org/10.1085/jgp.8.6.519>
- Weidling I, Swerdlow RH (2019) Mitochondrial dysfunction and stress responses in Alzheimer's disease. *Biology (Basel)* 8(2):39
- World Cancer Research Fund International (2020) Worldwide cancer data. Available from: <https://www.wcrf.org/dietandcancer/worldwide-cancer-data/>
- World Health Organization (2023) Social determinants of health. Available from: <https://www.who.int/health-topics/social-determinants-of-health>. Accessed 31 July 2023

- Yuan Y, Ju YS, Kim Y et al (2020) Comprehensive molecular characterization of mitochondrial genomes in human cancers. *Nat Genet* 52:342–352. <https://doi.org/10.1038/s41588-019-0557-x>
- Zárate SC, Traetta ME, Codagnone MG et al (2019) Humanin, a mitochondrial-derived peptide released by astrocytes, prevents synapse loss in hippocampal neurons. *Front Aging Neurosci* 11. <https://doi.org/10.3389/fnagi.2019.00123>
- Zhou X, Ren Y, Kong L et al (2015) Targeting EZH2 regulates tumor growth and apoptosis through modulating mitochondria dependent cell-death pathway in HNSCC. *Oncotarget* 6:33720. <https://doi.org/10.18632/oncotarget.5606>
- Zhu Q, An YA, Scherer PE (2022) Mitochondrial regulation and white adipose tissue homeostasis. *Trends Cell Biol* 32:351
- Zimmermann FA, Mayr JA, Neureiter D et al (2009) Lack of complex i is associated with oncocytic thyroid tumours. *Br J Cancer* 100:1434–1437. <https://doi.org/10.1038/sj.bjc.6605028>

The Obesity Epidemic and Cancer Risk: Molecular Pathways, Risk Factors and Therapeutic Avenues

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Abstract

Obesity, characterized by the excessive and abnormal accumulation of body fat, has been classified as a global pandemic by the World Health Organization (WHO). This is an increasing concern globally due to the rising incidence of obesity and its severe clinical ramifications. While obesity is traditionally linked with conditions such as type 2 diabetes and cardiovascular diseases, recent epidemiological studies have reported an association between obesity and an increased risk of various cancers. A 2019 study estimated that 3.9% of all cancer cases globally could be attributed to excess body weight (overweight, obese, or severely obese). A growing number of studies over recent years have investigated the precise causal molecular mechanisms underlying the association between obesity and cancer, yet the understanding of this complex relationship remains limited. At its core, the biological mechanisms underlying the obesity-cancer

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connection hinge on the alterations induced by obesity itself. These alterations encompass shifts in adipocyte function, the metabolic signaling of adipokines, and the perpetuation of chronic inflammation/oxidative stress, collectively creating a microenvironment conducive to tumor growth. Additionally, changes in insulin signaling and lipid deregulation associated with excessive adiposity may also aid in the pathogenesis of cancer. Gut microbiome changes and microenvironmental perturbations are additional mechanisms. Unraveling the intricate web of obesity-cancer nexus is essential for the identification of potential therapeutic agents targeting these shared molecular pathways. This chapter will investigate the molecular interconnections, and each of the essential pathways and mechanisms correlated with obesity and cancer. Furthermore, it will address the shared risk factors that contribute to the development of both obesity and cancer, such as a sedentary lifestyle and an unhealthy diet. It will also identify potential therapeutic targets which will aid in developing effective therapeutic strategies, spanning both personalized and population-wide approaches, that aim to reduce the risk and mortality of cancer in an increasing obese population.

Keywords

Adipokines · Cancer · Chronic inflammation · Gut microbiome · Insulin signaling · Obesity · Obesity-cancer nexus · Oxidative stress · Therapeutic targets

Abbreviations

AHO	Albright hereditary osteodystrophy
APN	Adiponectin
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BER	Base excision repair
BMI	Body mass index
CAA	Cancer-associated adipocytes
CAP1	Adenylyl cyclase-associated protein
COX2	Cyclooxygenase 2
CRC	Colorectal cancer
DCA	Deoxycholic acid
EMT	Epithelia-mesenchymal transition
ER	Endoplasmic reticulum
GSCI	Glucose-sodium cotransporter inhibitors
HDL	High-density lipoprotein
IGF-I	Insulin-like growth factor
IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
JAK2	Janus kinase 2
LEP	Leptin

LEPR	Leptin receptor
LepRb	Leptin receptor
MAPK	Mitogen-activated protein kinase
MC4R	Melanocortin 4 receptor
MCP-1	Monocyte chemoattractant protein-1
MMPs	Matrix metalloproteinases
NCDs	Noncommunicable diseases
NTRK2	Neurotrophic tyrosine kinase receptor type 2 gene
OACs	Obesity-associated cancers
OR-NCDs	Obesity-related noncommunicable diseases
PCSK1	Prohormone convertase 1
POMC	Proopiomelanocortin
RCTs	Randomized controlled trials
ROS	Reactive oxygen species
SHBG	Sex hormone binding globulin
SIM1	Single-minded homolog 1
TNF- α	Tumor necrosis factor alpha
Treg	Regulatory T cells
VEGF	Vascular endothelial growth factor
8-Oxo-dG	8-Oxo-7,8-dihydro-2'-deoxyguanosine

1 Introduction

Obesity is an epidemic occurrence across the globe that the World Health Organization defines as excessive and abnormal adipose tissue accumulation from excess energy intake and/or decreased physical activity. Body mass index (BMI), a measure of adiposity and a tool for identifying obesity, is derived from a person's weight in kilograms and height in meters. Adults with a BMI of ≥ 25 kg/m² are categorized as overweight, while a BMI of ≥ 30 kg/m² is classified as obese. Obesity can further be divided into class 1 (BMI of 30.0–34.9 kg/m²), class 2 (BMI of 35.0–39.9 kg/m²), and class 3 (BMI of ≥ 40.0 kg/m²). The World Health Organization found that in 2016, more than 39% of people over the age of 18 worldwide (1.9 billion) were overweight, of which 13% were obese; this tripled the prevalence of obesity in 1975. Alarming, the prevalence of obesity is further increasing, especially in industrialized countries (Avgerinos et al. 2019).

This chronic disease has become a public health catastrophe in recent years due to the severity of its repercussions on individuals and society. Obese individuals are correlated with other severe conditions and comorbidities, higher rates of mortality, impaired quality of life, lower life expectancy, and economic burden for both the individuals and the society (Abdelaal et al. 2017). Additionally, the clinical complications associated with obesity are vast and severe; obese individuals are at an increased risk of not only developing cardiovascular disease and type 2 diabetes but

also at an increased risk of developing cancer (Bhaskaran et al. 2014; Hruby and Hu 2015; Abdelaal et al. 2017). Many observational studies reported that a higher BMI is associated with an increased risk of several cancers, such as endometrial cancer, esophageal adenocarcinomas, gallbladder, and kidney cancer (Bhaskaran et al. 2014; Lauby-Secretan et al. 2016). The strongest obesity-cancer correlation is observed in endometrial cancer, where obese and severely obese patients have an increased risk for cancer by 2.6-fold and 4.7-fold, respectively (Shaw et al. 2016; MacKintosh et al. 2019). Additionally, in certain cancers like breast and prostate cancer, obesity leads to poorer prognosis, higher rate of cancer recurrence, increased mortality, and a decrease in the overall cancer survivorship, which accounts for 20% of women's and 14% of men's deaths due to cancer (Schmitz et al. 2013; Krupa-Kotara and Dakowska 2021). In colorectal cancer, a mere 10% increase in body fat percentage leads to a 33% higher risk for cancer mortality (Parekh et al. 2012).

As the obesity rate is expected to continue rising, the adverse consequences and increase in future burdens of obesity-related cancers will become globally significant (Arnold et al. 2016). To reduce the global burden of these noncommunicable diseases, research into the causal association and molecular insight between excess weight and cancer is of great importance. This is to ensure the design and implementation of effective intervention strategies and public health policies focused on the prevention of obesity as well as weight management in obese cancer patients. For instance, reducing excess body weight would reduce the risk of developing cancer. It must be emphasized that obesity and cancer are complex diseases with various pathophysiological mechanisms. Thus, it cannot be solved with a one-size-fits-all solution (Lin and Li 2021). Hence, the interventions for this preventable cause of cancer need a multisystem approach and require a tailored approach according to the cancer type and mechanisms associated with it.

While epidemiological studies on the link between obesity and cancer are abundant, the mechanistic insight and precise molecular interconnections between the two are unclear. Pathomechanisms connecting obesity and cancer revolve around changes in sex steroids and adipocyte-derived cytokines (adipokines) secretion level, as well as chronic inflammation due to metabolic alterations in the adipose tissue associated with excess adiposity (Fig. 1) (Park et al. 2014). Additionally, activation of insulin-like growth factor-1 (IGF-1) and hyperinsulinemia related to insulin resistance also influence the cancer etiology through several mechanisms (Szablewski 2024). Accumulated effects from these create a pro-tumorigenic microenvironment (e.g., oxidative stress and DNA damage) that promotes cell proliferation, thus driving the pathogenesis of cancer at a cellular level.

After this chapter explores the associations of obesity with cancer risk, it will summarize all the obesity-associated alterations impacting cancer, such as metabolic perturbation, cellular changes, inflammation, and tumor microenvironment changes. It will also investigate the current understanding of the mechanisms implicated in the link between obesity and cancer. Additionally, it will discuss the shared risk factors and factors in obesity and cancer. Therapeutic interventions based on nodal points of control in the above mechanism cascade and other strategies for prevention and

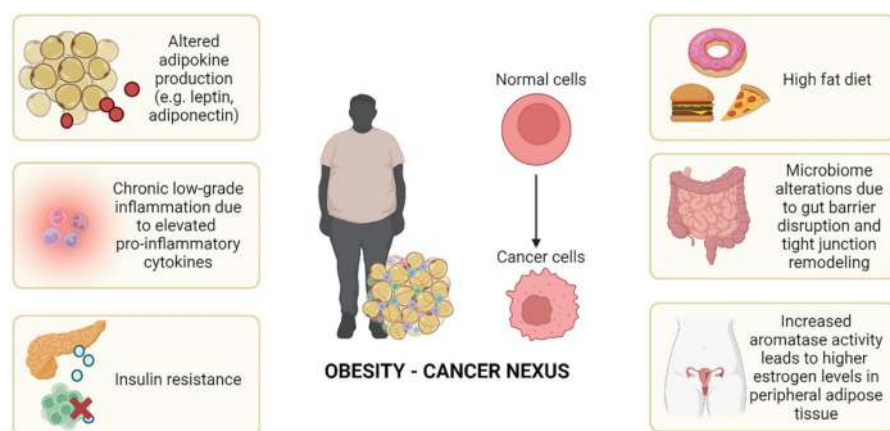


Fig. 1 Obesity-cancer nexus. This figure illustrates the multifaceted relationship between obesity and cancer development, emphasizing how obesity contributes to cancer risk through several biological mechanisms—metabolic, hormonal, and inflammatory

intervention will be discussed to develop new practical therapeutic approaches focused on preventing and treating obesity-related cancers.

2 The Complex Relationship Between Obesity and Cancer

Obesity has been increasingly linked to the risk of developing 13 types of cancer (Liu et al. 2021). In recent years, the correlation between obesity and cancer has become an area of intense research. The local and systemic environment created by excess adiposity, such as chronic inflammation, metabolic signaling of adipokines, as well as IGF-I alterations and insulin resistance, creates a pro-tumorigenic micro-environment that contributes to the complex relationship between these two conditions (Chait and den Hartigh 2020). Obesity-related alterations also involve changes in hormones, decreased anorexigenic leptin and increased orexigenic ghrelin, reduced resting metabolic rate (adaptive thermogenesis), heightened appetite sensations, and lower fat oxidation in weight-reduced states. Sex hormone-binding globulin (SHBG) regulates free estrogen bioavailability and, in turn, influences hormone-sensitive cancers (Friedenreich et al. 2021). Additionally, perturbations to other systems such as microenvironment and cellular (e.g., vascular, epithelial-mesenchymal transition, and endoplasmic reticulum (ER) stress) contribute to this nexus (Farahzadi et al. 2023). However, obesity also plays a protective role in certain cancers, such as premenopausal breast cancer and non-small cell lung cancer, whereby obesity is correlated with a reduced risk of these cancers (Avgerinos et al. 2019). Navigating this intricate connection, shedding light on the crucial link between obesity and cancer, will not only enhance our understanding of these two conditions but also open avenues for potential therapeutic strategies and the development of effective prevention strategies.

2.1 Exploring the Biological Mechanisms Linking Obesity and Cancer Development

There are intricate pathways through which obesity can lead to cancer, including chronic inflammation, insulin resistance, and hormone imbalances (Behrooz et al. 2024). One of the major contributors to the link between obesity and cancer development is adipose tissue. Adipose tissue plays a major role in promoting a tumor-friendly microenvironment with significant implications for inflammation, hormonal signaling, and cancer progression (Kim et al. 2024). In obesity, the adipose tissue undergoes substantial remodeling, leading to adipocyte hypertrophy (Spyrou et al. 2018). Excess adiposity consequently increases the secretion of adipokines, a variety of bioactive molecules secreted by adipocytes and immune cells within the adipose tissue. These adipokines include pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), which contribute to a state of chronic low-grade inflammation, a hallmark of obesity (Louie et al. 2013; Kim et al. 2024). This inflammatory state is further exacerbated by the infiltration of immune cells, particularly macrophages, into the adipose tissue (Guria et al. 2023). These macrophages form crown-like structures around dead or dying adipocytes, secreting additional pro-inflammatory cytokines and perpetuating the inflammatory cycle (Thomas and Apovian 2017).

Moreover, adipose tissue, a metabolic organ, is a crucial producer of adipokines (such as leptin, adiponectin, resistin, and visfatin) and cytokines. While these molecules are crucial for metabolic regulation, they also significantly contribute to tumor growth and metastasis, thereby worsening cancer progression (Bocian-Jastrzebska et al. 2023). In obese individuals, dysregulations in leptin secretion lead to leptin resistance, as its secretions are directly proportional to the amount of body fat (Obradovic et al. 2021). Resistance to leptin in obesity reduces its effects on appetite and energy expenditure and prolongs inflammation. High leptin levels can stimulate cancer cell proliferation, inhibit apoptosis, and induce angiogenesis, thus promoting tumor growth. These factors contribute to developing an adipose microenvironment that directly influences cancer progression. The adipocyte-rich environment can supply rapidly proliferating cancer cells with an energy source (Lengyel et al. 2018). Additionally, peritumoral adipocytes within a tumor microenvironment can become cancer-associated adipocytes (CAA) through a phenotypic switch, which would secrete pro-inflammatory cytokines and adipokines that promote cancer growth as well as metastasis (Wu et al. 2023). In the presence of cancer cells, the CAA, with the assistance of cytokines, generates fibroblast-like adipocyte-derived fibroblasts. These cells are involved in the tumorigenesis process by increasing their migration and invasiveness (Yao and He 2021). Another instance shows that leptin and hypoxic conditions in adipose tissue of obese individuals stimulate vascular endothelial growth factor (VEGF) production and matrix metalloproteinases (MMPs), one of the potent factors for the formation of new blood vessels (angiogenesis), thus promoting invasiveness of cancer cells via epithelial-to-mesenchymal transition in the tumor microenvironment (Herold and Kalucka 2020). Consequently, this provides a route for cancer cells to invade surrounding tissues and metastasize.

Adipose tissue is also the site of estrogen production via the enzyme aromatase (Pico et al. 2022). In obesity, increased aromatase activity leads to higher estrogen levels in peripheral adipose tissue. Elevated estrogen levels in women with high BMI are linked to a higher risk of ER+/PR+ postmenopausal breast cancer (Johansson et al. 2022). In endometrial cancer, estrogen drives tumorigenesis by promoting cell proliferation and inhibiting apoptosis via the production of IGF-I (Tian et al. 2019).

Furthermore, alterations in insulin-like growth factors (IGFs, such as IGF-I and IGF-II) are crucial in the obesity-cancer nexus associated with hyperinsulinemia and insulin resistance (Szablewski 2024). In obese patients, chronic low-grade inflammation in adipose tissue and pro-inflammatory adipokine secretion alterations interfere with insulin signaling, resulting in overexpression of IGF-1 receptors and insulin resistance, necessitating increased insulin secretion to maintain normal glucose levels (Zatterale et al. 2019). Insulin and insulin-like growth factor (IGF)-I have crucial importance in glucose metabolism and cell processes related to increased cancer risks, such as prostate, breast, liver, and colorectal cancers (Szablewski 2024). Elevated insulin levels activate target cells through insulin receptors or IGF mediation, triggering tumor-promoting mechanisms such as proliferation, angiogenesis, and anti-apoptosis (Hua et al. 2020). These processes involve the activation of the PI3K-AKT-mTOR pathway, which regulates cell growth and differentiation, and the Ras-Raf-MEK-MAPK pathway, which induces proliferation and inhibition of apoptosis via steroid hormones and integrins (Nepstad et al. 2019).

Another factor linking obesity and cancer is reactive oxygen species (ROS), produced due to hyperglycemia and elevated free fatty acid levels that are associated with obesity (Manna and Jain 2015). ROS drives tumor promotion, causing mitochondrial and DNA damage. It does so by oxidizing proteins, which subsequently increase the number of unfolded proteins in the ER, resulting in ER stress in adipocytes and inducing inflammatory response (Zhao et al. 2023).

2.2 Obesity and Chronic Low-Grade Inflammation: An Intricate Connection

Obesity is not merely a disorder of energy balance but also a state of chronic low-grade inflammation (Khanna et al. 2022). The complex mechanisms through which obesity promotes a persistent inflammatory state involve adipocyte hypertrophy and immune cell infiltration. Hypertrophic adipocytes and tissue-resident immune cells undergo phenotypic changes in an obese state, ceasing the secretion of anti-inflammatory and protective cytokines while secreting pro-inflammatory cytokines (Kawai et al. 2021). Additionally, adipokine imbalance, adipose tissue hypoxia, and insulin resistance contribute to this state (Zatterale et al. 2019). These factors create a favorable environment for cancer development and progression, as the inflammatory components present in obesity are also established components of the tumor microenvironment.

One of the key features of obesity-induced inflammation is the infiltration of immune cells into the fatty tissue. In a lean state, the adipose tissue primarily

contains anti-inflammatory immune cells such as M2 macrophages and regulatory T cells (Kawai et al. 2021). However, with obesity, there is a shift toward pro-inflammatory immune cells, including M1 macrophages, neutrophils, and Th1 cells (Savulescu-Fiedler et al. 2024). These immune cells secrete pro-inflammatory cytokines such as TNF- α , IL-6, and interleukin-1 beta (IL-1 β) that recruit more immune cells, perpetuating the inflammatory state (Hirano 2021).

The role of adipokines in obesity-induced inflammation cannot be overstated. Leptin, an adipokine whose levels increase with adiposity, has pro-inflammatory properties and can stimulate the secretion of pro-inflammatory cytokines such as IL-1, IL-6, IL-12, TNF- α , and cyclooxygenase 2 (COX2) (La Cava 2017). Leptin also triggers T cell proliferation and TH1 phenotype while suppressing regulatory T (Treg) cells via the mTOR pathway (Perez-Perez et al. 2020). On the other hand, adiponectin, which has anti-inflammatory effects and insulin-sensitizing properties, is downregulated in obesity (Luo and Liu 2022). Downregulation of adiponectin is correlated with increased inflammatory cytokines such as TNF- α and IL-6, contributing to hypoadiponectinemia and increased tumorigenesis (Huang et al. 2022). Elevated adiponectin is associated with the inhibition of some cancer cell proliferation, such as breast and colorectal cancer, via inhibition of signaling pathways AMPK, mTOR, MAPK, and PI3K/Akt (Obeid and Hebbard 2012). Thus, this imbalance in adipokine secretion further exacerbates the inflammatory state.

Insulin resistance associated with obesity can also drive inflammation. Those with elevated homeostatic model assessment for insulin resistance have more pronounced cancer mortality rates, with systemic inflammation being a major cause of it (Avgerinos et al. 2019).

Additionally, there are alterations in intestinal microbiota in individuals with obesity or high-fat diet, as bacteria with higher levels of lactate, acetate, and butyrate feed into fatty acid synthesis. Obesity leads to gut barrier dysfunction that favors carcinogenesis and tumor progression (Breton et al. 2022). Specific gut microbiota provoke the activation of pro-inflammatory components (COX2, IL-1, and IL-6) (Potrykus et al. 2021). Moreover, bile acid metabolism involving deoxycholic acid (DCA) increased. This results in p53 suppression via degradation in the proteasome system, as well as DNA damage via ROS production (Rezen et al. 2022).

2.3 Genetic and Epigenetic Factors: Shaping the Obesity-Cancer Relationship

A complex interplay of genetic, epigenetic, and environmental factors influences the relationship between obesity and cancer. Genetically, specific inherited mutations can predispose individuals to both obesity and cancer. Specific genetic variants are associated with an increased propensity for weight gain and obesity. These same genetic predispositions can also influence cancer susceptibility, either directly or

indirectly, through their impact on obesity. Epigenetically, modifications such as DNA methylation and histone changes, often influenced by environmental factors, can alter gene expression in ways that promote cancer development. Environmental factors, including diet, physical activity, and exposure to toxins, can further modulate these genetic and epigenetic processes (Pati et al. 2023). Together, these factors create a multifaceted network that can drive the progression from obesity to cancer.

The connection between obesity and cancer is influenced by various mechanisms that lead to genetic instability, which is a crucial element in the development of tumors. Obesity causes elevated levels of reactive oxygen species (ROS) due to metabolic imbalances and inflammation, leading to oxidative stress and DNA damage. This includes oxidative DNA lesions such as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG), which can result in mutations (Kompella and Vasquez 2019). Zhang et al. found that the altered genes common to both colon cancer and obese patients suggest involvement in inflammatory pathways (Zhang et al. 2023). The oxidative degradation of lipids in obese individuals produces harmful by-products, such as malondialdehyde and 4-hydroxynonenal, that form adducts with DNA, leading to further mutations (Ayala et al. 2014). Additionally, obesity can impair DNA repair mechanisms, such as the base excision repair (BER) pathway, resulting in the accumulation of unrepaired DNA lesions (Wlodarczyk and Nowicka 2019). The obesogenic state can also cause epigenetic alterations, including changes in DNA methylation patterns, affecting the expression of genes related to tumor suppression and metabolic regulation (Long et al. 2024). For instance, inflammation associated with obesity results in hypomethylation of oncogene promoter regions, such as the MYC proto-oncogene in colon cells, leading to overexpression. These obesity-related modulations in DNA damage and repair pathways contribute to genetic instability, a hallmark of cancer, thereby increasing cancer risk.

The dynamic and reversible nature of epigenetic modifications serves as an advantage, as these can be reversed—meaning that therapeutic strategies can be aimed at reversing the development of obesity-associated cancer.

3 Shared Risk Factors and Pathways Linking Obesity and Cancer

Obesity, characterized by excess body fat, is a significant health concern linked to various conditions such as diabetes mellitus and cardiovascular diseases. Additionally, it is a major risk factor for the development and mortality associated with several types of cancer. The mechanisms linking obesity to cancer risk and recurrence are multifaceted, involving various biological processes such as altered fatty acid metabolism, extracellular matrix remodeling, adipokine secretion, anabolic and sex hormone activity, immune dysregulation, and chronic inflammation.

3.1 Common Risk Factors Contributing to Obesity and Cancer

3.1.1 Sedentary Lifestyle and Physical Inactivity

Physical activity, defined as any bodily movement that requires energy expenditure, has been extensively studied in epidemiology. This research considers various types of activity (occupational, recreational, household, and transport), as well as the volume (frequency, duration, and intensity) and timing of these activities.

The biological mechanism linking physical inactivity, a sedentary lifestyle, obesity, and cancer risk is as follows: a sedentary lifestyle causes significant molecular-level changes in the body. Lack of physical activity impairs metabolic functions, reducing insulin sensitivity and elevated levels of insulin-like growth factor-1 and fasting glucose. This inactivity often results in weight gain, particularly in adipose tissue, which increases chronic low-grade inflammation and disrupts levels of bioavailable sex hormones.

Thus, the benefit of physical activity cannot be overstated. As physical activity increases, adipose tissue decreases, and adipokines and inflammatory cytokines secretion also decreases. This results in a notable reduction in molecules like leptin, IL-6, IL-1 β , TNF- α , C-reactive protein, and serum amyloid while adiponectin levels rise. Increased physical activity also improves insulin sensitivity and induces weight loss. Additionally, the elevation of sex hormone-binding globulin occurs due to reduced adipose tissue, leading to lower estrogen and androgen levels. Collectively, these molecular changes contribute to a lowered risk of cancer (Pati et al. 2023).

Recent updates published by the Physical Activity Guidelines for Americans (PAGA) Committee 2018, along with the World Cancer Research Fund (WCRF) and American Institute of Cancer Research (AICR), stated that there is strong evidence that physical activity reduces the risk of bladder, breast, colon, endometrial, esophageal, adenocarcinoma, and gastric cancers. There exists moderate evidence of an association between higher levels of physical activity and lower occurrence of renal, ovarian, pancreatic, and lung cancers. Obesity leads to an imbalance between antioxidants and oxidative stress, decreases insulin sensitivity, and leads to insulin resistance. The hypothesis regarding physical activities is based on the balance between ROS and antioxidants. ROS can cause chromosomal abnormalities, DNA damage, and mutations in tumor-suppressing genes. While acute physical activities induce oxidative stress and a pro-oxidant environment, regular physical activity leads to adaptation to stress and the generation of antioxidant defenses. The dose-response relationship between physical activity and reduced cancer risk is clear. However, quantifying and combining physical activity in studies is challenging, leading to inconsistent conclusions about the exact amount needed to achieve the desired effect. This inconsistency is a significant drawback (Friedenreich et al. 2021).

3.1.2 Unhealthy Diet and High Calorie Intake

Dietary patterns refer to the composition and consumption frequency of various foods and beverages within a diet. These patterns are typically categorized using three primary methods. First, an a priori index is created based on established dietary

guidelines or recommendations, often determined through scientific consensus or evidence-based approaches. Second, data-driven methods are employed to identify associations between different diet components, revealing food and beverage intake patterns that explain variations across dietary profiles independent of study outcomes. Lastly, individuals' nutritional preferences are examined by considering the inclusion and exclusion of specific foods and drinks in their diets, highlighting their unique intake patterns (Kerr et al. 2017).

Dietary factors play an essential role in a healthy person. High-fat diet (e.g., red and processed meat) associated with obesity is strongly associated with stomach and colorectal cancers (Aykan 2015). In contrast, a diet with high fiber decreases the risk of developing colorectal cancer (Kunzmann et al. 2015). A diet rich in fruits, vegetables, fiber, whole grains, animal products, and refined carbohydrates may reduce the risk of postmenopausal breast cancer and colorectal cancer. It is also linked to a decreased risk of estrogen receptor–negative breast cancer. Intake of alcohol consistently is associated with a higher rate of breast cancer and colorectal cancer risk (Fig. 2) (Kerr et al. 2017). Regular exercises with diet and routine lifestyle are effective interventions in these cancer treatments.

The impact of dietary factors such as whole grains, fiber, high-protein diets, high-fat diets, and caloric restriction on cancer risk is closely linked to intestinal microbiota composition. For instance, high-protein diets can reduce beneficial microbiota and metabolites essential for immune protection. In contrast, high-fat diets may drive intestinal progenitor cells toward a more stem cell-like state, increasing the likelihood of tumor formation. Interestingly, these effects are attributed to specific dietary fatty acids rather than solely to obesity. Caloric restriction, on the other hand, tends to

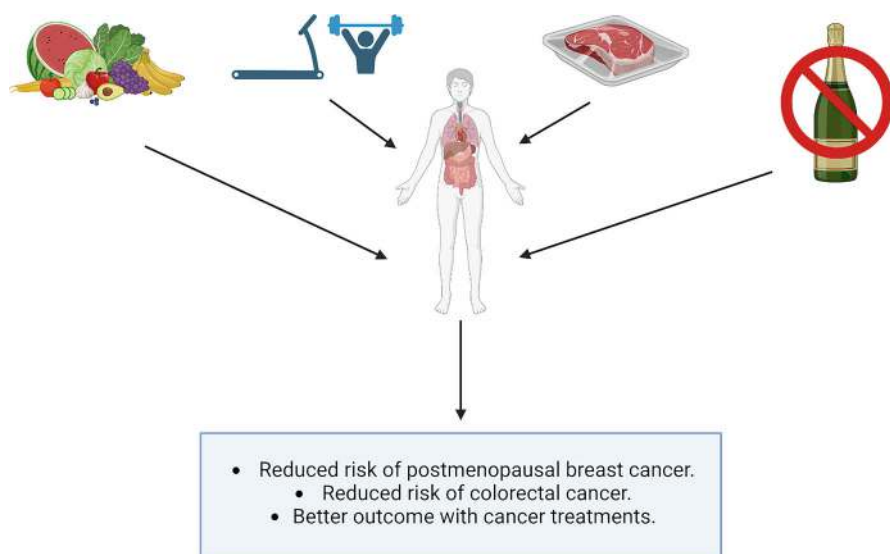


Fig. 2 Lifestyle modifications and cancer risk reduction. This figure highlights key lifestyle interventions that can help reduce cancer risk and improve outcomes

decrease the risk of tumor initiation. The ongoing debate on cancer development revolves around the relative contributions of obesity versus high-fat diets. Studies using mouse models have indicated that high-fat diets can promote cancer growth independently of obesity, highlighting a complex interplay involving dietary fat quality and quantity, obesity, inflammation, genetic factors, and oncogenesis. Dietary shifts in populations, such as rural Africans and African Americans, have led to significant changes in microbiota, metabolites, and cancer risk. The intestinal microbiome's role in promoting estrogen receptor–positive breast cancer is also noteworthy. Furthermore, plasma carotenoids have been inversely associated with high-grade prostate cancer incidence, with this association possibly influenced by specific DNA repair gene variations. Emerging research suggests that despite having the same caloric content, fructose and glucose are metabolized differently, with fructose being a major contributor to obesity and metabolic syndrome (Kerr et al. 2017).

In conclusion, structured exercise, in combination with dietary support and behavioral therapy, is an effective intervention to prevent cancer.

3.1.3 Metabolic Syndrome and Related Disorders

Metabolic syndrome, characterized by the presence of three out of five risk factors (hyperglycemia, hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol, and enlarged waist circumference), represents a multifactorial pathological condition. Individuals with metabolic syndrome often exhibit a prothrombotic and pro-inflammatory state. In cases of obesity, particularly visceral obesity resulting from a sedentary lifestyle, there is an excessive release of free fatty acids into circulation due to altered adipokine levels, such as increased resistin and decreased adiponectin production. This adipokine imbalance contributes to lipolysis, insulin resistance, and increased angiotensinogen production. In addition, it also leads to the release of inflammatory cytokines, affecting liver and kidney metabolism and inducing endothelial dysfunction (Venkatesh et al. 2022). Hsing et al. conducted a study linking obesity, metabolic syndrome, and prostate cancer, finding that obesity is associated with an increased risk of high-grade prostate cancer, a decreased risk of low-grade prostate cancer, and a consistently higher risk of mortality related to prostate cancer. The authors suggested that these varying effects stem from diversity in tumor origins and interactions involving androgen metabolism, insulin resistance, diabetes, inflammation, and genetic factors.

There are precisely three primary biological mechanisms implicated in the increased cancer risk among obese individuals.

The first mechanism revolves around adipose tissue acting as an “organ,” releasing various chemical mediators and enzymes. This includes the conversion of androgens into estradiol by aromatase in peripheral adipose tissue. Elevated levels of estradiol, a potent estrogen, are associated with higher risks of breast, endometrial, ovarian, and other cancers.

The second mechanism arises from hyperinsulinemia, frequently observed in individuals with a higher BMI. This condition leads to elevated levels of insulin and IGF-1, which promote cell growth and proliferation. Insulin resistance,

commonly found in obese individuals, further increases this risk, contributing to the development of colon, renal, prostate, and endometrial cancers. Xue and Michel conducted a meta-analysis to explore the relationship between type 2 diabetes and breast cancer risk. Their findings supported the association, particularly among postmenopausal women compared to premenopausal women. Similarly, Giovannucci reviewed epidemiological studies to examine the link between metabolic syndrome and colon cancer risk. This chapter indicated that elevated BMI, physical inactivity, visceral adiposity, and type 2 diabetes are consistent risk factors for colon cancer and adenomas (Zhou et al. 2007).

The third mechanism involves the pro-inflammatory environment resulting from altered adipokine secretion by adipose tissue. Elevated levels of inflammatory molecules such as leptin and decreased levels of anti-inflammatory molecules like adiponectin contribute to chronic inflammation. This inflammation, oxidative stress, and changes in the immune system can promote the initiation and progression of various cancers, including those of the biliary tract and liver. Additionally, obesity can affect tumor immunity and alter tissue characteristics, further increasing cancer susceptibility and metastatic potential.

The accumulation of free fatty acids in the liver leads to decreased cholesterol content in high-density lipoprotein (HDL) and hypertriglyceridemia, which also contributes to hyperglycemia by promoting gluconeogenesis. Lipolysis and insulin resistance impair muscle glucose utilization, creating hyperglycemia and promoting hyperinsulinemia. Hyperinsulinemia, resulting from consistently elevated blood glucose levels, can potentially promote tumorigenesis. In postmenopausal women, hyperinsulinemia may alter sex hormone bioavailability by reducing SHBG and increasing estrogen and androgen levels, which in turn contributes to tumorigenesis (Patel et al. 2019). The free fatty acid (FFA) theory suggests that elevated levels of intracellular fatty acid metabolites, such as diacylglycerol, fatty acyl-CoAs, or ceramides, trigger a cascade of serine-threonine kinase activation. This cascade results in the phosphorylation of serine-threonine sites on insulin receptor substrates. The phosphorylated forms of these proteins cannot interact with or activate phosphoinositide-3-kinase, leading to reduced activation of glucose transport and other downstream insulin signaling events. Consequently, there is an accumulation of triacylglycerols and intracellular fatty acid metabolites in insulin-responsive tissues, contributing to acquired insulin signaling defects and insulin resistance. Elevated plasma FFA concentrations are commonly associated with various insulin-resistant conditions, such as obesity and type 2 diabetes. Research by Bergman et al. demonstrated that nocturnal lipolysis, the breakdown of fats during nighttime, raises free fatty acids (FFAs) levels in the liver and muscles, leading to insulin resistance. They proposed that this nocturnal increase in lipolysis not only contributes to insulin resistance but also plays a role in hyperinsulinemia (Zhou et al. 2007).

Sex hormone-binding globulin (SHBG) plays a crucial role in regulating the activity of free estrogen, which is highly active and linked to increased risks of hormone-sensitive cancers, such as breast cancer. Studies, including both observational and randomized controlled trials (RCTs), focusing on women at risk of breast

cancer, have shown that higher levels of physical activity led to notable reductions in estradiol, free estradiol, and estrone while simultaneously increasing SHBG levels, regardless of menopausal status. However, the evidence for these effects is stronger in postmenopausal women than in premenopausal women.

Androgens, predominantly produced in men and, to a lesser extent, in women, have also been associated with tumorigenesis. In men, androgens and their receptors play crucial roles in regulating cell growth and death in the prostate, with implications for the development of prostate cancer. However, the relationship between androgen levels and prostate cancer risk in epidemiological studies has yielded inconsistent results.

In men, a noticeable negative correlation exists between adiposity and free, bioavailable, and total testosterone levels. However, the relationship between physical activity and testosterone levels remains inconclusive. Conversely, in women, obesity is linked to elevated androgen levels, while physical activity is associated with modest but statistically significant reductions in free testosterone and other androgens (Friedenreich et al. 2021).

Furthermore, obese adipocytes can contribute to hypertension through various mechanisms. These include increased kidney reabsorption of sodium and urate, elevated angiotensinogen release with subsequent upregulation of the renin-angiotensin pathway, and the production of inflammatory cytokines. These cytokines cause endothelial dysfunction and oxidative stress and impair nitric oxide-dependent vasodilation.

3.2 Role of Obesity-Related Hormones in Carcinogenesis

3.2.1 Adiponectin

Adiponectin (APN) is a hormone secreted by adipose tissue that plays a crucial role in maintaining glucose and lipid balance. It also exhibits insulin-sensitizing, anti-apoptotic, and immune regulatory effects. However, its levels are drastically decreased in obese individuals, potentially contributing to tumor growth. The role of APN in endocrine cancers is linked to the activation of AMPK, a regulator of energy balance that inhibits anabolic and proliferative pathways while increasing ATP production. This activation negatively influences cancer development by inducing the expression of molecules involved in cell cycle arrest and apoptosis, such as p53 and p21. APN's effects extend to various endocrine cancers, including breast, prostate, and endometrial cancers (Tumminia et al. 2019).

In breast cancer, adiponectin (APN) has demonstrated antiproliferative and proapoptotic effects by suppressing cell growth and proliferation and inhibiting migration and invasion capabilities. Adipokines, like adiponectin, exert diverse functions locally and systemically, and their dysregulation in obesity creates a pro-tumorigenic environment. Adiponectin protects against colorectal tumorigenesis through its insulin-sensitizing properties and modulation of signaling pathways involved in cell growth and proliferation. Understanding these complex interactions between adipose tissue, adipokines, and cancer risk provides valuable insights for

potential therapeutic interventions and preventive strategies. Potential therapeutic applications of APN involve developing APN receptor agonists and increasing endogenous APN concentrations. These approaches have shown promising results in suppressing the development of breast cancer xenografts and modulating signaling pathways.

Several studies over the past two decades suggest that APN exerts its antineoplastic effects on endocrine cancers via two mechanisms. First, it affects endocrine tumor growth by acting directly on cancer cells through receptor-mediated pathways. Second, it indirectly influences cancer biology by modulating insulin sensitivity, inflammation, and tumor angiogenesis. Brakenhielm et al. characterized the potent inhibition of angiogenesis by APN, highlighting specific signaling pathways like the mitogen-activated protein kinase (MAPK) and cAMP-PKA pathways. However, there is strong molecular evidence suggesting that APN might also have a significant pro-angiogenic effect, which could potentially promote cancer development, especially in murine mammary tumor models (Tumminia et al. 2019).

3.2.2 Resistin

Resistin, extensively studied in murine models with 60% homology to its human counterpart, plays a diverse role in cancer signaling. It activates various pathways, notably MAPK and PI3K/AKT, promoting cell survival and proliferation. Also, resistin activates NF κ B, increasing IL-6 secretion, thereby inducing epithelial-mesenchymal transition (EMT) and metastasis.

In ovarian cancer cells (HO-8910), resistin influences VEGF expression via the PI3K/AKT-Sp1 pathway. In human gastric cancer cells, resistin induces stromal cell-derived factor-1 (SDF-1) expression, leading to angiogenesis. Osteosarcoma cells exhibit VEGF-mediated angiogenesis through resistin-induced activation of ERK, JNK, and p38 pathways. In chondrosarcoma cells, resistin treatment enhances cellular invasion and MMP-2 secretion via the AMPK and p38 pathways (Garcia-Miranda et al. 2022). Previous studies have shown that serum resistin levels are significantly elevated in patients with colorectal cancer compared to healthy controls. Additionally, overexpression of the resistin receptor, adenylyl cyclase-associated protein 1 (CAP1), has been observed in epithelial ovarian cancer and breast cancer, both of which are recognized as obesity-related malignancies (Kawashima et al. 2017).

Breast cancer cells treated with resistin show increased phosphorylation of signaling molecules such as c-Src, PP2A, PKC α , ezrin, radixin, and moesin. This phosphorylation cascade and elevated vimentin expression promote cell invasion and metastasis. Additionally, resistin treatment in chondrosarcoma cells suppresses miR-519d.

The complex signaling network activated by resistin in various cancer contexts highlights its diverse impact on cell behavior. Understanding these molecular mechanisms is crucial for elucidating resistin's role in cancer progression and exploring its potential as a therapeutic target.

3.2.3 Leptin

In obesity, resistance to key metabolic hormones like leptin and ghrelin is crucial for the neuroendocrine regulation of energy balance.

Leptin, a 16 kDa cytokine mainly produced by adipose tissue, is released into the bloodstream in proportion to body fat mass. In individuals with normal body weight, circulating leptin crosses the blood-brain barrier (BBB) and binds to the “long” form of the leptin receptor (LepRb). This binding triggers the phosphorylation of Janus kinase 2 (JAK2) and multiple tyrosine residues in the intracellular domain of LepRb (Cui et al. 2017).

In obesity, increased circulating leptin levels are accompanied by reduced transport of leptin across the blood-brain barrier (BBB) and the activation of inhibitory feedback systems, ultimately leading to diminished LepRb signaling. Elevated levels of free fatty acids and chronic overnutrition associated with obesity result in lipotoxicity and endoplasmic reticulum (ER) stress. These conditions trigger inflammatory responses that may contribute to cancer development by promoting angiogenesis and immune modulation, creating a pro-inflammatory microenvironment.

Adipokines play a significant role in modulating autophagy within tumor cells during obesity. Elevated levels of leptin, resistin, apelin, and visfatin in obese individuals with cancer have been linked to poorer prognosis and have been shown to induce autophagy in tumor cells. This adipokine-induced autophagy is associated with pro-tumoral effects, including evasion of apoptosis, increased proliferation, and enhanced cell migration (Garcia-Miranda et al. 2022). Blanquer-Rosselló et al. observed that leptin enhances the co-localization of lysosomes with mitochondria, indicating an increase in mitophagy to maintain the mitochondrial fraction in MCF-7 breast cancer cells (Kawashima et al. 2017).

In conclusion, the intertwined risk factors and pathways linking obesity and cancer are intricate and multifaceted. Genetic predisposition, lifestyle choices, and hormonal influences contribute to the complex relationship between these two health challenges. Understanding these interconnected factors is crucial for developing effective prevention and intervention strategies to reduce the risk of obesity-related cancers. A comprehensive approach that addresses lifestyle modifications, hormonal imbalances, dietary intake, and the major cancer risks associated with obesity is essential for promoting overall health and well-being.

4 Mitigating the Impact: Strategies for Prevention and Intervention

4.1 Public Health Strategies to Address Obesity and Cancer Risk

Obesity is a rapidly growing global public health concern. The prevalence of obesity has significantly increased in numerous nations across the globe over the past 30 years, potentially as a result of urbanization, sedentary behavior, and the rising intake of high-calorie processed food. The significant rise in juvenile obesity indicates a substantial challenge in preventing chronic diseases in future global

public healthcare systems. Preventing obesity is crucial in managing obesity-related noncommunicable diseases (OR-NCDs), such as cancer. This section examines the public health factors related to obesity. It emphasizes the involvement of a team of professionals from different disciplines in creating public health strategies to address and prevent this disease. Both diabetes and obesity have been linked to an elevated cancer risk. Given the escalating prevalence of obesity and diabetes on a global scale, this development in cancer rates is a cause for concern (Giovannucci et al. 2010; Garg et al. 2014; Bendor et al. 2020). Potential mechanisms underlying the relationship, such as hyperinsulinemia, chronic inflammation, antihyperglycemic medications, and shared risk factors, have all been identified. Malignancies that arise most frequently as a result of obesity and diabetes are colorectal, endometrial, and postmenopausal breast malignancies (Lega and Lipscombe 2019). The International Agency for Research on Cancer has recently classified 13 malignancies as obesity-associated cancers (OACs) due to the presence of substantial evidence (Polednak 2003; Koroukian et al. 2019; Bruno and Berger 2020).

4.1.1 Importance of Weight Management and Healthy Lifestyle Promotion

Numerous lifestyle factors are significant in reducing the morbidity and mortality associated with medical and psychiatric disorders as well as their associated effects. These include consuming a balanced diet, getting more exercise, giving up smoking, abstaining from alcohol, and abusing illegal drugs. Other lifestyle elements for a healthy lifestyle include less stress and safe atmosphere, enough sleep, fun and anxiety-relieving activities, supportive social networks, and constructive mental pursuits. Without fully comprehending its scientific foundation, doctors have advocated for adopting healthy lifestyle practices like food and exercise to treat medical and psychological illnesses since antiquity and into the early twentieth century (Zaman et al. 2019).

Losing weight reduces cardiometabolic risk factors in obese or overweight individuals. The best noninvasive weight loss strategies are medication and intensive lifestyle modification (Wadden et al. 2021). Whatever the differences in weight, increased physical activity reduces the risk of cardiovascular disease. Exercise plays a significant role in improving metabolic control in individuals with type 2 diabetes and is included in lifestyle interventions for the disease's prevention (Bezner 2015; Hamasaki 2016). These goals may include maintaining lean body mass, preventing weight gain, preventing cardio-metabolic comorbidities, improving quality of life, or fostering social connections (Luan et al. 2019; Oppert et al. 2021).

Healthy lifestyle practices can influence several cardiovascular disease risk variables, including smoking, diabetes, and obesity, as well as cardiorespiratory fitness, which is less typically assessed. Reduced physical activity is linked to deteriorating cardiovascular disease risk factor profiles, such as increased obesity and lower cardiorespiratory fitness levels (Powell-Wiley et al. 2021; Kaminsky et al. 2022).

Also, a previous study found that women who experienced weight loss of 5% or more had a significantly lower risk of endometrial cancer ($HR = 0.71$,

95% CI: 0.54–0.95) compared to women whose weight remained steady (change within $\pm 5\%$). The correlation was most pronounced among obese women who deliberately lost weight (HR = 0.44, 95% CI: 0.25–0.78) (Luo et al. 2017).

4.1.2 Implementing Policies for Healthier Food Environments

One way to prevent obesity is by implementing policies that create healthier food environments. According to the World Health Organization, monitoring the success of these policies can help foster healthier diets and reduce the prevalence of obesity and noncommunicable diseases (NCDs) (Swinburn et al. 2013). A comprehensive and integrated approach is necessary to tackle the problem of noncommunicable diseases (NCDs) caused by diet and to enhance the overall nutrition of the population. An extensive intervention package should be implemented, including a diverse array of policy activities at the population level focusing on structural changes. Public health nutrition interventions should function efficiently within the broader framework of the food system and the national, European, and global economies. These interventions should also be in line with policies that govern food systems. Both national and local governments must create and enforce efficient policies to enhance food environments and diminish socioeconomic disparities (Wang et al. 2022). These policies should also ensure fair and equal access to nutritious food options for all individuals. Finland had the largest percentage (32%, $n = 7/22$) of policies that effectively influenced food environments. Slovenia and Poland exhibited the highest percentage of policies regarded as having a very low level of implementation, with 42% ($n = 10/24$) and 36% ($n = 9/25$), respectively. Healthier food environment implementing policies must include the composition, labeling, promotion, prices, provision, retail, trade, and investment of food, as well as a few domains related to infrastructure support: leadership, governance, finance and resources, monitoring and intelligence, platforms for engagement, and health in all policies have to be investigated to improve the policies and get healthier food environment (Pineda et al. 2022).

4.1.3 Encouraging Physical Activity and Reducing Sedentary Behavior

Physical activity is a key factor in preventing obesity. Therefore, governments and health organizations should launch comprehensive public awareness campaigns to educate individuals about the importance of maintaining an active lifestyle and the negative impacts of sedentary behaviors. Schools and universities must implement physical education programs to instill healthy behaviors in students early on. Promotion of workplace wellness initiatives and provision of break times as opportunities for physical activity can have a substantial influence on the overall health of employees. Additionally, this may enhance the efficiency of the institution. An all-community effort to promote physical activity has the potential to inspire each individual to join the collective effort. For such group activities to be conducted, public areas such as bike lanes, parks, and pedestrian paths must be accessible and secure. Advocate for increased utilization of public transportation, cycling, and walking to enhance daily physical activity while decreasing dependence on sedentary transportation modalities. A positive application of technology would be to

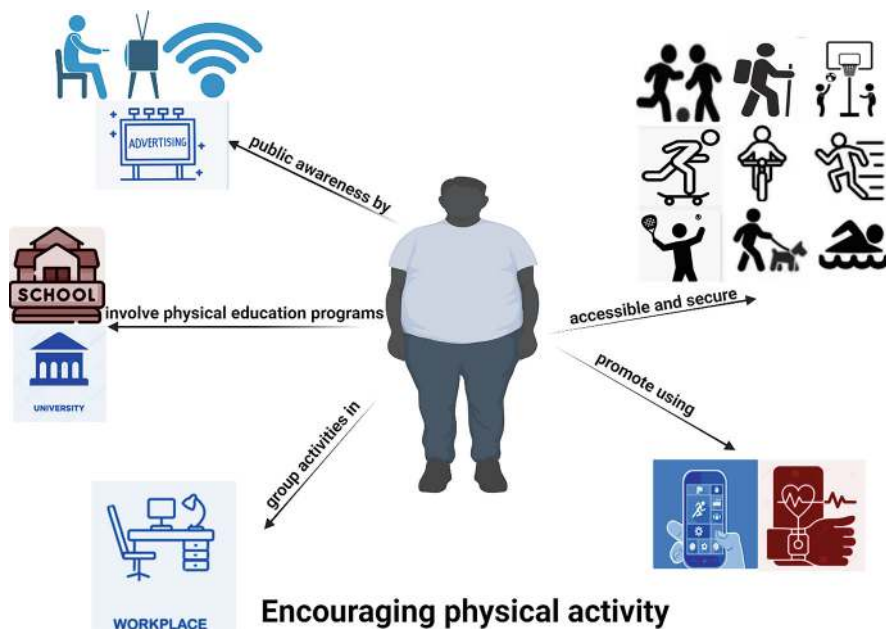


Fig. 3 Promotion of physical activity and reduction of sedentary behavior for obesity prevention. This figure highlights various strategies to encourage an active lifestyle, including public awareness campaigns, implementation of physical education in schools, workplace wellness programs, and development of safe public spaces like parks and bike lanes and walking. It illustrates the role of technology, such as fitness apps and wearable devices, in enhancing daily physical activity

promote the use of fitness apps and wearable devices that monitor physical activity, thereby inspiring people to achieve their exercise objectives (Fig. 3). Due to the proliferation of digital platforms, individuals' varied schedules and preferences can be accommodated by providing online fitness classes that are both accessible and affordable (Buckingham et al. 2019).

4.2 Personalized Approaches to Obesity-Cancer Prevention

Recent research has found that obesity can have genetic origins, and obesity genetics can be categorized into syndromic and non-syndromic types. Syndromic obesity includes conditions such as Prader-Willi, fragile X, Bardet-Biedl, Cohen, and Albright hereditary osteodystrophy (AHO) syndromes. These syndromes are characterized by developmental delay and the occurrence of obesity at an early age. Non-syndromic obesity may stem from monogenic, polygenic, or chromosomal causes. Monogenic obesity results from specific variations in individual genes, whereas polygenic obesity involves multiple genes, including those from gene families. Recent advancements in genetic testing have facilitated the discovery of genes associated with obesity. Leptin (LEP), leptin receptor (LEPR),

proopiomelanocortin (POMC), prohormone convertase 1 (PCSK1), melanocortin 4 receptor (MC4R), single-minded homolog 1 (SIM1), brain-derived neurotrophic factor (BDNF), and neurotrophic tyrosine kinase receptor type 2 gene (*NTRK2*) have been identified as genes that can cause obesity. Next-generation sequencing (NGS) is currently being utilized and is emerging as a valuable method for identifying potential genes associated with obesity in clinical situations (Mahmoud et al. 2022). So, personalized medicine according to patient gene mutation and target can help prevent obesity-cancer.

4.2.1 Identifying High-Risk Populations and Tailored Interventions

There are populations with a higher predisposition for obesity. For instance, school students may face a high risk of obesity, but this can be mitigated by implementing changes in school lunch programs, such as offering healthier meals, reducing sugary drinks, and promoting more physical activity. Additionally, an individual's health is influenced by culture and social interactions, so maintaining an active social life can help motivate healthy behaviors and lower obesity risk.

The nature of work and urban design often reduces physical activity by incorporating conveniences like elevators, escalators, and other labor-saving devices. Therefore, it is beneficial for employers to periodically organize physical activities or team events to encourage movement and well-being among employees. For teenagers, sedentary leisure activities such as video games, watching television, and Internet use for socializing or news can be managed and limited by parents. Additionally, since a stressful work environment can contribute to obesity, implementing stress management techniques and arranging regular check-ins with a counselor or mental health professional can be valuable for employee health (Meldrum et al. 2017).

4.2.2 Potential Role of Pharmacological Interventions

A large portion of affected patients do not achieve or maintain weight loss through mainstream approaches alone. It is widely accepted that individuals who do not see weight improvement after 6 months of lifestyle interventions and have a BMI over 30 kg/m² or a BMI over 27 kg/m² with obesity-related health conditions may qualify for anti-obesity medication treatment (May et al. 2020). The field of obesity pharmacotherapy is currently prioritizing the development of new therapeutic drugs that have enhanced safety and effectiveness characteristics. These trends also involve evaluating the efficacy of medications previously utilized for other ailments regarding their weight-reducing capabilities. Presently, various medications employ several methods to achieve their effects. These methods directly reduce calorie absorption, as seen with orlistat, which acts as a pancreatic lipase inhibitor. By blocking this enzyme, approximately 30% of daily dietary fat intake is not absorbed. For a person consuming 2000 cal daily, with 30% of fat, this results in a calorie deficit of around 200 cal per day.

Medications like bupropion with naltrexone and phentermine with topiramate act on the central nervous system. These medications, currently being researched for

treating eating disorders and obesity, include topiramate, bupropion, and naltrexone, all of which have shown promising clinical outcomes. Bupropion, an antidepressant, works by inhibiting the reuptake of dopamine and noradrenaline. At the same time, phentermine is considered an indirect sympathomimetic as it increases levels of norepinephrine and dopamine and indirectly impacts serotonin. Some studies suggest that phentermine may inhibit the activity of neuropeptide Y, a key signaling pathway that triggers hunger.

On the other hand, medications such as glucose-sodium cotransporter Inhibitors (GSCI) and exenatide inhibitors have recently been studied extensively. GSCI directly reduces body weight by eliminating glucose (calories) in the kidneys but only works when glucose is present. They can eliminate approximately 60–100 g of glucose daily through urine. The current focus in obesity pharmacotherapy is on developing new therapeutic drugs that are safer and more effective. This also involves assessing the weight-lowering efficacy of medications already used for other conditions (Kosmalski et al. 2023).

4.2.3 Bariatric Surgery and Its Impact on Cancer Risk

At present, bariatric surgery is a successful therapeutic approach for those who have morbid obesity or obesity with related co-morbidities and are unable to lose weight through a medically supervised diet and exercise program. Bariatric surgery methods facilitate weight loss and enhance the resolution of comorbidities by restricting food intake and reducing nutrient absorption. Traditionally, bariatric surgery has been categorized as either restrictive or hybrid procedures, which combine both methods. Restrictive techniques curtail food intake by limiting the size of the stomach, while hybrid approaches curtail nutrient absorption by bypassing sections of the intestine (Piché et al. 2015).

The contentious correlation between bariatric surgery and the heightened risk of colorectal cancer (CRC) is acknowledged and deliberated. Although bariatric surgery should not be regularly suggested as a cancer prevention method, it does have the potential to reduce the probability of developing specific types of malignancies as an additional positive outcome (Bruno and Berger 2020).

The meta-analysis found that bariatric surgery was linked to a lower occurrence of cancer overall (RR 0.62, 95% CI 0.46–0.84, $p < 0.002$), cancer-related to obesity (RR 0.59, 95% CI 0.39–0.90, $p = 0.01$), and death associated with cancer (RR 0.51, 95% CI 0.42–0.62, $p < 0.00001$). Researchers have found that bariatric surgery reduces the risk of developing specific cancers. These include hepatocellular carcinoma (RR 0.35, 95% CI 0.22–0.55, $p < 0.00001$), colorectal cancer (RR 0.63, CI 0.50–0.81, $p = 0.0002$), pancreatic cancer (RR 0.52, 95% CI 0.29–0.93, $p = 0.03$), and ovarian cancer (RR 0.45, 95% CI 0.31–0.64, $p < 0.0001$), etc. Bariatric surgery leads to sustained weight loss in individuals with severe obesity and enhances the metabolic syndrome. Bariatric surgery has the potential to reduce the occurrence and death rates of certain types of cancer, including seven malignancies that are associated with obesity (Wilson et al. 2023).

5 Conclusion and Future Directions

Obesity has emerged as a significant risk factor for various cancers, driven by a complex interplay of metabolic, hormonal, and inflammatory mechanisms. Adipose tissue dysfunction in obese individuals leads to altered adipokine production, chronic low-grade inflammation, insulin resistance, and hormonal imbalances, which together create a pro-tumorigenic environment. Moreover, dietary patterns, gut microbiome alterations, and increased aromatase activity further contribute to cancer progression. While the biological connections between obesity and cancer are well established, addressing obesity through lifestyle modifications such as improved diet, regular physical activity, and reduced alcohol consumption can mitigate these risks. Additionally, maintaining a healthy weight has been shown to improve cancer treatment outcomes, particularly in postmenopausal breast cancer and colorectal cancer patients. However, given the increasing global prevalence of obesity, understanding and addressing its impact on cancer risk is crucial for public health.

Future research should focus on identifying personalized prevention strategies based on genetic, metabolic, and environmental factors to reduce obesity-related cancer risks. Investigating the molecular pathways linking adipose tissue dysfunction to cancer progression will provide new therapeutic targets. Furthermore, the role of the gut microbiome and its alterations in cancer risk and treatment response deserves greater attention, potentially leading to microbiome-modifying interventions.

Clinically, developing and testing pharmacological interventions targeting obesity-related pathways, such as inflammation and insulin resistance, could complement existing cancer treatments. Public health initiatives must focus on obesity prevention through education, policy changes, and community-based programs that promote healthier diets and lifestyles. Early intervention in at-risk populations, particularly those with genetic predispositions to both obesity and cancer, will be essential. By combining molecular, clinical, and public health approaches, we can create a comprehensive strategy to reduce the cancer burden linked to obesity.

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Consent for Publication All authors consent to publication.

Availability of Data Not applicable.

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References

- Abdelaal M, le Roux CW, Docherty NG (2017) Morbidity and mortality associated with obesity. *Ann Transl Med* 5:161
- Arnold M, Leitzmann M, Freisling H, Bray F, Romieu I, Renehan A, Soerjomataram I (2016) Obesity and cancer: an update of the global impact. *Cancer Epidemiol* 41:8–15
- Avgerinos KI, Spyrou N, Mantzoros CS, Dalamaga M (2019) Obesity and cancer risk: emerging biological mechanisms and perspectives. *Metabolism* 92:121–135
- Ayala A, Munoz MF, Arguelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Med Cell Longev* 2014:360438
- Aykan NF (2015) Red meat and colorectal cancer. *Oncol Rev* 9:288
- Behrooz AB, Cordani M, Fiore A, Donadelli M, Gordon JW, Klionsky DJ, Ghavami S (2024) The obesity-autophagy-cancer axis: mechanistic insights and therapeutic perspectives. *Semin Cancer Biol* 99:24–44
- Bendor CD, Bardugo A, Pinhas-Hamiel O, Afek A, Twig G (2020) Cardiovascular morbidity, diabetes and cancer risk among children and adolescents with severe obesity. *Cardiovasc Diabetol* 19:79
- Bezner JR (2015) Promoting health and wellness: implications for physical therapist practice. *Phys Ther* 95:1433–1444
- Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L (2014) Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. *Lancet* 384:755–765
- Bocian-Jastrzebska A, Malczewska-Herman A, Kos-Kudla B (2023) Role of leptin and adiponectin in carcinogenesis. *Cancers (Basel)* 15
- Breton J, Galmiche M, Dechelotte P (2022) Dysbiotic gut bacteria in obesity: an overview of the metabolic mechanisms and therapeutic perspectives of next-generation probiotics. *Microorganisms* 10
- Bruno DS, Berger NA (2020) Impact of bariatric surgery on cancer risk reduction. *Ann Transl Med* 8:S13
- Buckingham SA, Williams AJ, Morrissey K, Price L, Harrison J (2019) Mobile health interventions to promote physical activity and reduce sedentary behaviour in the workplace: a systematic review. *Digital Health* 5:2055207619839883
- Chait A, den Hartigh LJ (2020) Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. *Front Cardiovasc Med* 7:22
- Cui H, Lopez M, Rahmouni K (2017) The cellular and molecular bases of leptin and ghrelin resistance in obesity. *Nat Rev Endocrinol* 13:338–351
- Farahzadi R, Valipour B, Fathi E, Pirmoradi S, Molavi O, Montazersaheb S, Sanaat Z (2023) Oxidative stress regulation and related metabolic pathways in epithelial-mesenchymal transition of breast cancer stem cells. *Stem Cell Res Ther* 14:342
- Friedenreich CM, Ryder-Burbidge C, McNeil J (2021) Physical activity, obesity and sedentary behavior in cancer etiology: epidemiologic evidence and biologic mechanisms. *Mol Oncol* 15:790–800
- Garcia-Miranda A, Garcia-Hernandez A, Castaneda-Saucedo E, Navarro-Tito N, Maycotte P (2022) Adipokines as regulators of autophagy in obesity-linked. *Cancer Cells*:11
- Garg SK, Maurer H, Reed K, Selagamsetty R (2014) Diabetes and cancer: two diseases with obesity as a common risk factor. *Diabetes Obes Metab* 16:97–110
- Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D (2010) Diabetes and cancer: a consensus report. *Diabetes Care* 33:1674–1685
- Guria S, Hoory A, Das S, Chattopadhyay D, Mukherjee S (2023) Adipose tissue macrophages and their role in obesity-associated insulin resistance: an overview of the complex dynamics at play. *Biosci Rep* 43

- Hamasaki H (2016) Daily physical activity and type 2 diabetes: a review. *World J Diabetes* 7:243–251
- Herold J, Kalucka J (2020) Angiogenesis in adipose tissue: the interplay between adipose and endothelial cells. *Front Physiol* 11:624903
- Hirano T (2021) IL-6 in inflammation, autoimmunity and cancer. *Int Immunol* 33:127–148
- Hruby A, Hu FB (2015) The epidemiology of obesity: a big picture. *PharmacoEconomics* 33:673–689
- Hua H, Kong Q, Yin J, Zhang J, Jiang Y (2020) Insulin-like growth factor receptor signaling in tumorigenesis and drug resistance: a challenge for cancer therapy. *J Hematol Oncol* 13:64
- Huang K, Liang Y, Ma Y, Wu J, Luo H, Yi B (2022) The variation and correlation of serum adiponectin, Nesfatin-1, IL-6, and TNF-alpha levels in prediabetes. *Front Endocrinol (Lausanne)* 13:774272
- Johansson A, Schmitz D, Hoglund J, Hadizadeh F, Karlsson T, Ek WE (2022) Investigating the effect of estradiol levels on the risk of breast, endometrial, and ovarian cancer. *J Endocr Soc* 6: bvac100
- Kaminsky LA, German C, Imboden M, Ozemek C, Peterman JE, Brubaker PH (2022) The importance of healthy lifestyle behaviors in the prevention of cardiovascular disease. *Prog Cardiovasc Dis* 70:8–15
- Kawai T, Autieri MV, Scalia R (2021) Adipose tissue inflammation and metabolic dysfunction in obesity. *Am J Physiol Cell Physiol* 320:C375–C391
- Kawashima K, Maeda K, Saigo C, Kito Y, Yoshida K, Takeuchi T (2017) Adiponectin and Intelectin-1: important Adipokine players in obesity-related colorectal carcinogenesis. *Int J Mol Sci* 18
- Kerr J, Anderson C, Lippman SM (2017) Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. *Lancet Oncol* 18:e457–e471
- Khanna D, Khanna S, Khanna P, Kahar P, Patel BM (2022) Obesity: a chronic low-grade inflammation and its markers. *Cureus* 14:e22711
- Kim JW, Kim JH, Lee YJ (2024) The role of Adipokines in tumor progression and its association with obesity. *Biomedicines* 12
- Kompella P, Vasquez KM (2019) Obesity and cancer: a mechanistic overview of metabolic changes in obesity that impact genetic instability. *Mol Carcinog* 58:1531–1550
- Koroukian SM, Dong W, Berger NA (2019) Changes in age distribution of obesity-associated cancers. *JAMA Netw Open* 2:e199261–e199261
- Kosmalski M, Deska K, Bąk B, Różycka-Kosmalska M, Pietras T (2023) Pharmacological support for the treatment of obesity—present and future. *Healthcare* 11:433
- Krupa-Kotara K, Dakowska D (2021) Impact of obesity on risk of cancer. *Cent Eur J Public Health* 29:38–44
- Kunzmann AT, Coleman HG, Huang WY, Kitahara CM, Cantwell MM, Berndt SI (2015) Dietary fiber intake and risk of colorectal cancer and incident and recurrent adenoma in the prostate, lung, colorectal, and ovarian cancer screening trial. *Am J Clin Nutr* 102:881–890
- La Cava A (2017) Leptin in inflammation and autoimmunity. *Cytokine* 98:51–58
- Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K, International Agency for Research on Cancer Handbook Working G (2016) Body Fatness and Cancer—Viewpoint of the IARC Working Group. *N Engl J Med* 375:794–798
- Lega IC, Lipscombe LL (2019) Review: diabetes, obesity, and cancer—pathophysiology and clinical implications. *Endocr Rev* 41:33–52
- Lengyel E, Makowski L, DiGiovanni J, Kolonin MG (2018) Cancer as a matter of fat: the crosstalk between adipose tissue and tumors. *Trends Cancer* 4:374–384
- Lin X, Li H (2021) Obesity: epidemiology, pathophysiology, and therapeutics. *Front Endocrinol (Lausanne)* 12:706978
- Liu XZ, Pedersen L, Halberg N (2021) Cellular mechanisms linking cancers to obesity. *Cell Stress* 5:55–72

- Long Y, Mao C, Liu S, Tao Y, Xiao D (2024) Epigenetic modifications in obesity-associated diseases. *MedComm* 5:e496
- Louie SM, Roberts LS, Nomura DK (2013) Mechanisms linking obesity and cancer. *Biochim Biophys Acta* 1831:1499–1508
- Luan X, Tian X, Zhang H, Huang R, Li N, Chen P, Wang R (2019) Exercise as a prescription for patients with various diseases. *J Sport Health Sci* 8:422–441
- Luo L, Liu M (2022) Adiponectin: friend or foe in obesity and inflammation. *Med Rev* 2:349–362
- Luo J, Hendryx M, Chlebowski RT (2017) Intentional weight loss and cancer risk. *Oncotarget* 8:81719–81720
- MacKintosh ML, Derbyshire AE, McVey RJ, Bolton J, Nickkho-Amiry M, Higgins CL, Kamieniorz M, Pemberton PW, Kirmani BH, Ahmed B, Syed AA, Ammori BJ, Renehan AG, Kitchener HC, Crosbie EJ (2019) The impact of obesity and bariatric surgery on circulating and tissue biomarkers of endometrial cancer risk. *Int J Cancer* 144:641–650
- Mahmoud R, Kimonis V, Butler MG (2022) Genetics of obesity in humans: a clinical review. *Int J Mol Sci* 23
- Manna P, Jain SK (2015) Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metab Syndr Relat Disord* 13:423–444
- May M, Schindler C, Engeli S (2020) Modern pharmacological treatment of obese patients. *Ther Adv Endocrinol Metab* 11:2042018819897527
- Meldrum DR, Morris MA, Gambone JC (2017) Obesity pandemic: causes, consequences, and solutions—but do we have the will? *Fertil Steril* 107:833–839
- Nepstad I, Hatfield KJ, Gronningsaeter IS, Aasebo E, Hernandez-Valladares M, Hagen KM, Rye KP, Berven FS, Selheim F, Reikvam H, Bruserud O (2019) Effects of insulin and pathway inhibitors on the PI3K-Akt-mTOR phosphorylation profile in acute myeloid leukemia cells. *Signal Transduct Target Ther* 4:20
- Obeid S, Hebbard L (2012) Role of adiponectin and its receptors in cancer. *Cancer Biol Med* 9:213–220
- Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, Gojobori T, Isenovic ER (2021) Leptin and obesity: role and clinical implication. *Front Endocrinol (Lausanne)* 12:585887
- Oppert JM, Bellicha A, Ciangura C (2021) Physical activity in management of persons with obesity. *Eur J Intern Med* 93:8–12
- Parekh BS, Berger E, Sibley S, Cahya S, Xiao L, LaCerte MA, Vaillancourt P, Wooden S, Gately D (2012) Development and validation of an antibody-dependent cell-mediated cytotoxicity-reporter gene assay. *MAbs* 4:310–318
- Park J, Morley TS, Kim M, Clegg DJ, Scherer PE (2014) Obesity and cancer--mechanisms underlying tumour progression and recurrence. *Nat Rev Endocrinol* 10:455–465
- Patel AV, Friedenreich CM, Moore SC, Hayes SC, Silver JK, Campbell KL, Winters-Stone K, Gerber LH, George SM, Fulton JE, Denlinger C, Morris GS, Hue T, Schmitz KH, Matthews CE (2019) American College of Sports Medicine roundtable report on physical activity, sedentary behavior, and cancer prevention and control. *Med Sci Sports Exerc* 51:2391–2402
- Pati S, Irfan W, Jameel A, Ahmed S, Shahid RK (2023) Obesity and cancer: a current overview of epidemiology, pathogenesis, outcomes, and management. *Cancers (Basel)* 15
- Perez-Perez A, Sanchez-Jimenez F, Vilarino-Garcia T, Sanchez-Margalet V (2020) Role of leptin in inflammation and vice versa. *Int J Mol Sci* 21(16):5887
- Piché M-È, Auclair A, Harvey J, Marceau S, Poirier P (2015) How to choose and use bariatric surgery in 2015. *Can J Cardiol* 31:153–166
- Pico C, Palou M, Pomar CA, Rodriguez AM, Palou A (2022) Leptin as a key regulator of the adipose organ. *Rev Endocr Metab Disord* 23:13–30
- Pineda E, Poelman MP, Aaspõllu A, Bica M, Bouzas C, Carrano E, De Miguel-Etayo P, Djojosoeparto S, Blenkuš MG, Graca P (2022) Policy implementation and priorities to create healthy food environments using the healthy food environment policy index (food-EPI): a pooled level analysis across eleven European countries. *Lancet Reg Health Eur* 23:100522

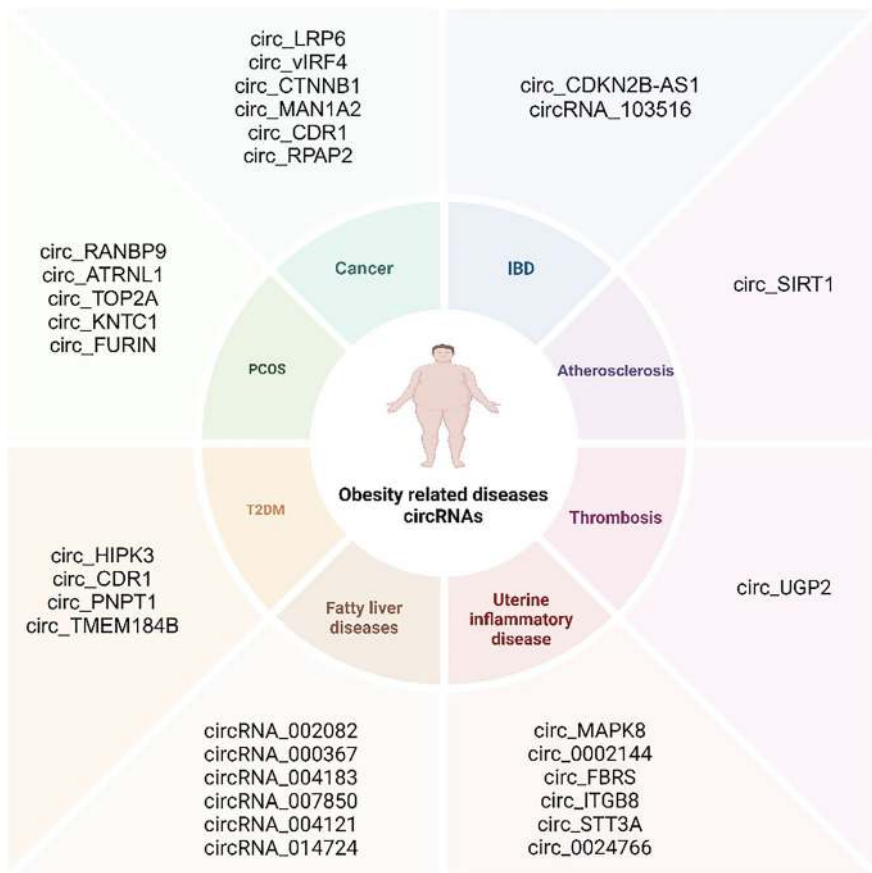
- Polednak AP (2003) Trends in incidence rates for obesity-associated cancers in the US. *Cancer Detect Prev* 27:415–421
- Potrykus M, Czaja-Stolc S, Stankiewicz M, Kaska L, Malgorzewicz S (2021) Intestinal microbiota as a contributor to chronic inflammation and its potential modifications. *Nutrients* 13(11):3839
- Powell-Wiley TM, Poirier P, Burke LE, Després J-P, Gordon-Larsen P, Lavie CJ, Lear SA, Ndumele CE, Neeland IJ, Sanders P, St-Onge M-P (2021) Obesity and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation* 143:e984–e1010
- Rezen T, Rozman D, Kovacs T, Kovacs P, Sipos A, Bai P, Miko E (2022) The role of bile acids in carcinogenesis. *Cell Mol Life Sci* 79:243
- Savulescu-Fiedler I, Mihalcea R, Dragosloveanu S, Scheau C, Baz RO, Caruntu A, Scheau AE, Caruntu C, Benea SN (2024) The interplay between obesity and inflammation. *Life (Basel)* 14
- Schmitz KH, Neuhouwer ML, Agurs-Collins T, Zanetti KA, Cadmus-Bertram L, Dean LT, Drake BF (2013) Impact of obesity on cancer survivorship and the potential relevance of race and ethnicity. *J Natl Cancer Inst* 105:1344–1354
- Shaw E, Farris M, McNeil J, Friedenreich C (2016) Obesity and endometrial cancer. *Recent Results Cancer Res* 208:107–136
- Spyrou N, Avgerinos KI, Mantzoros CS, Dalamaga M (2018) Classic and novel Adipocytokines at the intersection of obesity and cancer: diagnostic and therapeutic strategies. *Curr Obes Rep* 7:260–275
- Swinburn B, Vandevijvere S, Kraak V, Sacks G, Snowdon W, Hawkes C, Barquera S, Friel S, Kelly B, Kumanyika S, L'Abbe M, Lee A, Lobstein T, Ma J, Macmullan J, Mohan S, Monteiro C, Neal B, Rayner M, INFORMAS (2013) Monitoring and benchmarking government policies and actions to improve the healthiness of food environments: a proposed government healthy food environment policy index. *Obes Rev* 14(Suppl 1):24–37
- Szablewski L (2024) Insulin resistance: the increased risk of cancers. *Curr Oncol* 31:998–1027
- Thomas D, Apovian C (2017) Macrophage functions in lean and obese adipose tissue. *Metabolism* 72:120–143
- Tian W, Teng F, Gao J, Gao C, Liu G, Zhang Y, Yu S, Zhang W, Wang Y, Xue F (2019) Estrogen and insulin synergistically promote endometrial cancer progression via crosstalk between their receptor signaling pathways. *Cancer Biol Med* 16:55–70
- Tumminia A, Vinciguerra F, Parisi M, Graziano M, Sciacca L, Baratta R, Frittitta L (2019) Adipose tissue, obesity and adiponectin: role in endocrine cancer risk. *Int J Mol Sci* 20
- Venkatesh SS, Ferreira T, Benonisdottir S, Rahmioglu N, Becker CM, Granne I, Zondervan KT, Holmes MV, Lindgren CM, Wittemans LBL (2022) Obesity and risk of female reproductive conditions: a Mendelian randomisation study. *PLoS Med* 19:e1003679
- Wadden TA, Bailey TS, Billings LK, Davies M, Frias JP, Koroleva A, Lingvay I, O'Neil PM, Rubino DM, Skovgaard D, Wallenstein SOR, Garvey WT (2021) Effect of subcutaneous Semaglutide vs placebo as an adjunct to intensive behavioral therapy on body weight in adults with overweight or obesity: the STEP 3 randomized clinical trial. *JAMA* 325:1403–1413
- Wang J, Ding X, Gao H, Fan S (2022) Reshaping food policy and governance to incentivize and empower disadvantaged groups for improving nutrition. *Nutrients* 14
- Wilson RB, Lathigara D, Kaushal D (2023) Systematic review and meta-analysis of the impact of bariatric surgery on future cancer risk. *Int J Mol Sci* 24:6192
- Włodarczyk M, Nowicka G (2019) Obesity, DNA damage, and development of obesity-related diseases. *Int J Mol Sci* 20
- Wu C, Dong S, Huang R, Chen X (2023) Cancer-associated adipocytes and breast cancer: intertwining in the tumor microenvironment and challenges for cancer therapy. *Cancers (Basel)*:15
- Yao H, He S (2021) Multi-faceted role of cancer-associated adipocytes in the tumor microenvironment (review). *Mol Med Rep* 24
- Zaman R, Hankir A, Jemni M (2019) Lifestyle factors and mental health. *Psychiatr Danub* 31:217–220

- Zatterale F, Longo M, Naderi J, Raciti GA, Desiderio A, Miele C, Beguinot F (2019) Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. *Front Physiol* 10:1607
- Zhang XL, Zhang XF, Fang Y, Li ML, Shu R, Gong Y, Luo HY, Tian Y (2023) A possible genetic association between obesity and colon cancer in females. *Front Endocrinol (Lausanne)* 14: 1189570
- Zhao Y, Ye X, Xiong Z, Ihsan A, Ares I, Martinez M, Lopez-Torres B, Martinez-Larranaga MR, Anadon A, Wang X, Martinez MA (2023) Cancer metabolism: the role of ROS in DNA damage and induction of apoptosis in cancer cells. *Meta* 13
- Zhou JR, Blackburn GL, Walker WA (2007) Symposium introduction: metabolic syndrome and the onset of cancer. *Am J Clin Nutr* 86:817–819

CircRNAs: Orchestrating Obesity, Thermogenesis, and Obesity-Associated Cancer Pathways

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Graphical Abstract



Abstract

Circular RNAs (circRNAs) have been linked to the pathophysiology of metabolic disorders such as dyslipidemia, non-alcoholic fatty liver diseases (NAFLD), and type 2 diabetes (T2D). Dysregulated circRNA levels impact inflammation, insulin sensitivity (IS), and the metabolism of fats and carbohydrates. Hence, targeting these circRNAs can be beneficial in modulating adipogenesis, fat metabolism, and insulin resistance (IR) associated with obesity. Moreover, obesity-associated malignancies, such as colon, liver, and breast malignancies, were also associated with altered circRNA expression. Dysregulated circRNAs can impact tumor growth, metastasis, and chemoresistance via signaling pathways, cell cycle, and apoptosis affection. In this book chapter, we comprehensively discuss circRNAs in the context of obesity, their involvement in thermogenesis, and their role in mediating obesity-associated malignancies.

Keywords

Adipogenesis · Brown adipose tissue · Cancer · Circular RNAs (circRNAs) · Epigenetics · Insulin resistance · Metabolic diseases

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Abbreviations

(ob/ob and db/db)	Genetically obese and diabetes
AMPK	AMP-activated protein kinase
BAT	Brown adipose tissue
BMI	Body mass index
C/EBP α	CCAAT enhancer binding protein alpha
CDC	Centers for Disease Control
circRNAs	Circular RNAs
CRC	Colorectal cancer
CVDs	Cardiovascular disorders
EVs	Extracellular vesicles
FMNL2	Formin-like 2
FOXO1	Factor forkhead box protein O1
FTO	Fat mass and obesity-associated protein
GLUT4	Insulin-regulated glucose transporter
hADSC	Human adipose-derived stem cells
hBMSCs	Human bone marrow-derived mesenchymal stem cells
HFD	High-fat diet
HIF	Hypoxia-inducible factor
IGF	Insulin-like growth factor
IR	Insulin resistance
IRESs	Internal ribosome entry sites
IRS	Insulin receptor substrate
IS	Insulin sensitivity
miRNAs or miR	MicroRNAs
mRNA	Messenger RNA
mTOR	Mammalian target of rapamycin
ncRNA	Noncoding RNAs
NMF	Non-negative matrix factorization
O-GlcNAc	O linked N-acetyl glucosamine
PCOS	Polycystic ovary syndrome
PGC1 α	PPAR- γ coactivator-1 α
PPAR γ	Peroxisome proliferator-activated receptor
PTBP1	Polypyrimidine tract binding protein 1
RNA-Seq	RNA sequencing
ROS	Reactive oxygen species
siRNAs	Small interfering RNAs
Sterol regulatory element binding transcription factor1	SREBP-1c
T2D	Type 2 diabetes
T2DM	Type 2 diabetes mellitus
TIME	Tumor immune microenvironment

TME	Tumor microenvironment
UCP1	Uncoupling protein 1
WAT	White adipose tissue

1 Introduction

1.1 Background

Circular RNAs (circRNAs) are a relatively new class of noncoding RNAs (ncRNA) previously considered pre-messenger RNA (mRNA) splicing errors (Sanger et al. 1976). However, with high-throughput sequencing technologies and bioinformatics improvements, circRNAs have emerged as important regulators of crucial biological functions, including maintaining metabolic homeostasis (Szabo and Salzman 2016). The CircBank Database has fulfilled the requirement for a consistent nomenclature in circRNA research by implementing a methodology that is predicated on host genes and precise start/end positions. The database contains a vast amount of information regarding 140,790 human circRNAs (Bagheri Moghaddam et al. 2022). CircRNAs are classified by their origin: exonic circRNAs regulate genes by sequestering microRNAs (miRNAs), and circular intronic RNAs are located in the nucleus and are responsible for coordinating transcription (Mahmoud et al. 2021a, b). Exon-intron circRNAs interact with RNA polymerase II to regulate gene expression (Jeck and Sharpless 2014; Li et al. 2015; Chu et al. 2017). CircRNAs are essential in physiology and pathology, as they serve as miRNA absorbers, regulate gene transcription, control RNA-binding proteins, and synthesize functional peptides (Chen et al. 2022; Zhang et al. 2022b). They are synthesized via “Exons Back Splicing,” forming a single-stranded closed structure distinct from typical mRNA splicing (Zhang et al. 2013). This unique structure makes them more stable and resistant to exonuclease degradation, allowing them to exert long-lasting effects on cellular processes (Chen 2016). The biogenesis and mechanisms of actions of the circRNAs are comprehensively discussed and reviewed in Youness et al. (2024). CircRNAs are well expressed across the different human tissues. In Fig. 1, we show the top 30 expressed circRNAs in human tissues retrieved from **circAtlas 3.0** “updated June 30th, 2023” https://ngdc.cncb.ac.cn/circatlas/top_exp1.php and accessed July 6th, 2024.

1.2 Rationale for Study CircRNAs in the Context of Obesity and Obesity-Related Diseases and Malignancies

Several lines of evidence suggest circRNAs orchestrate various aspects of adipocyte differentiation, adipogenesis, and lipid metabolism (Jiang et al. 2020; Liu et al. 2020a, b; Ding et al. 2021). Recognizing the functions of circRNAs in these

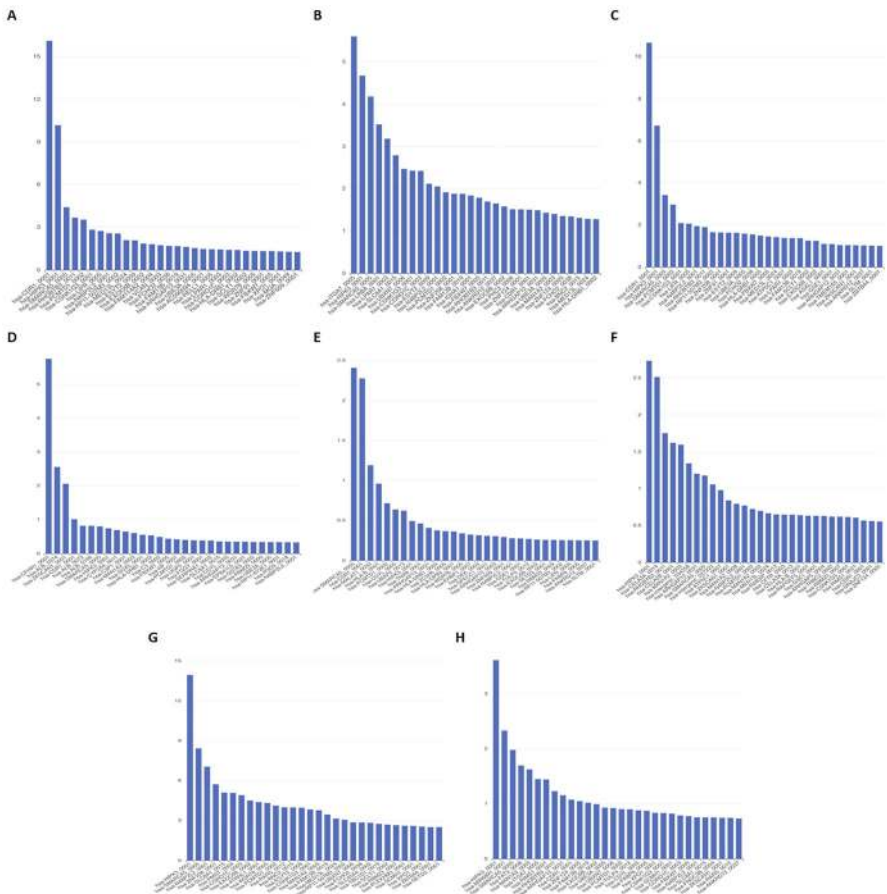


Fig. 1 Top 30 highly expressed circRNAs from pre-analyzed datasets of human tissues (a) adipose tissue, (b) colon tissue, (c) breast tissue, (d) liver tissue, (e) human mammary gland tissue, (f) pancreas tissue, (g) uterus tissue, and (h) stomach tissue; one-step analysis, expressed as the CPM count per million on the y-axis, retrieved from circAtlas 3.0 Accessed Jan. 4th, 2024. https://ngdc.cncb.ac.cn/circatlas/top_exp1.php

pathways may provide light on the molecular bases of obesity, which in turn could pave the way for their therapeutic use in the treatment of lipid-related disease (Chen et al. 2021). Obesity is frequently linked to chronic inflammation and insulin resistance (IR), which are significant factors that contribute to the development of obesity-associated cancers. CircRNAs have been suggested to regulate inflammatory pathways and insulin signaling, indicating their potential involvement in metabolic disorders related to obesity (Fang et al. 2018; Wu et al. 2022a). Moreover, circRNAs are stably expressed in various body fluids, making them attractive candidates as noninvasive biomarkers. Investigating circRNA profiles in obesity-associated diseases can help identify potential biomarkers for early detection, prognosis, and

therapeutic targets (Zaiou 2019; Dandare et al. 2023; Sun et al. 2023). In addition to that, the unique structure of circRNAs and their lower negative effect on triggering an immune response are all characteristics that render circRNAs advantageous over other RNA forms for developing RNA-based drugs. Indeed, intron-derived circRNAs have less impact on triggering an immune response than other RNAs (Zaiou 2019, Dandare et al. 2023, Sun et al. 2023). For example, the circRNA-RBD-Delta vaccine was designed to combat the COVID-19 pandemic (Qu et al. 2022). CircRNA therapeutics are currently the focus of drug discovery companies and are considered one of six academic spinouts of Nature Biotechnology 2021 (Eisenstein et al. 2022).

Collectively, our aim in this chapter is to emphasize the dual role of circRNAs in mediating obesity-associated cancer, the involvement of circRNAs in thermogenesis, and the expected roles of circRNAs in obesity.

2 CircRNA Role in Developmental Reprogramming and Early-Life Exposure

CircRNAs play a role related to the capacity of cells to experience abrupt changes in their cellular identity and gene expression patterns throughout development or in response to particular stimuli (Abd El Fattah et al. 2023; Hamdy et al. 2024). Below, we discuss the involvement of circRNA in both developmental reprogramming and early-life exposure.

2.1 The Role of CircRNAs in Developmental Reprogramming

CircRNA can play a pivotal role in either stem cell differentiation or embryonic development. Regarding stem cell differentiation, Mazziotta et al. report that circRNAs control how stem cells differentiate into distinct cell lineages, including neurons, cardiomyocytes, and adipocytes (Mazziotta et al. 2024). By adjusting the availability of microRNAs and affecting the expression of essential transcription factors and signaling pathways involved in lineage commitment, they can function as miR sponges. On the other hand, Di Agostino et al. (2020) show that circRNAs have been linked to controlling key embryonic developmental processes, including gastrulation, organogenesis, tissue patterning, and neurodevelopmental processes. They are dynamically expressed during embryonic development and are linked to various developmental disorders, such as autism spectrum disorder, intellectual disability, and developmental delays. They can interact with transcriptional regulators, chromatin remodeling complexes, and RNA-binding proteins to coordinate gene expression patterns necessary for healthy embryonic development. CircRNA in single-cell transcriptome experiments were performed to identify circRNAs in embryonic tissues, germ cells, cord blood, and fibroblasts or stem cells using various technologies ranging from Smart-seq2 to Tang, accessed July 4th, 2024 <https://ngdc.cncb.ac.cn/circatlas/circSC/data.html> as shown in Table 1.

Table 1 In silico search for CircRNA in single-cell transcriptome experiments

Study	Project ID	Description
Investigating the presence and patterns of circRNAs in human pre-implantation embryos	GSE71318	Following the expression of circRNAs in human embryos prior to implantation
An analysis of the transcriptome and DNA methylome of human primordial germ cells is presented	GSE63818	The transcriptome and DNA Methylome landscapes of human primordial germ cells
An investigation of the transcriptome of linear and circular RNAs in mouse preimplantation embryos was conducted using single-cell RNA sequencing (RNA-Seq)	GSE53386	Single-cell transcriptome analysis of circRNAs in mice embryos using RNA-Seq
Single-cell RNA-Seq uncovers previously unknown factors that control the process of human embryonic stem cell transformation into definitive endoderm	GSE75748	/
Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos investigates the lineage and X chromosome dynamics in human preimplantation embryos using single-cell RNA sequencing	E-MTAB-3929	Lineage formation and X-chromosome dosage compensation in human preimplantation embryos are revealed by single-cell RNA-Seq
Single-cell RNA-Seq characterization of human preimplantation embryos and embryonic stem cells	GSE36552	Tracing the pluripotency of human embryonic stem cells and early embryos through single-cell RNA-Seq
An investigation of the formation of human interneurons using an in vitro model demonstrates the temporal progression of cell type generation and maturation by single-cell profiling	GSE93593	Characterization of cell types produced over time in an in vitro model of human inhibitory interneuron differentiation using single-cell RNA-Seq
Analysis at the single-cell level uncovers the spectrum of human lympho-myeloid progenitor cells	GSE100618	Research on human lympho-myeloid progenitor populations using single-cell RNA-Seq (LMPP, MLP, and GMP)
Oscope is a tool used to detect genes that exhibit oscillatory behavior in unsynchronized single-cell RNA-Seq experiments	GSE64016	Human hESCs RNA-seq data collected from single cells for the purpose of testing Oscope, a statistical pipeline for the detection of oscillatory genes in non-synchronized RNA-seq data
Single-cell RNA-Seq analysis unveils the genetic programs present in early embryos of humans and mice	GSE44183	By single-cell RNA-Seq reveals genetic programs in human and rodent early embryos
Uncovering sparsely encoded transcription factor patterns that regulate cell states and transitions in the course of development	GSE86982	Single-cell RNA-Seq from human embryonic stem cells reveals region-specific neural stem cell lineages [smartseq]
Investigation of allelic expression patterns in clonal somatic cells using single-cell RNA-Seq	GSE75659	/

(continued)

Table 1 (continued)

Study	Project ID	Description
A non-negative matrix factorization (NMF) method is used to identify previously unnoticed gene areas that show differential expression in single-cell RNA-Seq data	GSE125288	The identification of differentially expressed gene regions that were previously neglected in single-cell RNA-Seq data using neural stem cells derived from human-induced pluripotent stem cells
Aligning single-cell developmental and reprogramming trajectories identifies molecular determinants of myogenic reprogramming outcome	GSE105211	The molecular determinants of the reprogramming outcome are identified by aligning single-cell developmental and reprogramming trajectories

<https://ngdc.cncb.ac.cn/circatlas/circSC/data.html> Accessed July 6th 2024

2.2 The Role of CircRNAs in Early Life Exposure to Health and Disease Outcomes

CircRNAs may help generate and maintain modified epigenetic landscapes of DNA methylation and histone alterations in response to early life exposures and experiences by controlling the expression or activity of epigenetic regulators (Lee et al. 2019). Some circRNAs may be passed through generations and may have a role in inheriting traits or susceptibilities to diseases linked to early life experiences (Gu et al. 2023). This finding emphasizes the possibility that environmental influences and their long-term, multigenerational effects could be linked molecularly by circRNAs. Stress response pathways or cascades and cell adaptation to early-life stresses are controlled by circRNAs through the regulation of gene expression to influence an individual’s susceptibility or resilience to the long-term consequences of early-life adversity and the negative consequences of adverse childhood experiences, enhancing an individual’s ability to cope with and adapt to early-life stressors (Hotamisligil and Davis 2016). Exposure to undernutrition, obesity in mothers, or gestational diabetes during infancy has been linked to a greater likelihood of developing metabolic and cardiovascular disorders (CVDs) later in life (Inzani and Ozanne 2022; Kereliuk and Dolinsky 2022). CircRNA signature profiles may identify people or subgroups more likely to experience unfavorable health outcomes due to early life or development exposure. To prevent or lessen the long-term effects of early-life adversity, these data may be used for the creation of personalized and targeted early-life treatments, such as dietary, nutritional or behavioral, and pharmaceutical therapies (He et al. 2021; Faraj et al. 2024). The role of circRNAs in early life exposure to health and disease outcomes has been illustrated in Fig. 2.

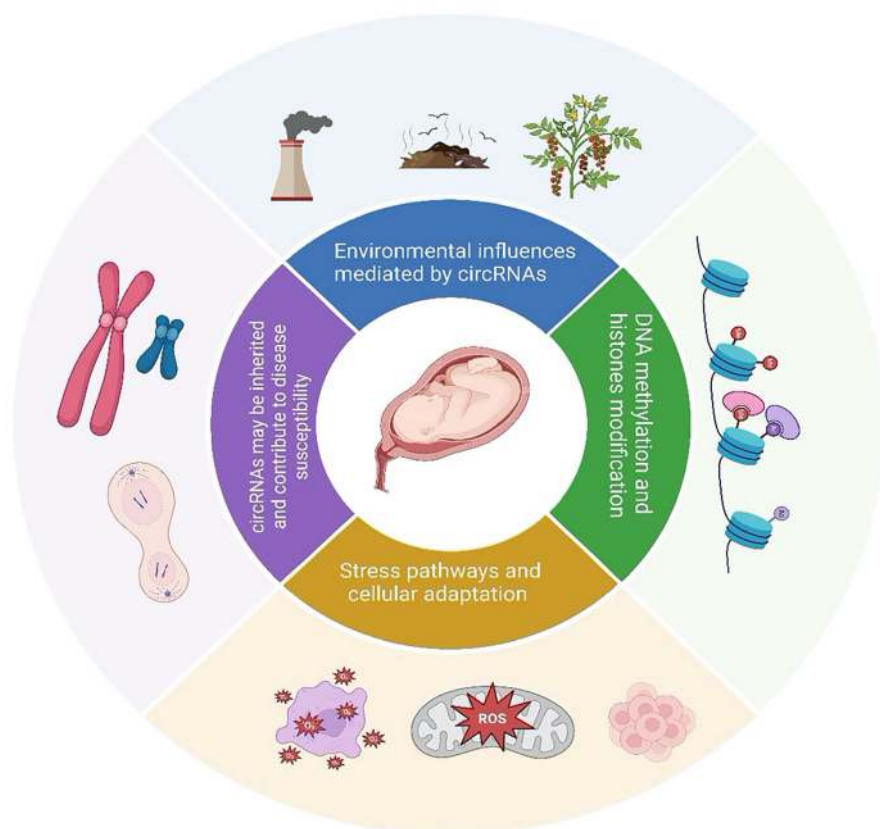


Fig. 2 The role of circRNAs in early life exposure to health and disease outcomes

3 The Relationship Among Thermogenesis, Obesity, and Cancer

Types of cancer that are linked to obesity include endometrial, breast, liver, and colon cancers (Mahmoud et al. 2021a, b; Eldosoky et al. 2023; Shaker et al. 2024; Youness et al. 2024). Severe metabolic disorders, such as obesity, type 2 diabetes mellitus (T2DM), and a number of malignancies, arise when calorie intake exceeds energy expenditure. One subset of fat cells, known as brown adipose tissue (BAT), is highly involved in the processes of thermogenesis and energy expenditure. Unlike white adipose tissue (WAT), which uses triglycerides as an energy storage mechanism, brown adipose tissue (BAT) has many mitochondria and is rich in tiny lipid droplets, which allow for the quick mobilization of stored components for energy production (Aboouf et al. 2023). A key factor in controlling BAT's overall activity is the careful regulation of its fatty acid and lipid metabolism (Din et al. 2018; Saito et al. 2020). The Krebs cycle metabolites and branched-chain amino acids are

consumed to a lesser degree than glucose and other metabolic substrates (Cannon and Nedergaard 2004; Bartelt et al. 2011; Mills et al. 2018) to fuel uncoupling protein 1 (UCP1)-dependent respiration (Golozoubova et al. 2001) to ultimately convert chemical energy to heat. There is evidence that BAT significantly contributes to small mammal whole-body energy expenditure via regulating core body temperature.

At the present time, cancer ranks first among the causes of death in industrialized countries and second among those in poorer nations (Siegel et al. 2024). There is a dose-dependent association between obesity and an increased risk of leukemia, lymphoma, and myeloma, according to multiple studies (Lichtman 2010). Additionally, obese adults face increased susceptibility to pancreatic (Bracci 2012), prostate (Lavalette et al. 2018), breast (Agurs-Collins et al. 2019), colon, endometrial, liver, kidney, esophageal, gastric, and gallbladder cancers (reviewed in (Scherubl 2021)). As childhood obesity rates mostly mirror those of adults (Collaboration 2024), there is a substantial increase in the risk of developing cancers later in life (Nuotio et al. 2022). While the epidemiological connections between cancer progression and prognosis are firmly established, the precise link between obesity and the initiation of cancer, along with the molecular mechanisms orchestrating these associations, is an ongoing area of exploration.

Obesity is a proven significant risk factor for several cancers, primarily colorectal cancer (CRC) (Frezza et al. 2006; Liu et al. 2019; Seo et al. 2023). Remarkably, the weight loss that occurs before the diagnosis of colon cancer might mask the strong association between obesity and colon cancer (Mandic et al. 2023). IR triggered by obesity can lead to hyperglycemia in a multiple inflammation pathway-mediated fashion (El-Mesallamy et al. 2011a, b, c) in relation to adiponectin (El-Mesallamy et al. 2012a, b). The Warburg effect makes glucose uptake crucial for glycolysis in cancer (Vander Heiden et al. 2009). Most cancers use glycolysis to harness energy for their infinite growth, invasion, and metastasis (Hanahan and Weinberg 2011; Bergers and Fendt 2021). Indeed, high-glucose environments promote the migration and invasion of cancer cells by increasing the levels of global O-linked N-acetyl glucosamine (O-GlcNAc) proteins and the expression of vimentin, hexokinase, glucosamine-fructose-6-phosphate amidotransferase and O-GlcNAc transferase in cancer cells (Phoomak et al. 2017). Significantly, T2DM patients are at high risk of developing liver, pancreatic, colon, bladder, and breast cancer (Linkeviciute-Ulinskiene et al. 2019).

Blood glucose absorption in adipocytes is enhanced when cold and medicines activate thermogenic metabolism in BAT (Cannon and Nedergaard 2004; Hankir and Klingenspor 2018). Specifically, BAT activation via cold exposure decreases blood glucose and favors glucose distribution to BAT instead of to tumor cells, leading to impaired glycolysis and reduced tumor growth (Seki et al. 2022; Chen and Kang 2023). Furthermore, BAT has recently been proven not to be associated with cachexia or overall mortality, as has been believed before (Eljalby et al. 2023). Interestingly, the aberrant expression of the brown fat-like phenotype in colon cancer cells was associated with better overall survival, suggesting the prognostic value of the UCP1 biomarker in cancer cells (Alnabulsi et al. 2019). Moreover, increased expression of UCP1, along with the expansion of stem cell differentiation into brown

adipocytes, was demonstrated in mice xenografted with breast cancer cell lines (Singh et al. 2016). Stimulation of this differentiation was associated with a larger tumor volume, which decreased after the depletion of UCP1-positive cells. Furthermore, higher BAT volume increases the likelihood of tumor recurrence or cancer-associated death (Chu et al. 2020).

The potential role of BAT in cancer progression is an area of active research, as it is complex, not fully understood and context-dependent.

While BAT is primarily known for its role in thermogenesis and energy expenditure, emerging evidence suggests that it may have broader effects on metabolism, inflammation, and the tumor microenvironment. Glucose uptake is essential for cancer glycolysis, and most cancers use glycolysis to harness energy for their infinite growth, invasion, and metastasis (Hanahan and Weinberg 2011; Bergers and Fendt 2021). The effects of BAT on cancer progression may vary depending on factors such as tumor type, stage, genetics, and the broader physiological context. To better understand the potential role of BAT in cancer, further research using rigorous experimental models is needed. Investigating the mechanisms through which BAT activation affects tumor behavior and the underlying molecular pathways involved could lead to valuable insights and potentially new cancer treatment or prevention strategies. Figure 3 depicts an illustrative scheme of how obesity, thermogenesis,

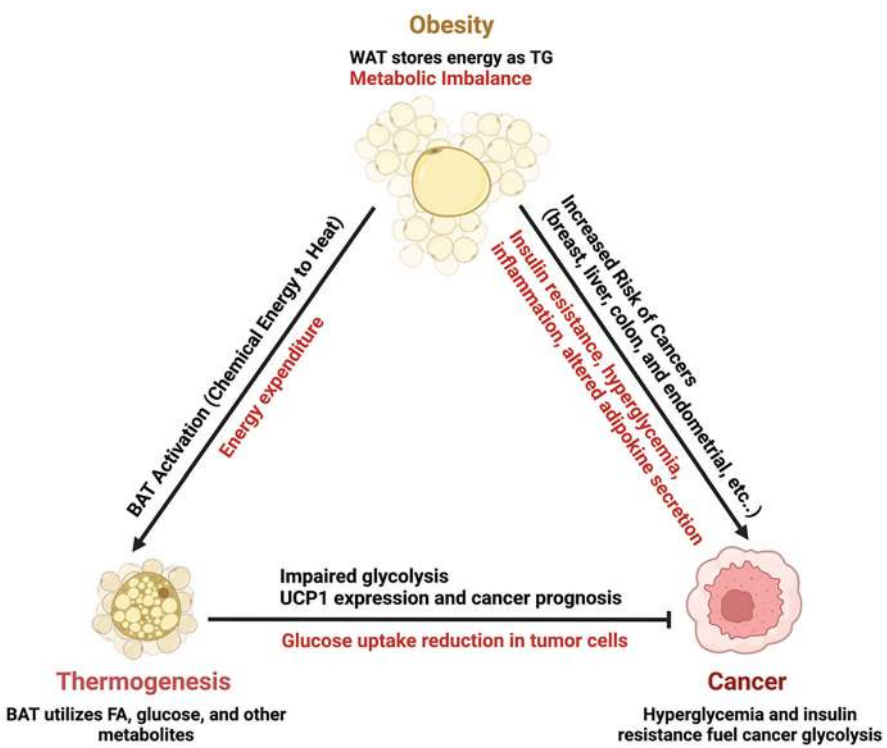


Fig. 3 Illustrative scheme of crosstalk among obesity, thermogenesis, and cancer

and cancer can be linked together, with more emphasis discussed in the upcoming chapter describing the intricate molecular interplay between the three corners of our triad of interest.

4 The Underlying Molecular Interplay Between Obesity, Thermogenesis, and Cancer

Next, we will discuss the different factors implicated in the relationship between obesity, thermogenesis, and cancer. Understanding these molecular mechanisms is crucial for developing targeted therapies and interventions to prevent or treat obesity-related cancers. It is important to note that the relationships among obesity, thermogenesis, and cancer are multifaceted, and ongoing research continues to uncover new insights into these complex interactions.

Adipokines and inflammatory adipokines. Produced by fat cells, these signaling chemicals are vital for controlling metabolism, inflammation, immunological response, heart function, and cancer. Obesity causes changes in the release of adipokines like leptin and adiponectin because of the excess fat tissue (Fasshauer and Bluher 2015). Leptin resistance is often observed in obese individuals, and this can contribute to increased inflammation. Chronic inflammation is associated with cancer development, and various cytokines and inflammatory mediators play a role in promoting tumor growth (Zhao et al. 2021).

Insulin resistance and hyperinsulinemia. Obesity is frequently linked to IR, where cells become less responsive to insulin signaling. Hyperinsulinemia, an elevated level of insulin in the blood, is common in individuals with obesity. The insulin and insulin-like growth factor (IGF) pathways are implicated in promoting cell proliferation and inhibiting apoptosis, potentially contributing to cancer development (Gallagher and LeRoith 2010).

Alterations in sex hormone. Obesity is associated with changes in sex hormone levels, including increased estrogen production in adipose tissue (Mair et al. 2020). Some malignancies, such as breast and endometrial cancers, are more likely to develop in women with elevated estrogen levels (Rodriguez et al. 2019; Al-Shami et al. 2023).

The intracellular signaling pathways. To maintain a steady internal energy level in cells, proteins including AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) play vital roles. The development of cancer may be influenced by the dysregulation of several pathways in obesity, which impact cell proliferation, metabolism, and survival (Grisouard et al. 2011; Donohoe et al. 2020).

Mitochondrial dysfunction and oxidative stress. Obesity can lead to mitochondrial dysfunction and increased production of reactive oxygen species (ROS) (de Mello et al. 2018). ROS can cause DNA damage and promote cellular transformation, contributing to the initiation and progression of obesity-related diseases (El-Mesallamy et al. 2010, 2011a, b, c, 2012a, b; Abaza et al. 2023) or cancer (Zhao et al. 2023).

As explained previously, BAT is involved in thermogenesis, a process of heat production. Activation of BAT may have protective effects against obesity-related metabolic disorders (Kim and Plutzky 2016) and certain cancers (Yin et al. 2022). UCP1 is the key molecule in BAT that not only dissipates energy as heat but also mediates the cold-triggered anticancer effect (Seki et al. 2022).

Hypoxia-inducible factor (HIF). Obesity can lead to adipose tissue expansion and inadequate vascularization, resulting in hypoxia (Trayhurn 2013). HIF is a transcription factor activated in response to hypoxia along with other genes involved in inflammation and fibrosis (Cifarelli et al. 2020) and is also involved in various tumor (Radwan et al. 2016) angiogenesis and metastasis (Lv et al. 2017).

5 Overview of CircRNAs and List of Their Regulatory Mechanisms

A class of ncRNAs known as circRNAs are characterized by the formation of covalently closed continuous loops that are distinct from the linear structure of traditional RNA molecules. These loops are devoid of poly (A) tails and 5'-3' ends (Chen and Yang 2015). Initially, considered splicing errors or byproducts, circRNAs have emerged as important regulators of gene expression and are implicated in various cellular processes (Salzman et al. 2012). CircRNAs are correlated with the pathogenesis of different human diseases, including diabetes (Fan et al. 2022) and cancer (Bach et al. 2019; Pisignano et al. 2023). Cancer progression, metastasis, stemness, and treatment resistance are all profoundly impacted by circRNAs (Guarnerio et al. 2016; Liang et al. 2017). Before we discuss the roles of the different circRNA in obesity and its related comorbidities, we will briefly present below an overview of the regulatory functions of the different circRNAs:

- 5.1 *Sponging miRNAs*: circRNAs can function as “miRNA sponges” by binding to miRNAs, which subsequently sequesters them and prevents them from repressing their target mRNAs. By altering the availability of miRNAs, this competitive binding regulates gene expression (Thomas and Sætrom 2014; Zhang and Xin 2018; Zhong et al. 2018).
- 5.2 *Interacting with RNA-binding proteins*: CircRNAs can function as substrates for interacting with RNA-binding proteins, thereby affecting their activity, stability, or subcellular localization. This interaction has the potential to influence the metabolism, splicing, or translation of RNA (Abdelmohsen et al. 2017).
- 5.3 *Regulating transcription*: Some circRNAs have been demonstrated to regulate the transcription of their ancestral genes by interacting with RNA polymerase complexes or transcription factors. They have the potential to either enhance or inhibit transcription, thereby influencing the levels of gene expression (Chen 2016).

- 5.4 *Modulating alternative splicing*: CircRNAs can regulate alternative splicing by sequestering splicing factors or directly interacting with pre-mRNA splice sites. This can influence the production of different mRNA isoforms and protein diversity (Ashwal-Fluss et al. 2014).
- 5.5 *Scaffolding complexes*: CircRNAs can serve as scaffolds to bring together multiple molecules or proteins within a ribonucleoprotein complex. This organization facilitates specific molecular interactions and signaling pathways (Zhang et al. 2017; Zhang and Xin 2018).
- 5.6 *Regulating translation*: Some circRNAs have been reported to contain internal ribosome entry sites (IRESs) or to interact with ribosomes directly, enabling them to be translated into proteins. This mechanism expands the coding potential of circRNAs (Legnini et al. 2017; Yang et al. 2018; Zhang et al. 2018).
- 5.7 *Stabilizing mRNAs*: CircRNAs can bind to and stabilize their target mRNAs, protecting them from ribonuclease degradation. This interaction enhances mRNA stability and increases protein expression levels.
- 5.8 *Modulating protein function*: CircRNAs can regulate protein function by acting as decoys, inhibitors, or enhancers of protein activity (Abdelmohsen et al. 2017). They can compete with other RNA molecules or directly interact with proteins to modulate their function.

6 Genetics of Obesity

Before delving into the roles of circRNAs in obesity, it is essential to understand simple but crucial facts regarding obesity. Obesity is a multifactorial polygenic disorder that mostly involves more than one genetic abnormality. Notably, in Table 2, we show the main genes with variants associated with obesity as retrieved from the Centers for Disease Control (CDC) Genes and Obesity <https://www.cdc.gov/genomics/resources/diseases/obesity/obesedit.htm#table> Accessed Jan. 5th, 2024.

7 In Silico Analysis of Adipose Tissue-Derived circRNA

We utilized bioinformatic databases to demonstrate the involvement of adipose tissue molecular signatures in the different body fluids and a myriad of human tissues. We used exoRBase 2.0 <http://www.exorbase.org/exoRBaseV2/toIndex> which is a repository of extracellular vesicles (EVs) long RNAs (exLRs) derived from around 1,000 RNA-Seq data analyses. It encompasses the integration and visualization of RNA expression profiles, as well as the heterogeneity of circulating-EV origins and the functional pathway-level changes. Figure 4 depicts the relative abundance of adipose tissue in many samples, including several human cancer tissues, retrieved from <http://www.exorbase.org/exoRBaseV2/cell/proportion> Accessed Jan. 5th, 2024.

Table 2 Main genes associated with obesity

Gene symbol	Name	Gene product’s role in energy balance
<i>ADIPOQ</i>	Adipocyte-, C1q-, and collagen domain-containing	Adiponectin, which is synthesized by adipose cells, stimulates energy expenditure
<i>FTO</i>	Fat mass- and obesity-associated	Encourages the consumption of food
<i>LEP</i>	Leptin	Generated by adipose tissue
<i>LEPR</i>	Leptin receptor	It suppresses appetite when bound to leptin
<i>INSIG2</i>	Insulin-induced gene 2	The regulation of the synthesis of fatty acids and cholesterol
<i>MC4R</i>	Melanocortin 4 receptor	It stimulates appetite when bound by alpha-melanocyte-stimulating hormone
<i>PCSK1</i>	Proprotein convertase subtilisin/kexin type 1	Regulates the biosynthesis of insulin
<i>PPARG</i>	Peroxisome proliferator-activated receptor gamma	Encourages the acquisition of lipids and the growth of adipose tissue

<https://www.cdc.gov/genomics/resources/diseases/obesity/obesedit.htm#table> Accessed Jan. 5th, 2024. *Obesity-related cancer gene together with tenomodulin (TNMD) as per <https://www.proteinatlas.org/search/obesity+related+cancer+>



Fig. 4 Relative abundance of adipose tissue in various samples (urine, CSF, bile), healthy and benign or different cancer cohorts. <http://www.exorbase.org/exoRBaseV2/cell/proportion> Accessed Jan. 5th, 2024

8 The Roles of CircRNAs in Obesity-Related Processes

Recent studies have attempted to pinpoint the functional roles of multiple circRNAs in obesity and its underlying pathways, as discussed below.

8.1 Fat Mass and Obesity-Associated Protein (FTO)

Fat mass and obesity-associated protein (FTO) are pivotal proteins in obesity. The regulation of its expression implicates an important circRNA, circSCMH1. This regulation can promote vascular repair when studied in the context of photothrombotic stroke (Li et al. 2023a). Similarly, circMAPK9 promotes adipogenesis by absorbing hsa-miR-1,322, abolishing its inhibitory effect on FTO (Chen et al. 2023). Interestingly, maternal circRNAs were shown to regulate lipid metabolism in offspring (Chen et al. 2020b). If we can use adipogenesis-modulating treatment this would constitute a treatment option (Mostafa et al. 2016).

Chen et al. fed female mice a high-fat diet (HFD) for 12 weeks before mating. An HFD leads to the differential expression of many circRNAs (Chen et al. 2020b). Deeper network analysis revealed that circRNA_0000660-miR_693-Igfbp1 is a potential mechanism for regulating lipid metabolism in offspring (Chen et al. 2020b).

8.2 The Effects of circRNAs

The effects of circRNAs on insulin secretion and resistance have been highlighted by *in vitro* and *in vivo* studies. Both ciRS-7 (also known as CDR1as or hsa_circ_0001946) and circHIPK3 are implicated in the regulation of β -cell function and diabetes development (Stoll et al. 2018). ciRS-7 sponges miR-7, promoting β -cell proliferation and insulin secretion in diabetes (Shoelson et al. 2006; Jones et al. 2017). In another study, insulin secretion was improved by activating the ciRS-7-miR-7-Pax6/Myrip axis in murine pancreatic β cells (Xu et al. 2015). These results increase the potential of these circRNAs as biomarkers for diabetes and adiposity.

8.2.1 The significance of circRNAs in patients or animal models of obesity and diabetes has not been investigated due to the comorbidity of these conditions. Obesity and diabetes are linked by an IR pathophysiology, and certain circRNAs are associated with IR (Zaiou 2020). For instance, circHIPK3 upregulates the expression of factor forkhead box protein O1 (FOXO1) by sponging miR-192-5p, contributing to hyperglycemia (Cai et al. 2020). IR is also associated with circRNA-TFRC, which causes overexpression of the transferrin receptor, increasing the risk of T2DM and metabolic disease (Jager et al. 2017).

8.2.2 Mouse and human adipose tissue circRNA content analysis revealed that thousands of circRNAs are unique to adipose tissue and regulate adipogenesis (1988). An emerging role for circRNAs in adipogenesis and adipocyte differentiation has been documented in the literature. Since circRNAs are tissue-specific and, in

some cases, can be more abundant than linear mRNAs (Jeck et al. 2013), studying adipose tissue-associated circRNAs can provide insight into their functional role in this specific tissue. Many circRNAs have been identified in adipose tissue and are linked to the pathophysiology of obesity. A loss-of-function study revealed that knocking out circRNA H19 resulted in excessive adipogenic differentiation of human adipose-derived stem cells (hADSC) by targeting polypyrimidine tract binding protein 1 (PTBP1) (Zhu et al. 2020). Deep sequencing was used to identify circRNAs in visceral and subcutaneous fat (Arcinas et al. 2019). They play a crucial role in adipogenesis. For example, circArhgap5-2 orchestrates the global transcriptional program of adipocyte genes (Arcinas et al. 2019). Inhibition of circArhgap5-2 inhibited lipid droplet accumulation and reduced the expression of adipogenic markers in vivo (Arcinas et al. 2019). Other circRNAs are associated with fat deposition in livestock, such as cattle, pigs, and chickens (Tian et al. 2021; Li et al. 2022b; Yang et al. 2022). Intramuscular adipogenesis in pigs is promoted by circPPARA through the promotion of intramuscular preadipocyte differentiation (Li et al. 2022b). The conserved nature of circRNAs hints at the role of these circRNAs in human adipogenesis as well. Pigs serve as a good model organism for studying circRNAs in adipogenesis. In the subcutaneous adipose tissue of Laiwu pigs and Large White pigs, many circRNAs are uniquely expressed (Li et al. 2018). The most significantly upregulated circRNA was circRNA_26852, while the most significantly downregulated circRNA was circRNA_11897, underpinning their importance in lipid metabolism and adipocyte differentiation (Li et al. 2018).

To further understand the involvement of circRNAs in adipogenesis, circRNAs were extracted from preadipocytes before and after differentiation (Sun et al. 2020). The investigators identified 4080 circRNAs that were differentially expressed, including 2215 upregulated and 1865 downregulated circRNAs (Sun et al. 2020). Among these circRNAs, the most significantly associated with visceral adipogenesis are hsa-circ-0136134, hsa-circ-0017650, and hsa-circRNA9227-1 (Sun et al. 2020). Adipogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs) was also promoted by increased levels of hsa_circ_0006859 extracted from plasma exosomes of osteoporosis patients (Zhi et al. 2021). This effect is mediated by inhibiting ROCK1 through upregulation of miR-431-5p (Zhi et al. 2021).

8.2.3 Another potential mechanism linking circRNAs to obesity involves leptin and leptin receptors. It was shown that genetically obese and diabetes (ob/ob and db/db) mice, which exhibit an obese phenotype due to the lack of leptin or the leptin receptor, had lower levels of ciRS-7 (Stoll et al. 2018).

8.2.4 One of the major circRNAs associated with obesity is circSAMD4A. circSAMD4A and its murine homolog mmu_circ_0000529 are other key regulators of adipogenesis. circSAMD4A binds to miR-138-5p to regulate adipogenesis, and upon knockdown of its homolog in mice, mmu_circ_0000529 reversed weight gain in HFD-fed mice, reduced body fat, and increased glucose tolerance and insulin sensitivity (Liu et al. 2020a, b). The proposed mechanism involves circSAMD4A activating the miR-138-5p/EZH2 axis to promote adipogenesis (Liu et al. 2020a, b).

Other circRNAs, such as circCDR1as, which absorbs miR-7-5p to activate Wnt5b expression, are involved in promoting the adipogenic differentiation of bone marrow mesenchymal stem cells (Chen et al. 2020a). Similarly, circTshz2-1 and circArhgap5-2 are essential for adipogenesis by organizing lipid biosynthesis and metabolism (Arcinas et al. 2019). Another obesity-associated circRNA, circGlis3, is believed to play a protective role in obesity. Its overexpression in obesity is believed to be involved in enhancing insulin secretion and inhibiting obesity-induced apoptosis. These functions are mediated through sponging miR-124-3p, regulating the expression of NeuroD1 and Creb1 during insulin secretion and interacting with SCOTIN to inhibit apoptosis (Liu et al. 2023).

8.2.5 CircRNAs regulate the expression of transcription factors involved in adipogenesis. circRNF111 sponges miR-27a-3p, activating proliferator-activated receptor (PPAR γ) transcription and eventually promoting adipogenesis (Zhang et al. 2022a). Another circRNA, circPPARA, also promotes adipocyte differentiation and inhibits preadipocyte differentiation in porcine tissue (Li et al. 2022b). In addition to PPAR- γ , circFUT10 upregulates the expression of PPAR- γ coactivator-1 α (PGC1 α), inhibiting adipogenic differentiation in Qinchuan bovine preadipocytes (Jiang et al. 2020). Interestingly, the proliferation and differentiation of preadipocytes can be activated by circBDP1, another circRNA (Zhang et al. 2022a).

The summary of these circRNA and their corresponding role in mediating obesity features are depicted in Fig. 5.

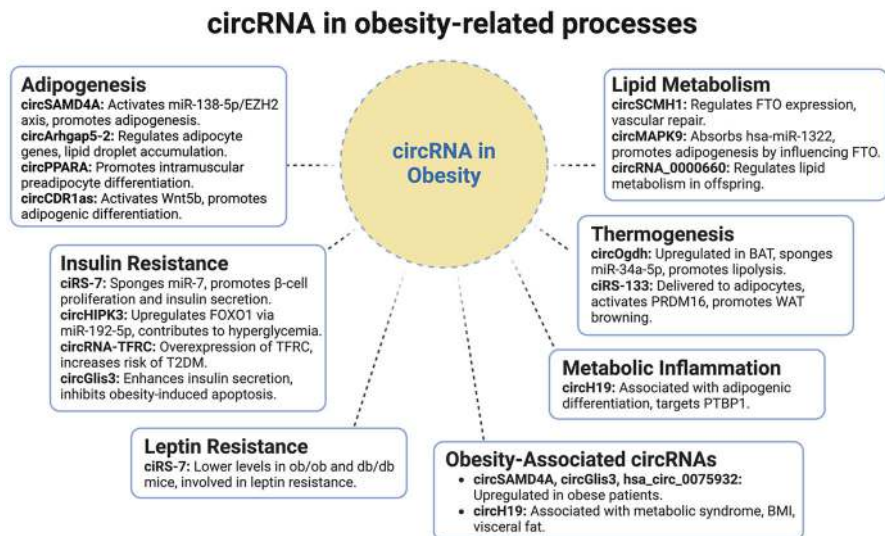


Fig. 5 Roles of circRNAs in obesity. This figure illustrates the involvement of different circRNAs in various obesity-related processes, highlighting their regulatory mechanisms and target pathways

8.3 CircRNAs Linked to Obesity-Related Metabolic Disorders, Lipolysis, and Thermogenesis

There are two types of adipose tissues, BAT and WAT. While BAT is involved in nonshivering thermogenesis, which is important for maintaining body temperature and energy balance, WAT acts as a storage for excess calories and has roles in hormone secretion and immune responses (Kershaw and Flier 2004; Peirce et al. 2014). Interestingly, BAT can be converted to WAT in a thermoneutral environment, a condition known as BAT whitening, exacerbating obesity-associated complications (Kotzbeck et al. 2018). The circRNA profiles of WAT and BAT also differ, which has been confirmed in different species, including mice, rabbits, and goats. In mice, 12,183 circRNAs were identified in adipose tissue, 165 of which were upregulated and 79 of which were downregulated in BAT versus WAT (Liu et al. 2022). Functional annotation of the differentially expressed circRNAs revealed mitochondrial pathways, thermogenesis, and lipid metabolism, including the AMPK and HIF-1 signaling pathways (Liu et al. 2022). An interesting circRNA stands out among the differentially expressed circRNAs: circOgdh. It is upregulated in BAT, localized in the cytoplasm and has a role in sponging miR-34a-5p, leading to Atgl overexpression and lipolysis. This mechanism of action of circOgdh ultimately involves lipolysis and suppression of lipid droplet accumulation (Liu et al. 2022). Many circRNAs are involved in the MAPK and VEGF signaling pathways as well as the thyroid hormone pathway (Zhang et al. 2021c). In primary BAT SVFs isolated from C57BL/6 J mice, 117 circRNAs were involved in brown adipogenesis (Zhang et al. 2021a). They were shown to play a role in cellular proliferation and thermogenesis (Zhang et al. 2021a). Finally, in plasma exosomes, ciRS-133 was shown to be upregulated in patients with gastric cancer (GC) compared to healthy controls. In GC cells, ciRS-133 is delivered to adipocytes, activates PRDM16, and is believed to promote white adipose browning (Zhang et al. 2019b).

8.4 CircRNAs that Are Specifically Linked to Obesity and Are Associated with Anti-obesity Treatment, if Applicable

Over the past few years, an upsurge in the number of studies investigating the association of circRNAs with obesity and their mechanisms of action has occurred. The deregulation of circRNAs in adipocytes has been linked to obesity. Upregulation of circSAMD4A in obese patients was reported in a recent study by Liu et al. (2020a, b). Another key circRNA in obesity is circGlis3, which has been reported to be abnormally upregulated in the pancreatic islets of obese male mice and in the serum of people with obesity (Liu et al. 2023). Similarly, hsa_circ_0075932 was shown to be more abundant in the adipose tissue-associated exosomes of patients with obesity than in those of lean subjects (Zhang et al. 2019a).

In a recent study, RNA sequencing was performed on visceral adipose tissues obtained from 30 obese patients ($\text{BMI} > 32.5 \text{ kg/m}^2$) and 30 lean subjects ($\text{BMI} < 20 \text{ kg/m}^2$) (Li et al. 2022a). The results revealed 52 differentially expressed

circRNAs between obese and lean subjects. Moreover, competing endogenous (ce) RNA networks between lncRNAs, circRNAs, and miRNAs revealed 2 key networks strongly associated with obesity, namely, circORC5/miR-197-5p/TNFRSF10D and circNTRK2/miR-760/LAT (Li et al. 2022a).

According to reports, patients with metabolic syndrome exhibit elevated levels of hsa_circH19. Its concentrations are substantially correlated with BMI, waist circumference, fat percentage, high-density lipoprotein cholesterol, and visceral fat area (Zhu et al. 2020). An essential gene associated with increased risks for T2DM, CVD, coronary artery diseases, myocardial infarction, and obesity is ANRIL (Cunnington et al. 2010; Congrains et al. 2013; Kong et al. 2016). The circRNA circANRIL regulates the expression of this gene. Nevertheless, the relationship between obesity and circANRIL has not yet been investigated. hsa_circ_0054633 was discovered to be differentially expressed in the blood of patients with T2DM compared to healthy controls in the context of T2DM, a condition that is associated with metabolic impairment and obesity (Zhao et al. 2017). hsa_circ_0054633 acts by regulating high glucose-induced human vascular endothelial cell dysfunction (Zaiou 2020). circSAMD4A is another circRNA correlated with obesity. Its levels are increased in obese patients compared to lean subjects (Liu et al. 2020a, b).

In a pediatric obese cohort, a study of 75 children with obesity and 75 control subjects revealed that hsa_circ_0046367 is downregulated while hsa_circ_0000284 is upregulated in pediatric obese individuals. These circRNAs are involved in neural plasticity mechanisms, cell secretion, and signal regulation (Li et al. 2023b).

CircRNAs are associated not only with obesity but also with complications such as obesity-related endometrial cancer. The overall circRNA abundance was shown to be lower in cancerous tissue than in adjacent noncancerous endometrial tissue (Takenaka et al. 2023). Differential expression analysis revealed 172 downregulated and 2 upregulated circRNAs (Takenaka et al. 2023).

9 CircRNAs Contribution to the Development and Progression of Cancer in Obese Individuals

The potential mechanisms by which circRNAs may bridge obesity, thermogenesis, and obesity-associated cancer via cancer hallmark pathways, as elucidated below and illustrated in Fig. 6.

9.1 Dysregulated Gene/Epigene Expression/Regulation

CircRNAs can function as miRNA sponges or ceRNAs, preventing miRNAs from binding to their target mRNAs by sequestering them. This can result in the upregulation of target genes that would otherwise be suppressed by miRNAs. Obesity and malignancy have been associated with dysregulated miRNA-mediated gene regulation. In obese individuals, the balance of miRNA regulation may be disrupted by specific circRNAs, resulting in altered expression of genes that are

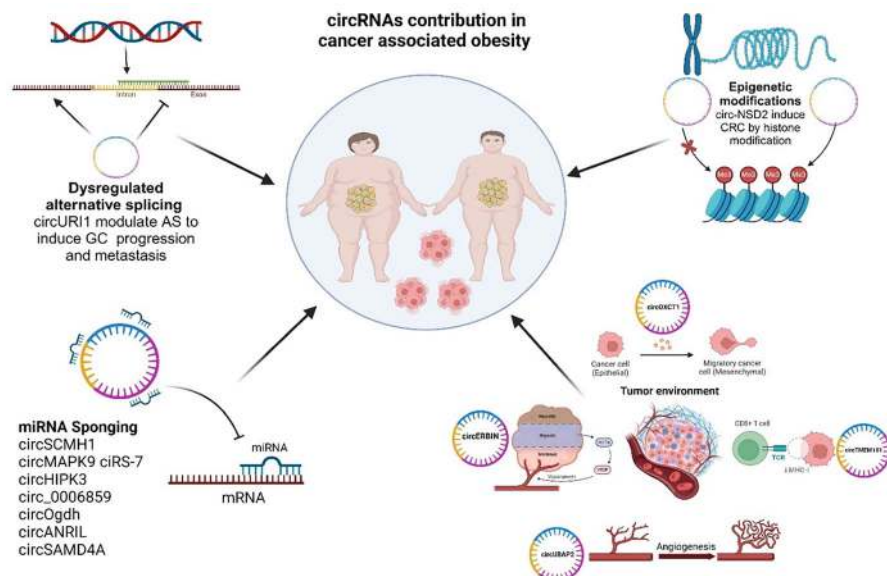


Fig. 6 circRNAs contribution in cancer-associated obesity

implicated in the development and progression of cancer. Many circRNAs that act as sponges for miRNAs have been mentioned in this chapter (circSCMH1, circMAPK9, ciRS-7, circHIPK3, circ_0006859, circOgdh, circANRIL, and circSAMD4A). The epigenetic landscape of genes involved in cancer-related processes may be influenced by circRNAs, which may also affect the recruitment of chromatin-modifying proteins such as histone methyltransferases and interactions with DNA methyltransferases. For instance, Chen et al. discovered that circ-NSD2 functions as a histone methyltransferase in CRC (Zhang et al. 2021d). This can contribute to epigenetic changes that promote oncogenic transformation and increased tumor progression (Emam et al. 2022; El-Sheikh et al. 2023).

9.2 Dysregulated Alternative Splicing

Dysregulated alternative splicing where circRNAs can interact with splicing factors and regulate their activity. This process is a characteristic of cancer and can contribute to tumor heterogeneity and altered protein functions, resulting in the generation of oncogenic isoforms or the loss of tumor-suppressive isoforms. It generates multiple mRNA isoforms from a single gene. Moreover, certain circRNAs have been identified as oncogenes or tumor suppressors in a variety of malignancies. The development and progression of cancer can be facilitated by the dysregulation of these genes, which can disrupt normal cellular processes and contribute to tumorigenesis, invasion, or metastasis, or by inhibiting apoptosis and enhancing

cell proliferation in obese individuals (Zhang et al. 2021b). For instance, Wang et al. have found that circURI1 could modulate alternative splicing to engage in cancer progression and metastasis (Wang et al. 2022).

9.3 Modulation of Various Signaling Pathways

The Wnt/ β -catenin pathway, for example, is involved in the progression of tumors, differentiation, and cell proliferation. Glucose homeostasis/metabolism is influenced by insulin signaling alteration, insulin-responsive gene dysregulation, insulin receptor substrate (IRS), insulin-regulated glucose transporter (GLUT4), and IGF (El-Mesallamy et al. 2013a, b) and its receptor (Almouh et al. 2022).

9.4 Adipose Tissue Dysfunction with Metabolic Alterations

Adipose tissue dysfunction with metabolic alterations is a hallmark of obesity and is associated with chronic inflammation and altered secretion of adipokines like FTO (Khella et al. 2017), leptin and its receptor (Swellam and Hamdy 2012), apelin (El-Mesallamy et al. 2011a, b, c; Aboouf et al. 2015), lipocalins (El-Mesallamy et al. 2012, 2013a, b), and related LX receptors (Mostafa et al. 2015), insulin receptor (El-Mesallamy et al. 2014).

Dysregulation of circRNAs in obesity can disrupt normal functioning adipose tissue and thermogenesis or energy homeostasis, leading to an inflammatory micro-environment and altered adipogenesis that promotes cancer development and progression via alterations in the peroxisome PPAR γ , CCAAT enhancer binding protein alpha (C/EBP α), and sterol regulatory element binding transcription factor 1 (SREBP-1c) UCP1 genes, which mediate miRNAs involved in such processes. Moreover, these metabolic disturbances are accompanied by IR, hyperinsulinemia, leptin resistance, apelin resistance, and dyslipidemia, creating an environment conducive to cancer development and progression (Almouh et al. 2022).

9.5 Modulation of the Tumor Microenvironment (TME) and Tumor Immune Microenvironment (TIME)

The TME is influenced by exosomal circRNAs through altered communication between cancer cells and adjacent stromal cells, which in turn affects tumor growth, invasion, and metastasis. CircRNAs upregulate the expression of immune-related genes or directly interact with immune cells, thereby contributing to the progression of cancer, through the dysregulation of the immune response through chronic low-grade inflammation in the cancer milieu. The TIME, tumor immune evasion, and resistance may be facilitated by dysregulated circRNAs in obese patients, which may contribute to the establishment of an immunosuppressive or proinflammatory

TME (Ramos et al. 2022). For instance, circ(UBAP2, OXCT1, ERBIN, and TMEM181) contribute to TME modulation (Wu et al. 2022b).

Moreover, studying immunomodulator effects and natural killer cells or LAIR-1 on the TIME or tumor cells reprogramming would constitute a hot area of research in relation to circRNAs and exosomal circRNAs (Anwar et al. 2022; Hammad et al. 2022; Elanany et al. 2023), respectively.

10 Specific circRNAs Identified as Mediators of Obesity-Associated Cancer

CircRNAs are becoming increasingly recognized for their roles in a variety of diseases, such as obesity-associated cancers, and have surfaced as critical regulators of gene expression (Su et al. 2019) (Zaiou 2020). While research in this area is ongoing and evolving, several circRNAs have been identified as potential mediators of obesity-associated cancer. Below, we list some of these circRNAs.

10.1 CircHIPK3

Circular RNA homeodomain-interacting protein kinase 3 (circHIPK3) has been implicated in a variety of malignancies that are associated with obesity, such as breast cancer (Luo et al. 2021), lung (Chen et al. 2020c), and CRC (Yan et al. 2020). CircHIPK3 expression was elevated in breast cancer, while miR-326 expression was diminished. The inhibition of cell proliferation, invasion, and migration was the consequence of circHIPK3 knockdown (Luo et al. 2021). There was a negative connection between the expression of circHIPK3 and MiR-326, suggesting that the latter is a direct target of the former. The increase of Ki-67 expression and the promotion of proliferation, migration, invasion, and apoptosis resistance were both brought about by the overexpression of circHIPK3 (Luo et al. 2021). In addition, E-cadherin, cleaved caspase-3, and Bcl-2 were downregulated, while vimentin, N-cadherin, and Bcl-2 were upregulated. When miR-326 was overexpressed, these changes in expression were partially undone (Luo et al. 2021).

Regarding circHIPK3 and CRC, Yan et al. revealed compelling insights into its role and mechanisms underlying CRC progression (Yan et al. 2020). They observed an upregulation of circHIPK3 in CRC and established a significant association between circHIPK3 expression and unfavorable clinicopathological features in CRC patients. Functional assays, including Transwell and proliferation assays, provided evidence supporting the function of circHIPK3 as an oncogene that promotes the migration, invasion, and proliferation of CRC cells. Moreover, their investigation identified formin-like 2 (FMNL2) as a crucial downstream molecule responsible for circHIPK3-induced metastasis and proliferation in CRC cells (Yan et al. 2020). Furthermore, the regulatory network involving miR-1,207-5p in CRC is further illuminated by these results.

One circRNA found in adipocytes, CircZNF609, encourages adipogenesis and adipocyte differentiation; it may have a role in the onset of obesity and obesity-related malignancies including breast cancer (Su et al. 2019; Zaiou 2020).

10.2 **CircRNAs in Inflammation and IR**

CircANRIL has been associated with inflammation and atherosclerosis (Shi et al. 2020) conditions closely linked to obesity and may contribute to cancer risk in obese individuals.

10.3 **CircRNAs in Energy Metabolism**

Insulin signaling and glucose metabolism have both been linked to circRNA 100290 (Chen et al. 2019) suggesting a potential role in linking obesity to cancer development.

10.4 **The Hypoxia Response of circRNAs**

The hypoxia response of circRNAs results in the growth of fat cells, which may hasten the development of cancer. There may be a connection between fat and cancer through HIF signaling and circRNAs that are involved in the cellular reaction to hypoxia.

The functions of several circRNAs in the onset and advancement of malignancies linked to obesity are shown in Fig. 7. At its core, the network is “Obesity-Associated Cancer,” which links to a number of circRNAs that have an impact on cancer.

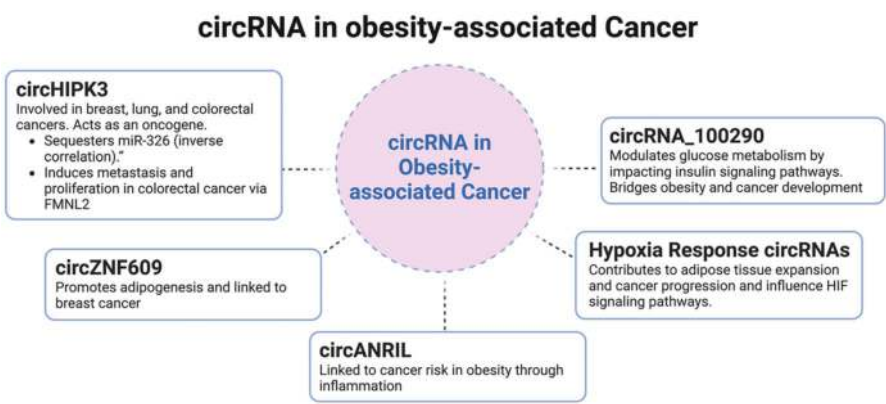


Fig. 7 CircRNAs mediating obesity-associated cancer

11 Clinical Implications

1. The potential mechanisms by which circRNAs may bridge the gap between obesity, thermogenesis, and obesity-associated cancer are the same as how these circRNAs contribute to the development and progression of cancer in obese individuals.
2. Studies have uncovered shared circRNA signatures or networks in these interconnected pathways. Therefore, no current studies are available, and more studies are required to study obesity-associated tumor-promoting immune signaling in obesity-related cancer as one side downstream of various tumor-specific circRNA-miR axes. These findings constitute the basis for cancer treatment involving these signaling pathways.
3. Potential clinical applications of circRNAs as diagnostic, prognostic, or therapeutic targets in obesity-associated cancer and metabolic disorders related to thermogenesis. Because they have no tail, are stable, do not encode protein, and have no function, they are thought to be implicated in various noncommunicable diseases, such as CVD (Zaiou 2020) and breast cancer (Gopikrishnan et al. 2023).

Because of their regulatory function, circRNAs may regulate genes involved in or related to treatment resistance, namely, genes involved in activating or metabolizing drugs or genes related to drug efflux, thereby affecting the overall response to therapy tolerance.

Because of their roles in disease etiology, ncRNAs have the potential to serve as therapeutic targets in addition to their current roles as disease molecular markers, diagnostic tools, and prognostic indicators. On Jan. 5th, 2024, the circRNADisease v2.0 database was viewed at the following URL: <http://cgga.org.cn:9091/circRNADisease/> from <http://cgga.org.cn:9091/circRNADisease/>. Tables 3, 4, 5 and 6 detail the names of circRNAs, the patterns of their expression (whether upregulated, downregulated, or dysregulated), the host genes connected with circRNAs, and sponged miRNAs in a variety of disorders related to obesity.

CircRNAs that are commonly dysregulated in both obesity-related diseases and obesity-associated cancer are presented in Fig. 8. There is one common circRNA, hsa_circ_CDR1, between obesity, some obesity-related diseases, some inflammatory diseases, and cancer and circRNAs in individuals with T2DM. Hsa_circ_MTO1 is common in obesity, some obesity-related diseases, some inflammatory diseases, and cancer and circRNA in PCOS patients.

11.1 Ongoing or Finished Registered Clinical Trials (CTs) on circRNAs in Obesity or Obesity-Associated Cancer

To present an overview of the current advances in the clinical translation of circRNAs, we searched the [ClinicalTrials.gov](https://clinicaltrials.gov) database for ongoing or finished clinical trials that focused on circRNAs in relation to obesity, obesity-related cancer,

Table 3 CircRNAs in obesity, some obesity-related diseases, some inflammatory diseases, and cancer

DO name	Name/circBase ID	HGene	miRNA	Method	Pattern in D.
Obesity-related diseases	Abdominal obesity-metabolic syndrome	hsa_circ_ARF3/-	hsa-miR-103	Q	DN
	Lipid metabolism disorder	hsa_circ_RNF111/-	hsa-miR-143-3p	Q	DN
	Acne	hsa_circ_FTO/ hsa_circ_0005941	-	Q S	DN
	Atherosclerosis	hsa_circ_SIRT1/-	hsa-miR-132, -212	Q	DN
	Thrombosis	hsa_circ_UGP2/ hsa_circ_0001020	hsa-miR-29c-3p	Q	UP
	Ovarian cancer	hsa_circ_AARS/ hsa_circ_0000714	hsa-miR-370-3p	Q	UP
Inflammatory diseases	Uterine inflammatory disease	hsa_circ_MAPK8/ hsa_circ_0002144	-	S	UP
		hsa_circ_FBRs/ hsa_circ_0005806	-	S	UP
		hsa_circ_ITGB8/ hsa_circ_0079536	-	S	DN
	Irritable bowel disease	hsa_circ_STT3A/ hsa_circ_0024766	-	S	DN
		hsa_circ_CDKN2B-AS1/- hsa-circRNA_103516/-	-	Q	DN
		-	hsa-miR-19b-1-5p	Q	UP

Obesity	hsa_circ_LPL/ hsa_circ_0136134	LPL	–	Q	M	UP
	hsa_circ_ITH5/ hsa_circ_0017650	ITH5	–	Q	M	UP
	hsa_circRNA9227-1/-	–	–	Q	M	UP
	hsa_circ_0004846/ hsa_circ_0004846	–	hsa-miR-138-5p	Q	M	UP
	hsa_circ_Tshz2-2/-	Tshz2-2	-	Q		UP
	exo_circ_24054*/ hsa_circ_0041568	ALOX15		-		UP
	hsa_circ_LRP6/-	LRP6	hsa-miR-455	S		UP
	hsa_circ_vIRF4/-	vIRF4	–	S		UP
	hsa_circ_PANs/-	PANs	–	S		UP
	hsa_circ008913/-	–	hsa-miR-889	S		DN
Cancer	hsa_circ_CTNNB1/ hsa_circ_0123778	CTNNB1	–	Q		UP
	hsa_circ_MAN1A2/-	MAN1A2	–	Q		UP
	hsa_circ_CDR1/ hsa_circ_0001946	CDR1	–	S		UP
	hsa_circRNA_001160/- -hsa_circ_0002570	–	hsa-miR-195-5p	Q		UP
	hsa_circ_RPAP2 circ_RPAP2/hsa_circ_000091	RPAP2	–	Q		DN
	hsa_circ_MTO1/-	MTO1	–	Q		UP

The pattern is either upregulated (UP) or downregulated (DN) and dysregulated (DS), and the methods of detection are RT-qPCR (Q), RNA-seq (S), and microarray (M). [DO, disease ontology.] <http://cgga.org.cn:9091/circRNADisease/> Accessed Jan. 5th, 2024

*<http://www.exorbase.org/exorBaseV2/detail/detailInfo?id=ALOX15&kind=longRNA>

Table 4 circRNAs in fatty liver diseases

DO name	Name/circBase ID	HGene	miRNA	Method	Pattern in D.
Fatty liver D.	hsa_circRNA_002082/-	–	–	Q M	DN
	hsa_circRNA_000367/-	–	–	Q M	DN
	hsa_circRNA_004183/-	–	–	Q M	DN
	hsa_circRNA_007850/-	–	–	Q M	UP
	hsa_circRNA_004121/-	–	–	Q M	UP
	hsa_circRNA_014724/-	–	–	Q M	UP
	has-circRNA_0046367/-	–	hsa-miR-34a	Q	DN
	circRNA_0046366/-	–	hsa-miR-34a	Q	DN
Nonalcoholic fatty liver D.	hsa_circ_Scd1/-	Scd1	–	Q M	DN
	-/hsa_circ_0057558	–	hsa-miR-206	Q	UP
	hsa_circ_CSPP1/ hsa_circ_0001805	CSPP1	hsa-miR-106a-5p, -320a	Q	DN
	hsa_circ_LDLR/-	LDLR	hsa-miR-667-5p	Q	DN
	hsa_circ_PI4KB/ hsa_circ_0006892	PI4KB	hsa-miR-122	Q	UP
Nonalcoholic steatohepatitis	hsa_circ_ZNF207/ hsa_circ_0043021	ZNF207	–	Q M	UP
	hsa_circ_ZNF462/ hsa_circ_0009029	ZNF462	–	Q	DS
	hsa_circ_RAI14/ hsa_circ_0004572	RAI14	–	Q	DS
	hsa_circ_AGRN/ hsa_circ_0009212	AGRN	–	Q	DS
	hsa_circ_NPHP4/ hsa_circ_0009453	NPHP4	–	Q	DS
	hsa_circ_SCAR/-	SCAR	–	Q M	DN
	hsa_circ_None/ hsa_circ_0089761	None	–	Q M	DS
	hsa_circ_None/ hsa_circ_0089763	None	–	Q M	DS
	hsa_circ_FASN/ hsa_circ_0046367	FASN	–	Q	UP

The pattern is either upregulated (UP) or downregulated (DN) and dysregulated (DS), and the methods of detection are RT-qPCR (Q) and microarray (M). [DO; disease ontology.] <http://cgga.org.cn:9091/circRNADisease/> Accessed Jan. 5th, 2024

or related types of cancer. Currently, no clinical trials have focused solely on circRNAs related to obesity, metabolic syndrome, T2DM, or CRC. However, one clinical trial was registered, but not yet recruiting, for liver cancer <https://clinicaltrials.gov/search?cond=Liver%20Cancer&intr=circular%20RNA> and one for breast cancer <https://clinicaltrials.gov/search?cond=Breast%20Cancer&intr=circular%20RNA> (Accessed July 6th, 2024). These two latter registered trial studies

Table 5 circRNAs in T2DM

Name/circBase ID	HGene	miRNA	Method	Pattern in D.
hsa_circRNA_003251/-	—	—	Q M	UP
hsa_circRNA_015115/-	—	—	Q M	UP
hsa_circRNA_100918/-	—	—	Q M	UP
hsa_circRNA_005019/-	—	—	Q M	DN
hsa_circRNA_003251/-	—	—	Q M	UP
hsa_circRNA_015115/-	—	—	Q M	UP
hsa_circRNA_100918/-	—	—	Q M	UP
hsa_circRNA_005019/-	—	—	Q M	DN
hsa_circ_HIPK3/ hsa_circ_0000284	HIPK3	hsa-miR-124-3p, -338-3p	M	DN
hsa_circ_CDR1/ hsa_circ_0001946	CDR1	hsa-miR-124-3p, -338-3p	M	DN
hsa_circRNA_1030	—	—	Q S	UP
hsa_circRNA_5333	—	—	Q S	UP
hsa_circRNA_33299	—	—	Q S	UP
hsa_circRNA_20159	—	—	Q S	DN
hsa_circRNA_37134	—	—	Q S	DN
hsa_circRNA_22054	—	—	Q S	DN
hsa_circ_HIPK3/ hsa_circ_0000284	HIPK3	hsa-miR-124	Q M	UP
hsa_circ_PNPT1/ hsa_circ_0054633	PNPT1	—	Q	UP
hsa_circ_TMEM184B/ hsa_circ_0005243	TMEM184B	—	Q S	DN
hsa_circ_3636/-	—	—	Q S	DN
hsa_circ_0395/-	—	—	Q S	DN
hsa_circ_XPNPEP3/ hsa_circ_0008360	XPNPEP3	—	Q S	DS
hsa_circ_FBXW4/ hsa_circ_0005741	FBXW4	—	Q S	DS
hsa_circ_MRRF/ hsa_circ_0003250	MRRF	—	Q S	DS
hsa_circ_BPTF/ hsa_circ_0045462	BPTF	—	Q S	DS
hsa_circ_CLASP2/ hsa_circ_0064772	CLASP2	—	Q S	DS
hsa_circ_HIF1A/ hsa_circ_0007976	HIF1A	—	Q S	DS
hsa_circ_FNDC3A/ hsa_circ_0005263	FNDC3A	—	Q S	DS
hsa_circ_TMEM184B/ hsa_circ_0005243	TMEM184B	—	Q S	DN
hsa_circ_VEGFC/-	VEGFC	hsa-miR-338-3p	Q M	UP
-/hsa_circ_0084043	ADAM9	hsa-miR-128-3p	Q	UP
-/hsa_circ_0022742	ARL2	hsa-miR-503-5p	Q	DN
hsa_circ_ARHGAP12/-	ARHGAP12	hsa-miR-301b-3p	Q S	DN
Exosomal RNA	—	—	Q M	DS

(continued)

Table 5 (continued)

Name/circBase ID	HGene	miRNA	Method	Pattern in D.
hsa_circ_SHOC2/-	SHOC2	hsa-miR-145	Q	UP
-/hsa_circ_0008028	–	hsa-miR-182-5p	Q	UP
hsa_circ_ADAM32/ hsa_circ_0084054	ADAM32	hsa-miR-508-3p	Q S	UP

The pattern is either upregulated (UP) or downregulated (DN) and dysregulated (DS), and the methods of detection are RT-qPCR (Q), RNA-seq (S) and microarray (M). <http://cgga.org.cn:9091/circRNADisease/> Accessed Jan. 5th, 2024

Table 6 circRNAs in polycystic ovary syndrome (PCOS)

Name/circBase ID	HGene	miRNA	Method	Pattern in D.
hsa_ciRNA-1880_RHOA/-	–	–	Q S	DN
hsa_ciRNA-2003_BECN1/-	–	–	Q S	DN
hsa_circRNA-6335_MAN1A2/-	–	–	Q S	DN
hsa_circRNA-12884_BPTF/-	–	–	Q S	DN
hsa_ciRNA-7323_TIAM1/-	–	–	Q S	DN
hsa_ciRNA-10775_RUVBL1/-	–	–	Q S	DN
hsa_circRNA-9160_CLEC16A/-	–	–	Q S	DN
hsa_circRNA-15112_UBAP2/-	–	–	Q S	DN
hsa_ciRNA-785_RELA/-	–	–	Q S	UP
hsa_circRNA-14867_ASH1L/-	–	–	Q S	DN
hsa_circ_RANBP9/ hsa_circ_0001577	RANBP9	–	S	UP
hsa_circ_ATRNL1/ hsa_circ_0020093	ATRNL1	–	S	DN
hsa_circ_TOP2A/ hsa_circ_0043533	TOP2A	–	M	UP
hsa_circ_KNTC1/ hsa_circ_0097636	KNTC1	–	M	DN
hsa_circ_TOP2A/ hsa_circ_0043532	TOP2A	–	M	UP
-/hsa_circ_0023942	–	–	Q M	DN
hsa_circ_PUM1/hsa_circ_0000043	PUM1	hsa-miR-760	Q	UP
hsa_circ_ASPH/-	ASPH	hsa-miR-375	Q	UP
hsa_circ_MTO1/-	MTO1	hsa-miR-320b	Q	DN
hsa_circ_RHBG/-	RHBG	hsa-miR-515	Q M	UP
hsa_circ_RANBP9/-	RANBP9	hsa-miR-136-5p	Q	UP
-/hsa_circ_0043532	TOP2A	hsa-miR-182	Q	UP
hsa_circ_FURIN/-	FURIN	hsa-miR-195-5p	Q	UP
hsa_circ_FURIN/-	FURIN	hsa-miR-423-5p	Q	UP
-/hsa_circ_0030018	–	hsa-miR-136	Q	UP
-/hsa_circ_0043533	–	hsa-miR-1179	Q	UP
-/hsa_circ_0005925	–	hsa-miR-324-3p	Q	UP
hsa_circ_SAMHD1/ hsa_circ_0115118	SAMHD1	hsa-miR-138-1-3p	Q	UP
hsa_circ_CDYL/hsa_circ_0008285	CDYL	hsa-miR-4644	Q	DN

The pattern was either upregulated (UP) or downregulated (DN), and the methods used for detection were RT-qPCR (Q) and microarray (M). <http://cgga.org.cn:9091/circRNADisease/> Accessed Jan. 5th, 2024

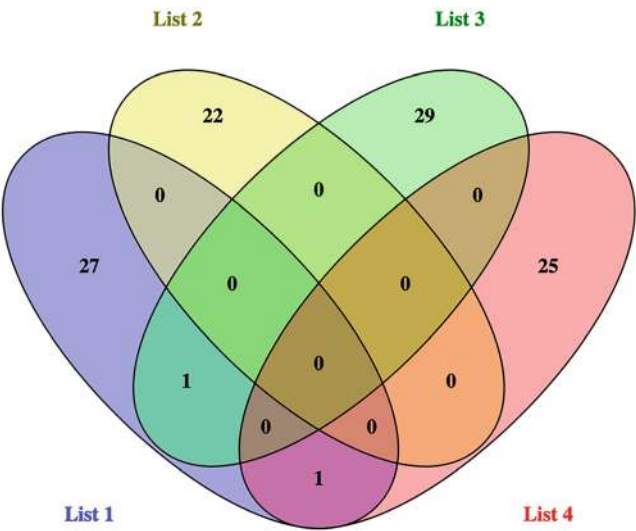


Fig. 8 Venn diagram of common circRNAs between various circRNAs in obesity and obesity-related diseases listed in the current review tables, calculated via the Venn diagram generator Venny ^{2,1} <https://bioinfogp.cnb.csic.es/tools/venny/index.html> Accessed April 12th, 2024. Where, list 1 is 29 circRNA in obesity, some obesity-related diseases, some inflammatory diseases, and cancer, list 2 consists of 22 circRNA in fatty liver diseases, list 3 is 30 circRNA in T2DM, and finally, list 4 are 26 circRNA listed in PCOS, from the previous Tables 4–7. One common circRNA in “List 1” and “List 3” was identified to be hsa_circ_CDR1, and 1 common circRNA in “List 1” and “List 4” was identified as hsa_circ_MTO1 (Two circRNAs hot areas worth further studying)

investigated the potential of certain circRNAs as diagnostic and/or predictive biomarkers.

11.2 Potential Therapeutic Strategies Targeting circRNAs in the Context of Both Obesity and Obesity-Associated Cancers and Metabolic Disorders

1. Oncogenic circRNAs were knocked down with antisense oligonucleotides or small interfering RNAs (siRNAs).
2. CircRNA sponges are created to trap circRNAs and interfere with their regulatory function.
3. CircRNA mimics were used to restore the expression of downregulated tumor suppressor circRNAs.
4. For better synergistic results, circRNA-targeted treatments should be used in conjunction with other anticancer medications or with existing drugs for metabolic disorders (He, Liu et al. 2021, Liu et al. 2021, Mao et al. 2021).

12 Challenges in Translating CircRNA Findings to Clinical Practice

Despite significant advancements, our knowledge of the precise functions and contribution mechanisms of circRNAs in various diseases is still evolving, and several mechanisms still need to be elucidated. For instance, the selective splicing mechanism that differs between circRNAs and linear RNAs, the integral factors that control the expression of circRNAs and their localization, and the functional consequences of the overlapping sequence between circRNAs and their linear mRNA counterparts (Li et al. 2021). Addressing these poorly understood molecular details could help reveal the precise downstream effects of circRNAs on cellular processes and signaling pathways.

The heterogeneity of the diseases necessitates the importance of personalized studies. As obesity is a complex issue with various mechanisms and manifestations, its role in cancer development is multifaceted (Pati et al. 2023). This heterogeneity has the potential to obscure modest alterations in circRNA expression, making it difficult to identify consistent expression patterns across individuals or disease types.

Measurements of circRNA abundance and copy number are necessary for elucidating the probable protein/miRNA binding mechanism in functional mechanism investigations. Whether we want to know whether circRNAs play a role in cooperative, decoy, or competitive regulation, we need to look at the circRNA-to-miRNA ratio. Research should focus on quantifying the amount of the protein that binds to the circRNA, since this method was also utilized to confirm the circRNA-protein binding relationships (Li et al. 2021). Some circRNAs may have difficulty affecting proteins through binding mechanisms due to their lower abundance compared to highly abundant proteins such as structural and housekeeping proteins (Ulshöfer et al. 2021). The significance of copy number measurements has been emphasized in several research, which have used this data to inform experimental approaches. For instance, Huang et al. (2019) reported that circumferential ERBB2 enhances the development of gallbladder cancer. The authors of this work counted the possible miRNA binding sites and the number of circERBB2 copies. The discovery that circERBB2 only has two binding sites for its potential miRNA targets raises the possibility that circERBB2 does not work by ensnaring miRNAs but rather by some other means.

Technical hurdles regarding the standardization of approaches related to circRNA analysis are a great challenge. Various algorithms and methodologies are employed to identify and measure circRNAs. Nevertheless, standard operating procedures for the detection and analysis of circRNAs are still lacking. The inconsistency of techniques hinders the ability to replicate and compare results between different studies, thereby limiting their therapeutic relevance (Nielsen et al. 2022). Hansen and his colleagues (Hansen et al. 2016) performed a comparative study. They noted significant discrepancies across 5 different circRNA prediction algorithms (circRNA_finder, find_circ, CIRCexplorer, CIRI, and MapSplice), particularly in relation to highly expressed circRNAs and circRNAs originating from nearby

splicing sites, highlighting the importance of cautious handling of circRNA annotation and the need to combine multiple algorithms for accurate predictions.

Moreover, developing optimization strategies for laboratory work on clinical samples is essential for the accurate and efficient assessment of circRNAs for diagnostic and therapeutic purposes (Nielsen et al. 2022). Researchers studying circRNAs are currently addressing the lack of standardized nomenclature for these molecules. The lack of uniformity in naming makes it difficult to validate experimental results and hinders a comprehensive understanding of the functional roles of circRNAs. Although several research groups have made efforts to create standardized resources for compiling circRNA data, these available databases are incomplete and not connected, forming independent entities with their own unique nomenclatures for annotating circRNAs. With this lack of uniformity, research articles that describe screenings or individual characterization of circRNAs could be filled with multiple previously recognized names and new names. As a result, replicating functional outcomes for a specific circRNA or even acquiring its sequence could be difficult (Costa and Enguita 2020).

13 Challenges in Therapeutic Applications of CircRNAs

One interesting line of inquiry might be the identification of circRNAs as possible therapeutic targets. The therapeutic promise of these techniques, however, remains unfulfilled due to the many outstanding challenges.

As circRNAs are relatively stable and resistant to degradation, current methods may not be specific or effective at targeting circRNAs (Pisignano et al. 2023). For instance, it is important to target oncogenic circRNAs in a way that does not affect the corresponding linear mRNA levels. However, using RNAi-based strategies against circRNA can result in off-target effects, which is a significant concern (He et al. 2021). Designing siRNAs to reduce off-target effects is a primary research focus for RNAi techniques against circRNAs (Zhang et al. 2018). The CRISPR/Cas13 system has demonstrated minimal mismatch tolerance and has the potential to knock down circRNAs with greater precision than RNAi (Li et al. 2022c). However, further research is needed to determine whether this approach would be effective *in vivo*.

Another obstacle that needs fixing is the creation of circular RNA for strong and consistent translation. It is common practice to pair intronic complementary sequences when using overexpression vectors. Linear RNAs or circular RNAs may be mis-spliced by this method. The unintended consequences of the mis-spliced byproducts may be harmful and vague. The lack of vectors capable of producing target circRNAs free of mis-spliced products is a current limitation. Making very pure circRNA molecules in a lab might be the key to solving this problem. Nevertheless, there are possible constraints on the therapeutic use of synthetic circRNAs produced on a wide scale. (He et al. 2021). Moreover, creating lengthy *in vitro* transcribed (IVT) RNA, ensuring sufficient protein expression, and adequately purifying circRNA is a challenging task that needs to be addressed.

However, there are some differences regarding circular splice variants of lncRNAs that can occur in some diseases.

Nuclear ciRNA knockdown and minimal enrichment of microRNA target sites may have led to decreased expression of the parent genes according to Fang et al. (2018).

CircRNAs can encode proteins according to circRNADb version 1.0 http://reprod.njmu.edu.cn/cgi-bin/circrnadb/broProtein_front.php (accessed Jan. 4th, 2024).

There are 6,608 circRNAs with protein-coding potential. There were 72 circRNAs with protein expression evidence, and there are 21 circRNAs with protein-coding potential and expression evidence.

Intricate regulatory networks in which circRNAs interact with other ncRNAs, such as lncRNAs and microRNAs, affect obesity gene expression and cellular processes.

However, insufficient data concerning all circRNA-potential axes and various new cancer hallmark aspects of differentially expressed circRNAs in various obesity-related cancers in clinical cases remain to be fully elucidated. CircRNAs involved in the TIME as promoters of inflammation and/or resistance are still lacking.

14 Sustainability and Future Directions

Several downstream signaling targets of circRNA-miRs have been identified for multiple fatty liver diseases, PCOS, cancer, obesity, T2DM, and inflammatory diseases. They could be further studied across cancer types to highlight their potential utility (future perspectives).

Hitting the hsa-circRNA-miR-downstream signaling pathway target axis using drug repurposing by molecular computations followed by proving the chosen drug (s) experimentally, as well as designing targeted nano formulas or loaded into exosomes, to fulfill the precision treatment of ncRNAs for improved health (SDG #3).

15 Summary

Knowledge of the relationships between obesity-related circRNAs and obesity-related cancer circRNAs via in silico databases, bioinformatics analysis, and extensive literature searches has provided comprehensive information, especially concerning circRNA biological functions in obesity, molecular mechanisms of circRNAs participating in obesity-related cancer genes, hallmarks, and target miR sponges as well as their final downstream genes or signaling pathway(s).

They play active roles in the progression of obesity and carcinogenesis, metastasis, and resistance. In the context of cancer, the circRNA/miRNA/ mRNA axis or circRNA/lncRNA/miRNA interaction arm plays a major role in cancer promotion or

resistance. CircRNAs sponge microRNAs to function as oncogenes or activators of tumor suppressors, deregulating pathways contributing to gene expression related to drug resistance. Gaining insight into circRNA dysregulation in various obesity-related cancer diseases could aid in identifying effective therapeutic targets.

References

- Abaza T, El-Aziz MKA, Daniel KA, Karousi P, Papatsirou M, Fahmy SA, Hamdy NM, Kontos CK, Youness RA (2023) Emerging role of circular RNAs in hepatocellular carcinoma immunotherapy. *Int J Mol Sci* 24(22):16484
- Abd El Fattah YK, Abulsoud AI, AbdelHamid SG, AbdelHalim S, Hamdy NM (2023) CCDC144NL-AS1/hsa-miR-143-3p/HMGA2 interaction: in-silico and clinically implicated in CRC progression, correlated to tumor stage and size in case-controlled study; step toward ncRNA precision. *Int J Biol Macromol* 253:126739
- Abdelmohsen K, Panda AC, Munk R, Grammatikakis I, Dudekula DB, De S, Kim J, Noh JH, Kim KM, Martindale JL, Gorospe M (2017) Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1. *RNA Biol* 14(3):361–369
- Aboouf MA, Hamdy NM, Amin AI, El-Mesallamy HO (2015) Genotype screening of APLN rs3115757 variant in Egyptian women population reveals an association with obesity and insulin resistance. *Diabetes Res Clin Pract* 109(1):40–47
- Aboouf MA, Gorr TA, Hamdy NM, Gassmann M, Thiersch M (2023) Myoglobin in Brown adipose tissue: a multifaceted player in thermogenesis. *Cells* 12(18):2240
- Agurs-Collins T, Ross SA, Dunn BK (2019) The many faces of obesity and its influence on breast cancer risk. *Front Oncol* 9:765
- Almouh M, Razmara E, Bitaraf A, Ghazimoradi MH, Hassan ZM, Babashah S (2022) Circular RNAs play roles in regulatory networks of cell signaling pathways in human cancers. *Life Sci* 309:120975
- Alnabulsi A, Cash B, Hu Y, Silina L, Alnabulsi A, Murray GI (2019) The expression of brown fat-associated proteins in colorectal cancer and the relationship of uncoupling protein 1 with prognosis. *Int J Cancer* 145(4):1138–1147
- Al-Shami K, Awadi S, Khamees A, Alsheikh AM, Al-Sharif S, Ala' Bereshy R, Al-Eitan SF, Banikhaled SH, Al-Qudimat AR, Al-Zoubi RM, Al Zoubi MS (2023) Estrogens and the risk of breast cancer: a narrative review of literature. *Heliyon* 9(9):e20224
- Anwar MM, Albanese C, Hamdy NM, Sultan AS (2022) Rise of the natural red pigment 'prodigiosin' as an immunomodulator in cancer. *Cancer Cell Int* 22(1):419
- Arcinas C, Tan W, Fang W, Desai TP, Teh DCS, Degirmenci U, Xu D, Foo R, Sun L (2019) Adipose circular RNAs exhibit dynamic regulation in obesity and functional role in adipogenesis. *Nat Metab* 1(7):688–703
- Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evantal N, Memczak S, Rajewsky N, Kadener S (2014) circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell* 56(1):55–66
- Bach DH, Lee SK, Sood AK (2019) Circular RNAs in cancer. *Mol Ther Nucleic Acids* 16:118–129
- Bagheri Moghaddam M, Maleki M, Oveisee M, Bagheri Moghaddam M, Arabian M, Malakootian M (2022) Circular RNAs: new players in cardiomyopathy. *Genes (Basel)* 13(9)
- Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychemuller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B, Nielsen P, Merkel M, Heeren J (2011) Brown adipose tissue activity controls triglyceride clearance. *Nat Med* 17(2):200–205
- Bergers G, Fendt SM (2021) The metabolism of cancer cells during metastasis. *Nat Rev Cancer* 21(3):162–180

- Bracci PM (2012) Obesity and pancreatic cancer: overview of epidemiologic evidence and biologic mechanisms. *Mol Carcinog* 51(1):53–63
- Cai H, Jiang Z, Yang X, Lin J, Cai Q, Li X (2020) Circular RNA HIPK3 contributes to hyperglycemia and insulin homeostasis by sponging miR-192-5p and upregulating transcription factor forkhead box O1. *Endocr J* 67(4):397–408
- Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. *Physiol Rev* 84(1):277–359
- Chen L-L (2016) The biogenesis and emerging roles of circular RNAs. *Nat Rev Mol Cell Biol* 17(4):205–211
- Chen Z, Kang Y (2023) Cold snap for cancer: cold-induced brown fat thermogenesis starves tumor growth. *Signal Transduct Target Ther* 8(1):10
- Chen LL, Yang L (2015) Regulation of circRNA biogenesis. *RNA Biol* 12(4):381–388
- Chen X, Yu J, Tian H, Shan Z, Liu W, Pan Z, Ren J (2019) Circle RNA hsa_circRNA_100290 serves as a ceRNA for miR-378a to regulate oral squamous cell carcinoma cells growth via glucose transporter-1 (GLUT1) and glycolysis. *J Cell Physiol* 234(11):19130–19140
- Chen G, Wang Q, Li Z, Yang Q, Liu Y, Du Z, Zhang G, Song Y (2020a) Circular RNA CDR1as promotes adipogenic and suppresses osteogenic differentiation of BMSCs in steroid-induced osteonecrosis of the femoral head. *Bone* 133:115258
- Chen Q, Liu M, Luo Y, Yu H, Zhang J, Li D, He Q (2020b) Maternal obesity alters circRNA expression and the potential role of mmu_circRNA_0000660 via sponging miR_693 in offspring liver at weaning age. *Gene* 731:144354
- Chen X, Mao R, Su W, Yang X, Geng Q, Guo C, Wang Z, Wang J, Kresty LA, Beer DG, Chang AC, Chen G (2020c) Circular RNA circHIPK3 modulates autophagy via MIR124-3p-STAT3-PRKAA/AMPKalpha signaling in STK11 mutant lung cancer. *Autophagy* 16(4):659–671
- Chen C, Zhang X, Deng Y, Cui Q, Zhu J, Ren H, Liu Y, Hu X, Zuo J, Peng Y (2021) Regulatory roles of circRNAs in adipogenesis and lipid metabolism: emerging insights into lipid-related diseases. *FEBS J* 288(12):3663–3682
- Chen L, Huang C, Shan G (2022) Circular RNAs in physiology and non-immunological diseases. *Trends Biochem Sci* 47(3):250–264
- Chen S, Song P, Wang Y, Wang Z, Xue J, Jiang Y, Zhou Y, Zhao J, Tang L (2023) CircMAPK9 promotes adipogenesis through modulating hsa-miR-1322/FTO axis in obesity. *iScience* 26(10):107756
- Chu Q, Zhang X, Zhu X, Liu C, Mao L, Ye C, Zhu QH, Fan L (2017) PlantcircBase: a database for plant circular RNAs. *Mol Plant* 10(8):1126–1128
- Chu K, Bos SA, Gill CM, Torriani M, Bredella MA (2020) Brown adipose tissue and cancer progression. *Skeletal Radiol* 49(4):635–639
- Cifarelli V, Beeman SC, Smith GI, Yoshino J, Morozov D, Beals JW, Kayser BD, Watrous JD, Jain M, Patterson BW, Klein S (2020) Decreased adipose tissue oxygenation associates with insulin resistance in individuals with obesity. *J Clin Invest* 130(12):6688–6699
- Collaboration, N. C. D. R. F (2024) Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *Lancet* 403(10431):1027–1050
- Congrains A, Kamide K, Ohishi M, Rakugi H (2013) ANRIL: molecular mechanisms and implications in human health. *Int J Mol Sci* 14(1):1278–1292
- Costa MC, Enguita FJ (2020) Towards a universal nomenclature standardization for circular RNAs. *Non-coding RNA Investig* 4
- Cunnington MS, Santibanez Koref M, Mayosi BM, Burn J, Keavney B (2010) Chromosome 9p21 SNPs associated with multiple disease phenotypes correlate with ANRIL expression. *PLoS Genet* 6(4):e1000899
- Dandare A, Khan MJ, Naeem A, Liaquat A (2023) Clinical relevance of circulating non-coding RNAs in metabolic diseases: emphasis on obesity, diabetes, cardiovascular diseases and metabolic syndrome. *Genes & Diseases* 10(6):2393–2413

- de Mello AH, Costa AB, Engel JDG, Rezin GT (2018) Mitochondrial dysfunction in obesity. *Life Sci* 192:26–32
- Di Agostino S, Riccioli A, De Cesaris P, Fontemaggi G, Blandino G, Filippini A, Fazi F (2020) Circular RNAs in embryogenesis and cell differentiation with a focus on cancer development. *Front Cell Dev Biol* 8:389
- Din MU, Saari T, Raiko J, Kudomi N, Maurer SF, Lahesmaa M, Fromme T, Amri EZ, Klingenspor M, Solin O, Nuutila P, Virtanen KA (2018) Postprandial oxidative metabolism of human Brown fat indicates thermogenesis. *Cell Metab* 28(2):207–+
- Ding Z, Sun D, Han J, Shen L, Yang F, Sah S, Sui X, Wu G (2021) Novel noncoding RNA CircPTK2 regulates lipolysis and adipogenesis in cachexia. *Mol Metabol* 53:101310
- Donohoe F, Wilkinson M, Baxter E, Brennan DJ (2020) Mitogen-activated protein kinase (MAPK) and obesity-related cancer. *Int J Mol Sci* 21(4)
- Eisenstein M, Garber K, Landhuis E, DeFrancesco L (2022) Nature biotechnology's academic spinouts 2021. *Nat Biotechnol* 40(11):1551
- Elanany MM, Mostafa D, Hamdy NM (2023) Remodeled tumor immune microenvironment (TIME) parade via natural killer cells reprogramming in breast cancer. *Life Sci* 330:121997
- Eldosoky MA, Hammad R, Elmadbouly AA, Aglan RB, AbdelHamid SG, Alboraie M, Hassan DA, Shaheen MA, Rushdi A, Ahmed RM, Abdelbadea A, Abdelmageed NA, Elshafei A, Ali E, Abo-Elkheir OI, Zaky S, Hamdy NM, Lambert C (2023) Diagnostic significance of hsa-miR-21-5p, hsa-miR-192-5p, hsa-miR-155-5p, hsa-miR-199a-5p panel and ratios in hepatocellular carcinoma on top of liver cirrhosis in HCV-infected patients. *Int J Mol Sci* 24(4):3157
- Eljalby M, Huang X, Becher T, Wibmer AG, Jiang CS, Vaughan R, Schoder H, Cohen P (2023) Brown adipose tissue is not associated with cachexia or increased mortality in a retrospective study of patients with cancer. *Am J Physiol Endocrinol Metab* 324(2):E144–E153
- El-Mesallamy H, Hamdy N, Suwailem S, Mostafa S (2010) Oxidative stress and platelet activation: markers of myocardial infarction in type 2 diabetes mellitus. *Angiology* 61(1):14–18
- El-Mesallamy HO, Hamdy NM, Rizk HH, El-Zayadi A-R (2011a) Apelin serum level in Egyptian patients with chronic hepatitis C. *Mediat Inflamm* 2011(1):703031
- El-Mesallamy H, Hamdy N, Salman T, Mahmoud S (2011b) Adiponectin and E-selectin concentrations in relation to inflammation in obese type 2 diabetic patients with coronary heart disease (s). *Minerva Endocrinol* 36(3):163–170
- El-Mesallamy HO, Hamdy NM, Ibrahim SM (2011c) Adiponectin and pro-inflammatory cytokines in obese diabetic boys. *Ind Pediatr* 48(10):815–816
- El-Mesallamy HO, Hamdy NM, Zaghloul AS, Sallam AM (2012a) Serum retinol binding protein-4 and neutrophil gelatinase-associated lipocalin are interrelated in pancreatic cancer patients. *Scand J Clin Lab Invest* 72(8):602–607
- El-Mesallamy HO, Hamdy NM, Salman TM, Ibrahim SM (2012b) Adiponectin and sE-selectin concentrations in relation to inflammation in obese type 2 diabetic patients with coronary heart disease. *Angiology* 63(2):96–102
- El-Mesallamy HO, Hamdy NM, Zaghloul AS, Sallam AM (2013a) Clinical value of circulating lipocalins and insulin-like growth factor axis in pancreatic cancer diagnosis. *Pancreas* 42(1):149–154
- El-Mesallamy HO, Hamdy NM, Sallam A-AM (2013b) Effect of obesity and glycemic control on serum lipocalins and insulin-like growth factor axis in type 2 diabetic patients. *Acta Diabetol* 50:679–685
- El-Mesallamy HO, Hamdy NM, Mostafa DM, Amin AI (2014) The serine protease granzyme B as an inflammatory marker, in relation to the insulin receptor cleavage in human obesity and type 2 diabetes mellitus. *J Interf Cytokine Res* 34(3):179–186
- El-Sheikh NM, Abulsoud AI, Fawzy A, Wasfey EF, Hamdy NM (2023) LncRNA NNT-AS1/hsa-miR-485-5p/HSP90 axis in-silico and clinical prospect correlated-to histologic grades-based CRC stratification: a step toward ncRNA precision. *Pathol Res Pract* 247:154570
- Emam O, Wasfey EF, Hamdy NM (2022) Notch-associated lncRNAs profiling circuiting epigenetic modification in colorectal cancer. *Cancer Cell Int* 22(1):316

- Fan W, Pang H, Xie Z, Huang G, Zhou Z (2022) Circular RNAs in diabetes mellitus and its complications. *Front Endocrinol (Lausanne)* 13:885650
- Fang Y, Wang X, Li W, Han J, Jin J, Su F, Zhang J, Huang W, Xiao F, Pan Q (2018) Screening of circular RNAs and validation of circANKRD36 associated with inflammation in patients with type 2 diabetes mellitus. *Int J Mol Med* 42(4):1865–1874
- Faraj GSH, Hussien BM, Abdullah SR, Rasul MF, Hajiesmaeili Y, Baniahmad A, Taheri M (2024) Advanced approaches of the use of circRNAs as a replacement for cancer therapy. *Non-coding RNA Res*
- Fasshauer M, Bluher M (2015) Adipokines in health and disease. *Trends Pharmacol Sci* 36(7):461–470
- Frezza EE, Wachtel MS, Chiriva-Internati M (2006) Influence of obesity on the risk of developing colon cancer. *Gut* 55(2):285–291
- Gallagher EJ, LeRoith D (2010) The proliferating role of insulin and insulin-like growth factors in cancer. *Trends Endocrinol Metab* 21(10):610–618
- Golozoubova V, Hohtola E, Matthias A, Jacobsson A, Cannon B, Nedergaard J (2001) Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *FASEB J* 15(9):2048–+
- Gopikrishnan M, Ashour HM, Pintus G, Hammad M, Kashyap MK, Zayed H (2023) Therapeutic and diagnostic applications of exosomal circRNAs in breast cancer. *Funct Integr Genomics* 23(2):184
- Grisoaurd J, Dembinski K, Mayer D, Keller U, Muller B, Christ-Crain M (2011) Targeting AMP-activated protein kinase in adipocytes to modulate obesity-related adipokine production associated with insulin resistance and breast cancer cell proliferation. *Diabetol Metab Syndr* 3:16
- Gu A, Jaijyan DK, Yang S, Zeng M, Pei S, Zhu H (2023) Functions of circular RNA in human diseases and illnesses. *Non-coding RNA* 9(4):38
- Guarnerio J, Bezzi M, Jeong JC, Paffenholz SV, Berry K, Naldini MM, Lo-Coco F, Tay Y, Beck AH, Pandolfi PP (2016) Oncogenic role of fusion-circRNAs derived from cancer-associated chromosomal translocations. *Cell* 165(2):289–302
- Hamdy NM, Zaki MB, Rizk NI, Abdelmaksoud NM, Abd-Elmawla MA, Ismail RA, Abulsoud AI (2024) Unraveling the ncRNA landscape that governs colorectal cancer: a roadmap to personalized therapeutics. *Life Sci* 354:122946
- Hammad R., R. B. Aglan, S. A. Mohammed, E. A.-e. Awad, M. A. Elsaid, H. M. Bedair, S. K. Khirala, M. A. Selim, A. A. Abo Elqasem and A. Rushdi (2022). "Cytotoxic T cell expression of leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) in viral hepatitis C-mediated hepatocellular carcinoma." *Int J Mol Sci* 23(20): 12541
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674
- Hankir MK, Klingenspor M (2018) Brown adipocyte glucose metabolism: a heated subject. *EMBO Rep* 19(9)
- Hansen TB, Venø MT, Damgaard CK, Kjems J (2016) Comparison of circular RNA prediction tools. *Nucleic Acids Res* 44(6):e58–e58
- He AT, Liu J, Li F, Yang BB (2021) Targeting circular RNAs as a therapeutic approach: current strategies and challenges. *Signal Transduct Target Ther* 6(1):185
- Hotamisligil GS, Davis RJ (2016) Cell signaling and stress responses. *Cold Spring Harb Perspect Biol* 8(10):a006072
- Huang X, He M, Huang S, Lin R, Zhan M, Yang D, Shen H, Xu S, Cheng W, Yu J (2019) Circular RNA circERBB2 promotes gallbladder cancer progression by regulating PA2G4-dependent rDNA transcription. *Mol Cancer* 18:1–19
- Inzani I, Ozanne SE (2022) Programming by maternal obesity: a pathway to poor cardiometabolic health in the offspring. *Proc Nutr Soc* 81(3):227–242
- Jager S, Wahl S, Kroger J, Sharma S, Hoffmann P, Floegel A, Pischon T, Prehn C, Adamski J, Muller-Nurasyid M, Waldenberger M, Strauch K, Peters A, Gieger C, Suhre K, Grallert H, Boeing H, Schulze MB, Meidtner K (2017) Genetic variants including markers from the exome chip and metabolite traits of type 2 diabetes. *Sci Rep* 7(1):6037

- Jeck WR, Sharpless NE (2014) Detecting and characterizing circular RNAs. *Nat Biotechnol* 32(5): 453–461
- Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE (2013) Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 19(2):141–157
- Jiang R, Li H, Yang J, Shen X, Song C, Yang Z, Wang X, Huang Y, Lan X, Lei C (2020) circRNA profiling reveals an abundant circFUT10 that promotes adipocyte proliferation and inhibits adipocyte differentiation via sponging let-7. *Mol Ther Nucl Acids* 20:491–501
- Jones A, Danielson KM, Benton MC, Ziegler O, Shah R, Stubbs RS, Das S, Macartney-Coxson D (2017) miRNA signatures of insulin resistance in obesity. *Obesity (Silver Spring)* 25(10): 1734–1744
- Kereliuk SM, Dolinsky VW (2022) Recent experimental studies of maternal obesity, diabetes during pregnancy and the developmental origins of cardiovascular disease. *Int J Mol Sci* 23(8):4467
- Kershaw EE, Flier JS (2004) Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89(6): 2548–2556
- Khella MS, Hamdy NM, Amin AI, El-Mesallamy HO (2017) The (FTO) gene polymorphism is associated with metabolic syndrome risk in Egyptian females: a case-control study. *BMC Med Genet* 18:1–8
- Kim SH, Plutzky J (2016) Brown fat and Browning for the treatment of obesity and related metabolic disorders. *Diabetes Metab J* 40(1):12–21
- Kong Y, Sharma RB, Nwosu BU, Alonso LC (2016) Islet biology, the CDKN2A/B locus and type 2 diabetes risk. *Diabetologia* 59(8):1579–1593
- Kotzbeck P, Giordano A, Mondini E, Murano I, Severi I, Venema W, Cecchini MP, Kershaw EE, Barbatelli G, Haemmerle G, Zechner R, Cinti S (2018) Brown adipose tissue whitening leads to brown adipocyte death and adipose tissue inflammation. *J Lipid Res* 59(5):784–794
- Lavalette C, Tretarre B, Rebillard X, Lamy PJ, Cenee S, Menegaux F (2018) Abdominal obesity and prostate cancer risk: epidemiological evidence from the EPICAP study. *Oncotarget* 9(77): 34485–34494
- Lee ECS, Elhassan SAM, Lim GPL, Kok WH, Tan SW, Leong EN, Tan SH, Chan EWL, Bhattamisra SK, Rajendran R (2019) The roles of circular RNAs in human development and diseases. *Biomed Pharmacother* 111:198–208
- Legnini I, Di Timoteo G, Rossi F, Morlando M, Briganti F, Sthandier O, Fatica A, Santini T, Andronache A, Wade M (2017) Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. *Mol Cell* 66(1):22–37. e29
- Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H, Shan G (2015) Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol* 22(3):256–264
- Li A, Huang W, Zhang X, Xie L, Miao X (2018) Identification and characterization of CircRNAs of two pig breeds as a new biomarker in metabolism-related diseases. *Cell Physiol Biochem* 47(6): 2458–2470
- Li F, Yang Q, He AT, Yang BB (2021) Circular RNAs in cancer: limitations in functional studies and diagnostic potential. Elsevier, *Seminars in cancer biology*
- Li G, Zhang H, Cao K, Zhai Z, Wang Z, Yang L, Han J (2022a) Transcriptome of visceral adipose tissue identifies an inflammation-related ceRNA network that regulates obesity. *Mol Cell Biochem* 477(4):1095–1106
- Li B, He Y, Wu W, Tan X, Wang Z, Irwin DM, Wang Z, Zhang S (2022b) Circular RNA profiling identifies novel circPPARA that promotes intramuscular fat deposition in pigs. *J Agric Food Chem* 70(13):4123–4137
- Li S, Wu H, Chen L-L (2022c) Screening circular RNAs with functional potential using the RfxCas13d/BSJ-gRNA system. *Nat Protoc* 17(9):2085–2107
- Li B, Xi W, Bai Y, Liu X, Zhang Y, Li L, Bian L, Liu C, Tang Y, Shen L, Yang L, Gu X, Xie J, Zhou Z, Wang Y, Yu X, Wang J, Chao J, Han B, Yao H (2023a) FTO-dependent m(6)a modification of Plpp3 in circSCMH1-regulated vascular repair and functional recovery following stroke. *Nat Commun* 14(1):489

- Li G, Xu X, Yang L, Cai Y, Sun Y, Guo J, Lin Y, Hu Y, Chen M, Li H, Wu S (2023b) Exploring the association between circRNA expression and pediatric obesity based on a case-control study and related bioinformatics analysis. *BMC Pediatr* 23(1):561
- Liang HF, Zhang XZ, Liu BG, Jia GT, Li WL (2017) Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. *Am J Cancer Res* 7(7): 1566–1576
- Lichtman MA (2010) Obesity and the risk for a hematological malignancy: leukemia, lymphoma, or myeloma. *Oncologist* 15(10):1083–1101
- Linkeviciute-Ulinskiene D, Patasius A, Zabuliene L, Stukas R, Smailyte G (2019) Increased risk of site-specific cancer in people with type 2 diabetes: a national cohort study. *Int J Environ Res Public Health* 17(1)
- Liu PH, Wu K, Ng K, Zauber AG, Nguyen LH, Song M, He X, Fuchs CS, Ogino S, Willett WC, Chan AT, Giovannucci EL, Cao Y (2019) Association of Obesity with Risk of early-onset colorectal cancer among women. *JAMA Oncol* 5(1):37–44
- Liu Y, Liu H, Li Y, Mao R, Yang H, Zhang Y, Zhang Y, Guo P, Zhan D, Zhang T (2020a) Circular RNA SAMD4A controls adipogenesis in obesity through the miR-138-5p/EZH2 axis. *Theranostics* 10(10):4705–4719
- Liu Y, Liu H, Li Y, Mao R, Yang H, Zhang Y, Zhang Y, Guo P, Zhan D, Zhang T (2020b) Circular RNA SAMD4A controls adipogenesis in obesity through the miR-138-5p/EZH2 axis. *Theranostics* 10(10):4705
- Liu C, Li N, Dai G, Cavdar O, Fang H (2021) A narrative review of circular RNAs as potential biomarkers and therapeutic targets for cardiovascular diseases. *Ann Transl Med* 9(7)
- Liu K, Liu X, Deng Y, Li Z, Tang A (2022) CircRNA-mediated regulation of brown adipose tissue adipogenesis. *Front Nutr* 9:926024
- Liu Y, Yang Y, Xu C, Liu J, Chen J, Li G, Huang B, Pan Y, Zhang Y, Wei Q, Pandol SJ, Zhang F, Li L, Jin L (2023) Circular RNA circGlis3 protects against islet beta-cell dysfunction and apoptosis in obesity. *Nat Commun* 14(1):351
- Luo N, Liu S, Li X, Hu Y, Zhang K (2021) Circular RNA circHIPK3 promotes breast cancer progression via sponging MiR-326. *Cell Cycle* 20(13):1320–1333
- Lv X, Li J, Zhang C, Hu T, Li S, He S, Yan H, Tan Y, Lei M, Wen M, Zuo J (2017) The role of hypoxia-inducible factors in tumor angiogenesis and cell metabolism. *Genes Dis* 4(1):19–24
- Mahmoud MM, Sanad EF, Elshimy RAA, Hamdy NM (2021a) Competitive endogenous role of the LINC00511/miR-185-3p Axis and miR-301a-3p from liquid biopsy as molecular markers for breast cancer diagnosis. *Front Oncol* 11
- Mahmoud MM, Sanad EF, Hamdy NM (2021b) MicroRNAs' role in the environment-related non-communicable diseases and link to multidrug resistance, regulation, or alteration. *Environ Sci Pollut Res* 28(28):36984–37000
- Mair KM, Gaw R, MacLean MR (2020) Obesity, estrogens and adipose tissue dysfunction – implications for pulmonary arterial hypertension. *Pulm Circ* 10(3):2045894020952019
- Mandic M, Safizadeh F, Niedermaier T, Hoffmeister M, Brenner H (2023) Association of Overweight, obesity, and recent weight loss with colorectal cancer risk. *JAMA Netw Open* 6(4): e239556
- Mao X, Cao Y, Guo Z, Wang L, Xiang C (2021) Biological roles and therapeutic potential of circular RNAs in osteoarthritis. *Mol Ther Nucl Acids* 24:856–867
- Mazziotta C, Badiale G, Cervellera CF, Tognon M, Martini F, Rotondo JC (2024) Regulatory mechanisms of circular RNAs during human mesenchymal stem cell osteogenic differentiation. *Theranostics* 14(1):143–158
- Mills EL, Pierce KA, Jedrychowski MP, Garrity R, Winther S, Vidoni S, Yoneshiro T, Spinelli JB, Lu GZ, Kazak L, Banks AS, Haigis MC, Kajimura S, Murphy MP, Gygi SP, Clish CB, Chouchani ET (2018) Accumulation of succinate controls activation of adipose tissue thermogenesis. *Nature* 560(7716):102–106
- Mostafa AM, Hamdy NM, El-Mesallamy HO, Abdel-Rahman SZ (2015) Glucagon-like peptide 1 (GLP-1)-based therapy upregulates LXR-ABCA1/ABCG1 cascade in adipocytes. *Biochem Biophys Res Commun* 468(4):900–905

- Mostafa AM, Hamdy NM, Abdel-Rahman SZ, El-Mesallamy HO (2016) Effect of vildagliptin and pravastatin combination on cholesterol efflux in adipocytes. *IUBMB Life* 68(7):535–543
- Nielsen AF, Bindereif A, Bozzoni I, Hanan M, Hansen TB, Irimia M, Kadener S, Kristensen LS, Legnini I, Morlando M (2022) Best practice standards for circular RNA research. *Nat Methods* 19(10):1208–1220
- Nuotio J, Laitinen TT, Sinaiko AR, Woo JG, Urbina EM, Jacobs DR Jr, Steinberger J, Prineas RJ, Sabin MA, Burgner DP, Minn H, Burns TL, Bazzano LA, Venn AJ, Viikari JSA, Hutri-Kahonen N, Daniels SR, Raitakari OT, Magnussen CG, Juonala M, Dwyer T (2022) Obesity during childhood is associated with higher cancer mortality rate during adulthood: the i3C consortium. *Int J Obes* 46(2):393–399
- Pati S, Irfan W, Jameel A, Ahmed S, Shahid RK (2023) Obesity and cancer: a current overview of epidemiology, pathogenesis, outcomes, and management. *Cancers* 15(2):485
- Peirce V, Carobbio S, Vidal-Puig A (2014) The different shades of fat. *Nature* 510(7503):76–83
- Phoomak C, Vaeteewoottacharn K, Silsirivanit A, Saengboonmee C, Seubwai W, Sawanyawisuth K, Wongkham C, Wongkham S (2017) High glucose levels boost the aggressiveness of highly metastatic cholangiocarcinoma cells via O-GlcNAcylation. *Sci Rep* 7:43842
- Pisignano G, Michael DC, Visal TH, Pirlog R, Ladomery M, Calin GA (2023) Going circular: history, present, and future of circRNAs in cancer. *Oncogene* 42(38):2783–2800
- Qu L, Yi Z, Shen Y, Lin L, Chen F, Xu Y, Wu Z, Tang H, Zhang X, Tian F (2022) Circular RNA vaccines against SARS-CoV-2 and emerging variants. *Cell* 185(10):1728–1744. e1716
- Radwan SM, Hamdy NM, Hegab HM, El-Mesallamy HO (2016) Beclin-1 and hypoxia-inducible factor-1 α genes expression: potential biomarkers in acute leukemia patients. *Cancer Biomark* 16(4):619–626
- Ramos RN, Amano MT, Paes Leme AF, Fox JW, de Oliveira AK (2022) Tumor microenvironment (TME) and tumor immune microenvironment (TIME): new perspectives for prognosis and therapy. *Frontiers Media SA* 10:971275
- Rodriguez AC, Blanchard Z, Maurer KA, Gertz J (2019) Estrogen signaling in endometrial cancer: a key oncogenic pathway with several open questions. *Horm Cancer* 10(2–3):51–63
- Saito M, Matsushita M, Yoneshiro T, Okamatsu-Ogura Y (2020) Brown adipose tissue, diet-induced thermogenesis, and Thermogenic food ingredients: from mice to men. *Front Endocrinol (Lausanne)* 11:222
- Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO (2012) Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 7(2)
- Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK (1976) Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *Proc Natl Acad Sci* 73(11):3852–3856
- Scherubl H (2021) Excess body weight and gastrointestinal cancer risk. *Visc Med* 37(4):261–266
- Seki T, Yang Y, Sun X, Lim S, Xie S, Guo Z, Xiong W, Kuroda M, Sakaue H, Hosaka K, Jing X, Yoshihara M, Qu L, Li X, Chen Y, Cao Y (2022) Brown-fat-mediated tumour suppression by cold-altered global metabolism. *Nature* 608(7922):421–428
- Seo JY, Jin EH, Chung GE, Kim YS, Bae JH, Yim JY, Han KD, Yang SY (2023) The risk of colorectal cancer according to obesity status at four-year intervals: a nationwide population-based cohort study. *Sci Rep* 13(1):8928
- Shaker FH, Sanad EF, Elghazaly H, Hsia S-M, Hamdy NM (2024) piR-823 tale as emerging cancer-hallmark molecular marker in different cancer types: a step-toward ncRNA-precision. *Naunyn-Schmiedeberg's Arch Pharmacol*
- Shi P, Ji H, Zhang H, Yang J, Guo R, Wang J (2020) circANRIL reduces vascular endothelial injury, oxidative stress and inflammation in rats with coronary atherosclerosis. *Exp Ther Med* 20(3):2245–2251
- Shoelson SE, Lee J, Goldfine AB (2006) Inflammation and insulin resistance. *J Clin Invest* 116(7):1793–1801
- Siegel RL, Giaquinto AN, Jemal A (2024) Cancer statistics, 2024. *CA Cancer J Clin* 74(1):12–49

- Singh R, Parveen M, Basgen JM, Fazel S, Meshesha MF, Thames EC, Moore B, Martinez L, Howard CB, Vergnes L, Reue K, Pervin S (2016) Increased expression of beige/brown adipose markers from host and breast cancer cells influence xenograft formation in mice. *Mol Cancer Res* 14(1):78–92
- Stoll L, Sobel J, Rodriguez-Trejo A, Guay C, Lee K, Veno MT, Kjems J, Laybutt DR, Regazzi R (2018) Circular RNAs as novel regulators of beta-cell functions in normal and disease conditions. *Mol Metab* 9:69–83
- Su M, Xiao YH, Ma JL, Tang YY, Tian B, Zhang YQ, Li X, Wu ZN, Yang DS, Zhou Y, Wang H, Liao QJ, Wang WX (2019) Circular RNAs in cancer: emerging functions in hallmarks, stemness, resistance and roles as potential biomarkers. *Mol Cancer* 18
- Sun W, Sun X, Chu W, Yu S, Dong F, Xu G (2020) CircRNA expression profiles in human visceral preadipocytes and adipocytes. *Mol Med Rep* 21(2):815–821
- Sun, X., X. Wan, M. A. Khan, K. Zhang, X. Yi, Z. Wang and K. Chen (2023). "Comprehensive analysis of circRNA expression profiles in human brown adipose tissue." *Diabet Metab Syndr Obesit*: 469–478
- Swellam M, Hamdy NM (2012) Association of nonalcoholic fatty liver disease with a single nucleotide polymorphism on the gene encoding leptin receptor. *IUBMB Life* 64(2):180–186
- Szabo L, Salzman J (2016) Detecting circular RNAs: bioinformatic and experimental challenges. *Nat Rev Genet* 17(11):679–692
- Takenaka K, Olzomer EM, Hoehn KL, Curry-Hyde A, Jun Chen B, Farrell R, Byrne FL, Janitz M (2023) Investigation of circular RNA transcriptome in obesity-related endometrial cancer. *Gene* 855:147125
- Thomas LF, Sætrom P (2014) Circular RNAs are depleted of polymorphisms at microRNA binding sites. *Bioinformatics* 30(16):2243–2246
- Tian W, Zhang B, Zhong H, Nie R, Ling Y, Zhang H, Wu C (2021) Dynamic expression and regulatory network of circular RNA for abdominal Preadipocytes differentiation in chicken (*Gallus gallus*). *Front Cell Dev Biol* 9:761638
- Trayhurn P (2013) Hypoxia and adipose tissue function and dysfunction in obesity. *Physiol Rev* 93(1):1–21
- Ulshöfer CJ, Pfafenrot C, Bindereif A, Schneider T (2021) Methods to study circRNA-protein interactions. *Methods* 196:36–46
- Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324(5930):1029–1033
- Wang X, Hua J, Li J, Zhang J, Dzakah EE, Cao G, Lin W (2022) Mechanisms of non-coding RNA-modulated alternative splicing in cancer. *RNA Biol* 19(1):541–547
- Wu Y-L, Li H-F, Chen H-H, Lin H (2022a) Emergent roles of circular RNAs in metabolism and metabolic disorders. *Int J Mol Sci* 23(3):1032
- Wu Z, Yu X, Zhang S, He Y, Guo W (2022b) Mechanism underlying circRNA dysregulation in the TME of digestive system cancer. *Front Immunol* 13:951561
- Xu H, Guo S, Li W, Yu P (2015) The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells. *Sci Rep* 5:12453
- Yan Y, Su M, Qin B (2020) CircHIPK3 promotes colorectal cancer cells proliferation and metastasis via modulating of miR-1207-5p/FMNL2 signal. *Biochem Biophys Res Commun* 524(4):839–846
- Yang Y, Gao X, Zhang M, Yan S, Sun C, Xiao F, Huang N, Yang X, Zhao K, Zhou H (2018) Novel role of FBXW7 circular RNA in repressing glioma tumorigenesis. *JNCI J Natl Cancer Inst* 110(3):304–315
- Yang X, Ma X, Mei C, Zan L (2022) A genome-wide landscape of mRNAs, lncRNAs, circRNAs and miRNAs during intramuscular adipogenesis in cattle. *BMC Genomics* 23(1):691
- Yin X, Chen Y, Ruze R, Xu R, Song J, Wang C, Xu Q (2022) The evolving view of thermogenic fat and its implications in cancer and metabolic diseases. *Signal Transduct Target Ther* 7(1):324
- Youness RA, Hassan HA, Abaza T, Hady AA, El Magdoub HM, Ali M, Vogel J, Thiersch M, Gassmann M, Hamdy NM (2024) A comprehensive insight and in Silico analysis of CircRNAs in hepatocellular carcinoma: a step toward ncRNA-based precision medicine. *Cells* 13(15):1245

- Zaiou M (2019) Circular RNAs as potential biomarkers and therapeutic targets for metabolic diseases. *Rev Biomark Stud Metab Metab Relat Disord*:177–191
- Zaiou M (2020) The emerging role and promise of circular RNAs in obesity and related metabolic disorders. *Cells* 9(6):1473
- Zhang M, Xin Y (2018) Circular RNAs: a new frontier for cancer diagnosis and therapy. *J Hematol Oncol* 11(1):21
- Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, Zhu S, Yang L, Chen LL (2013) Circular intronic long noncoding RNAs. *Mol Cell* 51(6):792–806
- Zhang Y, Liang W, Zhang P, Chen J, Qian H, Zhang X, Xu W (2017) Circular RNAs: emerging cancer biomarkers and targets. *J Exp Clin Cancer Res* 36(1):152
- Zhang M, Huang N, Yang X, Luo J, Yan S, Xiao F, Chen W, Gao X, Zhao K, Zhou H (2018) A novel protein encoded by the circular form of the SHPRH gene suppresses glioma tumorigenesis. *Oncogene* 37(13):1805–1814
- Zhang X, Chen L, Xiao B, Liu H, Su Y (2019a) Circ_0075932 in adipocyte-derived exosomes induces inflammation and apoptosis in human dermal keratinocytes by directly binding with PUM2 and promoting PUM2-mediated activation of AuroraA/NF-kappaB pathway. *Biochem Biophys Res Commun* 511(3):551–558
- Zhang H, Zhu L, Bai M, Liu Y, Zhan Y, Deng T, Yang H, Sun W, Wang X, Zhu K, Fan Q, Li J, Ying G, Ba Y (2019b) Exosomal circRNA derived from gastric tumor promotes white adipose browning by targeting the miR-133/PRDM16 pathway. *Int J Cancer* 144(10):2501–2515
- Zhang P, Sheng M, Du C, Chao Z, Xu H, Cheng X, Li C, Xu Y (2021a) Assessment of CircRNA expression profiles and potential functions in Brown Adipogenesis. *Front Genet* 12:769690
- Zhang Y, Qian J, Gu C, Yang Y (2021b) Alternative splicing and cancer: a systematic review. *Signal Transduct Target Ther* 6(1):78
- Zhang X, Zhan S, Yang S, Zhong T, Guo J, Cao J, Wang Y, Li L, Zhang H, Wang L (2021c) Dynamic expression profiles of circular RNAs during Brown to white adipose tissue transformation in goats (*Capra hircus*). *Animals (Basel)* 11(5)
- Zhang S, Sun J, Gu M, Wang G, Wang X (2021d) Circular RNA: a promising new star for the diagnosis and treatment of colorectal cancer. *Cancer Med* 10(24):8725–8740
- Zhang S, Jiang E, Kang Z, Bi Y, Liu H, Xu H, Wang Z, Lei C, Chen H, Lan X (2022a) CircRNA profiling reveals an abundant circBDP1 that regulates bovine fat development by sponging miR-181b/miR-204 targeting Sirt1/TRARG1. *J Agric Food Chem* 70(44):14312–14328
- Zhang J, Wang C, Jia C, Zhang Y, Qing X, Zhang Y, Liu J, Xu S, Pan Z (2022b) The role of circular RNAs in the physiology and pathology of the mammalian ovary. *Int J Mol Sci* 23(23)
- Zhao Z, Li X, Jian D, Hao P, Rao L, Li M (2017) Hsa_circ_0054633 in peripheral blood can be used as a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus. *Acta Diabetol* 54(3):237–245
- Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, Li Y (2021) Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduct Target Ther* 6(1):263
- Zhao Y, Ye X, Xiong Z, Ihsan A, Ares I, Martinez M, Lopez-Torres B, Martinez-Larranaga MR, Anadon A, Wang X, Martinez MA (2023) Cancer metabolism: the role of ROS in DNA damage and induction of apoptosis in cancer cells. *Meta* 13(7)
- Zhi F, Ding Y, Wang R, Yang Y, Luo K, Hua F (2021) Exosomal hsa_circ_0006859 is a potential biomarker for postmenopausal osteoporosis and enhances adipogenic versus osteogenic differentiation in human bone marrow mesenchymal stem cells by sponging miR-431-5p. *Stem Cell Res Ther* 12(1):157
- Zhong YX, Du YJ, Yang X, Mo YZ, Fan CM, Xiong F, Ren DX, Ye X, Li CW, Wang YM, Wei F, Guo C, Wu X, Li XL, Li Y, Li GY, Zeng ZY, Xiong W (2018) Circular RNAs function as ceRNAs to regulate and control human cancer progression. *Mol Cancer* 17
- Zhu Y, Gui W, Lin X, Li H (2020) Knock-down of circular RNA H19 induces human adipose-derived stem cells adipogenic differentiation via a mechanism involving the polypyrimidine tract-binding protein 1. *Exp Cell Res* 387(2):111753



Mutagenic Properties of Urban Road Dust: Different Approaches Using Bioindicators

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Abstract

Urban road dust (URD) is one of the greatest threats when it comes to air pollution, and there is still a lack of a cheap and effective tool for monitoring and assessing the health risks resulting from contact with URD. This work is devoted to the issue of mutagenicity testing of urban road dust with bioindicators.

Overall, the chapter summarizes the current state of knowledge on the topic. It creates an understanding of the topic for the reader by discussing the findings presented in recent research papers. This is not a frequently discussed issue, but it is definitely worth broader attention and recognition, because the application of common and available research biological materials or living organisms such as lichens, mosses or their products i.e. spider webs and feathers, is simple and much less expensive than traditional research and provides valuable information wherever traditional studies are difficult or even impossible to conduct.

Therefore, this chapter presents cheap alternatives that can help estimate the risk of exposure to URD in everyday life. In the summary, the possible future research prospects and method development were also presented.

Keywords

Air · Biomonitoring · Mutagenicity · Pollution · Spider webs · Urban road dust

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1 Introduction

The world population is increasing day by day, and it is expected to reach 9.8 billion in 2050 (United Nation 2017). This continuous growth of the global population intensifies the urbanization (Zhang 2008). The percentage of residents in urban areas is projected to exceed by 2050, and therefore, an increasing number of people will face exposure to environmental pollution in urban regions. The accumulation of both organic and inorganic matters on streets and roads poses a significant worldwide challenge, which requires scientifically robust approaches to assess risks. This will guide effective management strategies (Haynes et al. 2020).

1.1 Urban Road Dust (URD)

The extensive use of motor vehicles fuelled by fossil fuels and the expansion of urban areas are defining features of contemporary society. Consequently, urbanization leads to an increase in the impervious spaces such as roads, roofs and parking slots and a decline in the open spaces: forests, aquatic systems and meadows, which leads to derive urban road dust (Hwang et al. 2016). In literature, different terms have been used to introduce particles that accumulate on the roadway surfaces: road dust, street dust, road-deposited sediments, sweepsand, urban sediments, urban surface deposited sediments, etc. (Nguyen et al. 2021; Vanegas et al. 2021; Kim et al. 2021; Yarmoshenko et al. 2020; Hanfi et al. 2021). Urban road dust refers to particulate matter composed of fine particles and debris that accumulate on road surfaces in urban areas. It is an important carrier of urban runoff and comprises various pollutants arising from both man-made and natural processes (Khanal et al. 2014). URD serves as a significant contributor to coarse, fine and ultrafine particles in the air, as it is a mixture of toxic elements and other artefacts like heavy metals, polycyclic aromatic hydrocarbons (PAHs), mould spores, pollen, etc. derived from industrial activities, exhaust and non-exhaust traffic emissions, residential burning of fossil fuels and biomass, etc. This is particularly evident in industrial zones and cities characterized by a dense road network and wide areas covered by road pavements (Rybak et al. 2022; Vlasov et al. 2022; Semerjian et al. 2021).

Knowledge of the factors influencing the toxicity of urban road dust is important for the efficient management of this environmental issue. URD stands out as a major source of airborne particulate matter (PM) within urban environments (Hwang et al. 2016). It is believed that the distribution of particle sizes in URD is thought to significantly affect processes such as entrainment, sorption of pollutant metals, ingestion by biota and bioavailability. Generally, these particles have a size smaller than 0.92 mm (Shahab et al. 2020). Many studies have found that, when examining the distribution of toxicants, higher concentrations tend to be associated with finer particles rather than coarser ones (Khanal et al. 2014). URD can be resuspended through road movement and wind. Particularly, the fine dust particles may remain airborne for long periods, influenced by the weather conditions (Rybak et al. 2022). Investigation of sediment cores from watersheds has proved a direct correlation

between the rise in pollutants present in urban road dust and increase in traffic volume. This poses a significant risk for aquatic organisms. With the storm water runoff, contaminated urban road dust is dispersed into nearby receiving water bodies such as streams, rivers, lakes, and estuaries (Hwang et al. 2016).

1.2 Composition and Sources of URD

The chemical composition of URD is influenced by various anthropogenic sources and natural processes. This includes deflation and erosion of roadside soils during summer, particularly in relatively dry climates; dispersion of de-icing agents during winter and after snowmelt; transportation of particles through storm water runoff; the deposition of suspended atmospheric particles; and precipitation. Moreover, the composition of the URD differed according to the size and type of land use areas such as residential, industrial, commercial, recreational, educational, etc. in mega cities, medium cities and paved and unpaved roads between cities and settlements (Nguyen et al. 2021; Vanegas et al. 2021; Vlasov et al. 2022). URD is highly enriched with crustal dust and soil materials, heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), perfluorinated surfactants (PFSs), platinum group elements (PGEs), inorganic ions, mould spores, pollen, etc., which are derived from industrial activities, exhaust and non-exhaust traffic emissions, residential burning of fossil fuels and biomass, etc. (Rybak et al. 2022; Vlasov et al. 2022; Khanal et al. 2014; Hwang et al. 2016; Vanegas et al. 2021). The load of road dust and its chemical composition vary across different geographic locations, influenced by factors such as meteorological conditions, regional geology, and the specific type of pavement being sampled (Alves et al. 2020; Vanegas et al. 2021). For instance, particles deposited on the snow during the winter begin to be resuspended by traffic as the snow melts in the spring.

The primary components of road dust consist mainly of crustal dust and soil material. Therefore, it abundantly includes crust elements such as Si, Al, Fe, Mg, Na, P and Ti. It also contains lower concentrations of heavy metals such as Pb, V, Cd, Cu, and Sb (Amato et al. 2016; Vanegas et al. 2021). During the last few decades, heavy metal input has increased due to the emissions from both stationary (industrial and residential emissions) and mobile sources (automobile- and transportation-related activities on the road). Most importantly, road dust has a potential to adsorb a higher quantity of heavy metals as its surface area is higher (Nakajima and Aryal 2018; Murakami and Takada 2008). PAHs represent a group of organic compounds containing multiple fused rings. They are widely recognized as hazardous, carcinogenic, mutagenic, teratogenic and common pollutants of anthropogenic origin. Exhaust emissions from vehicles, tyre wear, fuel combustion activities, residential heating and asphalt are considered the contributors to PAHs in URD (Khpalwak et al. 2019; Murakami et al. 2005). Few studies have revealed the presence of PCBs, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans (PCDDs/PCDFs) in URD, which are highly toxic and categorized under persistent organic pollutants (POPs) (Klees et al. 2015). These compounds are released into the

environment from different industrial applications and as a by-product of thermal processes. PFSs are water-soluble, bioaccumulative, environmentally persistent and exhibit chronic toxicity and carcinogenic properties (Conder et al. 2008; Apelberg et al. 2007). They are commonly used in surfactants, automotive interiors, paper and textile coatings, fire retardants, etc. According to Murakami and Takada (2008), PFS concentration is very high in heavily trafficked areas. Platinum Group Elements (PGEs) include platinum (Pt), palladium (Pd), rhodium (Rh), ruthenium (Ru), iridium (Ir) and osmium (Os). PGEs have been emitted from catalytic converters since the mid of 1970s (Hwang et al. 2016). The predominant ionic components in URD consist mainly of Ca^{2+} , SO_4^{2-} , Cl^- , NO_3^- , and K^+ , originating from construction activities, emissions from chemical industries, and vehicle emissions (Othman and Latif 2020).

As road-deposited dust is a complex blend of pollutants originating from diverse sources, it requires the application of various techniques to accurately pinpoint the source of emissions. These are valuable for implementing targeted and effective control measures at the source. Hierarchical cluster analysis (HCA), enrichment factor analysis (EFA), correlation analysis (CA) and principal component analysis (PCA) are some of the statistical techniques that are used to identify the sources of complex mixtures (Mummullage et al. 2016; Zhao et al. 2021). Conferring to Mummullage et al. (2016), integrated utilization of EF, HCA and PCA, enhances the effectiveness of identification of sources of URD. Table 1 summarizes the potential sources of each component of the URD.

2 Mutagenic Properties of URD

The unprecedented surge in urbanization and the expansion of human needs and wants cause intensive resource utilization and chemical emissions, leading to various environmental issues (Liu et al. 2014). The development of travel and transportation, as well as the expansion of manufacturing and construction industries, introduces many pollutants to the environment (Petrović et al. 2016; Aminzadegan et al. 2022). URD is one of the most significant contributors of atmospheric particles, particularly in industrial zones and urban environments. It is a complex mixture of organic compounds, acid aerosols, trace metals, and large amounts of airborne dust with diameters less than $10\text{ }\mu\text{m}$ (PM_{10}) and $2.5\text{ }\mu\text{m}$ ($\text{PM}_{2.5}$). Not only the chemical components present in the URD, but also these fine particles, are capable of inducing the biological changes in living organisms and affecting their wellbeing. It revealed that URD could lead to mutations (Hannigan et al. 2005). Mutagenicity is defined as a property of a substance or agent to induce genetic mutations in the DNA of living organisms. Mutagenic substances can alter the DNA sequence, which may cause genetic mutation. These genetic alterations can be inheritable or affect the individual who has been exposed (Sundar et al. 2018). Many studies have conducted mutagenicity tests to evaluate the genetic effects of various substances. Ames test is one of the most widely used tests and is performed on different strains of *Salmonella typhimurium* and in *Escherichia coli* (Kumar et al. 2018). Moreover, several indices

Table 1 Sources of URD

Component of URD	Source	References
Heavy metals (Cu, Pb, Zn, Cd, Cr, As, Co, Ni)	<i>Natural</i> : weathering of rocks and minerals and volcanic eruption <i>Anthropogenic</i> : industrial production (thermal power plants, steel plants and metal manufacturing plants), traffic emissions (auto-mobile exhaust, tyre wear, brake wear and car shell corrosion), urban construction and domestic waste	Nakajima and Aryal (2018), Zhao et al. (2021), Murakami and Takada (2008)
PAHs	<i>Anthropogenic</i> : exhaust emission from vehicles, fuel and biomass combustion, oil spill and paving materials (coal tar-based asphalt road seal coat)	Khpalwak et al. (2019), Pashin and Bakhitova (1979)
PCBs, PCDDs/PCDFs	<i>Anthropogenic</i> : industrial applications (transformer oil and hydraulic fluid, paints, sealants and plastics), thermal processes in the presence of organochlorine (waste incineration, metallurgical industry), corrosion of construction materials and vehicle parts	Ossai et al. (2023)
PFSs (perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorocarboxylates (PFCAs)	<i>Anthropogenic</i> : traffic/vehicular emissions, industrial applications, consumer products (grease repellents in carpets and clothing etc)	Murakami and Takada (2008), Stahl et al. (2011)
PGEs (Pt, Pd, Rh, Ru, Ir, Os)	<i>Anthropogenic</i> : Catalytic converters	Hwang et al. (2016)
Inorganic ions (Ca^{2+} , SO_4^{2-} , Cl^- , NO_3^- , and K^+)	<i>Natural</i> : volcanic ash <i>Anthropogenic</i> : vehicular exhaust emissions, non-exhaust emissions, construction activities, chemical industrial emissions and secondary gas-to-particle conversion	Han et al. (2017), Othman and Latif (2020), Vlasov et al. (2022)

are used to assess the carcinogenic/non-carcinogenic health risks from the contaminants in URD: Hazard Quotient (HQ), Target Hazard Quotient (THQ), Hazard Index (HI), Index of Geo-accumulation (Igeo), Risk Assessment Code (RAC), Air Quality Index (AQI) etc. For instance, if there is a non-carcinogenic health risk in a particular substance, HI values indicate less than one ($\text{HI} < 1$), and

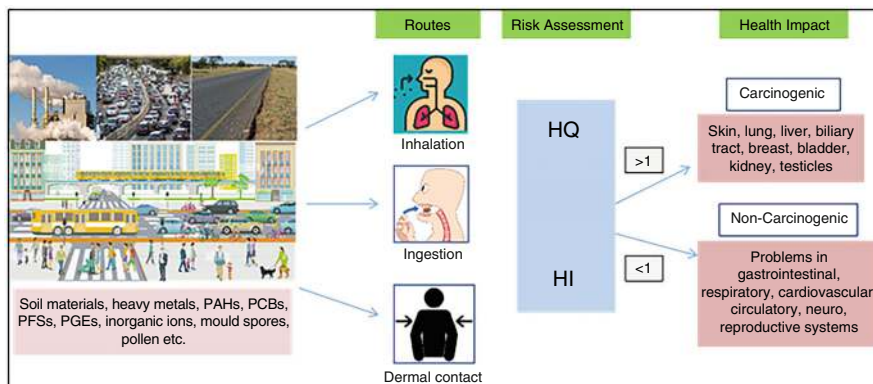


Fig. 1 Components and the health impacts of URD

vice versa (Zgłobicki and Telecka 2021; Faisal et al. 2021; Ossai et al. 2023). Figure 1 shows the components and origins of URD, the routes and the health impacts of the components according to the values of HQ and HI indices.

2.1 Mutagenic and Carcinogenic Elements and Compounds in URD

Mutagenic elements or compounds have the potential to alter the genetic materials and affect the organism's characteristics or functioning, while carcinogenic elements or compounds promote the development of cancer by inducing changes in genetic materials, which leads to uncontrolled cell growth and tumour formation. The correlation between mutagenicity and carcinogenicity is complex, as the ability of a substance to cause cancer is influenced by several factors rather than just its mutagenic potential. Moreover, some elements and compounds show both mutagenic and carcinogenic properties (Ames 1989). Most of the elements and compounds contained in URD depict both mutagenic and carcinogenic properties (Rybak et al. 2022).

The potentially toxic metal fraction in URD is a main concern for its mutagenic and carcinogenic properties. Mainly, the pollution of Cr, Ni, As, Cd, Co, Pb, Cu, Zn, Mn, Ba, Fe and Ca is resulted from anthropogenic sources such as vehicular exhaust and non-exhaust emissions, coal combustion, smelting, industrial emissions, domestic emissions, atmospheric emissions etc. (Liu et al. 2014; Nakajima and Aryal 2018; Zhao et al. 2021; Murakami and Takada 2008). Exposure to the URD, which is enriched with these metals, leads to numerous health concerns (Liu et al. 2014). Most importantly, heavy metals can be entered into the human body through dermal contact, ingestion and inhalation (Mahmoud et al. 2023). With long-term exposure to heavy metals, they tend to be bioaccumulated, implying that the heavy metal concentration within the organisms can exceed that present in the surrounding

environment (Du et al. 2013). Heavy metals can be mutagenic, carcinogenic, teratogenic and neurotoxic (Du et al. 2013; Vlasov et al. 2022). Cr exhibits both mutagenic and carcinogenic effects, and prolonged exposure to Cr causes lung cancer (Vlasov et al. 2022; Mahmoud et al. 2023). The primary pathway of Ni exposure is through inhalation, which tends to develop lung and nasal cancers in addition to involving various epigenetic changes (Genchi et al. 2020). Studies have found that long-term exposure to As causes cancers of the skin, liver, lungs, kidney, colon, and urinary bladder (Tchounwou et al. 2003). According to the studies, chronic exposure to Cd, Co, and Pb causes the development of cancer (Godt et al. 2006; Leyssens et al. 2017; Aendo et al. 2022). Particularly it has been reported that insoluble Pb is responsible for cancers developed in the respiratory tract (Khan and Strand 2018). As per the categorizations established by the International Agency for Research on Cancer (IARC), Cu, Zn and Mn are designated as non-carcinogenic metals (IARC 2012; Liu et al. 2014).

Polycyclic aromatic hydrocarbons are mainly formed by the incomplete combustion of carbon-based materials as they are components of petroleum products (gasoline, diesel, tar, etc.). Moreover, microbial mycotoxins and plant-produced alkaloids are the sources of some PACs that could be found in URD (Wei et al. 2015). PAH, a typical organic component present as fine particles in URD, leads to adverse human and environmental effects (Rybak et al. 2022). Studies have proved the mutagenic and teratogenic properties of PAHs. PAHs require metabolic activation to function as mutagens or carcinogens, and this activation is happening inside the body cells with the involvement of DNA. Metabolites of PAHs are carcinogenic, and due to the activation process, they have mutated the DNA (Pashin and Bakhitova 1979). PAHs lead to the development of cancers in the lung, bladder, skin and gastrointestinal. Breast cancer, which is the most commonly diagnosed among women, is influenced by PAHs exist in URD (Korsh et al. 2015; Khan and Strand 2018). Studies have found that PAHs with 4–6 rings are carcinogenic and PAHs with 2–3 rings are non-carcinogenic (Kumar et al. 2016).

Polychlorinated biphenyls are emitted from vehicular exhaust, industries, corrosion of construction materials and vehicle parts, etc. PCBs are commercially produced for different industrial applications such as paints, sealants, transformer oil and hydraulic fluid, plastics etc. (Ranjbaran et al. 2021). They are extremely toxic to humans and the environment. Due to its adverse impacts on both human health and environment, trading and applying it in production have been forbidden. PCBs have been implicated as mutagenic and carcinogenic. These are caused by cancers of the liver and biliary tract (Ossai et al. 2023). PFSS are another component of URD and are resistant to metabolic or environmental degradation, bioaccumulative, chronic toxic and environmentally persistent organic pollutants (Murakami and Takada 2008; Eriksen et al. 2010). According to Murakami and Takada (2008), URD is considered the origin of the PFSSs, and through the surface runoff it contributes to contaminating urban waters. Most importantly, long-term exposure to PFSSs leads to the development of testicular and kidney cancers, with one potential mechanism of their effects is the induction of oxidative stress (Steenland and Winquist 2021).

The elevated presence of platinum group elements (Pt, Pd, Rh, Ru, Ir and Os) in URD in heavily trafficked zones is attributed to their utilization as catalysts in catalytic converters. Mainly, PGEs are emitted in metal forms, which have a low toxicity, while some PGEs, such as Pt, Rh and Pd are soluble. Soluble compounds of Pt, Rh and Pd chlorides exhibit mutagenic and carcinogenic properties, and certain compounds of PGEs are potent allergens. Particularly, Pd absorbs from soil through roots and translocates up to vegetative parts of the plant. This poses a significant threat to human health (Vasile et al. 2019).

2.2 Possible Non-carcinogenic Health Impacts of URD

URD contains toxic concentrations in different fractions from anthropogenic sources, which can be hazardous to both humans and the environment (Othman and Latif 2020). URD functions as an adsorbent (for heavy metals, organic pollutants, etc) and mobile substrate for the pollutants (Jayarathne et al. 2018; Kormoker et al. 2022). Apart from the risks of mutagens and carcinogens, a great health risk exists due to their persistency, bioavailability, bioaccumulation and toxicity (Jayarathne et al. 2018). Ingestion, Inhalation and dermal contact are the major routes of exposure for URD. Mainly, non-carcinogenic health risks result from exposure to the potentially toxic elements and compounds in URD. The elderly and children are regarded as the most susceptible populations to these health risks due to their weakened or underdeveloped immune systems (Faisal et al. 2021). Table 2 summarizes the non-carcinogenic health effects of URD along with the particular pollutant.

3 Bioindicators Used in Studies of URD

Bioindicators such as lichens, mosses and higher plants are particularly suitable and most commonly used for different purposes in monitoring. As URD is one of the biggest contributors to airborne PM in urban environments, lichens, mosses and higher plants are applied most commonly to assess the concentration of selected elements in PM (Cabrerizo et al. 2016; De Nicola et al. 2014; Stojanowska et al. 2020; Zechmeister et al. 2006; van Laaten et al. 2020). It has been proved that lichens and mosses reflect pollutants existent in the air, both in gas- and PM-phases (Augusto et al. 2013). Few studies applied other products of animals, such as bird feathers, bird eggs, human hair or animal hair (Ackerman et al. 2016; Appenzeller and Tsatsakis 2012; Appenzeller et al. 2017; Dmowski 1999; Esteban and Castaño 2009; Jaspers et al. 2007, 2019; Toriba et al. 2003; Zhang and Ma 2011).

All the methods mentioned above have their advantages and disadvantages. The application of the bioindicators, i.e. higher plants, mosses and lichens, is limited to the duration of the vegetative season. The use of animals and human products (eggs, feathers and hair) also have some limitations. The bird eggs, the product of females, represent only a female part of the population. On the other hand, the distribution

Table 2 Non-carcinogenic health effects of different pollutants in URD

Component of URD	Health effect	Reference
Heavy metals		
Ni	Dermatitis, gastrointestinal manifestation, lung fibrosis, cardiovascular diseases and low birth weight (LBW)	Genchi et al. (2020), Mahmoud et al. (2023)
As	Cardiovascular diseases, birth defects, neurologic and cognitive disorders, diabetes, ototoxicity, peripheral vascular diseases and disorders of blood cells such as anemia and leukopenia	Tchounwou et al. (2003), Khan and Strand (2018)
Cd	Causes for detrimental effect on the respiratory system, liver and gastrointestinal tract, damages the kidneys and bone	Du et al. (2013), Godt et al. (2006)
Co	Asthma, hearing and visual impairment	Leyssens et al. (2017), Mahmoud et al. (2023)
Pb	Neuro toxin, toxic effects on cardiovascular system (hypertension) and kidney	Faisal et al. (2021), Kopp et al. (1988)
Cr	Respiratory distress, pulmonary and heart-related toxicity	Mitra et al. (2022)
Cu	Gastric upset, nausea, vomiting, coughing and sneezing, pulmonary fibrosis, Wilson disease (due to accumulation of copper in the liver), immune deficiency	Khan and Strand (2018)
PAHs	Asthma, reproductive toxicity, toxicity damages to circulatory system, digestive tract, cardiovascular system, and central nervous system	Kumar et al. (2016), Rybak et al. (2022), Khpalwak et al. (2019)
PCBs	Attention deficit/hyperactivity disorder (ADHD), autism and neuro toxicity	Ossai et al. (2023)
PFSs	Liver toxicity, reproductive toxicity, neurotoxic, thyroid disorder, immunosuppression	Stahl et al. (2011), Yamazaki et al. (2023)
Potentially toxic metals		
Al	LBW, Alzheimer disease, asthma, cardiac failure, anaemia	Nayak (2002), Miu et al. (2003)
Ba	Cardiac and renal failure, respiratory arrest and intestinal bleeding	Kravchenko et al. (2014)
PGEs (Pt, Rh, Pd)	Dermatitis, eye irritation and respiratory tract problems (sneezing, wheezing and asthma)	Rinkovec (2019), Colombo et al. (2008)

pattern of different pollutants in bird feathers is different depending on the stage of the bird's life and the pollutant type. Furthermore, the content of pollutants in human or animal hair depends on many factors, such as age, medical history, etc. Thus, different factors influence the interpretation of the results.

In general, studies using bioindicators focus mainly on the assessment of pollutant concentrations. The majority of papers are related to the assessment of the

concentrations of selected elements bound to PM. Furthermore, few works have focused on organic compounds, mainly dioxins and furans (PCDD/Fs) and polycyclic aromatic hydrocarbons (PAHs). Most frequently, lichens, mosses, pine needles and other vegetation have been used in such studies (Augusto et al. 2013). Researchers used mosses to test the presence of organic pollutants in the atmosphere, including organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) (Lim et al. 2006). Although these studies were restricted mainly to the assessment of the concentrations of organic compounds. If the health risk is assessed, it is based on the concentrations of organic compounds or elements (metals and metalloids) revealed in bioindicators; rarely is a comprehensive mutagenicity assessment based on bioindicators done. The quite complex approach to this issue was presented in studies where spider webs as bioindicators were used.

4 Spider Webs in Studies of URD

Spider webs are ubiquitous. They accumulate pollutants, therefore are an excellent source of information on the environment quality.

The following advantages of the application of spider webs as bioindicators are presented below.

- common availability
- convenient location of webs (in secluded places) preventing them from destroying by weather conditions (rain, wind, etc.)
- low cost and easy sampling
- non-invasiveness of studies
- non-specific and universal tool,
- organic, natural and environmentally friendly products (no waste production e.g. used sorbents)
- spiders can be bred under laboratory conditions and obtained webs can be used in any place
- easy dating of samples
- Long-term sampling

Recently, the use of spider webs has become more and more popular. The first research using spider webs to assess air pollution was published in 2002 (Hose et al. 2002). Further research was carried out in China, where it was used as an indicator of road traffic-related pollution (Xiao-li et al. 2006). The following years brought the development of the biomonitoring using spider webs, a large part of which was carried out in Poland (Rybak 2007, 2015; Rybak and Olejniczak 2014; Rybak et al. 2015, 2019). Spiders that are the most numerous and do not have the habit of eating old spider webs (such as Araneidae) are usually used in biomonitoring studies. The advantage of spider web applications over other commonly used bioindicators such as mosses or lichens is that the latter are dependent on sunlight and the vegetation season; therefore, it is difficult to use them in specific conditions such as city parking

lots or traffic tunnels when studying traffic pollution. Each family weaves a unique, characteristic web, and all spider webs can be divided into two basic types: sticky and dry. They usually belong to the following families: Agelenidae, Pholcidae, Theridiidae and Amaurobiidae. In various studies the concentrations of selected metals and organic compounds were determined, confirming the possibility of effective use of spider webs as an indicator for assessing the level of air pollution. The highest values of these compounds/elements were recorded at sites associated with increased car traffic and high emissions (underground parking lots, busy roads, viaducts and tunnels) (Rybak 2007, 2015; Rybak and Olejniczak 2014; Rybak et al. 2015). The advantages of research carried out at this type of site include a high concentration of pollutants, no deposition washed away by rain and minimization of the share of other sources of pollution; hence, it is easier to determine the pollution level and its sources. During various studies, it was found that the webs of the Agelenidae family are the best for the bioindication because their web is characterized by relatively high density and compactness, which facilitates the accumulation of pollutants on their surface (Fig. 2). Moreover, representatives of this family are common and weave large webs all year round (also in winter), which is an undoubted advantage over other bioindicators whose use is limited only to the growing season.

While examining the mechanism of accumulation of pollutants in the webs, it was found that the accumulation of pollutants probably takes place by external deposition and, to a lesser extent, endogenously by contaminated food eaten by spiders. Comparing the methods of accumulation of PAH compounds, metals and metalloids, it was found that webs are very suitable for the bioindication of organic compounds such as PAHs. Due to their chemical affinity, these compounds probably can enter, penetrate deeper into the web and bind permanently to the protein matrix, which immobilizes and makes them difficult to wash out. Spider silk is a protein (fibroin), which, through intermolecular interactions, may, like any protein, have chemical affinity for organic compounds emitted during combustion. On the other hand, other pollutants such as elements do not bind and can therefore be easily washed out from the meshes, e.g. under the influence of atmospheric precipitation. Although, in the studies of Rybak and Olejniczak (2014), it has been shown that the indication of PAHs with spider webs has certain limitations. The authors observed that more volatile compounds with low molecular weight are not retained in the meshes of

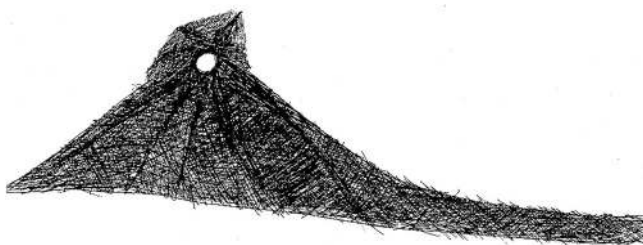


Fig. 2 Web of *Tegenaria* sp. (Agelenidae). (Author: J. Rybak)

webs, as they easily pass into the gas phase and then disperse. The research demonstrated the ability to immobilize high-molecular-weight PAHs in spider webs, including those that are already considered markers of traffic pollutants (benzo[a]pyrene, pyrene, fluoranthene and phenanthrene) and new ones (benzo[a]anthracene and indeno[1,2,3-c,d]pyrene), which may play such a role in the future.

In the studies of Rybak et al. (2019), PAHs were identified in spider webs indoors. It was the first study of this type. A high ability of spider webs to bind PAH compounds was observed again, and this was not restricted only to compounds with high molecular weight, which is a big advantage compared to outdoor monitoring using spider webs, where it has been reported that the webs are able to retain only selected PAH compounds due to their rapid photodegradation and microbiological decomposition. It has been proved that the use of spider webs for indoor monitoring is very convenient, economical and reliable method, especially compared to other passive sampling techniques (e.g. sorbents), because spider webs can be used simultaneously for a wide range of pollutants. The advantages of such studies include a high concentration of pollutants indoor, no deposition being washed away by rain, and minimization of the share of other sources of pollution, which makes it easier to determine the level of pollution and its sources. It is also important that the tool can be used to pre-select indoor pollutants, because conventional measurements are expensive and usually inaccessible, and spider webs naturally occur in our homes. On the other hand, the quantitative determination of PAHs on spider webs requires standardization in the laboratory based on comparison with conventional methods. The issue of standardization applies to all bioindicators, and it is not an easy task, especially in the current situation where there are no standards or recommendations defining a universal method of monitoring air quality indoors.

5 Studies on Mutagenicity of URD

The first studies on mutagenicity used spider webs (Rutkowski et al. 2019). The authors identified the concentration of 15 PAHs and 8 nitro-PAH compounds in dust deposited in spider webs and showed that the concentrations of these compounds are connected with the functions of rooms where studies have been conducted. The important factors influencing the results were also location, the heating method in a studied house/apartment and the individual habits of residents (e.g. smoking). It was demonstrated that these compounds are mutagenic, and spider webs can be used to assess the mutagenicity with the application of Ames test. There are many benefits of such studies. What is particularly important is that spider webs can be used in mutagenicity testing and risk assessment. This method is simple and effective because many species of spiders (e.g. *Pholcus phalangioides*) inhabit people's houses. They weave webs in the corners of rooms; therefore, instead of removing webs during standard cleaning, they can be used for monitoring and mutagenicity studies. Even in the case of people who are afraid of spiders, ex situ method can be used: i.e. to apply the clean webs obtained from the laboratory, which were

previously stretched on Petri dishes and delivered to the monitoring site and exposed to pollutants.

To date there are no other studies that use bioindicators for these purposes.

In the paper of Rybak et al. (2022) the authors assessed the mutagenicity of URD without the application of bioindicators. Dust was collected in Poland (Lower and Upper Silesia), two regions known for elevated levels of air pollution. The aim of the study was to determine the concentration of selected PAH compounds in URD in both regions, identify possible sources of pollution based on the PAH diagnostic ratio, and assess the possible mutagenic properties of URD using the Ames test, and determine the possible cancer risk associated with contact with URD in both studied regions. It has been proved that people are exposed to carcinogenic factors and/or compounds that may have an adverse impact on health through URD, deriving mainly from communication, heating systems and industrial activities in both agglomerations. Importantly, it has been revealed that both regions have a major problem with pollution, as almost all URD samples were characterized by high concentrations of PAHs, a high participation of five- and six-ring PAHs. Furthermore, the mutagenic properties of URD in both regions were revealed, which suggests that the impact of urban transport and home heating (low emissions) on both agglomerations is significant and should not be ignored, as both regions have 'problematic' areas that can be considered 'hotspots'. Therefore, the increased health risks have been demonstrated, which is particularly important for people who are exposed to long-term contact with pollutants due to the nature of their work outdoors (e.g. construction workers, etc.). The calculated ILCR values and the revealed mutagenic properties of URD indicated a high potential risk of the inhabitants of these regions for developing cancer and other serious diseases.

To date the mutagenic properties of URD have not been studied. There is also insufficient universal data on this topic in the international literature, as research mainly focuses on ultrafine particulates or soil rather than URD. As URD is one of the biggest contributors to airborne PM in urban environment, many studies were devoted to the mutagenicity of various PM fractions (André et al. 2011).

As we know, however, a comprehensive approach to the risk connected to urban road dust and examining direct exposure to URD, which is everywhere, is crucial in assessing the real health risk to humans. Due to the presence of high concentrations of pollutants, URD is considered toxic. The composition of the URD reflects the composition of the soil including industrial emissions, the deposition of vehicle exhaust components, the abraded road surface, abraded materials of car tires, brake fluids and paints used to paint streets and infrastructure roads (Trojanowska and Świetlik 2017). URD containing metals, metalloids and organic compounds poses a potential threat to the health of city residents. Due to the movement of vehicles or wind, URD is carried away and forms secondary source of air pollution. Collected on street surfaces, it is sent to landfills as part of cleaning work. The amount of stored street dust increases every day, which is related to the increase in vehicle traffic and the expansion of the urban area (Trojanowska and Świetlik 2017).

Apart from the above-mentioned studies, the mutagenic properties of URD were not directly examined i.e. using direct tests (Ames test). Other papers assessed the

health risk indirectly using USEPA formulas based on the identification of organic compounds, metals and metalloids. A direct approach to the problem and direct assessment of the impact of URD on human health provide reliable information about the real threat and possible solutions.

6 Conclusion

Meanwhile, the lack of complex research on the impact of URD on the environment is in contradiction with the implementation of various programs and activities worldwide aimed at limiting the impact of environmental pollution on human health.

On the other hand, identifying the relationship between the origin of URD, its chemical properties and its direct impact on health and the environment expressed in mutagenicity and carcinogenicity may provide an universal information on the impact strength of anthropogenic emissions on the environment in various urbanized areas and help avoid dangers related to this threat.

Simple, fast and cheap solutions are needed to provide reliable information about the risk connected with URD load in a studied area. Moreover, there is also a need for such assessments in the remote areas such as mountains or heavily forested areas, as URD may overcome long distances and influence the remote areas. Additionally, in such places, it is not possible to organize a classic campaign and employ the specialized equipment, which, due to its economic value or the need for maintenance, cannot be left unattended for a long time. The solution to this problem is the application of biomonitoring, i.e. the use of living organisms to assess the level of URD, which allows the risk assessment and gives the complex information about its mutagenicity and carcinogenicity, which in the future will reduce such threats or help to avoid the exposure and related diseases.

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References

- Ackerman JT, Eagles-Smith CA, Herzog MP, Hartman CA (2016) Maternal transfer of contaminants in birds: mercury and selenium concentrations in parents and their eggs. *Environ Pollut* 210:145–154. <https://doi.org/10.1016/j.envpol.2015.12.016>
- Aendo P, Netvichian R, Thienedsakul P, Khaodhiar S, Tulayakul P (2022) Carcinogenic risk of Pb, cd, Ni, and Cr and critical ecological risk of Cd and Cu in soil and groundwater around the municipal solid waste open dump in Central Thailand. *J Environ Public Health* 28:2022–3062215. <https://doi.org/10.1155/2022/3062215>
- Alves CA, Vicente ED, Vicente AM, Rienda IC, Tomé M, Querol X, Amato F (2020) Loadings, chemical patterns and risks of inhalable road dust particles in an Atlantic city in the north of Portugal. *Sci Total Environ* 737:139596. <https://doi.org/10.1016/j.scitotenv.2020.139596>

- Amato F, Favez O, Pandolfi M, Alastuey A, Quero X, Moukhtar S, Sciare J (2016) Traffic induced particle resuspension in Paris: emission factors and source contributions. *Atmos Environ* 129: 114–124. <https://doi.org/10.1016/j.atmosenv.2016.01.022>
- Ames BN (1989) Mutagenesis and carcinogenesis: endogenous and exogenous factors. *Environ Mol Mutagen* 14(Suppl 16):66–77. <https://doi.org/10.1002/em.2850140614>
- Aminzadegan S, Shahriari M, Mehranfar F, Abramović B (2022) Factors affecting the emission of pollutants in different types of transportation: a literature review. *Energy Rep* 8:2508–2529. <https://doi.org/10.1016/j.egyr.2022.01.161>
- André V, Billet S, Pottier D, Le Goff J, Pottier I, Garçon G, Shirali P, Sichel F (2011) Mutagenicity and genotoxicity of PM_{2.5} issued from an urbano-industrialized area of Dunkerque (France). *J Appl Toxicol* 31(2):131–138. <https://doi.org/10.1002/jat.1572>
- Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR (2007) Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ Health Perspect* 115(11):1670–1676. <https://doi.org/10.1289/ehp.10334>
- Appenzeller BMR, Tsatsakis AM (2012) Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: state of the art, critical review and future needs. *Toxicol Lett* 210(2):119–140. <https://doi.org/10.1016/j.toxlet.2011.10.021>
- Appenzeller BMR, Hardy EM, Grova N, Chata C, Faÿs F, Briand O, Schroeder H, Duca RC (2017) Hair analysis for the biomonitoring of pesticide exposure: comparison with blood and urine in a rat model. *Arch Toxicol*. <https://doi.org/10.1007/s00204-016-1910-9>
- Augusto S, Máguas C, Branquinho C (2013) Guidelines for biomonitoring persistent organic pollutants (POPs), using lichens and aquatic mosses—a review. *Environ Pollut* 180:330–338. <https://doi.org/10.1016/j.envpol.2013.05.019>
- Cabrerizo A, Tejado P, Dachs J, Benayas J (2016) Anthropogenic and biogenic hydrocarbons in soils and vegetation from the South Shetland Islands (Antarctica). *Sci Total Environ* 569–570: 1500–1509. <https://doi.org/10.1016/j.scitotenv.2016.06.240>
- Colombo C, Monhemius AJ, Plant JA (2008) Platinum, palladium and rhodium release from vehicle exhaust catalysts and road dust exposed to simulated lung fluids. *Ecotoxicol Environ Saf* 71(3):722–730. <https://doi.org/10.1016/j.ecoenv.2007.11.011>
- Conder JM, Hoke RA, Wolf WD, Russell MH, Buck RC (2008) Are PFCA's bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. *Environ Sci Technol* 42(4):995–1003. <https://doi.org/10.1021/es070895g>
- De Nicola F, Alfani A, Maisto G (2014) Polycyclic aromatic hydrocarbon contamination in an urban area assessed by *Quercus ilex* leaves and soil. *Environ Sci Pollut Res Int* 21(12): 7616–7623. <https://doi.org/10.1007/s11356-014-2665-6>
- Dmowski K (1999) Birds as bioindicators of heavy metal pollution: review and examples concerning European species. *Acta Ornithol* 34(1):1–25
- Du Y, Gao B, Zhou H, Ju X, Hao H, Yin S (2013) Health risk assessment of heavy metals in road dusts in urban parks of Beijing, China. *Procedia Environ Sci* 18:299–309. <https://doi.org/10.1016/j.proenv.2013.04.039>
- Eriksen KT, Raaschou-Nielsen O, Sørensen M, Roursgaard M, Loft S, Møller P (2010) Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. *Mutat Res* 700(1–2):39–43. <https://doi.org/10.1016/j.mrgentox.2010.04.024>
- Esteban M, Castaño A (2009) Non-invasive matrices in human biomonitoring: a review. *Environ Int* 35(2):438–449. <https://doi.org/10.1016/j.envint.2008.09.003>
- Faisal M, Wu Z, Wang H, Hussain Z, Azam MI (2021) Human health risk assessment of heavy metals in the urban road dust of Zhengzhou Metropolis, China. *Atmosphere* 12(9):1213. <https://doi.org/10.3390/atmos12091213>
- Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A (2020) Nickel: human health and environmental toxicology. *Int J Environ Res Public Health* 7(3):679. <https://doi.org/10.3390/ijerph17030679>

- Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A, Groneberg DA (2006) The toxicity of cadmium and resulting hazards for human health. *J Occup Med Toxicol* 10(1): 22. <https://doi.org/10.1186/1745-6673-1-22>
- Han X, Lu X, Qinggeletu; Wu Y. (2017) Health risks and contamination levels of heavy metals in dusts from parks and squares of an industrial city in semi-arid area of China. *Int J Environ Res Public Health* 14(8):886. <https://doi.org/10.3390/ijerph14080886>. PMID: 28783109
- Hanfi MY, Yarmoshenko I, Seleznev AA (2021) Gross alpha and gross beta activity concentrations in the dust fractions of urban surface-deposited sediment in Russian cities. *Atmos* 12(5):571. <https://doi.org/10.3390/atmos12050571>
- Hannigan MP, Busby WF, Cass GR (2005) Source contributions to the mutagenicity of urban particulate air pollution. *J Air Waste Manag Assoc* 55(4):399–410. <https://doi.org/10.1080/10473289.2005.10464633>
- Haynes HM, Taylor KG, Rothwell J, Byrne P (2020) Characterisation of road-dust sediment in urban systems: a review of a global challenge. *J Soils Sediments* 20:4194–4217. <https://doi.org/10.1007/s11368-020-02804-y>
- Hose GC, James JM, Gray MR (2002) Spider webs as environmental indicators. *Environ Pollut* 120(3):725–733. [https://doi.org/10.1016/S0269-7491\(02\)00171-9](https://doi.org/10.1016/S0269-7491(02)00171-9)
- Hwang HL, Fiala MJ, Park D, Wade TL (2016) Review of pollutants in urban road dust and stormwater runoff: part 1. Heavy metals released from vehicles. *Int J Urban Sci* 20(3):334–360. <https://doi.org/10.1080/12265934.2016.1193041>
- International Agency for Research on Cancer (2012) IARC monographs on the identification of carcinogenic hazards to humans. IOP Publishing Physics Web, Bristol, pp 1–125
- Jaspers VLB, Voorspoels S, Covaci A, Lepoint G, Eens M (2007) Evaluation of the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants: a comparative and meta-analytical approach. *Environ Int* 33(3):328–337. <https://doi.org/10.1016/j.envint.2006.11.011>
- Jaspers VLB, Covaci A, Herzke D, Eulaers I, Eens M (2019) Bird feathers as a biomonitor for environmental pollutants: prospects and pitfalls. *TrAC* 118:223–226. <https://doi.org/10.1016/j.trac.2019.05.019>
- Jayarathne A, Egodawatta P, Ayoko GA, Goonetilleke A (2018) Assessment of ecological and human health risks of metals in urban road dust based on geochemical fractionation and potential bioavailability. *Sci Total Environ* 635:1609–1619. <https://doi.org/10.1016/j.scitotenv.2018.04.098>
- Khan RK, Strand MA (2018) Road dust and its effect on human health: a literature review. *Epidemiol Health* 40:e2018013. <https://doi.org/10.4178/epih.e2018013>
- Khanal R, Furumai H, Nakajima F (2014) Toxicity assessment of size-fractionated urban road dust using ostracod *Heterocypris incongruens* direct contact test. *J Hazard Mater* 264:53–64. <https://doi.org/10.1016/j.jhazmat.2013.10.058>
- Khpalwak W, Jadoon WA, Abdel-Dayem SM, Sakugawa H (2019) Polycyclic aromatic hydrocarbons in urban road dust, Afghanistan: implications for human health. *Chemosphere* 218:517–526. <https://doi.org/10.1016/j.chemosphere.2018.11.087>
- Kim M-S, Kim J-Y, Park J, Yeon S-H, Shin S, Choi J (2021) Assessment of pollution sources and contribution in urban dust using metal concentrations and multi-isotope ratios (^{13}C , $^{207/206}\text{Pb}$) in a complex industrial Port Area, Korea. *Atmos* 12(7):840. <https://doi.org/10.3390/atmos12070840>
- Klees M, Hiester E, Bruckmann P, Molt K, Schmidt TC (2015) Polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans in street dust of North Rhine-Westphalia, Germany. *Sci Total Environ* 511:72–81. <https://doi.org/10.1016/j.scitotenv.2014.12.018>
- Kopp SJ, Barron JT, Tow JP (1988) Cardiovascular actions of lead and relationship to hypertension: a review. *Environ Health Perspect* 78:91–99. <https://doi.org/10.1289/ehp.887891>
- Kormoker T, Kabir MH, Khan R, Islam MS, Shammi RS, Al MA, Proshad R, Tamim U, Sarker ME, Taj MTI, Akter A, Idris AM (2022) Road dust-driven elemental distribution in megacity Dhaka, Bangladesh: environmental, ecological, and human health risks assessment. *Environ Sci Pollut Res Int* 29(15):22350–22371. <https://doi.org/10.1007/s11356-021-17369-7>

- Korsh J, Shen A, Aliano K, Davenport T (2015) Polycyclic aromatic hydrocarbons and breast cancer: a review of the literature. *Breast Care (Basel)* 10(5):316–318. <https://doi.org/10.1159/000436956>
- Kravchenko J, Darrah TH, Miller RK, Lyerly HK, Vengosh A (2014) A review of the health impacts of barium from natural and anthropogenic exposure. *Environ Geochem Health* 36(4): 797–814. <https://doi.org/10.1007/s10653-014-9622-7>
- Kumar V, Kothiyal NC, Saruchi Vikas P, Sharma R (2016) Sources, distribution, and health effect of carcinogenic polycyclic aromatic hydrocarbons (PAHs)—current knowledge and future directions. *J Chin Adv Mater Soc* 4(4):302–321. <https://doi.org/10.1080/22243682.2016.1230475>
- Kumar A, Shanker R, Dhawan A (2018) Detection of mutation in prokaryotic cells. In: *Mutagenicity: assays and applications*. Academic Press, London, pp 35–48
- Leyssens L, Vinck B, Van Der Straeten C, Wuyts F, Maes L (2017) Cobalt toxicity in humans –a review of the potential sources and systemic health effects. *Toxicology* 387:43–56. <https://doi.org/10.1016/j.tox.2017.05.015>
- Lim TB, Xu R, Tan B, Obbard JP (2006) Persistent organic pollutants in moss as bioindicators of atmospheric pollution in Singapore. *Chemosphere* 64(4):596–602. <https://doi.org/10.1016/j.chemosphere.2005.11.007>
- Liu E, Yan T, Birch G, Zhu Y (2014) Pollution and health risk of potentially toxic metals in urban road dust in Nanjing, a mega-city of China. *Sci Total Environ* 476–477:522–531. <https://doi.org/10.1016/j.scitotenv.2014.01.055>
- Mahmoud N, Al-Shahwani D, Al-Thani H, Isaifan RJ (2023) Risk assessment of the impact of heavy metals in urban traffic dust on human health. *Atmos* 14(6):1049. <https://doi.org/10.3390/atmos14061049>
- Mitra S, Chakraborty AJ, Tareq AM, Emran TB, Khusro FNA, Idris AM, Khandaker MU, Osman H, Alhumaydhi FA, Simal-Gandara J (2022) Impact of heavy metals on the environment and human health: novel therapeutic insights to counter the toxicity. *J King Saud Univ Sci* 34(3):101865. <https://doi.org/10.1016/j.jksus.2022.101865>
- Miu AC, Andreescu CE, Vasiu R, Olteanu AI (2003) A behavioral and histological study of the effects of long-term exposure of adult rats to aluminum. *Int J Neurosci* 113(9):1197–1211. <https://doi.org/10.1080/00207450390232292>
- Mummullage S, Egodawatta P, Ayoko GA, Goonetilleke A (2016) Use of physicochemical signatures to assess the sources of metals in urban road dust. *Sci Total Environ* 541:1303–1309. <https://doi.org/10.1016/j.scitotenv.2015.10.032>
- Murakami M, Takada H (2008) Perfluorinated surfactants (PFSS) in size-fractionated street dust in Tokyo. *Chemosphere* 73(8):1172–1177. <https://doi.org/10.1016/j.chemosphere.2008.07.063>
- Murakami M, Nakajima F, Furumai H (2005) Size-and density-distributions and sources of polycyclic aromatic hydrocarbons in urban road dust. *Chemosphere* 61(6):783–791. <https://doi.org/10.1016/j.chemosphere.2005.04.003>
- Nakajima F, Aryal R (2018) Heavy metals in urban dust. In: Saleh HM, Aglan RFES (eds) *Heavy metals*. IntechOpen
- Nayak P (2002) Aluminum: impacts and disease. *Environ Res* 89(2):101–115. <https://doi.org/10.1006/enrs.2002.4352>
- Nguyen V-T, Duy Dat N, Vo T-D-H, Nguyen D-H, Nguyen T-B, Nguyen L-SP, Nguyen XC, Dinh V-C, Nguyen T-H-H, Huynh T-M-T et al (2021) Characteristics and risk assessment of 16 metals in street dust collected from a highway in a densely populated metropolitan area of Vietnam. *Atmos* 12(12):1548. <https://doi.org/10.3390/atmos12121548>
- Ossai CJ, Iwegbue CMA, Tesi GO, Olisah C, Egobueze FE, Nwajei GE, Martincigh BS (2023) Spatial characteristics, sources and exposure risk of polychlorinated biphenyls in dusts and soils from an urban environment in The Niger Delta of Nigeria. *Sci Total Environ* 883:163513. <https://doi.org/10.1016/j.scitotenv.2023.163513>

- Othman M, Latif MT (2020) Pollution characteristics, sources, and health risk assessments of urban road dust in Kuala Lumpur City. *Environ Sci Pollut Res Int* 27(10):11227–11245. <https://doi.org/10.1007/s11356-020-07633-7>
- Pashin YV, Bakhitova LM (1979) Mutagenic and carcinogenic properties of polycyclic aromatic hydrocarbons. *Environ Health Perspect*:185–189. <https://doi.org/10.1289/ehp.7930185>
- Petrović N, Bojović N, Petrović J (2016) Appraisal of urbanization and traffic on environmental quality. *J CO2 Util* 16:428–430. <https://doi.org/10.1016/j.jcou.2016.10.010>
- Ranjbaran S, Sobhanardakani S, Cheraghi M, Lorestani B, Sadr MK (2021) Ecological and human health risks assessment of some polychlorinated biphenyls (PCBs) in surface soils of central and southern parts of city of Tehran, Iran. *J Environ Health Sci Eng* 19(2):1491–1503. <https://doi.org/10.1007/s40201-021-00705-x>
- Rinkovec J (2019) Platinum, palladium, and rhodium in airborne particulate matter. *Arh Hig Rada Toksikol* 70(4):224–231. <https://doi.org/10.2478/aiht-2019-70-3293>
- Rutkowski R, Rybak J, Rogula-Kozłowska W, Belcik M, Piekarska K, Jureczko I (2019) Mutagenicity of indoor air pollutants adsorbed on spider webs. *Ecotoxicol Environ Saf* 171:549–557. <https://doi.org/10.1016/j.ecoenv.2019.01.019>
- Rybak J (2007) Structure and function of the web of *Bathypantes similimus* (Araneae: Linyphiidae) in an isolated population in the Stołowe Mountains, SW Poland. *Arachnology*. <https://doi.org/10.13156/arac.2007.14.1.33>
- Rybak J (2015) Accumulation of major and trace elements in spider webs. *Water Air Soil Pollut* 226(4). <https://doi.org/10.1007/s11270-015-2369-7>
- Rybak J, Olejniczak T (2014) Accumulation of polycyclic aromatic hydrocarbons (PAHs) on the spider webs in the vicinity of road traffic emissions. *Environ Sci Pollut Res* 21(3). <https://doi.org/10.1007/s11356-013-2092-0>
- Rybak J, Sówka I, Zwodziak A, Fortuna M, Trzepla-Nabagło K (2015) Evaluation of the usefulness of spider webs as an air quality monitoring tool for heavy metals. *Ecol Chem Eng S* 22(3). <https://doi.org/10.1515/eces-2015-0021>
- Rybak J, Rogula-Kozłowska W, Jureczko I, Rutkowski R (2019) Monitoring of indoor polycyclic aromatic hydrocarbons using spider webs. *Chemosphere*:758–766. <https://doi.org/10.1016/j.chemosphere.2018.11.174>
- Rybak J, Wróbel M, Krzyżyńska R, Rogula-Kozłowska W, Olszowski, T (2022) Is Poland at risk of urban road dust? Comparison studies on mutagenicity of dust. *Environ Pollut* 314:120337. <https://doi.org/10.1016/j.envpol.2022.120337>
- Semerjian L, Okaiyeto K, Ojemaye MO, Ekundayo TC, Igwaran A, Okoh AI (2021) Global systematic mapping of road dust research from 1906 to 2020: research gaps and future direction. *Sustain For* 13(20):11516. <https://doi.org/10.3390/su132011516>
- Shahab A, Zhang H, Ullah H, Rashid A, Rad S, Li J, Xiao H (2020) Pollution characteristics and toxicity of potentially toxic elements in road dust of a tourist city, Guilin, China: ecological and health risk assessment. *Environ Pollut* 266(Pt 3):115419. <https://doi.org/10.1016/j.envpol.2020.115419>
- Stahl T, Mattern D, Brunn H (2011) Toxicology of perfluorinated compounds. *Environ Sci Eur* 23:38. <https://doi.org/10.1186/2190-4715-23-38>
- Steenland K, Winquist A (2021) PFAS and cancer, a scoping review of the epidemiologic evidence. *Environ Res* 194:110690. <https://doi.org/10.1016/j.envres.2020.110690>
- Stojanowska A, Rybak J, Bożym M, Olszowski T, Białowicz JS (2020) Spider webs and lichens as bioindicators of heavy metals: a comparison study in the vicinity of a copper smelter (Poland). *Sustain For* 12(19). <https://doi.org/10.3390/su12198066>
- Sundar R, Jain MR, Valani D (2018) Mutagenicity testing: regulatory guidelines and current needs. In: *Mutagenicity: assays and applications*. Academic Press, pp 191–228
- Tchounwou PB, Patlolla AK, Centeno JA (2003) Invited reviews: carcinogenic and systemic health effects associated with arsenic exposure – a critical review. *Toxicol Pathol* 31(6):575–588. <https://doi.org/10.1080/01926230390242007>

- Toriba A, Kuramae Y, Chetianukornkul T, Kizu R, Makino T, Nakazawa H, Hayakawa K (2003) Quantification of polycyclic aromatic hydrocarbons (PAHs) in human hair by HPLC with fluorescence detection: a biological monitoring method to evaluate the exposure to PAHs. *Biomed Chromatogr* 17(2–3):126–132. <https://doi.org/10.1002/bmc.222>
- Trojanowska M, Świetlik R (2017) Ocena narażenia środowiskowego na Cr, Cu, Pb, Ni oraz Zn obecne w pyłach miejskich. *Zielona Góra: Oficyna Wydawnicza Uniwersytetu Zielonogórskiego* 67:88–100
- United Nation (2017) Department of Economic and Social Affairs. The World Population Prospects: The 2017 Revision. [Online] <https://www.un.org/en/desa/world-population-projected-reach-98-billion-2050-and-112-billion-2100>. Accessed 8 Dec 2023
- van Laaten N, Merten D, von Tümpling W, Schäfer T, Pirrung M (2020) Comparison of spider web and moss bag biomonitoring to detect sources of airborne trace elements. *Water Air Soil Pollut* 231:512. (2020). <https://doi.org/10.1007/s11270-020-04881-8>
- Vanegas S, Trejos EM, Aristizábal BH, Pereira GM, Hernández JM, Murillo JH, Ramírez O, Amato F, Silva LFO, Rojas NY et al (2021) Spatial distribution and chemical composition of road dust in two high-altitude Latin American cities. *Atmos* 12(9):1109. <https://doi.org/10.3390/atmos12091109>
- Vasile G, Dinu C, Kim L, Tenea AG, Simion M, Ene C, Spinu C, Ungureanu EM, Manolache D (2019) Platinum group elements in road dust and vegetation from some European and national roads with intensive car traffic in Romania. *Revista de Chimie (Rev Chim)* 70, 1:286–292. <https://doi.org/10.37358/RC.19.1.6901>
- Vlasov D, Ramírez O, Luhar A (2022) Road dust in urban and industrial environments: sources, pollutants, impacts, and management. *Atmos* 13(4):607. <https://doi.org/10.3390/atmos13040607>
- Wei C, Bandowe BAM, Han Y, Cao J, Zhan C, Wilcke W (2015) Polycyclic aromatic hydrocarbons (PAHs) and their derivatives (alkyl-PAHs, oxygenated-PAHs, nitrated-PAHs and azaarenes) in urban road dusts from Xi'an, Central China. *Chemosphere* 134:512–520. <https://doi.org/10.1016/j.chemosphere.2014.11.052>
- Xiao-li S, Yu P, Hose GC, Jian C, Feng-xiang L (2006) Spider webs as indicators of heavy metal pollution in air. *Bull Environ Contamin Toxicol* 76(2):271–277. <https://doi.org/10.1007/s00128-006-0917-y>
- Yamazaki E, Lalwani D, Ruan Y, Taniyasu S, Hanari N, Kumar NJI, Lam PKS, Yamashita N (2023) Nationwide distribution of per- and polyfluoroalkyl substances (PFAS) in road dust from India. *Sci Total Environ* 892:164538. <https://doi.org/10.1016/j.scitotenv.2023.164538>
- Yarmoshenko I, Malinovsky G, Baglaeva E, Seleznev A (2020) A landscape study of sediment formation and transport in the urban environment. *Atmos* 11(12):1320. <https://doi.org/10.3390/atmos11121320>
- Zechmeister HG, Dullinger S, Hohenwallner D, Riss A, Hanus-Ilmar A, Scharf S (2006) Pilot study on road traffic emissions (PAHs, heavy metals) measured by using mosses in a tunnel experiment in Vienna, Austria. *Environ Sci Pollution Res* 13(6):398–405. <https://doi.org/10.1065/espr2006.01.292>
- Zglobicki W, Telecka M (2021) Heavy metals in urban street dust: health risk assessment (Lublin City, E Poland). *Appl Sci* 11(9):4092. <https://doi.org/10.3390/app11094092>
- Zhang W (2008) A forecast analysis on world population and urbanization process. *Environ Dev Sustain* 10:717–730. <https://doi.org/10.1007/s10668-007-9081-8>
- Zhang WW, Ma JZ (2011) Waterbirds as bioindicators of wetland heavy metal pollution. *Procedia Environ Sci* 10C:2769–2774. <https://doi.org/10.1016/j.proenv.2011.09.429>
- Zhao G, Zhang R, Han Y, Meng J, Qiao Q, Li H (2021) Pollution characteristics, spatial distribution, and source identification of heavy metals in road dust in a central eastern city in China: a comprehensive survey. *Environ Monit Assess* 193:1–13



The Relationship Between Genotoxicity and Cancer

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Abstract

Genotoxicity is described as the potential of xenobiotics to cause DNA damage by resulting in changes in the structure or information in genetic material. In addition, the formation of reactive oxygen species (ROS), which are by-products of oxidative respiration within the cell, Fenton reactions, and oxidative stress also cause DNA damage. Damage to biomolecules such as lipids, proteins, and DNA due to oxidative stress paves the way for the formation of many chronic diseases, especially cancer. In vitro, in vivo, and in silico models are used to detect all agents, including chemical and physical agents, that cause genetic damage and to evaluate their genotoxic potential. DNA damage that causes genotoxicity is frequently investigated in cancer and its treatments because it causes physical or chemical changes in DNA in cells that affect the interpretation and transmission of genetic information. Carcinogenesis is a process that includes the malignant transformation of cells after exposure to xenobiotics, changes in genome integrity due to gene mutations and chromosome damage, and eventually cancer development. This relationship between genotoxicity and cancer is particularly important for preclinical safety assessment of drugs and chemicals. It has also been reported that environmental and occupational exposure may cause genotoxic damage and pose a risk for cancer. This chapter aims to evaluate the mechanism of genotoxicity and cancer and the relationship between them, and the in vitro and in vivo studies conducted in this field.

Keywords

Cancer · Carcinogenesis · DNA damage · Genotoxicity

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1 Introduction

Each of the 10^{13} cells in the human body undergoes tens of thousands of DNA lesions during the day. DNA damage is also caused by reactive oxygen species (ROS) within the cell, which are by-products of oxidative respiration, redox cycles, or Fenton reactions mediated by heavy metals. Damage to biomolecules such as lipids, proteins, and DNA due to oxidative stress, which occurs due to the disruption of antioxidant and oxidant balance within cells, paves the way for the formation of many chronic diseases, especially cancer (Bacanlı et al. 2017).

Various chemical, physical, and biological agents have the potential to induce mutations ranging from single-nucleotide changes in the DNA sequence to major chromosomal alterations (Marchetti et al. 2023). These xenobiotics can interact with genetic material and lead to genomic instability and cancer-related mutations (Araldi et al. 2015). Therefore, toxicological evaluation especially genotoxicity determination is of great importance for the examination and approval of various factors (Marchetti et al. 2023). For this purpose, regulatory agencies such as the Food and Drug Administration (FDA), the European Medicines Agency (EMA), and Agência Nacional de Vigilância Sanitária (ANVISA, Brazil) emphasize genotoxicity testing as an important part of drug validation (Araldi et al. 2015).

Genotoxicity is defined as the ability of substances to cause DNA damage by altering the structure or information content of genetic material in cells (Baderna et al. 2020). In vitro and in vivo genotoxicity tests are designed to detect all chemical and physical agents that can cause genetic damage (Baderna et al. 2020; Yan et al. 2013). In addition to these methods, in silico approaches are available to evaluate the genotoxic potential of the different factors (Baderna et al. 2020).

Genotoxicity testing involves the measurement of primary DNA damage, which can be repaired and therefore reversed, and the detection of stable and irreversible damage, such as gene mutations and chromosome abnormalities, which can be passed on to the next generation due to their existence in the germ cells. Since gene mutation, structural chromosome aberrations, and numerical chromosome aberrations are all associated with carcinogenesis and inherited diseases, the assessment of genotoxicity should include these three main endpoints. Since mutations are known to be involved in the cancer process, mutagenicity tests are applied to identify substances that can trigger mutations and initiate cancer. These tests include in vitro mammalian chromosomal aberration testing (OECD TG 473), bacterial reverse mutation testing (OECD TG 471), in vitro mammalian cell gene mutation testing (OECD TG 476 and TG 490 [MLA/tk]), and in vitro mammalian cell micronucleus (MN) testing (OECD TG 487) (Corvi and Madia 2017).

2 Mechanisms of Genotoxicity

DNA damage, which causes genotoxicity, is physical or chemical changes in DNA in cells that affect the interpretation and transmission of genetic information, leading to many diseases, including cancer (Srinivas et al. 2019). Genotoxic effects mainly occur through direct or indirect genotoxicity mechanisms (Fig. 1).

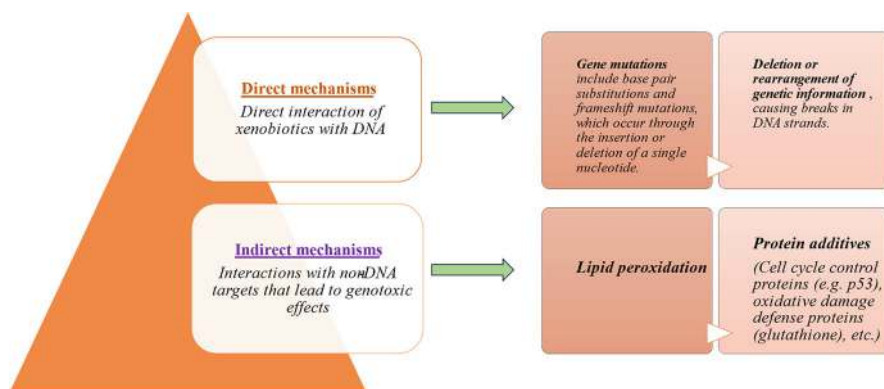


Fig. 1 Mechanisms of genotoxicity

Direct genotoxicity results from the direct interaction of xenobiotics and their metabolites with DNA. Induction of gene mutations and structural chromosomal damage (clastogens) constitute these genotoxic mechanisms. Gene mutations include base pair substitutions and frameshift mutations, which occur through the insertion or deletion of a single nucleotide, whereas failure to repair clastogens leads to insertion, deletion, or rearrangement of genetic information, causing breaks in DNA strands (Graziano and Jacobson-Kram 2015).

Interactions with non-DNA targets that lead to genotoxic effects constitute indirect mechanisms of genotoxicity. These interactions include lipid peroxidation and production of protein adducts. For this reason, research focuses on the inhibition of repair enzymes, cell cycle control proteins (e.g., p53), apoptosis-related gene products (e.g., p53, bax, bcl-2), nuclear lamins, oxidative damage defense proteins (glutathione (GSH)), metabolizing enzymes, and mitotic/mitotic miline tubulins (Kirsch-Volders et al. 2003).

Exogenous and endogenous factors such as chemicals, radiation, and free radicals can cause different forms of DNA damage. Of these, reactive oxygen species (ROS) are a group of reactive oxygen-containing species that can induce DNA damage and affect the DNA damage response. There are many studies showing that ROS affects genotoxic stress caused by chemotherapeutic agents and ionizing radiation (Srinivas et al. 2019). Disruption of the balance between antioxidant and DNA repair mechanisms and ROS levels can cause oxidative stress and oxidative damage in cells. Formed oxidative stress can modulate gene expression pathways (Klaunig et al. 2011). Mutagenic changes, like the hydroxylation of adenine and guanine, are caused by oxidative damage to DNA (Foldbjerg et al. 2011). It is seen that oxidative stress and oxidative genotoxic damage can play a role in all stages of cancer, especially in the beginning and changing of the process. Also, studies have shown the relationship between polymorphisms in oxidative DNA repair, antioxidant genes, and cancer susceptibility in humans (Klaunig et al. 2011).

Type II topoisomerases, which play a role in the DNA process, are enzymes that organize the upper and lower strands of DNA and untangle knots and tangles in the genetic material by passing it through a transient double-stranded break where they form an intact double helix. In their catalytic cycle, these enzymes create breaks in DNA strands and therefore have the potential to fragment the genome. Due to these properties, type II topoisomerases have significant genotoxic effects and have been used in the development of anticancer drugs for the clinical treatment of human malignancies (McClendon and Osheroff 2007).

Cellular responses to DNA damage are mediated by two different kinases, the ATM-Chk2 and ATR-Chk1 pathways, which are activated by DNA double-strand breaks and single-stranded DNA. The ATR-Chk1 pathway is essential for the survival of many cell types as it is a direct effector of DNA damage and replication checkpoints. In the case of DNA double-strand breaks, ATM promotes the formation of single-stranded DNA at the sites of damage through ATR-Chk1 activation and nucleolytic resection. It is also required to initiate DNA repair through homologous recombination (HRR). Furthermore, cells survive with mutations in ATM or other components required for HRR, such as *BRCA1* and *BRCA2*. However, this can lead to genomic instability and cancer susceptibility. HRR deficiency in *BRCA1*- and *BRCA2*-deficient tumors leads to sensitivity to inhibitors of poly (ADP-ribose) polymerase (PARP), an enzyme required for the repair of endogenous DNA damage (Smith et al. 2010).

3 Genotoxicity and Cancer

Genotoxicity is associated with carcinogenesis as it includes small abnormalities such as single base pair substitutions and large chromosomal abnormalities. For this reason, it is frequently investigated in cancer and its treatments. Cells are constantly exposed to potential mutagenic attacks due to exposure to environmental carcinogens. If genetic damage is beyond repair, the cell dies (apoptosis) to prevent the emergence of abnormal cell populations (Doak et al. 2017).

Carcinogenesis is a process that includes malignant transformation of cells upon exposure to chemicals, alterations in genome integrity due to gene mutations and chromosome damage, and eventually the development of cancer. This relationship between DNA damage and cancer development is important for preclinical safety assessment of drugs and chemicals. This process involves the evaluation of the genotoxicity of xenobiotics and their potential to cause DNA damage and carcinogenicity. It also contains the ability to form tumors in animals with long-term exposure (Ellinger-Ziegelbauer et al. 2009).

It has been suggested that carcinogens can be divided into two groups: genotoxic and non-genotoxic (Fig. 2). This will provide a theoretical basis for an accurate evaluation of cancer risk. A chemical that can cause cancer by directly modifying the genetic information of target cells is referred to as a *genotoxic carcinogen*, whereas a chemical that can cause cancer through a secondary mechanism unrelated to direct gene damage is referred to as a *non-genotoxic carcinogen* (Hayashi 1992).

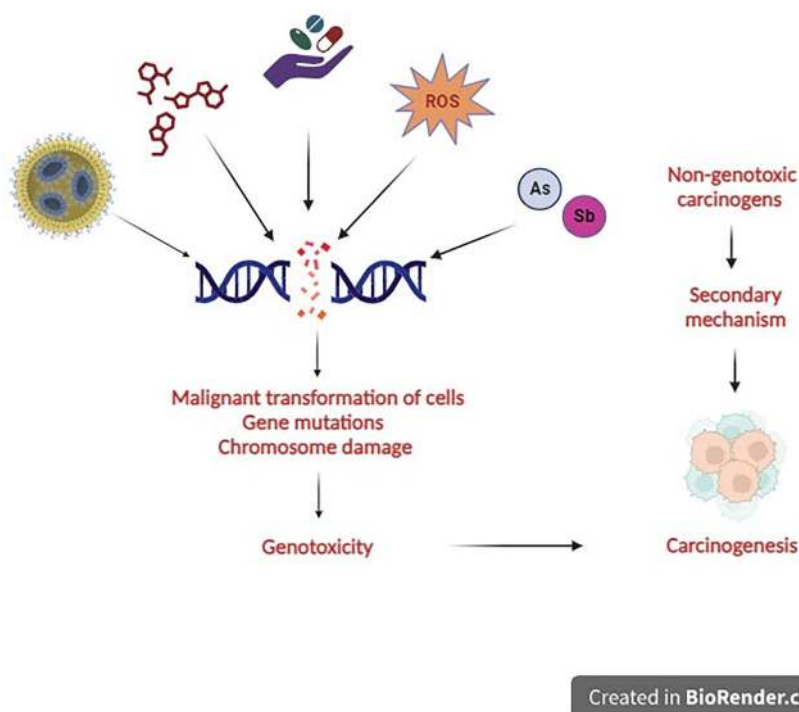


Fig. 2 Mechanisms of carcinogenesis

Chemicals known as genotoxic carcinogens cause cancer by creating mutations in cells. Since they interact with DNA, it is believed that there is no safe threshold or dosage for exposure. Regulations pertaining to genotoxic carcinogens are predicated on the idea that, even at very low concentrations, they could cause cancer in people. On the other hand, non-genotoxic carcinogens are thought to have a safe exposure threshold or dose; as a result, their use in society is allowed unless the exposure or intake level exceeds the threshold. These carcinogens cause cancer through mechanisms other than mutations, such as hormonal effects, cytotoxicity, cell proliferation, or epigenetic changes (Nohmi 2018).

The first step in tumor formation involves a carcinogen interacting with DNA and creating a permanent change in the genome of cells. Tumors can be started by a single mutation in a gene within a single cell as a result of an exogenous attack, such as carcinogens, or an endogenous attack, such as ROS. Further mutational events can result in malignant transformation if the initiating genetic abnormalities occur in vital genes (Doak et al. 2017).

Genomic instability is a hallmark of cancer, and loss of genes involved in DNA repair can be termed tumour suppressors because they lead to cancer-promoting mutations and genomic rearrangements. In the cases studied, the presence of *BRCA1* and *BRCA2* mutations in familial breast and ovarian cancer inactivate mismatch repair in colon cancer (Lecona and Fernandez-Capetillo 2018).

As one of the most common types of cancer, 5–10% of colon cancer cases are caused by hereditary and DNA mutations that occur during life (Ristori et al. 2022). Colon cancer develops over many years as a result of the accumulation of DNA damage and mutations, leading to loss of control of cell proliferation and failure of damaged cells to go through apoptosis (Pot et al. 2010). Animal models to investigate the treatment of chemically and genetically induced colon cancer provide much more information, whereas such evidence is more difficult to obtain in humans (Ristori et al. 2022).

3.1 Anticancer Drug Studies

Cancer is a disease characterized by the hallmarks of proliferation and resistance to cell death (including apoptosis) and is often caused by changes in the genome. Since most cancer cells divide more frequently than normal cells, the process of cell division is usually targeted in cancer therapy. DNA replication is an important stage of the cell cycle and one of the main targets of these therapies. Most of the cytotoxic agents commonly used in cancer treatment cause high levels of DNA damage that initiates the cell cycle, leading to cell cycle stop and/or cell death (Swift and Golsteyn 2014). The anticancer effect mechanism of frequently used anticancer drugs is shown in Table 1.

Table 1 Frequently used anticancer drugs and their anticancer effect mechanisms (Carr et al. 2008; Toolaram et al. 2014)

Anticancer drugs	Mechanism of action
Platinum analogs (e.g., carboplatin, cisplatin)	Causes selective inhibition of tumor DNA synthesis
Alkylating agents (e.g., cyclophosphamide, busulfan)	Replaces a hydrogen atom with an alkyl group that can slow or block DNA replication
Antimetabolites (e.g., methotrexate, mercaptopurine, fluorouracil)	Affects DNA synthesis and cell proliferation by being involved in metabolic pathways instead of endogenous purines and pyrimidines due to structural similarity
Agents of plant origin (e.g., vinblastine, vincristine, paclitaxel, docetaxel, etoposide)	It interacts with microtubules. This leads to the inhibition of synthesis of proteins and nucleic acids, inhibition of mitosis, and cell death
Topoisomerase inhibitors (e.g., irinotecan, topotecan)	Inhibits DNA replication
Hormones (e.g., estrogens)	Direct cytotoxic effect on tumor cells
Antiandrogens (e.g., cyproterone)	Causes tumor flare inhibition
Other antineoplastic agents (e.g., procarbazine, imatinib)	Procarbazine causes free radical formation. Imatinib is a protein kinase inhibitor and can inhibit proliferation by blocking Abl tyrosine kinase. Thus, apoptosis can be induced in myeloid leukemia cells

Antineoplastic drugs affect the structure and functions of DNA directly or indirectly. However, their toxicity is important as they show their effects not only on target cells but also on non-tumor cells (Parrella et al. 2015). Genotoxic chemotherapeutics such as cisplatin are the mainstay of cancer treatment. If the DNA damage caused by them is not corrected, the DNA damage response (DDR) is activated, and cell death occurs. In addition, altered responses to DNA-damaging agents are frequently observed in cancer patients.

It is known that many anticancer drugs show their anticancer effect by increasing DNA damage in cells and creating a cytotoxic effect in the cell. At this point, there are studies evaluating the genotoxic effects of various anticancer drugs on different cancer cell lines (Karamese et al. 2023; Erdoğan et al. 2023; Bacanlı et al. 2021).

In a study, the genotoxicity of gemcitabine and topotecan was examined in mouse bone marrow cells using MN and chromosomal aberration test systems. Gemcitabine was shown to increase the frequency of micronuclei at an average dose, to have cytotoxic effects on bone marrow, and to decrease the polychromatic/normochromatic erythrocyte ratio in a dose-dependent manner. It has also been observed to increase the number of abnormal cells and cause a significant increase in total chromosomal abnormalities. Topotecan caused high levels of micronuclei and decreased the polychromatic/normochromatic erythrocyte ratio and showed cytotoxic effects in bone marrow. It was also shown that topotecan increased the number of abnormal metaphases and total chromosomal abnormalities in a dose-dependent manner (Aydemir and Bilaloğlu 2003).

DNA repair gene polymorphisms are known to be associated with genotoxicity as a result of exposure to antineoplastics. Base excision repair (BER) and homologous recombination (HR) pathways are DNA repair mechanisms required to maintain chromosome stability and prevent chromosomal fragmentation, translocations, and deletions. X-ray repair cross-complement 1 (XRCC1) and X-ray repair cross-complement 3 (XRCC3) proteins are required for these DNA repair mechanisms. Single-nucleotide polymorphisms in XRCC1 and XRCC3 genes may lead to genetic imbalance and increase susceptibility to cancer (Aristizabal-Pachon and Castillo 2020).

In a study investigating occupational exposure, the genotoxicity of antineoplastic drugs (5-fluorouracil, ifosfamide, cytarabine, and gemcitabine) was investigated in nurses and pharmacy workers working in an oncology hospital. The MN assay with lymphocytes did not show significant differences compared to the control group, while the test with buccal cells showed higher values in nurses administering these drugs compared to pharmacy workers. In the analysis of chromosomal aberrations (CA), a significant increase in structural CA (especially breaks) was found in the exposed groups (Cavallo et al. 2005). Another study investigated the genotoxicity of occupational exposure to these drugs and the effects of XRCC1 and XRCC3 polymorphisms on individual response. A significant increase was seen in the DNA damage in the COMET and MN tests in the exposed group compared to the control group. The study showed that there was no significant relationship between XRCC1 and XRCC3 genotype, allele, or haplotype (Aristizabal-Pachon and Castillo 2020).

Although anticancer drugs are administered in hospitals under controlled conditions, their environmental toxicology is also important. The main environmental source of these drugs is patients' excretion in urine and feces. In addition, hospital wastes are usually mixed into the municipal sewage system, and municipal wastewater is another important source for the spread of these drugs into the environment. Information on the effects of these drugs on humans and the environment in terms of environmental toxicology is limited (Toolaram et al. 2014). In a study the environmental chronic toxicity of six anticancer drugs (5-fluorouracil, capecitabine, cisplatin, doxorubicin, etoposide, and imatinib), selected from five classes of the anatomical therapeutic classification (ATC), was investigated. The genotoxicity of these drugs was studied by in vivo comet assay in cells from *Daphnia magna* and *Ceriodaphnia dubia*. The further genotoxic/mutagenic effects of the selected anticancer drugs were also examined by SOS-chromotest and Salmonella mutagenicity tests. The COMET test showed that all drugs caused DNA damage. SOS chromotest showed that all drugs except imatinib activated the repair system in *Escherichia coli* PQ37 in *D. magna*. In the *Salmonella* mutagenicity test, doxorubicin was reported to cause direct and indirect frameshift and base pair substitution mutations (Parrella et al. 2015).

3.2 Nanoparticle Studies

Nanotechnology is a newly developing and advanced research area that is devoted to controlling matter on atomic and molecular levels while focusing on designing and producing a wide range of materials. Nanomaterials (NMs) are substances manufactured with nanotechnology and used in medicine, cosmetics, electronics, etc. (Girigoswami 2018). Nanomaterials (NMs) are of great interest, especially in cancer therapy, due to their unique optical properties, simple surface chemistry, and convenient size scale (Jain et al. 2007). In addition to these remarkable properties, NMs are of concern for human health as their toxicity is not fully known.

Nanomaterials (NMs) or nanoparticles (NPs) can cause genotoxicity through different mechanisms: (I) primary mechanisms through direct interaction with DNA; (II) primary indirect mechanisms (interaction of NMs with molecules involved in DNA replication, transcription and repair, and cell division); (III) secondary mechanisms such as inflammation (damage to DNA as a result of ROS generation) (Landsiedel et al. 2022). Furthermore, it has been reported that NPs of size 8–10 nm can reach the nucleus through nuclear pores, while large-sized NPs can access DNA in cells (Huo et al. 2014; Yadav et al. 2018; Sharma 2023). NMs in the environment can be affected by air, water, and soil. This may change the toxicity or activity of NMs. Asbestos and air pollution are some of the important examples of environmental effects on genotoxicity of NMs (Elsaesser and Howard 2012).

Among NPs, metallic NPs are of interest for their unique, size-dependent properties in food, cosmetics, and biomedical fields (Paino et al. 2012). In particular, gold nanoparticles (AuNPs) are of great importance for cancer diagnosis and treatment due to their improved light scattering and absorption by surface plasmon

resonance (SPR), binding to ligands that specifically target biomarkers on cancer cells, molecular specific imaging, and cancer detection (Jain et al. 2007). In a study, the genotoxicity of different types of AuNPs against human hepatocellular carcinoma cells (HepG2) and peripheral blood mononuclear cells (PBMC) was investigated. The results showed that AuNPs interact with HepG2 and PBMC cells and can exhibit genotoxicity at very low concentrations. In addition, PBMC were found to be less sensitive to the effects of DNA damage toxicity than HepG2 cells (Paino et al. 2012).

In a study, the toxicity of silver nanoparticles (AgNPs) was investigated in normal human lung fibroblast (IMR-90) and human glioblastoma cells (U251). AgNPs were shown to increase ROS production in a dose-dependent manner. In addition, DNA damage was also shown by single-cell gel electrophoresis and cytokinesis blocked MN assay in dose-dependent, especially in cancer cells. NPs are thought to cause cell cycle stop in the G2/M phase due to repair of damaged DNA. Transmission electron microscopy (TEM) analysis used in the study showed the presence of AgNPs in mitochondria and nucleus and their direct involvement in mitochondrial toxicity and DNA damage (AshaRani et al. 2009).

The toxicity of AgNPs was investigated in human lung carcinoma cells (A549). As a result of the study, it was shown that AgNPs were taken up by the cells, causing oxidative stress by increasing ROS formation and leading to apoptotic and necrotic cell death. ROS formation was shown to cause direct and indirect damage to DNA. It has also been shown that there is a dose-dependent increase in the level of volumetric DNA inserts after 24 h exposure of cells to AgNPs (Foldbjerg et al. 2011).

Nanoplastics (NPPs), one of the leading environmental pollutants, are 100–1,000 nm in size and are formed by the breakdown of plastics. While NPPs are widely used, they are considered pollutants since their toxicity is not fully known. In a study investigating this issue, the genotoxic effects of NPPs on colorectal adenocarcinoma cells (HCT116) were investigated by cytokinesis-block micronucleus (CBMN) assay. As a result of the study, it was reported that NPPs showed moderate toxicity in terms of ROS formation and DNA damage (Vecchiotti et al. 2021).

Recent work in the field of nanotechnology has focused on NPs specifically designed for the simultaneous treatment and imaging of tumors (theragnostic NPs). Among these, zinc oxide (ZnO) NPs are a wide-gap (3.3 eV) semiconductor with important properties suitable for use in cancer therapy, bioimaging, and drug delivery. It also shows biocompatibility and selective toxicity against cancer cells and strong photocatalytic properties under UV radiation. Due to these properties, in a study, gadolinium (Gd) element was used in NP formation to improve the imaging visualization capabilities of ZnO NPs. As a result of the study, it was reported that cancer cells were blocked in G1, apoptosis and micronuclei formation increased, and DNA repair efficiency was impaired due to the downregulation of mRNA levels of XRCC2 and XRCC4 genes (Zangeneh et al. 2019).

In another study, the theragnostic use of dipeptide (Phe-Phe) structures containing mercury (Hg^{2+}) was evaluated, and it was shown that these structures

were promising due to their high biocompatibility properties and low genotoxic effects (Bacanli et al. 2023).

Titanium dioxide (TiO_2) is one of the most produced NMs in the world. Because TiO_2 is used worldwide in large numbers, it is highly possible to be exposed to it daily. TiO_2 genotoxicity can be classified as primary and secondary genotoxicity. As mentioned previously, direct genotoxicity occurs when there is an interaction between NMs and genetic materials like DNA and chromosomes. Indirect genotoxicity occurs from the NM-affected genetic materials, like proteins that are involved in DNA replication, ROS, or free radicals (Raja et al. 2021; Wani and Shadab 2020).

Researchers have studied the genotoxicity of TiO_2 NM in in vitro and in vivo models. In in vitro studies, it showed its genotoxicity by inhibiting DNA repair mechanisms, changing the morphology of cells, and inducing different immune responses. The agglomeration of TiO_2 may induce some unwanted immune responses, thus leading to health problems (Armand et al. 2016).

With all the developments on the medical side of nanoparticles, mesoporous silica nanoparticles (MSNs) have been found appropriate for delivering drugs, biomolecules, and biomaterials. As ROS increases and accumulates in the body, causing DNA and biomolecule damage, many diseases such as cancer occur as a result of this (Choudante et al. 2022).

The genotoxicity of metal oxide NMs is affected by certain factors like chemical properties, experimental animals, and exposure time/mode/concentration. In the future with more understanding of the genotoxicity of NMs, many chronic diseases including cancer can be treated.

3.3 Phytochemical Studies

The most common cancer treatments are chemotherapy, radiotherapy, immunotherapy, and surgery. However, these treatment methods can be harsh on the body and cells or alleviate cancer. The main aim of cancer treatment is to terminate tumors while prolonging life and enhancing life quality (Bhuia et al. 2023). Thus, ever since cancer has been found, scientists have been looking for better treatment methods (Zhang et al. 2023). Worldwide cancer incidence is increasing as years go by; therefore, more people are complaining about chemotherapy. As the complaints increase and people try to avoid chemicals, many people started to use nature and natural products. Natural chemotherapeutic agents have more than one remedial effect (Deshpande et al. 2022).

Polyphenolic compounds, especially those with antioxidant activity, are promising anticancer agents due to their pro-apoptotic activity and chemopreventive potential. The genotoxicity of three flavonoid glycosides (naringin, diosmin, and hesperidin) was investigated in the prostate cancer cell line (DU145). The results showed that the compounds differentially reduced cancer cell number and proliferative activity, induced oxidative stress, and stimulated DNA double-strand breaks and micronuclei formation. Furthermore, the compounds were found to be

DNA hypomethylating agents that modulate the cancer cell epigenome, leading to changes in gene expression patterns (Lewinska et al. 2015). Similarly, Secerli et al. showed that two different phytochemicals, naringin and berberine, can be useful in the treatment of colon cancer in PMMA NP form with low toxic effects (Secerli et al. 2023).

One of the beneficial agents in cancer therapy is curcumin (Cur). Cur is a natural chemotherapeutic agent obtained from the rhizome of *Curcuma longa*. It has a large variety of remedial effects like antitumor, antibacterial, anti-inflammatory, antidiabetic, chemopreventive, and antiangiogenic effects. However, Cur is not soluble in water and has bad bioavailability. This makes it hard for Cur to reach its target; thus, some of the nanotechnology products are used as delivery systems (Deshpande et al. 2022).

Deshpande et al. used chitosan-coated ZnO-curcumin nanocomposite (ZnCurNC) as a co-precipitation method in one of their studies. They used ZnO nanoparticles because of their advantages like stability, low toxicity, and easy synthesis. Their study showed that ZnCurNC is more biocompatible with healthy mammalian cells. They conducted in vitro and in vivo genotoxicity tests to confirm the anticancer activity of ZnCurNC, and the results were satisfactory in both biocompatibility and genotoxicity tests. ZnCurNC showed enhanced therapeutic effect and bioavailability and no significant genotoxicity; thus, tumor growth was inhibited (Deshpande et al. 2022).

Another important phytochemical with beneficial health effects is gracillin. Gracillin, triterpenoid steroidal saponin, is obtained from *Dioscorea nipponica*, which has various remedial effects including anticancer effects. It shows its effects by causing apoptotic cell death, autophagy, induction of ROS, regulation of cell cycle, and antiproliferative, anti-inflammatory, genotoxic, and cytotoxic effects. Gracillin shows its effectiveness against colorectal cancer, lung cancer, etc. with different mechanisms. Li et al. showed that gracillin induced DNA damage in some cancer cell types, thus inducing cellular damage in cancerous cells. This suggests that gracillin showed no genotoxicity against healthy cell lines. Various studies deduced that gracillin showed an anticancer effect against different types of cancers. Gracillin has the potential qualities to serve as an anticancer agent (Bhuia et al. 2023; Li et al. 2022).

Glycyrrhizic acid (GL) is a triterpenoid saponin obtained from *Glycyrrhiza uralensis* Fisch. It is used as a native Chinese herbal remedy for many diseases. It has anti-inflammatory, antitumor, antiviral, antioxidant, immunoregulatory, and liver protector effects, and it is also used for alleviating the adverse effects of chemotherapy. It is also used as a blending agent because it has a diminishing toxicity effect (Zhang et al. 2023).

Like most antitumor agents, GL also has genotoxic effects affecting growth inhibition with the induction of ROS production. Also, GL is a chemoprotective agent due to anti-genotoxic properties. Arjumand and Sultana showed that GL can significantly reverse the genotoxicity of cisplatin, thus increasing glutathione and antioxidant activities (Arjumand and Sultana 2011).

Ptychotis verticillata, also known as Nûnkha, is a medicinal plant endemic to Morocco. This plant is known for its remedial effects; thus, it has been used in traditional medicine for years. *Ptychotis verticillata* essential oil (PVEO) demonstrated antioxidant properties and cytotoxicity against some breast cancer cell lines. The plant is mostly used for its healing effects on digestive system disorders, especially gastric and intestinal problems. It also has various other remedial effects, such as analgesic, diuretic, antimicrobial, and anti-asthmatic effects (Taibi et al. 2023).

Many natural medicinal remedies have genotoxicity in their effect systems, either as genotoxic agents or anti-genotoxic agents. They cause genotoxicity by coming in contact with target organs or cells and creating DNA damage like DNA sequence alternations, DNA strand breaks, etc. Taibi et al. focused on this part and examined the genotoxic effects of the PVEO on rat leukocytes in predetermined concentrations. The results of the study did not show any DNA damage caused by PVEO. According to this finding, PVEO can be considered non-genotoxic in tested concentrations and also showed selectivity toward some breast cancer cell lines (Taibi et al. 2023; Sharifi-Rad et al. 2018).

3.4 Metal Studies

A metal is an inorganic substance that has high electrical and thermal conductivity, has reflectivity of light, can be shaped, and is prone to forming cations (Minay and Boccaccini 2005). Metalloids are substances that possess similarities with the physical and chemical properties of metals and nonmetals (Wysocki et al. 2023).

Arsenic (As) is a metalloid that can easily enter the ecological cycle with either soil or water. Actually, As mainly enters our environment through geological from the Earth's crust, and anthropogenic activities. In the biological cycle, it can be present at any step. It is a highly toxic element; therefore, it is important to take precautions. As is used in mining, chemical industries, cosmetics, insecticides, pigments, etc. (Ozturk et al. 2022). Arsenic is a highly mutagenic, and genotoxic metalloid element. Arsenic mutagenicity originates from its interference with the DNA repairing system (Ozturk et al. 2022). A study investigated the possible genotoxic effects of arsenic trioxide on DNA synthesis and on human colon cancer (HT-29) cells. The proliferative response (DNA synthesis) to arsenic trioxide, [3H] thymidine incorporation, and genotoxic effects were evaluated by alkaline single-cell gel electrophoresis. The results showed that arsenic trioxide affected DNA synthesis in HT-29 cells, showing a slight increase in cell proliferation at low exposure levels and a significant inhibition at higher doses. The study also reported that exposure to arsenic trioxide induced genotoxicity by causing significant increases in DNA damage, comet tail lengths, and tail moment compared to unexposed cells (Stevens et al. 2010).

In the human body, especially, As shows its effects on growth factors, DNA damage, and ROS. It affects the human body by creating DNA adducts, cross-linked

DNA, and proteins. The main source of the genotoxic effects of As is ROS. Through ROS, it triggers DNA damage, such as nucleotide, base excision repair, and mismatch repair; increases gluconeogenesis; and forms carbonyl derivatives. With As-induced oxidative stress in long-term exposure, the activities of glutathione peroxidase (GPx) and catalase (CAT) decrease; as these enzymes cannot continue their activities, the level of oxidative stress and free radicals increase, resulting in various disease, thus showing As genotoxicity (Ozturk et al. 2022).

Antimony (Sb) is a genotoxic metalloid element. After exposure, Sb shows toxic effects on the skin, in the respiratory, cardiovascular, and gastrointestinal systems. Its highest concentrations can be found in the kidneys and liver, and lungs, if inhaled. Sb is environmentally important as its toxicity is seen mostly in occupational exposure (Wysocki et al. 2023).

Sb and As are generally thought to be related structurally. Genotoxic effects of Sb mainly result from oxidative stress–originated DNA damage. Antimonite (Sb(III)) shows its effects by cellular redox balance and disrupting some enzyme functions, thus causing DNA damage. Metalloid toxicity especially depends on metabolic transformations. Depending on the form, both elements can change target and toxicity. As and Sb may undergo dimethylation, thus changing properties and behavior, resulting in changed toxic effects (Wysocki et al. 2023).

Many occupational and environmental exposure studies for Sb showed little to no increase in cancer incidence. Studies also stated that at low concentrations of Sb(III), there is a potential for increased oxidative stress caused by indirect DNA damage. There are also studies on the effects of Sb effect on DNA repair. In some of the studies, it was demonstrated that Sb can inhibit DNA repair in sub-cytotoxic and cytotoxic concentrations, therefore causing genotoxicity (Wysocki et al. 2023).

3.5 Other Studies

Tobacco usage is a risk factor that kills over 8.7 million people all around the world, with 1.3 million of these deaths being vulnerable to secondhand smoke exposure, including nonsmokers, children, and infants (WHO, 2003).

Electronic cigarettes (e-cigarettes) are an alternative way to tobacco smoking and have been around since 2003 (WHO 2023). Even though there are many studies stating otherwise, many people believe that e-cigarettes are more innocent and riskless compared to conventional cigarettes (CC). Even if it cannot be said that e-cigarettes are carcinogenic, their smoke is (Hikisz and Jacenik 2022; Platel et al. 2022). An e-cigarette is a portable complex electronic device that mimics smoking and includes a wide range of chemicals that do not stray further than CC (Hikisz and Jacenik 2022). It is used with e-liquids which have nicotine, flavoring, and a matrix that contains glycerin, propylene glycol, or a mixture in their formulations. Many ingredients of e-cigarettes are either genotoxic or carcinogenic as many of them are products of tobacco-specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs) (Zhang and Wen 2023).

E-cigarettes do not contain tobacco leaves and do not undergo combustion; thus, it can be said that e-cigarettes are less harmful to living beings. But while using it, a vapor comes out of the device which contains some toxicants such as acrolein, which has genotoxic properties. Acrolein is also considered a group 2A carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 2021). It shows its toxicity by producing DNA adducts. It is known that some of these DNA adducts have mutagenic abilities.

Cheng et al. reported that with the usage of e-cigarettes, levels of acrolein metabolites in buccal cells increase to a high degree. Acrolein-DNA adducts formed in buccal/oral cells increase cancer risk and can be used as an acrolein exposure biomarker (Cheng et al. 2022).

4 Conclusion

Xenobiotics causing changes in genetic material and resulting in DNA damage are defined as genotoxicity. In vitro, in vivo, and in silico models are used to determine genotoxicity and to evaluate genotoxic potential. This DNA damage, which causes genotoxicity, leads to many diseases, especially cancer. Xenobiotics can show their genotoxicity in two ways: direct and indirect. Direct genotoxicity occurs when there is interaction between xenobiotic and genetic materials. Indirect genotoxicity occurs from the xenobiotic-affected genetic material-induced ROS or free radicals. Genotoxicity is closely related to carcinogenesis, as it includes large chromosomal abnormalities as well as small abnormalities. Therefore, it forms the basis of cancer studies. Tumor formation begins when a carcinogen interacts with DNA and creates a permanent change in the genome of cells. Tumors can be initiated by a mutation in a gene within a single cell as a result of an exogenous or endogenous carcinogens.

Studies have reported that environmental and occupational exposure may cause genotoxic damage and pose risks to human health such as cancer. Although anticancer drugs are administered in a controlled manner in hospitals, this exposure is important. These studies reported a relationship between DNA repair gene polymorphisms and genotoxicity, especially occupational exposure to antineoplastics.

In addition to the classical methods used in cancer treatment, recent studies also focus on plant-derived compounds and NPs. In particular, NPs attract attention because they have unique properties that other compounds do not have. However, genotoxicity and carcinogenicity of these nanoparticles need to be well evaluated.

In this chapter, genotoxicity caused by many carcinogens such as metals, plant-derived compounds, nanoparticles, batteries, and the relationship between genotoxicity and cancer are emphasized. Understanding the chronic and acute toxicity of all these xenobiotics considering both human health and environmental exposure is important for many diseases, especially cancer. Therefore, further in vitro and in vivo studies are needed to determine the genotoxic effects of such xenobiotics on the mechanism of cancer formation.

References

- Araldi RP, de Melo TC, Mendes TB, de Sá Júnior PL, Nozima BHN, Ito ET, de Carvalho RF, de Souza EB, de Cassia Stocco R (2015) Using the comet and micronucleus assays for genotoxicity studies: a review. *Biomed Pharmacother* 72:74–82. <https://doi.org/10.1016/J.BIOPHA.2015.04.004>
- Aristizabal-Pachon AF, Castillo WO (2020) Genotoxic evaluation of occupational exposure to antineoplastic drugs. *Toxicol Res* 36(1):29–36. <https://doi.org/10.1007/S43188-019-00003-7/TABLES/3>
- Arjumand W, Sultana S (2011) Glycyrrhizic acid: a phytochemical with a protective role against cisplatin-induced genotoxicity and nephrotoxicity. *Life Sci* 89(13–14):422–429. <https://doi.org/10.1016/j.lfs.2011.06.016>
- Armand L, Tarantini A, Beal D, Biola-Clier M, Bobyk L, Sorieul S, Pernet- Gallay K, Marie-Desvergne C, Lynch I, Herlin-Boime N, Carriere M (2016) Long-term exposure of A549 cells to titanium dioxide nanoparticles induces DNA damage and sensitizes cells towards genotoxic agents. *Nanotoxicology* 10(7):913–923. <https://doi.org/10.3109/17435390.2016.1141338>
- AshaRani PV, Mun GLK, Hande MP, Valiyaveetil S (2009) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3(2):279–290. https://doi.org/10.1021/NN800596W/SUPPL_FILE/NN800596W_SI_001.PDF
- Aydemir N, Bilaloglu R (2003) Genotoxicity of two anticancer drugs, gemcitabine and topotecan, in mouse bone marrow in vivo. *Mutat Res Genet Toxicol Environ Mutagen* 537(1):43–51. [https://doi.org/10.1016/S1383-5718\(03\)00049-4](https://doi.org/10.1016/S1383-5718(03)00049-4)
- Bacanlı M, Aydın S, Başaran AA, Başaran N (2017) Are all phytochemicals useful in the preventing of DNA damage? *Food Chem Toxicol* 109:210–217
- Bacanlı M, Eşim O, Erdoğan H, Sarper M, Erdem O, Özkan Y (2021) Evaluation of cytotoxic and genotoxic effects of paclitaxel-loaded PLGA nanoparticles in neuroblastoma cells. *Food Chem Toxicol* 154:112323
- Bacanlı M, Secerli I, Karayavuz B, Erdem O, Erdoğan H (2023) Is a non-cytotoxic and non-genotoxic novel bioinspired dipeptide structure synthesis possible for theragnostic applications? *Drug Chem Toxicol* 46(5):1015–1023
- Baderna D, Gadaleta D, Lostaglio E, Selvestrel G, Raitano G, Golbamaki A, Lombardo A, Benfenati E (2020) New in silico models to predict in vitro micronucleus induction as marker of genotoxicity. *J Hazard Mater* 385:121638. <https://doi.org/10.1016/J.JHAZMAT.2019.121638>
- Bhuia MS, Chowdhury R, Sonia FA, Kamli H, Shaikh A, El-Nashar S, El-Shazly M, Islam MT (2023) Anticancer potential of the plant-derived Saponin Gracillin: a comprehensive review of mechanistic approaches. *Chem Biodivers* 20(9):e202300847. <https://doi.org/10.1002/cbdv.202300847>
- Carr C, Ng J, Wigmore T (2008) The side effects of chemotherapeutic agents. *Curr Anaesth Critic Care* 19(2):70–79. <https://doi.org/10.1016/J.CACC.2008.01.004>
- Cavallo D, Ursini CL, Perniconi B, Di Francesco A, Giglio M, Rubino FM, Marinaccio A, Iavicoli S (2005) Evaluation of genotoxic effects induced by exposure to antineoplastic drugs in lymphocytes and exfoliated buccal cells of oncology nurses and pharmacy employees. *Mutat Res Genet Toxicol Environ Mutagen* 587(1–2):45–51. <https://doi.org/10.1016/J.MRGENTOX.2005.07.008>
- Cheng G, Guo J, Carmella SG, Lindgren B, Ikuemonisan J, Niesen B, Jensen J, Hatsukami DK, Balbo S, Hecht SS (2022) Increased acrolein-DNA adducts in buccal brushings of e-cigarette users. *Carcinogenesis* 43(5):437–444. <https://doi.org/10.1093/carcin/bgac026>
- Choudante PC, Nethi SK, Díaz-García D, Prashar S, Misra S, Gómez-Ruiz S, Patra CR (2022) Tin-loaded mesoporous silica nanoparticles: antineoplastic properties and genotoxicity assessment. *Biomater Adv* 137:212819. <https://doi.org/10.1016/j.bioadv.2022.212819>
- Corvi R, Madia F (2017) In vitro genotoxicity testing—can the performance be enhanced? *Food Chem Toxicol* 106:600–608. <https://doi.org/10.1016/J.FCT.2016.08.024>

- Deshpande SS, Veeragoni D, Rachamalla HK, Misra S (2022) Anticancer properties of ZnO-curcumin nanocomposite against melanoma cancer and its genotoxicity profiling. *J Drug Del Sci Technol* 75:103703. <https://doi.org/10.1016/j.jddst.2022.103703>
- Doak SH, Liu Y, Chen C (2017) Genotoxicity and cancer. Adverse effects of engineered nanomaterials: exposure, toxicology, and impact on human health, end edn, pp 423–445. <https://doi.org/10.1016/B978-0-12-809199-9.00018-5>
- Ellinger-Ziegelbauer H, Aubrecht J, Kleinjans JC, Ahr HJ (2009) Application of toxicogenomics to study mechanisms of genotoxicity and carcinogenicity. *Toxicol Lett* 186(1):36–44
- Elsaesser A, Howard CV (2012) Toxicology of nanoparticles. *Adv Drug Deliv Rev* 64(2):129–137. <https://doi.org/10.1016/j.addr.2011.09.001>
- Erdoğan H, Karayavuz B, Bacanlı M, Sarper M, Esim O, Altuntaş S et al (2023) Low-molecular-weight dipeptide Nanogel containing Plasmonic gold nanoparticles for drug release applications. *ACS Appl Nano Mater* 6(8):6700–6714
- Foldbjerg R, Dang DA, Autrup H (2011) Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. *Arch Toxicol* 85(7):743–750. <https://doi.org/10.1007/S00204-010-0545-5/FIGURES/5>
- Girigoswami K (2018) Toxicity of metal oxide nanoparticles. In: Saquib Q, Faisal M, Al-Khedhairi A, Alatar A (eds) *Cellular and molecular toxicology of nanoparticles*, Advances in experimental medicine and biology, vol 1048. Springer, Cham. https://doi.org/10.1007/978-3-319-72041-8_7
- Graziano MJ, Jacobson-Kram D (eds) (2015) *Genotoxicity and carcinogenicity testing of pharmaceuticals*, vol 11763. Springer
- Hayashi Y (1992) Overview of genotoxic carcinogens and non-genotoxic carcinogens. *Exp Toxicol Pathol* 44(8):465–471
- Hikisz P, Jacenik D (2022) The tobacco smoke component, Acrolein, as a major culprit in lung diseases and respiratory cancers: molecular mechanisms of acrolein cytotoxic activity. *Cells* 12(6):879. <https://doi.org/10.3390/cells12060879>
- Huo S, Jin S, Ma X, Xue X, Yang K, Kumar A et al (2014) Ultrasmall gold nanoparticles as carriers for nucleus-based gene therapy due to size-dependent nuclear entry. *ACS Nano* 8(6):5852–5862
- IARC Monographs Volume 128: Acrolein, Crotonaldehyde, and Arecoline (n.d.) Retrieved November 17, 2023, from The International Agency for Research on Cancer (IARC). <https://www.iarc.who.int/news-events/iarc-monographs-volume-128-acrolein-crotonaldehyde-and-arecoline/>
- Jain PK, ElSayed IH, El-Sayed MA (2007) Au nanoparticles target cancer. *Nano Today* 2(1):18–29. [https://doi.org/10.1016/S1748-0132\(07\)70016-6](https://doi.org/10.1016/S1748-0132(07)70016-6)
- Karamese M, Atalay N, Kugu S, Dinc O, Kati A, Bacanlı M et al (2023) FOLFIRINOX-loaded immunoliposome-like particles for localized pancreatic cancer treatment. *J Drug Deliv Sci Technol* 104595:104595
- Kirsch-Volders M, Vanhauwaert A, Eichenlaub-Ritter U, Decordier I (2003) Indirect mechanisms of genotoxicity. *Toxicol Lett* 140–141:63–74. [https://doi.org/10.1016/S0378-4274\(02\)00498-8](https://doi.org/10.1016/S0378-4274(02)00498-8)
- Klaunig JE, Wang Z, Pu X, Zhou S (2011) Oxidative stress and oxidative damage in chemical carcinogenesis. *Toxicol Appl Pharmacol* 254(2):86–99. <https://doi.org/10.1016/J.TAAP.2009.11.028>
- Landsiedel R, Honarvar N, Seiffert SB, Oesch B, Oesch F (2022) Genotoxicity testing of nanomaterials. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 14(6):e1833. <https://doi.org/10.1002/WNAN.1833>
- Lecona E, Fernandez-Capetillo O (2018) Targeting ATR in cancer. *Nat Rev Cancer* 18(9):586–595. <https://doi.org/10.1038/s41568-018-0034-3>
- Lewinska A, Siwak J, Rzeszutek I, Wnuk M (2015) Diosmin induces genotoxicity and apoptosis in DU145 prostate cancer cell line. *Toxicol In Vitro* 29(3):417–425. <https://doi.org/10.1016/J.TIV.2014.12.005>

- Li J, Zhu P, Wang Y, Jiang X, Zhang Z, Zhang Z, Yung K (2022) Gracillin exerts anti-melanoma effects in vitro and in vivo: role of DNA damage, apoptosis and autophagy. *Phytomedicine* 108: 154526. <https://doi.org/10.1016/j.phymed.2022.154526>
- Marchetti F, Cardoso R, Chen CL, Douglas GR, Elloway J, Escobar PA, Harper T, Heflich RH, Kidd D, Lynch AM, Myers MB, Parsons BL, Salk JJ, Settivari RS, Smith-Roe SL, Witt KL, Yauk CL, Young R, Zhang S, Minocherhomji S (2023) Error-corrected next generation sequencing—promises and challenges for genotoxicity and cancer risk assessment. *Mutat Res Rev Mutat Res* 792:108466. <https://doi.org/10.1016/J.MRREV.2023.108466>
- McClendon AK, Osheroff N (2007) DNA topoisomerase II, genotoxicity, and cancer. *Mutat Res Fundament Mol Mechan Mutagen* 623(1–2):83–97. <https://doi.org/10.1016/J.MRFMMM.2007.06.009>
- Minay EJ, Boccaccini AR (2005) Metals. *Biomaterials, Artificial Organs and Tissue Engineering* 15–25. <https://doi.org/10.1533/9781845690861.1.15>
- No tobacco, W. H. O (ed) (2023). (publication) WHO report on the global tobacco epidemic, 2023: protect people from tobacco smoke. WHO. Acrolein is an Group 2A carcinogen
- Nohmi T (2018) Thresholds of genotoxic and non-genotoxic carcinogens. *Toxicol Res* 34:281–290
- Ozturk M, Metin M, Altay V, Bhat RA, Ejaz M, Gul A et al (2022) Arsenic and human health: genotoxicity, epigenomic effects, and cancer signaling. *Biol Trace Elem Res* 1-14:988. <https://doi.org/10.1007/s12011-021-02719-w>
- Paino IMM, Marangoni VS, de Oliveira R, de CS, Antunes LMG, Zucolotto V (2012) Cyto and genotoxicity of gold nanoparticles in human hepatocellular carcinoma and peripheral blood mononuclear cells. *Toxicol Lett* 215(2):119–125. <https://doi.org/10.1016/J.TOXLET.2012.09.025>
- Parrella A, Lavorgna M, Criscuolo E, Russo C, Isidori M (2015) Eco-genotoxicity of six anticancer drugs using comet assay in daphnids. *J Hazard Mater* 286:573–580. <https://doi.org/10.1016/J.JHAZMAT.2015.01.012>
- Platel A, Dusautoir R, Kervoaze G, Dourdin G, Gateau E, Talahari S, Huot L, Simar S, Ollivier A, Laine W, Kluza J, Gosset P, Garçon G, Anthérieu S, Guidice JL, Nesslany F (2022) Comparison of the in vivo genotoxicity of electronic and conventional cigarettes aerosols after subacute, subchronic and chronic exposures. *J Hazard Mater* 423:127246. <https://doi.org/10.1016/j.jhazmat.2021.127246>
- Pot GK, Habermann N, Majsak-Newman G, Harvey LJ, Geelen A, Przybylska-Philips K, Nagegast FM, Witterman BJM, van de Meeberg PC, Hart AR, Schaafsma G, Hooiveld G, Gleij M, Lund EK, Pool-Zobel BL, Kampman E (2010) Increasing fish consumption does not affect genotoxicity markers in the colon in an intervention study. *Carcinogenesis* 31(6): 1087–1091. <https://doi.org/10.1093/CARCIN/BGP255>
- Raja IS, Lee JH, Hong SW, Shin D, Lee JH, Han D (2021) A critical review on genotoxicity potential of low dimensional nanomaterials. *J Hazard Mater* 409:124915. <https://doi.org/10.1016/j.jhazmat.2020.124915>
- Ristori S, Scavone F, Bartolozzi C, Giovannelli L (2022) The comet assay for the evaluation of gut content genotoxicity: use in human studies as an early biomarker of colon cancer risk. *Mutat Res Genet Toxicol Environ Mutagen* 878:503477. <https://doi.org/10.1016/J.MRGENTOX.2022.503477>
- Secerli J, Adatepe Ş, Altuntas S, Topal GR, Erdem O, Bacanlı M (2023) *In vitro* toxicity of naringin and berberine alone, and encapsulated within PMMA nanoparticles. *Toxicol In Vitro* 89:105580
- Sharifi-Rad M, Varoni EM, Iriti M, Martorell M, Setzer WN, Del Mar Contreras M, Salehi B, Soltani-Nejad A, Rajabi S, Tajbakhsh M, Sharifi-Rad J (2018) Carvacrol and human health: a comprehensive review. *Phytother Res PTR* 32(9):1675–1687. <https://doi.org/10.1002/ptr.6103>
- Sharma P (2023) Genotoxicity of the nanoparticles. *Impact Nanopart Agricult Soil* 115–128. <https://doi.org/10.1016/B978-0-323-91703-2.00017-8>
- Smith J, Mun Tho L, Xu N, Gillespie A, D. (2010) The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. *Adv Cancer Res* 108(C):73–112. <https://doi.org/10.1016/B978-0-12-380888-2.00003-0>

- Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD (2019) ROS and the DNA damage response in cancer. *Redox Biol* 25:101084. <https://doi.org/10.1016/J.REDOX.2018.101084>
- Stevens JJ, Graham B, Walker AM, Tchounwou PB, Rogers C (2010) The effects of arsenic trioxide on DNA synthesis and genotoxicity in human colon cancer cells. *Intl J Environ Res Public Health* 7(5):2018–2032. <https://doi.org/10.3390/IJERPH7052018>
- Swift LH, Golsteyn RM (2014) Genotoxic anti-cancer agents and their relationship to DNA damage, mitosis, and checkpoint adaptation in proliferating cancer cells. *Intl J Mol Sci* 15(3): 3403–3431. <https://doi.org/10.3390/IJMS15033403>
- Taibi M, Elbouzidi A, Ouahhoud S, Loukili EH, Ouahabi S, Alqahtani AS, Noman OM, Addi M, Bellaouchi R, Asehraoui A, Saalaoui E, Guerrouj BE, Chaabane K (2023) Evaluation of antioxidant activity, cytotoxicity, and genotoxicity of *Ptychotis verticillata* essential oil: towards novel breast cancer therapeutics. *Life* 13(7):1586. <https://doi.org/10.3390/life13071586>
- Toolaram AP, Kümmerer K, Schneider M (2014) Environmental risk assessment of anti-cancer drugs and their transformation products: a focus on their genotoxicity characterization-state of knowledge and shortcomings. *Mutat Res/Rev Mutat Res* 760:18–35. <https://doi.org/10.1016/J.MRREV.2014.02.001>
- Vecchiotti G, Colafarina S, Aloisi M, Zarivi O, Di Carlo P, Poma A (2021) Genotoxicity and oxidative stress induction by polystyrene nanoparticles in the colorectal cancer cell line HCT116. *PLoS One* 16(7):e0255120. <https://doi.org/10.1371/JOURNAL.PONE.0255120>
- Wani MR, Shadab G (2020) Titanium dioxide nanoparticle genotoxicity: a review of recent in vivo and in vitro studies. *Toxicol Ind Health* 36:514. <https://doi.org/10.1177/0748233720936835>
- World Health Organization. Available online: <https://www.who.int/news-room/fact-sheets/detail/tobacco>. Accessed on 17 Nov 2023
- Wysocki R, Rodrigues JI, Litwin I, Tamás MJ (2023) Mechanisms of genotoxicity and proteotoxicity induced by the metalloids arsenic and antimony. *Cell Mol Life Sci* 80(11):342. <https://doi.org/10.1007/s00018-023-04992-5>
- Yadav M, Verma MK, Chauhan NS (2018) A review of metabolic potential of human gut microbiome in human nutrition. *Arch Microbiol* 200:203–217
- Yan JS, Chen M, Wang Q (2013) New drug regulation and approval in China. A comprehensive guide to toxicology in preclinical drug development, pp 713–723. <https://doi.org/10.1016/B978-0-12-387815-1.00029>
- Zangeneh M, Nedaei HA, Mozdarani H, Mahmoudzadeh A, Salimi M (2019) Enhanced cytotoxic and genotoxic effects of gadolinium-doped ZnO nanoparticles on irradiated lung cancer cells at megavoltage radiation energies. *Mater Sci Eng C* 103:109739. <https://doi.org/10.1016/J.MSEC.2019.109739>
- Zhang Q, Wen C (2023) The risk profile of electronic nicotine delivery systems, compared to traditional cigarettes, on oral disease: a review. *Front Public Health* 11:1146949. <https://doi.org/10.3389/fpubh.2023.1146949>
- Zhang Y, Sheng Z, Xiao J, Li Y, Huang J, Jia J, Zeng X, Li L (2023) Advances in the roles of glycyrrhizic acid in cancer therapy. *Front Pharmacol* 14:1265172. <https://doi.org/10.3389/fphar.2023.1265172>



Sour Consequences of Aberrant Glycosylation in Cancer Development

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Abstract

Glycans mediate different functions: maintenance of cell structure, cell-cell and cell-extracellular matrix interactions, regulation of protein structural integrity and function, receptor signaling, and modulation of immune responses.

In epithelial cancer cells, dysregulation of glycosylation leads to aberrant tumor-associated glycan or carbohydrate structures. Altered glycosylation promotes cell proliferation, adhesion, survival, migration, invasion, and trigger immune responses.

In this review, we describe aberrant glycan features of cancer cells and glycosylation pathways. We also highlight glycosylation as a fundamental mechanistic concept associated with the hallmarks of cancer, focusing on the role of glycans in promoting genome instability, maintenance of cell proliferative signaling, evasion of growth suppressors, resistance to cell death, epithelial-to-mesenchymal transition, cell adhesion, migration and invasion, glucose metabolism, and angiogenesis, among other processes that promote cancer growth and metastasis.

Keywords

Aberrant · Cancer · Glycan · Glycosylation · Lectin · Metastasis

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1 Introduction

Glycans are the most diverse and flexible molecules that not only decorate molecules, but also mediate specific functions that rely on the structure and function of the carrier protein or lipid to which they are attached.

Glycans can have a structural role, such as the maintenance of cell and tissue organization, and constitute physical barriers through the glycocalyx or the polysaccharide coat found in eukaryotic and prokaryotic cells, respectively (Varki 2017). In addition, numerous constituents of the extracellular matrix (ECM) in vertebrates consist of vast glycan polymers that give rise to structures like basement membranes and cartilage (Farach-Carson et al. 2014). Glycans also serve as a protective storage reservoir for biologically important molecules, such as heparin-binding growth factors that bind to glycosaminoglycan (GAG) chains within the ECM hindering their diffusion away from the secretion site and extending their active lifespan.

Glycans can also affect the folding of proteins (Jayaprakash and Surolia 2017), protect them against proteolysis, regulate protein structural integrity, solubility, trafficking, or function (Amore et al. 2017; Gao et al. 2021; Guan et al. 2020; Kuribara and Totani 2021; Sun et al. 2020; Varki 2017; Zhou and Qiu 2019). For instance, Fc glycosylation is required to elicit effector functions of human IgG1 (Hristodorov et al. 2013) since it increases its affinity for FcγR (Dekkers et al. 2018). Moreover, glycosylation can significantly influence the catalytic activity of enzymes by altering substrate recognition, specificity, and binding affinity (Goettig 2016). Last, glycans are also carriers of information that are decoded by specific carbohydrate-binding proteins known as lectins (Gabius and Kayser 2014). Glycan-lectin binding mediates interactions within cells and to microbes, triggering host immune responses (Prado Acosta and Lepenies 2019; Varki 2017).

Given the complexity of malignant transformation and the significant role of glycans in nearly all pathophysiological processes, oncogenesis is inevitably accompanied by glycosylation alterations. These modifications include the loss or excessive expression of certain glycan structures, the increased presence of incomplete or truncated glycans, and, less frequently, the emergence of novel glycans, all of which provide molecular diversity in the tumor microenvironment (TME) (Freire-de-Lima 2014). Given that cancer represents a “microevolutionary” process in which only the fittest cells survive (Wu et al. 2016) and considering that tumors are constantly under immune surveillance pressure (Galon et al. 2013), it is plausible that these specific glycan changes are selectively advantageous during tumor progression. Indeed, altered glycosylation promotes cell proliferation and survival and controls tumor-associated immune responses, leading to tumor growth and metastasis (Stowell et al. 2015).

The study of the different glycosylation patterns found in tumor cells with respect to healthy tissues can lead to the identification of cancer biomarkers, both for diagnostic purposes and for evaluating therapeutic responses. In this section, we provide an overview of the key glycosylation characteristics of cancer cells and discuss how glycoconjugates foster cancer development.

2 Glycosylation Pathways and Glycan Structures

Glycosyltransferases, the enzymes that catalyze glycosylation reactions, use activated forms of monosaccharides (nucleotide sugars) (Pucci et al. 2022). Monosaccharide precursors are imported into the cell, salvaged from degraded glycans, or created enzymatically from other sugars within the cells. The donors are synthesized in the cytosol from these precursors and from nucleoside triphosphates, and further transported into the Golgi and endoplasmic reticulum (ER) (Mikkola 2020). Glycosylation relies on the generation of the sugar donor uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) by the hexosamine biosynthetic pathway (HBP), which is required for O-GlcNAcylation in the cytoplasm, nucleus, and mitochondria (Paneque et al. 2023) and for O- and N-linked glycosylation of proteins occurring in the ER and the Golgi apparatus, respectively (Varki et al. 2022). To this end, UDP-GlcNAc is used to produce UDP-N-acetylgalactosamine (UDP-GalNAc), cytidine monophosphate sialic acid (CMP-SiaA), guanosine diphosphate mannose (GDP-Man), and GDP-Fucose (GDP-Fuc) for further glycosylation. Thus, cancer-associated glycosylation is the result of changes in the HBP flux, metabolic reprogramming, and alterations in the expression and activity of enzymes involved in the glycosylation processes (Akella et al. 2019), among others. We describe below the glycosylation pathways of secreted and membrane proteins and glycolipids as well as intracellular glycosylation that serves as a nutritional sensor.

2.1 Glycosylation of Secreted and Membrane Proteins

Two primary classes of glycans are present in membrane and extracellular mammalian glycoproteins:

- (i) *N*-glycans linked to an asparagine (Asn) residue located within a peptide consensus sequence known as Asn-X-Ser/Thr (with X representing any amino acid except proline) (Breitling and Aebi 2013).
- (ii) *O*-GalNAc glycans attached to the hydroxyl groups of serine (Ser) or threonine (Thr) residues (Steentoft et al. 2013).

Both *O*- and *N*-glycan chains typically exhibit branching and elongation, often presenting sialic acids, Lewis blood group-related antigens, or ABO(H) blood group determinants as terminal structures. Moreover, additional glycan diversity arises from various modifications to individual sugars, including O-acetylation of sialic acids and O-sulfation of galactose (Gal) and GlcNAc residues. Mature glycans can also undergo structural remodeling at the cell surface, facilitated by extracellular glycosyltransferases and glycosidases circulating in the plasma or carried by platelets. This further enhances the structural complexity and dynamic nature of the glycome.

2.1.1 N-glycosylation of Proteins

N-glycosylation begins with the synthesis of a dolichol lipid-linked oligosaccharide precursor, which is then transferred *en bloc* to nascent proteins by the oligosaccharyltransferase (OST) enzyme complex located in the ER. Subsequently, the oligosaccharide undergoes processing, involving the addition (by glycosyltransferases) and removal (by glycosidases) of monosaccharides. Throughout this processing, three primary structures of *N*-glycans emerge (Fig. 1):

- (i). The high-mannose type, synthesized within the ER, represents the initial stage of processing.
- (ii). The hybrid type, synthesized within the Golgi apparatus, exhibits characteristics of both high-mannose and complex-type glycans.
- (iii). The complex type, also synthesized within the Golgi apparatus, involves the addition of N-acetylglucosamine (GlcNAc), enabling the elongation of chains known as antennae (bi-, tri-, or tetra-antennary structures).

N-glycan modifications, such as fucosylation (the addition of a fucose residue), sialylation (the addition of a sialic acid residue), or increased synthesis of polylactosamine chains including Lewis x (Le^x), Lewis y (Le^y), sialyl-Lewis x (sLe^x), and sialyl-Lewis a (sLe^a), are usually associated with tumors (Fig. 2).

Altered *N*-glycosylation in cancer is often due to a dysregulation of different glycosyltransferase gene expression. For instance, in the normal mammary gland, the expression of N-acetylglucosaminyltransferase V (GnTV), an enzyme that

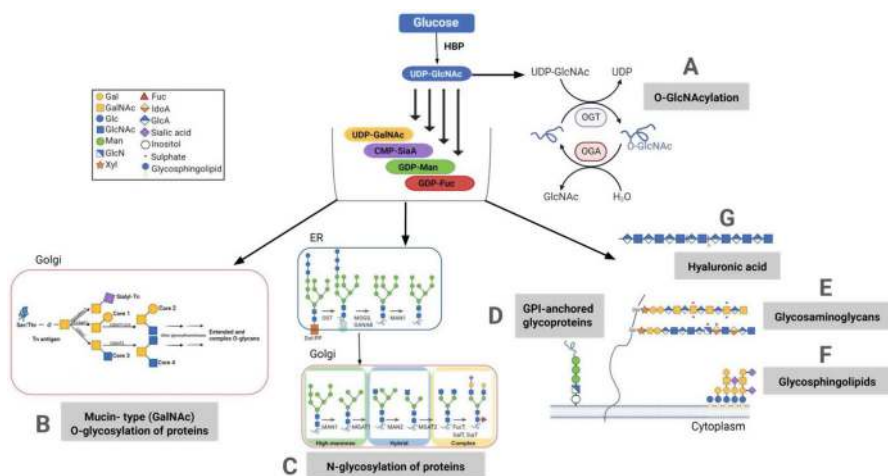
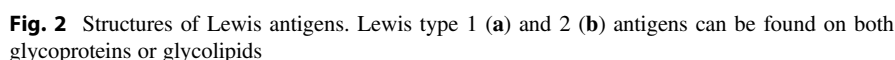


Fig. 1 Glycosylation pathways. The hexosamine biosynthetic pathway (HBP) converts UDP-GlcNAc from glucose, which can turn into UDP-GalNAc, CMP-Sialic acid, GDP-Man, or GDP-Fuc. Activated carbohydrates can be used for intracellular GlcNAcylation (a) of proteins or for the production of secreted or membrane-anchored glycoconjugates, including much-like O-glycosylated proteins (b), N-glycosylated proteins (c), GPI-anchored glycoproteins (d), glycosaminoglycans (e), glycosphingolipids (f), or hyaluronic acid (g)



2.1.2 Mucin-Type O-Glycosylation of Proteins

Unlike *N*-glycans, *O*-linked glycans are added individually and sequentially to the peptide core of a protein (Wandall et al. 2021). The biosynthesis of *O*-linked glycans is considered to be a more straightforward and heterogeneous process than *N*-glycosylation, as it does not require consensus sequences or dolichol precursors for synthesis (van Tol et al. 2019). *O*-GalNAc glycosylation, also termed mucin-type *O*-glycosylation, is the most common type of *O*-glycosylation in mammals and is highly conserved throughout the evolutionary history of many species. The first step, the transfer of GalNAc to a Ser or Thr residue (forming the Tn antigen, GalNAc- α 1-O-Ser/Thr), is an enzymatic reaction catalyzed by a family of at least 20 polypeptide GalNAc transferases (GALNTs). Then, the T synthase, also known as core 1 β 1,3-galactosyltransferase (C1GALT1), catalyzes the galactosylation of Tn to form core 1 (Gal β 1 $\rightarrow$ 3GalNAc, T antigen) (Vasconcelos-Dos-Santos et al. 2015). Core 2 is created by the addition of a branching GlcNAc to core 1 by core 2 β 1,6-N-acetylglucosaminyltransferases (GCNTs 1, 2, and 3 in humans) (Gao et al. 2013). Alternatively, GlcNAc can be transferred in a β 1-3 linkage to generate the core

3 structure, a reaction catalyzed by β 1,3-N-acetylglucosaminyltransferase 6 (B3GNT6) (Gupta et al. 2020). Core 3 can serve as a substrate for the core 4 enzyme, GCNT3, which adds GlcNAc in a β 1-6 linkage to GalNAc (Fig. 1). While core structures 5–8 have a very limited occurrence, core 7 has not been identified in humans (Varki et al. 2022).

In many human cancers, incomplete *O*-glycosylation results in surface expression of truncated *O*-glycans that are usually intermediates in the secretory pathway (Cervoni et al. 2020). These include the Tn antigen, its sialylated version the sialyl-Tn (sTn) antigen, and the elongated T antigen. These have been detected in various carcinomas affecting multiple organs, including the lungs, colon, stomach, and pancreas, and are closely linked to malignant transformation, cancer proliferation, and the development of metastatic capabilities (da Costa et al. 2021; Kaur et al. 2013; Liu et al. 2019b; Schultz et al. 2012; Thomas et al. 2019). Since these structures are typically extended by other glycosyltransferases in normal cells, their expression is considered a hallmark of cancer cells (Radhakrishnan et al. 2014). Additionally, the presence of Tn and sTn antigens is associated with unfavorable patient survival outcomes (Hofmann et al. 2015).

The presence of truncated *O*-glycans in cancer is believed to arise from various factors, including the overexpression of GALNT (Hussain et al. 2016) or sialyltransferase (Hugonnet et al. 2021), mislocation of GALNTs from the Golgi to the ER (Gill et al. 2013; Nguyen et al. 2017), fluctuations in pH levels (Kellokumpu 2019), and hypermethylation or mutations in the T-synthase associated chaperone COSMC (core 1 β 3-Gal-T-specific molecular chaperone) (Radhakrishnan et al. 2014; Sun et al. 2018), among others. Cosmc chaperone is essential for T synthase activity. In the absence of the functional Cosmc, T-synthase misfolds, rendering it incapable of elongating the Tn antigen on glycoproteins (Ju et al. 2011) and leading to a substantial accumulation of Tn and sTn antigens on the cell surface (Schjoldager et al. 2012).

O-glycosylation can also be regulated by the spatial localization of GALNTs, a process known as the GALA pathway (GALNT activation). The activation of the proto-oncogene Src specifically relocates GALNTs from Golgi to ER and allows the *O*-glycosylation of ER-resident proteins (Chia et al. 2014). Since C1GALT1 is not relocated to the ER via GALA, the Tn antigen generated by GALNTs cannot be further extended within the same compartment (Chia et al. 2019; Ju et al. 2014). Moreover, cancer cells exhibit an elevated pH in the Golgi, causing its disruption and fragmentation and in turn, the misplacement of glycosyltransferases from the Golgi to the endosomes, thereby changing glycosylation patterns in malignant cells and increasing the presence of the Tn antigen (Kellokumpu 2019).

2.1.3 GPI-Anchor

A particular glycosylation known as the glycosylphosphatidylinositol (GPI) anchor is a unique type of glycoconjugate added to certain cell surface glycoproteins that contain a C-terminal signal sequence in addition to the N-terminal signal sequence. The GPI anchor consists of a core structure that is assembled and added *en bloc* by the GPI transamidase (GPIT) multi-subunit complex. The expression of this enzyme

is increased in cancers (Dolezal et al. 2014) leading to an accumulation of GPI-anchored proteins in the cell membrane (Tutanov et al. 2023) and favors cell tumorigenicity (Zhao et al. 2012).

2.1.4 Terminal Glycan Structures

The behavior of cells is largely influenced by the end parts of glycoconjugates. These end structures relevant in cancer consist of α 2,3- and α 2,6-linked sialic acids and other fucosylated structures like the Lewis (Le) antigens, such as Le^x and Le^a, as described below.

- (i) Fucosylation: Fucosylation alterations can be categorized into two main types: (i) core fucosylation and (ii) outer-arm fucosylation. These modifications are catalyzed by fucosyltransferases (FUT 1–11), each responsible for adding a Fuc residue at a specific linkage within the *N*-glycan structure. FUT8 is the only enzyme capable of introducing a fucose residue at the α 1–6 linkage of the *N*-glycan structure, which is referred to as core fucosylation. In contrast, the remaining FUTs can add fucose residues at various linkages located on the antenna portion of the *N*-glycan structure, known as outer-arm fucosylation (Jia et al. 2018).
- (ii) Lewis-related antigens: Lewis antigens are terminal fucosylated carbohydrate epitopes belonging to the histo-blood group antigen system (Blanas et al. 2018a, b), that can be found on *O*-glycans, *N*-glycans, and glycolipids (Fig. 2). The synthesis of these antigens requires specific enzymatic machinery (Kudelka et al. 2015). The α -(1, 2) fucosyltransferase is responsible for forming the H antigen. UDP-GalNAc-transferase and Gal-transferase are responsible for forming the A and B antigens, respectively, by adding a GalNAc or Gal residue on the β -galactose of the H antigen (Kudelka et al. 2015). In most human carcinomas, there is a decrease in the expression of A and B antigens on cancer cells due to a reduction or absence of AB transferase activity (Abegaz 2021; Kominato et al. 2020).
- (iii) Sialylation: Sialylation involves attaching of sialic acids, which are negatively charged sugar residues, to the terminal position of glycan chains on glycoproteins and glycolipids. Sialic acid is crucial in cellular interactions involving the ECM, immune cells, epithelial cells, antibodies, and intercellular processes and has profound implications in cancer (Bassaganas et al. 2014; Bull et al. 2014; Hauselmann and Borsig 2014; Lustig et al. 2023). Indeed, sialic acid accumulation (Munkley 2016; Rodrigues and Macauley 2018) promotes tumor metastasis through the stimulation of tumor invasion and migration (Wang et al. 2016a; Zhao et al. 2017; Zhou et al. 2020), inhibition of apoptosis (Swindall and Bellis 2011), and evasion of immune surveillance (Macauley et al. 2014; Rodrigues and Macauley 2018). Cancer-associated hypersialylation is the result of the differential expression and activity of sialyltransferases, which transfer sialic acid (Hugonnet et al. 2021), and neuraminidases, which

cleave sialic acid from glycan chains (Rodrigues and Macauley 2018). Alterations in sialylated glycans in cancer involve the overexpression of sialylated Lewis antigens (sLe^a and sLe^x) (Natoni et al. 2016), elevated levels of sTn (Munkley 2016), and an increase in the sialylated ganglioside GM2 (Bull et al. 2014; Ishihara et al. 2018; Peixoto et al. 2019).

When the same monosaccharide units are linked with other glycosidic bonds, they give rise to their related structures Lewis a, b, x, and y. Further sialylation forms sLe^a and sLe^x (Fig. 2). The expression of both sLe^x and/or sLe^a is progressively observed in various cancers and indicates poor prognosis (Pinho and Reis 2015). The ability of sLe^x and sLe^a to bind with selectins, a family of lectins whose functions are well characterized as mediators of leukocyte trafficking (Wright and Cooper 2014) and homing (McEver 2015), allows tumor cells to spread and colonize distant organs during the metastatic cascade (Natoni et al. 2016; Reymond et al. 2013). Sialyl-Lewis-related structures also have immunosuppressive functions by interacting with Siglecs (Huang et al. 2019), as will be discussed in the next chapter.

2.1.5 Proteoglycans

Proteoglycans (PGs) are a class of heavily glycosylated proteins that consist of a protein core that is covalently linked to one or several anionic high molecular weight glycosaminoglycan (GAG) chains (Walimbe and Panitch 2019). The GAG chains are typically assembled from a tetrasaccharide linker region that is covalently attached to Ser residues within the protein core (Prydz 2015) and include chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and heparan sulfate (HS) (Nikitovic and Perez 2021). In general, GAGs consist of long linear repeats of disaccharide units containing hexuronic acids and hexosamines. Some proteoglycans, like syndecans, may be modified with different GAG types simultaneously, depending on the structure of the ectodomain. Syndecans can carry both CS/DS and HS (Iozzo and Schaefer 2015). The structures of GAGs are extremely diverse, as their synthesis in the Golgi apparatus depends on the highly regulated expression of enzymes (Chen et al. 2018; Mikami and Kitagawa 2013).

PGs significantly participate in intracellular and ECM interactions and cellular signaling, acting as co-receptors for growth factors and tyrosine kinases (Baghy et al. 2016; Iozzo and Schaefer 2015; Nikitovic et al. 2018). Their role in cancer development depends on factors like concentration, spatial distribution, size, and post-translational modifications. Indeed, GAGs have varying sulfation patterns (Huang et al. 2021) that determine their biological function and are used as recognition motifs for various growth factors, cytokines, and chemokines (Mizumoto et al. 2015; Pinho and Reis 2015; Xu and Esko 2014). Interestingly, high expression of CS or HS sulfotransferases has been reported in epithelial cancers (Li et al. 2017; Oliveira-Ferrer et al. 2015).

2.2 Hyaluronan

Unlike other GAGs, hyaluronic acid (HA) is not covalently attached to a protein core. It is synthesized as an unmodified polysaccharide at the plasma membrane, extruded from the cell surface and cleaved off into the ECM (Litwiniuk et al. 2016). It is an anionic, linear, non-sulfated GAG consisting of repeated disaccharide units of glucuronic acid and GlcNAc by hyaluronan synthases (HAS1-3) and hyaluronidases (HYAL1-3) (Cyphert et al. 2015; Monslow et al. 2015). HA is a structural component of the glycocalyx found in most cells. It interacts with receptors and surface PGs through their hyaluronan-binding motifs (Amorim et al. 2021; Mereiter et al. 2019; Senbanjo and Chellaiah 2017), and regulates the glycocalyx's viscosity (Dogne and Flamion 2020). When it binds, HA activates various signaling pathways involved in cell proliferation, differentiation, motility, and adhesion (Liu et al. 2019a; Wolf et al. 2020; Yan et al. 2021). The degree of HA accumulation strongly correlates with malignancy and unfavorable prognosis for patients with many cancers, such as breast and ovarian carcinomas (Auvinen et al. 2013; Rangel et al. 2015). The expression of hyaluronidases and HA synthases has also been linked with reduced prognosis, therapy response, and tumor pathology in various tumors, including prostate, ovarian, head and neck, and breast carcinoma (Auvinen et al. 2013; Heldin et al. 2019; McAtee et al. 2014).

2.3 O-glycosylation of Intracellular Proteins

O-linked GlcNAc is the most common type of glycoconjugate in the cytosol and nucleus of cells. O-GlcNAcylation is a non-canonical glycosylation consisting of a unique (non-elongated) GlcNAc residue linked to a Ser or Thr in intracellular proteins (van der Laarse et al. 2018). O-GlcNAcylation is a reversible and highly dynamic process, in a fashion analogous to phosphorylation. The reversible addition of GlcNAc is catalyzed by the O-GlcNAc transferase, while O-GlcNAcase (OGA) removes GlcNAc. In combination with phosphorylation, O-GlcNAc regulates a great variety of cellular processes such as cell signaling and gene regulation (Hardiville and Hart 2014; Yang and Qian 2017).

Since O-GlcNAcylation is activated as a response to nutrient deprivation and cellular stress (Hart et al. 2011), it is not unexpected that it plays a pivotal role in cancer (Lee et al. 2021). Indeed, it participates in cell proliferation, angiogenesis, and metastasis of cancer cells by regulating transcription, post-translational modification, cell signal transduction, and inducing epigenetic modifications (Lee et al. 2021). As nutrient sensor, since O-GlcNAc is an end product of the glucose metabolic pathway consuming around 2–5% of the total glucose (Gonzalez-Rellan et al. 2022), O-GlcNAcylation increases under hyperglycemic conditions, a state that favors the HBP and the production of UDP-GlcNAc (Taylor et al. 2008, 2009). Furthermore, during nutrient deprivation, a usual condition of the TME (Ahmadiankia et al. 2019), there is an increase in cellular O-GlcNAcylation due to upregulation of OGT expression, even though UDP-GlcNAc levels are decreased (Lee et al. 2021).

2.4 Glycosylation of Lipids

Glycosphingolipids (GSL), the major glycolipids anchor to the outer cell plasma membrane, are glycosylated ceramides attached to a fatty acid. Depending on their carbohydrate content they are classified into three major series: globo, ganglio, and (neo)lacto-series (Sandhoff et al. 2018). The core structure is synthesized in the Golgi by glucosylceramide synthase, which is then modified by a series of Golgi glycosyltransferases to produce higher-order GSL structures (Fig. 1).

There is increasing evidence showing that GSLs are a class of biomolecules involved in signal transduction, cell-cell communication, and differentiation. In cancer, hypersialylation of gangliosides and hyperfucosylation are common and regulate cell adhesion and motility, further resulting in the initiation of metastasis (Rodrigues and Macauley 2018; Schneider et al. 2017).

3 Functional Properties of Altered Glycans in Cancer

In order to develop malignant tumors able to thrive and spread in the body, cells must acquire a set of functional properties (Hanahan 2022) that confers them with the capability of a sustained proliferation, immortality, migration, and invasion. These are the result of a combination of processes that include genome instability, tumor-promoting inflammation, non-mutational epigenetic reprogramming, and polymorphic microbiomes (Hanahan 2022). In this section, we will highlight glycosylation as a fundamental mechanistic concept associated with the well-acknowledged hall-mark characteristics (Fig. 3). The immunological properties of tumor-associated glycans will be discussed in the next chapter.

3.1 Genome Instability and Mutations

The process of acquiring cancer hallmarks is partly due to the development of genomic instability in cancer cells. This instability generates random mutations and rearrangements in the chromosomes. To prevent instability, a set of sensor molecules actively monitors the genome for DNA damage. When mutations are detected, DNA repair pathways are activated. However, if these repair pathways are mutated or deactivated, mutations will accumulate over time (Stead and Bjedov 2021). As some of these molecules are glycosylated, carbohydrate structures may play important roles in the regulation of the pathways in which they participate.

In particular, O-GlcNAc regulates DNA damage signaling or repair (Na et al. 2020). The p53 tumor suppressor is responsible for maintaining a stable genome (Yao and Dai 2014; Yeo et al. 2016). Its stability and activity are regulated by O-GlcNAc and O-phosphate modifications (de Queiroz et al. 2016). O-GlcNAcsylation also affects the activation and recovery state of ATM in cancer (Zhong et al. 2015), another important regulator of DNA damage repair.

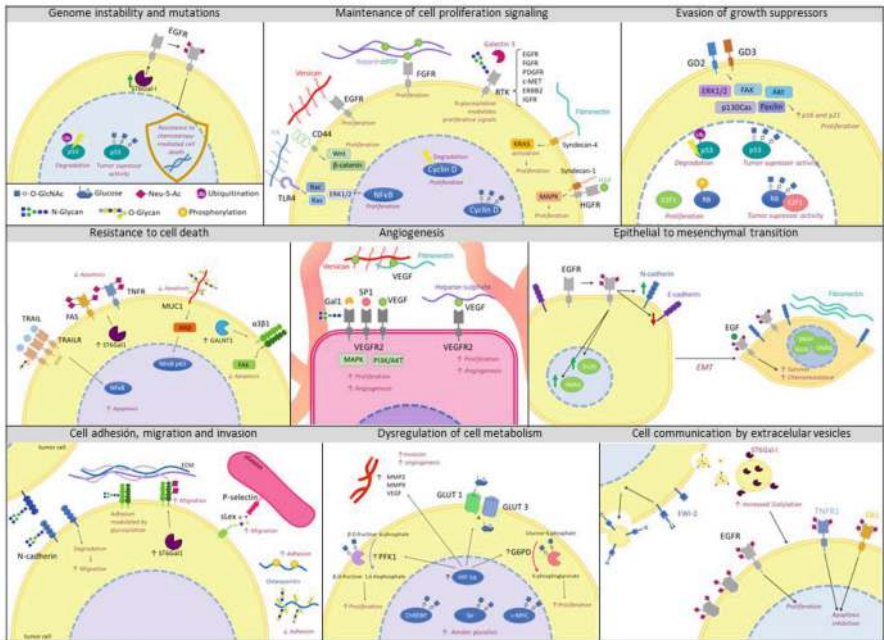


Fig. 3 Molecular and cellular mechanisms of aberrant glycosylation in cancer. Altered glycosylation plays a key role in several mechanisms that promote cancer, such as genome instability and mutations, maintenance of cell proliferation signaling, evasion of growth suppressors, resistance to cell death, angiogenesis, epithelial to mesenchymal transition, cell adhesion, migration and invasion, dysregulation of cell metabolisms and cell communication by extracellular vesicles

Sialylation has also been implicated in the repair of DNA damage and genome stability. The sialylation of epidermal growth factor receptor (EGFR) by ST6GAL1, an upstream regulator of DNA repair pathways in ovarian cancer cells, triggers receptor signaling and resistance to chemotherapy (Britain et al. 2018).

3.2 Maintenance of Cell Proliferative Signaling

Cancer cells are distinguished by uncontrolled proliferation, primarily resulting from the dysregulation of the generation, release, and responsiveness to growth factors (Liu and Ashour Ahmed 2017). In addition, tumor cells can induce ECM remodeling and promote the disruption of growth-inhibiting adhesion complexes, thereby sustaining the proliferative capacity of cancer cells (Yuan et al. 2023). Glycosylation regulates cell proliferation and ECM remodeling by mediating receptor activation, turnover, ligand binding, oligomerization or interaction with adaptor molecules, influencing receptor signaling (Takahashi et al. 2016; Wu et al. 2013).

One of the mechanisms that fosters cell proliferation in multiple cancer types (Kim et al. 2016; Liu et al. 2016) is hyper O-GlcNAcylation, induced by increased

expression of OGT (Hanover et al. 2018; Itkonen et al. 2013; Makwana et al. 2019; Ortiz-Meoz et al. 2014). For instance, O-GlcNAcylation stabilizes transcription factors that are critical in cell cycle progression, such as forkhead protein M1 (FoxM1), cyclin D1, and c-MYC (Itkonen et al. 2013).

Glycosylation also modulates the activation of growth factor receptors, particularly tyrosine kinase receptors (RTKs) (Ferreira et al. 2018; Neill et al. 2015; Wei et al. 2021). For instance, the branching of *N*-glycans in ERBB3 can induce or inhibit cell proliferation (Peixoto et al. 2019) by regulating ERBB3 heterodimerization with ERBB2 (Duarte et al. 2022). Moreover, extensive glycosylation facilitates the interaction of RTKs with galectins, β -galactoside-specific multivalent lectins (Bertuzzi et al. 2020), modulating RTK activation, interaction with ligands, endocytosis and cellular transport of RTKs (Porebska et al. 2021). *N*-glycosylation motifs on ERBB receptors, for example, form binding sites for galectins that modulate activation of ERBB receptors by most of the mentioned mechanisms (Ferreira et al. 2018). Last, gangliosides on cancer cells can also interact with RTKs and modulate the induction of signal transduction pathways has also been demonstrated (Julien et al. 2013).

GAGs also play a crucial role in the regulation of tumor cell proliferation by interacting with growth factors and affecting proliferation signaling (Bat-Erdene et al. 2018). GAGs can bind to and sequester growth factors and cytokines, to regulate their activity by either acting as a co-factor or by limiting their bioavailability (Smock and Meijers 2018). In general, the biological activity and binding affinity of GAGs is dictated by their sulfation pattern, disaccharide unit sequence, and 3D conformation (Miller et al. 2014). In the TME, growth factors like basic fibroblast growth factor (bFGF) become gradually released from entrapment by GAGs and stimulate the proliferation of neighboring cells and promote vascular growth and tissue regeneration (Hao et al. 2018; Li et al. 2018). Last, CS can also inhibit tumor suppressors, such as PTEN, leading to the activation of melanoma cell proliferation (Lin et al. 2018).

PGs exert their influence on tumor growth by interacting with signaling molecules or ECM proteins associated with cell proliferation. For example, perlecan significantly enhances the binding and mitogenic activity of bFGF (Dituri et al. 2022). On the other hand, glypican-3 (GPC3) promotes the Wnt/Frizzled and insulin-like growth factor (IGF) signaling axis (Cheng et al. 2017) while CS proteoglycan 4 (CSPG4) enhances the MAPK cascade (Hsu et al. 2018; Ilieva et al. 2017).

Interestingly, versican, a large CSPG, promotes tumor cell proliferation by inducing EGF-like signaling (Islam and Watanabe 2020), disrupting cell adhesion (Wight et al. 2014), or by interacting with CD44 (Roedig et al. 2020). CD44 can also be regulated by aberrant *O*- and *N*-glycosylation inducing Wnt/ β -catenin signaling pathway (Du et al. 2020; Leon et al. 2022; Lin et al. 2023a; Schmitt et al. 2015; Scott et al. 2023; Zhang et al. 2022). This non-kinase transmembrane glycoprotein overexpressed in several cell types can also interact with HA. In addition, HA binds to TLR4 and layilin and activates NF- κ B (Berdiaki et al. 2023; Ferrandez

et al. 2018) pathways or induces assembly of tight junctions in epithelial cells (Kim et al. 2018), respectively.

Syndecans are transmembrane PGs with CS and HS attached to their extracellular domain that regulate cell proliferation. Syndecan-1, functioning as a coreceptor and collaborator with hepatocyte growth factor (HGF) (Szatmari et al. 2015) and HER2 (Wang et al. 2014), respectively, activates proliferative signals in cancer cells. Furthermore, syndecan-1 is crucial for the growth of tumors, as it acts as an activator of KRAS (Yao et al. 2019). Syndecan-2 expression is linked to the proliferation of malignant tumor cells, particularly in colon carcinoma cells (Hua et al. 2020). Based on a growing body of research, specific PGs, characterized by different core proteins and GAG chains, can serve as oncoproteins and promote cell growth. Syndecan-4, acting as a sensor for the mechanical properties of the ECM, binds to fibronectin and initiates a series of signaling events, including the MAPK pathways, which ultimately lead to cell proliferation (Elfenbein and Simons 2013).

3.3 Evasion of Growth Suppressors

Cancer cells not only induce and receive stimulatory growth signals but also develop the ability to evade negative regulation of tumor suppressor genes (Amin et al. 2015). Interestingly, some canonical suppressors of cell proliferation, such as p53 and retinoblastoma (RB), are regulated by O-GlcNAcylation (Wells et al. 2011; Yang et al. 2006).

p53 is a tumor suppressor involved in repairing DNA damage and inducing apoptosis. Interestingly, the addition of GlcNAc on Ser 149 of p53 favors its stabilization by avoiding ubiquitination and degradation (Soesanto et al. 2008; Yang et al. 2006) and leading to higher tumor suppressive activity. However, mutations on p53 in cancer cells prevent the addition of O-GlcNAc (de Queiroz et al. 2014), and thus, p53 loses its function as a tumor suppressor (Wang et al. 2015a).

RB is a classical tumor suppressor that is critical for cell replication (Fischer and Muller 2017). In G1 phase, RB is hypophosphorylated and binds E2F-1, an essential transcription factor for many S-phase genes. In late G1 phase, hyperphosphorylation of RB releases E2F-1 to transcribe downstream S-phase genes and allows cells to enter the S phase (Fischer and Muller 2017). Furthermore, an increase of O-GlcNAcylation in RB during G1 phase inactivates the transcription factor E2F1, which targets genes encoding proteins that regulate cell cycle progression, DNA repair, and apoptosis (Wells et al. 2011). In addition, the transcript levels of OGT and OGA are also regulated by RB/E2F1 (Muthusamy et al. 2015).

More recently, the role of *N*-glycosylation on tumor suppressors has been reported. Indeed, *N*-glycosylation of KIAA1324, a tumor suppressor and prognostic indicator in several cancer types, plays an essential role in its capacity to act as a tumor suppressor (Kang et al. 2015; Yun et al. 2023).

In glioma cells, the overexpression of gangliosides GD3 and GD2 on the cell surface enhances the activation of signaling molecules such as Erk1/2, FAK, Akt,

p130Cas, and paxillin. This grants the tumor cells an increased motility, invasion, and proliferation, avoiding cell cycle arrest (Iwasawa et al. 2018).

3.4 Resistance to Cell Death

Research suggests that glycosylation has a significant role in the regulation of cell death through various pathways. Certain glycans, glycosyltransferases, and glycosidases are crucial in programmed cell death (Lichtenstein and Rabinovich 2013) as they affect ligand-receptor interactions, which in turn influence the formation of signaling complexes (Kumar Das et al. 2023; Zhang et al. 2019a). Furthermore, the altered glycosylation of cell death receptors may prevent their internalization, as seen in the case of the Fas and TNFR1 receptors (Holdbrooks et al. 2018; Swindall and Bellis 2011).

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family that can cause the expression of pro-inflammatory mediators dependent on NF- κ B and trigger apoptosis when it binds to its specific receptors, TRAIL-Rs (Ashkenazi and Salvesen 2014; Dai et al. 2015). However, many tumors develop resistance to TRAIL-induced apoptosis (Zhang et al. 2019a). Interestingly, *O*- and *N*-glycosylation of TRAIL-R makes cancer cells more receptive to TRAIL-induced apoptosis by promoting the receptor clustering and, consequently, the assembly of the death-inducing signaling complex (DISC) (Dufour et al. 2017; Wagner et al. 2007).

Altered glycosylation in cancer cells can also have an impact on integrin-mediated signaling related to anti-apoptotic functions (Li et al. 2014; Kong et al. 2015). Furthermore, sialylation of integrins prevents colon tumor cells from Gal-3-induced apoptosis (Santos et al. 2016; Zhuo et al. 2008). In the same line, hypersialylation of Fas and TNFR1 death receptor can prevent apoptosis and increase survival of cancer cells. Fas has two *N*-glycosylation sites at N136 and N118. When they are sialylated by ST6Gal-I, colon tumor cells develop resistance against Fas-mediated apoptosis by decreasing activation of caspases 8 and 3, which blocks the association of Fas-associated death domain (FADD) with Fas cytoplasmic tails, and inhibits Fas internalization (Swindall and Bellis 2011). It was also found that α 2-6 sialylation of TNFR1 inhibits TNF-induced TNFR1 internalization. Therefore, by restraining TNFR1 at the cell surface through sialylation, ST6Gal-I acts as a functional switch to divert signaling toward survival (Holdbrooks et al. 2018).

The resistance of cancer cells to apoptosis is also associated with the expression of certain highly *O*-glycosylated proteins (mucins) such as MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC13, MUC16, and MUC21 (Reynolds et al. 2019). For instance, MUC1 overexpression results in sustained induction of the IKK β -NF- κ B p65 pathway (Ahmad et al. 2009). Also, *O*-glycosylation of the MUC1 extracellular domain contributes to anoikis resistance in epithelial cancer cells (Piyush et al. 2017; Zhao et al. 2014). *O*-glycans rich in Gal are also essential for MUC21-mediated resistance to apoptosis (Tian et al. 2022).

Finally, hyper-O-GlcNAcylation can play an anti-apoptotic role in cancer. For instance, in human pancreatic ductal adenocarcinoma cells, oncogenic NF- κ B transcriptional activity is supported by O-GlcNAcylation. By reducing O-GlcNAcylation, NF- κ B transcriptional activity decreases and drives apoptosis (Ma et al. 2013). Moreover, increasing O-GlcNAc in pancreatic cancer cells protects against suspension-induced apoptosis (Ma et al. 2013). Recent studies have reported that OGT promotes fatty liver-associated liver cancer by regulating palmitic acid metabolism and inducing ER stress, thereby activating the oncogenic NF- κ B pathway (Xu et al. 2017a). These data suggest that in cancer cells, regulation of the NF- κ B pathway by O-GlcNAcylation might be multifaceted. Furthermore, hyper-O-GlcNAcylation could contribute to cancer cell survival by mitigating ER stress through the inhibition of the folding enzyme chaperone CHOP (Ferrer et al. 2014). Several other apoptosis- and cycle-related proteins, such as CREB (cAMP response element-binding protein), p53, HIF-1 α (hypoxia-inducible factor 1 α), Id2 (inhibitor of differentiation 2), FoxO1 (forkhead box O1), and AKT (protein kinase B), are regulated by O-GlcNAc. This, in turn, allows cancer cells to escape the fate of death and proliferate endlessly (Ferrer et al. 2014; Ozcan et al. 2010).

3.5 Epithelial to Mesenchymal Transition

Epithelial to mesenchymal transition (EMT), a mechanism that promotes cell invasion and migration, is characterized by a change in cell morphology from a cobblestone epithelial-type shape to an elongated spindle-shaped fibroblast-like appearance (Zhang et al. 2021). Aberrant glycosylation of cancer cells, such as hypersialylation or incomplete glycosylation, induces EMT. Indeed, sialylation of EGFR (Britain et al. 2021) by overexpression of ST6Gal-I increases the expression of mesenchymal markers (N-cadherin, slug, snail, fibronectin) and cell invasiveness whereas it diminishes epithelial markers (E-cadherin, occludin) (Britain et al. 2021). Furthermore, sialylation on EGFR induces EGF-mediated signaling and therefore modulates survival and chemoresistance (Britain et al. 2021; Zhang et al. 2019b).

In the same line, incomplete *O*-glycosylation leading to the expression of Tn-antigen is associated with EMT and metastasis by activation of the cell division control protein 42 (Cdc42) signaling pathway. This results in augmented expression of N-cadherin, Vimentin, and Snail and in the reduction of the epithelial marker ZO-1 (Li et al. 2023b).

Fucosylation also affects the EMT of cancer cells. Downregulation of protein fucosylation by fucosyltransferase 6 (FUT6) promotes cell proliferation, migration, and invasion and relates to a mesenchymal phenotype of cancer cells, suggesting the role of FUT6 in the EMT process (Wang et al. 2023).

3.6 Cell Adhesion, Migration, and Invasion

Cell-cell and cell-ECM interactions are key elements in cancer progression and they are essential in invasion and metastasis. To be able to metastasize, cells need to detach from the primary tumor, migrate to another organ, and reattach to the ECM at the metastatic niche. Since these interactions take place by glycoproteins or glycan-binding proteins, it is foreseen that as a result of aberrant glycosylation, the ability of tumor cells to interact with other cells and ECM proteins could be affected.

Cell adhesion molecules can be grouped into five categories: integrins, selectins, cadherins, members of the immunoglobulin superfamily (IgSF), and mucins (Harjunpaa et al. 2019). These groups differ both structurally and in their binding specificities. While integrins usually bind to ECM; selectins, cadherins, and IgSF bind to ligands on other cells (Harjunpaa et al. 2019).

Selectins are one of the major adhesion proteins on platelets (P-selectin), endothelium (E-selectin), and leukocytes (L-selectin) that bind sLe^x and sLe^a. P-selectins may bind tumor cells in circulation and promote metastasis by formation of a physical shield protecting the tumor cells, enhancing extravasation by increasing endothelium permeability, and signaling directly on the tumor cell (Thurin and Kieber-Emmons 2002).

Integrins are cell-adhesion molecules that consist of non-covalently associated α/β heterodimers. When they interact with their ligands they trigger signaling and integrin clustering, strengthening adhesiveness (Park et al. 2020). Interestingly, the adhesion properties of a cell depend on both integrin expression and its glycosylation status (Singh et al. 2018). In fact, N-glycosylation of some integrins, such as $\alpha v \beta 3$ and $\alpha v \beta 6$, is essential for the attachment of cells to ECM proteins (Singh et al. 2018). The ganglioside GD2 interacts with integrin $\beta 1$ and enhances the malignant phenotype of melanoma cells (Yesmin et al. 2023).

Cadherins mediate cell-cell adhesion and maintenance of tissue architecture. The classical cadherins are epithelial-cadherin, placental-cadherin, and neural-cadherin (E-, P- and N-cadherin, respectively) that interact with catenin to form adherens junctions (Lin et al. 2023b). Like with integrins, N-glycosylation of N-cadherin is essential for migration of cancer cells (Xu et al. 2017b). Particularly, the $\beta 1,6\text{GlcNAc}$ branching on N-cadherin confers protein stability and promotes cell-cell adhesion (Xu et al. 2017b).

Hypersialylation is another key mechanism involved in cell adhesion. Inhibition of sialylation leads to a reduction of cell adhesion and migration mediated by an attenuated activation of integrin downstream signaling (Chiang et al. 2010). Furthermore, increased sialylation of integrins facilitates cell migration across ECM components, which enhances two facets of metastasis, tethering and rolling, and firm adhesion in the extravasation process (Venturi et al. 2019). Upregulation of ST6Gal-1 by colon cancer cells promotes the attachment to collagen I and laminin (Venturi et al. 2019) whereas cleavage of sialic acid from integrins $\alpha 2$, $\alpha 5$, and $\beta 1$ in breast cancer increases adhesion ECM (Yuan et al. 2016).

Finally, another example on how glycosylation has a role in cell adhesion properties is in the case of osteopontin, whose O-glycans are essential for its cell adhesion activity (Kariya et al. 2014).

3.7 Cell Communication and Modulation by Extracellular Vesicles

As a mechanism of intercellular communication, cells relay in extracellular vesicles (EVs) that act as mediators of the crosstalk between cells. These nanoparticles transport different cargoes, such as proteins, lipids, DNA, and different types of RNAs (Konoshenko et al. 2018). EVs are formed by a lipid bilayer that contains surface receptors and membrane molecules.

Tumor cells take advantage of this universal mechanism of communication to reprogram recipient cells and favor the microenvironment for cancer progression (Konoshenko et al. 2018; Rocha et al. 2019). Moreover, glycosylation patterns of exosomes reflect the glycosylation status of the secreting cell, therefore EVs from tumor and non-tumor origin may be differentiated by their glycans, leading to a potential source for biomarkers and diagnostic tools (Li et al. 2023a; Lin et al. 2020; Martins et al. 2021).

Alterations in the glycosylation may change the proteomic content of secreted EVs, since glycans are capable of modulating EV cargo sorting. Furthermore, some proteins are selectively enriched in EVs depending on the profile and architecture of the originating cell (Martins et al. 2023; Rocha et al. 2019). An example is EWI-2, a member of the immunoglobulin superfamily that is N-glycosylated and is involved in cell migration. Inhibition of N-glycosylation on EWI-2 results in a proportional reduction in the recruitment of this protein to EVs, correlating with the number of N-glycan sites removed (Liang et al. 2014).

EVs may also carry proteins involved in the glycosylation process. For instance, soluble and anchored forms of ST6Gal1 are present in EVs derived from cancer cells and can alter the recipient cell phenotype, inducing hypersialylation of its surface glycans and mediating pro-neoplastic properties (Zhang et al. 2019b). As mentioned before, ST6Gal1 favors EGFR-induced signaling and promotes cell proliferation. In addition, ST6Gal1 is capable of sialylating integrins like $\beta 1$ and the death receptors FAS and TNFR1, inhibiting apoptosis signaling (Britain et al. 2021; Zhang et al. 2019b).

Aberrant glycosylation in cancer cells can modify the cargo of EVs. For instance, disruption of core 1 O-glycosylation in colon cancer cells upregulates expression of CD44 in EVs carrying the Tn and sTn antigens (Gaiteiro et al. 2022; Shuvalova et al. 2020). Moreover, CD44 in EVs can promote cell motility, invasion, and cancer-initiating cell marker expression in recipient cells (Wang et al. 2016b). A similar effect is observed in GD2 derived from melanoma cells that promotes cell growth, invasion, and adhesion (Yesmin et al. 2023).

3.8 Dysregulation of Glucose Metabolism

The ability of a tumor cell to adapt and survive depends on its capacity to modify its metabolism in response to changes in its microenvironment. When a tumor experiences hypoxia (reduction in the oxygen levels) the cells shift from using oxidative phosphorylation to anaerobic glycolysis for energy production (Warburg 1956), a process known as the ‘Warburg effect’. This allows tumor cells to increase the uptake of glucose and glutamine to meet the tumor’s energy and biosynthetic demands under oxygen deficiency. This process depends on the activation of hypoxia-inducible transcription factor 1 α (HIF-1 α) by elevated GlcNAcylation (Akella et al. 2019; Ferrer et al. 2014), which in turn upregulates glucose transporters 1 and 3 (GLUT1 and GLUT3) and glycolytic enzymes (Jaskiewicz and Townson 2019; Singh et al. 2015; Yi et al. 2012) to respond to the demands of tumor cells in nutrient status (Ferrer et al. 2014). Indeed, the addition of GlcNAc to glucose-6-phosphate dehydrogenase (G6PD) or phosphofructokinase 1 (PFK1) favors tumor cell proliferation and tumor formation (Ferrer et al. 2014; Yi et al. 2012).

The increased uptake of glucose and glutamine drives an increment in the hexosamine biosynthesis pathway and therefore an increase in O-GlcNAcylation (Cheng and Oon 2018). The transcription factor FoxM1 needs to be O-GlcNAcylated to induce the gene expression of VEGF in prostate cancer (Lynch et al. 2012). The absence of O-GlcNAc in FoxM1 leads to its degradation and to an overall inhibition of invasion and angiogenesis (Lynch et al. 2012).

In addition to regulating glycolytic enzymes under hypoxia, O-GlcNAcylation also favors activity and stability of other transcription factors, such as ChREBP, Sp, and c-MYC, which lead to an increase in aerobic glycolysis to resupply intermediates required for nucleotide metabolism and lipogenesis (Itkonen et al. 2013; Penque et al. 2013).

3.9 Angiogenesis

Sustained tumor angiogenesis is also considered a hallmark of cancer since it is essential to supply nutrients and oxygen to tumor cells (Cheng and Oon 2018). It consists of the propagation of endothelial cells leading to the sprouting and maturation of new blood vessels that infiltrate the TME (Cheng and Oon 2018). This process is followed by degradation of the ECM and extension of the endothelial network resulting from the balance between pro- and anti-angiogenic factors, many of which correspond to PGs, glycoproteins, and GAGs (Chandler et al. 2019). Indeed, aberrant glycosylation enhances tumor angiogenesis (Chandler et al. 2019; da Costa et al. 2021; Varone et al. 2022).

Angiogenesis is driven by the vascular endothelial growth factor (VEGF), which can induce cell proliferation and survival (Eguchi et al. 2022). While VEGF-A is important in angiogenesis, VEGF-C and D are important in lymphangiogenesis (Shibuya 2011). The interaction between VEGF-A and its receptors (VEGFR1 and VEGFR2) and co-receptors (neuropilin-1 and 2) results in the phosphorylation of its

tyrosine residues, triggering MAPK and PI3K/Akt pathway activation (Eguchi et al. 2022).

There are other molecular factors that promote angiogenesis in a VEGF-A-independent pathway. For instance, sphingosine-1 phosphate (S1P), a lipid mediator, is capable of interacting with both S1P and VEGF receptors, triggering its signaling (Eguchi et al. 2022). In addition, galectin-1, a lectin that specifically binds to β -galactosides, triggers VEGFR2-induced signaling in a glycan-dependent manner in absence of VEGF (Croci et al. 2014a, b; Croci and Rabinovich 2014; Hosaka et al. 2020).

Among ECM components, PGs and GAGs are involved in vasculogenesis (Wei et al. 2020). For instance, the sulfate PG versican forms aggregates with the GAG HA, via HA receptors such as CD44 (Asano et al. 2017) and boosts recruitment of host stromal cells. Versican also interacts directly with fibronectin and VEGF, forming a complex that promotes angiogenic-related functions in the endothelial cells and therefore promoting extensive vasculature formation (Papadas and Asimakopoulos 2020). Furthermore, versican is a substrate for several members of a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs (ADAMTS) (Sandy et al. 2001). Particularly, one of versican cleavage products, versikine, has been described to relocate to endothelial cells and contribute to angiogenesis (Asano et al. 2017). Moreover, ADAMTS1 absence has been involved in alteration of the vasculature of tumors and tumor progression (Fernandez-Rodriguez et al. 2016). Although versican is extensively O- and N-glycosylated (Martin et al. 2021), how cancer-associated aberrant glycosylation affects versican cleavage by ADAMTS has not been investigated so far. Nevertheless, it is known that it is overexpressed in breast, ovarian, and gastric cancer (Ghosh et al. 2010; Tian et al. 2012; Zhai et al. 2021) and preferentially sialylated in breast cancer tissues (Tian et al. 2012). Moreover, it has been well established that N-glycosylation allows proper trafficking and stability of one of versican isoforms, V3 (Harten et al. 2020).

Considering the role of HS proteoglycans in angiogenesis, it has been reported they aid in growth factor signaling by stabilizing the complex formed between the growth factor and its receptor, including VEGF (De Zutter et al. 2021). This allows the formation of a more potent and prolonged signaling that mediates differentiation, proliferation, and migration of endothelial cells. Indeed, binding of VEGF165, the predominant VEGF isoform, to endothelial cells is dependent on heparan sulfate, as it facilitates signaling through VEGFR2 (van Wijk and van Kuppevelt 2014). Particularly, when using a high-affinity GAG-binding peptide, it interferes with angiogenesis by inhibiting multiple angiogenic factor-mediated processes, such as proliferation, chemotaxis, adhesion, and spheroid sprouting of endothelial cells (De Zutter et al. 2021). Similar effect was reported when using a chemically modified heparin, evidencing the role of ECM on the angiogenic process (Chung et al. 2015). The targeting of PGs in the ECM as anti-angiogenic therapeutic strategies is of particular interest as it targets multiple growth factors simultaneously, which have been reported to upregulate and compensate each other when targeting VEGF-A alone (De Zutter et al. 2021).

4 Concluding Remarks

In conclusion, all forms of glycosylation described in mammalian cells participate in essential functions for the cell, from structure to functional roles. The dysregulation of glycosylation pathways that take place with cell transformation leads to the expression of tumor-associated aberrant glycans. They promote cancer growth and metastasis by fostering sustained proliferation, immortality, migration, invasion, glucose metabolism, angiogenesis, and modulation of neighboring cells. Thus, tumor-associated glycans constitute both precise cancer biomarkers and potential targets for novel immunotherapeutic approaches as will be discussed in the following chapter.

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References

- Abegaz SB (2021) Human ABO blood groups and their associations with different diseases. *Biomed Res Int* 2021:6629060. <https://doi.org/10.1155/2021/6629060>
- Ahmad R, Raina D, Joshi MD, Kawano T, Ren J, Kharbanda S, Kufe D (2009) MUC1-C oncoprotein functions as a direct activator of the nuclear factor-kappaB p65 transcription factor. *Cancer Res* 69(17):7013–7021. <https://doi.org/10.1158/0008-5472.CAN-09-0523>
- Ahmadiankia N, Bagheri M, Fazli M (2019) Nutrient deprivation modulates the metastatic potential of breast cancer cells. *Rep Biochem Mol Biol* 8(2):139–146
- Akella NM, Ciraku L, Reginato MJ (2019) Fueling the fire: emerging role of the hexosamine biosynthetic pathway in cancer. *BMC Biol* 17(1):52. <https://doi.org/10.1186/s12915-019-0671-3>
- Amin A, Karpowicz PA, Carey TE, Arbiser J, Nahta R, Chen ZG, Dong JT, Kucuk O, Khan GN, Huang GS, Mi S, Lee HY, Reichrath J, Honoki K, Georgakilas AG, Amedei A, Amin A, Helferich B, Boosani CS, Ciriolo MR, Chen S, Mohammed SI, Azmi AS, Keith WN, Bhakta D, Halicka D, Niccolai E, Fujii H, Aquilano K, Ashraf SS, Nowsheen S, Yang X, Bilsland A, Shin DM (2015) Evasion of anti-growth signaling: a key step in tumorigenesis and potential target for treatment and prophylaxis by natural compounds. *Semin Cancer Biol* 35(Suppl):S55–S77. <https://doi.org/10.1016/j.semcancer.2015.02.005>
- Amore A, Knott BC, Supekar NT, Shajahan A, Azadi P, Zhao P, Wells L, Linger JG, Hobday SE, Vander Wall TA, Shollenberger T, Yarbrough JM, Tan Z, Crowley MF, Himmel ME, Decker SR, Beckham GT, Taylor LE II (2017) Distinct roles of N- and O-glycans in cellulase activity and stability. *Proc Natl Acad Sci USA* 114(52):13667–13672. <https://doi.org/10.1073/pnas.1714249114>
- Amorim S, Reis CA, Reis RL, Pires RA (2021) Extracellular matrix mimics using Hyaluronan-based biomaterials. *Trends Biotechnol* 39(1):90–104. <https://doi.org/10.1016/j.tibtech.2020.06.003>
- Anugraham M, Jacob F, Nixdorf S, Everest-Dass AV, Heinzelmann-Schwarz V, Packer NH (2014) Specific glycosylation of membrane proteins in epithelial ovarian cancer cell lines: glycan structures reflect gene expression and DNA methylation status. *Mol Cell Proteomics* 13(9):2213–2232. <https://doi.org/10.1074/mcp.M113.037085>

- Asano K, Nelson CM, Nandadasa S, Aramaki-Hattori N, Lindner DJ, Alban T, Inagaki J, Ohtsuki T, Oohashi T, Apte SS, Hirohata S (2017) Stromal Versican regulates tumor growth by promoting angiogenesis. *Sci Rep* 7(1):17225. <https://doi.org/10.1038/s41598-017-17613-6>
- Ashkenazi A, Salvesen G (2014) Regulated cell death: signaling and mechanisms. *Annu Rev Cell Dev Biol* 30:337–356. <https://doi.org/10.1146/annurev-cellbio-100913-013226>
- Auvinen P, Tammi R, Kosma VM, Sironen R, Soini Y, Mannermaa A, Tumelius R, Uljas E, Tammi M (2013) Increased hyaluronan content and stromal cell CD44 associate with HER2 positivity and poor prognosis in human breast cancer. *Int J Cancer* 132(3):531–539. <https://doi.org/10.1002/ijc.27707>
- Baghy K, Tatnai P, Regos E, Kovalszky I (2016) Proteoglycans in liver cancer. *World J Gastroenterol* 22(1):379–393. <https://doi.org/10.3748/wjg.v22.i1.379>
- Bassaganas S, Perez-Garay M, Peracaula R (2014) Cell surface sialic acid modulates extracellular matrix adhesion and migration in pancreatic adenocarcinoma cells. *Pancreas* 43(1):109–117. <https://doi.org/10.1097/MPA.0b013e31829d9090>
- Bat-Erdene U, Quan E, Chan K, Lee BM, Matook W, Lee KY, Rosales JL (2018) Neutrophil TLR4 and PKR are targets of breast cancer cell glycosaminoglycans and effectors of glycosaminoglycan-induced APRIL secretion. *Oncogenesis* 7(6):45. <https://doi.org/10.1038/s41389-018-0058-2>
- Berdiaki A, Neagu M, Spyridaki I, Kuskov A, Perez S, Nikitovic D (2023) Hyaluronan and reactive oxygen species signaling-novel cues from the matrix? *Antioxidants (Basel)* 12(4):824. <https://doi.org/10.3390/antiox12040824>
- Bertuzzi S, Quintana JJ, Arda A, Gimeno A, Jimenez-Barbero J (2020) Targeting galectins with glycomimetics. *Front Chem* 8:593. <https://doi.org/10.3389/fchem.2020.00593>
- Blanas A, Sahasrabudhe NM, Rodriguez E, van Kooyk Y, van Vliet SJ (2018a) Corrigendum: Fucosylated antigens in cancer: an Alliance toward tumor progression, metastasis, and resistance to chemotherapy. *Front Oncol* 8:150. <https://doi.org/10.3389/fonc.2018.00150>
- Blanas A, Sahasrabudhe NM, Rodriguez E, van Kooyk Y, van Vliet SJ (2018b) Fucosylated antigens in cancer: an Alliance toward tumor progression, metastasis, and resistance to chemotherapy. *Front Oncol* 8:39. <https://doi.org/10.3389/fonc.2018.00039>
- Breitling J, Aepli M (2013) N-linked protein glycosylation in the endoplasmic reticulum. *Cold Spring Harb Perspect Biol* 5(8):a013359. <https://doi.org/10.1101/cshperspect.a013359>
- Britain CM, Holdbrooks AT, Anderson JC, Willey CD, Bellis SL (2018) Sialylation of EGFR by the ST6Gal-I sialyltransferase promotes EGFR activation and resistance to gefitinib-mediated cell death. *J Ovarian Res* 11(1):12. <https://doi.org/10.1186/s13048-018-0385-0>
- Britain CM, Bhalariao N, Silva AD, Chakraborty A, Buchsbaum DJ, Crowley MR, Crossman DK, Edwards YJK, Bellis SL (2021) Glycosyltransferase ST6Gal-I promotes the epithelial to mesenchymal transition in pancreatic cancer cells. *J Biol Chem* 296:100034. <https://doi.org/10.1074/jbc.RA120.014126>
- Bull C, Stoel MA, den Brok MH, Adema GJ (2014) Sialic acids sweeten a tumor's life. *Cancer Res* 74(12):3199–3204. <https://doi.org/10.1158/0008-5472.CAN-14-0728>
- Cervoni GE, Cheng JJ, Stackhouse KA, Heimburg-Molinaro J, Cummings RD (2020) O-glycan recognition and function in mice and human cancers. *Biochem J* 477(8):1541–1564. <https://doi.org/10.1042/BCJ20180103>
- Chandler KB, Costello CE, Rahimi N (2019) Glycosylation in the tumor microenvironment: implications for tumor angiogenesis and metastasis. *Cells* 8(6):544. <https://doi.org/10.3390/cells8060544>
- Chen YH, Narimatsu Y, Clausen TM, Gomes C, Karlsson R, Steentoft C, Spliid CB, Gustavsson T, Salanti A, Persson A, Malmstrom A, Willen D, Ellervik U, Bennett EP, Mao Y, Clausen H, Yang Z (2018) The GAGome: a cell-based library of displayed glycosaminoglycans. *Nat Methods* 15(11):881–888. <https://doi.org/10.1038/s41592-018-0086-z>
- Cheng WK, Oon CE (2018) How glycosylation aids tumor angiogenesis: an updated review. *Biomed Pharmacother* 103:1246–1252. <https://doi.org/10.1016/j.biopha.2018.04.119>

- Cheng W, Huang PC, Chao HM, Jeng YM, Hsu HC, Pan HW, Hwu WL, Lee YM (2017) Glypican-3 induces oncogenicity by preventing IGF-1R degradation, a process that can be blocked by Grb10. *Oncotarget* 8(46):80429–80442. <https://doi.org/10.18632/oncotarget.19035>
- Chia J, Tham KM, Gill DJ, Bard-Chapeau EA, Bard FA (2014) ERK8 is a negative regulator of O-GalNAc glycosylation and cell migration. *eLife* 3:e01828. <https://doi.org/10.7554/eLife.01828>
- Chia J, Tay F, Bard F (2019) The GalNAc-T activation (GALA) pathway: drivers and markers. *PLoS One* 14(3):e0214118. <https://doi.org/10.1371/journal.pone.0214118>
- Chiang CH, Wang CH, Chang HC, More SV, Li WS, Hung WC (2010) A novel sialyltransferase inhibitor AL10 suppresses invasion and metastasis of lung cancer cells by inhibiting integrin-mediated signaling. *J Cell Physiol* 223(2):492–499. <https://doi.org/10.1002/jcp.22068>
- Chung SW, Bae SM, Lee M, Al-Hilal TA, Lee CK, Kim JK, Kim IS, Kim SY, Byun Y (2015) LHT7, a chemically modified heparin, inhibits multiple stages of angiogenesis by blocking VEGF, FGF2 and PDGF-B signaling pathways. *Biomaterials* 37:271–278. <https://doi.org/10.1016/j.biomaterials.2014.10.004>
- Croci DO, Rabinovich GA (2014) Linking tumor hypoxia with VEGFR2 signaling and compensatory angiogenesis: glycans make the difference. *Onco Targets Ther* 3:e29380. <https://doi.org/10.4161/onci.29380>
- Croci DO, Cerliani JP, Dalotto-Moreno T, Mendez-Huergo SP, Mascanfroni ID, Dergan-Dylon S, Toscano MA, Caramelo JJ, Garcia-Vallejo JJ, Ouyang J, Mesri EA, Junttila MR, Bais C, Shipp MA, Salatino M, Rabinovich GA (2014a) Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. *Cell* 156(4):744–758. <https://doi.org/10.1016/j.cell.2014.01.043>
- Croci DO, Cerliani JP, Pinto NA, Morosi LG, Rabinovich GA (2014b) Regulatory role of glycans in the control of hypoxia-driven angiogenesis and sensitivity to anti-angiogenic treatment. *Glycobiology* 24(12):1283–1290. <https://doi.org/10.1093/glycob/cwu083>
- Cyphert JM, Trempus CS, Garantziotis S (2015) Size matters: molecular weight specificity of Hyaluronan effects in cell biology. *Int J Cell Biol* 2015:563818. <https://doi.org/10.1155/2015/563818>
- da Costa V, van Vliet SJ, Carasi P, Frigerio S, Garcia PA, Croci DO, Festari MF, Costa M, Landeira M, Rodriguez-Zraquia SA, Cagnoni AJ, Cutine AM, Rabinovich GA, Osinaga E, Marino KV, Freire T (2021) The Tn antigen promotes lung tumor growth by fostering immunosuppression and angiogenesis via interaction with macrophage galactose-type lectin 2 (MGL2). *Cancer Lett* 518:72–81. <https://doi.org/10.1016/j.canlet.2021.06.012>
- Dai X, Zhang J, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA, Kumar AP, Ahn KS, Sethi G (2015) Targeting TNF-related apoptosis-inducing ligand (TRAIL) receptor by natural products as a potential therapeutic approach for cancer therapy. *Exp Biol Med (Maywood)* 240(6):760–773. <https://doi.org/10.1177/1535370215579167>
- de Queiroz RM, Carvalho E, Dias WB (2014) O-GlcNAcylation: the sweet side of the cancer. *Front Oncol* 4:132. <https://doi.org/10.3389/fonc.2014.00132>
- de Queiroz RM, Madan R, Chien J, Dias WB, Slawson C (2016) Changes in O-linked N-Acetylglucosamine (O-GlcNAc) homeostasis activate the p53 pathway in ovarian cancer cells. *J Biol Chem* 291(36):18897–18914. <https://doi.org/10.1074/jbc.M116.734533>
- De Zutter A, Crijns H, Berghmans N, Garcia-Caballero M, Vanbrabant L, Portner N, Vanheule V, Verscheure P, Siddiquei MM, Abu El-Asrar AM, Carmeliet P, Van Wielendaele P, De Meester I, Van Damme J, Proost P, Struyf S (2021) The chemokine-based peptide, CXCL9 (74–103), inhibits angiogenesis by blocking heparan sulfate proteoglycan-mediated signaling of multiple endothelial growth factors. *Cancers (Basel)* 13(20):5090. <https://doi.org/10.3390/cancers13205090>
- Dekkers G, Rispens T, Vidarsson G (2018) Novel concepts of altered immunoglobulin G Galactosylation in autoimmune diseases. *Front Immunol* 9:553. <https://doi.org/10.3389/fimmu.2018.00553>

- Dituri F, Gigante G, Scialpi R, Mancarella S, Fabregat I, Giannelli G (2022) Proteoglycans in cancer: friends or enemies? A special focus on hepatocellular carcinoma. *Cancers (Basel)* 14(8): 1902. <https://doi.org/10.3390/cancers14081902>
- Dogne S, Flamion B (2020) Endothelial Glycocalyx impairment in disease: focus on Hyaluronan shedding. *Am J Pathol* 190(4):768–780. <https://doi.org/10.1016/j.ajpath.2019.11.016>
- Dolezal S, Hester S, Kirby PS, Nairn A, Pierce M, Abbott KL (2014) Elevated levels of glycosylphosphatidylinositol (GPI) anchored proteins in plasma from human cancers detected by C. Septicum alpha toxin. *Cancer Biomark* 14(1):55–62. <https://doi.org/10.3233/CBM-130377>
- Du T, Jia X, Dong X, Ru X, Li L, Wang Y, Liu J, Feng G, Wen T (2020) Cosmc disruption-mediated aberrant O-glycosylation suppresses breast cancer cell growth via impairment of CD44. *Cancer Manag Res* 12:511–522. <https://doi.org/10.2147/CMAR.S234735>
- Duarte HO, Reis CA, Gomes J (2022) Insights on ErbB glycosylation – contributions to precision oncology. *Trends Cancer* 8(6):448–455. <https://doi.org/10.1016/j.trecan.2022.02.003>
- Dufour F, Rattier T, Shirley S, Picarda G, Constantinescu AA, Morle A, Zakaria AB, Marcion G, Causse S, Szegezdi E, Zajonc DM, Seigneuric R, Guichard G, Gharbi T, Picaud F, Herlem G, Garrido C, Schneider P, Benedict CA, Micheau O (2017) N-glycosylation of mouse TRAIL-R and human TRAIL-R1 enhances TRAIL-induced death. *Cell Death Differ* 24(3):500–510. <https://doi.org/10.1038/cdd.2016.150>
- Eguchi R, Kawabe JI, Wakabayashi I (2022) VEGF-independent angiogenic factors: beyond VEGF/VEGFR2 signaling. *J Vasc Res* 59(2):78–89. <https://doi.org/10.1159/000521584>
- Elfenbein A, Simons M (2013) Syndecan-4 signaling at a glance. *J Cell Sci* 126(Pt 17):3799–3804. <https://doi.org/10.1242/jcs.124636>
- Farach-Carson MC, Warren CR, Harrington DA, Carson DD (2014) Border patrol: insights into the unique role of perlecan/heparan sulfate proteoglycan 2 at cell and tissue borders. *Matrix Biol* 34: 64–79. <https://doi.org/10.1016/j.matbio.2013.08.004>
- Fernandez-Rodriguez R, Rodriguez-Baena FJ, Martino-Echarri E, Peris-Torres C, Del Carmen Plaza-Calonge M, Rodriguez-Manzanque JC (2016) Stroma-derived but not tumor ADAMTS1 is a main driver of tumor growth and metastasis. *Oncotarget* 7(23):34507–34519. <https://doi.org/10.18632/oncotarget.8922>
- Ferrandez E, Gutierrez O, Segundo DS, Fernandez-Luna JL (2018) NFkappaB activation in differentiating glioblastoma stem-like cells is promoted by hyaluronic acid signaling through TLR4. *Sci Rep* 8(1):6341. <https://doi.org/10.1038/s41598-018-24444-6>
- Ferreira IG, Pucci M, Venturi G, Malagolini N, Chiricolo M, Dall’Olio F (2018) Glycosylation as a Main regulator of growth and death factor receptors signaling. *Int J Mol Sci* 19(2):580. <https://doi.org/10.3390/ijms19020580>
- Ferrer CM, Lynch TP, Sodi VL, Falcone JN, Schwab LP, Peacock DL, Vocadlo DJ, Seagroves TN, Reginato MJ (2014) O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway. *Mol Cell* 54(5):820–831. <https://doi.org/10.1016/j.molcel.2014.04.026>
- Fischer M, Muller GA (2017) Cell cycle transcription control: DREAM/MuvB and RB-E2F complexes. *Crit Rev Biochem Mol Biol* 52(6):638–662. <https://doi.org/10.1080/10409238.2017.1360836>
- Freire-de-Lima L (2014) Sweet and sour: the impact of differential glycosylation in cancer cells undergoing epithelial-mesenchymal transition. *Front Oncol* 4:59. <https://doi.org/10.3389/fonc.2014.00059>
- Gabius HJ, Kayser K (2014) Introduction to glycopathology: the concept, the tools and the perspectives. *Diagn Pathol* 9:4. <https://doi.org/10.1186/1746-1596-9-4>
- Gaiteiro C, Soares J, Relvas-Santos M, Peixoto A, Ferreira D, Paulo P, Brandao A, Fernandes E, Azevedo R, Palmeira C, Freitas R, Miranda A, Osorio H, Prieto J, Lima L, Silva AMN, Santos LL, Ferreira JA (2022) Glycoproteogenomics characterizes the CD44 splicing code associated with bladder cancer invasion. *Theranostics* 12(7):3150–3177. <https://doi.org/10.7150/thno.67409>

- Galon J, Angell HK, Bedognetti D, Marincola FM (2013) The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. *Immunity* 39(1): 11–26. <https://doi.org/10.1016/j.immuni.2013.07.008>
- Gao Y, Aryal RP, Ju T, Cummings RD, Gahlay G, Jarvis DL, Matta KL, Vlahakis JZ, Szarek WA, Brockhausen I (2013) Acceptor specificities and selective inhibition of recombinant human Gal- and GlcNAc-transferases that synthesize core structures 1, 2, 3 and 4 of O-glycans. *Biochim Biophys Acta* 1830(8):4274–4281. <https://doi.org/10.1016/j.bbagen.2013.04.001>
- Gao Y, Luan X, Melamed J, Brockhausen I (2021) Role of Glycans on key cell surface receptors that regulate cell proliferation and cell death. *Cells* 10(5):1252. <https://doi.org/10.3390/cells10051252>
- Ghosh S, Albitar L, LeBaron R, Welch WR, Samimi G, Birrer MJ, Berkowitz RS, Mok SC (2010) Up-regulation of stromal versican expression in advanced stage serous ovarian cancer. *Gynecol Oncol* 119(1):114–120. <https://doi.org/10.1016/j.ygyno.2010.05.029>
- Gill DJ, Tham KM, Chia J, Wang SC, Steentoft C, Clausen H, Bard-Chapeau EA, Bard FA (2013) Initiation of GalNAc-type O-glycosylation in the endoplasmic reticulum promotes cancer cell invasiveness. *Proc Natl Acad Sci USA* 110(34):E3152–E3161. <https://doi.org/10.1073/pnas.1305269110>
- Goettig P (2016) Effects of glycosylation on the enzymatic activity and mechanisms of proteases. *Int J Mol Sci* 17(12):1969. <https://doi.org/10.3390/ijms17121969>
- Gonzalez-Rellan MJ, Fondevila MF, Dieguez C, Nogueiras R (2022) O-GlcNAcylation: a sweet hub in the regulation of glucose metabolism in health and disease. *Front Endocrinol (Lausanne)* 13:873513. <https://doi.org/10.3389/fendo.2022.873513>
- Guan N, Liu Z, Zhao Y, Li Q, Wang Y (2020) Engineered biomaterial strategies for controlling growth factors in tissue engineering. *Drug Deliv* 27(1):1438–1451. <https://doi.org/10.1080/10717544.2020.1831104>
- Gupta R, Leon F, Rauth S, Batra SK, Ponnusamy MP (2020) A systematic review on the implications of O-linked glycan branching and truncating enzymes on cancer progression and metastasis. *Cells* 9(2):446. <https://doi.org/10.3390/cells9020446>
- Hanahan D (2022) Hallmarks of cancer: new dimensions. *Cancer Discov* 12(1):31–46. <https://doi.org/10.1158/2159-8290.CD-21-1059>
- Hanover JA, Chen W, Bond MR (2018) O-GlcNAc in cancer: an oncometabolism-fueled vicious cycle. *J Bioenerg Biomembr* 50(3):155–173. <https://doi.org/10.1007/s10863-018-9751-2>
- Hao W, Han J, Chu Y, Huang L, Zhuang Y, Sun J, Li X, Zhao Y, Chen Y, Dai J (2018) Collagen/heparin Bi-affinity multilayer modified collagen scaffolds for controlled bFGF release to improve angiogenesis in vivo. *Macromol Biosci* 18(11):e1800086. <https://doi.org/10.1002/mabi.201800086>
- Hardiville S, Hart GW (2014) Nutrient regulation of signaling, transcription, and cell physiology by O-GlcNAcylation. *Cell Metab* 20(2):208–213. <https://doi.org/10.1016/j.cmet.2014.07.014>
- Harjunpaa H, Lloret Asens M, Guenther C, Fagerholm SC (2019) Cell adhesion molecules and their roles and regulation in the immune and tumor microenvironment. *Front Immunol* 10:1078. <https://doi.org/10.3389/fimmu.2019.01078>
- Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O (2011) Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu Rev Biochem* 80:825–858. <https://doi.org/10.1146/annurev-biochem-060608-102511>
- Harten IA, Kaber G, Agarwal KJ, Kang I, Ibarrientos SR, Workman G, Chan CK, Nivison MP, Nagy N, Braun KR, Kinsella MG, Merrilees MJ, Wight TN (2020) The synthesis and secretion of versican isoform V3 by mammalian cells: a role for N-linked glycosylation. *Matrix Biol* 89: 27–42. <https://doi.org/10.1016/j.matbio.2020.01.002>
- Hauselmann I, Borsig L (2014) Altered tumor-cell glycosylation promotes metastasis. *Front Oncol* 4:28. <https://doi.org/10.3389/fonc.2014.00028>
- Heldin P, Lin CY, Koliopoulos C, Chen YH, Skandalis SS (2019) Regulation of hyaluronan biosynthesis and clinical impact of excessive hyaluronan production. *Matrix Biol* 78-79:100–117. <https://doi.org/10.1016/j.matbio.2018.01.017>

- Hofmann BT, Schluter L, Lange P, Mercanoglu B, Ewald F, Folster A, Picksak AS, Harder S, El Gammal AT, Grupp K, Gungor C, Drenckhan A, Schluter H, Wagener C, Izbicki JR, Jucker M, Bockhorn M, Wolters-Eisfeld G (2015) COSMC knockdown mediated aberrant O-glycosylation promotes oncogenic properties in pancreatic cancer. *Mol Cancer* 14:109. <https://doi.org/10.1186/s12943-015-0386-1>
- Holdbrooks AT, Britain CM, Bellis SL (2018) ST6Gal-I sialyltransferase promotes tumor necrosis factor (TNF)-mediated cancer cell survival via sialylation of the TNF receptor 1 (TNFR1) death receptor. *J Biol Chem* 293(5):1610–1622. <https://doi.org/10.1074/jbc.M117.801480>
- Hosaka K, Yang Y, Seki T, Du Q, Jing X, He X, Wu J, Zhang Y, Morikawa H, Nakamura M, Scherzer M, Sun X, Xu Y, Cheng T, Li X, Liu X, Li Q, Liu Y, Hong A, Chen Y, Cao Y (2020) Therapeutic paradigm of dual targeting VEGF and PDGF for effectively treating FGF-2 off-target tumors. *Nat Commun* 11(1):3704. <https://doi.org/10.1038/s41467-020-17525-6>
- Hristodorov D, Fischer R, Joerissen H, Muller-Tiemann B, Apeler H, Linden L (2013) Generation and comparative characterization of glycosylated and aglycosylated human IgG1 antibodies. *Mol Biotechnol* 53(3):326–335. <https://doi.org/10.1007/s12033-012-9531-x>
- Hsu SC, Nadesan P, Puviindran V, Stallcup WB, Kirsch DG, Alman BA (2018) Effects of chondroitin sulfate proteoglycan 4 (NG2/CSPG4) on soft-tissue sarcoma growth depend on tumor developmental stage. *J Biol Chem* 293(7):2466–2475. <https://doi.org/10.1074/jbc.M117.805051>
- Hua R, Yu J, Yan X, Ni Q, Zhi X, Li X, Jiang B, Zhu J (2020) Syndecan-2 in colorectal cancer plays oncogenic role via epithelial-mesenchymal transition and MAPK pathway. *Biomed Pharmacother* 121:109630. <https://doi.org/10.1016/j.biopha.2019.109630>
- Huang HC, Chao CC, Wu PH, Chung HY, Lee HY, Suen CS, Hwang MJ, Cai BH, Kannagi R (2019) Epigenetic silencing of the synthesis of immunosuppressive Siglec ligand glycans by NF-kappaB/EZH2/YY1 axis in early-stage colon cancers. *Biochim Biophys Acta Gene Regul Mech* 1862(2):173–183. <https://doi.org/10.1016/j.bbagr.2019.01.002>
- Huang YF, Mizumoto S, Fujita M (2021) Novel insight into glycosaminoglycan biosynthesis based on gene expression profiles. *Front Cell Dev Biol* 9:709018. <https://doi.org/10.3389/fcell.2021.709018>
- Hugonnet M, Singh P, Haas Q, von Gunten S (2021) The distinct roles of Sialyltransferases in cancer biology and Onco-immunology. *Front Immunol* 12:799861. <https://doi.org/10.3389/fimmu.2021.799861>
- Hussain MR, Hoessli DC, Fang M (2016) N-acetylgalactosaminyltransferases in cancer. *Oncotarget* 7(33):54067–54081. <https://doi.org/10.18632/oncotarget.10042>
- Ilieva KM, Cheung A, Mele S, Chiaruttini G, Crescioli S, Griffin M, Nakamura M, Spicer JF, Tsoka S, Lacy KE, Tutt ANJ, Karagiannis SN (2017) Chondroitin sulfate proteoglycan 4 and its potential as an antibody immunotherapy target across different tumor types. *Front Immunol* 8:1911. <https://doi.org/10.3389/fimmu.2017.01911>
- Iozzo RV, Schaefer L (2015) Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. *Matrix Biol* 42:11–55. <https://doi.org/10.1016/j.matbio.2015.02.003>
- Ishihara S, Aoki K, Mizutani T, Amano M, Nishimura SI, Haga H (2018) Glycosphingolipid GM2 induces invasiveness in irradiation-tolerant lung cancer cells. *Cell Struct Funct* 43(2):177–185. <https://doi.org/10.1247/csf.18026>
- Islam S, Watanabe H (2020) Versican: a dynamic regulator of the extracellular matrix. *J Histochem Cytochem* 68(11):763–775. <https://doi.org/10.1369/0022155420953922>
- Itkonen HM, Minner S, Guldvik IJ, Sandmann MJ, Tsourlakis MC, Berge V, Svindland A, Schlomm T, Mills IG (2013) O-GlcNAc transferase integrates metabolic pathways to regulate the stability of c-MYC in human prostate cancer cells. *Cancer Res* 73(16):5277–5287. <https://doi.org/10.1158/0008-5472.CAN-13-0549>
- Iwasawa T, Zhang P, Ohkawa Y, Momota H, Wakabayashi T, Ohmi Y, Bhuiyan RH, Furukawa K, Furukawa K (2018) Enhancement of malignant properties of human glioma cells by ganglioside GD3/GD2. *Int J Oncol* 52(4):1255–1266. <https://doi.org/10.3892/ijo.2018.4266>

- Jaskiewicz NM, Townson DH (2019) Hyper-O-GlcNAcylation promotes epithelial-mesenchymal transition in endometrial cancer cells. *Oncotarget* 10(30):2899–2910. <https://doi.org/10.18632/oncotarget.26884>
- Jayaprakash NG, Surolia A (2017) Role of glycosylation in nucleating protein folding and stability. *Biochem J* 474(14):2333–2347. <https://doi.org/10.1042/BCJ20170111>
- Jia L, Zhang J, Ma T, Guo Y, Yu Y, Cui J (2018) The function of Fucosylation in progression of lung cancer. *Front Oncol* 8:565. <https://doi.org/10.3389/fonc.2018.00565>
- Ju T, Otto VI, Cummings RD (2011) The Tn antigen-structural simplicity and biological complexity. *Angew Chem Int Ed Engl* 50(8):1770–1791. <https://doi.org/10.1002/anie.201002313>
- Ju T, Aryal RP, Kudelka MR, Wang Y, Cummings RD (2014) The Cosmc connection to the Tn antigen in cancer. *Cancer Biomark* 14(1):63–81. <https://doi.org/10.3233/CBM-130375>
- Julien S, Bobowski M, Steenackers A, Le Bourhis X, Delannoy P (2013) How do gangliosides regulate RTKs signaling? *Cells* 2(4):751–767. <https://doi.org/10.3390/cells2040751>
- Kang JM, Park S, Kim SJ, Kim H, Lee B, Kim J, Park J, Kim ST, Yang HK, Kim WH, Kim SJ (2015) KIAA1324 suppresses gastric cancer progression by inhibiting the Oncoprotein GRP78. *Cancer Res* 75(15):3087–3097. <https://doi.org/10.1158/0008-5472.CAN-14-3751>
- Kariya Y, Kanno M, Matsumoto-Morita K, Konno M, Yamaguchi Y, Hashimoto Y (2014) Osteopontin O-glycosylation contributes to its phosphorylation and cell-adhesion properties. *Biochem J* 463(1):93–102. <https://doi.org/10.1042/BJ20140060>
- Kaur S, Kumar S, Momi N, Sasson AR, Batra SK (2013) Mucins in pancreatic cancer and its microenvironment. *Nat Rev Gastroenterol Hepatol* 10(10):607–620. <https://doi.org/10.1038/nrgastro.2013.120>
- Kellokumpu S (2019) Golgi pH, ion and redox homeostasis: how much do they really matter? *Front Cell Dev Biol* 7:93. <https://doi.org/10.3389/fcell.2019.00093>
- Kim M, Kim YS, Kim H, Kang MY, Park J, Lee DH, Roh GS, Kim HJ, Kang SS, Cho GJ, Park JK, Cho JW, Shin JK, Choi WS (2016) O-linked N-acetylglucosamine transferase promotes cervical cancer tumorigenesis through human papillomaviruses E6 and E7 oncogenes. *Oncotarget* 7(28):44596–44607. <https://doi.org/10.18632/oncotarget.10112>
- Kim Y, West GA, Ray G, Kessler SP, Petrey AC, Fiocchi C, McDonald C, Longworth MS, Nagy LE, de la Motte CA (2018) Layilin is critical for mediating hyaluronan 35kDa-induced intestinal epithelial tight junction protein ZO-1 in vitro and in vivo. *Matrix Biol* 66:93–109. <https://doi.org/10.1016/j.matbio.2017.09.003>
- Kominato Y, Sano R, Takahashi Y, Hayakawa A, Ogasawara K (2020) Human ABO gene transcriptional regulation. *Transfusion* 60(4):860–869. <https://doi.org/10.1111/trf.15760>
- Kong D, Chen F, Sima NI (2015) Inhibition of focal adhesion kinase induces apoptosis in bladder cancer cells via Src and the phosphatidylinositol 3-kinase/Akt pathway. *Exp Ther Med* 10(5):1725–1731. <https://doi.org/10.3892/etm.2015.2745>
- Konoshenko MY, Lekchnov EA, Vlassov AV, Laktionov PP (2018) Isolation of extracellular vesicles: general methodologies and latest trends. *Biomed Res Int* 2018:8545347. <https://doi.org/10.1155/2018/8545347>
- Kudelka MR, Ju T, Heimburg-Molinaro J, Cummings RD (2015) Simple sugars to complex disease—mucin-type O-glycans in cancer. *Adv Cancer Res* 126:53–135. <https://doi.org/10.1016/bs.acr.2014.11.002>
- Kumar Das A, Ghosh N, Mandal A, Sil PC (2023) Glycobiology of cellular expiry: decrypting the role of glycan-lectin regulatory complex and therapeutic strategies focusing on cancer. *Biochem Pharmacol* 207:115367. <https://doi.org/10.1016/j.bcp.2022.115367>
- Kuribara T, Totani K (2021) Structural insights into N-linked glycan-mediated protein folding from chemical and biological perspectives. *Curr Opin Struct Biol* 68:41–47. <https://doi.org/10.1016/j.sbi.2020.11.004>
- Lee JB, Pyo KH, Kim HR (2021) Role and function of O-GlcNAcylation in cancer. *Cancers (Basel)* 13(21):5365. <https://doi.org/10.3390/cancers13215365>

- Leon F, Seshacharyulu P, Nimmakayala RK, Chugh S, Karmakar S, Nallasamy P, Vengoji R, Rachagani S, Cox JL, Mallya K, Batra SK, Ponnusamy MP (2022) Reduction in O-glycome induces differentially glycosylated CD44 to promote stemness and metastasis in pancreatic cancer. *Oncogene* 41(1):57–71. <https://doi.org/10.1038/s41388-021-02047-2>
- Li C, Yang Z, Du Y, Tang H, Chen J, Hu D, Fan Z (2014) BCMab1, a monoclonal antibody against aberrantly glycosylated integrin $\alpha 3\beta 1$, has potent antitumor activity of bladder cancer in vivo. *Clin Cancer Res* 20(15):4001–4013. <https://doi.org/10.1158/1078-0432.CCR-13-3397>
- Li G, Li L, Joo EJ, Son JW, Kim YJ, Kang JK, Lee KB, Zhang F, Linhardt RJ (2017) Glycosaminoglycans and glycolipids as potential biomarkers in lung cancer. *Glycoconj J* 34(5):661–669. <https://doi.org/10.1007/s10719-017-9790-7>
- Li R, Li Y, Wu Y, Zhao Y, Chen H, Yuan Y, Xu K, Zhang H, Lu Y, Wang J, Li X, Jia X, Xiao J (2018) Heparin-Poloxamer Thermosensitive hydrogel loaded with bFGF and NGF enhances peripheral nerve regeneration in diabetic rats. *Biomaterials* 168:24–37. <https://doi.org/10.1016/j.biomaterials.2018.03.044>
- Li D, Jia S, Wang S, Hu L (2023a) Glycoproteomic analysis of urinary extracellular vesicles for biomarkers of hepatocellular carcinoma. *Molecules* 28(3):1293. <https://doi.org/10.3390/molecules28031293>
- Li R, Dong X, Chen S, Tan J, Chen X, Liu J, Wen T, Ru X (2023b) Tn antigen promotes breast cancer metastasis via impairment of CASC4. *Cell Biol Int* 47(11):1854–1867. <https://doi.org/10.1002/cbin.12077>
- Liang Y, Eng WS, Colquhoun DR, Dinglasan RR, Graham DR, Mahal LK (2014) Complex N-linked glycans serve as a determinant for exosome/microvesicle cargo recruitment. *J Biol Chem* 289(47):32526–32537. <https://doi.org/10.1074/jbc.M114.606269>
- Lichtenstein RG, Rabinovich GA (2013) Glycobiology of cell death: when glycans and lectins govern cell fate. *Cell Death Differ* 20(8):976–986. <https://doi.org/10.1038/cdd.2013.50>
- Lin R, Xia S, Shan C, Chen D, Liu Y, Gao X, Wang M, Kang HB, Pan Y, Liu S, Chung YR, Abdel-Wahab O, Merghoub T, Rossi M, Kudchadkar RR, Lawson DH, Khuri FR, Lonial S, Chen J (2018) The dietary supplement Chondroitin-4-sulfate exhibits oncogene-specific pro-tumor effects on BRAF V600E melanoma cells. *Mol Cell* 69(6):923–937 e928. <https://doi.org/10.1016/j.molcel.2018.02.010>
- Lin S, Zhou S, Yuan T (2020) The “sugar-coated bullets” of cancer: tumor-derived exosome surface glycosylation from basic knowledge to applications. *Clin Transl Med* 10(6):e204. <https://doi.org/10.1002/ctm2.204>
- Lin NY, Lee JJ, Chen ST, Lin JA, Lin CH, Lin HY, Su YH, Chen CC, Lin MC, Kuo CY, Huang MC (2023a) Truncation of GalNAc-type O-glycans suppresses CD44-mediated Osteoclastogenesis and bone metastasis in breast cancer. *Mol Cancer Res* 21(7):664–674. <https://doi.org/10.1158/1541-7786.MCR-22-0907>
- Lin WH, Cooper LM, Anastasiadis PZ (2023b) Cadherins and catenins in cancer: connecting cancer pathways and tumor microenvironment. *Front Cell Dev Biol* 11:1137013. <https://doi.org/10.3389/fcell.2023.1137013>
- Litwiniuk M, Krejner A, Speyrer MS, Gauto AR, Grzela T (2016) Hyaluronic acid in inflammation and tissue regeneration. *Wounds* 28(3):78–88
- Liu S, Ashour Ahmed A (2017) Growth factors and uncontrolled proliferation. In: Kerr DJ et al. (eds) Oxford textbook of oncology, 3rd edn. (Oxford, 2016; online edn, Oxford Academic, 25 May 2017). In *Essentials of glycobiology*, 4th edn. Cold Spring Harbor, New York
- Liu Q, Tao T, Liu F, Ni R, Lu C, Shen A (2016) Hyper-O-GlcNAcylation of YB-1 affects Ser102 phosphorylation and promotes cell proliferation in hepatocellular carcinoma. *Exp Cell Res* 349(2):230–238. <https://doi.org/10.1016/j.yexcr.2016.10.011>
- Liu X, Taftaf R, Kawaguchi M, Chang YF, Chen W, Entenberg D, Zhang Y, Gerratana L, Huang S, Patel DB, Tsui E, Adorno-Cruz V, Chirieleison SM, Cao Y, Harney AS, Patel S, Patsialou A, Shen Y, Avril S, Gilmore HL, Lathia JD, Abbott DW, Cristofanilli M, Condeelis JS, Liu H (2019a) Homophilic CD44 interactions mediate tumor cell aggregation and polyclonal metastasis in patient-derived breast cancer models. *Cancer Discov* 9(1):96–113. <https://doi.org/10.1158/2159-8290.CD-18-0065>

- Liu Z, Liu J, Dong X, Hu X, Jiang Y, Li L, Du T, Yang L, Wen T, An G, Feng G (2019b) Tn antigen promotes human colorectal cancer metastasis via H-Ras mediated epithelial-mesenchymal transition activation. *J Cell Mol Med* 23(3):2083–2092. <https://doi.org/10.1111/jcmm.14117>
- Lustig M, Chan C, Jansen JHM, Brautigam M, Kolling MA, Gehlert CL, Baumann N, Mester S, Foss S, Andersen JT, Bastian L, Sondermann P, Peipp M, Burger R, Leusen JHW, Valerius T (2023) Disruption of the sialic acid/siglec-9 axis improves antibody-mediated neutrophil cytotoxicity towards tumor cells. *Front Immunol* 14:1178817. <https://doi.org/10.3389/fimmu.2023.1178817>
- Lynch TP, Ferrer CM, Jackson SR, Shahriari KS, Vosseller K, Reginato MJ (2012) Critical role of O-linked beta-N-acetylglucosamine transferase in prostate cancer invasion, angiogenesis, and metastasis. *J Biol Chem* 287(14):11070–11081. <https://doi.org/10.1074/jbc.M111.302547>
- Ma Z, Vocadlo DJ, Vosseller K (2013) Hyper-O-GlcNAcylation is anti-apoptotic and maintains constitutive NF-kappaB activity in pancreatic cancer cells. *J Biol Chem* 288(21):15121–15130. <https://doi.org/10.1074/jbc.M113.470047>
- Macauley MS, Crocker PR, Paulson JC (2014) Siglec-mediated regulation of immune cell function in disease. *Nat Rev Immunol* 14(10):653–666. <https://doi.org/10.1038/nri3737>
- Makwana V, Ryan P, Patel B, Dukie SA, Rudrawar S (2019) Essential role of O-GlcNAcylation in stabilization of oncogenic factors. *Biochim Biophys Acta Gen Subj* 1863(8):1302–1317. <https://doi.org/10.1016/j.bbagen.2019.04.002>
- Martin DR, Santamaria S, Koch CD, Ahnstrom J, Apte SS (2021) Identification of novel ADAMTS1, ADAMTS4 and ADAMTS5 cleavage sites in versican using a label-free quantitative proteomics approach. *J Proteome* 249:104358. <https://doi.org/10.1016/j.jprot.2021.104358>
- Martins AM, Ramos CC, Freitas D, Reis CA (2021) Glycosylation of cancer extracellular vesicles: capture strategies, functional roles and potential clinical applications. *Cells* 10(1):109. <https://doi.org/10.3390/cells10010109>
- Martins AM, Lopes TM, Diniz F, Pires J, Osorio H, Pinto F, Freitas D, Reis CA (2023) Differential protein and glycan packaging into extracellular vesicles in response to 3D gastric cancer cellular organization. *Adv Sci (Weinh)* 10(24):e2300588. <https://doi.org/10.1002/advs.202300588>
- McAtee CO, Barycki JJ, Simpson MA (2014) Emerging roles for hyaluronidase in cancer metastasis and therapy. *Adv Cancer Res* 123:1–34. <https://doi.org/10.1016/B978-0-12-800092-2.00001-0>
- McEver RP (2015) Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. *Cardiovasc Res* 107(3):331–339. <https://doi.org/10.1093/cvr/cvv154>
- Mereiter S, Martins AM, Gomes C, Balmana M, Macedo JA, Polom K, Roviello F, Magalhaes A, Reis CA (2019) O-glycan truncation enhances cancer-related functions of CD44 in gastric cancer. *FEBS Lett* 593(13):1675–1689. <https://doi.org/10.1002/1873-3468.13432>
- Mikami T, Kitagawa H (2013) Biosynthesis and function of chondroitin sulfate. *Biochim Biophys Acta* 1830(10):4719–4733. <https://doi.org/10.1016/j.bbagen.2013.06.006>
- Mikkola S (2020) Nucleotide sugars in chemistry and biology. *Molecules* 25(23):5755. <https://doi.org/10.3390/molecules25235755>
- Miller T, Goude MC, McDevitt TC, Temenoff JS (2014) Molecular engineering of glycosaminoglycan chemistry for biomolecule delivery. *Acta Biomater* 10(4):1705–1719. <https://doi.org/10.1016/j.actbio.2013.09.039>
- Mizumoto S, Yamada S, Sugahara K (2015) Molecular interactions between chondroitin-dermatan sulfate and growth factors/receptors/matrix proteins. *Curr Opin Struct Biol* 34:35–42. <https://doi.org/10.1016/j.sbi.2015.06.004>
- Monslow J, Govindaraju P, Pure E (2015) Hyaluronan – a functional and structural sweet spot in the tissue microenvironment. *Front Immunol* 6:231. <https://doi.org/10.3389/fimmu.2015.00231>
- Munkley J (2016) The role of Sialyl-Tn in cancer. *Int J Mol Sci* 17(3):275. <https://doi.org/10.3390/ijms17030275>
- Muthusamy S, Hong KU, Dassanayaka S, Hamid T, Jones SP (2015) E2F1 transcription factor regulates O-linked N-acetylglucosamine (O-GlcNAc) transferase and O-GlcNAcase expression. *J Biol Chem* 290(52):31013–31024. <https://doi.org/10.1074/jbc.M115.677534>

- Na HJ, Akan I, Abramowitz LK, Hanover JA (2020) Nutrient-driven O-GlcNAcylation controls DNA damage repair signaling and stem/progenitor cell homeostasis. *Cell Rep* 31(6):107632. <https://doi.org/10.1016/j.celrep.2020.107632>
- Natoni A, Macauley MS, O'Dwyer ME (2016) Targeting selectins and their ligands in cancer. *Front Oncol* 6:93. <https://doi.org/10.3389/fonc.2016.00093>
- Neill T, Schaefer L, Iozzo RV (2015) Decoding the matrix: instructive roles of proteoglycan receptors. *Biochemistry* 54(30):4583–4598. <https://doi.org/10.1021/acs.biochem.5b00653>
- Nguyen AT, Chia J, Ros M, Hui KM, Saltel F, Bard F (2017) Organelle specific O-glycosylation drives MMP14 activation, tumor growth, and metastasis. *Cancer Cell* 32(5):639–653.e636. <https://doi.org/10.1016/j.ccell.2017.10.001>
- Nikitovic D, Perez S (2021) Preface for the special issue on the exploration of the multifaceted roles of glycosaminoglycans: GAGs. *Biomolecules* 11(11):1630. <https://doi.org/10.3390/biom11111630>
- Nikitovic D, Berdiaki A, Spyridaki I, Krasanakis T, Tsatsakis A, Tzanakakis GN (2018) Proteoglycans-biomarkers and targets in cancer therapy. *Front Endocrinol (Lausanne)* 9:69. <https://doi.org/10.3389/fendo.2018.00069>
- Oliveira-Ferrer L, Hessling A, Trillsch F, Mahner S, Milde-Langosch K (2015) Prognostic impact of chondroitin-4-sulfotransferase CHST11 in ovarian cancer. *Tumour Biol* 36(11):9023–9030. <https://doi.org/10.1007/s13277-015-3652-3>
- Ortiz-Meoz RF, Merbl Y, Kirschner MW, Walker S (2014) Microarray discovery of new OGT substrates: the medulloblastoma oncogene OTX2 is O-GlcNAcylated. *J Am Chem Soc* 136(13):4845–4848. <https://doi.org/10.1021/ja500451w>
- Ozcan S, Andrali SS, Cantrell JE (2010) Modulation of transcription factor function by O-GlcNAc modification. *Biochim Biophys Acta* 1799(5–6):353–364. <https://doi.org/10.1016/j.bbaggm.2010.02.005>
- Paneque A, Fortus H, Zheng J, Werlen G, Jacinto E (2023) The Hexosamine biosynthesis pathway: regulation and function. *Genes (Basel)* 14(4):933. <https://doi.org/10.3390/genes14040933>
- Papadas A, Asimakopoulos F (2020) Versican in the tumor microenvironment. *Adv Exp Med Biol* 1272:55–72. https://doi.org/10.1007/978-3-030-48457-6_4
- Park EJ, Myint PK, Ito A, Appiah MG, Darkwah S, Kawamoto E, Shimaoka M (2020) Integrin-ligand interactions in inflammation, cancer, and metabolic disease: insights into the multifaceted roles of an emerging ligand Irisin. *Front Cell Dev Biol* 8:588066. <https://doi.org/10.3389/fcell.2020.588066>
- Peixoto A, Relvas-Santos M, Azevedo R, Santos LL, Ferreira JA (2019) Protein glycosylation and tumor microenvironment alterations driving cancer hallmarks. *Front Oncol* 9:380. <https://doi.org/10.3389/fonc.2019.00380>
- Penque BA, Hoggatt AM, Herring BP, Elmendorf JS (2013) Hexosamine biosynthesis impairs insulin action via a cholesterologenic response. *Mol Endocrinol* 27(3):536–547. <https://doi.org/10.1210/me.2012-1213>
- Pinho SS, Reis CA (2015) Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 15(9):540–555. <https://doi.org/10.1038/nrc3982>
- Piyush T, Rhodes JM, Yu LG (2017) MUC1 O-glycosylation contributes to anoikis resistance in epithelial cancer cells. *Cell Death Discov* 3:17044. <https://doi.org/10.1038/cddiscovery.2017.44>
- Porebska N, Pozniak M, Matynia A, Zukowska D, Zakrzewska M, Otlewski J, Opalinski L (2021) Galectins as modulators of receptor tyrosine kinases signaling in health and disease. *Cytokine Growth Factor Rev* 60:89–106. <https://doi.org/10.1016/j.cytogfr.2021.03.004>
- Prado Acosta M, Lepenies B (2019) Bacterial glycans and their interactions with lectins in the innate immune system. *Biochem Soc Trans* 47(6):1569–1579. <https://doi.org/10.1042/BST20170410>
- Prydz K (2015) Determinants of glycosaminoglycan (GAG) structure. *Biomol Ther* 5(3):2003–2022. <https://doi.org/10.3390/biom5032003>

- Pucci M, Duca M, Malagolini N, Dall'Olio F (2022) Glycosyltransferases in cancer: prognostic biomarkers of survival in patient cohorts and impact on malignancy in experimental models. *Cancers (Basel)* 14(9):2128. <https://doi.org/10.3390/cancers14092128>
- Radhakrishnan P, Dabelsteen S, Madsen FB, Francavilla C, Kopp KL, Steentoft C, Vakhrushev SY, Olsen JV, Hansen L, Bennett EP, Woetmann A, Yin G, Chen L, Song H, Bak M, Hlady RA, Peters SL, Opavsky R, Thode C, Qvortrup K, Schjoldager KT, Clausen H, Hollingsworth MA, Wandall HH (2014) Immature truncated O-glycophenotype of cancer directly induces oncogenic features. *Proc Natl Acad Sci USA* 111(39):E4066–E4075. <https://doi.org/10.1073/pnas.1406619111>
- Rangel MP, de Sa VK, Martins V, Martins JR, Parra ER, Mendes A, Andrade PC, Reis RM, Longatto-Filho A, Oliveira CZ, Takagaki T, Carraro DM, Nader HB, Capelozzi VL (2015) Tissue hyaluronan expression, as reflected in the sputum of lung cancer patients, is an indicator of malignancy. *Braz J Med Biol Res* 48(6):557–567. <https://doi.org/10.1590/1414-431X20144300>
- Reymond N, d'Agua BB, Ridley AJ (2013) Crossing the endothelial barrier during metastasis. *Nat Rev Cancer* 13(12):858–870. <https://doi.org/10.1038/nrc3628>
- Reynolds IS, Fichtner M, McNamara DA, Kay EW, Prehn JHM, Burke JP (2019) Mucin glycoproteins block apoptosis; promote invasion, proliferation, and migration; and cause chemoresistance through diverse pathways in epithelial cancers. *Cancer Metastasis Rev* 38(1–2):237–257. <https://doi.org/10.1007/s10555-019-09781-w>
- Rocha S, Carvalho J, Oliveira P, Voglstaetter M, Schvartz D, Thomsen AR, Walter N, Khanduri R, Sanchez JC, Keller A, Oliveira C, Nazarenko I (2019) 3D cellular architecture affects MicroRNA and protein cargo of extracellular vesicles. *Adv Sci (Weinh)* 6(4):1800948. <https://doi.org/10.1002/adv.201800948>
- Rodrigues E, Macauley MS (2018) Hypersialylation in cancer: modulation of inflammation and therapeutic opportunities. *Cancers (Basel)* 10(6):207. <https://doi.org/10.3390/cancers10060207>
- Roedig H, Damiescu R, Zeng-Brouwers J, Kutija I, Trebicka J, Wygrecka M, Schaefer L (2020) Danger matrix molecules orchestrate CD14/CD44 signaling in cancer development. *Semin Cancer Biol* 62:31–47. <https://doi.org/10.1016/j.semcancer.2019.07.026>
- Sandhoff R, Schulze H, Sandhoff K (2018) Ganglioside metabolism in health and disease. *Prog Mol Biol Transl Sci* 156:1–62. <https://doi.org/10.1016/bs.pmbts.2018.01.002>
- Sandy JD, Westling J, Kenagy RD, Iruela-Arispe ML, Verscharen C, Rodriguez-Mazaneque JC, Zimmermann DR, Lemire JM, Fischer JW, Wight TN, Clowes AW (2001) Versican V1 proteolysis in human aorta in vivo occurs at the Glu441-Ala442 bond, a site that is cleaved by recombinant ADAMTS-1 and ADAMTS-4. *J Biol Chem* 276(16):13372–13378. <https://doi.org/10.1074/jbc.M009737200>
- Santos SN, Junqueira MS, Francisco G, Vilanova M, Magalhaes A, Dias Baruffi M, Chammass R, Harris AL, Reis CA, Bernardes ES (2016) O-glycan sialylation alters galectin-3 subcellular localization and decreases chemotherapy sensitivity in gastric cancer. *Oncotarget* 7(50):83570–83587. <https://doi.org/10.18632/oncotarget.13192>
- Schjoldager KT, Vakhrushev SY, Kong Y, Steentoft C, Nudelman AS, Pedersen NB, Wandall HH, Mandel U, Bennett EP, Levery SB, Clausen H (2012) Probing isoform-specific functions of polypeptide GalNAc-transferases using zinc finger nuclease glycoengineered SimpleCells. *Proc Natl Acad Sci USA* 109(25):9893–9898. <https://doi.org/10.1073/pnas.1203563109>
- Schmitt M, Metzger M, Gradl D, Davidson G, Orian-Rousseau V (2015) CD44 functions in Wnt signaling by regulating LRP6 localization and activation. *Cell Death Differ* 22(4):677–689. <https://doi.org/10.1038/cdd.2014.156>
- Schneider M, Al-Shareffi E, Haltiwanger RS (2017) Biological functions of fucose in mammals. *Glycobiology* 27(7):601–618. <https://doi.org/10.1093/glycob/cwx034>
- Schultz MJ, Swindall AF, Bellis SL (2012) Regulation of the metastatic cell phenotype by sialylated glycans. *Cancer Metastasis Rev* 31(3–4):501–518. <https://doi.org/10.1007/s10555-012-9359-7>

- Scott E, Archer Goode E, Garnham R, Hodgson K, Orozco-Moreno M, Turner H, Livermore K, Putri Nangkana K, Frame FM, Bermudez A, Jose Garcia Marques F, McClurg UL, Wilson L, Thomas H, Buskin A, Hepburn A, Duxfield A, Bastian K, Pye H, Arredondo HM, Hysenaj G, Heavey S, Stopka-Farooqui U, Haider A, Freeman A, Singh S, Johnston EW, Punwani S, Knight B, McCullagh P, McGrath J, Crundwell M, Harries L, Heer R, Maitland NJ, Whitaker H, Pitteri S, Troyer DA, Wang N, Elliott DJ, Drake RR, Munkley J (2023) ST6GAL1-mediated aberrant sialylation promotes prostate cancer progression. *J Pathol* 261(1):71–84. <https://doi.org/10.1002/path.6152>
- Senbanjo LT, Chellaiah MA (2017) CD44: a multifunctional cell surface adhesion receptor is a regulator of progression and metastasis of cancer cells. *Front Cell Dev Biol* 5:18. <https://doi.org/10.3389/fcell.2017.00018>
- Shibuya M (2011) Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-Angiogenic therapies. *Genes Cancer* 2(12):1097–1105. <https://doi.org/10.1177/1947601911423031>
- Shuvalova ML, Kopylov AT, Mazurov DV, Pichugin AV, Bovin NV, Filatov AV (2020) CD44-associated Tn antigen as a new biomarker of tumor cells with aberrant glycosylation. *Biochemistry (Mosc)* 85(9):1064–1081. <https://doi.org/10.1134/S0006297920090060>
- Singh JP, Zhang K, Wu J, Yang X (2015) O-GlcNAc signaling in cancer metabolism and epigenetics. *Cancer Lett* 356(2 Pt A):244–250. <https://doi.org/10.1016/j.canlet.2014.04.014>
- Singh C, Shyanti RK, Singh V, Kale RK, Mishra JPN, Singh RP (2018) Integrin expression and glycosylation patterns regulate cell-matrix adhesion and alter with breast cancer progression. *Biochem Biophys Res Commun* 499(2):374–380. <https://doi.org/10.1016/j.bbrc.2018.03.169>
- Smock RG, Meijers R (2018) Roles of glycosaminoglycans as regulators of ligand/receptor complexes. *Open Biol* 8(10):180026. <https://doi.org/10.1098/rsob.180026>
- Soesanto YA, Luo B, Jones D, Taylor R, Gabrielsen JS, Parker G, McClain DA (2008) Regulation of Akt signaling by O-GlcNAc in euglycemia. *Am J Physiol Endocrinol Metab* 295(4):E974–E980. <https://doi.org/10.1152/ajpendo.90366.2008>
- Stead ER, Bjedov I (2021) Balancing DNA repair to prevent ageing and cancer. *Exp Cell Res* 405(2):112679. <https://doi.org/10.1016/j.yexcr.2021.112679>
- Steenfot C, Vakhruhev SY, Joshi HJ, Kong Y, Vester-Christensen MB, Schjoldager KT, Lavrsen K, Dabelsteen S, Pedersen NB, Marcos-Silva L, Gupta R, Bennett EP, Mandel U, Brunak S, Wandall HH, Lavery SB, Clausen H (2013) Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology. *EMBO J* 32(10):1478–1488. <https://doi.org/10.1038/emboj.2013.79>
- Stowell SR, Ju T, Cummings RD (2015) Protein glycosylation in cancer. *Annu Rev Pathol* 10:473–510. <https://doi.org/10.1146/annurev-pathol-012414-040438>
- Sun X, Ju T, Cummings RD (2018) Differential expression of Cosmc, T-synthase and mucins in Tn-positive colorectal cancers. *BMC Cancer* 18(1):827. <https://doi.org/10.1186/s12885-018-4708-8>
- Sun X, Tie HC, Chen B, Lu L (2020) Glycans function as a Golgi export signal to promote the constitutive exocytic trafficking. *J Biol Chem* 295(43):14750–14762. <https://doi.org/10.1074/jbc.RA120.014476>
- Swindall AF, Bellis SL (2011) Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. *J Biol Chem* 286(26):22982–22990. <https://doi.org/10.1074/jbc.M110.211375>
- Szatmari T, Otvos R, Hjerpe A, Dobra K (2015) Syndecan-1 in cancer: implications for cell signaling, differentiation, and prognostication. *Dis Markers* 2015:796052. <https://doi.org/10.1155/2015/796052>
- Takahashi M, Hasegawa Y, Gao C, Kuroki Y, Taniguchi N (2016) N-glycans of growth factor receptors: their role in receptor function and disease implications. *Clin Sci (Lond)* 130(20):1781–1792. <https://doi.org/10.1042/CS20160273>

- Taylor RP, Parker GJ, Hazel MW, Soesanto Y, Fuller W, Yazzie MJ, McClain DA (2008) Glucose deprivation stimulates O-GlcNAc modification of proteins through up-regulation of O-linked N-acetylglucosaminyltransferase. *J Biol Chem* 283(10):6050–6057. <https://doi.org/10.1074/jbc.M707328200>
- Taylor RP, Geisler TS, Chambers JH, McClain DA (2009) Up-regulation of O-GlcNAc transferase with glucose deprivation in HepG2 cells is mediated by decreased hexosamine pathway flux. *J Biol Chem* 284(6):3425–3432. <https://doi.org/10.1074/jbc.M803198200>
- Thomas D, Sagar S, Caffrey T, Grandgenett PM, Radhakrishnan P (2019) Truncated O-glycans promote epithelial-to-mesenchymal transition and stemness properties of pancreatic cancer cells. *J Cell Mol Med* 23(10):6885–6896. <https://doi.org/10.1111/jcmm.14572>
- Thurin M, Kieber-Emmons T (2002) SA-lea and tumor metastasis: the old prediction and recent findings. *Hybrid Hybridomics* 21(2):111–116. <https://doi.org/10.1089/153685902317401708>
- Tian Y, Esteve FJ, Song J, Zhang H (2012) Altered expression of sialylated glycoproteins in breast cancer using hydrazide chemistry and mass spectrometry. *Mol Cell Proteomics* 11(6): M111.011403. <https://doi.org/10.1074/mcp.M111.011403>
- Tian Y, Denda-Nagai K, Tsukui T, Ishii-Schrade KB, Okada K, Nishizono Y, Matsuzaki K, Hafley M, Bresalier RS, Irimura T (2022) Mucin 21 confers resistance to apoptosis in an O-glycosylation-dependent manner. *Cell Death Discov* 8(1):194. <https://doi.org/10.1038/s41420-022-01006-4>
- Tutanov OS, Glass SE, Coffey RJ (2023) Emerging connections between GPI-anchored proteins and their extracellular carriers in colorectal cancer. *Extracell Vesicles Circ Nucl Acids* 4(2): 195–217. <https://doi.org/10.20517/evcna.2023.17>
- van der Laarse SAM, Leney AC, Heck AJR (2018) Crosstalk between phosphorylation and O-GlcNAcylation: friend or foe. *FEBS J* 285(17):3152–3167. <https://doi.org/10.1111/febs.14491>
- van Tol W, Wessels H, Lefeber DJ (2019) O-glycosylation disorders pave the road for understanding the complex human O-glycosylation machinery. *Curr Opin Struct Biol* 56:107–118. <https://doi.org/10.1016/j.sbi.2018.12.006>
- van Wijk XM, van Kuppevelt TH (2014) Heparan sulfate in angiogenesis: a target for therapy. *Angiogenesis* 17(3):443–462. <https://doi.org/10.1007/s10456-013-9401-6>
- Varki A (2017) Biological roles of glycans. *Glycobiology* 27(1):3–49. <https://doi.org/10.1093/glycob/cww086>
- Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Mohnen D, Kinoshita T, Packer NH, Prestegard JH, Schnaar RL, Seeberger PH (2022) In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Mohnen D, Kinoshita T, Packer NH, Prestegard JH, Schnaar RL, Seeberger PH (eds) *Essentials of glycobiology*, 4th edn. Cold Spring Harbor, New York
- Varone E, Chernorudskiy A, Cherubini A, Cattaneo A, Bachi A, Fumagalli S, Erol G, Gobbi M, Lenardo MJ, Borgese N, Zito E (2022) ERO1 alpha deficiency impairs angiogenesis by increasing N-glycosylation of a proangiogenic VEGFA. *Redox Biol* 56:102455. <https://doi.org/10.1016/j.redox.2022.102455>
- Vasconcelos-Dos-Santos A, Oliveira IA, Lucena MC, Mantuano NR, Whelan SA, Dias WB, Todeschini AR (2015) Biosynthetic machinery involved in aberrant glycosylation: promising targets for developing of drugs against cancer. *Front Oncol* 5:138. <https://doi.org/10.3389/fonc.2015.00138>
- Venturi G, Gomes Ferreira I, Pucci M, Ferracin M, Malagolini N, Chiricolo M, Dall'Olio F (2019) Impact of sialyltransferase ST6GAL1 overexpression on different colon cancer cell types. *Glycobiology* 29(10):684–695. <https://doi.org/10.1093/glycob/cwz053>
- Wagner KW, Punnoose EA, Januario T, Lawrence DA, Pitti RM, Lancaster K, Lee D, von Goetz M, Yee SF, Totpal K, Huw L, Katta V, Cavet G, Hymowitz SG, Amler L, Ashkenazi A (2007) Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nat Med* 13(9):1070–1077. <https://doi.org/10.1038/nm1627>
- Walimbe T, Panitch A (2019) Proteoglycans in biomedicine: resurgence of an underexploited class of ECM molecules. *Front Pharmacol* 10:1661. <https://doi.org/10.3389/fphar.2019.01661>

- Wandall HH, Nielsen MAI, King-Smith S, de Haan N, Bagdonaite I (2021) Global functions of O-glycosylation: promises and challenges in O-glycobiology. *FEBS J* 288(24):7183–7212. <https://doi.org/10.1111/febs.16148>
- Wang H, Jin H, Beauvais DM, Rapraeger AC (2014) Cytoplasmic domain interactions of syndecan-1 and syndecan-4 with alpha6beta4 integrin mediate human epidermal growth factor receptor (HER1 and HER2)-dependent motility and survival. *J Biol Chem* 289(44):30318–30332. <https://doi.org/10.1074/jbc.M114.586438>
- Wang X, Simpson ER, Brown KA (2015a) p53: protection against tumor growth beyond effects on cell cycle and apoptosis. *Cancer Res* 75(23):5001–5007. <https://doi.org/10.1158/0008-5472.CAN-15-0563>
- Wang Y, Fukuda T, Isaji T, Lu J, Im S, Hang Q, Gu W, Hou S, Ohtsubo K, Gu J (2015b) Loss of alpha1,6-fucosyltransferase inhibits chemical-induced hepatocellular carcinoma and tumorigenesis by down-regulating several cell signaling pathways. *FASEB J* 29(8):3217–3227. <https://doi.org/10.1096/fj.15-270710>
- Wang X, Li X, Zeng YN, He F, Yang XM, Guan F (2016a) Enhanced expression of polysialic acid correlates with malignant phenotype in breast cancer cell lines and clinical tissue samples. *Int J Mol Med* 37(1):197–206. <https://doi.org/10.3892/ijmm.2015.2395>
- Wang Z, von Au A, Schnolzer M, Hackert T, Zoller M (2016b) CD44v6-competent tumor exosomes promote motility, invasion and cancer-initiating cell marker expression in pancreatic and colorectal cancer cells. *Oncotarget* 7(34):55409–55436. <https://doi.org/10.18632/oncotarget.10580>
- Wang Q, Liao C, Tan Z, Li X, Guan X, Li H, Tian Z, Liu J, An J (2023) FUT6 inhibits the proliferation, migration, invasion, and EGF-induced EMT of head and neck squamous cell carcinoma (HNSCC) by regulating EGFR/ERK/STAT signaling pathway. *Cancer Gene Ther* 30(1):182–191. <https://doi.org/10.1038/s41417-022-00530-w>
- Warburg O (1956) On the origin of cancer cells. *Science* 123(3191):309–314. <https://doi.org/10.1126/science.123.3191.309>
- Wei J, Hu M, Huang K, Lin S, Du H (2020) Roles of proteoglycans and Glycosaminoglycans in cancer development and progression. *Int J Mol Sci* 21(17):5983. <https://doi.org/10.3390/ijms21175983>
- Wei KC, Lin YC, Chen CH, Chu YH, Huang CY, Liao WC, Liu CH (2021) Fucosyltransferase 8 modulates receptor tyrosine kinase activation and temozolomide resistance in glioblastoma cells. *Am J Cancer Res* 11(11):5472–5484
- Wells L, Slawson C, Hart GW (2011) The E2F-1 associated retinoblastoma-susceptibility gene product is modified by O-GlcNAc. *Amino Acids* 40(3):877–883. <https://doi.org/10.1007/s00726-010-0709-x>
- Wight TN, Kinsella MG, Evanko SP, Potter-Perigo S, Merrilees MJ (2014) Versican and the regulation of cell phenotype in disease. *Biochim Biophys Acta* 1840(8):2441–2451. <https://doi.org/10.1016/j.bbagen.2013.12.028>
- Wolf KJ, Shukla P, Springer K, Lee S, Coombes JD, Choy CJ, Kenny SJ, Xu K, Kumar S (2020) A mode of cell adhesion and migration facilitated by CD44-dependent microtentacles. *Proc Natl Acad Sci USA* 117(21):11432–11443. <https://doi.org/10.1073/pnas.1914294117>
- Wright RD, Cooper D (2014) Glycobiology of leukocyte trafficking in inflammation. *Glycobiology* 24(12):1242–1251. <https://doi.org/10.1093/glycob/cwu101>
- Wu YM, Liu CH, Huang MJ, Lai HS, Lee PH, Hu RH, Huang MC (2013) C1GALT1 enhances proliferation of hepatocellular carcinoma cells via modulating MET glycosylation and dimerization. *Cancer Res* 73(17):5580–5590. <https://doi.org/10.1158/0008-5472.CAN-13-0869>
- Wu CI, Wang HY, Ling S, Lu X (2016) The ecology and evolution of cancer: the ultra-microevolutionary process. *Annu Rev Genet* 50:347–369. <https://doi.org/10.1146/annurev-genet-112414-054842>
- Xu D, Esko JD (2014) Demystifying heparan sulfate-protein interactions. *Annu Rev Biochem* 83:129–157. <https://doi.org/10.1146/annurev-biochem-060713-035314>

- Xu W, Zhang X, Wu JL, Fu L, Liu K, Liu D, Chen GG, Lai PB, Wong N, Yu J (2017a) O-GlcNAc transferase promotes fatty liver-associated liver cancer through inducing palmitic acid and activating endoplasmic reticulum stress. *J Hepatol* 67(2):310–320. <https://doi.org/10.1016/j.jhep.2017.03.017>
- Xu Y, Chang R, Xu F, Gao Y, Yang F, Wang C, Xiao J, Su Z, Bi Y, Wang L, Zha X (2017b) N-glycosylation at Asn 402 stabilizes N-cadherin and promotes cell-cell adhesion of glioma cells. *J Cell Biochem* 118(6):1423–1431. <https://doi.org/10.1002/jcb.25801>
- Yan T, Chen X, Zhan H, Yao P, Wang N, Yang H, Zhang C, Wang K, Hu H, Li J, Sun J, Dong Y, Lu E, Zheng Z, Zhang R, Wang X, Ma J, Gao M, Ye J, Wang X, Teng L, Liu H, Zhao S (2021) Interfering with hyaluronic acid metabolism suppresses glioma cell proliferation by regulating autophagy. *Cell Death Dis* 12(5):486. <https://doi.org/10.1038/s41419-021-03747-z>
- Yang X, Qian K (2017) Protein O-GlcNAcylation: emerging mechanisms and functions. *Nat Rev Mol Cell Biol* 18(7):452–465. <https://doi.org/10.1038/nrm.2017.22>
- Yang WH, Kim JE, Nam HW, Ju JW, Kim HS, Kim YS, Cho JW (2006) Modification of p53 with O-linked N-acetylglucosamine regulates p53 activity and stability. *Nat Cell Biol* 8(10):1074–1083. <https://doi.org/10.1038/ncb1470>
- Yao Y, Dai W (2014) Genomic instability and cancer. *J Carcinog Mutagen* 5:1000165. <https://doi.org/10.4172/2157-2518.1000165>
- Yao W, Rose JL, Wang W, Seth S, Jiang H, Taguchi A, Liu J, Yan L, Kapoor A, Hou P, Chen Z, Wang Q, Nezi L, Xu Z, Yao J, Hu B, Pettazzoni PF, Ho IL, Feng N, Ramamoorthy V, Jiang S, Deng P, Ma GJ, Den P, Tan Z, Zhang SX, Wang H, Wang YA, Deem AK, Fleming JB, Carugo A, Heffernan TP, Maitra A, Viale A, Ying H, Hanash S, DePinho RA, Draetta GF (2019) Syndecan 1 is a critical mediator of macropinocytosis in pancreatic cancer. *Nature* 568(7752):410–414. <https://doi.org/10.1038/s41586-019-1062-1>
- Yeo CQX, Alexander I, Lin Z, Lim S, Aning OA, Kumar R, Sangthongpitag K, Pendharkar V, Ho VHB, Cheok CF (2016) p53 maintains genomic stability by preventing interference between transcription and replication. *Cell Rep* 15(1):132–146. <https://doi.org/10.1016/j.celrep.2016.03.011>
- Yesmin F, Furukawa K, Kambe M, Ohmi Y, Bhuiyan RH, Hasnat MA, Mizutani M, Tajima O, Hashimoto N, Tsuchida A, Kaneko K, Furukawa K (2023) Extracellular vesicles released from ganglioside GD2-expressing melanoma cells enhance the malignant properties of GD2-negative melanomas. *Sci Rep* 13(1):4987. <https://doi.org/10.1038/s41598-023-31216-4>
- Yi W, Clark PM, Mason DE, Keenan MC, Hill C, Goddard WA 3rd, Peters EC, Driggers EM, Hsieh-Wilson LC (2012) Phosphofructokinase 1 glycosylation regulates cell growth and metabolism. *Science* 337(6097):975–980. <https://doi.org/10.1126/science.1222278>
- Yuan Y, Wu L, Shen S, Wu S, Burdick MM (2016) Effect of alpha 2,6 sialylation on integrin-mediated adhesion of breast cancer cells to fibronectin and collagen IV. *Life Sci* 149:138–145. <https://doi.org/10.1016/j.lfs.2016.02.071>
- Yuan Z, Li Y, Zhang S, Wang X, Dou H, Yu X, Zhang Z, Yang S, Xiao M (2023) Extracellular matrix remodeling in tumor progression and immune escape: from mechanisms to treatments. *Mol Cancer* 22(1):48. <https://doi.org/10.1186/s12943-023-01744-8>
- Yun R, Hong E, Kim J, Park B, Kim SJ, Lee B, Song YS, Kim SJ, Park S, Kang JM (2023) N-linked glycosylation is essential for anti-tumor activities of KIAA1324 in gastric cancer. *Cell Death Dis* 14(8):546. <https://doi.org/10.1038/s41419-023-06083-6>
- Zhai L, Chen W, Cui B, Yu B, Wang Y, Liu H (2021) Overexpressed versican promoted cell multiplication, migration and invasion in gastric cancer. *Tissue Cell* 73:101611. <https://doi.org/10.1016/j.tice.2021.101611>
- Zhang B, van Roosmalen IAM, Reis CR, Sestroikromo R, Quax WJ (2019a) Death receptor 5 is activated by fucosylation in colon cancer cells. *FEBS J* 286(3):555–571. <https://doi.org/10.1111/febs.14742>
- Zhang Q, Higginbotham JN, Jeppesen DK, Yang YP, Li W, McKinley ET, Graves-Deal R, Ping J, Britain CM, Dorsett KA, Hartman CL, Ford DA, Allen RM, Vickers KC, Liu Q, Franklin JL, Bellis SL, Coffey RJ (2019b) Transfer of functional cargo in exomeres. *Cell Rep* 27(3):940–954 e946. <https://doi.org/10.1016/j.celrep.2019.01.009>

- Zhang J, Ten Dijke P, Wuhrer M, Zhang T (2021) Role of glycosylation in TGF-beta signaling and epithelial-to-mesenchymal transition in cancer. *Protein Cell* 12(2):89–106. <https://doi.org/10.1007/s13238-020-00741-7>
- Zhang J, Wang H, Wu J, Yuan C, Chen S, Liu S, Huo M, Zhang C, He Y (2022) GALNT1 enhances malignant phenotype of gastric cancer via modulating CD44 glycosylation to activate the Wnt/beta-catenin signaling pathway. *Int J Biol Sci* 18(16):6068–6083. <https://doi.org/10.7150/ijbs.73431>
- Zhao P, Nairn AV, Hester S, Moremen KW, O'Regan RM, Oprea G, Wells L, Pierce M, Abbott KL (2012) Proteomic identification of glycosylphosphatidylinositol anchor-dependent membrane proteins elevated in breast carcinoma. *J Biol Chem* 287(30):25230–25240. <https://doi.org/10.1074/jbc.M112.339465>
- Zhao Q, Piyush T, Chen C, Hollingsworth MA, Hilkens J, Rhodes JM, Yu LG (2014) MUC1 extracellular domain confers resistance of epithelial cancer cells to anoikis. *Cell Death Dis* 5(10):e1438. <https://doi.org/10.1038/cddis.2014.421>
- Zhao Y, Wei A, Zhang H, Chen X, Wang L, Zhang H, Yu X, Yuan Q, Zhang J, Wang S (2017) α 2,6-sialylation mediates hepatocellular carcinoma growth in vitro and in vivo by targeting the Wnt/beta-catenin pathway. *Oncogenesis* 6(5):e343. <https://doi.org/10.1038/oncsis.2017.40>
- Zhong J, Martinez M, Sengupta S, Lee A, Wu X, Chaerkady R, Chatterjee A, O'Meally RN, Cole RN, Pandey A, Zachara NE (2015) Quantitative phosphoproteomics reveals crosstalk between phosphorylation and O-GlcNAc in the DNA damage response pathway. *Proteomics* 15(2–3): 591–607. <https://doi.org/10.1002/pmic.201400339>
- Zhou Q, Qiu H (2019) The mechanistic impact of N-glycosylation on stability, pharmacokinetics, and immunogenicity of therapeutic proteins. *J Pharm Sci* 108(4):1366–1377. <https://doi.org/10.1016/j.xphs.2018.11.029>
- Zhou X, Yang G, Guan F (2020) Biological functions and analytical strategies of sialic acids in tumor. *Cells* 9(2):273. <https://doi.org/10.3390/cells9020273>
- Zhuo Y, Chammas R, Bellis SL (2008) Sialylation of beta1 integrins blocks cell adhesion to galectin-3 and protects cells against galectin-3-induced apoptosis. *J Biol Chem* 283(32): 22177–22185. <https://doi.org/10.1074/jbc.M8000015200>



Melatonin and Cancer: New Insights

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Abstract

Melatonin is a ubiquitous molecule on the phylogenetic scale, and cancer genes, inherent to the coexistence and cooperation between cells, appeared with multicellularity in ancestral metazoans. It is not surprising that the functional network of the indoleamine intersects with the malfunction of pathways responsible for cancer during evolution. Attention paid to melatonin over the past decades has revealed extraordinary pleiotropy involving the synchronization of endogenous rhythms, control of redox metabolism, receptor-mediated downstream signaling, and (epi)genetic regulation, among others. This multiplicity of actions outlines indoleamine as a protective agent that prevents both damage to cellular structures and the alteration of homeostasis. Instead, cancer represents the tectonic convulsion of cellular stabilization mechanisms to put them at the service of uncontrolled growth and propagation. From this perspective, it is clear that melatonin biochemistry is placed at the center of the cancer scene and, more importantly, that its functionalities can be potentially antagonistic. Research on the functional biology of melatonin has given rise to an important volume of epidemiological, in vitro, and animal studies that all together point directly and indirectly to its ability to inhibit critical carcinogenesis steps. However, there is still much basic and translational knowledge to be achieved, namely systematic research programs in animal models and clinical trials that would clarify the possibilities of melatonin in the prevention and treatment of the disease. Information continues to

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accumulate and ongoing research needs regular state-of-the-art updates. This chapter attempts to answer this concern by summarizing the latest findings on the mechanistic actions of melatonin in critical processes of tumor development. In addition, recent evidence on the therapeutic capacity of melatonin in the clinical setting of cancer patients is presented.

Keywords

Adjuvant therapy · Angiogenesis · Apoptosis · Cancer · Melatonin · Signaling pathways

1 Introduction

Cancer exemplifies the great failure and, at the same time, the great achievement of contemporary society. After decades of intense research and investment in new treatments, cancer continues to be responsible for approximately 10 million deaths in 2020, almost one in six, and an estimated 19.3 million new diagnoses per year, which they are expected to increase to 28.4 million by 2040 (Sung et al. 2021; WHO 2023). Mortality, in addition to morbidity and prolonged health interventions, unparalleled among highly prevalent pathologies, is frustrating and represents a colossal innovation challenge for biomedicine. However, this disheartening facet of cancer arises, in part, from our era's success in accessing a way of life that ensures survival rates that are high enough for an insidious, usually slow-progressing disease to reach such an epidemiological dimension.

The gnoseological unification of cancer as a genetic disease, augured 50 years ago by the first chromosomal abnormalities and cancer genes (Knudson 2002), was progressively disproved by molecular signatures and biochemical singularities of primary tissues, patients, and tumors. Correspondingly, the optimism raised by the hypothetical simplification of anticancer approaches faded as the paradigmatic complexity of cancer became evident. Indeed, molecular oncology gradually made clear that cancer encompasses more than 200 clinical entities with notable differences in multifactorial etiology, stage-specific mutational burdens, deregulated signaling pathways, and/or microenvironmental determinants for tumor growth and spread. Nevertheless, more difficult than conceptual polysemy was the heterogeneous presentation of cancer types that, together with the possible involvement of any organic structure, eclipsed the hope for therapeutic formulations that would fully address tumor progression. On the contrary, clinical diversity entails that different modalities are diagnosed individually and, similarly, variable expressivity means that the effectiveness of conventional therapy is reduced. Consequently, the need to stratify the management of different phenotypes according to patient and/or tumor type was revealed. Thus, although multimodal treatments based on surgery, neoadjuvant or first-line radiochemotherapy, hormone therapy, immunotherapy, and/or new generation biological therapies have considerably increased palliative efficacy (Falzone et al. 2018), as well as blinded or targeted screenings allow earlier

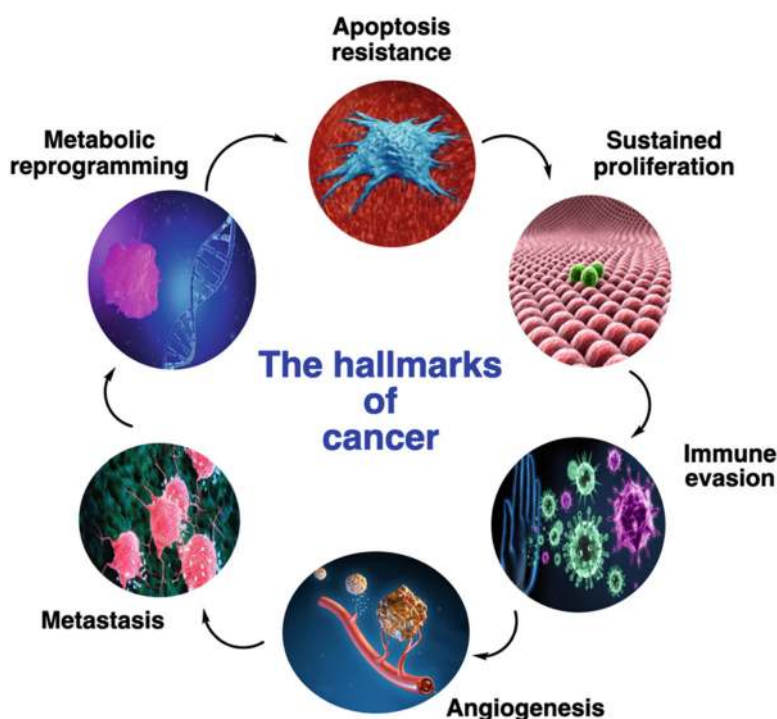


Fig. 1 Functional components for the survival and propagation of cancer cells throughout the tumor cycle

detection, detrimental side effects (Wang et al. 2022a, b) and 5-year survival rates (Crosby et al. 2022) remain disappointing. At the same time, the insufficient success of standardized regimens stimulated cancer researchers to subsume tumor dyshomeostasis to a narrow set of basal processes, generally unbalanced in different cancer types (Fig. 1) (Hanahan 2022). This step forward furthered the goal of theoretical carcinogenesis being predictive, in order to select preferential targets to direct the therapeutic armamentarium. However, current clinical consensus continues to indicate that the ecosystem perspective should be the pivotal axis in any decision-making (Grizzi et al. 2006; Du and Elemento 2015), so that more case- and tumor-specific approaches may improve outcomes and the quality of life of cancer patients (Sandhu et al. 2018; Praus et al. 2023).

Despite great recent advances, cancer research is still in the exploratory phase of detailing the molecular pathogenesis and strengthening the available oncolytic pharmacopeia through more specific and less toxic options. In this regard, melatonin (*N*-acetyl-5-methoxytryptamine), initially known to be the master synchronizer of central and peripheral circadian clocks, as well as the chronoregulator of seasonal cyclicality, has been progressively associated with a variety of other physiological functions such as the response to oxidative stress, immunomodulation, and

inflammation (Tordjman et al. 2017). Likewise, accumulated evidence has also reported that melatonin can modulate fundamental oncostatic mechanisms throughout the different phases of the tumor cycle, thus achieving synergies in protection against the cytotoxicity of standard schedules and enhancing radiochemotherapy lethality (Talib et al. 2021). In addition, several prospective studies have indicated that melatonin suppression, due to excess of artificial light-at-night or night-shift occupations, is linked to a higher incidence of cancer, particularly breast, prostate, and colon tumors (Jones 2021). In this sense, the International Agency for Research on Cancer (IARC) has classified night work as “probably carcinogenic to humans” since 2007, revalidating this association at 2019 based on epidemiological evidence, mechanistic feasibility and animal studies (2019). Therefore, resynchronization of circadian rhythmicity opens an important research avenue to determine if melatonin administration can be a possible preventive and/or palliative strategy to reduce the burden of cancer.

A recent *Nature* editorial summarized directions in which clinical cancer research could raise patients’ expectations in the near term (2022). In this context, it is essential to achieve the following two objectives; to reduce the side effects of current therapies and to eliminate asymmetries, whether for political and/or socioeconomic reasons, in access to clinical trials and treatments. It should be noted that melatonin could play a huge role in achieving both goals. The antioxidant, immunostimulant, and anti-inflammatory effects of this indoleamine have been shown repeatedly to mitigate some harmful effects of current management schemes. In addition, melatonin is inexpensive and prescription could ameliorate the social and regional distribution of research benefits. Consequently, we consider that thoroughly investigating the anticancer suitability of this ancient biomolecule should be seriously considered from a cost-benefit point of view. The coevolution of this indoleamine with the entire spectrum of living beings has linked it to essential mechanisms of cell and tissue homeostasis (Fig. 2). Therefore, elucidating the overlap of melatonin functional network with cancer pathophysiology will broaden our knowledge of how we get sick but, importantly, how we can inhibit cancer progression and/or reduce debilitating side effects. In this sense, the medicinal indication of melatonin is committed to a more naturalistic alternative for cancer therapy and patient support, although its feasibility is not free of controversies and technical difficulties (Wang et al. 2022a, b). Both will only find a definitive answer through more basic investigation, which clarifies the biochemistry of the molecule beyond its demonstrated actions on biological and metabolic rhythms, and more clinical research to overcome the low solubility and rule out possible reactions of melatonin at pharmacological doses.

In the effort to unravel the antineoplastic capabilities of this multitasking indoleamine, we present an update of the latest findings on the impact of melatonin and its downstream signaling on three critical milestones of tumor progression: apoptosis, angiogenesis, and metastasis. In the second part, we summarize the adjuvant potential of melatonin combined regimens. Hopefully, these advances and the new prevention and treatment perspectives they open will be the starting

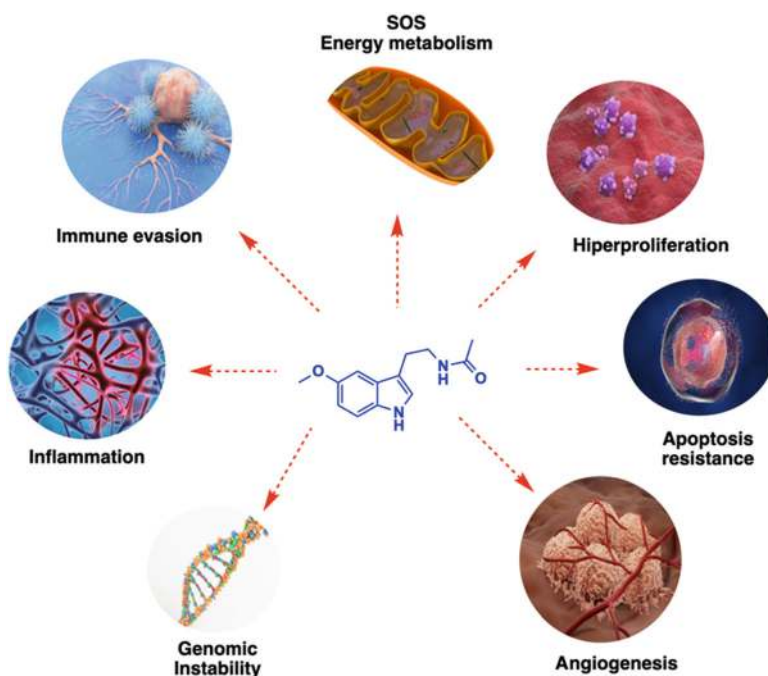


Fig. 2 Some of the cross actions of melatonin and central carcinogenic processes

point for future improvements in the course of the disease and the well-being of patients.

2 Cancer-Related Involvement of Melatonin Receptors and Signaling Pathways

In recent years, the compelling anti-cancer properties of melatonin have captivated researchers, prompting extensive research into its workings. These investigations delve into identifying melatonin's membrane receptors and their binding sites, deciphering their precise modes of action and unraveling how melatonin operates independently of receptors as a scavenger of free radicals.

In humans, the main melatonin-binding sites correspond to the cell membrane receptors, MT1, MT2, and MT3, according to the classification of the International Union of Pharmacology (Dubocovich et al. 2010). Of picomolar affinity, MT1 and MT2 belong to the largest superfamily of G protein-linked receptors (Pavlos and Friedman 2017) and are expressed in a plethora of tissues (Pandi-Perumal et al. 2008; Slominski et al. 2012; Soderquist et al. 2015). Moreover, they share a high sequence homology (55% overall homology in amino acid sequence and 70% within transmembrane domain) (Jockers et al. 2016), show a quaternary composition as

homodimeric and heterodimeric forms, and have different melatonin affinity (Hay and Pioszak 2016). The activation of MT1/MT2 receptors triggers their associated G-protein-coupled second messenger pathways that subsequently inhibit adenylate cyclase (in the case of MT2, also the guanylyl cyclase) (Comai and Gobbi 2014), resulting in lower cytosolic cAMP (and cGMP) and the final modulation of different intracellular signaling pathways (von Gall et al. 2002; Witt-Enderby et al. 2003; Hardeland et al. 2006; Legros et al. 2014). Additionally, a third receptor, MT3, corresponding to a melatonin-sensitive form of the cytosolic quinone reductase 2 enzyme (Legros et al. 2022), and whose real existence and transduction cascade have not yet been definitely accredited in humans (Slominski et al. 2012), has been confirmed independently by several groups. The highly lipophilic nature allows melatonin to widely spread in the organism and easily diffuse through the cell membranes, reaching intracellular organelles and eventually activating nuclear receptor signaling. Internalization in the nucleus allows melatonin to modulate gene expression by binding with high affinity to transcription factors, which recognize specific response elements in the promoter of their target genes. Accordingly, nanomolar or subnanomolar melatonin may accumulate in the nucleus and bind specifically to the ligand-inducible and transcriptionally active retinoid Z receptor Z (RZR) or retinoid orphan receptor (ROR) (Ma et al. 2021; Kulsoom et al. 2023), an orphan member of the nuclear receptor superfamily (Ekmekcioglu 2006). Adding complexity to the melatonin-mediated signaling, some experimental data suggest a functional link between MT1/MT2 and the transcription factor ROR/RZR, since changes in the expression of both types of melatonin-binding sites evolve in the same direction in blood mononuclear cells (Chen et al. 2011). Remarkably, ROR enables melatonin to resynchronize the intracellular rhythmicity network through clock control genes (Jetten 2009) and participate in immunomodulation (Calvo et al. 2013), as well as coordinate anti-tumor processes (Karasek et al. 2002; Winczyk et al. 2002).

Therefore, the involvement of melatonin receptors in cancer is multifaceted, modulating critical cellular functions like cell proliferation, apoptosis, and/or immune response. Thus, studies indicate that reduced expression or dysfunction of melatonin receptors, particularly MT1, is associated with increased cancer risk (Bicer et al. 2023; Cuciello et al. 2023). The MT1 receptor, when activated by melatonin, exerts anti-proliferative effects, slowing down cancer cell growth and promoting programmed cell death (apoptosis) (Sun et al. 2022a, b). Its presence on immune cells also suggests a role in enhancing immune surveillance against cancer cells (Targhazeh et al. 2022a, b; Silva et al. 2023). The involvement of melatonin receptors in cancer underscores the intricate interplay between circadian rhythm, melatonin signaling, and cellular processes regulating cancer development, which opens doors for novel treatment strategies in order to enhance treatment efficacy by modulating cellular responses and reducing the adverse effects of chemotherapy. In this sense, further research into these receptors and their mechanisms holds promise in devising targeted therapies and interventions against various types of cancer.

Melatonin influences various signaling pathways by binding to cytoplasmic and intra-nuclear receptors, enabling the regulation of crucial mediators. This

exceptional capability has spurred scholarly exploration into melatonin's potential to reverse carcinogenesis (a complex process involving numerous pathways) and its role in modulating multiple signaling pathways pivotal in cancer progression. It's important to note that the specific signaling pathways affected may vary depending on the cell type and the context of the cancer under investigation.

NF- κ B (nuclear factor- κ B), is an important transcription factor involved in inflammation and immunity, which can suppress pro-survival signals in cancer cells (Karin and Greten 2005), constitutively active in most cancers and induced by carcinogens (Wang et al. 2009; Oh et al. 2023; Pavitra et al. 2023). It regulates the expression of many members of the TNF superfamily (IL-1 α / β , IL-6/8/18, chemokines, metalloproteinase-9, VEGF, COX-2, etc.) that recruit leucocytes and initiate a feed-forward loop, which is why the anti-inflammatory agents that suppress NF- κ B, or NF- κ B-regulated genes, should have chemopreventive and therapeutic utility in the treatment of neoplasia. In this regard, melatonin has been shown to inhibit the NF- κ B signaling pathway, decreasing the tumor size and growth in MDA-MB-231 cell line xenograft in Balb/c nude athymic mice (Colombo et al. 2018), as well as inhibiting angiogenesis in HUVEC cells (Qin et al. 2012).

The “guardian of the genome,” p53, is a tumor suppressor protein that plays a central role in preventing cancer, regulating cell division, and DNA repair. When cells face stress or damage, p53 orchestrates responses, either initiating repairs or triggering programmed cell death (apoptosis) to prevent the propagation of damaged cells. Studies suggest that melatonin positively influences p53 activity, enhancing its tumor-suppressive functions (Proietti et al. 2014; Nabih et al. 2023). High p53 levels often result in increased activation of its downstream pathways (Song et al. 2018), intensifying the cell's ability to arrest proliferation or induce apoptosis in those damaged or malignant (Nabih et al. 2023).

Nuclear factor-E2-related factor 2 (Nrf2) serves as a critical regulator of cellular defense mechanisms against oxidative stress and it plays a complex and significant role in cancer development and progression (Zimta et al. 2019). Under normal conditions, Nrf2 activation is essential for balancing cells by controlling protective gene expression. It manages how cells react to oxidative stress, neutralizing damaging free radicals and ROS, potentially preventing the onset of cancer. However, dysregulation or aberrant activation of Nrf2 has been implicated in cancer (Basak et al. 2017). Additionally, the overactivation of Nrf2 can contribute to the promotion of tumor growth, metastasis, and resistance to therapies (Basak et al. 2017). It may facilitate cancer cell proliferation by regulating genes involved in cell cycle progression and anti-apoptotic pathways. Therefore, some studies suggest that Nrf2 activity might have a dual role in cancer, inhibiting tumor growth by enhancing the antioxidant defense mechanisms of normal cells and counteracting oxidative stress-induced damage, thus preventing malignant transformation. In this sense, numerous studies had shown the promising influence of melatonin in modulating Nrf2 activity to restore its normal function or selectively inhibit its hyperactivation in cancer cells hold promise for improving treatment outcomes (Kilic et al. 2013; Vriend and Reiter 2015; Ahmadi and Ashrafizadeh 2020; Tascioglu Aliyev et al. 2021).

Yes-associated protein (YAP) and PDZ-binding motif (TAZ) signaling system (YAP/TAZ and Hippo pathway) are two structural components of the β -catenin destruction complex that regulate the Wnt signaling pathway and also downstream transcriptional coactivators of the highly conserved Hippo pathway (Azzolin et al. 2014). The most prominent and best-known physiological function of Hippo signaling is to restrict uncontrolled cell proliferation and migration when cells are contact inhibited (Zhao et al. 2007; Meng et al. 2016). In this way, excessive tissue growth is prevented and the appropriate organ size in higher eukaryotes, including mammals, is assured (Zhao et al. 2010). This control is achieved by mechanical stress and/or cell density-dependent Hippo pathway activation (Aragona et al. 2013), which signals the subsequent phosphorylation and nuclear expulsion of YAP/TAZ transcriptional coactivators (Dong et al. 2007). In this regard, evidence contributes to the conviction of clinically relevant crosstalk between melatonin and YAP/TAZ signaling pathways (Lo Sardo et al. 2017) such as the following: *i*) both the intracellular signaling of melatonin and the Hippo system are positively or negatively modulated by G-protein coupled receptors; *ii*) melatonin stimulation rises cytosolic cAMP pool and this second messenger activates the phosphorylation and subsequent inhibition of YAP/TAZ; or *iii*) melatonin downregulates TAZ expression through the inhibition of its transcriptional enhancer, the NF- κ B. While melatonin-receptor-dependent signaling exhibits specificity across several cell types and tissues (Pandi-Perumal et al. 2008), it is essential to consider its implications in non-colonic solid tumors, such as pancreatic or breast tumors, among others. Research has evidenced that in this context, the oncogenic role of YAP/TAZ in tumor progression and therapeutic resistance manifests through the following mechanisms. First, by the hyperactivation of the pro-proliferative PI3K/Akt/mTOR pathway that expedites vasculature and angiogenesis, facilitating tumor growth and dissemination. Second, YAP/TAZ transcriptionally activates the genes involved in remodeling the extracellular matrix, responding to mechanical stimuli from cell-matrix interactions during the proliferative phases. Lastly, the loss of cell junctions and cell polarity proteins, which typically act as negative regulators of the YAP/TAZ pathway, further exacerbates its oncogenic effects.

The PI3K/Akt/mTOR pathway is a crucial signaling cascade that regulates various cellular processes like growth, survival, metabolism, and proliferation. Dysregulation of this pathway is a hallmark in many cancers, contributing significantly to their initiation, progression, and resistance to therapies (Porta et al. 2014). At its core, the pathway involves several key players: phosphatidylinositol 3-kinase (PI3K), Akt (also known as protein kinase B), and mammalian target of rapamycin (mTOR). When a cell receives external signals, such as growth factors, PI3K is activated leading to the production of a molecule called PIP3. This triggers Akt's recruitment to the cell membrane, where it gets activated and initiates a cascade of events impacting cellular functions (Glaviano et al. 2023). Melatonin has been found to interact with the PI3K/Akt/mTOR pathway, exerting regulatory effects that can impede cancer cell growth and proliferation (Chen et al. 2021a, b) in several tumors, such as hepatoma (Kimball et al. 2008) and melanoma (Kim et al. 2014).

Mitogen-activated protein kinase (MAPK) pathways, including ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase), and p38, are key components influencing cell proliferation, survival, differentiation, and migration. In this sense, the p38/MAPK/JNK/ERK pathway, comprising multiple signaling cascades, plays a critical role in regulating various cellular processes, and its dysregulation is implicated in cancer initiation, progression, and metastasis across diverse cancer types (Dhillon et al. 2007). p38 MAPK pathway regulates responses to stress and inflammation. It impacts cell cycle regulation, apoptosis, and the production of inflammatory cytokines, influencing tumor growth and the tumor microenvironment. JNK is involved in apoptosis, and its dysregulation can lead to both inhibition of cell death (apoptosis resistance) and promotion of cell migration and invasion, fostering tumor aggressiveness. Constitutive activation of ERK promotes uncontrolled cell proliferation by regulating genes involved in the cell cycle (Sun et al. 2015). Melatonin has been found to regulate these pathways in lung cancer cells, reducing cell migration (Zhou et al. 2014). Additionally, melatonin exhibits the ability to modulate JNK and p38 pathways, impacting cell death (apoptosis) and cellular stress responses, potentially impeding cancer progression (Cabrera et al. 2010; Li et al. 2015). Furthermore, melatonin's antioxidant and anti-inflammatory properties contribute to the regulation of these pathways. By scavenging free radicals and reducing oxidative stress, melatonin might indirectly impact the activation of MAPK (Motilva et al. 2011).

However, further research is necessary to elucidate the precise mechanisms and optimize strategies for employing melatonin as an adjunctive therapy in cancer treatment, especially in modulating the signaling pathways dysregulation seen in various cancer types.

3 Melatonin's Role in Regulating Cell Cycle and Apoptosis in Cancer

One of the main issues with chemotherapy regimens is that they are not selective in their activity, thus they can harm healthy cells while attempting to kill cancerous cells. To create effective chemotherapy regimens, it would be ideal to have precise control over the cell cycle and apoptosis of the tumor cells. In this section, we will summarize the current evidence on how melatonin can selectively arrest the tumor cell cycle and promote apoptosis.

Numerous studies conducted on tumor cell lines and animal models have demonstrated that melatonin can reduce tumor growth. However, the exact mechanisms behind this action are not yet fully understood. Since the end of the last century, it has been frequently reported that melatonin is capable of precisely reducing cancer cell viability (Blask and Hill 1986; Hill and Blask 1988; Cucina et al. 2009; Mao et al. 2010; Novotny et al. 2011; Sanchez-Hidalgo et al. 2012; Perdomo et al. 2013; Rodriguez et al. 2013; Jardim-Perassi et al. 2014; Plaimée et al. 2015; Gelaleti et al. 2017). In cancer therapy, a key target is the regulation of the cell cycle and related proteins to ensure stopping uncontrolled growth and repairing

damaged cells (Vermeulen et al. 2003). Melatonin can influence the four phases of the cell cycle; G1, S, G2, and M. When cells are in G1, they can enter into the G0 phase arresting state where cells do not grow or proliferate. Melatonin can arrest, at this point, the tumor cell cycle, preventing uncontrolled growth. In vitro studies show that melatonin arrests the cell cycle at the G0/G1 phase delaying the progression to phase S in tumoral cancer cells (Liu et al. 2017, 2021; Huang et al. 2021; Li et al. 2024) as well as in cancer models in vivo (Sanchez et al. 2017). Most of these studies identified that G2 and M phases are also arrested by melatonin, inhibiting the progress of the cell cycle (Martin-Renedo et al. 2008). This cell cycle control, exerted by melatonin, involves the inhibition of cell cycle progression, avoiding tumor growth and, therefore, resulting in an oncostatic profile (Targhazeh et al. 2022a, b).

Besides, proteins related to the cell cycle are also modulated by melatonin, contributing to the oncostatic role of melatonin. The cyclin-dependent kinases (CDK) are involved in cell cycle progression and their activation, together with the CDK inhibitors playing a crucial role in cell cycle regulation (Santo et al. 2015). The succession of the activation and inactivation of the CDK family members ensures the correct progression along the cell cycle phases. Cancer cells frequently have CDK dysregulation, promoting cell cycle progression and inhibiting apoptosis (Peyressatre et al. 2015). Moreover, CDK inhibitors have been designed as anticancer drugs (Otto and Sicinski 2017). Melatonin displays an inhibitory action downregulating the CDK family (Yun et al. 2014; Xiao and Dong 2023), CDK members are closely related to particular phases of the cell cycle progression. Melatonin downregulates CDK2 and CDK4, arresting the cell cycle in phases G1 and S in ovarian cancer lines, neuroblastoma (Pizarro et al. 2008; Shen et al. 2016), and osteoblastic and osteosarcoma cell lines, inhibiting CDK4, as well as CDK1, which is related to phases G2 and M (Liu et al. 2011, 2012, 2013; Huang et al. 2021). Additionally, there is in vivo evidence indicating the ability of melatonin, at 10 mg/kg, to inhibit CDK4 and CDK6 expression in a mice hepatocarcinogenesis model (Sanchez et al. 2017). This melatonin profile is reinforced by the upregulation of several CDK inhibitors in several tumor cell lines (Milicevic et al. 1986).

Apoptosis, programmed cell death, is essential in the regulation of the cell cycle to eliminate damaged cells. It progresses by two main pathways, intrinsic (mitochondrial) and extrinsic (receptor) (Hengartner 2000). Accumulation of mutated cells and proliferation of uncontrolled cell occur when apoptosis is deregulated, which has been related to oncologic events. The anti-apoptotic effects of melatonin have been widely studied in healthy cells (Chen et al. 2021a, b; Luchetti et al. 2023; Mafi et al. 2023), and, on the contrary, when examined in tumor cells, an opposite effect has been observed, displaying a pro-apoptotic effect (Sawe et al. 1985; Liu et al. 2017; Bhattacharya et al. 2019; Targhazeh et al. 2022a, b). This exceptional feature makes melatonin a meaningful molecule to study in cancer therapy. Melatonin promotes apoptosis of tumoral cells regulating cell death pathways and apoptotic mediators (Gelaleti et al. 2017). Particularly, melatonin activates the caspase signaling pathway inducing apoptosis (Rubio et al. 2007; Bejarano et al. 2009; Sanchez-Hidalgo et al. 2012; Kosar et al. 2016; Liao et al. 2020) as well as coordinates the

BAX/BCL-2 ratio, which determines the apoptosis regulation (Hengartner 2000), downregulating the anti-apoptotic BCL-2 and upregulating the pro-apoptotic BAX via the intrinsic mitochondrial pathway (Talib 2020; Minocha et al. 2022). Another interesting finding is that melatonin can inhibit other main cell dead pathways, such as ERK and AKT (Lu et al. 2016; Gao et al. 2017; Li et al. 2024).

In several cancer types, microRNA (miRNA), short molecules that commonly regulate RNA messengers, are deregulated, which is closely related to cell cycle and apoptosis disruption. Melatonin can additionally interact with several miRNAs, acting as an antiproliferative and tumor suppressor factor in cancer (Maleki et al. 2021).

Complementary to the aforementioned findings, the activity of melatonin seems to be dual, not only arresting the cancerogenic cell cycle but also preventing cancer onset (Subramanian et al. 2007). Therefore, based on the evidence mentioned above, we can conclude that melatonin arrests the cell cycle and induces apoptosis in a wide variety of tumor cell lines and malignant neoplasms by several mechanisms of action revealing melatonin's therapeutic potential as an oncostatic treatment.

4 Melatonin Modulation of Angiogenesis and Metastasis in Cancer

The process of metastasis involves the spread of neoplastic cells from the site of origin to surrounding tissues and distant organs (Viallard and Larrivee 2017). This process occurs in a stepwise manner, starting with the infiltration of adjacent tissues, followed by the entry of neoplastic cells into the bloodstream, their journey through the vessels, extravasation of neoplastic cells, and, finally, proliferation in distant organs (Gupta and Massague 2006). In the literature, it has been described that melatonin is capable of reducing the metastatic potential of tumors by acting at different levels: modulating cell-cell interaction, cell-extracellular matrix interaction, inhibiting the action of metalloproteinases responsible for remodeling the extracellular matrix and promoting the infiltration of neoplastic cells, inhibiting the epithelial-mesenchymal transition mechanism, and inhibiting the process of angiogenesis, which is one of the most studied mechanisms in different tumors (Su et al. 2017).

The process of angiogenesis is defined as the formation of blood vessels from preexisting arterial blood vessels. It is a complex process that occurs both under physiological conditions, such as during the healing process, and under pathological conditions, such as in cancer (Hanahan and Weinberg 2011; Saeidnia and Abdollahi 2013). In the case of solid tumors, Judah Folkman formulated, in 1971, the hypothesis that angiogenesis is necessary for tumor cells, which control the process to obtain oxygen and nutrients for their progression (Folkman 1971).

The angiogenesis process is regulated by both pro- and anti-angiogenic factors, which remain in balance during tumor progression, until a point where this balance tilts toward a pro-angiogenic outcome ("angiogenesis switch"), resulting in a vascularized tumor that grows excessively (Bergers and Benjamin 2003).

The main activators of angiogenesis are the VEGF (vascular endothelial growth factor) and the VEGF receptor family (VEGFR) (Bergers and Benjamin 2003). VEGF, also known as VEGF-A, was first identified by Senger et al. in 1983 (Senger et al. 1983). It belongs to a family of homodimeric polypeptides, including VEGF-B, C, D, and PlGF (placental growth factor), which act as membrane receptors. VEGF-A activates the angiogenesis through its interaction with VEGFR-2 (Roskoski 2007).

Under hypoxic conditions, the lack of oxygen leads to an increase in HIF-1 α levels (Boddy et al. 2005). Since it is not hydroxylated, it accumulates as a heterodimer bound to the HIF-1 β subunit. This heterodimer migrates to the nucleus and activates genes involved in cellular adaptation to hypoxia, such as VEGF (Wykoff et al. 2004; Melillo 2006).

The first evidence regarding the antiangiogenic effect of melatonin on tumors dates back to the year 2001. Lissoni et al. (2001) investigated the effect of administering 20 mg/day of melatonin for 2 months in 20 patients diagnosed with metastatic cancer unresponsive to conventional antineoplastic treatments. After the completion of the treatment, patients who did not exhibit tumor progression showed a significant decrease in VEGF levels, while VEGF levels remained unchanged in those patients with tumor progression.

In relation to the antitumor effect of melatonin, this is mainly due to its antiangiogenic effect. Gonzalez et al. (2021) published a review article in 2021 about the relationship between melatonin and antiangiogenic mechanisms through which this hormone is able to sensitize tumor cells to treatments such as radiotherapy or chemotherapy (Gonzalez et al. 2021). After the review of several clinical trials, these authors concluded that, although the specific antiangiogenic mechanisms of melatonin are not well understood, it safely increases response rates and survival (up to 1 year) when administered in combination with standard oncological treatments. They also concluded that numerous studies associate melatonin with the inhibition of VEGF and the inactivation of HIF-1 α , indicating that melatonin neutralizes proangiogenic effects and enhances antiangiogenic effects induced by radiotherapy and chemotherapy.

There are numerous *in vitro* and *in vivo* studies investigating the antiangiogenic effect of melatonin on different types of tumors. For instance, concerning malignant breast tumors, *in vitro* studies on the MCF-7 cell line (cells with estrogen, progesterone, and glucocorticoid receptors) showed that the administration of 1 mM melatonin for 24 h was associated with a reduction in the gene expression of VEGF (Alvarez-Garcia et al. 2013a, b). The antiangiogenic effect of melatonin has also been evaluated on the MDA-MB-231 cell line (triple-negative breast cancer) in both *in vitro* studies (1 mM for 24 h) and *in vivo* studies (40 mg/kg/day) in mice for 21 days. These studies found a decrease in the gene expression of VEGFR-2 and a reduction in microvascular density (Jardim-Perassi et al. 2014; Jardim-Perassi et al. 2016).

The antiangiogenic effect of melatonin has also been studied in other types of tumors, such as colon cancer. This type of tumor has been studied using the HCT116 colon cancer cell line, which has been incubated with 1 mM of melatonin for 16 h,

inducing a reduction in both VEGF and HIF-1, mRNA, and protein levels (Park et al. 2010).

Different studies have evaluated the impact of melatonin administration in the HepG2 hepatocarcinoma cell line. All the studies use 1 mM of melatonin to treat the different cell lines for 24 or 48 h (Colombo et al. 2016), finding the same antiangiogenic effect, described as a reduction in VEGF and HIF-1 α gene expression and a reduction in HIF-1 α nuclear translocation.

The analysis of the effect of melatonin on prostate cancer showed different results in *in vitro* and *in vivo* studies. In *in vitro* studies on the PC-3 cell line, the administration of 1 mM melatonin for 1, 2, 4, and 12 h revealed a decrease in mRNA levels of both VEGF and HIF-1 α , as well as a decrease in protein levels of VEGF and the gene expression of HIF-1 α (Cho et al. 2011; Sohn et al. 2015). However, when studies on the effect of melatonin on prostate cancer were conducted in mice, the administration of 1 mg/kg/day of melatonin via intraperitoneal injection for 41 days, the results were opposite to those observed *in vitro*. An increase in gene expression of VEGF, HIF-1 α , and VEGFR-2 was identified (Paroni et al. 2014).

Two *in vivo* studies have been carried out in mice to evaluate the effect of melatonin on the process of angiogenesis in both renal and gastric cancers. Mice, that were evaluated for renal cancer (Kim et al. 2013), were intraperitoneally injected with 20 mg/kg/day melatonin for 7 days and those that were assessed for gastric cancer (Wang et al. 2016) were intraperitoneally injected with 50, 100, and 150 mg/kg/day melatonin for 7 days. In both cases, a decrease in VEGF gene expression, HIF-1 α gene expression, and the levels of HIF-1 α were found.

5 Melatonin as an Adjuvant Therapy in Cancer: Promising Insights and Applications

For more than 50 years, the anticancer activity of melatonin alone and in combination with other antitumoral drugs has been reported from *in vitro* experiments to clinical trials. It has been determined that melatonin acts as a tumor suppressor preventing cell proliferation, arresting the cell cycle, and inhibiting metastasis. When administered concurrently with other medications, it has the potential to enhance therapeutic efficacy, sensitize drug-resistant tumors, and decrease chemotherapy toxicity (Lu et al. 2019; Kvietkauskas et al. 2020; Najafi et al. 2020; Baburina et al. 2021; Talib et al. 2021; Hagstrom et al. 2022; Maleki Dana et al. 2022; Dana et al. 2023; Rafiyan et al. 2023).

There is substantial evidence supporting melatonin synergisms in combination with numerous chemotherapeutic agents *in vitro*. Melatonin, when combined with peroxisome proliferator-activated receptor- γ (PPAR γ) agonists, as Alpelisib (PI3K inhibitor), and docetaxel (taxane), is able to improve the effectiveness of these chemotherapeutic agents in breast cancer cell lines (Korkmaz et al. 2009; Alonso-Gonzalez et al. 2018; de Godoy et al. 2023), in gastric cancer cell lines, melatonin plus thapsigargin intensify the apoptotic effect (Huang et al. 2021), in human

anaplastic thyroid cancer cells, melatonin synergizes dabrafenib anti-tumor effect (Liao et al. 2020) providing a promising improvement in cancer treatment.

Moreover, it has been established that melatonin may act as a potent chemotherapeutic agent by sensitizing resistant cell lines to anticancer drugs, i.e., melatonin sensitizes TKI-resistant H1975 cells to gefitinib (Yun et al. 2014). Melatonin demonstrated its capacity to sensitize esophageal squamous cell carcinoma to 5-FU (Lu et al. 2016), and additionally, melatonin exhibited the ability to prevent insensitivity to docetaxel and cisplatin and act as an oncostatic agent in cervical cancer cells (Pariante et al. 2017; Chen et al. 2018a, b; Song and Wang 2023). Recently, the therapeutic role of melatonin in hepatocarcinoma cells resistant to doxorubicin (Hamed et al. 2023) and sorafenib (Prieto-Dominguez et al. 2017) chemotherapy was assessed, revealing that its combination may improve cancer outcomes and reduce drug resistance in both cases; likewise, melatonin may improve sorafenib cytotoxicity in leukemia cells (Tian et al. 2019). In rats, coadministration of melatonin with several chemotherapies demonstrated to improve antitumor response, such as cyclophosphamide, adriamycin, plus 5-FU, and adriamycin plus docetaxel cytotoxic drug combinations (Panchenko et al. 2020), and etoposide (Chen et al. 2018a, b).

Melatonin has also demonstrated to be effective reducing chemotherapy resistance in vitro, it is able to diminish cisplatin drug resistance and to improve the efficacy of numerous drugs in several cancer cell lines (Zemla et al. 2017; Jadid et al. 2021; Hosseini et al. 2022; Wang et al. 2022a, b), reducing vorinostat cell resistance in glioblastoma (Sung et al. 2019), in colorectal cancer cells melatonin diminishes 5-fluorouracil (5-FU) (Pariante et al. 2018; Sakatani et al. 2019) and doxorubicin (Fic et al. 2017) resistance, etoposide resistance in hepatocellular carcinoma cells (Chen et al. 2018a, b), and oxaliplatin resistance in colorectal cancer cells (Lee et al. 2018) among others.

The ability of melatonin reducing the toxic effects of many chemotherapeutic agents is also a remarkable feature in vitro, melatonin reduces mitoxantrone toxicity in HepG2 cells (Zamani et al. 2020) and decreases vincristine and busulfan toxicity both in vivo and in vitro (Cui et al. 2017; Soliman et al. 2020). A recent systematic review of animal studies, which includes a wide range of radio and chemotherapies, revealed that all the induced toxicity outcomes were improved when melatonin was co-administered with cancer therapy (Dehdari Ebrahimi et al. 2023). The protective role of melatonin versus the cognitive impairment induced by chemotherapeutic treatments, usually detected in cancer survivors, was also reviewed recently in animals (Kuyl et al. 2023). Doxorubicin administration in cancer patients is related to an increased risk of cardiac dysfunction and congestive heart failure; in vivo, melatonin coadministration resulted in protective effects preventing doxorubicin-induced cardiotoxicity (Arinno et al. 2021; Ma et al. 2023) and the negative effects of methotrexate on memory and neurogenesis in a rat model (Sirichoat et al. 2019).

Cisplatin is one of the first-line treatments against cancers; despite its efficacy, it is highly toxic (Romani 2022). Reportedly, when coadministered with melatonin, it enhances its oncostatic properties in vitro, in vivo, and in humans (Rafiiyan et al. 2023). Furthermore, this association in clinical trials was efficient in preventing

nephrotoxicity, mucositis, neurotoxicity, thrombocytopenia, myelosuppression, neuropathy, ototoxicity, and testes and ovarian toxicity (Lissoni et al. 1997a, b; 2003a, b; De Araujo et al. 2019; Lozano et al. 2021; Al-Shahat et al. 2022; Karvan et al. 2022; Zhang et al. 2022). There is increasing evidence of these melatonin protective effects in numerous in vivo cancer models (Moradi et al. 2021; Rodrigues et al. 2022; Rafiyan et al. 2023).

Cyclophosphamide chemotherapy has been related to an increased risk of premature ovarian insufficiency. In female mice, a dose of 50 mg/kg of melatonin before chemotherapy initiation and maintained 14 days after significantly prevented ovarian granulosa cell loss, which may have important implications for young women's fertility after chemotherapy (Feng et al. 2022). Melatonin in mice was able to protect against ovarian damage caused by Epirubicin and preserve fertility during chemotherapy (Wang et al. 2020).

These findings, both in vivo and in vitro, reveal that melatonin has the potential to diminish chemotoxicity as well as improve therapeutical efficacy. These results reinforce the idea that melatonin may be an exceptional and safe chemotherapy toxicity protector for cancer patients.

In clinical trials, melatonin administered at 20–40 mg/day, alone or in combination with other treatments, is safe and improves 1-year survival for solid cancers (Lissoni et al. 1999; Mills et al. 2005). Several clinical trials point to melatonin as a potential tool to obtain better clinical results. In metastatic colorectal cancer patients treated with 5-FU and folates, interleukin-2 (IL-2) is also administered to generate an effective immune response, and the main concern of IL-2 is that only high doses are effective, as well as toxic. A clinical trial observed an improvement in the survival rate after administering a low dose of IL-2 with melatonin (40 mg/day), improving tumor regression in metastatic colon cancer patients which progressed in response to 5-FU plus folates (Barni et al. 1995). Additionally, for metastatic colorectal cancer patients who had previous 5-FU treatment, further treatment with melatonin (20 mg/day) and irinotecan resulted in a higher partial response than using irinotecan alone, although a complete response was not observed (Cerea et al. 2003). Likewise, a subsequent clinical study in non-small cell lung cancer (NSCLC) patients compared IL-2 plus melatonin (40 mg/day) versus chemotherapy (cisplatin and etoposide), and the differences observed between treatments were a higher percentage survival at 1 year and lower toxicity in IL-2 plus melatonin treatment; however, no complete response was achieved and no differences in partial response were either observed (Lissoni et al. 1994). In NSCLC patients, cisplatin and etoposide chemotherapy alone was compared to this same chemotherapy plus melatonin (20 mg/day). After 1 year, the tumor response rate was higher in patients receiving melatonin, but no statistically significant differences were found. On the contrary, a higher 1-year survival rate and lower toxicity were observed in the melatonin-treated group (Lissoni et al. 1997a, b). After a 5-year observation period, the survival rate and quality of life in NSCLC patients cotreated with melatonin were higher than in those treated with chemotherapy alone (Lissoni et al. 2003a, b). The life quality of advanced NSCLC patients was also observed to be enhanced when melatonin was administered at 10 mg/day; however, this dose did not affect survival or

chemotherapy toxicity (Sookprasert et al. 2014). These results suggest a better disease prognosis in melatonin-treated patients at higher doses.

In the early 1990s for patients with NSCLC who did not progress under first-line chemotherapy containing cisplatin, there was no effective subsequent chemotherapy protocol described. In a clinical trial, where NSCLC patients were included, melatonin administered at 10 mg/day was compared to supportive care alone, and significant changes in percentages of disease stabilization and 1-year survival were observed (Lissoni et al. 1992). Noteworthy, higher doses of melatonin (40 mg/day) for 21 days in advanced inoperable lung cancer patients did not protect against the myelotoxic effect of carboplatin and etoposide (Ghielmini et al. 1999).

As mentioned above, drug resistance is a major concern as it limits the effectiveness of chemotherapy. It has been suggested that melatonin could be a potential co-treatment to reduce resistance (Gonzalez et al. 2021; Targhazeh et al. 2022a, b). The effect of melatonin (20 mg/day) on clinical response in patients with locally advanced oral squamous cell carcinoma (OSCC) was determined after neoadjuvant chemotherapy. Results indicated that, despite melatonin reduced chemoresistance and decreased tumor residue, the results were not statistically significant, but it may be effective in reducing hypoxia-inducible factor-1 α expression (Kartini et al. 2020, 2021). In an observational study of 15 osteosarcoma patients, those who received melatonin as a co-adjuvant therapy showed better results in neoplastic progression (Di Bella et al. 2022). Several models have, hence, been studied showing promising results in reducing multidrug resistance with the coadministration of melatonin.

Several clinical studies have been conducted on coadministration of chemotherapy and melatonin (18–20 mg/day) in breast cancer women, revealing that this association was able to decrease chemotherapy toxicity, increase partial response and 1-year survival rate, and enhance the quality of life of patients by reducing fatigue levels and improving cognitive function, sleep quality, and depressive symptoms associated with the treatments (Lissoni et al. 1995, 1997a, b, 1999; Palmer et al. 2020; Sedighi Pashaki et al. 2021; Mukhopadhyay et al. 2023). In several other studies, anxiety and depression were also mitigated by the coadministration of melatonin in breast cancer patients (Chen et al. 2014; Hansen et al. 2014). Notwithstanding in clinical trials that administered lower doses of melatonin (3 mg/day), only benefits in fatigue and sleep quality were observed, not detecting other interesting effects (Ramos et al. 2023).

In pediatric oncology patients, melatonin was well tolerated at 0.3 mg/kg (maximum 20 mg), preventing weight loss (Johnston et al. 2019). As no significant adverse effects were observed in this study, further investigation of the administration of melatonin at high dose in pediatric oncology patients would be interesting.

These results suggest that adjusting the dose is crucial to achieving the promising results obtained in vivo in humans (Ramos et al. 2023). The administration of higher doses of melatonin is possible due to its wide safety profile. Most human clinical trials that have included melatonin have tested doses around 20 mg/day, with favorable but not conclusive results. In 2018, a clinical study with 14 cancer patients revealed that it is essential to start melatonin therapy at a dose of 100 mg/day or at least 1 mg/kg of body weight/day to achieve clinical antitumor efficacy (Lissoni

Table 1 Translational doses of effective melatonin coadministration from in vivo studies to clinical trials

Animal model	References	Melatonin daily dose (mg/kg)	Outcomes	Daily HED for a 60 kg adult
Rat	Soliman et al. (2020)	10 mg/kg	Reduces <i>vincristine</i> -induced liver and kidney toxicity and peripheral neuropathy of the sciatic nerve	97 mg/kg
Rat	Arinno et al. (2021)	10 mg/kg	Reduces <i>doxorubicin</i> cardiotoxicity	97 mg/kg
Rat	Sirichoat et al. (2019)	8 mg/kg	Diminishes the negative effects of <i>methotrexate</i> on memory and neurogenesis and improves chemotherapy treatment	78 mg/kg
Rat	Zhuo and Jiang (2022)	10 mg/kg	Reduces <i>cisplatin</i> cardiotoxicity	97 mg/kg
Rat	Bayraktar et al. (2022)	20 mg/kg	Reduces <i>cisplatin</i> neurotoxicity	195 mg/kg
Rat	Bilir-Yildiz et al. (2022)	10 mg/kg	Reduces <i>cisplatin</i> -induced neuropathy	97 mg/kg
Rat	Abdel-Rahman Mohamed et al. (2022)	10 mg/kg	Reduces <i>cisplatin</i> nephrotoxicity	97 mg/kg
Mice	Zhang et al. (2020)	25 mg/kg	Coadministered with <i>cisplatin</i> , inhibits metastasis of advanced nasopharyngeal carcinoma	122 mg/kg
Mice	Ma et al. (2023)	20 mg/kg	Reduces <i>doxorubicin</i> cardiotoxicity	97 mg/kg
Mice	Sun et al. (2022a, b)	20 mg/kg	Reduces <i>cisplatin</i> -induced acute kidney injury	97 mg/kg
Mice	Al Za’abi et al. (2021)	10 mg/kg	Reduces <i>cisplatin</i> nephrotoxicity	49 mg/kg

et al. 2018). Accurately translating effective doses from animal models to humans is key to achieving success (Reagan-Shaw et al. 2008). If we apply the dose translation from most preclinical studies, the suggested doses are closer to 100 mg/kg than to 20 mg/kg (Ramos et al. 2023) (Table 1).

6 Conclusion

The current state of cancer exemplifies a paradox of humanity’s great achievements and failures. Despite prolonged health interventions and significant investment in cancer treatment, it remains responsible for an alarming mortality rate and an increasing number of new diagnoses. Over 200 different types of cancer have been identified, each of them with unique characteristics and behaviors, making the

development of effective treatments a daunting task. In this context, melatonin has been the subject of extensive research regarding its potential role in cancer prevention and treatment. While numerous studies have suggested a correlation between melatonin levels and cancer risk, the relationship is complex and multifaceted, presenting both challenges and promising future directions.

One challenge lies in deciphering the intricate interplay between melatonin and various types of cancer. Melatonin has been shown to possess antioxidant, immunomodulatory, and anti-inflammatory properties, which may contribute to its protective effects against cancer. Its toxicity is low, even when administered for long periods, and, therefore, the challenge in using it as an adjuvant in oncological treatments lies in the discrepancy among the different studies when defining the dosage and exposure times necessary to achieve a therapeutic benefit. However, the specific mechanisms through which melatonin influences cancer development and progression remain a topic of ongoing investigation. Furthermore, the variations in study designs, patient populations, and methodologies contribute to the difficulty in drawing universal conclusions.

Future research directions involve exploring the optimal dosage and timing of melatonin supplementation, as well as its potential synergies with conventional cancer therapies. Clinical trials are needed to determine the efficacy of melatonin in preventing cancer in high-risk populations and enhancing the outcomes of cancer treatments.

In conclusion, the relationship between melatonin and cancer presents challenges rooted in its diversity and the complexity of its biological systems. However, ongoing research efforts and clinical trials hold the promise of elucidating the mechanisms involved and establishing melatonin as a valuable tool in the prevention and treatment of cancer.

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References

- Abdel-Rahman Mohamed A, Khater SI, Metwally MMM, Bin Emran T, Nassan MA, Abd El-Emam MM, Mostafa-Hedeab G, El-Shetry ES (2022) TGF-beta1, NAG-1, and antioxidant enzymes expression alterations in cisplatin-induced nephrotoxicity in a rat model: comparative modulating role of melatonin, Vit. E and ozone. *Gene* 820:146293. <https://doi.org/10.1016/j.gene.2022.146293>
- Ahmadi Z, Ashrafizadeh M (2020) Melatonin as a potential modulator of Nrf2. *Fundam Clin Pharmacol* 34(1):11–19. <https://doi.org/10.1111/fcp.12498>
- Al Za'abi M, Ali H, Al Sabahi M, Ali BH (2021) The salutary action of melatonin and betaine, given singly or concomitantly, on cisplatin-induced nephrotoxicity in mice. *Naunyn Schmiedebert's Arch Pharmacol* 394(8):1693–1701. <https://doi.org/10.1007/s00210-021-02097-z>
- Alonso-Gonzalez C, Menendez-Menendez J, Gonzalez-Gonzalez A, Gonzalez A, Cos S, Martinez-Campa C (2018) Melatonin enhances the apoptotic effects and modulates the changes in gene expression induced by docetaxel in MCF-7 human breast cancer cells. *Int J Oncol* 52(2): 560–570. <https://doi.org/10.3892/ijo.2017.4213>

- Al-Shahat A, Hulail MAE, Soliman NMM, Khamis T, Fericean LM, Arisha AH, Moawad RS (2022) Melatonin mitigates cisplatin-induced ovarian dysfunction via altering steroidogenesis, inflammation, apoptosis, oxidative stress, and PTEN/PI3K/Akt/mTOR/AMPK signaling pathway in female rats. *Pharmaceutics* 14(12). <https://doi.org/10.3390/pharmaceutics14122769>
- Alvarez-Garcia V, Gonzalez A, Alonso-Gonzalez C, Martinez-Campa C, Cos S (2013a) Antiangiogenic effects of melatonin in endothelial cell cultures. *Microvasc Res* 87:25–33. <https://doi.org/10.1016/j.mvr.2013.02.008>
- Alvarez-Garcia V, Gonzalez A, Alonso-Gonzalez C, Martinez-Campa C, Cos S (2013b) Regulation of vascular endothelial growth factor by melatonin in human breast cancer cells. *J Pineal Res* 54(4):373–380. <https://doi.org/10.1111/jpi.12007>
- Aragona M, Panciera T, Manfrin A, Giullitti S, Michielin F, Elvassore N, Dupont S, Piccolo S (2013) A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* 154(5):1047–1059. <https://doi.org/10.1016/j.cell.2013.07.042>
- Arinno A, Manechote C, Khuanjing T, Ongnok B, Prathumsap N, Chunchai T, Arunsak B, Kerdphoo S, Shinlapawittayatorn K, Chattipakorn SC, Chattipakorn N (2021) Cardioprotective effects of melatonin and metformin against doxorubicin-induced cardiotoxicity in rats are through preserving mitochondrial function and dynamics. *Biochem Pharmacol* 192:114743. <https://doi.org/10.1016/j.bcp.2021.114743>
- Azzolin L, Panciera T, Soligo S, Enzo E, Bicciato S, Dupont S, Bresolin S, Frasson C, Basso G, Guzzardo V, Fassina A, Cordenonsi M, Piccolo S (2014) YAP/TAZ incorporation in the beta-catenin destruction complex orchestrates the Wnt response. *Cell* 158(1):157–170. <https://doi.org/10.1016/j.cell.2014.06.013>
- Baburina Y, Lomovsky A, Krestinina O (2021) Melatonin as a potential multitherapeutic agent. *J Pers Med* 11(4). <https://doi.org/10.3390/jpm11040274>
- Barni S, Lissoni P, Cazzaniga M, Ardizzoia A, Meregalli S, Fossati V, Fumagalli L, Brivio F, Tancini G (1995) A randomized study of low-dose subcutaneous interleukin-2 plus melatonin versus supportive care alone in metastatic colorectal cancer patients progressing under 5-fluorouracil and folates. *Oncology* 52(3):243–245. <https://doi.org/10.1159/000227465>
- Basak P, Sadhukhan P, Sarkar P, Sil PC (2017) Perspectives of the Nrf-2 signaling pathway in cancer progression and therapy. *Toxicol Rep* 4:306–318. <https://doi.org/10.1016/j.toxrep.2017.06.002>
- Bayraktar UA, Arihan O, Atalay O, Gok M, Cicek C, Bodur E, Tuncer M (2022) Melatonin is effective in attenuating cisplatin-induced neurotoxicity. *J Biochem Mol Toxicol* 36(7):e23075. <https://doi.org/10.1002/jbt.23075>
- Bejarano I, Redondo PC, Espino J, Rosado JA, Paredes SD, Barriga C, Reiter RJ, Pariente JA, Rodriguez AB (2009) Melatonin induces mitochondrial-mediated apoptosis in human myeloid HL-60 cells. *J Pineal Res* 46(4):392–400. <https://doi.org/10.1111/j.1600-079X.2009.00675.x>
- Bergers G, Benjamin LE (2003) Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3(6):401–410. <https://doi.org/10.1038/nrc1093>
- Bhattacharya S, Patel KK, Dehari D, Agrawal AK, Singh S (2019) Melatonin and its ubiquitous anticancer effects. *Mol Cell Biochem* 462(1–2):133–155. <https://doi.org/10.1007/s11010-019-03617-5>
- Bicer E, Bese T, Tuzun DD, Ilvan S, Kayan BO, Demirkiran F (2023) The relationship between melatonin 1-2 receptor expression in patients with epithelial ovarian cancer and survival. *Int J Gynecol Pathol*. <https://doi.org/10.1097/PGP.0000000000000968>
- Bilir-Yildiz B, Sunay FB, Yilmaz HF, Bozkurt-Girit O (2022) Low-intensity low-frequency pulsed ultrasound ameliorates sciatic nerve dysfunction in a rat model of cisplatin-induced peripheral neuropathy. *Sci Rep* 12(1):8125. <https://doi.org/10.1038/s41598-022-11978-z>
- Blask DE, Hill SM (1986) Effects of melatonin on cancer: studies on MCF-7 human breast cancer cells in culture. *J Neural Transm Suppl* 21:433–449
- Boddy JL, Fox SB, Han C, Campo L, Turley H, Kanga S, Malone PR, Harris AL (2005) The androgen receptor is significantly associated with vascular endothelial growth factor and hypoxia sensing via hypoxia-inducible factors HIF-1a, HIF-2a, and the prolyl hydroxylases in human prostate cancer. *Clin Cancer Res* 11(21):7658–7663. <https://doi.org/10.1158/1078-0432.CCR-05-0460>

- Cabrera J, Negrin G, Estevez F, Loro J, Reiter RJ, Quintana J (2010) Melatonin decreases cell proliferation and induces melanogenesis in human melanoma SK-MEL-1 cells. *J Pineal Res* 49(1):45–54. <https://doi.org/10.1111/j.1600-079X.2010.00765.x>
- Calvo JR, Gonzalez-Yanes C, Maldonado MD (2013) The role of melatonin in the cells of the innate immunity: a review. *J Pineal Res* 55(2):103–120. <https://doi.org/10.1111/jpi.12075>
- Cerea G, Vaghi M, Ardizzoia A, Villa S, Bucovec R, Mengo S, Gardani G, Tancini G, Lissoni P (2003) Biomodulation of cancer chemotherapy for metastatic colorectal cancer: a randomized study of weekly low-dose irinotecan alone versus irinotecan plus the oncostatic pineal hormone melatonin in metastatic colorectal cancer patients progressing on 5-fluorouracil-containing combinations. *Anticancer Res* 23(2C):1951–1954
- Chen CQ, Fichna J, Bashashati M, Li YY, Storr M (2011) Distribution, function and physiological role of melatonin in the lower gut. *World J Gastroenterol* 17(34):3888–3898. <https://doi.org/10.3748/wjg.v17.i34.3888>
- Chen WY, Giobbie-Hurder A, Gantman K, Savoie J, Scheib R, Parker LM, Schernhammer ES (2014) A randomized, placebo-controlled trial of melatonin on breast cancer survivors: impact on sleep, mood, and hot flashes. *Breast Cancer Res Treat* 145(2):381–388. <https://doi.org/10.1007/s10549-014-2944-4>
- Chen CC, Chen CY, Wang SH, Yeh CT, Su SC, Ueng SH, Chuang WY, Hsueh C, Wang TH (2018a) Melatonin sensitizes hepatocellular carcinoma cells to chemotherapy through long non-coding RNA RAD51-AS1-mediated suppression of DNA repair. *Cancers (Basel)* 10(9). <https://doi.org/10.3390/cancers10090320>
- Chen L, Liu L, Li Y, Gao J (2018b) Melatonin increases human cervical cancer HeLa cells apoptosis induced by cisplatin via inhibition of JNK/Parkin/mitophagy axis. *In Vitro Cell Dev Biol Anim* 54(1):1–10. <https://doi.org/10.1007/s11626-017-0200-z>
- Chen K, Zhu P, Chen W, Luo K, Shi XJ, Zhai W (2021a) Melatonin inhibits proliferation, migration, and invasion by inducing ROS-mediated apoptosis via suppression of the PI3K/Akt/mTOR signaling pathway in gallbladder cancer cells. *Aging (Albany NY)* 13(18):22502–22515. <https://doi.org/10.18632/aging.203561>
- Chen Z, Zhao C, Liu P, Huang H, Zhang S, Wang X (2021b) Anti-apoptosis and autophagy effects of melatonin protect rat chondrocytes against oxidative stress via regulation of AMPK/Foxo3 pathways. *Cartilage* 13(2_suppl):1041S–1053S. <https://doi.org/10.1177/19476035211038748>
- Cho SY, Lee HJ, Jeong SJ, Lee HJ, Kim HS, Chen CY, Lee EO, Kim SH (2011) Sphingosine kinase 1 pathway is involved in melatonin-induced HIF-1 α inactivation in hypoxic PC-3 prostate cancer cells. *J Pineal Res* 51(1):87–93. <https://doi.org/10.1111/j.1600-079X.2011.00865.x>
- Colombo J, Maciel JM, Ferreira LC, Silva DARF, Zuccari DA (2016) Effects of melatonin on HIF-1 α and VEGF expression and on the invasive properties of hepatocarcinoma cells. *Oncol Lett* 12(1):231–237. <https://doi.org/10.3892/ol.2016.4605>
- Colombo J, Jardim-Perassi BV, Ferreira JPS, Braga CZ, Sonehara NM, Junior RP, Moschetta MG, Girol AP, Zuccari D (2018) Melatonin differentially modulates NF- κ B, Cyr11cB expression in breast and liver cancer cells. *Anti Cancer Agents Med Chem* 18(12):1688–1694. <https://doi.org/10.2174/1871520618666180131112304>
- Comai S, Gobbi G (2014) Unveiling the role of melatonin MT2 receptors in sleep, anxiety and other neuropsychiatric diseases: a novel target in psychopharmacology. *J Psychiatry Neurosci* 39(1):6–21. <https://doi.org/10.1503/jpn.130009>
- Crosby D, Bhatia S, Brindle KM, Coussens LM, Dive C, Emberton M, Esener S, Fitzgerald RC, Gambhir SS, Kuhn P, Rebbeck TR, Balasubramanian S (2022) Early detection of cancer. *Science* 375(6586):eaay9040. <https://doi.org/10.1126/science.aay9040>
- Cucielo MS, Freire PP, Emilio-Silva MT, Romagnoli GG, Carvalho RF, Kaneno R, Hiruma-Lima CA, Delella FK, Reiter RJ, Chuffa LGA (2023) Melatonin enhances cell death and suppresses the metastatic capacity of ovarian cancer cells by attenuating the signaling of multiple kinases. *Pathol Res Pract* 248:154637. <https://doi.org/10.1016/j.prp.2023.154637>

- Cucina A, Proietti S, D'Anselmi F, Coluccia P, Dinicola S, Frati L, Bizzarri M (2009) Evidence for a biphasic apoptotic pathway induced by melatonin in MCF-7 breast cancer cells. *J Pineal Res* 46(2):172–180. <https://doi.org/10.1111/j.1600-079X.2008.00645.x>
- Cui Y, Ren L, Li B, Fang J, Zhai Y, He X, Du E, Miao Y, Hua J, Peng S (2017) Melatonin relieves busulfan-induced spermatogonial stem cell apoptosis of mouse testis by inhibiting endoplasmic reticulum stress. *Cell Physiol Biochem* 44(6):2407–2421. <https://doi.org/10.1159/000486165>
- Dana PM, Sadoughi F, Reiter RJ, Yousefi B, Asemi Z (2023) Potential mechanisms of melatonin in osteosarcoma and bone-related neoplasms: updated review. *Mini Rev Med Chem* 23(3):290–297. <https://doi.org/10.2174/1389557522666220619232941>
- De Araujo JG, Serra LSM, Lauand L, Kuckelhaus SAS, Sampaio ALL (2019) Protective effect of melatonin on cisplatin-induced ototoxicity in rats. *Anticancer Res* 39(5):2453–2458. <https://doi.org/10.21873/anticancer.13364>
- de Godoy BLV, Moschetta-Pinheiro MG, Chuffa LGA, Ponde NF, Reiter RJ, Colombo J, Zuccari D (2023) Synergistic actions of Alpelisib and melatonin in breast cancer cell lines with PIK3CA gene mutation. *Life Sci* 324:121708. <https://doi.org/10.1016/j.lfs.2023.121708>
- Dehdari Ebrahimi N, Sadeghi A, Shojaei-Zarghani S, Shahlaee MA, Taherifard E, Rahimian Z, Eghlidis Z, Azarpira N, Safarpour AR (2023) Protective effects of exogenous melatonin therapy against oxidative stress to male reproductive tissue caused by anti-cancer chemical and radiation therapy: a systematic review and meta-analysis of animal studies. *Front Endocrinol (Lausanne)* 14:1184745. <https://doi.org/10.3389/fendo.2023.1184745>
- Dhillon AS, Hagan S, Rath O, Kolch W (2007) MAP kinase signalling pathways in cancer. *Oncogene* 26(22):3279–3290. <https://doi.org/10.1038/sj.onc.1210421>
- Di Bella G, Di Bella L, Borghetto V, Moscato I, Costanzo E (2022) A retrospective observational study on cases of osteosarcomas treated with a multitherapy: the rationale and effectiveness. *Neuro Endocrinol Lett* 43(3):173–179
- Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D (2007) Elucidation of a universal size-control mechanism in drosophila and mammals. *Cell* 130(6):1120–1133. <https://doi.org/10.1016/j.cell.2007.07.019>
- Du W, Elemento O (2015) Cancer systems biology: embracing complexity to develop better anticancer therapeutic strategies. *Oncogene* 34(25):3215–3225. <https://doi.org/10.1038/onc.2014.291>
- Dubocovich ML, Delagrange P, Krause DN, Sugden D, Cardinali DP, Olcese J (2010) International union of basic and clinical pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. *Pharmacol Rev* 62(3):343–380. <https://doi.org/10.1124/pr.110.002832>
- Ekmekcioglu C (2006) Melatonin receptors in humans: biological role and clinical relevance. *Biomed Pharmacother* 60(3):97–108. <https://doi.org/10.1016/j.biopha.2006.01.002>
- Falzone L, Salomone S, Libra M (2018) Evolution of cancer pharmacological treatments at the turn of the third millennium. *Front Pharmacol* 9:1300. <https://doi.org/10.3389/fphar.2018.01300>
- Feng J, Ma WW, Li HX, Pei XY, Deng SL, Jia H, Ma WZ (2022) Melatonin prevents cyclophosphamide-induced primordial follicle loss by inhibiting ovarian granulosa cell apoptosis and maintaining AMH expression. *Front Endocrinol (Lausanne)* 13:895095. <https://doi.org/10.3389/fendo.2022.895095>
- Fic M, Gomulkiewicz A, Grzegorzolka J, Podhorska-Okolow M, Zabel M, Dziegiel P, Jablonska K (2017) The impact of melatonin on colon cancer cells' resistance to doxorubicin in an in vitro study. *Int J Mol Sci* 18(7). <https://doi.org/10.3390/ijms18071396>
- Folkman J (1971) Tumor angiogenesis: therapeutic implications. *N Engl J Med* 285(21):1182–1186. <https://doi.org/10.1056/NEJM197111182852108>
- Gao Y, Xiao X, Zhang C, Yu W, Guo W, Zhang Z, Li Z, Feng X, Hao J, Zhang K, Xiao B, Chen M, Huang W, Xiong S, Wu X, Deng W (2017) Melatonin synergizes the chemotherapeutic effect of 5-fluorouracil in colon cancer by suppressing PI3K/AKT and NF-kappaB/iNOS signaling pathways. *J Pineal Res* 62(2). <https://doi.org/10.1111/jpi.12380>

- Gelaleti GB, Borin TF, Maschio-Signorini LB, Moschetta MG, Jardim-Perassi BV, Calvinho GB, Facchini MC, Vilorio-Petit AM, de Campos Zuccari DAP (2017) Efficacy of melatonin, IL-25 and siIL-17B in tumorigenesis-associated properties of breast cancer cell lines. *Life Sci* 183: 98–109. <https://doi.org/10.1016/j.lfs.2017.06.013>
- Ghielmini M, Pagani O, de Jong J, Pampallona S, Conti A, Maestroni G, Sessa C, Cavalli F (1999) Double-blind randomized study on the myeloprotective effect of melatonin in combination with carboplatin and etoposide in advanced lung cancer. *Br J Cancer* 80(7):1058–1061. <https://doi.org/10.1038/sj.bjc.6690463>
- Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, Eng H, Nair MG, Makvandi P, Georger B, Kulke MH, Baird RD, Prabhu JS, Carbone D, Pecoraro C, Teh DBL, Sethi G, Cavalieri V, Lin KH, Javidi-Sharifi NR, Toska E, Davids MS, Brown JR, Diana P, Stebbing J, Fruman DA, Kumar AP (2023) PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol Cancer* 22(1):138. <https://doi.org/10.1186/s12943-023-01827-6>
- Gonzalez A, Alonso-Gonzalez C, Gonzalez-Gonzalez A, Menendez-Menendez J, Cos S, Martinez-Campa C (2021) Melatonin as an adjuvant to antiangiogenic cancer treatments. *Cancers (Basel)* 13(13). <https://doi.org/10.3390/cancers13133263>
- Grizzi F, Di Ieva A, Russo C, Frezza EE, Cobos E, Muzzio PC, Chiriva-Internati M (2006) Cancer initiation and progression: an unsimplifiable complexity. *Theor Biol Med Model* 3:37. <https://doi.org/10.1186/1742-4682-3-37>
- Gupta GP, Massague J (2006) Cancer metastasis: building a framework. *Cell* 127(4):679–695. <https://doi.org/10.1016/j.cell.2006.11.001>
- Hagstrom A, Kal Omar R, Williams PA, Stalhammar G (2022) The rationale for treating uveal melanoma with adjuvant melatonin: a review of the literature. *BMC Cancer* 22(1):398. <https://doi.org/10.1186/s12885-022-09464-w>
- Hamed AR, Yahya SMM, Nabih HK (2023) Anti-drug resistance, anti-inflammation, and anti-proliferation activities mediated by melatonin in doxorubicin-resistant hepatocellular carcinoma: in vitro investigations. *Naunyn Schmiedeberg's Arch Pharmacol* 396(6):1117–1128. <https://doi.org/10.1007/s00210-023-02385-w>
- Hanahan D (2022) Hallmarks of cancer: new dimensions. *Cancer Discov* 12(1):31–46. <https://doi.org/10.1158/2159-8290.CD-21-1059>
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- Hansen MV, Andersen LT, Madsen MT, Hageman I, Rasmussen LS, Bokmand S, Rosenberg J, Gogenur I (2014) Effect of melatonin on depressive symptoms and anxiety in patients undergoing breast cancer surgery: a randomized, double-blind, placebo-controlled trial. *Breast Cancer Res Treat* 145(3):683–695. <https://doi.org/10.1007/s10549-014-2962-2>
- Hardeland R, Pandi-Perumal SR, Cardinali DP (2006) Melatonin. *Int J Biochem Cell Biol* 38(3): 313–316. <https://doi.org/10.1016/j.biocel.2005.08.020>
- Hay DL, Pioszak AA (2016) Receptor activity-modifying proteins (RAMPs): new insights and roles. *Annu Rev Pharmacol Toxicol* 56:469–487. <https://doi.org/10.1146/annurev-pharmtox-010715-103120>
- Hengartner MO (2000) The biochemistry of apoptosis. *Nature* 407(6805):770–776. <https://doi.org/10.1038/35037710>
- Hill SM, Blask DE (1988) Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. *Cancer Res* 48(21):6121–6126
- Hosseini F, Shانهbandi D, Soleimanpour J, Yousefi B, Alemi F (2022) Melatonin increases the sensitivity of osteosarcoma cells to chemotherapy drug cisplatin. *Drug Res (Stuttg)* 72(6): 312–318. <https://doi.org/10.1055/a-1830-8716>
- Huang Y, Yuan K, Tang M, Yue J, Bao L, Wu S, Zhang Y, Li Y, Wang Y, Ou X, Gou J, Zhao Q, Yuan L (2021) Melatonin inhibiting the survival of human gastric cancer cells under ER stress involving autophagy and Ras-Raf-MAPK signalling. *J Cell Mol Med* 25(3):1480–1492. <https://doi.org/10.1111/jcmm.16237>

- IARC Monographs Vol 124 group (2019) Carcinogenicity of night shift work. *Lancet Oncol* 20(8): 1058–1059. [https://doi.org/10.1016/S1470-2045\(19\)30455-3](https://doi.org/10.1016/S1470-2045(19)30455-3)
- Jadid MFS, Aghaei E, Taheri E, Seyyedsani N, Chavoshi R, Abbasi S, Khorrami A, Goleij P, Hajazimian S, Taefehshokr S, Isazadeh A, Shanehbandi D (2021) Melatonin increases the anticancer potential of doxorubicin in Caco-2 colorectal cancer cells. *Environ Toxicol* 36(6): 1061–1069. <https://doi.org/10.1002/tox.23105>
- Jardim-Perassi BV, Arbab AS, Ferreira LC, Borin TF, Varma NR, Iskander AS, Shankar A, Ali MM, de Campos Zuccari DA (2014) Effect of melatonin on tumor growth and angiogenesis in xenograft model of breast cancer. *PLoS One* 9(1):e85311. <https://doi.org/10.1371/journal.pone.0085311>
- Jardim-Perassi BV, Lourenco MR, Doho GM, Grigolo IH, Gelaleti GB, Ferreira LC, Borin TF, Moschetta MG, Pires de Campos Zuccari DA (2016) Melatonin regulates angiogenic factors under hypoxia in breast cancer cell lines. *Anti Cancer Agents Med Chem* 16(3):347–358. <https://doi.org/10.2174/1871520615666150511094201>
- Jetten AM (2009) Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. *Nucl Recept Signal* 7:e003. <https://doi.org/10.1621/nrs.07003>
- Jockers R, Delagrange P, Dubocovich ML, Markus RP, Renault N, Tosini G, Cecon E, Zlotos DP (2016) Update on melatonin receptors: IUPHAR review 20. *Br J Pharmacol* 173(18): 2702–2725. <https://doi.org/10.1111/bph.13536>
- Johnston DL, Zupanec S, Nicksy D, Morgenstern D, Narendran A, Deyell RJ, Samson Y, Wu B, Baruchel S (2019) Phase I dose-finding study for melatonin in pediatric oncology patients with relapsed solid tumors. *Pediatr Blood Cancer* 66(6):e27676. <https://doi.org/10.1002/pbc.27676>
- Jones RR (2021) Exposure to artificial light at night and risk of cancer: where do we go from here? *Br J Cancer* 124(9):1467–1468. <https://doi.org/10.1038/s41416-020-01231-7>
- Karasek M, Carrillo-Vico A, Guerrero JM, Winczyk K, Pawlikowski M (2002) Expression of melatonin MT(1) and MT(2) receptors, and ROR alpha(1) receptor in transplantable murine colon 38 cancer. *Neuro Endocrinol Lett* 23(Suppl 1):55–60
- Karin M, Greten FR (2005) NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 5(10):749–759. <https://doi.org/10.1038/nri1703>
- Kartini D, Taher A, Panigoro SS, Setiabudy R, Jusman SW, Haryana SM, Abdullah M, Rustamadji P, Purwanto DJ, Sutandyo N, Suroyo I, Siregar BH, Maruli H, Sungkar S (2020) Effect of melatonin supplementation in combination with neoadjuvant chemotherapy to miR-210 and CD44 expression and clinical response improvement in locally advanced oral squamous cell carcinoma: a randomized controlled trial. *J Egypt Natl Canc Inst* 32(1):12. <https://doi.org/10.1186/s43046-020-0021-0>
- Kartini D, Taher A, Panigoro SS, Setiabudy R, Jusman SW, Haryana SM, Murdani A, Rustamadji P, Karisyah A, Rasyid SH (2021) Melatonin effect on hypoxia inducible factor-1alpha and clinical response in patients with oral squamous cell carcinoma receiving neoadjuvant chemotherapy: a randomized controlled trial. *J Carcinog* 20:5. https://doi.org/10.4103/jcar.JCar_19_20
- Karvan S, Sadeghi A, Farrokhi P, Nekouee A, Sharifi M, Moghaddas A (2022) Melatonin in the prevention of cisplatin-induced acute nephrotoxicity: a randomized, controlled clinical trial. *Res Pharm Sci* 17(2):176–188. <https://doi.org/10.4103/1735-5362.335176>
- Kilic U, Kilic E, Tuzcu Z, Tuzcu M, Ozercan IH, Yilmaz O, Sahin F, Sahin K (2013) Melatonin suppresses cisplatin-induced nephrotoxicity via activation of Nrf-2/HO-1 pathway. *Nutr Metab (Lond)* 10(1):7. <https://doi.org/10.1186/1743-7075-10-7>
- Kim KJ, Choi JS, Kang I, Kim KW, Jeong CH, Jeong JW (2013) Melatonin suppresses tumor progression by reducing angiogenesis stimulated by HIF-1 in a mouse tumor model. *J Pineal Res* 54(3):264–270. <https://doi.org/10.1111/j.1600-079X.2012.01030.x>
- Kim HS, Kim TJ, Yoo YM (2014) Melatonin combined with endoplasmic reticulum stress induces cell death via the PI3K/Akt/mTOR pathway in B16F10 melanoma cells. *PLoS One* 9(3): e92627. <https://doi.org/10.1371/journal.pone.0092627>

- Kimball SR, Abbas A, Jefferson LS (2008) Melatonin represses oxidative stress-induced activation of the MAP kinase and mTOR signaling pathways in H4IIE hepatoma cells through inhibition of Ras. *J Pineal Res* 44(4):379–386. <https://doi.org/10.1111/j.1600-079X.2007.00539.x>
- Knudson AG (2002) Cancer genetics. *Am J Med Genet* 111(1):96–102. <https://doi.org/10.1002/ajmg.10320>
- Korkmaz A, Tamura H, Manchester LC, Ogden GB, Tan DX, Reiter RJ (2009) Combination of melatonin and a peroxisome proliferator-activated receptor-gamma agonist induces apoptosis in a breast cancer cell line. *J Pineal Res* 46(1):115–116. <https://doi.org/10.1111/j.1600-079X.2008.00635.x>
- Kosar PA, Naziroglu M, Ovey IS, Cig B (2016) Synergic effects of doxorubicin and melatonin on apoptosis and mitochondrial oxidative stress in MCF-7 breast cancer cells: involvement of TRPV1 channels. *J Membr Biol* 249(1–2):129–140. <https://doi.org/10.1007/s00232-015-9855-0>
- Kuil LE, Varkevisser T, Huisman MH, Jansen M, Bunt J, Compter A, Ket H, Schagen SB, Meeteren A, Partanen M (2023) Artificial and natural interventions for chemotherapy- and / or radiotherapy-induced cognitive impairment: a systematic review of animal studies. *Neurosci Biobehav Rev* 157:105514. <https://doi.org/10.1016/j.neubiorev.2023.105514>
- Kulsoom K, Ali W, Saba ZH, Hussain S, Zahra S, Sadia SH, Irshad M, Yasin M, Ramzan MS (2023) Revealing melatonin's mysteries: receptors, signaling pathways, and therapeutics applications. *Horm Metab Res*. <https://doi.org/10.1055/a-2226-3971>
- Kvietkauskas M, Zitkute V, Leber B, Strupas K, Stiegler P, Schemmer P (2020) The role of melatonin in colorectal cancer treatment: a comprehensive review. *Ther Adv Med Oncol* 12: 1–14. <https://doi.org/10.1177/1758835920931714>
- Lee JH, Yoon YM, Han YS, Yun CW, Lee SH (2018) Melatonin promotes apoptosis of oxaliplatin-resistant colorectal cancer cells through inhibition of cellular prion protein. *Anticancer Res* 38(4):1993–2000. <https://doi.org/10.21873/anticancer.12437>
- Legros C, Devavry S, Caignard S, Tessier C, Delagrangé P, Ouvre C, Boutin JA, Nosjean O (2014) Melatonin MT(1) and MT(2) receptors display different molecular pharmacologies only in the G-protein coupled state. *Br J Pharmacol* 171(1):186–201. <https://doi.org/10.1111/bph.12457>
- Legros C, Dupuis P, Ferry G, Boutin JA (2022) Measuring binding at the putative melatonin receptor MT3. *Methods Mol Biol* 2550:283–289. https://doi.org/10.1007/978-1-0716-2593-4_30
- Li W, Fan M, Chen Y, Zhao Q, Song C, Yan Y, Jin Y, Huang Z, Lin C, Wu J (2015) Melatonin induces cell apoptosis in AGS cells through the activation of JNK and P38 MAPK and the suppression of nuclear factor-kappa B: a novel therapeutic implication for gastric cancer. *Cell Physiol Biochem* 37(6):2323–2338. <https://doi.org/10.1159/000438587>
- Li L, Lin M, Luo J, Sun H, Zhang Z, Lin D, Chen L, Feng S, Lin X, Zhou R, Song J (2024) Loss of keratin 23 enhances growth inhibitory effect of melatonin in gastric cancer. *Mol Med Rep* 29(2). <https://doi.org/10.3892/mmr.2023.13145>
- Liao Y, Gao Y, Chang A, Li Z, Wang H, Cao J, Gu W, Tang R (2020) Melatonin synergizes BRAF-targeting agent dabrafenib for the treatment of anaplastic thyroid cancer by inhibiting AKT/hTERT signalling. *J Cell Mol Med* 24(20):12119–12130. <https://doi.org/10.1111/jcmm.15854>
- Lissoni P, Barni S, Ardizzoia A, Paolorossi F, Crispino S, Tancini G, Tisi E, Archili C, De Toma D, Pipino G et al (1992) Randomized study with the pineal hormone melatonin versus supportive care alone in advanced nonsmall cell lung cancer resistant to a first-line chemotherapy containing cisplatin. *Oncology* 49(5):336–339. <https://doi.org/10.1159/000227068>
- Lissoni P, Meregalli S, Fossati V, Paolorossi F, Barni S, Tancini G, Frigerio F (1994) A randomized study of immunotherapy with low-dose subcutaneous interleukin-2 plus melatonin vs chemotherapy with cisplatin and etoposide as first-line therapy for advanced non-small cell lung cancer. *Tumori* 80(6):464–467. <https://doi.org/10.1177/030089169408000611>
- Lissoni P, Ardizzoia A, Barni S, Paolorossi F, Tancini G, Meregalli S, Esposti D, Zubelewicz B, Braczowski R (1995) A randomized study of tamoxifen alone versus tamoxifen plus melatonin in estrogen receptor-negative heavily pretreated metastatic breast-cancer patients. *Oncol Rep* 2(5):871–873. <https://doi.org/10.3892/or.2.5.871>

- Lissoni P, Paolorossi F, Ardizzoia A, Barni S, Chilelli M, Mancuso M, Tancini G, Conti A, Maestroni GJ (1997a) A randomized study of chemotherapy with cisplatin plus etoposide versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state. *J Pineal Res* 23(1):15–19. <https://doi.org/10.1111/j.1600-079x.1997.tb00329.x>
- Lissoni P, Tancini G, Barni S, Paolorossi F, Ardizzoia A, Conti A, Maestroni G (1997b) Treatment of cancer chemotherapy-induced toxicity with the pineal hormone melatonin. *Support Care Cancer* 5(2):126–129. <https://doi.org/10.1007/BF01262569>
- Lissoni P, Barni S, Mandala M, Ardizzoia A, Paolorossi F, Vaghi M, Longarini R, Malugani F, Tancini G (1999) Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumour patients with poor clinical status. *Eur J Cancer* 35(12):1688–1692. [https://doi.org/10.1016/s0959-8049\(99\)00159-8](https://doi.org/10.1016/s0959-8049(99)00159-8)
- Lissoni P, Rovelli F, Malugani F, Bucovec R, Conti A, Maestroni GJ (2001) Anti-angiogenic activity of melatonin in advanced cancer patients. *Neuro Endocrinol Lett* 22(1):45–47
- Lissoni P, Chilelli M, Villa S, Cerizza L, Tancini G (2003a) Five years survival in metastatic non-small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial. *J Pineal Res* 35(1):12–15. <https://doi.org/10.1034/j.1600-079x.2003.00032.x>
- Lissoni P, Malugani F, Bukovec R, Bordin V, Perego M, Mengo S, Ardizzoia A, Tancini G (2003b) Reduction of cisplatin-induced anemia by the pineal indole 5-methoxytryptamine in metastatic lung cancer patients. *Neuro Endocrinol Lett* 24(1–2):83–85
- Lissoni P, Messina G, Rovelli F, Brivio F, Di Fede G (2018) Dose-dependency of antitumor effects of the pineal hormone melatonin in untreatable metastatic solid tumor patients. *Int J Immunol Immunobiol* 1(1). <https://doi.org/10.31021/ijii.20181104>
- Liu L, Zhu Y, Xu Y, Reiter RJ (2011) Melatonin delays cell proliferation by inducing G1 and G2/M phase arrest in a human osteoblastic cell line hFOB 1.19. *J Pineal Res* 50(2):222–231. <https://doi.org/10.1111/j.1600-079X.2010.00832.x>
- Liu L, Zhu Y, Xu Y, Reiter RJ (2012) Prevention of ERK activation involves melatonin-induced G (1) and G(2)/M phase arrest in the human osteoblastic cell line hFOB 1.19. *J Pineal Res* 53(1): 60–66. <https://doi.org/10.1111/j.1600-079X.2011.00971.x>
- Liu L, Xu Y, Reiter RJ (2013) Melatonin inhibits the proliferation of human osteosarcoma cell line MG-63. *Bone* 55(2):432–438. <https://doi.org/10.1016/j.bone.2013.02.021>
- Liu S, Liang B, Jia H, Jiao Y, Pang Z, Huang Y (2017) Evaluation of cell death pathways initiated by antitumor drugs melatonin and valproic acid in bladder cancer cells. *FEBS Open Bio* 7(6): 798–810. <https://doi.org/10.1002/2211-5463.12223>
- Liu D, Shi K, Fu M, Chen F (2021) Melatonin indirectly decreases gastric cancer cell proliferation and invasion via effects on cancer-associated fibroblasts. *Life Sci* 277:119497. <https://doi.org/10.1016/j.lfs.2021.119497>
- Lo Sardo F, Muti P, Blandino G, Strano S (2017) Melatonin and hippo pathway: is there existing cross-talk? *Int J Mol Sci* 18(9). <https://doi.org/10.3390/ijms18091913>
- Lozano A, Marruecos J, Rubio J, Farre N, Gomez-Millan J, Morera R, Planas I, Lanzuela M, Vazquez-Masedo MG, Cascallar L, Giral J, Escames G, Valenti V, Grima P, Bosser R, Tarrago C, Mesia R (2021) Randomized placebo-controlled phase II trial of high-dose melatonin mucoadhesive oral gel for the prevention and treatment of oral mucositis in patients with head and neck cancer undergoing radiation therapy concurrent with systemic treatment. *Clin Transl Oncol* 23(9):1801–1810. <https://doi.org/10.1007/s12094-021-02586-w>
- Lu YX, Chen DL, Wang DS, Chen LZ, Mo HY, Sheng H, Bai L, Wu QN, Yu HE, Xie D, Yun JP, Zeng ZL, Wang F, Ju HQ, Xu RH (2016) Melatonin enhances sensitivity to fluorouracil in oesophageal squamous cell carcinoma through inhibition of Erk and Akt pathway. *Cell Death Dis* 7(10):e2432. <https://doi.org/10.1038/cddis.2016.330>
- Lu KH, Lin RC, Yang JS, Yang WE, Reiter RJ, Yang SF (2019) Molecular and cellular mechanisms of melatonin in osteosarcoma. *Cells* 8(12). <https://doi.org/10.3390/cells8121618>

- Luchetti F, Carloni S, Nasoni MG, Reiter RJ, Balduini W (2023) Melatonin, tunneling nanotubes, mesenchymal cells, and tissue regeneration. *Neural Regen Res* 18(4):760–762. <https://doi.org/10.4103/1673-5374.353480>
- Ma H, Kang J, Fan W, He H, Huang F (2021) ROR: nuclear receptor for melatonin or not? *Molecules* 26(9). <https://doi.org/10.3390/molecules26092693>
- Ma Y, Ma J, Lu L, Xiong X, Shao Y, Ren J, Yang J, Liu J (2023) Melatonin restores autophagic flux by activating the Sirt3/TFEB signaling pathway to attenuate doxorubicin-induced cardiomyopathy. *Antioxidants (Basel)* 12(9). <https://doi.org/10.3390/antiox12091716>
- Mafi A, Rismanchi H, Gholinezhad Y, Mohammadi MM, Mousavi V, Hosseini SA, Milasi YE, Reiter RJ, Ghezelbash B, Rezaee M, Sheida A, Zarepour F, Asemi Z, Mansournia MA, Mirzaei H (2023) Melatonin as a regulator of apoptosis in leukaemia: molecular mechanism and therapeutic perspectives. *Front Pharmacol* 14:1224151. <https://doi.org/10.3389/fphar.2023.1224151>
- Maleki Dana P, Sadoughi F, Reiter RJ, Mohammadi S, Heidar Z, Mirzamoradi M, Asemi Z (2022) Melatonin as an adjuvant treatment modality with doxorubicin. *Biochimie* 202:49–55. <https://doi.org/10.1016/j.biochi.2022.06.007>
- Maleki M, Khelghati N, Alemi F, Younesi S, Asemi Z, Abolhasan R, Bazdar M, Samadi-Kafil H, Yousefi B (2021) Multiple interactions between melatonin and non-coding RNAs in cancer biology. *Chem Biol Drug Des* 98(3):323–340. <https://doi.org/10.1111/cbdd.13849>
- Mao L, Cheng Q, Guardiola-Lemaitre B, Schuster-Klein C, Dong C, Lai L, Hill SM (2010) In vitro and in vivo antitumor activity of melatonin receptor agonists. *J Pineal Res* 49(3):210–221. <https://doi.org/10.1111/j.1600-079X.2010.00781.x>
- Martin-Renedo J, Mauriz JL, Jorquera F, Ruiz-Andres O, Gonzalez P, Gonzalez-Gallego J (2008) Melatonin induces cell cycle arrest and apoptosis in hepatocarcinoma HepG2 cell line. *J Pineal Res* 45(4):532–540. <https://doi.org/10.1111/j.1600-079X.2008.00641.x>
- Melillo G (2006) Inhibiting hypoxia-inducible factor 1 for cancer therapy. *Mol Cancer Res* 4(9): 601–605. <https://doi.org/10.1158/1541-7786.MCR-06-0235>
- Meng Z, Moroishi T, Guan KL (2016) Mechanisms of hippo pathway regulation. *Genes Dev* 30(1): 1–17. <https://doi.org/10.1101/gad.274027.115>
- Milicevic Z, Vujic D, Isakovic K, Micic M, Milicevic NM (1986) Involution of bursa of fabricius in male and female chickens: a light microscopic histoquantitative study. *Poult Sci* 65(12): 2318–2323. <https://doi.org/10.3382/ps.0652318>
- Mills E, Wu P, Seely D, Guyatt G (2005) Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis. *J Pineal Res* 39(4):360–366. <https://doi.org/10.1111/j.1600-079X.2005.00258.x>
- Minocha T, Das M, Rai V, Verma SS, Awasthee N, Gupta SC, Haldar C, Yadav SK (2022) Melatonin induces apoptosis and cell cycle arrest in cervical cancer cells via inhibition of NF-kappaB pathway. *Inflammopharmacology* 30(4):1411–1429. <https://doi.org/10.1007/s10787-022-00964-6>
- Moradi M, Goodarzi N, Faramarzi A, Cheraghi H, Hashemian AH, Jalili C (2021) Melatonin protects rats testes against bleomycin, etoposide, and cisplatin-induced toxicity via mitigating nitro-oxidative stress and apoptosis. *Biomed Pharmacother* 138:111481. <https://doi.org/10.1016/j.biopha.2021.111481>
- Motilva V, Garcia-Maurino S, Talero E, Illanes M (2011) New paradigms in chronic intestinal inflammation and colon cancer: role of melatonin. *J Pineal Res* 51(1):44–60. <https://doi.org/10.1111/j.1600-079X.2011.00915.x>
- Mukhopadhyay ND, Khorasanchi A, Pandey S, Nemani S, Parker G, Deng X, Arthur DW, Urdaneta A, Del Fabbro E (2023) Melatonin supplementation for cancer-related fatigue in patients with early stage breast cancer receiving radiotherapy: a double-blind placebo-controlled trial. *Oncologist*. <https://doi.org/10.1093/oncolo/oyad250>
- Nabih HK, Hamed AR, Yahya SMM (2023) Anti-proliferative effect of melatonin in human hepatoma HepG2 cells occurs mainly through cell cycle arrest and inflammation inhibition. *Sci Rep* 13(1):4396. <https://doi.org/10.1038/s41598-023-31443-9>

- Najafi M, Hooshangi Shayesteh MR, Mortezaee K, Farhood B, Haghi-Aminjan H (2020) The role of melatonin on doxorubicin-induced cardiotoxicity: a systematic review. *Life Sci* 241:117173. <https://doi.org/10.1016/j.lfs.2019.117173>
- Novotny C, Svobodova K, Benada O, Kofronova O, Heissenberger A, Fuchs W (2011) Potential of combined fungal and bacterial treatment for color removal in textile wastewater. *Bioresour Technol* 102(2):879–888. <https://doi.org/10.1016/j.biortech.2010.09.014>
- Oh A, Pardo M, Rodriguez A, Yu C, Nguyen L, Liang O, Chorzalska A, Dubielecka PM (2023) NF-kappaB signaling in neoplastic transition from epithelial to mesenchymal phenotype. *Cell Commun Signal* 21(1):291. <https://doi.org/10.1186/s12964-023-01207-z>
- Otto T, Sicinski P (2017) Cell cycle proteins as promising targets in cancer therapy. *Nat Rev Cancer* 17(2):93–115. <https://doi.org/10.1038/nrc.2016.138>
- Palmer ACS, Zortea M, Souza A, Santos V, Biazus JV, Torres ILS, Fregni F, Caumo W (2020) Clinical impact of melatonin on breast cancer patients undergoing chemotherapy; effects on cognition, sleep and depressive symptoms: a randomized, double-blind, placebo-controlled trial. *PLoS One* 15(4):e0231379. <https://doi.org/10.1371/journal.pone.0231379>
- Panchenko AV, Tyndyk ML, Maydin MA, Balduva IA, Artemyeva AS, Kruglov SS, Kireeva GS, Golubev AG, Belyaev AM, Anisimov VN (2020) Melatonin administered before or after a cytotoxic drug increases mammary cancer stabilization rates in HER2/neu mice. *Chemotherapy* 65(1–2):42–50. <https://doi.org/10.1159/000509238>
- Pandi-Perumal SR, Trakht I, Srinivasan V, Spence DW, Maestroni GJ, Zisapel N, Cardinali DP (2008) Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. *Prog Neurobiol* 85(3):335–353. <https://doi.org/10.1016/j.pneurobio.2008.04.001>
- Pariente R, Bejarano I, Espino J, Rodriguez AB, Pariente JA (2017) Participation of MT3 melatonin receptors in the synergistic effect of melatonin on cytotoxic and apoptotic actions evoked by chemotherapeutics. *Cancer Chemother Pharmacol* 80(5):985–998. <https://doi.org/10.1007/s00280-017-3441-3>
- Pariente R, Bejarano I, Rodriguez AB, Pariente JA, Espino J (2018) Melatonin increases the effect of 5-fluorouracil-based chemotherapy in human colorectal adenocarcinoma cells in vitro. *Mol Cell Biochem* 440(1–2):43–51. <https://doi.org/10.1007/s11010-017-3154-2>
- Park SY, Jang WJ, Yi EY, Jang JY, Jung Y, Jeong JW, Kim YJ (2010) Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. *J Pineal Res* 48(2):178–184. <https://doi.org/10.1111/j.1600-079x.2009.00742.x>
- Paroni R, Terraneo L, Bonomini F, Finati E, Virgili E, Bianciardi P, Favero G, Frascini F, Reiter RJ, Rezzani R, Samaja M (2014) Antitumour activity of melatonin in a mouse model of human prostate cancer: relationship with hypoxia signalling. *J Pineal Res* 57(1):43–52. <https://doi.org/10.1111/jpi.12142>
- Pavitra E, Kancharla J, Gupta VK, Prasad K, Sung JY, Kim J, Tej MB, Choi R, Lee JH, Han YK, Raju GSR, Bhaskar L, Huh YS (2023) The role of NF-kappaB in breast cancer initiation, growth, metastasis, and resistance to chemotherapy. *Biomed Pharmacother* 163:114822. <https://doi.org/10.1016/j.biopha.2023.114822>
- Pavlos NJ, Friedman PA (2017) GPCR signaling and trafficking: the long and short of it. *Trends Endocrinol Metab* 28(3):213–226. <https://doi.org/10.1016/j.tem.2016.10.007>
- Perdomo J, Cabrera J, Estevez F, Loro J, Reiter RJ, Quintana J (2013) Melatonin induces apoptosis through a caspase-dependent but reactive oxygen species-independent mechanism in human leukemia Molt-3 cells. *J Pineal Res* 55(2):195–206. <https://doi.org/10.1111/jpi.12062>
- Peyressat M, Prevel C, Pellerano M, Morris MC (2015) Targeting cyclin-dependent kinases in human cancers: from small molecules to peptide inhibitors. *Cancers (Basel)* 7(1):179–237. <https://doi.org/10.3390/cancers7010179>
- Pizarro JG, Yeste-Velasco M, Esparza JL, Verdaguer E, Pallas M, Camins A, Folch J (2008) The antiproliferative activity of melatonin in B65 rat dopaminergic neuroblastoma cells is related to the downregulation of cell cycle-related genes. *J Pineal Res* 45(1):8–16. <https://doi.org/10.1111/j.1600-079X.2007.00548.x>

- Plaimee P, Weerapreeyakul N, Barusrux S, Johns NP (2015) Melatonin potentiates cisplatin-induced apoptosis and cell cycle arrest in human lung adenocarcinoma cells. *Cell Prolif* 48(1): 67–77. <https://doi.org/10.1111/cpr.12158>
- Porta C, Paglino C, Mosca A (2014) Targeting PI3K/Akt/mTOR signaling in cancer. *Front Oncol* 4: 64. <https://doi.org/10.3389/fonc.2014.00064>
- Praus F, Kunstner A, Sauer T, Kohl M, Kern K, Deichmann S, Vegvari A, Keck T, Busch H, Habermann JK, Gemoll T (2023) Panomics reveals patient individuality as the major driver of colorectal cancer progression. *J Transl Med* 21(1):41. <https://doi.org/10.1186/s12967-022-03855-0>
- Prieto-Dominguez N, Mendez-Blanco C, Carbajo-Pescador S, Fondevila F, Garcia-Palomo A, Gonzalez-Gallego J, Mauriz JL (2017) Melatonin enhances sorafenib actions in human hepatocarcinoma cells by inhibiting mTORC1/p70S6K/HIF-1 α and hypoxia-mediated mitophagy. *Oncotarget* 8(53):91402–91414. <https://doi.org/10.18632/oncotarget.20592>
- Proietti S, Cucina A, Dobrowolny G, D'Anselmi F, Dinicola S, Masiello MG, Pasqualato A, Palombo A, Morini V, Reiter RJ, Bizzarri M (2014) Melatonin down-regulates MDM2 gene expression and enhances p53 acetylation in MCF-7 cells. *J Pineal Res* 57(1):120–129. <https://doi.org/10.1111/jpi.12150>
- Qin W, Lu W, Li H, Yuan X, Li B, Zhang Q, Xiu R (2012) Melatonin inhibits IL1 β -induced MMP9 expression and activity in human umbilical vein endothelial cells by suppressing NF- κ B activation. *J Endocrinol* 214(2):145–153. <https://doi.org/10.1530/JOE-12-0147>
- Rafiiyan M, Davoodvandi A, Reiter RJ, Mansournia MA, Rasooli Manesh SM, Arabshahi V, Asemi Z (2023) Melatonin and cisplatin co-treatment against cancer: a mechanistic review of their synergistic effects and melatonin's protective actions. *Pathol Res Pract* 253:155031. <https://doi.org/10.1016/j.prp.2023.155031>
- Ramos E, Egea J, Lopez-Munoz F, Gil-Martin E, Romero A (2023) Therapeutic potential of melatonin counteracting chemotherapy-induced toxicity in breast cancer patients: a systematic review. *Pharmaceutics* 15(6). <https://doi.org/10.3390/pharmaceutics15061616>
- Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* 22(3):659–661. <https://doi.org/10.1096/fj.07-9574LSF>
- Rodrigues TD, Lima KR, Uggioni MLR, Ferraz SD, Cardoso HS, Colonetti T, da Rosa MI (2022) Effectiveness of melatonin adjuvant treatment in cisplatin to prevent depletion of ovarian follicles in mice: systematic reviewdagger. *Biol Reprod* 107(6):1386–1394. <https://doi.org/10.1093/biolre/iaoc164>
- Rodriguez C, Martin V, Herrera F, Garcia-Santos G, Rodriguez-Blanco J, Casado-Zapico S, Sanchez-Sanchez AM, Suarez S, Puente-Moncada N, Anitua MJ, Antolin I (2013) Mechanisms involved in the pro-apoptotic effect of melatonin in cancer cells. *Int J Mol Sci* 14(4):6597–6613. <https://doi.org/10.3390/ijms14046597>
- Romani AMP (2022) Cisplatin in cancer treatment. *Biochem Pharmacol* 206:115323. <https://doi.org/10.1016/j.bcp.2022.115323>
- Roskoski R Jr (2007) Vascular endothelial growth factor (VEGF) signaling in tumor progression. *Crit Rev Oncol Hematol* 62(3):179–213. <https://doi.org/10.1016/j.critrevonc.2007.01.006>
- Rubio S, Estevez F, Cabrera J, Reiter RJ, Loro J, Quintana J (2007) Inhibition of proliferation and induction of apoptosis by melatonin in human myeloid HL-60 cells. *J Pineal Res* 42(2): 131–138. <https://doi.org/10.1111/j.1600-079X.2006.00392.x>
- Saeidnia S, Abdollahi M (2013) Toxicological and pharmacological concerns on oxidative stress and related diseases. *Toxicol Appl Pharmacol* 273(3):442–455. <https://doi.org/10.1016/j.taap.2013.09.031>
- Sakatani A, Sonohara F, Goel A (2019) Melatonin-mediated downregulation of thymidylate synthase as a novel mechanism for overcoming 5-fluorouracil associated chemoresistance in colorectal cancer cells. *Carcinogenesis* 40(3):422–431. <https://doi.org/10.1093/carcin/bgy186>
- Sanchez DI, Gonzalez-Fernandez B, San-Miguel B, de Urbina JO, Crespo I, Gonzalez-Gallego J, Tunon MJ (2017) Melatonin prevents deregulation of the sphingosine kinase/sphingosine 1-phosphate signaling pathway in a mouse model of diethylnitrosamine-induced hepatocellular carcinoma. *J Pineal Res* 62(1). <https://doi.org/10.1111/jpi.12369>

- Sanchez-Hidalgo M, Lee M, de la Lastra CA, Guerrero JM, Packham G (2012) Melatonin inhibits cell proliferation and induces caspase activation and apoptosis in human malignant lymphoid cell lines. *J Pineal Res* 53(4):366–373. <https://doi.org/10.1111/j.1600-079X.2012.01006.x>
- Sandhu C, Qureshi A, Emili A (2018) Panomics for precision medicine. *Trends Mol Med* 24(1): 85–101. <https://doi.org/10.1016/j.molmed.2017.11.001>
- Santo L, Siu KT, Raje N (2015) Targeting cyclin-dependent kinases and cell cycle progression in human cancers. *Semin Oncol* 42(6):788–800. <https://doi.org/10.1053/j.seminoncol.2015.09.024>
- Sawe J, Svensson JO, Odar-Cederlof I (1985) Kinetics of morphine in patients with renal failure. *Lancet* 2(8448):211. [https://doi.org/10.1016/s0140-6736\(85\)91520-x](https://doi.org/10.1016/s0140-6736(85)91520-x)
- Sedighi Pashaki A, Mohammadian K, Afshar S, Gholami MH, Moradi A, Javadinia SA, Keshtpour Amlashi Z (2021) A randomized, controlled, parallel-group, trial on the effects of melatonin on fatigue associated with breast cancer and its adjuvant treatments. *Integr Cancer Ther* 20: 1534735420988343. <https://doi.org/10.1177/1534735420988343>
- Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF (1983) Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* 219(4587): 983–985. <https://doi.org/10.1126/science.6823562>
- Shen CJ, Chang CC, Chen YT, Lai CS, Hsu YC (2016) Melatonin suppresses the growth of ovarian cancer cell lines (OVCAR-429 and PA-1) and potentiates the effect of G1 arrest by targeting CDKs. *Int J Mol Sci* 17(2). <https://doi.org/10.3390/ijms17020176>
- Silva KMR, Franca DCH, de Queiroz AA, Fagundes-Triches DLG, de Marchi PGF, Morais TC, Honorio-Franca AC, Franca EL (2023) Polarization of melatonin-modulated colostrum macrophages in the presence of breast tumor cell lines. *Int J Mol Sci* 24(15). <https://doi.org/10.3390/ijms241512400>
- Sirichoat A, Krutsri S, Suwannakot K, Aranarochana A, Chaisawang P, Pannangrong W, Wigmore P, Welbat JU (2019) Melatonin protects against methotrexate-induced memory deficit and hippocampal neurogenesis impairment in a rat model. *Biochem Pharmacol* 163:225–233. <https://doi.org/10.1016/j.bcp.2019.02.010>
- Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT (2012) Melatonin membrane receptors in peripheral tissues: distribution and functions. *Mol Cell Endocrinol* 351(2):152–166. <https://doi.org/10.1016/j.mce.2012.01.004>
- Soderquist F, Hellstrom PM, Cunningham JL (2015) Human gastroenteropancreatic expression of melatonin and its receptors MT1 and MT2. *PLoS One* 10(3):e0120195. <https://doi.org/10.1371/journal.pone.0120195>
- Sohn EJ, Won G, Lee J, Lee S, Kim SH (2015) Upregulation of miRNA3195 and miRNA374b mediates the anti-angiogenic properties of melatonin in hypoxic PC-3 prostate cancer cells. *J Cancer* 6(1):19–28. <https://doi.org/10.7150/jca.9591>
- Soliman A, Wahid A, Wahby MM, Bassiouny A (2020) Study of the possible synergistic protective effects of Melatonin and Pregabalin in vincristine induced peripheral neuropathy Wistar albino rats. *Life Sci* 244:117095. <https://doi.org/10.1016/j.lfs.2019.117095>
- Song Y, Wang S (2023) Melatonin synergistically enhances docetaxel induced endoplasmic reticulum stress to promote apoptosis by suppressing NF-kappaB activation in cervical cancer. *Med Oncol* 40(8):219. <https://doi.org/10.1007/s12032-023-02087-6>
- Song J, Ma SJ, Luo JH, Zhang H, Wang RX, Liu H, Li L, Zhang ZG, Zhou RX (2018) Melatonin induces the apoptosis and inhibits the proliferation of human gastric cancer cells via blockade of the AKT/MDM2 pathway. *Oncol Rep* 39(4):1975–1983. <https://doi.org/10.3892/or.2018.6282>
- Sookprasert A, Johns NP, Phunmanee A, Pongthai P, Cheawchanwattana A, Johns J, Konsil J, Plaimee P, Porasuphatana S, Jitpimolmard S (2014) Melatonin in patients with cancer receiving chemotherapy: a randomized, double-blind, placebo-controlled trial. *Anticancer Res* 34(12): 7327–7337
- Su SC, Hsieh MJ, Yang WE, Chung WH, Reiter RJ, Yang SF (2017) Cancer metastasis: mechanisms of inhibition by melatonin. *J Pineal Res* 62(1). <https://doi.org/10.1111/jpi.12370>
- Subramanian P, Mirunalini S, Dakshayani KB, Pandi-Perumal SR, Trakht I, Cardinali DP (2007) Prevention by melatonin of hepatocarcinogenesis in rats injected with N-nitrosodiethylamine. *J Pineal Res* 43(3):305–312. <https://doi.org/10.1111/j.1600-079X.2007.00478.x>

- Sun Y, Liu WZ, Liu T, Feng X, Yang N, Zhou HF (2015) Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. *J Recept Signal Transduct Res* 35(6):600–604. <https://doi.org/10.3109/10799893.2015.1030412>
- Sun Q, Zhang J, Li X, Yang G, Cheng S, Guo D, Zhang Q, Sun F, Zhao F, Yang D, Wang S, Wang T, Liu S, Zou L, Zhang Y, Liu H (2022a) The ubiquitin-specific protease 8 antagonizes melatonin-induced endocytic degradation of MT(1) receptor to promote lung adenocarcinoma growth. *J Adv Res* 41:1–12. <https://doi.org/10.1016/j.jare.2022.01.015>
- Sun T, Wang D, Wang B, Liu X, Li N, Shi K (2022b) Melatonin attenuates cisplatin-induced acute kidney injury in mice: involvement of PPARalpha and fatty acid oxidation. *Food Chem Toxicol* 163:112970. <https://doi.org/10.1016/j.fct.2022.112970>
- Sung GJ, Kim SH, Kwak S, Park SH, Song JH, Jung JH, Kim H, Choi KC (2019) Inhibition of TFEB oligomerization by co-treatment of melatonin with vorinostat promotes the therapeutic sensitivity in glioblastoma and glioma stem cells. *J Pineal Res* 66(3):e12556. <https://doi.org/10.1111/jpi.12556>
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71(3):209–249. <https://doi.org/10.3322/caac.21660>
- Talib WH (2020) A ketogenic diet combined with melatonin overcomes cisplatin and vincristine drug resistance in breast carcinoma syngraft. *Nutrition* 72:110659. <https://doi.org/10.1016/j.nut.2019.110659>
- Talib WH, Alsayed AR, Abuawad A, Daoud S, Mahmod AI (2021) Melatonin in cancer treatment: current knowledge and future opportunities. *Molecules* 26(9). <https://doi.org/10.3390/molecules26092506>
- Targhazeh N, Hutt KJ, Winship AL, Reiter R, Yousefi B (2022a) Melatonin as an oncstatic agent: review of the modulation of tumor microenvironment and overcoming multidrug resistance. *Biochimie* 202:71–84. <https://doi.org/10.1016/j.biochi.2022.09.010>
- Targhazeh N, Reiter RJ, Rahimi M, Qujeq D, Yousefi T, Shahavi MH, Mir SM (2022b) Oncstatic activities of melatonin: roles in cell cycle, apoptosis, and autophagy. *Biochimie* 202:34–48. <https://doi.org/10.1016/j.biochi.2022.06.008>
- Tascioglu Aliyev A, Panieri E, Stepanic V, Gurer-Orhan H, Saso L (2021) Involvement of NRF2 in breast cancer and possible therapeutical role of polyphenols and melatonin. *Molecules* 26(7). <https://doi.org/10.3390/molecules26071853>
- (2022) The ‘war on cancer’ isn’t yet won. *Nature* 601(7893): 297. <https://doi.org/10.1038/d41586-022-00109-3>
- Tian T, Li J, Li Y, Lu YX, Tang YL, Wang H, Zheng F, Shi D, Long Q, Chen M, Garcia-Manero G, Hu Y, Qin L, Deng W (2019) Melatonin enhances sorafenib-induced cytotoxicity in FLT3-ITD acute myeloid leukemia cells by redox modification. *Theranostics* 9(13):3768–3779. <https://doi.org/10.7150/thno.34327>
- Tordjman S, Chokron S, Delorme R, Charrier A, Bellissant E, Jaafari N, Fougere C (2017) Melatonin: pharmacology, functions and therapeutic benefits. *Curr Neuropharmacol* 15(3): 434–443. <https://doi.org/10.2174/1570159X14666161228122115>
- Vermeulen K, Van Bockstaele DR, Berneman ZN (2003) The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell Prolif* 36(3):131–149. <https://doi.org/10.1046/j.1365-2184.2003.00266.x>
- Viallard C, Larrivee B (2017) Tumor angiogenesis and vascular normalization: alternative therapeutic targets. *Angiogenesis* 20(4):409–426. <https://doi.org/10.1007/s10456-017-9562-9>
- von Gall C, Stehle JH, Weaver DR (2002) Mammalian melatonin receptors: molecular biology and signal transduction. *Cell Tissue Res* 309(1):151–162. <https://doi.org/10.1007/s00441-002-0581-4>
- Vriend J, Reiter RJ (2015) The Keap1-Nrf2-antioxidant response element pathway: a review of its regulation by melatonin and the proteasome. *Mol Cell Endocrinol* 401:213–220. <https://doi.org/10.1016/j.mce.2014.12.013>

- Wang S, Liu Z, Wang L, Zhang X (2009) NF-kappaB signaling pathway, inflammation and colorectal cancer. *Cell Mol Immunol* 6(5):327–334. <https://doi.org/10.1038/cmi.2009.43>
- Wang RX, Liu H, Xu L, Zhang H, Zhou RX (2016) Melatonin downregulates nuclear receptor RZR/RORgamma expression causing growth-inhibitory and anti-angiogenesis activity in human gastric cancer cells in vitro and in vivo. *Oncol Lett* 12(2):897–903. <https://doi.org/10.3892/ol.2016.4729>
- Wang N, Li H, Zhu Y, Li N, Chen ZJ, Zhang C (2020) Melatonin protects against Epirubicin-induced ovarian damage. *J Reprod Dev* 66(1):19–27. <https://doi.org/10.1262/jrd.2019-085>
- Wang L, Wang C, Choi WS (2022a) Use of melatonin in cancer treatment: where are we? *Int J Mol Sci* 23(7). <https://doi.org/10.3390/ijms23073779>
- Wang Q, Zhang L, Xiao Y, Ghaemnezhad A (2022b) Diosgenin promotes cisplatin-induced apoptosis through oxidative DNA damage in A549 non-small cell lung cells. *Cell Biol Int* 46(10):1571–1576. <https://doi.org/10.1002/cbin.11862>
- WHO (World Health Organization) Fact sheet: Cancer (2023). <https://www.who.int/news-room/fact-sheets/detail/cancer>. Accessed 11 Nov 2023
- Winczyk K, Pawlikowski M, Guerrero JM, Karasek M (2002) Possible involvement of the nuclear RZR/ROR-alpha receptor in the antitumor action of melatonin on murine colon 38 cancer. *Tumour Biol* 23(5):298–302. <https://doi.org/10.1159/000068569>
- Witt-Enderby PA, Bennett J, Jarzynka MJ, Firestine S, Melan MA (2003) Melatonin receptors and their regulation: biochemical and structural mechanisms. *Life Sci* 72(20):2183–2198. [https://doi.org/10.1016/s0024-3205\(03\)00098-5](https://doi.org/10.1016/s0024-3205(03)00098-5)
- Wykoff CC, Sotiriou C, Cockman ME, Ratcliffe PJ, Maxwell P, Liu E, Harris AL (2004) Gene array of VHL mutation and hypoxia shows novel hypoxia-induced genes and that cyclin D1 is a VHL target gene. *Br J Cancer* 90(6):1235–1243. <https://doi.org/10.1038/sj.bjc.6601657>
- Xiao Y, Dong J (2023) Coming of age: targeting cyclin K in cancers. *Cells* 12(16). <https://doi.org/10.3390/cells12162044>
- Yun M, Kim EO, Lee D, Kim JH, Kim J, Lee H, Lee J, Kim SH (2014) Melatonin sensitizes H1975 non-small-cell lung cancer cells harboring a T790M-targeted epidermal growth factor receptor mutation to the tyrosine kinase inhibitor gefitinib. *Cell Physiol Biochem* 34(3):865–872. <https://doi.org/10.1159/000366305>
- Zamani M, Etebari M, Moradi SH (2020) The increment of genoprotective effect of melatonin due to "autoptotic" effect versus the genotoxicity of mitoxantrone. *J Biomed Phys Eng* 10(6):771–782. <https://doi.org/10.31661/jbpe.v0i0.508>
- Zemla A, Grzegorek I, Dziegiel P, Jablonska K (2017) Melatonin synergizes the chemotherapeutic effect of cisplatin in ovarian cancer cells independently of MT1 melatonin receptors. *In Vivo* 31(5):801–809. <https://doi.org/10.21873/in vivo.11133>
- Zhang J, Xie T, Zhong X, Jiang HL, Li R, Wang BY, Huang XT, Cen BH, Yuan YW (2020) Melatonin reverses nasopharyngeal carcinoma cisplatin chemoresistance by inhibiting the Wnt/beta-catenin signaling pathway. *Aging (Albany NY)* 12(6):5423–5438. <https://doi.org/10.18632/aging.102968>
- Zhang J, Fang Y, Tang D, Xu X, Zhu X, Wu S, Yu H, Cheng H, Luo T, Shen Q, Gao Y, Ma C, Liu Y, Wei Z, Chen X, Tao F, He X, Cao Y (2022) Activation of MT1/MT2 to protect testes and leydig cells against cisplatin-induced oxidative stress through the SIRT1/Nrf2 signaling pathway. *Cells* 11(10). <https://doi.org/10.3390/cells11101690>
- Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, Xie J, Ikenoue T, Yu J, Li L, Zheng P, Ye K, Chinnaiyan A, Halder G, Lai ZC, Guan KL (2007) Inactivation of YAP oncoprotein by the hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* 21(21):2747–2761. <https://doi.org/10.1101/gad.1602907>
- Zhao B, Li L, Tumaneng K, Wang CY, Guan KL (2010) A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP). *Genes Dev* 24(1):72–85. <https://doi.org/10.1101/gad.1843810>

- Zhou Q, Gui S, Zhou Q, Wang Y (2014) Melatonin inhibits the migration of human lung adenocarcinoma A549 cell lines involving JNK/MAPK pathway. *PLoS One* 9(7):e101132. <https://doi.org/10.1371/journal.pone.0101132>
- Zhuo X, Jiang H (2022) Protective effects of melatonin in cisplatin-induced cardiac toxicity: possible role of BDNF-TNF-alpha signaling pathway. *Acta Cir Bras* 37(2):e370208. <https://doi.org/10.1590/acb370208>
- Zimta AA, Cenariu D, Irimie A, Magdo L, Nabavi SM, Atanasov AG, Berindan-Neagoe I (2019) The role of Nrf2 activity in cancer development and progression. *Cancers (Basel)* 11(11). <https://doi.org/10.3390/cancers11111755>



Matrix Metalloproteinases as a Key Player in Cancer Progression

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Abstract

Cancer is a major health problem all over the world. One of the important key regulators of cancer development and pathogenesis is a group of enzymes called matrix metalloproteinases (MMPs). These proteolytic enzymes have an essential role in ECM remodeling, cell-cell communication, angiogenesis, metastasis, and cancer immunomodulation. The number of MMPs recognized till now is 23 enzymes. Some of them are released in a soluble form, and others exert their function through attachment to the plasma membrane. The regulation of MMPs in the body is maintained through a group of inhibitory enzymes called tissue inhibitor MMPs (TIMPs). These TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) are present in a balance with the MMPs, and any functional abnormality in the MMPs/TIMPs regulation can significantly affect tumor promotion or suppression as well. Therefore, MMPs together with TIMPs are considered a potential targeted therapy for cancer patients. In this review, we tried to illustrate each of the MMPs individually emphasizing their role in cancer progression. This could help provide a new diagnostic or prognostic marker that could serve as a target for a novel therapeutic modality for cancer patients.

Keywords

Cancer · Metalloproteinase · MMPs · MT-MMPs · TIMPs

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1 Introduction

Cancer is a global health problem that affects all age groups. Despite the different therapeutic modalities available for cancer treatment, it is the most common cause of death across both developing and developed countries (Vaghari-Tabari et al. 2021). The difficulty in cancer control is that it is a multistep disease with heterogeneous cell types (Vaghari-Tabari et al. 2021). Additionally, it has a multifactorial etiology including genetic and environmental causes (Goldar et al. 2015; Torgovnick and Schumacher 2015). Cancers are developed due to the accumulation of mutations that result from defective DNA repair mechanisms that occur following exposure to different carcinogens such as oxidative stress, chemicals, or ultraviolet (UV) irradiations (Iranzo et al. 2018). The key features that characterize cancerous cells are sustained cell proliferation, immortality, unresponsiveness to growth suppressor signals, invasion of the surrounding tissues, and metastasis (Hanahan and Weinberg 2011). A great body of evidence suggests that the process of carcinogenesis and the behavior of the tumor rely mainly on the interaction between the tumor cells and the surroundings namely the tumor microenvironment (TME) (Wang et al. 2018). The TME is formed of the nourishing vasculature, non-malignant cells (including inflammatory cells, immune cells, and cancer-associated fibroblast [CAF]), and the extracellular matrix (ECM) (Spill et al. 2016; Joyce and Fearon 2015). The tumor cells can interact with the surrounding cells of the TME resulting in the release of different immunomodulators, cytokines, and growth factors such as fibroblast growth factor (FGFs), transforming growth factor beta (TGF- β), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF). All those factors can support tumor evolution by maintaining cell growth, immunotolerance, angiogenesis, cell motility, degradation of the ECM, epithelial-mesenchymal transition (EMT), and metastasis (Korneev et al. 2017; Marsh et al. 2013; Weber and Kuo 2012; Stover et al. 2007).

The ECM constitutes the surrounding non-cellular structure that provides structural support for the tumor cells (de Almeida et al. 2022). It is formed of two main constituents: the basement membrane (BM) and the interstitial connective tissue matrix (Hynes and Naba 2012). The BM separates the epithelial cells from the surrounding stroma, while the connective tissue matrix serves to preserve tissue support and elasticity (Bonnans et al. 2014). Additionally, the ECM plays a major role in maintaining cell shape, motility, adhesion, proliferation, survival, and interaction with the surrounding environment (Bonnans et al. 2014; Sternlicht and Werb 2001). Also, it has an important role in sustaining tissue integrity and homeostasis, where it constitutes 4% of the total body proteome. Therefore, it is a dynamic structure that is continuously remodeled to maintain tissue functions (Cox 2021). Many distinct mechanisms are known to contribute to ECM remodeling and reassembling, and accordingly, any error that occurs in ECM reconstruction is associated with different pathological diseases including osteoarthritis, fibrosis, and cancer (Bonnans et al. 2014; Zhen and Cao 2014). The ECM is degraded intracellularly by the lysosomal enzymes present in the phagolysosomes or extracellularly by the secreted proteases (Dufour and Overall 2015). The extracellular

proteases that regulate the ECM remodeling include MMPs, plasmin, elastase, cathepsins, A disintegrins and metalloproteinases (ADAMs), and the ADAMs with thrombospondin motif (ADAMTSs), as well as the protease inhibitors including cysteine, tissue inhibitors metalloproteinases (TIMPs), and serpins (Cox 2021; Martin et al. 2021; Brassart-Pasco et al. 2020).

MMPs and their inhibitors TIMPs have a significant role in ECM remodeling (de Almeida et al. 2022). MMPs are concerned with the processing and releasing of many growth factors such as TGF- β and VEGF which are important mediators for tumor angiogenesis and metastasis (Schultz and Wysocki 2009; Mott and Werb 2004). They can also regulate the function of other mediators including chemokines, cytokines, metabolic proteins, growth factors, cell-surface receptors, and nuclear proteins (Chopra et al. 2019; Young et al. 2019; Dufour and Overall 2013).

Until now, 23 MMP members have been discovered, and accordingly, various classifications have emerged to categorize these MMPs (de Almeida et al. 2022). The most commonly used classification is developed according to their structure, function, and substrate specificity. These groups include the collagenases (MMP-1, MMP-8, MMP-13, and MMP-18) that are responsible mainly for the degradation of the helical fibrillar collagen in the bone structure (Vincenti and Brinckerhoff 2002). The gelatinases (MMP-2 and MMP-9) are mainly concerned with angiogenesis and neurogenesis. Other subgroups include the matrilysins (MMP-7 and MMP-26), the stromelysins (MMP-3, MMP-10, and MMP-11), and the membrane-type (MT) MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, and MMP-25) (de Almeida et al. 2022). In addition, other MMPs are not included in the previously mentioned group include MMP-12 (metalloelastase), MMP-19 (RASI-1), MMP-20 (enamelysin), MMP-21, MMP-22, MMP-23, and MMP-28 (epilysin) (de Almeida et al. 2022; Pittayaprupek et al. 2016).

Most MMPs have a general structure that is formed of an N-terminal propeptide domain composed of nearly 80 amino acids, a catalytic domain that consists of about 170 amino acids containing a zinc-binding site, a hinge region (a linker peptide) of variable length, and a C-terminal hemopexin-like domain formed of about 200 amino acids (Hofmann et al. 2000).

MMPs, together with their TIMPs, have a pivotal role in cancer pathogenesis and progression. Accordingly, any functional abnormality in MMPs/TIMPs regulation can significantly affect tumor promotion or suppression. Therefore, this chapter aims to review the involvement of 23 characterized MMPs in cancer development. In addition, we will illustrate each of the MMPs individually emphasizing their role in cancer pathogenesis. This will help in paving the way to recognize new diagnostic or prognostic markers that can serve as targets for novel anti-neoplastic therapeutic modalities.

1.1 Matrix Metalloproteinase-1 (MMP-1)

MMP-1 degrades interstitial collagen types I, II, and III. So that, it enables the cancer cells to invade the ECM and metastasize to different tissues (Wang and Kong 2015).

Additionally, it has been reported that MMP-1 can promote tumor angiogenesis and invasiveness through stimulating the endothelial protease-activated receptor-1 (PARP-1) (Trivedi et al. 2009). The PARP pathway has a key role in glucose and lipid metabolism, through which any dysregulated signaling leads to various metabolic disorders. Such metabolic dysregulations induce inflammatory reactions with the release of large quantities of interleukin (IL)-8, IL-6, leptin, and angiopoietin-like 4 which are associated with the development and metastasis of cancer (Cangemi et al. 2016; Zhu et al. 2012). Tian et al. (2018) demonstrated that MMP-1 is an independent factor for the metastasis of cervical cancer by inducing EMT in cervical cancer cells. They also concluded that MMP-1 overexpression is associated with lymph node metastasis through the dysregulation of the PARP-1 signaling pathway (Tian et al. 2018). Moreover, Anand et al. concluded that MMP-1 has an important role in glioma cell invasion by activating the mitogen-activated protein kinase (MAPK) pathway (Anand et al. 2011). Accordingly, increased expression of MMP-1 is a poor prognostic factor in various malignancies including bladder, gastric, esophageal, colorectal, and prostate cancer (Shin et al. 2015; Ozden et al. 2013; Cai et al. 2012; Murray et al. 1996, 1998).

The gene responsible for MMP-1 expression is located at 11q22.3 and is regulated by the activator protein-1 (AP1) which induces the promotor region of MAPKs (Biondi et al. 2000). These MAPKs activate the erythroblastosis 26-1 (Ets-1), activating transcription factor 2 (ATF2), Elk-1, and c-Jun transcription factors that result in MMP-1 transcription (Vincenti and Brinckerhoff 2002). It has been reported that MMP-1 polymorphisms are associated significantly with the risk of various cancers, especially the MMP1-1607 (1G > 2G) genotype (Ozden et al. 2013). In extensive research performed by Bau and his colleagues, they concluded that the MMP1-1607 (1G/2G) genotype is implicated in the increased risk of colorectal cancer (CRC) in the Taiwanese population, while it is associated with personal susceptibility to oral cancer in non-smokers compared to smoker patients (Wu et al. 2021; Sun et al. 2016). Additionally, they reported that the MMP-1 promoter 1607 (rs1799750) genotype has a limited role in promoting bladder and breast cancer in Taiwanese patients (Liao et al. 2021a, b; Su et al. 2016). In line with these results, several studies from Italy and Poland have proposed that MMP-1 promoter 1,607 polymorphism has no significant effect on breast cancer pathogenesis (Przybylowska et al. 2006; Ghilardi et al. 2002; Biondi et al. 2000). On the other hand, Srivastava et al. (2010) reported that MMP1-1607 2G is significantly associated with the increased risk of bladder cancer in the North Indian population. Also, other studies from India and Iran have reported significant associations of MMP-1 promoter 1607 (rs1799750) genotype with the risk of breast cancer pathogenesis and development (Balkhi et al. 2020; Padala et al. 2017). Therefore, the impact of MMP-1 promotor 1607 polymorphism on cancer progression varies according to many factors including the cancer type, personal habits such as smoking, as well as ethnic variations. In consistency with these data, a meta-analysis performed by Zhou et al. (2018) revealed that MMP1-1607 (1G/2G) polymorphism is associated with increased incidence of CRC, lung, renal, bladder, nervous system, and nasopharyngeal cancers, while there was no significant association of

MMP1–1607 (1G/2G) genotype and oral, gastric, hepatocellular, esophageal, breast, endometrial, ovarian, prostate, and head and neck cancers (Zhou et al. 2018). Moreover, they found that the association of MMP1–1607 polymorphism with cancer risk is more commonly detected in Asian and Caucasian populations, though the Asian population has a higher risk for the dominant, homozygous allele model compared to the Caucasian population (Zhou et al. 2018).

1.2 Matrix Metalloproteinase-2 (MMP-2)

Matrix metalloproteinase-2 (MMP-2), previously described as “gelatinase”, is a zinc-dependent endopeptidase that digests the ECM components such as collagen, elastin, fibronectin, and fibrillin (Henriet and Emonard 2019). The encoding gene for the MMP-2 consists of 13 exons and 12 introns at the long arm of chromosome 16 (Shipley et al. 1996). It is initially released as a 72 KD proenzyme (proMMP-2) in the cytosol, which is then activated by proteolytic cleavage or oxidative stress intracellularly (Jacob-Ferreira and Schulz 2013). Other different activating mechanisms have been proposed for proMMP-2 at the cell surface through the MT-MMPs including MT1-MMP, MT2-MMP, MT3-MMP, and MT5-MMP (Shamamian et al. 2001). The activation of proMMP-2 by the MT3-MMP depends on the interaction between the hemopexin (Hpx)-like domain of proMMP-2 and the chondroitin sulfate glycosaminoglycans present at the cytoplasmic surface (Iida et al. 2007), while the activation of proMMP-2 by MT2-MMP occurs through direct interaction with the Hpx-like domain (Morrison and Overall 2006). On the other hand, MT1-MMP is the most important activator for proMMP-2 which predominantly requires the presence of the TIMP-2 (Itoh et al. 2006). It had been previously reported that the inactivation of the TIMP-2 gene in genetically modified mice resulted in deficient activation of proMMP-2 by MT1-MMP (Caterina et al. 2000; Wang et al. 2000). Additionally, Itoh et al. (2001) found that cancer cell invasiveness is significantly associated with the increased MT1-MMP level mediated through proMMP-2 activation. Moreover, Chernov et al. concluded that cancer cell aggressiveness is significantly related to MMP-2 promotor hypomethylation and decreased histone H3 lysine27 trimethylation (Chernov et al. 2009).

Accordingly, MMP-2 has been reported to have an essential role in cancer initiation, progression, invasion, and dissemination (Jiang and Li 2021; Liu et al. 2018a, b; Liu 2016; Fu et al. 2015a, b; Ren et al. 2015; Wang et al. 2014a, b; Wen et al. 2014). Therefore, many recent studies have investigated the diagnostic role and the poor prognostic effect of MMP-2 overexpression in different types of cancer including breast cancer (Ren et al. 2015; Chernov et al. 2009), endometrial (Liu et al. 2018a, b), ovarian carcinoma (Liu 2016; Fu et al. 2015a, b), pituitary adenomas (Liu et al. 2016), HCC (Wang et al. 2014a), osteosarcoma (Wen et al. 2014), gastric cancer (Wang et al. 2014b), and oral cancer (Tsai et al. 2018). Han et al. (2020) reported that the increased MMP-2 level in lung cancer patients is an independent risk factor for poor clinical outcomes, shorter survival rates, more advanced tumor stage, and increased cancer aggressiveness. Similarly, other studies have concluded

that the decreased MMP-2 expression level is significantly associated with favorable patient outcomes, reduced tumor cell invasion, and metastasis (Lou et al. 2017; Li et al. 2016; Poudel et al. 2016). Additionally, it is found that MMP-2 overexpression is significantly associated with increased bone marrow blast cell count and minimal residual disease (MRD) in pediatric acute lymphoblastic leukemia (ALL) (Saleh et al. 2021).

An interesting study performed by Li and his colleagues illustrated the different roles of MMP-2 in bone metastasis and osteolysis (Li et al. 2021). They stated that MMP-2 overexpression is responsible for metastatic osteolysis through different mechanisms including collagen digestion in the bone matrix, increasing osteoclast activity, inhibiting osteoblast formation, and inducing certain osteolytic factors that promote bone resorption (such as IL-6 and TGF- β). In addition, MMP-2 has a proangiogenic activity through participating in ECM remodeling which facilitates the motility of pericytes and endothelial cells, together with the release of VEGF and TGF- β angiogenic factors (Quintero-Fabián et al. 2019). Additionally, MMP-2 stimulates the secretion of platelet-derived growth factor type BB (PDGF-BB), which induces the formation of type H vessels (Yang et al. 2018). Interestingly, MMP-2 downregulation could also induce osteolytic activity and skeletal developmental disorders (Li et al. 2021; Itoh et al. 1997), as MMP-2 regulates the expression of some mineralizing factors such as bone sialoprotein (BSP) and osteopontin (OPN) (Mosig and Martignetti 2013).

It is worth noting that MMP-2 is also detectable in the nucleus in addition to its function in the cytosol (Jobin et al. 2017; Sinha et al. 2014). Therefore, it is suggested that MMP-2 has a potential role in nuclear matrix remodeling and regulation of gene expression through involvement in the digestion of different nuclear proteins, transcription factors, as well as RNA processing (Jobin et al. 2017; Xie et al. 2017).

Many studies have reported several factors that could regulate the transcription of MMP-2 in different types of cancer. Teng et al. (2019) found that High Mobility Group AT-Hook 1 (HMGA1) can induce the transcription of MMP2 in HCC cells. Also, Guan et al. (2017) proposed that tumor-associated calcium signal transducer 2 (Trop2) is associated with thyroid cancer cell invasion and metastasis through stimulating MAPK ERK/JNK/AP1/MMP2 signaling pathway. Other studies have revealed that the MMP-2 promotor region could be regulated by ER α -36-STAT3 in breast cancer, and retinoic acid receptor α (RAR α) in CRC (Huang et al. 2019; Xiang et al. 2019). Sun et al. (2021) found that epidermal growth factor (EGF), latrophilin, and seven transmembrane domains containing 1 (ELTD1) can regulate the expression of MMP-2 in CRC cells in an indirect manner. Taken together, these studies identified different biomarkers for targeting MMP-2 which provide a different modality for cancer treatment.

1.3 Matrix Metalloproteinase-3 (MMP-3)

Matrix metalloproteinase-3 (MMP-3) is also a zinc-dependent protease. It belongs to the stromelysins group; however, it can degrade a wide variety of ECM elements including procollagen type-I, collagen type III, IV, IX, proteoglycans, laminin, fibronectin, IL-1b, and tumor necrosis factor α (TNF- α) precursor (Karaarslan et al. 2016; Visse and Nagase 2003). It is secreted by connective tissue cells, endothelial cells, macrophages, neutrophils, and cancer cells (Kofla-Dłubacz et al. 2014). It was incorporated in many physiological and pathological processes including inflammation, tissue repair, and morphogenesis. It was also found to have an important role in the pathogenesis of rheumatoid arthritis through its destructive function for the chondrocytes (Guerrero et al. 2021). Additionally, MMP-3 has a significant contribution to the pathogenesis of Parkinson's and Alzheimer's diseases (Lech et al. 2019). It can cleave the pro-MMP-1, -3, -7, -8, -9, and -13, as well as their upstream activators, such as urokinase-type plasminogen activator and plasminogen (McCawley and Matrisian 2001; Arza et al. 2000). As a member of MMPs with proteolytic activities, MMP-3 is reported to participate in cancer promotion (Mehner et al. 2015), progression (Frieling et al. 2020), angiogenesis, and metastasis (Shih et al. 2008) in different tumor types including HCC, glioblastoma, breast, lung, and cervical cancer (Humayun et al. 2021; Yu et al. 2021; Rajkumar et al. 2011; Fang et al. 2005; Biondi et al. 2000). Moreover, Fu and his colleagues noted a significant increase in the serum level of MMP-3 in metastatic and aggressive CRC using a highly sensitive time-resolved fluorescence immunoassay (TRFIA) technique (Fu et al. 2023).

Numerous studies have investigated the different pathways involved in MMP-3 regulation and function (Yu et al. 2021; Van Hove et al. 2012; Cauwe and Opdenakker 2010; Shih et al. 2008). Yu et al. (2021) demonstrated that human guanylate binding protein 5 (GBP5) could induce glioblastoma cell proliferation and invasion through the activation of the Src-ERK1/2-MAPK signaling pathway, which consequently increases MMP3 expression levels. Similarly, Shine et al. (2008) proposed that the autocrine motility factor/phosphoglucose isomerase (AMF/PGI) promotes liver cancer cell migration by inducing MMP-3 expression through the activation of the Src-RhoA-PI3K signaling pathway, which stimulates the MMP-3 promotor activation by the transcription factor activating protein-1 (AP-1). It was also reported that MMP-3 is present in the nucleus and associated with the Ras-related nuclear protein (RAN)-binding protein (Cauwe and Opdenakker 2010). These data indicated that MMP-3 could participate in the process of apoptosis, transcription, gene regulation, and protein synthesis (Van Hove et al. 2012; Cauwe and Opdenakker 2010). On the other side, McCawley et al. performed extensive research to investigate the effect of MMP-3 loss of function in genetic knockout mice. They observed that the loss of MMP-3 function could also increase tumor cell proliferation, dedifferentiation, and metastasis in a faster manner than that of mice with competent MMP-3 functions (McCawley et al. 2004, 2008).

Other series have studied the impacts of MMP-3 gene polymorphism on tumor progression (Balkhi et al. 2020; Li et al. 2018; Padala et al. 2017). They have

reported that MMP3 (rs632478) gene polymorphism is associated with the risk of breast cancer carcinogenesis and development (Balkhi et al. 2020; Padala et al. 2017). On the other hand, in a meta-analysis by Li et al. (2018), the MMP-2(−1306) C/T or MMP-3(−1171) 5A/6A polymorphisms did not correlate with oral cancer carcinogenesis.

1.4 Matrix Metalloproteinase-7 (MMP-7)

MMP-7, the smallest endopeptidase, is a zinc- and calcium-dependent metalloproteinase. It is recognized as matrilysin, matrilysin-1, punctuated metalloproteinase (PUMP1), and putative metalloproteinase (Abramson et al. 1995). The encoding gene for MMP-7 is located at 11q21- q22 and contains 13 exons. Its structure is characteristically different from other MMPs as it has four conservative regions of MMPs and does not have a C-terminal hemoglobin domain (Gaire et al. 1994).

Like other MMPs, MMP-7 can digest ECM components such as mucin, heparin, osteopontin, and elastin (Tan and Liu 2012; Surlin et al. 2011). However, it can also degrade other proteins including Fas ligand (FasL) and E-cadherin (Ii et al. 2006). In addition, it is incorporated into many physiological processes including wound healing, aging, and bone growth and remodeling (Liao et al. 2021a, b; Sbardella et al. 2012). Moreover, it was reported that increased MMP-7 level is a poor prognostic factor for advanced tumor stage, shorter overall survival (OS), inferior clinical outcomes, and increased metastatic rates in many cancers including gastric (Song et al. 2017), renal (Verma and Das 2018), urinary bladder (Singh et al. 2018), breast (Niu et al. 2019), melanoma, esophageal (Juchniewicz et al. 2017), liver (Yang et al. 2019a, b), colorectal (Chen and Miao 2022), pancreatic (Van Doren 2022), gallbladder, ovarian, and lung cancers (Liao et al. 2021a, b). Similarly, other studies have reported the association of MMP7-181 G promotor polymorphism with increased risk of bladder, gastric, ovarian, and colorectal cancers (Srivastava et al. 2010; Li et al. 2006; Zhang et al. 2005; Ghilardi et al. 2003).

Various studies have investigated the different roles of MMP-7 in cancer progression and metastasis, Yang et al. (2019a, b) reported that MMP-7 is a downstream regulator in the β -catenin signaling pathway, which has an essential role in cancer cell proliferation, invasion, and metastasis. Similarly, Chan et al. (2011) concluded that Keratin-15 (KRT15) could be a potential therapeutic target in patients with metastatic CRC, as it induces CRC cell migration and invasion via stimulating β -catenin/MMP7 signaling pathway. In addition, MMP-7 promotes the dissociation of tumor cells through the cleavage of the c-type lectin domain 3 member A (CLEC3A). The latter maintains tumor cell adhesion to the ECM by supporting the binding of the heparan sulfate proteoglycans located on the cell surface and the integrin ligands present in the ECM (Tsunezumi et al. 2009).

It had been previously reported that the cluster of differentiation (CD)-44 molecule mediates the formation of MMP-7 and HB-EGF precursor complexes on the surface of tumor cells. This complex formation results in the activation of HB-EGF

and its receptor ErbB4, which leads to the destruction of the BM, and consequently tumor cell invasion and metastasis (Hartmann-Petersen et al. 2009; Yokoyama et al. 2008). In addition, MMP-7 has an important role in angiogenesis as it induces endothelial cell proliferation together with the destruction of the basement membrane and degradation of the ECM (Huo et al. 2002). These processes finally result in neovascularization and tumor growth (Liao et al. 2021a, b).

Additionally, MMP-7 induces tumor cell proliferation and survival through binding to various insulin-like growth factor binding proteins (IGFBPs). This leads to the increased bioavailability and activity of IGFs, which stimulate tumor cell survival and immortality (Nakamura et al. 2005). Furthermore, MMP-7 could suppress apoptosis of tumor cells through splitting and shedding of the soluble Fas and its ligands as well as TNF- α , which results in inhibition of apoptosis and confers chemoresistance in cancer cells (Almendo et al. 2009; Karayiannakis et al. 2001). On the other hand, MMP-7 can activate other MMPs (including MMP-1, MMP-9, and MMP-13), which consequently reinvigorates cancer progression and survival (Scheau et al. 2019). Therefore, MMP-7 is considered an important oncogenic protein that could be a potential therapeutic target for various cancer types.

1.5 Matrix Metalloproteinase-8 (MMP-8)

MMP-8 is a collagenase-2 or neutrophil collagenase, as it has been found during inflammation (Van Lint and Libert 2006). It has proteolytic activities against a wide range of substrates including collagen type I, II, III, laminin-5, fibrinogen, fibronectin, proteoglycans, tenascin, angiotensin, IGFBP-5, and L-selectin (Van Lint and Libert 2006; Hemers et al. 2005). The role of MMP-8 in tumor progression is still a debatable issue (Sirniö et al. 2018). Some studies have noted the protective impact of increased MMP-8 levels in the prognosis of many cancers. For instance, it was found that increased MMP-8 levels are associated with negative lymph node metastasis in breast cancer patients, as well as with prolonged survival in patients with oral tongue squamous cell carcinoma (Åström et al. 2017; Decock et al. 2008). On the contrary, MMP-8 was found to be associated with cancer progression, poor overall survival, advanced tumor stage, and increased incidence of metastasis in CRC, HCC, and ovarian cancer (Sirniö et al. 2018; Lempinen et al. 2013; Stadlmann et al. 2003). Similarly, Böckelman et al. (2018) concluded that MMP-8 and TIMP-1 are negative prognostic markers in CRC patients, especially in those with systemic inflammatory response. Moreover, Qin et al. (2016) reported a positive interplay between MMP-8 and TGF- β that induces EMT and associates with HCC stage, aggressiveness, and metastasis through stimulating the PI3K/Akt/Rac1 signaling pathway. On the other hand, Laitinen et al. stated that MMP-8 alone has no prognostic role in gastric cancer; however, its molar ratio with TIMP-1 can serve as a poor prognostic marker for gastric cancer (Laitinen et al. 2018).

In a meta-analysis performed by Feng and his colleagues, they found that MMP-8 C-799 T (rs11225395) promoter polymorphism is associated with increased cancer risk in non-Asian (but not Asian) populations (Feng et al. 2019). On the other side,

Zhang et al. (2019) showed that MMP-8 polymorphic variants including C-799 T, Lys460Thr, and Lys87Glu have no significant impacts on cancer susceptibility in both Asian and Caucasian populations. Similarly, Shen et al. reported that MMP-8 C-799 T, Val436Ala, and Lys460Thr variants have no role in lung cancer risk in the Taiwanese population (Shen et al. 2017). Therefore, the role of MMP-8 in cancer risk and development is still controversial and varies according to the type of malignancy, the ethnic differences, the associated inflammation, and the underlying polymorphic variations.

1.6 Matrix Metalloproteinase-9 (MMP-9)

MMP-9 belongs to the gelatinase B group, where it can act against different substrates including gelatine, collagen, and elastin, in addition to other extracellular, intracellular, and plasma membrane proteins (Bronisz and Kurkowska-Jastrzębska 2016; Farina and Mackay 2014; Hill et al. 2012). The gene encoding for it is present on chromosome 20q13.12, which is formed of 13 exons and 12 introns (Vandooren et al. 2013). MMP-9 is synthesized and secreted by fibroblasts, neutrophils, macrophages, and endothelial cells (Vandooren et al. 2013). It is released as a pro-enzyme in the ECM where it is activated by proteolytic enzymes such as MMP-3 (Geurts et al. 2012).

Several studies have investigated the role of MMP-9 in the pathogenesis and development of cancers (Rashid and Bardaweel 2023; Dufour et al. 2010; Redondo-Muñoz et al. 2010). It was found that the binding of proMMP-9 to PEX to its receptors ($\beta 1$ integrin, $\alpha 4$, and CD44) results in the activation of various signaling pathways, including the Lyn kinase and the epidermal growth factor receptor (EGFR) pathways, which leads to the activation of the downstream kinase effectors PI3K/Akt, ERK, and focal adhesion kinase (FAK) that maintain cancer cell proliferation, survival, and migration (Dufour et al. 2010; Redondo-Muñoz et al. 2010). St-Pierre (2021) demonstrated in his review that the pro-tumorigenic effect of galectin-7 is maintained via the upregulation of MMP-9 in various cancers including breast, ovarian, cervical, gastric, head and neck, as well as oral squamous cell carcinomas (Wang et al. 2020a, b; Chen et al. 2018; Guo and Li 2017; Zhu et al. 2013). It was also demonstrated that MMP-9 overexpression is associated with lymph node metastasis, and advanced tumor grade and clinical stage in patients with breast cancer (Jiang and Li 2021). Additionally, Dong et al. proposed that MMP-9 maintains breast cancer aggressiveness and development through the activation of the TGF- β /SMAD signaling pathway (Dong et al. 2019). Moreover, it is reported that $\alpha_v\beta_6$ integrin-mediated CRC pathogenesis and progression is maintained through the increased MMP-9 expression, which leads to the activation of the protein kinase C (PKC) pathway (Agrez et al. 1999). The pivotal role of MMP-9 in cancer cell invasion, metastasis, and angiogenesis is mediated through its ability to degrade gelatine and collagens type IV, V, XI, and XVI (Bronisz and Kurkowska-Jastrzębska 2016). Furthermore, its level is found to be increased in metastatic lung cancer due to induction by VEGF (Hiratsuka et al. 2002). On the

other hand, the role of MMP-9 in leukemia is still unclear. MMP-9 is found to be associated with shorter overall survival in acute myeloid leukemia (AML) patients (Lin et al. 2002). However, only 25% of cases with adult acute lymphocytic leukemia (ALL) showed positive staining in blast cells for MMP-9, with a trend toward poor survival. MMP-9 has had no associations with the clinical course and survival in pediatric ALL (Saleh et al. 2021; Kuittinen et al. 2001).

1.7 Matrix Metalloproteinase-10 (MMP-10)

The encoding gene of MMP-10 (also recognized as tansin-2 or stromelysin-2) is located at chromosome 11q22.2 (Sun and Liu 2022). It is implicated in ECM remodeling through the degradation of various components including fibronectin, proteoglycans, gelatine, elastin, and collagens type III, IV, and V (Bäck et al. 2010). It also degrades heparin binding-epidermal growth factor (HB-EGF), zonula occludens-1 (ZO-1), pro-MMP-1, pro-MMP-7, pro-MMP-8, pro-MMP-9, and pro-MMP-13 (Sun and Liu 2022; Schlage et al. 2015; Barksby et al. 2006). The expression level of MMP-10 is scarcely detectable in normal physiological conditions, while its level is greatly increased during tissue injury (McMahan et al. 2016). It is suggested that MMP-10 mediates inflammatory reactions through the activation of the NF- κ B signaling pathway in peritoneal macrophages and mesothelial cells (Ishimura et al. 2023). MMP-10 is reported to have an important role in the pathogenesis and promotion of various types of cancers, including esophageal, bladder, lung, and tongue cancers (Juchniewicz et al. 2017; Upadhyay et al. 2017; Du et al. 2014; Justilien et al. 2012). Moreover, MMP-10 promotes invasion and metastasis of head and neck squamous cell carcinoma through the upregulation of periostin and Wnt-5b, with the inhibition of the p38 MAPK signaling pathway (Deraz et al. 2011). Similarly, MMP-10 is associated with tumor invasion and shorter survival rates in patients with RCC (Lin et al. 2019; Miyata et al. 2007).

1.8 Matrix Metalloproteinase-11 (MMP-11)

MMP11 is known as stromelysin-3. Unlike other MMPs, it is characteristically expressed as an active enzyme, and it does not require an activational proteolytic cleavage (Pei and Weiss 1995). It is mainly secreted by mesenchymal cells such as fibroblasts and adipocytes (Andarawewa et al. 2005). Therefore, it is detected in plenty in the TME where it is produced by the CAFs, cancer-associated adipocytes (CAAs), and intertumoral mononuclear inflammatory cells, chiefly the macrophage (Eiro et al. 2019; Andarawewa et al. 2005). Another unique feature of MMP-11 is the degradation of only a small number of substrates including IGF-BP1, the native alpha 3 chain of collagen VI, and the laminin receptor (Motrescu et al. 2008). The pro-tumorigenic effects of MMP-11 are evidenced in many cancers including gastric, colon, lung, esophageal, bladder, and pancreatic cancer (Chen et al. 2020; Yang et al. 2019a, b; Deng et al. 2005; Hourihan et al. 2003; Włodarczyk et al. 2001;

Von Marschall et al. 1998). Regarding its role in breast cancer, many studies have reported controversial functions for MMP-11 in its pathogenesis. In an experimental tumor model, MMP-11 was found to promote breast cancer progression in the early stages, while it inhibits tumor cell metastasis in the late stages (Andarawewa et al. 2003). Other studies have demonstrated that MMP-11 is a poor prognostic factor for breast cancer patients as it is associated with increased cell proliferation, advanced tumor stage, poor survival, and increased drug resistance (Han et al. 2017; Lee et al. 2016; Fu et al. 2015a, b). Other studies have specified the source of MMP-11 and have shown that MMP-11 expressed by the tumor cell and/or the fibroblast-like cells is an independent prognostic marker for shorter OS of the patients (Eiro et al. 2019; Cid et al. 2018; Min et al. 2013). On the contrary, Kang et al. (2022) performed a study on breast cancer tissues of 497 patients and concluded that MMP-11 expressed by the mononuclear inflammatory cells (mainly macrophages) is associated with inferior clinical outcomes. On the other hand, MMP-11 which was released from tumor cells or the fibroblast had no significant prognostic roles. MMP-11 was found to promote immune suppression by inducing programmed death-ligand 1 (PD-L1) expression, monocyte recruitment, and stimulation of CCL2–CCR2 pathway-mediated tube formation in endothelial cells (Kang et al. 2022). Tan et al. (2020) demonstrated the role of MMP-11 in promoting tumor cell survival and inhibition of cell death by activating the IGF1/AKT/FoxO1 signaling pathway (Tan et al. 2020).

Other studies have revealed that MMP-11 SNPs rs738792 and rs131451 polymorphic variants can have a potential role in the diagnosis of Taiwanese CRC patients (Huang et al. 2022). Similarly, the MMP-11 SNP rs131451 was found to be associated with the development of prostate cancer in Taiwanese patients with biochemical recurrence (Saad et al. 2020). While the MMP-11 rs738791 variant was associated with the incidence of HCC development in Egyptian patients, this association was not detected with the MMP-11 rs738792 (Hsieh et al. 2020).

1.9 The Membrane-Type Matrix Metalloproteinases (MT-MMPs)

The MT-MMPs are a group of six proteolytic enzymes that are not secreted in the soluble form as the other MMPs. They exert their functions through attachment to the plasma membrane (Turunen et al. 2017). Thereby, they have many functions other than the remodeling of the ECM proteins, which include the regulation of the intracellular signaling pathways and the modulation of tumor cell-cell communication (Itoh 2015). These functions are mediated through the shedding of cell surface receptors, adhesion molecules, growth factors, and surface ligands which are important for maintaining the TME (Kessenbrock et al. 2010). These MT-MMPs are bound to the cell membrane either through a transmembrane domain such as MMP-14 (MT1-MMP), MMP-15 (MT2-MMP), MMP-16 (MT3-MMP), and MMP-24 (MT5-MMP) or through a glycosylphosphatidylinositol (GPI) anchor such as MMP-17 (MT4-MMP) and MMP-25 (MT6-MMP) (Cui et al. 2017; Sohail et al. 2008). MMP-23 is characteristically a type II transmembrane proteinase that can be released and activated by a single proteolytic cleavage (Pei et al. 2000).

The MT1-MMP is also known as MMP-14. Though it was first identified as a cell surface protein, recently it is found in many subcellular compartments such as the nucleus, Golgi apparatus, endoplasmic reticulum, and caveolae (Knapinska and Fields 2019; Koziol et al. 2012). Previous studies have addressed the intracellular functions of MT1-MMP, as it is involved in the cleavage of centrosomal proteins and the formation of mitotic spindle. Therefore, MT1-MMP aberration could result in abnormal cell division, chromosomal instability, genomic mutations, and accordingly, tumorigenesis (Wali et al. 2014; Golubkov et al. 2006). Moreover, the nuclear MT1-MMP affects the transcription of more than 100 genes involved in metabolism regulation, chromatin remodeling, cell proliferation, apoptosis, regulation of immune response, and inflammatory reactions (Maybee et al. 2022; Jobin et al. 2017). It is also reported that MT1-MMP has significant relations with Smad-1, Dickkopf-3, VEGF-A, SPRY4, and PI3K/Akt/GSK3 signaling pathway, which implicate the important role of MT1-MMP in the tumor progression other than its proteolytic activity (Shaverdashvili et al. 2015; Shimizu-Hirota et al. 2012; Gingras and Béliveau 2010; Eisenach et al. 2010; Sounni et al. 2004). In addition to its role in invadopodia formation (which facilitates cell motility and migration), the MT1-MMP cytoplasmic-binding protein 1 (MTCBP-1) is reported to have a protective tumor suppressor role in glioblastoma cells through binding and blocking the function of MT1-MMP (Hoshino et al. 2013; Pratt et al. 2016).

Another important function of MT1-MMP is related to its localization in the Golgi apparatus which allows MT1-MMP to regulate the release of hypoxia-inducible factor 1 (HIF-1) through the formation of MT1-MMP/FIH-1/Mint-3 complex that activates glycolysis to produce ATP (Sakamoto and Seiki 2009; Pei et al. 2000). Accordingly, MT1-MMP is essential for the motility and migration of macrophages as well as cancer cells (Maybee et al. 2022).

MT2-MMP is found to be expressed in cancers of epithelial origin (such as cholangiocarcinoma), in addition to the testicular germ cell tumor and multiple myeloma (Fakhari et al. 2022; Turunen et al. 2017). MT3-MMP is expressed in tumors originating from cells of neural crest origin including glioblastoma and glioma of the brain, in addition to melanoma (Turunen et al. 2017; Szabova et al. 2005). Moreover, melanoma cancer cells have the highest expression of MT4-MMPs (Ferraro et al. 2011). Additionally, MT4-MMP is proven to have a premetastatic function in breast and prostate carcinomas (Truong et al. 2016; Riddick et al. 2005). MT5-MMP is greatly expressed in brain tumors and mesothelioma (Pei 1999a), while high MT6-MMP expression is detected in leukemias (Pei 1999b).

1.10 Other Matrix Metalloproteinases

Many researchers have investigated the role of different MMPs in cancer progression and metastasis. It is demonstrated that MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-14, and MMP-28 have important roles in EMT, and MMP-14, in particular, induces the mesenchymal phenotype in the tumor cells through degrading

E-cadherin and other BM components (Niland et al. 2021; Quintero-Fabián et al. 2019; Scheau et al. 2019; Liu et al. 2018a, b; Yan et al. 2015). It also induces the release of the receptor protein tyrosine kinase PTK7, which results in the activation of the Wnt/planar cell polarity pathway (Golubkov et al. 2010). MMP-12 mRNA is reported to have a useful prognostic function for evaluating tumor recurrence and overall survival in HCC patients after liver resection (Ng et al. 2011).

MMP-13 (collagenase-3) has a cleavage activity against ECM components including collagen, fibronectin, gelatine, osteonectin, fibrillin, and aggrecans (Ala-aho and Kähäri 2005). Characteristically, it can inactivate certain chemokines (such as stromal cell-derived factor-1 [SDF-1] and monocyte chemoattractant protein-3 [MCP-3]) (McQuibban et al. 2001). In addition to the activation of TGF- β 3 (Deng et al. 2000), it is found to be involved in the pathogenesis of many cancers; however, it was not detected in most tissues under normal physiological conditions (Ala-aho and Kähäri 2005). Therefore, MMP-13 is considered a pathological marker for cancer development, especially in the squamous epithelial cell transformation (Johansson et al. 2000).

MMP-28 or epylisin is the last defined member of MMPs, and it characteristically has a threonine residue in its catalytic domain (Lohi et al. 2001). Its upregulation is observed in bladder cancer, where it is associated with higher tumor grade, lymphatic metastasis, and poorer patient outcomes (Wang et al. 2020a, b). Moreover, MMP-28 and MMP-21 are reported to be independent negative prognostic factors for the aggressiveness and metastasis of gastric cancer (Zhang et al. 2018).

The MMP-19 gene is detected on chromosome 12q13.2, and it is expressed in many tissues including the placenta, breast, ovary, spleen, heart, and small intestine (Zhao et al. 2011). The exact role of MMP-19 has varied in different types of cancer (Zhai et al. 2016; Wang et al. 2013). Some studies have reported a significant downregulation of MMP-19 in uveal melanoma, skin, breast, and nasopharyngeal carcinoma (Chan et al. 2011; Delassus et al. 2008; Lai et al. 2008; Impola et al. 2003). On the other hand, MMP-19 is found to be upregulated in NSCLC, melanoma, and oropharyngeal squamous cell carcinoma (Yu et al. 2014; Müller et al. 2010; Velinov et al. 2007). Additionally, co-expression of MMP-19 and MMP-14 is found to be a diagnostic, as well as an independent prognostic marker for poor survival in glioma patients (Wang et al. 2013). In a study conducted by Bister et al. (2004), they reported that MMP-19 and MMP-28 are differentially downregulated during the neoplastic transformation of colon cancer, while MMP-26 is involved in enterocyte migration. On the contrary, Sena and his team found that MMP-19 and MMP-15 are significantly upregulated during the malignant transformation of colon cancer (Sena et al. 2012). Moreover, Zhai and his colleague concluded that MMP-19 and MMP-20 were significantly increased in patients with pancreatic ductal adenocarcinoma (PDAC) and were associated with poorer clinical outcomes (Zhai et al. 2016). MMP-20 is mainly formed by human odontoblasts, which are important for enamel synthesis and maintaining its adherence to dentin (Simmer and Hu 2002). MMP-20 upregulation was detected in many cancers, including laryngeal squamous cell carcinoma, odontogenic tumors, and tongue carcinoma (Liu et al. 2011; Väänänen et al. 2001, 2004).

The functional activity of MMPs is balanced by the expression of TIMPs (Brew and Nagase 2010). TIMP family of enzymes (TIMP-1, TIMP-2, TIMP-3, TIMP-4) can inhibit MMPs, ADAMs, and ADAMTSs through a reversible binding (Cabral-Pacheco et al. 2020; Brew and Nagase 2010). TIMPs can inhibit all MMP members according to the tissue of origin; however, they have varied affinities to some MMPs. For example, TIMP-1 has a special affinity to MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, and MMP-9; TIMP-2 inhibits MT1-MMP, MMP-2, MMP-9, and MMP-14; TIMP-3 inhibits MMP-2 and MMP-9; and TIMP-4 inhibits MMP-2, MMP-26, and MT1-MMP (Cabral-Pacheco et al. 2020). Additionally, TIMPs can be implicated in the activation of MMPs, and they can interact with the proenzyme form (Matchett et al. 2019), as TIMP-1 and TIMP-3 can interact with the pro-MMP-9, while TIMP-2 and TIMP-4 can interact with the pro-MMP-2 (Wang & Khalil 2018; Nagase et al. 2006). Therefore, TIMPs also have an essential role in the pathogenesis and malignant transformation of cancer cells (Cabral-Pacheco et al. 2020).

2 Summary

In summary, a great body of evidence suggests that MMPs are important modulators of cancer progression and metastasis (Table 1). Though the function of some MMPs is not well clear till now, their effect cannot be ignored, and further research is required to accurately illustrate their roles. Moreover, understanding the precise function of each MMP member could enhance our understanding of cancer pathogenesis, which will help in discovering a novel therapeutic modality for cancer patients. Also, studying the genetic polymorphism in each population could help in developing a potential personal targeted therapy according to the underlying MMPs genetic polymorphism, which accordingly affects the tumor behavior and patients' outcomes.

Table 1 Illustration of the main role of some MMPs in different types of cancer

MMPs	Main functions	Type of cancer
MMP-1	Angiogenesis and metastasis, stimulating the endothelial PARP pathway Inducing EMT Activation of the MAPK pathway	Cervical cancer Glioma cells
MMP-2	Cancer initiation, progression, invasion, and dissemination Increased BM blast cell count and MRD in pediatric ALL Bone metastasis and osteolysis Increased secretion of release of VEGF, TGF- β , and PDGF-BB	Endometrial cancer, ovarian carcinoma, pituitary adenomas, HCC, lung, osteosarcoma, gastric cancer, and oral cancer

(continued)

Table 1 (continued)

MMPs	Main functions	Type of cancer
MMP-3	Cleavage of the pro-MMP-1, -3, -7, -8, -9, -13, urokinase-type plasminogen activator and plasminogen Cancer promotion, progression, angiogenesis, and metastasis Participate in the process of apoptosis, transcription, gene regulation, and protein synthesis	HCC, glioblastoma, breast, lung, CRC, and cervical cancer
MMP-7	It is a downstream regulator in the β -catenin signaling pathway It promotes the dissociation of tumor cells through the cleavage of the c-type lectin domain 3 member A (CLEC3A) It promotes tumor cell growth and neovascularization through stimulation of endothelial cell proliferation, destruction of the basement membrane, and degradation of the ECM It binds to various insulin-like growth factor binding proteins (IGFBPs) It inhibits apoptosis of tumor cells through splitting and shedding of the soluble Fas and its ligands as well as TNF- α It is a poor prognostic factor for advanced tumor stage, shorter overall survival (OS) rate, inferior clinical outcomes, and increased metastatic rates It activates other MMPs including MMP-1, MMP-9, and MMP-13	Gastric, renal, urinary bladder, breast, melanoma, esophageal, liver, colorectal, pancreatic, gallbladder, ovarian, and lung cancers
MMP-8	It is associated with negative lymph node metastasis, and prolonged survival It is associated with cancer progression, poor overall survival, and advanced tumor stage Stimulation of the PI3K/Akt/Rac1 signaling pathway	Breast cancer, oral tongue squamous cell carcinoma CRC, HCC, and ovarian cancer
MMP-9	Activation of the Lyn kinase and the EGFR pathways, which leads to the activation of the downstream kinase effectors PI3K/Akt, ERK, and FAK that maintain cancer cell proliferation, survival, and migration It is associated with lymph node metastasis, advanced tumor grade, and advanced clinical stage in patients with breast cancer Activation of the TGF- β /SMAD signaling pathway Induction of VEGF in metastatic lung cancer	Breast, ovarian, cervical, gastric, oral squamous cell carcinomas, lung, head and neck cancers AML and ALL

(continued)

Table 1 (continued)

MMPs	Main functions	Type of cancer
MMP-10	It degrades heparin binding-epidermal growth factor (HB-EGF), zonula occludens-1 (ZO-1), pro-MMP-1, pro-MMP -7, pro-MMP -8, pro-MMP -9, and pro-MMP -13 It mediates inflammatory reactions through the activation of the NF-kB signaling pathway in peritoneal macrophages and mesothelial cells Upregulation of periostin and Wnt-5b, with the inhibition of the p38 MAPK signaling pathway It is associated with tumor invasion and shorter survival rates in patients with RCC	Esophageal, bladder, lung, and tongue cancers Head and neck squamous cell carcinoma RCC
MMP-11	It promotes breast cancer progression in the early stages, while it inhibits tumor cell metastasis in the late stages MMP-11 expressed by the mononuclear inflammatory cells (mainly macrophages) is associated with inferior clinical outcomes. On the other hand, MMP-11 which was released from tumor cells, or the fibroblast had no significant prognostic roles It induces PD-L1 expression, monocyte recruitment, and stimulation of CCL2–CCR2 pathway-mediated tube formation in the endothelial cells Activation of the IGF1/AKT/FoxO1 signaling pathway	Brast cancer Gastric, colon, lung, esophageal, bladder, and pancreatic cancer
MMP-12	MMP-12 mRNA has a prognostic role in tumor recurrence and overall survival in HCC patients after liver resection	HCC
MMP-13	Inactivation of stromal cell-derived factor-1 [SDF-1] and monocyte chemoattractant protein-3 [MCP-3] Activation of TGF-β3	Many cancers, especially the squamous epithelial cell
MT1-MMP	It is involved in the cleavage of centrosomal proteins and the formation of mitotic spindle It affects the transcription of more than 100 genes involved in metabolism regulation, chromatin remodeling, cell proliferation, apoptosis, regulation of immune response, and inflammatory reactions It is involved in the regulation of Smad-1, Dickkopf-3, VEGF-A, SPRY4, and PI3K/Akt/GSK3 signaling pathway	Brast cancer, gastric, colon, lung, and glioblastoma

(continued)

Table 1 (continued)

MMPs	Main functions	Type of cancer
MT2-MMP	ECM proteins, including which include the regulation of the intracellular signaling pathways and the modulation of tumor cell-cell communication	Cholangiocarcinoma, testicular germ cell tumor, and multiple myeloma
MT3-MMP	ECM proteins, including which include the regulation of the intracellular signaling pathways and the modulation of tumor cell-cell communication	Glioblastoma, glioma of the brain, and melanoma
MT4-MMP	It has a premetastatic function in breast and prostate carcinomas	Breast cancer, prostate carcinoma, and melanoma
MT5-MMP	ECM proteins, including which include the regulation of the intracellular signaling pathways and the modulation of tumor cell-cell communication	Brain tumors and mesothelioma
MT6-MMP	ECM proteins, including which include the regulation of the intracellular signaling pathways and the modulation of tumor cell-cell communication	Leukemias
MMP-19	It has protective roles in uveal melanoma, skin, breast, and nasopharyngeal carcinoma It is upregulated in NSCLC, melanoma, and oropharyngeal squamous cell carcinoma	Uveal melanoma, skin, breast, and nasopharyngeal Lung, melanoma, and oropharyngeal squamous cell carcinoma
MMP-20	Poor prognostic factor	Laryngeal squamous cell carcinoma, odontogenic tumors, and tongue carcinoma
MMP-28	It is associated with higher tumor grade, lymphatic metastasis, and poorer patient outcomes	Bladder cancer and gastric cancer

References

- Abramson SR, Conner GE, Nagase H, Neuhaus I, Woessner JF Jr (1995) Characterization of rat uterine matrilysin and its cDNA. Relationship to human pump-1 and activation of procollagenases. *J Biol Chem* 270(27):16016–16022. <https://doi.org/10.1074/jbc.270.27.16016>
- Agrez M, Gu X, Turton J, Meldrum C, Niu J, Antalis T, Howard EW (1999) The alpha v beta 6 integrin induces gelatinase B secretion in colon cancer cells. *Int J Cancer* 81(1):90–97
- Ala-aho R, Kähäri VM (2005) Collagenases in cancer. *Biochimie* 87(3–4):273–286. <https://doi.org/10.1016/j.biochi.2004.12.009>
- Almendo V, Ametller E, García-Recio S, Collazo O, Casas I, Augé JM, Maurel J, Gascón P (2009) The role of MMP7 and its cross-talk with the FAS/FASL system during the acquisition of chemoresistance to oxaliplatin. *PLoS One* 4(3):e4728. <https://doi.org/10.1371/journal.pone.0004728>
- Anand M, Van Meter TE, Fillmore HL (2011) Epidermal growth factor induces matrix metalloproteinase-1 (MMP-1) expression and invasion in glioma cell lines via the MAPK pathway. *J Neuro-Oncol* 104(3):679–687. <https://doi.org/10.1007/s11060-011-0549-x>

- Andarawewa KL, Boulay A, Masson R, Mathelin C, Stoll I, Tomasetto C, Chenard MP, Gintz M, Belloq JP, Rio MC (2003) Dual stromelysin-3 function during natural mouse mammary tumor virus-ras tumor progression. *Cancer Res* 63(18):5844–5849
- Andarawewa KL, Motrescu ER, Chenard MP, Gansmuller A, Stoll I, Tomasetto C, Rio MC (2005) Stromelysin-3 is a potent negative regulator of adipogenesis participating to cancer cell-adipocyte interaction/crosstalk at the tumor invasive front. *Cancer Res* 65(23):10862–10871. <https://doi.org/10.1158/0008-5472.CAN-05-1231>
- Arza B, Hoylaerts MF, Felez J, Collen D, Lijnen HR (2000) Prostromelysin-1 (proMMP-3) stimulates plasminogen activation by tissue-type plasminogen activator. *Eur J Biochem* 267: 6378–6384
- Åström P, Juurikka K, Hadler-Olsen ES, Svineng G, Cervigne NK, Coletta RD, Risteli J, Kauppila JH, Skarp S, Kuttner S, Oteiza A, Sutinen M, Salo T (2017) The interplay of matrix metalloproteinase-8, transforming growth factor- β 1 and vascular endothelial growth factor-C cooperatively contributes to the aggressiveness of oral tongue squamous cell carcinoma. *Br J Cancer* 117(7):1007–1016. <https://doi.org/10.1038/bjc.2017.249>
- Bäck M, Ketelhuth DF, Agewall S (2010) Matrix metalloproteinases in atherothrombosis. *Prog Cardiovasc Dis* 52(5):410–428. <https://doi.org/10.1016/j.pcad.2009.12.002>
- Balkhi S, Mashayekhi F, Salehzadeh A, Saedi HS (2020) Matrix metalloproteinase (MMP)-1 and MMP-3 gene variations affect MMP-1 and -3 serum concentration and associates with breast cancer. *Mol Biol Rep* 47(12):9637–9644. <https://doi.org/10.1007/s11033-020-05962-x>
- Barksby HE, Milner JM, Patterson AM, Peake NJ, Hui W, Robson T, Lakey R, Middleton J, Cawston TE, Richards CD, Rowan AD (2006) Matrix metalloproteinase 10 promotion of collagenolysis via procollagenase activation: implications for cartilage degradation in arthritis. *Arthritis Rheum* 54(10):3244–3253. <https://doi.org/10.1002/art.22167>
- Biondi ML, Turri O, Leviti S, Seminati R, Cecchini F, Bernini M, Ghilardi G, Guagnellini E (2000) MMP1 and MMP3 polymorphisms in promoter regions and cancer. *Clin Chem* 46(12): 2023–2024
- Bister VO, Salmela MT, Karjalainen-Lindsberg ML, Uria J, Lohi J, Puolakkainen P, Lopez-Otin C, Saarialho-Kere U (2004) Differential expression of three matrix metalloproteinases, MMP-19, MMP-26, and MMP-28, in normal and inflamed intestine and colon cancer. *Dig Dis Sci* 49(4): 653–661. <https://doi.org/10.1023/b:ddas.0000026314.12474.17>
- Böckelman C, Beilmann-Lehtonen I, Kaprio T, Koskensalo S, Tervahartiala T, Mustonen H, Stenman UH, Sorsa T, Haglund C (2018) Serum MMP-8 and TIMP-1 predict prognosis in colorectal cancer. *BMC Cancer* 18(1):679. <https://doi.org/10.1186/s12885-018-4589-x>
- Bonnans C, Chou J, Werb Z (2014) Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 15(12):786–801. <https://doi.org/10.1038/nrm3904>
- Brassart-Pasco S, Brézillon S, Brassart B, Ramont L, Oudart JB, Monboisse JC (2020) Tumor microenvironment: extracellular matrix alterations influence tumor progression. *Front Oncol* 10: 397. <https://doi.org/10.3389/fonc.2020.00397>
- Brew K, Nagase H (2010) The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochim Biophys Acta* 1803(1):55–71. <https://doi.org/10.1016/j.bbamcr.2010.01.003>
- Bronisz E, Kurkowska-Jastrzębska I (2016) Matrix metalloproteinase 9 in epilepsy: the role of neuroinflammation in seizure development. *Mediat Inflamm* 2016:7369020. <https://doi.org/10.1155/2016/7369020>
- Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodríguez JF, Martínez-Avila N, Martínez-Fierro ML (2020) The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci* 21(24):9739. <https://doi.org/10.3390/ijms21249739>
- Cai QW, Li J, Li XQ, Wang JQ, Huang Y (2012) Expression of STAT3, MMP-1 and TIMP-1 in gastric cancer and correlation with pathological features. *Mol Med Rep* 5(6):1438–1442. <https://doi.org/10.3892/mmr.2012.849>

- Cangemi A, Fanale D, Rinaldi G, Bazan V, Galvano A, Perez A, Barraco N, Massihnia D, Castiglia M, Vieni S, Bronte G, Mirisola M, Russo A (2016) Dietary restriction: could it be considered as speed bump on tumor progression road? *Tumour Biol* 37(6):7109–7118. <https://doi.org/10.1007/s13277-016-5044-8>
- Caterina JJ, Yamada S, Caterina NC, Longenecker G, Holmbäck K, Shi J, Yermovsky AE, Engler JA, Birkedal-Hansen H (2000) Inactivating mutation of the mouse tissue inhibitor of metalloproteinases-2(Timp-2) gene alters proMMP-2 activation. *J Biol Chem* 275(34):26416–26422. <https://doi.org/10.1074/jbc.M001271200>
- Cauwe B, Opdenakker G (2010) Intracellular substrate cleavage: a novel dimension in the biochemistry, biology and pathology of matrix metalloproteinases. *Crit Rev Biochem Mol Biol* 45:351–423
- Chan KC, Ko JM, Lung HL, Sedlacek R, Zhang ZF, Luo DZ, Feng ZB, Chen S, Chen H, Chan KW, Tsao SW, Chua DT, Zabarovsky ER, Stanbridge EJ, Lung ML (2011) Catalytic activity of matrix metalloproteinase-19 is essential for tumor suppressor and anti-angiogenic activities in nasopharyngeal carcinoma. *Int J Cancer* 129(8):1826–1837. <https://doi.org/10.1002/ijc.25855>
- Chen W, Miao C (2022) KRT15 promotes colorectal cancer cell migration and invasion through β -catenin/MMP-7 signaling pathway. *Med Oncol (Northwood, London, England)* 39(5):68. <https://doi.org/10.1007/s12032-021-01619-2>
- Chen YS, Chang CW, Tsay YG, Huang LY, Wu YC, Cheng LH, Yang CC, Wu CH, Teo WH, Hung KF, Huang CY, Lee TC, Lo JF (2018) HSP40 co-chaperone protein Tid1 suppresses metastasis of head and neck cancer by inhibiting Galectin-7-TCF3-MMP9 axis signaling. *Theranostics* 8(14):3841–3855. <https://doi.org/10.7150/thno.25784>
- Chen C, Liu X, Jiang J, Li S, Wang G, Ju L, Wang F, Liu T, Li S (2020) Matrix metalloproteinase 11 is a potential biomarker in bladder cancer diagnosis and prognosis. *Onco Targets Ther* 13:9059–9069. <https://doi.org/10.2147/OTT.S243452>
- Chernov AV, Sounni NE, Remacle AG, Strongin AY (2009) Epigenetic control of the invasion-promoting MT1-MMP/MMP-2/TIMP-2 axis in cancer cells. *J Biol Chem* 284(19):12727–12734. <https://doi.org/10.1074/jbc.M900273200>
- Chopra S, Overall CM, Dufour A (2019) Matrix metalloproteinases in the CNS: interferons get nervous. *Cell Mol Life Sci* 76(16):3083–3095. <https://doi.org/10.1007/s00018-019-03171-9>
- Cid S, Eiro N, Fernández B, Sánchez R, Andicoechea A, Fernández-Muñiz PI, González LO, Vizoso FJ (2018) Prognostic influence of tumor stroma on breast cancer subtypes. *Clin Breast Cancer* 18(1):e123–e133. <https://doi.org/10.1016/j.clbc.2017.08.008>
- Cox TR (2021) The matrix in cancer. *Nat Rev Cancer* 21(4):217–238. <https://doi.org/10.1038/s41568-020-00329-7>
- Cui N, Hu M, Khalil RA (2017) Biochemical and biological attributes of matrix metalloproteinases. *Prog Mol Biol Transl Sci* 147:1–73. <https://doi.org/10.1016/bs.pmbts.2017.02.005>
- de Almeida LGN, Thode H, Eslambolchi Y, Chopra S, Young D, Gill S, Devel L, Dufour A (2022) Matrix metalloproteinases: from molecular mechanisms to physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 74(3):712–768. <https://doi.org/10.1124/pharmrev.121.000349>
- Decock J, Hendrickx W, Vanleeuw U, Van Belle V, Van Huffel S, Christiaens MR, Ye S, Paridaens R (2008) Plasma MMP1 and MMP8 expression in breast cancer: protective role of MMP8 against lymph node metastasis. *BMC Cancer* 8:77. <https://doi.org/10.1186/1471-2407-8-77>
- Delassus GS, Cho H, Park J, Eliceiri GL (2008) New pathway links from cancer-progression determinants to gene expression of matrix metalloproteinases in breast cancer cells. *J Cell Physiol* 217(3):739–744. <https://doi.org/10.1002/jcp.21548>
- Deng SJ, Bickett DM, Mitchell JL, Lambert MH, Blackburn RK, Carter HL 3rd, Neugebauer J, Pahl G, Weiner MP, Moss ML (2000) Substrate specificity of human collagenase 3 assessed using a phage-displayed peptide library. *J Biol Chem* 275(40):31422–31427. <https://doi.org/10.1074/jbc.M004538200>
- Deng H, Guo RF, Li WM, Zhao M, Lu YY (2005) Matrix metalloproteinase 11 depletion inhibits cell proliferation in gastric cancer cells. *Biochem Biophys Res Commun* 326(2):274–281. <https://doi.org/10.1016/j.bbrc.2004.11.027>

- Deraz EM, Kudo Y, Yoshida M, Obayashi M, Tsunematsu T, Tani H, Siriwardena SB, Keikhaee MR, Qi G, Iizuka S, Ogawa I, Campisi G, Lo Muzio L, Abiko Y, Kikuchi A, Takata T (2011) MMP-10/stromelysin-2 promotes invasion of head and neck cancer. *PLoS One* 6(10):e25438. <https://doi.org/10.1371/journal.pone.0025438>
- Dong H, Diao H, Zhao Y, Xu H, Pei S, Gao J, Wang J, Hussain T, Zhao D, Zhou X, Lin D (2019) Overexpression of matrix metalloproteinase-9 in breast cancer cell lines remarkably increases the cell malignancy largely via activation of transforming growth factor beta/SMAD signalling. *Cell Prolif* 52(5):e12633. <https://doi.org/10.1111/cpr.12633>
- Du X, Lin BC, Wang QR, Li H, Ingalla E, Tien J, Rooney I, Ashkenazi A, Penuel E, Qing J (2014) MMP-1 and pro-MMP-10 as potential urinary pharmacodynamic biomarkers of FGFR3-targeted therapy in patients with bladder cancer. *Clin Cancer Res* 20(24):6324–6335. <https://doi.org/10.1158/1078-0432.CCR-13-3336>
- Dufour A, Overall CM (2013) Missing the target: matrix metalloproteinase antitargets in inflammation and cancer. *Trends Pharmacol Sci* 34(4):233–242. <https://doi.org/10.1016/j.tips.2013.02.004>
- Dufour A, Overall CM (2015) Subtracting matrix out of the equation: new key roles of matrix metalloproteinases in innate immunity and disease. *Matrix Metalloprot Biol*:131–152
- Dufour A, Zucker S, Sampson NS, Kescu C, Cao J (2010) Role of matrix metalloproteinase-9 dimers in cell migration: design of inhibitory peptides. *J Biol Chem* 285(46):35944–35956. <https://doi.org/10.1074/jbc.M109.091769>
- Eiro N, Cid S, Fernández B, Fraile M, Cernea A, Sánchez R, Andicoechea A, DeAndrés Galiana EJ, González LO, Fernández-Muñoz Z, Fernández-Martínez JL, Vizoso FJ (2019) MMP11 expression in intratumoral inflammatory cells in breast cancer. *Histopathology* 75(6):916–930. <https://doi.org/10.1111/his.13956>
- Eisenach PA, Roghi C, Fogarasi M, Murphy G, English WR (2010) MT1-MMP regulates VEGF-A expression through a complex with VEGFR-2 and Src. *J Cell Sci* 123(Pt 23):4182–4193. <https://doi.org/10.1242/jcs.062711>
- Fakhari S, Jalili A, Nikkhoo B, Ghaderi B, Boshagh MA, Mirzaie S, Moradzad M (2022) MT2-MMP is differentially expressed in multiple myeloma cells and mediates their growth and progression. *Cell Signal* 92:110248. <https://doi.org/10.1016/j.cellsig.2022.110248>
- Fang S, Jin X, Wang R, Li Y, Guo W, Wang N et al (2005) Polymorphisms in the MMP1 and MMP3 promoter and non-small cell lung carcinoma in North China. *Carcinogenesis* 26:481–486
- Farina AR, Mackay AR (2014) Gelatinase B/MMP-9 in tumour pathogenesis and progression. *Cancers* 6(1):240–296. <https://doi.org/10.3390/cancers6010240>
- Feng J, Chen Y, Hua W, Sun X, Chen Y, Liu Y, Fan J, Zhao Y, Zhao L, Xu X, Yang X (2019) The MMP-8 rs11225395 promoter polymorphism increases cancer risk of non-Asian populations: evidence from a meta-analysis. *Biomol Ther* 9(10):570. <https://doi.org/10.3390/biom9100570>
- Ferraro GB, Morrison CJ, Overall CM, Strittmatter SM, Fournier AE (2011) Membrane-type matrix metalloproteinase-3 regulates neuronal responsiveness to myelin through Nogo-66 receptor 1 cleavage. *J Biol Chem* 286(36):31418–31424. <https://doi.org/10.1074/jbc.M111.249169>
- Frieling JS, Li T, Tauro M, Lynch CC (2020) Prostate cancer-derived MMP-3 controls intrinsic cell growth and extrinsic angiogenesis. *Neoplasia* 22:511–521
- Fu J, Khaybullin R, Zhang Y, Xia A, Qi X (2015a) Gene expression profiling leads to discovery of correlation of matrix metalloproteinase 11 and heparanase 2 in breast cancer progression. *BMC Cancer* 15:473. <https://doi.org/10.1186/s12885-015-1410-y>
- Fu Z, Xu S, Xu Y, Ma J, Li J, Xu P (2015b) The expression of tumor-derived and stromal-derived matrix metalloproteinase 2 predicted prognosis of ovarian cancer. *Int J Gynecol Cancer* 25(3):356–362. <https://doi.org/10.1097/JG.C.0000000000000386>
- Fu Y, Wang X, Chen X, Hong J, Qin Y, Zhou Z, Zhou X, Wang Y, Zhou J, Fang H, Liu P, Huang B (2023) Establishment of matrix metalloproteinase 3 time-resolved immunoassay and some potential clinical applications. *Anal Biochem* 666:115072. <https://doi.org/10.1016/j.ab.2023.115072>

- Gaire M, Magbanua Z, McDonnell S, McNeil L, Lovett DH, Matrisian LM (1994) Structure and expression of the human gene for the matrix metalloproteinase matrilysin. *J Biol Chem* 269(3): 2032–2040
- Geurts N, Becker-Pauly C, Martens E, Proost P, Van den Steen PE, Stöcker W, Opdenakker G (2012) Meprips process matrix metalloproteinase-9 (MMP-9)/gelatinase B and enhance the activation kinetics by MMP-3. *FEBS Lett* 586(24):4264–4269. <https://doi.org/10.1016/j.febslet.2012.10.033>
- Ghilardi G, Biondi ML, Caputo M, Leviti S, DeMonti M, Guagnellini E, Scorza R (2002) A single nucleotide polymorphism in the matrix metalloproteinase-3 promoter enhances breast cancer susceptibility. *Clin Cancer Res* 8(12):3820–3823
- Ghilardi G, Biondi ML, Erario M, Guagnellini E, Scorza R (2003) Colorectal carcinoma susceptibility and metastases are associated with matrix metalloproteinase-7 promoter polymorphisms. *Clin Chem* 49(11):1940–1942. <https://doi.org/10.1373/clinchem.2003.018911>
- Gingras D, Béliveau R (2010) Emerging concepts in the regulation of membrane-type 1 matrix metalloproteinase activity. *Biochim Biophys Acta* 1803(1):142–150. <https://doi.org/10.1016/j.bbamcr.2009.04.011>
- Goldar S, Khaniani MS, Derakhshan SM, Baradaran B (2015) Molecular mechanisms of apoptosis and roles in cancer development and treatment. *Asian Pac J Cancer Prev* 16:2129–2144
- Golubkov VS, Chekanov AV, Savinov AY, Rozanov DV, Golubkova NV, Strongin AY (2006) Membrane type-1 matrix metalloproteinase confers aneuploidy and tumorigenicity on mammary epithelial cells. *Cancer Res* 66(21):10460–10465. <https://doi.org/10.1158/0008-5472.CAN-06-2997>
- Golubkov VS, Chekanov AV, Cieplak P, Aleshin AE, Chernov AV, Zhu W, Radichev IA, Zhang D, Dong PD, Strongin AY (2010) The Wnt/planar cell polarity protein-tyrosine kinase-7 (PTK7) is a highly efficient proteolytic target of membrane type-1 matrix metalloproteinase: implications in cancer and embryogenesis. *J Biol Chem* 285(46): 35740–35749. <https://doi.org/10.1074/jbc.M110.165159>
- Guan H, Guo Z, Liang W, Li H, Wei G, Xu L, Xiao H, Li Y (2017) Trop2 enhances invasion of thyroid cancer by inducing MMP2 through ERK and JNK pathways. *BMC Cancer* 17(1):486. <https://doi.org/10.1186/s12885-017-3475-2>
- Guerrero S, Sánchez-Tirado E, Agüí L, González-Cortés A, Yáñez-Sedeño P, Pingarrón JM (2021) Simultaneous determination of CXCL7 chemokine and MMP3 metalloproteinase as biomarkers for rheumatoid arthritis. *Talanta* 234:122705. <https://doi.org/10.1016/j.talanta.2021.122705>
- Guo JP, Li XG (2017) Galectin-7 promotes the invasiveness of human oral squamous cell carcinoma cells via activation of ERK and JNK signaling. *Oncol Lett* 13(3):1919–1924. <https://doi.org/10.3892/ol.2017.5649>
- Han J, Choi YL, Kim H, Choi JY, Lee SK, Lee JE, Choi JS, Park S, Choi JS, Kim YD, Nam SJ, Nam BH, Kwon MJ, Shin YK (2017) MMP11 and CD2 as novel prognostic factors in hormone receptor-negative, HER2-positive breast cancer. *Breast Cancer Res Treat* 164(1):41–56. <https://doi.org/10.1007/s10549-017-4234-4>
- Han L, Sheng B, Zeng Q, Yao W, Jiang Q (2020) Correlation between MMP2 expression in lung cancer tissues and clinical parameters: a retrospective clinical analysis. *BMC Pulm Med* 20(1): 283. <https://doi.org/10.1186/s12890-020-01317-1>
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- Hartmann-Petersen S, Tammi RH, Tammi MI, Kosma VM (2009) Depletion of cell surface CD44 in nonmelanoma skin tumours is associated with increased expression of matrix metalloproteinase 7. *Br J Dermatol* 160(6):1251–1257. <https://doi.org/10.1111/j.1365-2133.2009.09031.x>
- Hemers E, Duval C, McCaig C, Handley M, Dockray GJ, Varro A (2005) Insulin-like growth factor binding protein-5 is a target of matrix metalloproteinase-7: implications for epithelial-mesenchymal signaling. *Cancer Res* 65(16):7363–7369. <https://doi.org/10.1158/0008-5472.CAN-05-0157>

- Henriet P, Emonard H (2019) Matrix metalloproteinase-2: not (just) a “hero” of the past. *Biochimie* 166:223–232. <https://doi.org/10.1016/j.biochi.2019.07.019>
- Hill JW, Poddar R, Thompson JF, Rosenberg GA, Yang Y (2012) Intracellular matrix metalloproteinases promote DNA damage and apoptosis induced by oxygen-glucose deprivation in neurons. *Neuroscience* 220:277–290. <https://doi.org/10.1016/j.neuroscience.2012.06.019>
- Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, Shipley JM, Senior RM, Shibuya M (2002) MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. *Cancer Cell* 2(4):289–300. [https://doi.org/10.1016/s1535-6108\(02\)00153-8](https://doi.org/10.1016/s1535-6108(02)00153-8)
- Hofmann UB, Westphal JR, Van Muijen GN, Ruiter DJ (2000) Matrix metalloproteinases in human melanoma. *J Invest Dermatol* 115(3):337–344. <https://doi.org/10.1046/j.1523-1747.2000.00068.x>
- Hoshino D, Kirkbride KC, Costello K, Clark ES, Sinha S, Grega-Larson N, Tyska MJ, Weaver AM (2013) Exosome secretion is enhanced by invadopodia and drives invasive behavior. *Cell Rep* 5(5):1159–1168. <https://doi.org/10.1016/j.celrep.2013.10.050>
- Hourihan RN, O’Sullivan GC, Morgan JG (2003) Transcriptional gene expression profiles of oesophageal adenocarcinoma and normal oesophageal tissues. *Anticancer Res* 23(1A):161–165
- Hsieh CY, Chou YE, Lin CY, Wang SS, Chien MH, Tang CH, Lin JC, Wen YC, Yang SF (2020) Impact of matrix metalloproteinase-11 gene polymorphisms on biochemical recurrence and clinicopathological characteristics of prostate cancer. *Int J Environ Res Public Health* 17(22):8603. <https://doi.org/10.3390/ijerph17228603>
- Huang GL, Chen QX, Ma JJ, Sui SY, Wang YN, Shen DY (2019) Retinoic acid receptor α facilitates human colorectal cancer progression via Akt and MMP2 signaling. *Onco Targets Ther* 12:3087–3098. <https://doi.org/10.2147/OTT.S200261>
- Huang HC, Shiu BH, Su SC, Huang CC, Ting WC, Chang LC, Yang SF, Chou YE (2022) The impact of matrix metalloproteinase-11 polymorphisms on colorectal cancer progression and clinicopathological characteristics. *Diagnostics (Basel, Switzerland)* 12(7):1685. <https://doi.org/10.3390/diagnostics12071685>
- Humayun M, Ayuso JM, Brenneke RA et al (2021) Elucidating cancer-vascular paracrine signaling using a human organotypic breast cancer cell extravasation model. *Biomaterials* 270:120640
- Huo N, Ichikawa Y, Kamiyama M, Ishikawa T, Hamaguchi Y, Hasegawa S, Nagashima Y, Miyazaki K, Shimada H (2002) MMP-7 (matrilysin) accelerated growth of human umbilical vein endothelial cells. *Cancer Lett* 177(1):95–100. [https://doi.org/10.1016/s0304-3835\(01\)00772-8](https://doi.org/10.1016/s0304-3835(01)00772-8)
- Hynes RO, Naba A (2012) Overview of the matrisome—an inventory of extracellular matrix constituents and functions. *Cold Spring Harb Perspect Biol* 4(1):a004903. <https://doi.org/10.1101/cshperspect.a004903>
- Ii M, Yamamoto H, Adachi Y, Maruyama Y, Shinomura Y (2006) Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. *Exp Biol Med* (Maywood) 231(1):20–27. <https://doi.org/10.1177/153537020623100103>
- Iida J, Wilhelmson KL, Ng J, Lee P, Morrison C, Tam E, Overall CM, McCarthy JB (2007) Cell surface chondroitin sulfate glycosaminoglycan in melanoma: role in the activation of pro-MMP-2 (pro-gelatinase A). *Biochem J* 403(3):553–563. <https://doi.org/10.1042/BJ20061176>
- Impola U, Toriseva M, Suomalainen S, Jeskanen L, Hieta N, Jahkola T, Grenman R, Kähäri VM, Saarialho-Kere U (2003) Matrix metalloproteinase-19 is expressed by proliferating epithelium but disappears with neoplastic dedifferentiation. *Int J Cancer* 103(6):709–716. <https://doi.org/10.1002/ijc.10902>
- Iranzo J, Martincorena I, Koonin EV (2018) Cancer-mutation network and the number and specificity of driver mutations. *Proc Natl Acad Sci USA* 115:E6010–E6019
- Ishimura T, Ishii A, Yamada H, Osaki K, Toda N, Mori KP, Ohno S, Kato Y, Handa T, Sugioka S, Ikushima A, Nishio H, Yanagita M, Yokoi H (2023) Matrix metalloproteinase-10 deficiency has protective effects against peritoneal inflammation and fibrosis via transcription factor NF κ B pathway inhibition. *Kidney Int* 104(5):929–942. <https://doi.org/10.1016/j.kint.2023.08.010>

- Itoh Y (2015) Membrane-type matrix metalloproteinases: their functions and regulations. *Matrix Biol* 44-46:207–223. <https://doi.org/10.1016/j.matbio.2015.03.004>
- Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T, Itohara S (1997) Unaltered secretion of beta-amyloid precursor protein in gelatinase a (matrix metalloproteinase 2)-deficient mice. *J Biol Chem* 272(36):22389–22392. <https://doi.org/10.1074/jbc.272.36.22389>
- Itoh Y, Takamura A, Ito N, Maru Y, Sato H, Suenaga N, Aoki T, Seiki M (2001) Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion. *EMBO J* 20(17):4782–4793. <https://doi.org/10.1093/emboj/20.17.4782>
- Itoh Y, Ito N, Nagase H, Evans RD, Bird SA, Seiki M (2006) Cell surface collagenolysis requires homodimerization of the membrane-bound collagenase MT1-MMP. *Mol Biol Cell* 17(12):5390–5399. <https://doi.org/10.1091/mbc.e06-08-0740>
- Jacob-Ferreira AL, Schulz R (2013) Activation of intracellular matrix metalloproteinase-2 by reactive oxygen-nitrogen species: consequences and therapeutic strategies in the heart. *Arch Biochem Biophys* 540(1–2):82–93. <https://doi.org/10.1016/j.abb.2013.09.019>
- Jiang H, Li H (2021) Prognostic values of tumoral MMP2 and MMP9 overexpression in breast cancer: a systematic review and meta-analysis. *BMC Cancer* 21(1):149. <https://doi.org/10.1186/s12885-021-07860-2>
- Jobin PG, Butler GS, Overall CM (2017) New intracellular activities of matrix metalloproteinases shine in the moonlight. *Biochim Biophys Acta Mol Cell Res* 1864(11 Pt A):2043–2055. <https://doi.org/10.1016/j.bbamcr.2017.05.013>
- Johansson N, Ala-aho R, Uitto V, Grénman R, Fusenig NE, López-Otín C, Kähäri VM (2000) Expression of collagenase-3 (MMP-13) and collagenase-1 (MMP-1) by transformed keratinocytes is dependent on the activity of p38 mitogen-activated protein kinase. *J Cell Sci* 113(Pt 2):227–235. <https://doi.org/10.1242/jcs.113.2.227>
- Joyce JA, Fearon DT (2015) T cell exclusion, immune privilege, and the tumor microenvironment. *Science* 348(6230):74–80. <https://doi.org/10.1126/science.aaa6204>
- Juchniewicz A, Kowalczyk O, Milewski R, Ludański W, Dziegielewska P, Kozłowski M, Nikliński J (2017) MMP-10, MMP-7, TIMP-1 and TIMP-2 mRNA expression in esophageal cancer. *Acta Biochim Pol* 64(2):295–299. https://doi.org/10.18388/abp.2016_1408
- Justilien V, Regala RP, Tseng IC, Walsh MP, Batra J, Radisky ES, Murray NR, Fields AP (2012) Matrix metalloproteinase-10 is required for lung cancer stem cell maintenance, tumor initiation and metastatic potential. *PLoS One* 7(4):e35040. <https://doi.org/10.1371/journal.pone.0035040>
- Kang SU, Cho SY, Jeong H, Han J, Chae HY, Yang H, Sung CO, Choi YL, Shin YK, Kwon MJ (2022) Matrix metalloproteinase 11 (MMP11) in macrophages promotes the migration of HER2-positive breast cancer cells and monocyte recruitment through CCL2-CCR2 signaling. *Lab Invest* 102(4):376–390. <https://doi.org/10.1038/s41374-021-00699-y>
- Karaarslan N, Gurbuz MS, Caliskan T, Ayan E, Aker FV, Berkman MZ (2016) The effect of matrix metalloproteinase-3 on the prognosis and biological behaviour of meningiomas. *Turk Neurosurg* 26(5):678–683. <https://doi.org/10.5137/1019-5149.JTN.12807-14.1>
- Karayannakis AJ, Syrigos KN, Polychronidis A, Pitiakoudis M, Bounovas A, Simopoulos K (2001) Serum levels of tumor necrosis factor-alpha and nutritional status in pancreatic cancer patients. *Anticancer Res* 21(2B):1355–1358
- Kessenbrock K, Plaks V, Werb Z (2010) Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 141(1):52–67. <https://doi.org/10.1016/j.cell.2010.03.015>
- Knapinska AM, Fields GB (2019) The expanding role of MT1-MMP in cancer progression. *Pharmaceuticals (Basel, Switzerland)* 12(2):77. <https://doi.org/10.3390/ph12020077>
- Kofla-Dłubacz A, Matusiewicz M, Krzesiek E, Noga L, Iwańczak B (2014) Metalloproteinase-3 and -9 as novel markers in the evaluation of ulcerative colitis activity in children. *Adv Clin Exp Med* 23(1):103–110. <https://doi.org/10.17219/acem/37031>
- Korneev KV, Atretkhany KN, Drutskaya MS, Grivennikov SI, Kuprash DV, Nedospasov SA (2017) TLR-signaling and proinflammatory cytokines as drivers of tumorigenesis. *Cytokine* 89:127–135. <https://doi.org/10.1016/j.cyto.2016.01.021>

- Kozioł A, Martín-Alonso M, Clemente C, Gonzalo P, Arroyo AG (2012) Site-specific cellular functions of MT1-MMP. *Eur J Cell Biol* 91(11–12):889–895. <https://doi.org/10.1016/j.ejcb.2012.07.003>
- Kuittinen O, Savolainen ER, Koistinen P, Möttönen M, Turpeenniemi-Hujanen T (2001) MMP-2 and MMP-9 expression in adult and childhood acute lymphatic leukemia (ALL). *Leuk Res* 25(2):125–131. [https://doi.org/10.1016/s0145-2126\(00\)00104-1](https://doi.org/10.1016/s0145-2126(00)00104-1)
- Lai K, Conway RM, Crouch R, Jager MJ, Madigan MC (2008) Expression and distribution of MMPs and TIMPs in human uveal melanoma. *Exp Eye Res* 86(6):936–941. <https://doi.org/10.1016/j.exer.2008.03.010>
- Laitinen A, Hagström J, Mustonen H, Kokkola A, Tervahartiala T, Sorsa T, Böckelman C, Haglund C (2018) Serum MMP-8 and TIMP-1 as prognostic biomarkers in gastric cancer. *Tumour Biol* 40(9):1010428318799266. <https://doi.org/10.1177/1010428318799266>
- Lech AM, Wiera G, Mozrzymas JW (2019) Matrix metalloproteinase-3 in brain physiology and neurodegeneration. *Adv Clin Exp Med* 28(12):1717–1722. <https://doi.org/10.17219/acem/110319>
- Lee JY, Park K, Lee E, Ahn T, Jung HH, Lim SH, Hong M, Do IG, Cho EY, Kim DH, Kim JY, Ahn JS, Im YH, Park YH (2016) Gene expression profiling of breast cancer brain metastasis. *Sci Rep* 6:28623. <https://doi.org/10.1038/srep28623>
- Lempinen M, Lyytinen I, Nordin A, Tervahartiala T, Mäkisalo H, Sorsa T, Isoniemi H (2013) Prognostic value of serum MMP-8, -9 and TIMP-1 in patients with hepatocellular carcinoma. *Ann Med* 45(7):482–487. <https://doi.org/10.3109/07853890.2013.823779>
- Li Y, Jin X, Kang S, Wang Y, Du H, Zhang J, Guo W, Wang N, Fang S (2006) Polymorphisms in the promoter regions of the matrix metalloproteinases-1, -3, -7, and -9 and the risk of epithelial ovarian cancer in China. *Gynecol Oncol* 101(1):92–96. <https://doi.org/10.1016/j.ygyno.2005.09.058>
- Li C, Deng L, Shen H, Meng Q, Qian A, Sang H, Xia J, Li X (2016) O-6- methylguanine-DNA methyltransferase inhibits gastric carcinoma cell migration and invasion by downregulation of matrix metalloproteinase 2. *Anti Cancer Agents Med Chem* 16(9):1125–1132
- Li Y, Wang Y, Sun H, Zhang Y, Li H, Cong X, Yin W, Song W (2018) Association between matrix Metalloproteinase-1, 2, 3 polymorphisms and oral cancer risk: a meta-analysis. *Genet Test Mol Biomarkers* 22(8):456–464. <https://doi.org/10.1089/gtmb.2018.0089>
- Li X, Jin L, Tan Y (2021) Different roles of matrix metalloproteinase 2 in osteolysis of skeletal dysplasia and bone metastasis (review). *Mol Med Rep* 23(1):70. <https://doi.org/10.3892/mmr.2020.11708>
- Liao HY, Da CM, Liao B, Zhang HH (2021a) Roles of matrix metalloproteinase-7 (MMP-7) in cancer. *Clin Biochem* 92:9–18. <https://doi.org/10.1016/j.clinbiochem.2021.03.003>
- Liao CH, Tsai CW, Chang WS, Wang ZH, Gong CL, Wu HC, Wang BR, Hsu SW, Huang WC, Shen TC, Bau DT (2021b) Association of matrix metalloproteinase-1 genotypes with bladder cancer risk. In vivo (Athens, Greece) 35(5):2535–2540. <https://doi.org/10.21873/in vivo.12535>
- Lin LI, Lin DT, Chang CJ, Lee CY, Tang JL, Tien HF (2002) Marrow matrix metalloproteinases (MMPs) and tissue inhibitors of MMP in acute leukaemia: potential role of MMP-9 as a surrogate marker to monitor leukaemic status in patients with acute myelogenous leukaemia. *Br J Haematol* 117(4):835–841. <https://doi.org/10.1046/j.1365-2141.2002.03510.x>
- Lin TC, Yeh YM, Fan WL, Chang YC, Lin WM, Yang TY, Hsiao M (2019) Ghrelin upregulates oncogenic Aurora a to promote renal cell carcinoma invasion. *Cancers* 11(3):303. <https://doi.org/10.3390/cancers11030303>
- Liu C (2016) Pathological and prognostic significance of matrix metalloproteinase-2 expression in ovarian cancer: a meta-analysis. *Clin Exp Med* 16(3):375–382. <https://doi.org/10.1007/s10238-015-0369-y>
- Liu Y, Li Y, Liu Z, Zhang L, Anniko M, Duan M (2011) Prognostic significance of matrix metalloproteinase-20 overexpression in laryngeal squamous cell carcinoma. *Acta Otolaryngol* 131(7):769–773. <https://doi.org/10.3109/00016489.2011.560186>

- Liu HY, Gu WJ, Wang CZ, Ji XJ, Mu YM (2016) Matrix metalloproteinase-9 and -2 and tissue inhibitor of matrix metalloproteinase-2 in invasive pituitary adenomas: a systematic review and meta-analysis of case-control trials. *Medicine* 95(24):e3904. <https://doi.org/10.1097/MD.0000000000003904>
- Liu C, Li Y, Hu S, Chen Y, Gao L, Liu D, Guo H, Yang Y (2018a) Clinical significance of matrix metalloproteinase-2 in endometrial cancer: a systematic review and meta-analysis. *Medicine* 97(29):e10994. <https://doi.org/10.1097/MD.00000000000010994>
- Liu M, Qi Y, Zhao L, Chen D, Zhou Y, Zhou H, Lv Y, Zhang L, Jin S, Li S, Zou H, Jia W, Wang C, Jiang J, Liang W, Pang L, Li F (2018b) Matrix metalloproteinase-14 induces epithelial-to-mesenchymal transition in synovial sarcoma. *Hum Pathol* 80:201–209. <https://doi.org/10.1016/j.humpath.2017.12.031>
- Lohi J, Wilson CL, Roby JD, Parks WC (2001) Epilysin, a novel human matrix metalloproteinase (MMP-28) expressed in testis and keratinocytes and in response to injury. *J Biol Chem* 276:10134–10144
- Lou C, Zhu Z, Zhao Y, Zhu R, Zhao H (2017) Arctigenin, a lignan from *Arctium lappa* L., inhibits metastasis of human breast cancer cells through the downregulation of MMP-2/ -9 and heparanase in MDA-MB-231 cells. *Oncol Rep* 37(1):179–184
- Marsh T, Pietras K, McAllister SS (2013) Fibroblasts as architects of cancer pathogenesis. *Biochim Biophys Acta* 1832(7):1070–1078. <https://doi.org/10.1016/j.bbadis.2012.10.013>
- Martin DR, Santamaria S, Koch CD, Ahnström J, Apte SS (2021) Identification of novel ADAMTS1, ADAMTS4 and ADAMTS5 cleavage sites in versican using a label-free quantitative proteomics approach. *J Proteome* 249:104358. <https://doi.org/10.1016/j.jprot.2021.104358>
- Matchett EF, Wang S, Crawford BD (2019) Paralogues of Mmp11 and Timp4 interact during the development of the myotendinous junction in the zebrafish embryo. *J Dev Biol* 7(4):22. <https://doi.org/10.3390/jdb7040022>
- Maybee DV, Ink NL, Ali MAM (2022) Novel roles of MT1-MMP and MMP-2: beyond the extracellular milieu. *Int J Mol Sci* 23(17):9513. <https://doi.org/10.3390/ijms23179513>
- McCawley LJ, Matrisian LM (2001) Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol* 13:534–540
- McCawley LJ, Crawford HC, King LE, Mudgett J, Matrisian LM (2004) A protective role for matrix metalloproteinase-3 in squamous cell carcinoma. *Cancer Res* 64:6965–6972
- McCawley LJ, Wright J, LaFleur BJ, Crawford HC, Matrisian LM (2008) Keratinocyte expression of MMP3 enhances differentiation and prevents tumor establishment. *Am J Pathol* 173:1528–1539
- McMahan RS, Birkland TP, Smigiel KS, Vandivort TC, Rohani MG, Manicone AM, McGuire JK, Gharib SA, Parks WC (2016) Stromelysin-2 (MMP10) moderates inflammation by controlling macrophage activation. *J Immunol* 197(3):899–909. <https://doi.org/10.4049/jimmunol.1600502>
- McQuibban GA, Butler GS, Gong JH, Bendall L, Power C, Clark-Lewis I, Overall CM (2001) Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. *J Biol Chem* 276(47):43503–43508. <https://doi.org/10.1074/jbc.M107736200>
- Mehner C, Miller E, Nassar A, Bamlet WR, Radisky ES, Radisky DC (2015) Tumor cell expression of MMP3 as a prognostic factor for poor survival in pancreatic, pulmonary, and mammary carcinoma. *Genes Cancer* 6:480
- Min KW, Kim DH, Do SI, Pyo JS, Kim K, Chae SW, Sohn JH, Oh YH, Kim HJ, Choi SH, Choi YJ, Park CH (2013) Diagnostic and prognostic relevance of MMP-11 expression in the stromal fibroblast-like cells adjacent to invasive ductal carcinoma of the breast. *Ann Surg Oncol* 20 (Suppl 3):S433–S442. <https://doi.org/10.1245/s10434-012-2734-3>
- Miyata Y, Iwata T, Maruta S, Kanda S, Nishikido M, Koga S, Kanetake H (2007) Expression of matrix metalloproteinase-10 in renal cell carcinoma and its prognostic role. *Eur Urol* 52(3):791–797. <https://doi.org/10.1016/j.euro.2006.12.028>
- Morrison CJ, Overall CM (2006) TIMP independence of matrix metalloproteinase (MMP)-2-activation by membrane type 2 (MT2)-MMP is determined by contributions of both the MT2-MMP catalytic and hemopexin C domains. *J Biol Chem* 281(36):26528–26539. <https://doi.org/10.1074/jbc.M603331200>

- Mosig RA, Martignetti JA (2013) Loss of MMP-2 in murine osteoblasts upregulates osteopontin and bone sialoprotein expression in a circuit regulating bone homeostasis. *Dis Model Mech* 6(2):397–403. <https://doi.org/10.1242/dmm.007914>
- Motrescu ER, Blaise S, Etique N, Messaddeq N, Chenard MP, Stoll I, Tomasetto C, Rio MC (2008) Matrix metalloproteinase-11/stromelysin-3 exhibits collagenolytic function against collagen VI under normal and malignant conditions. *Oncogene* 27(49):6347–6355. <https://doi.org/10.1038/onc.2008.218>
- Mott JD, Werb Z (2004) Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol* 16(5):558–564. <https://doi.org/10.1016/j.ceb.2004.07.010>
- Müller M, Beck IM, Gadesmann J, Karschuk N, Paschen A, Proksch E, Djonov V, Reiss K, Sedlacek R (2010) MMP19 is upregulated during melanoma progression and increases invasion of melanoma cells. *Mod Pathol* 23(4):511–521. <https://doi.org/10.1038/modpathol.2009.183>
- Murray GI, Duncan ME, O’Neil P, Melvin WT, Fothergill JE (1996) Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. *Nat Med* 2(4):461–462
- Murray GI, Duncan ME, O’Neil P, McKay JA, Melvin WT, Fothergill JE (1998) Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. *J Pathol* 185(3):256–261
- Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 69(3):562–573. <https://doi.org/10.1016/j.cardiores.2005.12.002>
- Nakamura M, Miyamoto S, Maeda H, Ishii G, Hasebe T, Chiba T, Asaka M, Ochiai A (2005) Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. *Biochem Biophys Res Commun* 333(3):1011–1016. <https://doi.org/10.1016/j.bbrc.2005.06.010>
- Ng KT, Qi X, Kong KL, Cheung BY, Lo CM, Poon RT, Fan ST, Man K (2011) Overexpression of matrix metalloproteinase-12 (MMP-12) correlates with poor prognosis of hepatocellular carcinoma. *Eur J Cancer* 47(15):2299–2305. <https://doi.org/10.1016/j.ejca.2011.05.032>
- Niland S, Riscanevo AX, Eble JA (2021) Matrix metalloproteinases shape the tumor microenvironment in cancer progression. *Int J Mol Sci* 23(1):146. <https://doi.org/10.3390/ijms23010146>
- Niu J, Li XM, Wang X, Liang C, Zhang YD, Li HY, Liu FY, Sun H, Xie SQ, Fang D (2019) DKK1 inhibits breast cancer cell migration and invasion through suppression of β -catenin/MMP7 signaling pathway. *Cancer Cell Int* 19:168. <https://doi.org/10.1186/s12935-019-0883-1>
- Ozden F, Saygin C, Uzunaslan D, Onal B, Durak H, Aki H (2013) Expression of MMP-1, MMP-9 and TIMP-2 in prostate carcinoma and their influence on prognosis and survival. *J Cancer Res Clin Oncol* 139(8):1373–1382. <https://doi.org/10.1007/s00432-013-1453-x>
- Padala C, Tupurani MA, Puranam K et al (2017) Synergistic effect of collagenase-1 (MMP1), stromelysin-1 (MMP3) and gelatinase-B (MMP9) gene polymorphisms in BC. *PLoS One* 12(9):e0184448. <https://doi.org/10.1371/journal.pone.0184448>
- Pei D (1999a) Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. *J Biol Chem* 274(13):8925–8932. <https://doi.org/10.1074/jbc.274.13.8925>
- Pei D (1999b) Leukolysin/MMP25/MT6-MMP: a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. *Cell Res* 9(4):291–303. <https://doi.org/10.1038/sj.cr.7290028>
- Pei D, Weiss SJ (1995) Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature* 375(6528):244–247. <https://doi.org/10.1038/375244a0>
- Pei D, Kang T, Qi H (2000) Cysteine array matrix metalloproteinase (CA-MMP)/MMP-23 is a type II transmembrane matrix metalloproteinase regulated by a single cleavage for both secretion and activation. *J Biol Chem* 275(43):33988–33997. <https://doi.org/10.1074/jbc.M006493200>
- Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M (2016) Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *Int J Mol Sci* 17(6):868. <https://doi.org/10.3390/ijms17060868>

- Poudel B, Ki HH, Luyen BT, Lee YM, Kim YH, Kim DK (2016) Triticumoside induces apoptosis via caspase-dependent mitochondrial pathway and inhibits migration through downregulation of MMP2/9 in human lung cancer cells. *Acta Biochim Biophys Sin Shanghai* 48(2):153–160
- Pratt J, Iddir M, Bourgault S, Annabi B (2016) Evidence of MTCBP-1 interaction with the cytoplasmic domain of MT1-MMP: implications in the autophagy cell index of high-grade glioblastoma. *Mol Carcinog* 55(2):148–160. <https://doi.org/10.1002/mc.22264>
- Przybyłowska K, Kluczn A, Zadrozny M, Krawczyk T, Kulig A, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J, Blasiak J (2006) Polymorphisms of the promoter regions of matrix metalloproteinases genes MMP-1 and MMP-9 in breast cancer. *Breast Cancer Res Treat* 95(1): 65–72. <https://doi.org/10.1007/s10549-005-9042-6>
- Qin G, Luo M, Chen J, Dang Y, Chen G, Li L, Zeng J, Lu Y, Yang J (2016) Reciprocal activation between MMP-8 and TGF- β 1 stimulates EMT and malignant progression of hepatocellular carcinoma. *Cancer Lett* 374(1):85–95. <https://doi.org/10.1016/j.canlet.2016.02.001>
- Quintero-Fabián S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, Ramírez-Camacho MA, Alvarez-Sánchez ME (2019) Role of matrix metalloproteinases in angiogenesis and cancer. *Front Oncol* 9:1370. <https://doi.org/10.3389/fonc.2019.01370>
- Rajkumar T, Sabitha K, Vijayalakshmi N, Shirley S, Bose MV, Gopal G et al (2011) Identification and validation of genes involved in cervical tumorigenesis. *BMC Cancer* 11:80
- Rashid ZA, Bardaweel SK (2023) Novel matrix metalloproteinase-9 (MMP-9) inhibitors in cancer treatment. *Int J Mol Sci* 24(15):12133. <https://doi.org/10.3390/ijms241512133>
- Redondo-Muñoz J, Ugarte-Berzal E, Terol MJ, Van den Steen PE, Hernández del Cerro M, Roderfeld M, Roeb E, Opendakker G, García-Marco JA, García-Pardo A (2010) Matrix metalloproteinase-9 promotes chronic lymphocytic leukemia b cell survival through its hemopexin domain. *Cancer Cell* 17(2):160–172. <https://doi.org/10.1016/j.ccr.2009.12.044>
- Ren F, Tang R, Zhang X, Madushi WM, Luo D, Dang Y, Li Z, Wei K, Chen G (2015) Overexpression of MMP family members functions as prognostic biomarker for breast cancer patients: a systematic review and meta-analysis. *PLoS One* 10(8):e0135544. <https://doi.org/10.1371/journal.pone.0135544>
- Riddick AC, Shukla CJ, Pennington CJ, Bass R, Nuttall RK, Hogan A, Sethia KK, Ellis V, Collins AT, Maitland NJ, Ball RY, Edwards DR (2005) Identification of degradome components associated with prostate cancer progression by expression analysis of human prostatic tissues. *Br J Cancer* 92(12):2171–2180. <https://doi.org/10.1038/sj.bjc.6602630>
- Saad H, Zahran MA, Hendy O, Abdel-Samiee M, Bedair HM, Abdelsameea E (2020) Matrix metalloproteinase-11 gene polymorphisms as a risk for hepatocellular carcinoma development in Egyptian patients. *Asian Pac J Cancer Prev* 21(12):3725–3734. <https://doi.org/10.31557/APJCP.2020.21.12.3725>
- Sakamoto T, Seiki M (2009) Cytoplasmic tail of MT1-MMP regulates macrophage motility independently from its protease activity. *Genes Cells* 14(5):617–626. <https://doi.org/10.1111/j.1365-2443.2009.01293.x>
- Saleh M, Khalil M, Abdellateif MS, Ebeid E, Madney Y, Kandeel EZ (2021) Role of matrix metalloproteinase MMP-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP-1) in the clinical progression of pediatric acute lymphoblastic leukemia. *Hematology (Amsterdam, Netherlands)* 26(1):758–768. <https://doi.org/10.1080/16078454.2021.1978763>
- Sbardella D, Fasciglione GF, Gioia M, Ciaccio C, Tundo GR, Marini S, Coletta M (2012) Human matrix metalloproteinases: an ubiquitarian class of enzymes involved in several pathological processes. *Mol Asp Med* 33(2):119–208. <https://doi.org/10.1016/j.mam.2011.10.015>
- Scheau C, Badarau IA, Costache R, Caruntu C, Mihai GL, Didilescu AC, Constantin C, Neagu M (2019) The role of matrix metalloproteinases in the epithelial-mesenchymal transition of hepatocellular carcinoma. *Anal Cell Pathol (Amst)* 2019:9423907. <https://doi.org/10.1155/2019/9423907>

- Schlage P, Kockmann T, Sabino F, Kizhakkedathu JN, Auf dem Keller U (2015) Matrix metalloproteinase 10 degradomics in keratinocytes and epidermal tissue identifies bioactive substrates with pleiotropic functions. *Mol Cell Proteomics* 14(12):3234–3246. <https://doi.org/10.1074/mcp.M115.053520>
- Schultz GS, Wysocki A (2009) Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen* 17(2):153–162. <https://doi.org/10.1111/j.1524-475X.2009.00466.x>
- Sena P, Mariani F, Marzona L, Benincasa M, Ponz de Leon M, Palumbo C, Roncucci L (2012) Matrix metalloproteinases 15 and 19 are stromal regulators of colorectal cancer development from the early stages. *Int J Oncol* 41(1):260–266. <https://doi.org/10.3892/ijo.2012.1441>
- Shamamian P, Schwartz JD, Pocock BJ, Monea S, Whiting D, Marcus SG, Mignatti P (2001) Activation of progelatinase a (MMP-2) by neutrophil elastase, cathepsin G, and proteinase-3: a role for inflammatory cells in tumor invasion and angiogenesis. *J Cell Physiol* 189(2):197–206. <https://doi.org/10.1002/jcp.10014>
- Shaverdashvili K, Zhang K, Osman I, Honda K, Jobava R, Bedogni B (2015) MT1-MMP dependent repression of the tumor suppressor SPRY4 contributes to MT1-MMP driven melanoma cell motility. *Oncotarget* 6(32):33512–33522. <https://doi.org/10.18632/oncotarget.5258>
- Shen TC, Hsia TC, Chao CY, Chen WC, Chen CY, Chen WC, Lin YT, Hsiao CL, Chang WS, Tsai CW, Bau DT (2017) The contribution of MMP-8 promoter polymorphisms in lung cancer. *Anticancer Res* 37(7):3563–3567. <https://doi.org/10.21873/anticancer.11726>
- Shih WL, Liao MH, Yu FL, Lin PY, Hsu HY, Chiu SJ (2008) AMF/PGI transactivates the MMP-3 gene through the activation of Src-RhoA-phosphatidylinositol 3-kinase signaling to induce hepatoma cell migration. *Cancer Lett* 270(2):202–217. <https://doi.org/10.1016/j.canlet.2008.05.005>
- Shimizu-Hirota R, Xiong W, Baxter BT, Kunkel SL, Maillard I, Chen XW, Sabeh F, Liu R, Li XY, Weiss SJ (2012) MT1-MMP regulates the PI3K/Akt/mTOR-dependent control of macrophage immune function. *Genes Dev* 26(4):395–413. <https://doi.org/10.1101/gad.178749.111>
- Shin DH, Dier U, Melendez JA, Hempel N (2015) Regulation of MMP-1 expression in response to hypoxia is dependent on the intracellular redox status of metastatic bladder cancer cells. *Biochim Biophys Acta* 1852(12):2593–2602. <https://doi.org/10.1016/j.bbdis.2015.09.001>
- Shipley JM, Doyle GA, Fliszar CJ, Ye QZ, Johnson LL, Shapiro SD, Welgus HG, Senior RM (1996) The structural basis for the elastolytic activity of the 92-kDa and 72-kDa gelatinases. Role of the fibronectin type II-like repeats. *J Biol Chem* 271(8):4335–4341. <https://doi.org/10.1074/jbc.271.8.4335>
- Simmer JP, Hu JC (2002) Expression, structure, and function of enamel proteinases. *Connect Tissue Res* 43(2–3):441–449. <https://doi.org/10.1080/03008200290001159>
- Singh R, Mandhani A, Agrawal V, Garg M (2018) Positive correlation between matrix metalloproteinases and epithelial-to-mesenchymal transition and its association with clinical outcome in bladder cancer patients. *Cancer Microenviron* 11(1):23–39. <https://doi.org/10.1007/s12307-017-0199-4>
- Sinha SK, Asotra K, Uzui H, Nagwani S, Mishra V, Rajavashisth TB (2014) Nuclear localization of catalytically active MMP-2 in endothelial cells and neurons. *Am J Transl Res* 6(2):155–162
- Sirniö P, Tuomisto A, Tervahartiala T, Sorsa T, Klintrup K, Karhu T, Herzig KH, Mäkelä J, Karttunen TJ, Salo T, Mäkinen MJ, Väyrynen JP (2018) High-serum MMP-8 levels are associated with decreased survival and systemic inflammation in colorectal cancer. *Br J Cancer* 119(2):213–219. <https://doi.org/10.1038/s41416-018-0136-4>
- Sohail A, Sun Q, Zhao H, Bernardo MM, Cho JA, Fridman R (2008) MT4-(MMP17) and MT6-MMP (MMP25), a unique set of membrane-anchored matrix metalloproteinases: properties and expression in cancer. *Cancer Metastasis Rev* 27(2):289–302. <https://doi.org/10.1007/s10555-008-9129-8>
- Song Z, Wu Y, Yang J, Yang D, Fang X (2017) Progress in the treatment of advanced gastric cancer. *Tumour Biol* 39(7):1010428317714626. <https://doi.org/10.1177/1010428317714626>

- Sounni NE, Roghi C, Chabottaux V, Janssen M, Munaut C, Maquoui E, Galvez BG, Gilles C, Franken F, Murphy G, Foidart JM, Noel A (2004) Up-regulation of vascular endothelial growth factor- α by active membrane-type 1 matrix metalloproteinase through activation of Src-tyrosine kinases. *J Biol Chem* 279(14):13564–13574. <https://doi.org/10.1074/jbc.M307688200>
- Spill F, Reynolds DS, Kamm RD, Zaman MH (2016) Impact of the physical microenvironment on tumor progression and metastasis. *Curr Opin Biotechnol* 40:41–48. <https://doi.org/10.1016/j.copbio.2016.02.007>
- Srivastava P, Gangwar R, Kapoor R, Mittal RD (2010) Bladder cancer risk associated with genotypic polymorphism of the matrix metalloproteinase-1 and 7 in north Indian population. *Dis Markers* 29(1):37–46. <https://doi.org/10.3233/DMA-2010-0724>
- Stadlmann S, Pollheimer J, Moser PL, Raggi A, Amberger A, Margreiter R, Offner FA, Mikuz G, Dirnhofer S, Moch H (2003) Cytokine-regulated expression of collagenase-2 (MMP-8) is involved in the progression of ovarian cancer. *Eur J Cancer* 39(17):2499–2505. <https://doi.org/10.1016/j.ejca.2003.08.011>
- Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 17:463–516. <https://doi.org/10.1146/annurev.cellbio.17.1.463>
- Stover DG, Bierie B, Moses HL (2007) A delicate balance: TGF- β and the tumor microenvironment. *J Cell Biochem* 101(4):851–861. <https://doi.org/10.1002/jcb.21149>
- St-Pierre Y (2021) Towards a better understanding of the relationships between Galectin-7, p53 and MMP-9 during cancer progression. *Biomol Ther* 11(6):879. <https://doi.org/10.3390/biom11060879>
- Su CH, Lane HY, Hsiao CL, Liu LC, Ji HX, Li HT, Yen ST, Su CH, Hsia TC, Chang WS, Tsai CW, Bau DT (2016) Matrix metalloproteinase-1 genetic polymorphism in breast cancer in Taiwanese. *Anticancer Res* 36(7):3341–3345
- Sun X, Liu Y (2022) Matrix metalloproteinase-10 in kidney injury repair and disease. *Int J Mol Sci* 23(4):2131. <https://doi.org/10.3390/ijms23042131>
- Sun KT, Tsai CW, Chang WS, Shih LC, Chen LY, Tsai MH, Ji HX, Hsiao CL, Liu YC, Li CY, Bau DT (2016) The contribution of matrix metalloproteinase-1 genotype to oral cancer susceptibility in Taiwan. *In vivo (Athens, Greece)* 30(4):439–444
- Sun J, Zhang Z, Chen J, Xue M, Pan X (2021) ELTD1 promotes invasion and metastasis by activating MMP2 in colorectal cancer. *Int J Biol Sci* 17(12):3048–3058. <https://doi.org/10.7150/ijbs.62293>
- Surlin V, Ioana M, Pleşea IE (2011) Genetic patterns of metalloproteinases and their tissular inhibitors—clinicopathologic and prognostic significance in colorectal cancer. *Romanian J Morphol Embryol* 52(1 Suppl):231–236
- Szabova L, Yamada SS, Birkedal-Hansen H, Holmbeck K (2005) Expression pattern of four membrane-type matrix metalloproteinases in the normal and diseased mouse mammary gland. *J Cell Physiol* 205(1):123–132. <https://doi.org/10.1002/jcp.20385>
- Tan RJ, Liu Y (2012) Matrix metalloproteinases in kidney homeostasis and diseases. *Am J Physiol Renal Physiol* 302(11):F1351–F1361. <https://doi.org/10.1152/ajprenal.00037.2012>
- Tan B, Jaulin A, Bund C, Outilaft H, Wendling C, Chenard MP, Alpy F, Cicek AE, Namer IJ, Tomasetto C, Dali-Youcef N (2020) Matrix metalloproteinase-11 promotes early mouse mammary gland tumor growth through metabolic reprogramming and increased IGF1/AKT/FoxO1 signaling pathway, enhanced ER stress and alteration in mitochondrial UPR. *Cancers* 12(9):2357. <https://doi.org/10.3390/cancers12092357>
- Teng K, Wei S, Zhang C, Chen J, Chen J, Xiao K, Liu J, Dai M, Guan X, Yun J, Xie D (2019) KIFC1 is activated by TCF-4 and promotes hepatocellular carcinoma pathogenesis by regulating HMGA1 transcriptional activity. *J Exp Clin Cancer Res* 38(1):329. <https://doi.org/10.1186/s13046-019-1331-8>
- Tian R, Li X, Gao Y, Li Y, Yang P, Wang K (2018) Identification and validation of the role of matrix metalloproteinase-1 in cervical cancer. *Int J Oncol* 52(4):1198–1208. <https://doi.org/10.3892/ijo.2018.4267>

- Torgovnick A, Schumacher B (2015) DNA repair mechanisms in cancer development and therapy. *Front Genet* 6:157. <https://doi.org/10.3389/fgene.2015.00157>
- Trivedi V, Boire A, Tchernychev B, Kaneider NC, Leger AJ, O'Callaghan K, Covic L, Kuliopulos A (2009) Platelet matrix metalloprotease-1 mediates thrombogenesis by activating PAR1 at a cryptic ligand site. *Cell* 137(2):332–343. <https://doi.org/10.1016/j.cell.2009.02.018>
- Truong A, Yip C, Paye A, Blacher S, Munaut C, Deroanne C, Noel A, Sounni NE (2016) Dynamics of internalization and recycling of the prometastatic membrane type 4 matrix metalloproteinase (MT4-MMP) in breast cancer cells. *FEBS J* 283(4):704–722. <https://doi.org/10.1111/febs.13625>
- Tsai CW, Hsu HM, Wang YC, Chang WS, Shih LC, Sun KT, Hung YW, Yang YC, Gong CL, Bau DT (2018) Contribution of MMP2 promoter genotypes to oral cancer susceptibility, recurrence and metastasis in Taiwan. *Anticancer Res* 38(12):6821–6826. <https://doi.org/10.21873/anticancer.13055>
- Tsunezumi J, Higashi S, Miyazaki K (2009) Matrilysin (MMP-7) cleaves C-type lectin domain family 3 member a (CLEC3A) on tumor cell surface and modulates its cell adhesion activity. *J Cell Biochem* 106(4):693–702. <https://doi.org/10.1002/jcb.22062>
- Turunen SP, Tatti-Bugaeva O, Lehti K (2017) Membrane-type matrix metalloproteases as diverse effectors of cancer progression. *Biochim Biophys Acta, Mol Cell Res* 1864(11 Pt A):1974–1988. <https://doi.org/10.1016/j.bbamcr.2017.04.002>
- Upadhyay P, Gardi N, Desai S, Chandrani P, Joshi A, Dharavath B, Arora P, Bal M, Nair S, Dutt A (2017) Genomic characterization of tobacco/nut chewing HPV-negative early stage tongue tumors identify MMP10 as a candidate to predict metastases. *Oral Oncol* 73:56–64. <https://doi.org/10.1016/j.oraloncology.2017.08.003>
- Väänänen A, Srinivas R, Parikka M, Palosaari H, Bartlett JD, Iwata K, Grenman R, Stenman UH, Sorsa T, Salo T (2001) Expression and regulation of MMP-20 in human tongue carcinoma cells. *J Dent Res* 80(10):1884–1889. <https://doi.org/10.1177/00220345010800100501>
- Väänänen A, Tjäderhane L, Eklund L, Heljasvaara R, Pihlajaniemi T, Herva R, Ding Y, Bartlett JD, Salo T (2004) Expression of collagen XVIII and MMP-20 in developing teeth and odontogenic tumors. *Matrix Biol* 23(3):153–161. <https://doi.org/10.1016/j.matbio.2004.04.003>
- Vaghari-Tabari M, Ferns GA, Qujeq D, Andevani AN, Sabahi Z, Moein S (2021) Signaling, metabolism, and cancer: an important relationship for therapeutic intervention. *J Cell Physiol* 236(8):5512–5532. <https://doi.org/10.1002/jcp.30276>
- Van Doren SR (2022) MMP-7 marks severe pancreatic cancer and alters tumor cell signaling by proteolytic release of ectodomains. *Biochem Soc Trans* 50(2):839–851. <https://doi.org/10.1042/BST20210640>
- Van Hove I, Lemmens K, Van de Velde S, Verslegers M, Moons L (2012) Matrix metalloproteinase-3 in the central nervous system: a look on the bright side. *J Neurochem* 123(2):203–216. <https://doi.org/10.1111/j.1471-4159.2012.07900.x>
- Van Lint P, Libert C (2006) Matrix metalloproteinase-8: cleavage can be decisive. *Cytokine Growth Factor Rev* 17(4):217–223. <https://doi.org/10.1016/j.cytogfr.2006.04.001>
- Vandooren J, Van den Steen PE, Opdenakker G (2013) Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. *Crit Rev Biochem Mol Biol* 48(3):222–272. <https://doi.org/10.3109/10409238.2013.770819>
- Velinov N, Aebersold D, Haeni N, Hlushchuk R, Weinstein F, Sedlacek R, Djonov V (2007) Matrix metalloproteinase-19 is a predictive marker for tumor invasiveness in patients with oropharyngeal squamous cell carcinoma. *Int J Biol Markers* 22(4):265–273. <https://doi.org/10.5301/IJBM.2008.2632>
- Verma SP, Das P (2018) Monensin induces cell death by autophagy and inhibits matrix metalloproteinase 7 (MMP7) in UOK146 renal cell carcinoma cell line. *In Vitro Cell Dev Biol Anim* 54(10):736–742. <https://doi.org/10.1007/s11626-018-0298-7>
- Vincenti MP, Brinckerhoff CE (2002) Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res* 4(3):157–164. <https://doi.org/10.1186/ar401>

- Visse R, Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 92(8):827–839. <https://doi.org/10.1161/01.RES.0000070112.80711.3D>
- Von Marschall Z, Riecken EO, Rosewicz S (1998) Stromelysin 3 is over expressed in human pancreatic carcinoma and regulated by retinoic acid in pancreatic carcinoma cell lines. *Gut* 43(5):692–698. <https://doi.org/10.1136/gut.43.5.692>
- Wali N, Hosokawa K, Malik S, Saito H, Miyaguchi K, Imajoh-Ohmi S, Miki Y, Nakanishi A (2014) Centrosomal BRCA2 is a target protein of membrane type-1 matrix metalloproteinase (MT1-MMP). *Biochem Biophys Res Commun* 443(4):1148–1154. <https://doi.org/10.1016/j.bbrc.2013.12.103>
- Wang X, Khalil RA (2018) Matrix metalloproteinases, vascular remodeling, and vascular disease. *Adv Pharmacol (San Diego, Calif)* 81:241–330. <https://doi.org/10.1016/bs.apha.2017.08.002>
- Wang L, Kong B (2015) Analysis of the association of matrix metalloproteinase-1 gene promoter (rs1799750) polymorphism and risk of ovarian cancer. *Int J Gynecol Cancer* 25(6):961–967. <https://doi.org/10.1097/IGC.0000000000000463>
- Wang Z, Juttermann R, Soloway PD (2000) TIMP-2 is required for efficient activation of proMMP-2 in vivo. *J Biol Chem* 275(34):26411–26415. <https://doi.org/10.1074/jbc.M001270200>
- Wang L, Yuan J, Tu Y, Mao X, He S, Fu G, Zong J, Zhang Y (2013) Co-expression of MMP-14 and MMP-19 predicts poor survival in human glioma. *Clin Transl Oncol* 15(2):139–145. <https://doi.org/10.1007/s12094-012-0900-5>
- Wang B, Ding YM, Fan P, Wang B, Xu JH, Wang WX (2014a) Expression and significance of MMP2 and HIF-1 α in hepatocellular carcinoma. *Oncol Lett* 8(2):539–546. <https://doi.org/10.3892/ol.2014.2189>
- Wang HL, Zhou PY, Zhang Y, Liu P (2014b) Relationships between abnormal MMP2 expression and prognosis in gastric cancer: a meta-analysis of cohort studies. *Cancer Biother Radiopharm* 29(4):166–172. <https://doi.org/10.1089/cbr.2014.1608>
- Wang JJ, Lei KF, Han F (2018) Tumor microenvironment: recent advances in various cancer treatments. *Eur Rev Med Pharmacol Sci* 22(12):3855–3864. https://doi.org/10.26355/eurev_201806_15270
- Wang SF, Huang KH, Tseng WC, Lo JF, Li AF, Fang WL, Chen CF, Yeh TS, Chang YL, Chou YC, Hung HH, Lee HC (2020a) DNAJA3/Tid1 is required for mitochondrial DNA maintenance and regulates migration and invasion of human gastric cancer cells. *Cancers* 12(11):3463. <https://doi.org/10.3390/cancers12113463>
- Wang H, Wu JX, Chen XP, Zhang Q, Wei HB, Wang HJ, Yang X, Zhang DH (2020b) Expression and clinical significance of MMP-28 in bladder cancer. *Technol Cancer Res Treat* 19:1533033820974017. <https://doi.org/10.1177/1533033820974017>
- Weber CE, Kuo PC (2012) The tumor microenvironment. *Surg Oncol* 21(3):172–177. <https://doi.org/10.1016/j.suronc.2011.09.001>
- Wen X, Liu H, Yu K, Liu Y (2014) Matrix metalloproteinase 2 expression and survival of patients with osteosarcoma: a meta-analysis. *Tumour Biol* 35(1):845–848. <https://doi.org/10.1007/s13277-013-1116-1>
- Włodarczyk J, Stolte M, Mueller J (2001) E-cadherin, beta-catenin and stromelysin-3 expression in de novo carcinoma of the colorectum. *Pol J Pathol* 52(3):119–124
- Wu MH, Yueh TC, Chang WS, Tsai CW, Fu CK, Yang MD, Yu CC, Bau DT (2021) Contribution of matrix metalloproteinase-1 genotypes to colorectal cancer in Taiwan. *Cancer Genomics Proteomics* 18(3):245–251. <https://doi.org/10.21873/cgp.20255>
- Xiang Y, Li JP, Guo W, Wang DQ, Yao A, Zhang HM, Huang F, Li HH, Dai ZT, Zhang ZJ, Li H, Tan Y, Chen K, Bao LY, Liao XH (2019) Novel interactions between ER α -36 and STAT3 mediate breast cancer cell migration. *Cell Commun Signal* 17(1):93. <https://doi.org/10.1186/s12964-019-0409-4>
- Xie Y, Mustafa A, Yerzhan A, Merzhakupova D, Yerlan PN, Orakov A, Wang X, Huang Y, Miao L (2017) Nuclear matrix metalloproteinases: functions resemble the evolution from the intracellular to the extracellular compartment. *Cell Death Discov* 3:17036. <https://doi.org/10.1038/cddiscovery.2017.36>

- Yan T, Lin Z, Jiang J, Lu S, Chen M, Que H, He X, Que G, Mao J, Xiao J, Zheng Q (2015) MMP14 regulates cell migration and invasion through epithelial-mesenchymal transition in nasopharyngeal carcinoma. *Am J Transl Res* 7(5):950–958
- Yang P, Lv S, Wang Y, Peng Y, Ye Z, Xia Z, Ding G, Cao X, Crane JL (2018) Preservation of type H vessels and osteoblasts by enhanced preosteoclast platelet-derived growth factor type BB attenuates glucocorticoid-induced osteoporosis in growing mice. *Bone* 114:1–13. <https://doi.org/10.1016/j.bone.2018.05.025>
- Yang X, Du X, Sun L, Zhao X, Zhu J, Li G, Tian J, Li X, Wang Z (2019a) SULT2B1b promotes epithelial-mesenchymal transition through activation of the β -catenin/MMP7 pathway in hepatocytes. *Biochem Biophys Res Commun* 510(4):495–500. <https://doi.org/10.1016/j.bbrc.2019.01.034>
- Yang H, Jiang P, Liu D, Wang HQ, Deng Q, Niu X, Lu L, Dai H, Wang H, Yang W (2019b) Matrix metalloproteinase 11 is a potential therapeutic target in lung adenocarcinoma. *Mol Ther Oncolytics* 14:82–93. <https://doi.org/10.1016/j.omto.2019.03.012>
- Yokoyama Y, Grünebach F, Schmidt SM, Heine A, Häntschel M, Stevanovic S, Rammensee HG, Brossart P (2008) Matrilysin (MMP-7) is a novel broadly expressed tumor antigen recognized by antigen-specific T cells. *Clin Cancer Res* 14(17):5503–5511. <https://doi.org/10.1158/1078-0432.CCR-07-4041>
- Young D, Das N, Anowai A, Dufour A (2019) Matrix metalloproteases as influencers of the cells' social media. *Int J Mol Sci* 20(16):3847. <https://doi.org/10.3390/ijms20163847>
- Yu G, Herazo-Maya JD, Nukui T, Romkes M, Parwani A, Juan-Guardela BM, Robertson J, Gaudie J, Siegfried JM, Kaminski N, Kass DJ (2014) Matrix metalloproteinase-19 promotes metastatic behavior in vitro and is associated with increased mortality in non-small cell lung cancer. *Am J Respir Crit Care Med* 190(7):780–790. <https://doi.org/10.1164/rccm.201310-1903OC>
- Yu X, Jin J, Zheng Y, Zhu H, Xu H, Ma J, Lan Q, Zhuang Z, Chen CC, Li M (2021) GBP5 drives malignancy of glioblastoma via the Src/ERK1/2/MMP3 pathway. *Cell Death Dis* 12(2):203. <https://doi.org/10.1038/s41419-021-03492-3>
- Zhai LL, Wu Y, Cai CY, Huang Q, Tang ZG (2016) High-level expression and prognostic significance of matrix metalloprotease-19 and matrix metalloprotease-20 in human pancreatic ductal adenocarcinoma. *Pancreas* 45(7):1067–1072. <https://doi.org/10.1097/MPA.0000000000000569>
- Zhang J, Jin X, Fang S, Wang R, Li Y, Wang N, Guo W, Wang Y, Wen D, Wei L, Dong Z, Kuang G (2005) The functional polymorphism in the matrix metalloproteinase-7 promoter increases susceptibility to esophageal squamous cell carcinoma, gastric cardiac adenocarcinoma and non-small cell lung carcinoma. *Carcinogenesis* 26(10):1748–1753. <https://doi.org/10.1093/carcin/bgi144>
- Zhang J, Pan Q, Yan W, Wang Y, He X, Zhao Z (2018) Overexpression of MMP21 and MMP28 is associated with gastric cancer progression and poor prognosis. *Oncol Lett* 15(5):7776–7782. <https://doi.org/10.3892/ol.2018.8328>
- Zhang LF, Zhu LJ, Zhang W, Yuan W, Song NH, Zuo L, Mi YY, Wang ZJ, Zhang W (2019) MMP-8 C-799 T, Lys460Thr, and Lys87Glu variants are not related to risk of cancer. *BMC Med Genet* 20(1):162. <https://doi.org/10.1186/s12881-019-0890-z>
- Zhao S, Zhao Y, Niu P, Wang N, Tang Z, Zan L, Li K (2011) Molecular characterization of porcine MMP19 and MMP23B genes and its association with immune traits. *Int J Biol Sci* 7:1101–1113
- Zhen G, Cao X (2014) Targeting TGF β signaling in subchondral bone and articular cartilage homeostasis. *Trends Pharmacol Sci* 35(5):227–236. <https://doi.org/10.1016/j.tips.2014.03.005>
- Zhou Z, Ma X, Wang F, Sun L, Zhang G (2018) A matrix metalloproteinase-1 polymorphism, MMP1-1607 (1G>2G), is associated with increased cancer risk: a meta-analysis including 21,327 patients. *Dis Markers* 2018:7565834. <https://doi.org/10.1155/2018/7565834>
- Zhu P, Goh YY, Chin HF, Kersten S, Tan NS (2012) Angiopoietin-like 4: a decade of research. *Biosci Rep* 32(3):211–219. <https://doi.org/10.1042/BSR20110102>
- Zhu H, Wu TC, Chen WQ, Zhou LJ, Wu Y, Zeng L, Pei HP (2013) Roles of galectin-7 and S100A9 in cervical squamous carcinoma: clinicopathological and in vitro evidence. *Int J Cancer* 132(5):1051–1059. <https://doi.org/10.1002/ijc.27764>



The Vascular Endothelium in Cancer

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Abstract

The endothelium plays a key role in regulating the progression and metastasis of tumours. The blood vessels not only deliver oxygen and nutrients to tumours but also provide the pathway for cancerous cells to metastasise. The formation of new blood vessels (angiogenesis) is crucial for tumour growth, progression, and metastasis. Endothelial cells (ECs) form new blood vessels by migrating and proliferating towards the tumour, following pro-angiogenic signals. The crosstalk between ECs and tumour cells in the tumour microenvironment is essential for tumour development and progression. When compared to normal ECs, tumour-associated ECs exhibit different characteristics and could be a key therapeutic target. Understanding the role of ECs and their interactions with the tumour cells can help in the implementation of new therapeutic regimes for the management of tumours. Disruption of the EC-tumour cell interactions might be an effective strategy for cancer therapy. In this chapter, the role of ECs in tumour angiogenesis and cancer progression, endothelial-tumour cell communication in tumour microenvironment, and endothelial malignancies and dysregulation of EC signalling pathways are presented.

Keywords

Angiogenesis · Cancer progression · Endothelium · Metastasis

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1 Introduction

The role of the cardiovascular system (CVS) is to deliver oxygen, nutrients, and hormones to all tissues, remove waste products, and maintain homeostasis. All blood vessels transporting the blood throughout the body and connecting all organ systems are part of the vascular system. They must perform their functions while adapting continuously to acute and chronic changes in the tissues they perfuse. The vascular network is organised in a tree-like hierarchy structure based on its functions, vessels varying in size, wall structure, and shape. The arterial system carries blood away from the heart, with larger arteries feeding into gradually smaller vessels, namely arterioles and capillaries where the gas exchange is performed. The venous system collects deoxygenated blood from capillaries to venules, veins and finally to the heart. The capillaries are adapted for gas exchange by having a small diameter and narrow walls (Lugano et al. 2020).

The vascular endothelium is a monolayer of squamous epithelial cells that forms the inner lining of the vessels. Endothelial cells (ECs) line the entire CVS, from heart (endocardium) to arteries, veins, and the smallest capillaries. The endothelium is surrounded by a vascular basement membrane which is a specialised extracellular matrix (ECM) composed of proteins, glycoproteins, and collagen fibres that provide structural support. Vascular smooth muscle envelopes the larger-calibre vessels (arteries, veins, and arterioles), while the smaller-calibre vessels (capillaries and post-capillary venules) are sparsely supported by pericytes. These mural cells are contractile cells with role in blood flow regulation, stabilisation of vessel walls as well as vessel maturation in the final stages of angiogenesis. The vascular endothelium is in direct contact with the blood components flowing in the lumen and acts as a selective barrier. This is particularly important in capillaries, where the endothelium regulates the exchange of gases and nutrients, waste products, immune cells, and signalling molecules between the bloodstream and the surrounding tissue. The endothelium is adaptive to both physiological and pathological stimuli (Dight et al. 2022), this adaptability being indispensable for maintaining vascular homeostasis. Some of its functions include the control of blood fluidity and haemostasis, vascular tone, inflammation, leukocyte trafficking, and angiogenesis. Furthermore, the vascular endothelium can be regarded as an endocrine organ, as it secretes vasoactive peptide hormones, coagulation factors, growth factors, and other biologically active mediators with widespread effects on the body (Reglero-Real et al. 2016).

Angiogenesis, formation of new blood vessels, is essential for tumour growth and progression. Besides the supply of oxygen and nutrients to the tumour cells, the blood vessels provide the pathway for cancerous cells to metastasise (Yao and Zeng 2023). Recent research has indicated that tumour-associated ECs possess distinct characteristics compared to normal vascular ECs. These cells play a significant role in tumour progression and metastasis, making them a crucial focus for cancer treatment (Yao and Zeng 2023). ECs orchestrate angiogenesis within the tumour microenvironment and foster both proliferation and metastasis of cancer cells (Wagner et al. 2017). In addition, ECs are implicated in promoting tumour drug

resistance by secreting tumour necrosis factor alpha (TNF- α) and inducing the acquisition of drug resistance (Acharyya et al. 2012).

2 The Role of ECs in Vascular Morphogenesis and Function

ECs, forming the vascular endothelium, play a central role in the development and maintenance of the functions of the CVS. The development of the new vasculature in an organism is referred to as vascular morphogenesis and consists of two different processes, vasculogenesis and angiogenesis. Vasculogenesis is the *de novo* formation of blood vessels during early embryogenic development and is followed by angiogenesis which consists of the vasculature being expanded and remodelled. Initially, angioblasts (endothelial cell progenitors) differentiate into ECs, which aggregate into a network of tubules forming the primitive vascular plexus (Trimm and Red-Horse 2023). Through various mechanisms of angiogenesis, the vascular plexus is remodelled into branched, tree-like structures representing the future vascular system. Remodelling also involves regression of unnecessary vessels. Maturation occurs through recruitment of mural cells, namely pericytes and smooth muscle cells. Vascular endothelial growth factor (VEGF) and Notch signalling contribute to the regulation of vasculogenesis and angiogenesis (Trimm and Red-Horse 2023). After birth, angiogenesis assists the CVS in adapting to changes triggered by growth, reproduction, physical exercise, wound repair, and by pathological conditions such as cancer.

ECs are central to the functions exerted by the vascular endothelium, including transport and tissue-blood barrier functions, regulation of vascular tone, control of blood fluidity and haemostasis, inflammation, leukocyte trafficking, and angiogenesis. These cells are highly specialised and exhibit heterogeneous phenotypes (Dight et al. 2022), allowing them to play such roles. The continuous (skin), fenestrated (kidney glomeruli), or sinusoidal (liver) ECs are examples of epithelial cells that demonstrate their structural heterogeneity in various organs and tissues (Dudley and Griffioen 2023a). Synthesis and secretion of endothelium-derived factors, expression of adhesion molecules, proliferation and migration, and modulation of vascular tone are ways in which ECs participate in physiological and pathological processes. Dysfunction of ECs can lead to impairment of their regulatory function and is linked to various cardiovascular disorders such as hypertension, atherosclerosis, and thrombosis.

ECs synthesise and release vasoactive substances that modulate vascular tone (the degree of constriction or dilation of the blood vessel). These factors can be vasodilators (nitric oxide (NO) and prostacyclin) or vasoconstrictors (endothelin-1), regulating blood flow and pressure and influencing perfusion to specific tissues and organs. NO is a potent vasodilator and a key blood pressure regulator. ECs produce NO in response to various stimuli which include shear stress, neurotransmitters, and hormones. After secretion, NO diffuses into the smooth muscle cells of the blood vessel wall, promoting their relaxation. ECs also regulate the haemostatic balance. Healthy ECs promote blood fluidity and maintain the

normal antithrombotic state because their luminal surface is anticoagulant and anti-thrombotic. They prevent platelet adhesion, aggregation and activation by releasing substances, including prostacyclin and NO. When the endothelium is disrupted, activated ECs promote thrombogenesis through exposure of collagen fibres and secretion of a von Willebrand factor (vWF) and hence provoke the adhesion of circulating platelets. They also express tissue factor, initiating the extrinsic coagulation pathway. Moreover, ECs are essential in the initiation, regulation, and resolution of inflammation. For example, inflammatory signals (e.g. cytokines) activate ECs which express adhesion molecules, therefore recruiting leukocytes to undergo diapedesis (or transmigration), through which they cross the endothelium and migrate from the bloodstream into surrounding tissue (leukocyte trafficking). They release chemokines, signalling proteins, that create a concentration gradient guiding leukocytes to the site of inflammation. ECs also increase vascular permeability, facilitating diapedesis. Once migrated into the tissue, the leukocytes migrate through chemotaxis to the site of inflammation or infection to perform their immune function.

3 Angiogenesis

Angiogenesis is a physiological process in which new blood vessels are created from pre-existing ones to fulfil the demands of the tissues they perfuse (Dight et al. 2022). It is fundamental in biological processes such as embryonic development, growth, tissue repair (wound healing), reproduction (endometrial angiogenesis), and adaptation of skeletal muscle to physical exercise (Hillen and Griffioen 2007). Angiogenesis also plays an important role in progression of many pathological conditions such as cancer, diabetes, ischaemia, and immune and inflammatory disorders (Hillen and Griffioen 2007; Dudley and Griffioen 2023b). The onset of angiogenesis is stimulated by a series of pro-angiogenic factors that promote migration and proliferation of ECs and formation of new vasculature. The concept of angiogenic switch (Hanahan and Folkman 1996) is used to describe the process in which the expression of the pro-angiogenic (stimulating) factors dominates the expression of the anti-angiogenic (inhibiting) factors, pushing the balance in the favour of angiogenesis (Fig. 1). In other words, it is the shift from a relative quiescence and non-proliferative state of the ECs to a state of active proliferation.

Angiogenesis is associated with responses to hypoxia and inflammation. In wound healing, the existing microvasculature is disrupted leading to oedema, hypoxia, and inflammation. New vessels grow in a multistep process to bring oxygen, nutrients, and immune cells to the injured tissue. Over time, the newly formed vascular bed is trimmed back to the density of a normal tissue (Veith et al. 2019).

During angiogenesis, ECs in existing blood vessels proliferate, migrate, and remodel to create new vessels. This process of sprouting new vessels from existing ones represents the classical understanding of angiogenesis. Sprouting angiogenesis (Fig. 2) is a mechanism in which ECs from a capillary sprout to form a new capillary bed. The endothelial sprouting may be triggered by exposure to tissue hypoxia,

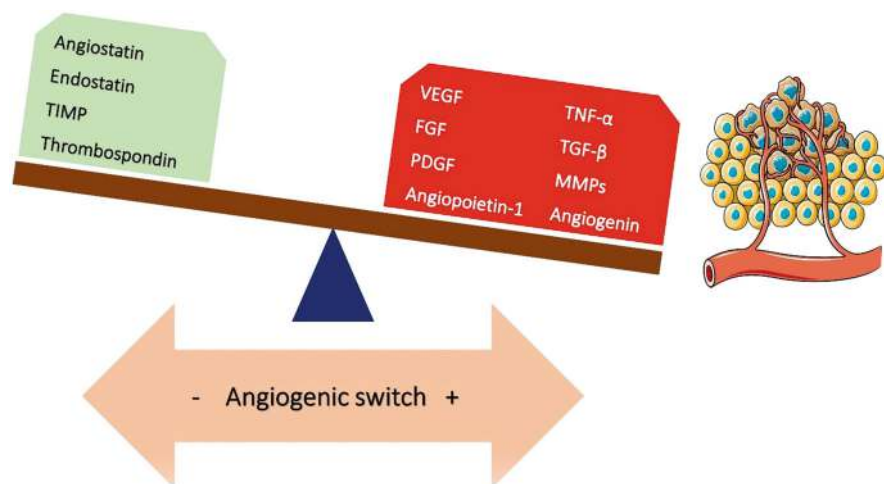


Fig. 1 Depiction of the angiogenic switch as an interplay between several pro-angiogenic and anti-angiogenic factors. Pro-angiogenic factors activate ECs, while anti-angiogenic factors suppress their activation. VEGF vascular endothelial growth factor, FGF fibroblast growth factor, PDGF platelet-derived growth factor, TNF- α tumour necrosis factor-alpha, TGF- β transforming growth factor-beta, MMPs matrix metalloproteinases, TIMP inhibitor of metalloproteinase. Parts of the figure were drawn by using pictures from Servier Medical Art (Servier Medical Art)

injury, or tumour growth (Dudley and Griffioen 2023a). In a poorly perfused tissue in need for new vasculature, cells may respond to the hypoxic environment by producing VEGF, one of the key pro-angiogenic growth factors. VEGF directs capillary growth towards the source of the angiogenic signal. ECs exposed to the stimulus become activated through specific receptors and develop a new functional blood vessel in the direction of the stimulus (Dudley and Griffioen 2023a). The newly created vasculature may undergo anastomosis by fusing with local vessels (Udan et al. 2013).

Additional mechanisms of angiogenesis have been identified over the last years and are less understood than the sprouting angiogenesis. These include intussusceptive angiogenesis, coalescent angiogenesis, vessel co-option, and vasculogenic mimicry (Hillen and Griffioen 2007; Dudley and Griffioen 2023a) (Fig. 2). In intussusceptive angiogenesis or splitting angiogenesis, the vascular bed is expanded by splitting of pre-existing vessels. It occurs in embryonic development, in physiological mechanisms such as adaptation to exercise or pathological conditions such as cancer (Makanya et al. 2009). Because intussusceptive angiogenesis is not initially dependent on endothelial proliferation and only requires reorganisation of existing ECs, it is thought to be faster than sprouting angiogenesis (Hillen and Griffioen 2007; Dudley and Griffioen 2023a). In contrast, coalescent angiogenesis is the fusion (or coalescence) of multiple small blood vessels to form larger blood vessels and has been identified in embryonic development (Dudley and Griffioen 2023a).

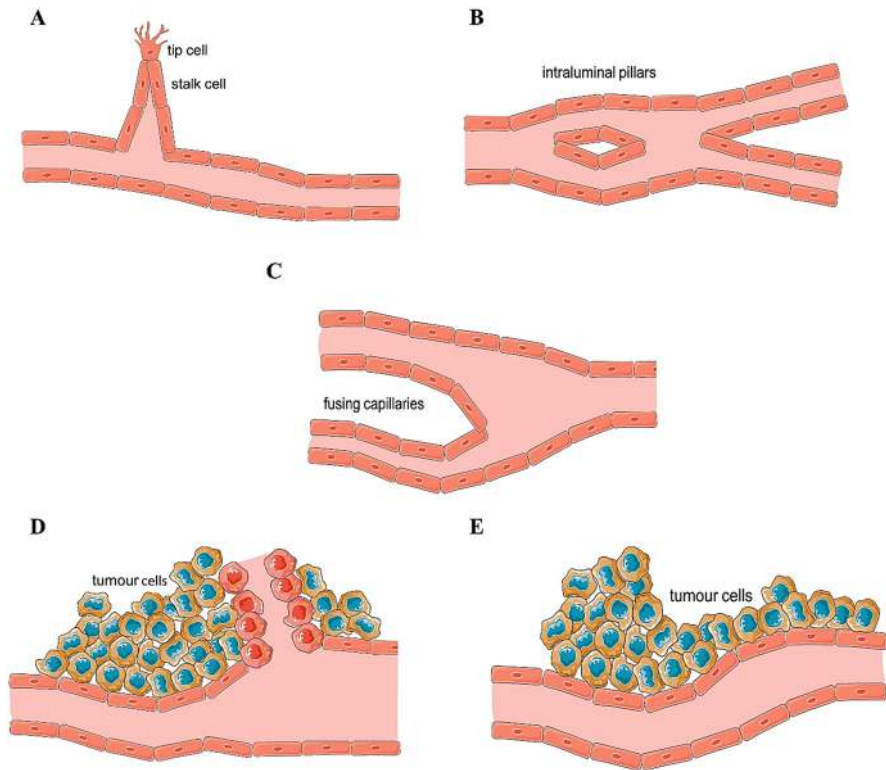


Fig. 2 Different modes of angiogenesis. (A) Sprouting angiogenesis. Pro-angiogenic factors activate quiescent ECs to produce new functional blood vessels. They secrete proteolytic enzymes to degrade the local ECM and the basement membrane to sprout through the blood vessel wall. Pericytes dissociate from the abluminal surface. One epithelial cell is selected to become a tip cell and migrates towards the source of the angiogenic stimulus while endothelial stalk cells proliferate behind it to extend the growing vessel. Further proliferation produces tube-like structures which will mature into a blood vessel lumen. Recruitment of pericytes and generation of ECM lead to the maturation and stabilisation of the newly formed capillary. (B) Intussusceptive angiogenesis. Protrusions of ECs called intraluminal pillars develop at opposite cells in the lumen of the capillary. These pillars grow and extend towards each other until they fuse, eventually dividing the capillary longitudinally into two vessels. (C) Coalescent angiogenesis. Blood vessels coalesce (merge) together to form larger blood vessels. (D) Vasculogenic mimicry. Tumour cells form vessel-like structures to secure their blood supply. They are often lined with vasculogenic tumour cells that acquire EC phenotype. (E) Vessel co-option. Malignant cells migrate along the abluminal surface of pre-existing blood vessels, co-opting them to meet their needs. Parts of the figure were drawn by using pictures from Servier Medical Art (Servier Medical Art)

Vessel co-option is a process in which cancer cells or other tissues exploit (co-opt) existing blood vessels to meet their needs, rather than stimulating the formation of new blood vessels through other angiogenesis mechanisms. Malignant cells migrate along pre-existing vessels (Zhang et al. 2020) and hence there is no need for vascular proliferation. Co-optive growth patterns have been observed in

multiple cancer types and metastasis (Zhang et al. 2020). In vasculogenic mimicry, cancer cells or tumour cells develop structures that mimic blood vessels. These channels are often lined with tumour cells that de-differentiate to an endothelial phenotype. While this is reported as a rare phenomenon in cancer (Pezzella and Ribatti 2022), it is associated with drug resistance and poor outcome (Hillen and Griffioen 2007; Dudley and Griffioen 2023a).

Some of these mechanisms do not require vascular proliferation, therefore some authors refer to them as non-angiogenic mechanisms. Co-option and vascular mimicry can initiate non-angiogenic tumour growth and are resistant to anti-VEGF treatment (Pezzella and Ribatti 2022). It has been suggested that anti-VEGF treatment results in increased vascular co-option as an adaptation of the tumour to the therapy (Zhang et al. 2020).

4 Tumour Angiogenesis

Genetic and epigenetic changes enable malignant cells to proliferate uncontrollably, resist cell death, and evade the immune system. While small tumours acquire nutrients through simple diffusion, larger tumours require access to the circulatory system for oxygen and nutrients in order to proliferate and survive (Lugano et al. 2020). They acquire vasculature as they grow by secreting factors that stimulate the formation of new vessels. The recruitment of local blood vessels through the process of pathological angiogenesis is essential for malignant tumour growth and progression. Tumour cells also use blood vessels to spread from the primary location to distant tissues or organs where they form metastases (Jiang et al. 2020).

The tumour microenvironment is the ecosystem surrounding cancer cells, comprised of various cell types, blood vessels, ECM, and signalling molecules. The continuous interaction between cancer, endothelial, stromal, immune, and inflammatory cells creates a pro-angiogenic environment. The microenvironment contributes to tumour growth and metastasis and influences the response to anticancer therapies (Lugano et al. 2020). As tumours grow, the oxygen requirements exceed the available blood supply leading to oxygen-deficient areas in the tumour and tumour microenvironment (Lugano et al. 2020). Hypoxia is a major inducer of angiogenesis and discoveries in the early 1990s provided insight into the adaptive response of cells to changing oxygen levels (Wang and Semenza 1993; Griffioen and Bischoff 2019). The regulatory mechanism is centred around the hypoxia-inducible factors (HIFs), a family of transcriptional regulators that control gene expression in response to changes in oxygen availability (Dudley and Griffioen 2023b). During normoxic conditions (normal oxygen tension), the von Hippel-Lindau (VHL) proteins promote the ubiquitination and proteasomal degradation of the isoform HIF-1 α . However, during hypoxia, stabilisation of HIF-1 α occurs, leading to its translocation to nucleus and the upregulation of several pro-angiogenic genes (Lugano et al. 2020). Mutations in the oxygen-sensing pathway can be observed in some tumours and result in the stabilisation of HIF-1 α even during normoxia, leading to increased expression of its target gene and thus contributing to excessive

angiogenesis. Such mutation is associated with poorer patient outcome (Lugano et al. 2020). The discovery of hypoxia as a driving force for neovascularisation has led to the development of therapeutic strategies that aim to inhibit angiogenesis by targeting oxygen-sensing molecules (directly or indirectly). However, tumours can develop resistance mechanisms, such as adaptation to hypoxic environments, resulting in limited success of such therapies (Lugano et al. 2020).

In contrast to physiological angiogenesis, a tightly regulated process that halts when the new vasculature is not required anymore, tumour angiogenesis involves persistent signalling of pro-angiogenic factors which can lead to chaotic and abnormal tumour vasculature. VEGF, FGF, PDGF, and angiopoietins are some of the pro-angiogenic factors that promote vasculature formation in tumours (Lugano et al. 2020). VEGF, initially named vascular permeability factor, is one of the most potent inducers of angiogenesis and historically the key target in anti-angiogenic cancer therapies (Lugano et al. 2020). VEGF activates ECs via paracrine signalling, primarily through the transmembrane receptor tyrosine kinases VEGFR-2 expressed by ECs and initiates signalling pathways that lead to cell proliferation, survival, migration, and vascular permeability (Udan et al. 2013). VEGF also promotes the expression of matrix metalloproteinases (MMP) and urokinase plasminogen activators that degrade the local basal membrane and ECM, allowing ECs to migrate and form capillary sprouts. The FGF family are glycoproteins sequestered in the ECM and secreted by various cell types, including tumour cells and macrophages (Hillen and Griffioen 2007). FGF ligands bind to the tyrosine kinase receptor FGFR and promote angiogenesis, proliferation, and migration of ECs (Veith et al. 2019). The PDGF family are secreted by a variety of cell types including activated platelets and ECs and target pericytes and other stromal cells. In the context of angiogenesis, PDGF promotes VEGF upregulation and pericyte recruitment (Uccelli et al. 2019). Angiopoietins are glycoproteins associated with blood vessels development, maintenance, and remodelling. Tyrosine kinase receptor Tie-2 on the surface of ECs mediates angiopoietin signalling. Angiopoietin-1 is Tie-2 receptor agonist, while angiopoietin-2 acts as a competitive antagonist. Angiopoietin 1, mostly produced by perivascular cells such as pericytes, is mainly associated with vessel maturation and stabilisation (Uccelli et al. 2019). In contrast, angiopoietin-2 is mainly secreted by ECs upon their stimulation with VEGF and is required in the angiogenesis initiation, inducing pericytes detachment and vessel destabilisation. Co-targeting angiopoietin-2 and VEGFR2 appear to have beneficial effects in cancer therapy (Lugano et al. 2020). Other cells in the tumour microenvironment play a role in angiogenesis and vascular remodelling. Tumour cells or cells in the tumour microenvironment secrete MMPs which contribute to the remodelling of the ECM and the release of pro-angiogenic growth factors (Lugano et al. 2020). Tumour-induced macrophages, typically of M2 phenotype, promote angiogenesis by secreting pro-angiogenic factors such as VEGF, FGF, and chemokines (Lugano et al. 2020).

Anti-angiogenic cancer treatments, a major area in cancer research, aim to target the tumour vasculature to induce tumour starvation and regression. Therapies focus on inhibition of key angiogenic signalling pathways by using antibodies or tyrosine kinase inhibitors. However, tumours can develop resistance, such as adapting to

hypoxic environments or producing alternative pathways to sprouting angiogenesis, therefore reducing the success of anti-angiogenic therapies. For example, tumours can co-op vasculature through vessel co-option or get nutrients via vasculogenic mimicry (Fig. 2), mechanisms that evade anti-angiogenic therapies such as anti-VEGF therapy (Lugano et al. 2020).

5 The Role of ECs in Cancer Progression

Endothelial cells form new blood vessels by migrating and proliferating towards the tumour, following pro-angiogenic signals. The new vasculature supplies the tumour with oxygen and nutrients, facilitating not only its growth and invasion into nearby tissue but also the metastasis process. Cancer metastasis refers to the spread of malignant cells from the primary tumour to other tissues or organs. ECs secrete pro-angiogenic factors themselves, which further stimulate angiogenesis and cancer progression (Baluk et al. 2005). Tumour blood vessels have multiple abnormalities most likely resulting from the excessive angiogenesis leading to chaotic blood vessel formation as well as the abnormal environment surrounding the tumour. Firstly, tumour blood vessels lack the conventional vascular hierarchy in which arterioles connect to capillaries and then to venules. Furthermore, they are variable in size and shape and appear more immature (Baluk et al. 2005).

Tumour ECs display increased fenestrations consistent with vascular hyperpermeability. The altered barrier function of the endothelium contributes to the intravasation of malignant cells, ECs being considered a gateway for metastasis. Pericytes appear to have abnormal shape and loosely attach to ECs (Baluk et al. 2005). Furthermore, basement membrane of tumour vessels is loosely associated with the ECs and presents multiple layers (Baluk et al. 2005). The aberrant structure of the tumour vasculature and the increased interstitial pressure (consequence of vascular hyperpermeability) lead to abnormal blood flow, reducing the efficacy of the anticancer therapies (Goel et al. 2011). Anti-angiogenic cancer therapies have had a modest outcome in clinical context, however they appear to normalise the tumour vasculature. This phenomenon refers to the mitigation of tumour vasculature abnormalities and modification of tumour microenvironment (towards a more normal phenotype) which improve the efficacy of the concurrently administered cancer therapy, e.g. chemotherapy (Goel et al. 2011).

6 Endothelial-Tumour Cell Communication in Tumour Microenvironment

Effective communication between stromal cells and tumour cells is essential for both tumour development and progression. Stromal cells in the tumour microenvironment include macrophages, fibroblasts, and the ECs. The latter is crucial for angiogenesis required for the progression and metastasis of tumours (Choi and Moon 2018). Cancer cells penetrate the normal epithelium and interact with the ECs to secrete

growth factors and cytokines, such as VEGF, and promote tube formation, leading to angiogenesis (Brenner et al. 2010). Tumour ECs exhibit rapid proliferation and migration when compared with normal ECs, and the induced changes in ECs by tumour cells occur via vesicles, gap junction, and adhesion receptors (Lopes-Bastos et al. 2016). Different signalling within the ECs is altered by interaction with tumour cells (Lopes-Bastos et al. 2016). The initial contact via carbohydrate–carbohydrate interactions initiates the activation of ECs and tumour cells via growth factors, free radicals, cytokines, and other molecules (Dube and Bertozzi 2005). Consequently, adhesion molecules, including cadherins, selectins, and integrins, are expressed on ECs and boost the initial contact (Kannagi 1997). For instance, the expression of P-selectin on the surface of ECs and platelets is implicated in metastasis (Reyes-Reyes et al. 2006) and the lack of L-selectin reduces it (Läubli and Borsig 2010). The binding of E-selectin to its receptors is also implicated in the adhesion of cancer cells and its inhibition resulted in attenuated metastasis (Kang et al. 2016). Regarding integrins, blood vessels associated with tumours express $\alpha v \beta 3$ and $\alpha v \beta 5$ and the inhibitors of these integrins, such as cilengitide have shown promising effects as anticancer agents (Desgrosellier and Cheresh 2010; Bendas and Borsig 2012). The signalling pathway induced by integrins is implicated in the migration of cells and both ECs and cancer cells showed activated focal adhesion kinases (Feng et al. 2017).

7 Endothelial Cell Malignancies

Malignancies of the ECs are uncommon in developed countries; yet, the tumours that do occur are typically characterised by their high aggressiveness and resistance to treatment (Wagner et al. 2017). EC malignancies could have intermediate or high malignant grade and occur in adults. Endovascular papillary haemangioendotheliomas, spindle cell, epithelioid, and pseudomyogenic neoplasms are among the intermediate-grade EC malignancies. The high potential EC malignancies include angiosarcomas and Kaposi sarcoma (KS) (Wagner et al. 2017). High-grade EC malignancies exhibit a favourable response to conventional chemotherapeutics; however, their long-term effectiveness is limited, since the tumours quickly develop resistance to these drugs (Fury et al. 2005). Anti-angiogenic therapeutics have had limited success in the treatment of EC malignancies and therefore understanding of the mechanisms underlying the resistance of these cells to chemotherapy and targeted therapies can improve the clinical outcomes (Wagner et al. 2017).

Dysregulation of EC signalling pathways provoked by chromosomal rearrangements, viral oncoproteins, paracrine signalling in the tumour microenvironment, and other mechanisms, represents the common feature of EC malignancies. The dysregulated pathways include VEGF/VEGFR, Notch, angiopoietin (Ang)/Tie2, PDGF, angiostatin and endostatin, endoglin/transforming growth factor beta (TGF- β), and PI3K/AKT/mTOR pathways (Wagner et al. 2017).

In normal angiogenesis, VEGF/VEGFR2 signalling plays a crucial role in the selection and movement of tip cells. Both VEGFR2 and VEGFR3 facilitate the

formation of tip cells, the breakdown of the basement membrane, and the reduction of pericyte coverage, so enabling the migration of ECs. In EC neoplasms, abnormal signalling in the VEGF/VEGFR pathway was observed (Wagner et al. 2017). Mutations, amplifications, and other alterations in VEGF and VEGFRs have been characterised in aggressive angiosarcomas. For instance, 65–94% increase in the expressions of VEGF-A and VEGFR (1–3) (Itakura et al. 2008) and mutations of VEGFR2 have been reported in angiosarcomas (Antonescu et al. 2009). In KS, VEGF contributes to its progression by acting as an autocrine growth factor and high levels of the receptors VEGFR1, 2, and 3 were detected (Masood et al. 1997; Stallone et al. 2009). Despite the implication of VEGF/VEGFR pathway in EC malignancies, the clinical application of anti-VEGF drugs and VEGFR2 inhibitors was disappointing. For example, only 9% response rate to bevacizumab was reported in patients with angiosarcoma (Agulnik et al. 2013) and sorafenib exhibited 14.6% response rate in patients with advanced angiosarcoma (Ray-Coquard et al. 2012).

Under physiological conditions, Notch signalling is active at the endothelial tip cells because of increased DLL4 expression, and in the neighbouring stalk cells to prevent the migration of stalk cells and therefore modulates organised branching (Hellström et al. 2007). Studies have demonstrated that altered Notch signalling is implicated in EC neoplasms. Proliferative vascular tumours were developed in mice after DLL4 inhibition (Yan et al. 2010), and Notch1 knockout resulted in angiosarcomas in the liver of 86% mice (Dill et al. 2012).

Ang/Tie signalling promotes the stability and survival of ECs and hence is not involved in the early development of the vasculature. Ang1 and Ang2 act as ligands for Tie and Ang2 is produced primarily by ECs (Maisonpierre et al. 1997). In angiogenesis in solid tumours, Ang2 is upregulated, and its high levels are linked to worse clinical outcomes (Huang et al. 2010). In 26% of angiosarcoma, loss-of-function mutations in receptor-like protein-tyrosine phosphatase (PTP) beta (PTPRB) which inactivates Tie2 via dephosphorylation were observed (Behjati et al. 2014). Like angiosarcoma, Tie1, Tie2, and Ang2 are highly expressed in KS (Brown et al. 2000).

PDGF is involved in the paracrine signalling between ECs and perivascular cells. To maintain the endothelial basement membrane integrity, ECs secrete PDGF-BB resulting in increased pericyte coverage (Lindahl et al. 1997). Activation of PDGFR activates phospholipase C-gamma (PLCG) which is also activated by VEGFR2 and provokes angiosarcoma and leads to resistance to VEGF/VEGFR targeted therapies (Kundra et al. 1994; Wagner et al. 2017).

The protein fragments angiostatin and endostatin inhibit the growth of tumours and the formation of new blood vessels (O'Reilly et al. 1997). In combination with chemotherapy, endostatin was used for the treatment of angiosarcoma (Ye et al. 2015). More than 90% of EC neoplasms, such as benign haemangiomas and angiosarcomas, express the receptor of angiostatin (annexin II). Therefore, angiostatin could be effective in suppressing the growth of EC malignancies. In this context, in vivo findings revealed the ability of angiostatin to inhibit haemangi endothelioma growth (Lannutti et al. 1997).

Endoglin is a component of the TGF- β receptor family that attracted great interest as a target for the development of drugs to target tumour angiogenesis (Wong et al. 2000). In angiogenesis under normal physiological conditions, endoglin mediates TGF- β signalling and promotes ECs migration and proliferation (Lebrin et al. 2004). In angiosarcomas, high expression levels of endoglin have been detected (Hara 2012), and the levels of TGF- β pathway increase significantly in angiosarcoma of bone than in soft tissue (Verbeke et al. 2013).

PI3K/AKT/mTOR signalling in ECs is normally activated by Ang/Tie2, VEGF, and other stimuli (Shiojima and Walsh 2002); however, in EC neoplasms, AKT phosphorylation increases (Phung et al. 2015). Different isoforms of AKT have been found to affect the cells paradoxically where AKT1 provoked cell growth and migration and AKT3 inhibited the growth of haemangioma and angiosarcoma (Phung et al. 2015). Besides AKT1, angiosarcomas exhibited S6-kinase and S6 ribosomal protein activation downstream to PI3K-mediated activation of mTOR complex 1 (mTORC1) and topical rapamycin inhibited the proliferation of angiosarcoma cell lines in vitro (Du et al. 2013).

8 Conclusion

ECs play a crucial role in tumour angiogenesis, growth, progression, and metastasis. ECs orchestrate angiogenesis within the tumour microenvironment and foster both proliferation and metastasis of cancer cells. The crosstalk between ECs and tumour cells represents an important target and understanding the mechanisms underlying these cellular interactions might pave the way to develop new therapeutic strategies for cancer management. Malignancies of the ECs exhibit dysregulation in numerous signalling pathways involved in vascular development along with other oncogenic pathways. Implementation of cell-lined and model systems representing angiosarcomas and other endothelial malignancies is needed to understand the implicated mechanism (s) and develop new therapeutic regimes for the management of these tumours.

References

- Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, Morris PG, Manova-Todorova K, Leversha M, Hogg N, Seshan VE, Norton L, Brogi E, Massagué J (2012) A CXCL1 paracrine network links cancer chemoresistance and metastasis. *Cell* 150:165–178
- Agulnik M, Yarber JL, Okuno SH, von Mehren M, Jovanovic BD, Brockstein BE, Evens AM, Benjamin RS (2013) An open-label, multicenter, phase II study of bevacizumab for the treatment of angiosarcoma and epithelioid hemangioendotheliomas. *Ann Oncol* 24:257–263
- Antonescu CR, Yoshida A, Guo T, Chang NE, Zhang L, Agaram NP, Qin LX, Brennan MF, Singer S, Maki RG (2009) KDR activating mutations in human angiosarcomas are sensitive to specific kinase inhibitors. *Cancer Res* 69:7175–7179
- Baluk P, Hashizume H, McDonald DM (2005) Cellular abnormalities of blood vessels as targets in cancer. *Curr Opin Genet Dev* 15:102–111

- Behjati S, Tarpey PS, Sheldon H, Martincorena I, Van Loo P, Gundem G, Wedge DC, Ramakrishna M, Cooke SL, Pillay N, Vollan HKM, Papaemmanuil E, Koss H, Bunney TD, Hardy C, Joseph OR, Martin S, Mudie L, Butler A, Teague JW, Patil M, Steers G, Cao Y, Gumbs C, Ingram D, Lazar AJ, Little L, Mahadeshwar H, Protopopov A, Al Sanna GA, Seth S, Song X, Tang J, Zhang J, Ravi V, Torres KE, Khatri B, Halai D, Roxanis I, Baumhoer D, Tirabosco R, Amary MF, Boshoff C, McDermott U, Katan M, Stratton MR, Futreal PA, Flanagan AM, Harris A, Campbell PJ (2014) Recurrent PTPRB and PLAGL1 mutations in angiosarcoma. *Nat Genet* 46:376–379
- Bendas G, Borsig L (2012) Cancer cell adhesion and metastasis: selectins, integrins, and the inhibitory potential of heparins. *Int J Cell Biol* 2012:676731
- Brenner W, Beitz S, Schneider E, Benzing F, Unger RE, Roos FC, Thüroff JW, Hampel C (2010) Adhesion of renal carcinoma cells to endothelial cells depends on PKC μ . *BMC Cancer* 10:183
- Brown LF, Dezube BJ, Tognazzi K, Dvorak HF, Yancopoulos GD (2000) Expression of Tie1, Tie2, and angiopoietins 1, 2, and 4 in Kaposi's sarcoma and cutaneous angiosarcoma. *Am J Pathol* 156:2179–2183
- Choi H, Moon A (2018) Crosstalk between cancer cells and endothelial cells: implications for tumor progression and intervention. *Arch Pharm Res* 41:711–724
- Desgrosellier JS, Chersesh DA (2010) Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer* 10:9–22
- Dight J, Zhao J, Styke C, Khosrotehrani K, Patel J (2022) Resident vascular endothelial progenitor definition and function: the age of reckoning. *Angiogenesis* 25:15–33
- Dill MT, Rothweiler S, Djonov V, Hlushchuk R, Tornillo L, Terracciano L, Meili-Butz S, Radtke F, Heim MH, Semela D (2012) Disruption of Notch1 induces vascular remodeling, intussusceptive angiogenesis, and angiosarcomas in livers of mice. *Gastroenterology* 142:967–977.e962
- Du W, Gerald D, Perruzzi CA, Rodriguez-Waitkus P, Enayati L, Krishnan B, Edmonds J, Hochman ML, Lev DC, Phung TL (2013) Vascular tumors have increased p70 S6-kinase activation and are inhibited by topical rapamycin. *Lab Invest* 93:1115–1127
- Dube DH, Bertozzi CR (2005) Glycans in cancer and inflammation—potential for therapeutics and diagnostics. *Nat Rev Drug Discov* 4:477–488
- Dudley AC, Griffioen AW (2023a) The modes of angiogenesis: an updated perspective. *Angiogenesis* 26:477–480
- Dudley AC, Griffioen AW (2023b) Pathological angiogenesis: mechanisms and therapeutic strategies. *Angiogenesis* 26:313–347
- Feng T, Yu H, Xia Q, Ma Y, Yin H, Shen Y, Liu X (2017) Cross-talk mechanism between endothelial cells and hepatocellular carcinoma cells via growth factors and integrin pathway promotes tumor angiogenesis and cell migration. *Oncotarget* 8:69577–69593
- Fury MG, Antonescu CR, Van Zee KJ, Brennan MF, Maki RG (2005) A 14-year retrospective review of angiosarcoma: clinical characteristics, prognostic factors, and treatment outcomes with surgery and chemotherapy. *Cancer J* 11:241–247
- Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, Jain RK (2011) Normalization of the vasculature for treatment of cancer and other diseases. *Physiol Rev* 91:1071–1121
- Griffioen AW, Bischoff J (2019) Oxygen sensing decoded: a Nobel concept in biology. *Angiogenesis* 22:471–472
- Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86:353–364
- Hara H (2012) Endoglin (CD105) and claudin-5 expression in cutaneous angiosarcoma. *Am J Dermatopathol* 34:779–782
- Hellström M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalén M, Gerhardt H, Betsholtz C (2007) Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature* 445:776–780
- Hillen F, Griffioen AW (2007) Tumour vascularization: sprouting angiogenesis and beyond. *Cancer Metastasis Rev* 26:489–502

- Huang H, Bhat A, Woodnutt G, Lappe R (2010) Targeting the ANGPT-TIE2 pathway in malignancy. *Nat Rev Cancer* 10:575–585
- Itakura E, Yamamoto H, Oda Y, Tsuneyoshi M (2008) Detection and characterization of vascular endothelial growth factors and their receptors in a series of angiosarcomas. *J Surg Oncol* 97: 74–81
- Jiang X, Wang J, Deng X, Xiong F, Zhang S, Gong Z, Li X, Cao K, Deng H, He Y, Liao Q, Xiang B, Zhou M, Guo C, Zeng Z, Li G, Li X, Xiong W (2020) The role of microenvironment in tumor angiogenesis. *J Exp Clin Cancer Res* 39:204
- Kang SA, Blache CA, Bajana S, Hasan N, Kamal M, Morita Y, Gupta V, Tsolmon B, Suh KS, Gorenstein DG, Razaq W, Rui H, Tanaka T (2016) The effect of soluble E-selectin on tumor progression and metastasis. *BMC Cancer* 16:331
- Kannagi R (1997) Carbohydrate-mediated cell adhesion involved in hematogenous metastasis of cancer. *Glycoconj J* 14:577–584
- Kundra V, Escobedo JA, Kazlauskas A, Kim HK, Rhee SG, Williams LT, Zetter BR (1994) Regulation of chemotaxis by the platelet-derived growth factor receptor-beta. *Nature* 367: 474–476
- Lannutti BJ, Gately ST, Quevedo ME, Soff GA, Paller AS (1997) Human angiostatin inhibits murine hemangioendothelioma tumor growth in vivo. *Cancer Res* 57:5277–5280
- Läubli H, Borsig L (2010) Selectins promote tumor metastasis. *Semin Cancer Biol* 20:169–177
- Lebrin F, Goumans MJ, Jonker L, Carvalho RL, Valdimarsdottir G, Thorikay M, Mummery C, Arthur HM, ten Dijke P (2004) Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *EMBO J* 23:4018–4028
- Lindahl P, Johansson BR, Levéen P, Betsholtz C (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* 277:242–245
- Lopes-Bastos BM, Jiang WG, Cai J (2016) Tumour-endothelial cell communications: important and indispensable mediators of tumour angiogenesis. *Anticancer Res* 36:1119–1126
- Lugano R, Ramachandran M, Dimberg A (2020) Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci* 77:1745–1770
- Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 277:55–60
- Makanya AN, Hlushchuk R, Djonov VG (2009) Intussusceptive angiogenesis and its role in vascular morphogenesis, patterning, and remodeling. *Angiogenesis* 12:113–123
- Masood R, Cai J, Zheng T, Smith DL, Naidu Y, Gill PS (1997) Vascular endothelial growth factor/vascular permeability factor is an autocrine growth factor for AIDS-Kaposi sarcoma. *Proc Natl Acad Sci USA* 94:979–984
- O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J (1997) Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88:277–285
- Pezzella F, Ribatti D (2022) Vascular co-option and vasculogenic mimicry mediate resistance to antiangiogenic strategies. *Cancer Rep (Hoboken)* 5:e1318
- Phung TL, Du W, Xue Q, Ayyaswamy S, Gerald D, Antonello Z, Nhek S, Perruzzi CA, Acevedo I, Ramanna-Valmiki R, Rodriguez-Waitkus P, Enayati L, Hochman ML, Lev D, Geeganage S, Benjamin LE (2015) Akt1 and akt3 exert opposing roles in the regulation of vascular tumor growth. *Cancer Res* 75:40–50
- Ray-Coquard I, Italiano A, Bompas E, Le Cesne A, Robin YM, Chevreau C, Bay JO, Bousquet G, Piperno-Neumann S, Isambert N, Lemaitre L, Fournier C, Gauthier E, Collard O, Cupissol D, Clisant S, Blay JY, Penel N (2012) Sorafenib for patients with advanced angiosarcoma: a phase II trial from the French sarcoma group (GSF/GETO). *Oncologist* 17:260–266
- Reglero-Real N, Colom B, Bodkin JV, Nourshargh S (2016) Endothelial cell junctional adhesion molecules: role and regulation of expression in inflammation. *Arterioscler Thromb Vasc Biol* 36:2048–2057

- Reyes-Reyes ME, George MD, Roberts JD, Akiyama SK (2006) P-selectin activates integrin-mediated colon carcinoma cell adhesion to fibronectin. *Exp Cell Res* 312:4056–4069
- Servier Medical Art. Retrieved 15/5, 2023, from <https://smart.servier.com/>
- Shiojima I, Walsh K (2002) Role of Akt signaling in vascular homeostasis and angiogenesis. *Circ Res* 90:1243–1250
- Stallone G, Infante B, Pontrelli P, Ranieri E, Loverre A, Schena A, Cormio L, Carrieri G, Schena FP, Grandaliano G, Gesualdo L (2009) ID2-VEGF-related pathways in the pathogenesis of Kaposi's sarcoma: a link disrupted by rapamycin. *Am J Transplant* 9:558–566
- Trimm E, Red-Horse K (2023) Vascular endothelial cell development and diversity. *Nat Rev Cardiol* 20:197–210
- Uccelli A, Wolff T, Valente P, Di Maggio N, Pellegrino M, Gurke L, Banfi A, Gianni-Barrera R (2019) Vascular endothelial growth factor biology for regenerative angiogenesis. *Swiss Med Wkly* 149:w20011
- Udan RS, Culver JC, Dickinson ME (2013) Understanding vascular development. *Wiley Interdiscip Rev Dev Biol* 2:327–346
- Veith AP, Henderson K, Spencer A, Sligar AD, Baker AB (2019) Therapeutic strategies for enhancing angiogenesis in wound healing. *Adv Drug Deliv Rev* 146:97–125
- Verbeke SL, Bertoni F, Bacchini P, Oosting J, Sciort R, Krenács T, Bovée JV (2013) Active TGF- β signaling and decreased expression of PTEN separates angiosarcoma of bone from its soft tissue counterpart. *Mod Pathol* 26:1211–1221
- Wagner MJ, Ravi V, Menter DG, Sood AK (2017) Endothelial cell malignancies: new insights from the laboratory and clinic. *NPJ Precis Oncol* 1:11
- Wang GL, Semenza GL (1993) General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Natl Acad Sci USA* 90:4304–4308
- Wong SH, Hamel L, Chevalier S, Philip A (2000) Endoglin expression on human microvascular endothelial cells association with betaglycan and formation of higher order complexes with TGF-beta signalling receptors. *Eur J Biochem* 267:5550–5560
- Yan M, Callahan CA, Beyer JC, Allamneni KP, Zhang G, Ridgway JB, Niessen K, Plowman GD (2010) Chronic DLL4 blockade induces vascular neoplasms. *Nature* 463:E6–E7
- Yao X, Zeng Y (2023) Tumour associated endothelial cells: origin, characteristics and role in metastasis and anti-angiogenic resistance. *Front Physiol* 14:1199225
- Ye J, Li XF, Wang YD, Yuan Y (2015) Long-term survival of a patient with scalp angiosarcoma and multiple metastases treated using combination therapy: a case report. *Oncol Lett* 9:1725–1728
- Zhang Y, Wang S, Dudley AC (2020) Models and molecular mechanisms of blood vessel co-option by cancer cells. *Angiogenesis* 23:17–25



Extracellular Vesicles as the Dynamic Structural and Functional Network in Aging-Related Diseases and Cancer Treatment

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Abstract

Extracellular vesicles (EVs) are membrane-encapsulated vesicles carrying various signal molecules, including metabolites, proteins, enzymes, genetic material, nucleic acids, mRNAs, noncoding RNAs, intact RNAs, lipids, organelles, ions, pathogenic microorganisms, and disease-specific molecules. The International Society for Extracellular Vesicles (ISEV) describes EVs as lipid-enveloped vesicles released from cells that do not contain a nucleus and cannot replicate themselves. EVs are categorized based on size and biogenesis as apoptotic bodies, exosomes, macrovesicles, and others. EVs are released from prokaryotic and eukaryotic cells, such as epithelial cells, mesenchymal stem cells, neurons, and endothelial cells, to regulate intracellular regulation and communication through blood circulation. Therefore, EVs are potential and promising biomarkers, drug delivery tools, and therapeutic approaches. These vesicles transfer knowledge of the parent cell's biochemical and physiological status to the neighbor cells. The information of the neighbor cell from the current healthy or pathological conditions can be used not only for intercellular conditions but also as a biomarker for many biological or pathological processes. Crosstalk between the cells may have many crucial functions in health, aging-related disease, and cancer cell signaling. Delayed diagnosis and treatment lead to poor prognosis and increased risk for mortality in cancer patients; thus, novel cancer biomarkers are urgently required for early prognosis, disease progression monitoring, and personalized therapies. Since cell-cell interactions between tumor cells and cancer cell communication with stromal and immune cells occur via EVs, they are considered promising biomarkers for the early detection of cancer

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since they are secreted from cells circulating in the blood and are easy to collect. EVs have superior properties to overcome chemotherapy-related limitations because they have extended circulation time, excellent tumor-targeting capabilities, lower immunogenicity, higher biocompatibility, and reduced systemic toxicity in the peripheral tissues. In this chapter, we discussed the biogenesis, role, functions, and challenges of various types of EVs in aging-related diseases and cancer treatment.

Keywords

Apoptotic bodies · Cancer treatment · Exosomes · Extracellular vesicles · Microvesicles (MVs)

1 Introduction

The International Society for Extracellular Vesicles (ISEV) describes EVs as lipid-enveloped vesicles released from cells that do not contain a nucleus and cannot replicate themselves. EVs are divided into three groups: microvesicles, exosomes, apoptotic bodies, and others carrying proteins, nucleic acids, lipids, metabolites, and organelles. Cells release varied (about 30–5000 nm in diameter) types of membrane vesicles into the extracellular environment for cell-cell signaling, various physiological purposes, and intracellular communications. The apoptotic bodies are released during apoptosis; however, their size is heterogeneous and ranges from 30 to 5000 nm (Lo Cicero et al. 2015; Raposo and Stoorvogel 2013; Bernardi and Balbi 2020). However, cancer cells are producing EVs in various sizes (30–150 nm) (Merchant et al. 2017; Whiteside et al. 2021).

EVs are released from prokaryotic and eukaryotic cells, such as epithelial cells, mesenchymal stem cells, neurons, and endothelial cells, to regulate intracellular regulation and communication through blood circulation. Therefore, EVs are considered potential and promising biomarkers, drug delivery tools, and therapeutic approaches (Wang et al. 2023).

The extracellular matrix (ECM) proteins and molecules have complex connections, communications, and networks, to regulate complex cellular functions. The molecules that are sent to the ECM can change many properties of the matrix such as tensile strength, cell adhesion, tissue development, cell migration, growth, and stabilization, as well as induce cell proliferation, etc. (Kusindarta and Wihadmadyatami 2018). ECM and extracellular vesicles (EVs) are in interaction, and these vesicles can modulate the content of ECM, including nucleic acids, proteins, lipids, and cell-to-cell communications (Patel et al. 2023). EVs have many interactions with the ECM proteins via cross-linking to the matrix proteins and cause ECM calcification and degradation (Gu et al. 2020; Lewin et al. 2020; Skotland et al. 2020). EVs can carry very vital cellular metabolites, organelles, bioactive proteins, enzymes, nucleic acids, lipids, and glycoconjugate components that have effects on ECM homeostasis, remodeling, stem cell homeostasis, neuronal

signaling, health and progression of the disease, cancer progression, wound healing, cell proliferation, blood vessel tube formation, immunity, tissue repair, collagen production, and ECM remodeling (Kim et al. 2017; Nawaz et al. 2018; Shimoda 2019; Shimoda and Khokha 2017). These vesicles can carry various viral components according to the type of viral infection. It has been clarified that COVID-19, cytomegalovirus, Epstein-Barr virus, hepatitis C virus, human immunodeficiency virus, herpes simplex virus, and Kaposi's sarcoma-associated herpesvirus infection EVs can affect the target cell function. These vesicles can modulate immune cell functions, and they play a key role in the spreading of infection (Hassanpour et al. 2020).

Nowadays, it has been clarified that they are biological nanosized cargos, and they are involved in many mechanisms, many cellular reactions, regulating various physiological and pathophysiological processes, cancer biogenesis, neuronal communication, immune response, and many complex mechanisms including cell death (Di Bella 2022). In general, these vesicles are released from donor cells via outward budding of the plasma membrane or inward budding. EVs can be released from the donor cells via cell activation or stress (Gołębiewska et al. 2021; Abels and Breakefield 2016; O'Neill et al. 2019).

Despite EVs being ideal and promising candidates for disease biomarkers and therapeutic approaches, several limitations should be overcome in EVs-related limitations. Storage, isolation, purification, biodistribution, thrombosis, alloimmune responses, elimination, and pharmacokinetic calculations are the significant drawbacks of EV-related applications. Thus, the indicated limitations should be overcome to achieve successful EV-related applications (Zeng et al. 2022). In this book chapter, we discussed the impact of EVs on cancer and aging-related diseases as biomarkers, drug delivery approaches, prognosis indicators, and therapeutic tools.

2 Roles of EVs in Cellular Communication

Cellular communication is one of the important ubiquitous physiological functions of cells, which is fundamental for all living organisms. EVs have key roles in the regulation of gene transcription and translation. Therefore, EVs are critically important in cell survival, proliferation, reproduction, development, angiogenesis, vascular barrier function, waste management, host-microbiome interaction, gut-brain communication, viral immunity, immune response, receptor-ligand signaling, apoptosis, cellular differentiation and neoplasia, cellular migration and metastatic disease, metabolomic reprogramming, senescence, and age-related pathologies (Chatterjee et al. 2020; Cuesta et al. 2021; Kalluri and LeBleu 2020; Pegtel and Gould 2019; Tang et al. 2020; Wallis et al. 2020). EVs are secreted due to an internal or external stimulus for biosynthesis or remodeling, environmental sensing, virulence, immunomodulation, etc. (Liebana-Jordan et al. 2021). EVs are supposed to have endosomal origin and are secreted from reticulocytes or the plasma membrane (Colombo et al. 2014; Robbins and Morelli 2014). There is a functional alteration in the size and density, composition, and secretion place of the urine (uEVs), according

to the pathological condition. EVs are approximately smaller than pores, and 0.6–3% is the protein in a healthy state. On the other hand, EV-based changes in kidney function or malignant cells were investigated in specific conditions (Merchant et al. 2017; Whiteside et al. 2021; Claridge et al. 2021).

3 Classification of EVs

In general, there are two different classifications of EVs named exosomes and ectosomes, and all the formation, mechanism, biogenesis, release, assembly, uptake by the target cells, composition, and regulation, are directly proportional to the biochemical and functional properties. These vesicles are also required for long- and short-distance communications (Cocucci and Meldolesi 2015). The membrane surface proteins of EV and exosomes are changing according to their source, routes of intracellular trafficking, and cellular uptake mechanisms (Bebelman et al. 2018; Kalluri and LeBleu 2020; van Niel et al. 2018). EVs have lipid bilayers and contain various types of proteins, lipid anchors, surface proteoglycans, and integrin, like plasma membranes. EV membrane proteins have various functions; immunomodulatory, antigen-presenting, multivesicular body biogenesis, tetraspanins, membrane transport, and cell adhesion. The outer surface membrane proteins of EVs change according to the health and pathological conditions of vesicle biogenesis, cell targeting, cellular uptake mechanism, cargo selection, protection against enzymatic degradation, and determining the functions of the vesicles (Kalluri and LeBleu 2020; Keller et al. 2011; Ludwig et al. 2019; Yáñez-Mó et al. 2015; Zhang and Yu 2019). Membrane lipids also have key properties for the protection of the encapsulated material; however, the composition and function of the EVs are very limited (Skotland et al. 2020). The production rate and the content of exosomes change according to the physiological and pathological conditions; for instance, cancer cells produce exosomes (Bebelman et al. 2018; Ludwig et al. 2019; L. Zhang and Yu 2019).

4 The Role of EVs in Cancer

Cancer is the second-leading cause of death worldwide, and its incidence is increasing every year. Since delayed diagnosis and treatment lead to poor prognosis and increased risk for mortality in cancer patients, novel cancer biomarkers are urgently needed for early prognosis, disease progression monitoring, and personalized therapies (Krzyszczuk et al. 2018). Liquid biopsy and biological fluids, especially blood, are considered the most critical strategies for cancer detection, treatment, screening, and management because blood samples are easy to collect without patient discomfort. Cell-cell interactions and communications between tumor, stromal, and immune cells occur via EVs. On the other hand, EVs have superior properties to overcome chemotherapy-related limitations because they have extended circulation time, excellent tumor-targeting capabilities, lower immunogenicity, higher biocompatibility, and

reduced systemic toxicity in the peripheral tissues. Therefore, they are considered promising biomarkers for early detection of cancers since they are secreted from cells and circulated through blood (Lone et al. 2022).

On the other hand, chemotherapeutic drugs have limitations for cancer treatment since they have poor bioavailability, uncontrolled cytotoxicity in peripheral tissues, systemic side effects, and poor targeting ability. However, EVs have superior properties to overcome the indicated limitations due to extended circulation time, excellent tumor-targeting capabilities, lower immunogenicity, higher biocompatibility, and reduced systemic toxicity in the peripheral tissues (Saari et al. 2015).

5 EVs and Therapeutic Properties

Nowadays, EVs are gaining novel therapeutic properties because they not only carry a selection of components from the cell for cell-to-cell signaling, promotion of differentiation, enhancement of homeostatic response, or garbage bags, but they are also giving some new perspectives about the cells' biological and pathological conditions. EVs can enter circulation and are found in serum, plasma, monocytes, macrophages, urine, platelets, cardiomyocytes, valvular interstitial cells, and endothelial cells. The EVs can be used as biomarkers for diagnosis, prognosis, therapeutic flow, and drug delivery vehicles by using various technologies, such as Western Blot, flow cytometry, ultracentrifugation, LC-MS/MS, Luminex-based multiplex panels, Bio-plex 200 systems, and ELISA (de Freitas et al. 2021; Kenneweg et al. 2019; Liu et al. 2019a, b; Matsumoto et al. 2013; Melnikov et al. 2020; Nguyen et al. 2018; Rogers et al. 2020; Sinning et al. 2011; Vida et al. 2017).

According to the traditional synthetic drug delivery nanosystems, EVs have superior advantages in carrying diverse biomolecules and provide various advantages in drug delivery systems (Gaurav et al. 2021; Meng et al. 2020). The content of transported material changes according to a specific function and to regulate signaling, and gene expression, leading to alterations in various diseases, for instance, cancer, cardiovascular diseases, neurological disorders, central nervous system diseases, and inflammation (Kwok et al. 2021). It has been proven that exosomes can be found in cerebrospinal fluid and body fluids, and can penetrate the blood-brain barrier. Natural EVs contain specific surface molecules for targeting, which can be used in nanocarriers/fused with cells, and in many clinical applications. From a nano-engineering perspective, EVs are stable in circulation and can overcome natural barriers to be used as drug delivery vehicles. EVs can be used in regenerative medicine for such things as musculoskeletal systems, myocardial repair, etc. (Abreu et al. 2021; He et al. 2021; Liu et al. 2021a; Vader et al. 2016).

The properties of EVs give them extra-important biomarker properties for early prognosis and severity judgment and for monitoring diseases. Exosomes can provide nutrition and promote neurogenesis, and they can be involved in brain reconstruction after stroke (Harishkumar et al. 2021; Liu et al. 2019a). There are many factors affecting health, and any change in health conditions can affect the components of EVs and exosomes. For example, cigarette smoke contains many chemicals that can

induce cigarette smoke-induced diseases. It has been investigated that EVs have various functions in lung diseases such as lung cancer, chronic obstructive pulmonary disease, etc. (Fujita et al. 2015; Ryu et al. 2018). The biochemical effects may be positive or negative effects on the organism since some EVs may have an important role in tumor progression, cell proliferation, escape from apoptosis, inducing cancer cell invasion, metastasis, reprogramming all energy metabolism pathways, transferring the knowledge of any type of mutation, changing the extracellular matrix, modifying the microenvironment, or sending messages for any type of disease. EVs have unique properties: for example, by aging, mtDNA levels would decrease (Lazo et al. 2021; Xavier et al. 2020). In acute lymphoblastic leukemia, ovarian serous carcinoma, thyroid cancer, cholangiocarcinoma, breast cancer, and pancreatic cancer, derived microvesicles contain microRNAs (miRNAs), and miRNAs have key roles in cell proliferation, intracellular signaling, communication, differentiation, apoptosis, and cell cycle regulation (Cappellesso et al. 2014; Kitdumrongthum et al. 2018; Lee et al. 2015; Lu et al. 2015; Xavier et al. 2020). The content of the EVs changes according to the aggressiveness of cancer; for instance, glioblastoma and neoplasms have a 100% recurrence rate (Kovalenko et al. 2021; Pavlyukov et al. 2018). EVs can be used for early biomarkers for cancer and neurodegenerative disease diagnosis. The usage detection of various biomarkers in blood or other body fluids by novel technologies for early diagnosis (Hu et al. 2021; Jiang et al. 2022; Martinez-Dominguez et al. 2021). EVs secreted from cancer cells can induce cell growth and cell survival, and the content of the EVs changes after chemotherapy. The EVs that are generated after chemotherapy can promote angiogenesis and chemoresistance (Chang et al. 2021). On the other side, it is well known that RNAs have various functions besides protein coding, such as regulatory functions, intercellular communication, and many other functions. RNA molecules in EVs derived from different cellular origins could have different roles that we don't know. (Karn et al. 2021; Kovalenko et al. 2021). The EVs are not new in the scientific research area, but with the help of novel researchers, we are learning additional properties of these vesicles. It will happen quite soon that EVs will be the key factor for diagnosis, therapy, and monitoring of diseases for treating a wide range of diseases.

6 Aging and EVs

Aging is the major risk factor for many age-related pathologies and diseases. Cellular senescence is characterized by a change in various biochemical mechanisms, including metabolism, decreased bioenergetics, destabilization of the electron transport chain, complexes, proteostatic and nutrient signaling dysfunction due to telomere erosion, stem cell exhaustion, altered intracellular communication, genomic instability, oncogene overexpression, increased reactive oxygen species (ROS)-mediated DNA damage, mitochondrial dysfunction, defective mitophagy, endoplasmic reticulum stress, inflammation, epigenetic factors, macrophage-aging, increased oxidative stress, other dysregulated cellular pathways and mechanisms,

etc. (Aunan et al. 2016; Aydemir et al. 2021; Grammatikakis et al. 2014; López-Otín et al. 2013; McHugh and Gil 2018; Prattichizzo et al. 2016; Ulusu 2015a; Ulusu et al. 2017; Ulusu and Tandogan 2006). By aging enzyme activities and kinetic mechanisms are also changing, which can cause enormous effects on many metabolic pathways, metabolism, and diseases (Ulusu 2015a, b, c; Ulusu and Tandogan 2006). The age-related decline or changes may affect the contents of EVs, and this would play a diverse physiological, biological, and biochemical processes (Lananna and Imai 2021).

Dysregulation of all mechanisms and pathways refers to a poor ability to control and manage all stresses that may cause various disorders, diseases, and cellular aging. Production of oxidants by endogenous sources or from exogenous sources can damage the biological systems if the antioxidant defenses are not at adequate levels in the system. (Flohé 2020; Sharifi-Rad et al. 2020). Investigating the sources of oxidants and antioxidants is very crucial. The free radicals, ROS, and reactive nitrogen species (RNS) are derived from exogenous sources such as cigarette smoke, air pollution, ozone, heavy metals, ultraviolet light, ionizing radiation, industrial solvents, pesticides, certain drugs, and from endogenous sources during the metabolism of various substrates via dual oxidases, lipoxygenase, and cyclooxygenase in organelles mitochondria, peroxisomes, endoplasmic reticulum, and phagocytic cells (Aydemir et al. 2019; Aydemir and Ulusu 2020a; Flohé 2020; Guz et al. 2007; Phaniendra et al. 2015; Sharifi-Rad et al. 2020; Tandogan and Ulusu 2010; Ulusu 2015b; Ulusu et al. 2005; Ulusu and Tandogan 2006). There must be a balance between the oxidants and antioxidants in the cells, and antioxidants have beneficial roles in the detoxification of radicals. But oxidants have disadvantageous roles in biological systems, and the various types of radicals can induce pathological, irreversible cell injury reactions in cell death mechanisms such as apoptosis, autophagy, ferroptosis, and necrosis. ROS have enormous roles in lipid peroxidation, and lipid peroxidation induces apoptosis (Su et al. 2019; Wood and Youle 2006; Zhang et al. 2022).

However, more antioxidants cause harm to metabolism and increase the risk of various diseases, such as high concentration of selenium, which may have adverse or pathological effects on the tissues (Aydemir et al. 2018; Ulusu et al. 2003; Vinceti et al. 2014; Wang et al. 2018). Selenium is an essential trace element and has a regulatory effect on glutathione peroxidase enzyme (GPx). This enzyme is accepted as a detoxification enzyme, can catalyze the hydrogen peroxide to water and oxygen, and is classified under the glutathione s-transferase family (GST) (Can et al. 2005; Turan et al. 2001). It has been investigated that small extracellular vesicles (sEVs) contain GST enzymes from the young cells, and it is accepted that the roles of these enzymes in the sEVs are for restoring antioxidant capacity. In another paper, it was suggested that sEVs secreted from young people can reduce oxidative stress in old individuals' cells (Fafián-Labora et al. 2020; Melidoni 2020). Furthermore, it has been considered that the vesicles that are secreted from senescent cells contain high amounts of inflammatory cytokines, chemokines, matrix metalloproteinase enzymes, ephrin type-A receptor 2 (EphA2), and DNA. These molecules can induce inflammation, DNA damage, and cancer cell proliferation and are associated with

cancer cell metastasis. But, in the EVs, decreased miR-219 concentrations have negative impacts on myelination, and increased concentrations of telomeric repeat-containing RNA (TERRA) can cause immune cell activation. Decreased galactin-3 and increased miR-183-5p reduce osteogenesis (Davis et al. 2017; Pusic and Kraig 2014; Takahashi et al. 2017; Takasugi 2018; Wang et al. 2015; Weilner et al. 2016). The molecules that are secreted from senescent cells are the confirmation that aging is an inevitable major risk factor for cancer development (Aunan et al. 2017).

The molecules that are associated with the energy status of cells aging. NAD^+ and its phosphorylated form NADP^+ are coenzymes of many regulatory enzymes. Both molecules are essential in energy metabolism, DNA damage repair, gene expression, and oxidative stress, and they play a critical role in aging. The NAD^+ is an important electron carrier in the electron transport chain, and depletion of NAD^+ and ATP can trigger cell death (Berger 2004; Huang 2019; Katsyuba and Auwerx 2017; Morales et al. 2014). The *de novo* biosynthesis of NAD^+ molecule is done by the nicotinamide phosphoribosyltransferase enzyme. It is investigated that EVs contain nicotinamide phosphoribosyltransferase enzyme (eNAMPT); however, the concentration of this enzyme decreases in circulation with aging (Garten et al. 2015; Yoshida et al. 2019). The reduced forms of NAD and NADPH concentrations have central roles in various cellular reactions and supporting cellular homeostasis (Koju et al. 2022). The aging content of the EVs is changing. EVs have enormous effects on their neighbor cells/target cells, and sending an EV to the neighboring cells means that you may get a response according to the content of the vesicle. The target cells' metabolism would be upregulated or downregulated, or various types of biochemical changes would occur after taking the EVs, which are sent from neighbor cells (Liu et al. 2021b).

7 Aging Is the Major Risk Factor for the Development of Diseases

The world's population is getting older every year, and the number of diseases is increasing (Divo et al. 2014). Biological aging is a significant process for increasing the risk factors for developing various diseases. The molecular mechanism of cellular senescence is accepted as the cells lose some of their biological and biochemical functions, change in response to intrinsic and extrinsic stimuli and regulatory protein functions, lose protein homeostasis, lose the ability to divide, and lose the ability to combat oxidative stress (Aydemir and Ulusu 2021; Becker and Haferkamp 2013; Kumari and Jat 2021; Squier 2001; Ulusu 2015c; Ulusu et al. 2017; Ulusu and Tandogan 2006).

Aging is one of the key factors in a group of neurodegenerative diseases. Mental and physical changes are associated with aging and Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) disease (Aydemir and Ulusu 2020b; Erbayraktar et al. 2017, 2018; Evlice and Ulusu 2017; McGinley and Ontaneda 2019; Sanai et al. 2016; Simon et al. 2020; Tartaglia et al. 2007; Ulusu 2015a, b, c; Ulusu et al. 2015). On the other hand, all these diseases are multifactorial, and genetic mutations, epigenetic factors, oxidative

stress, head injuries, protein aggregations, infections, trauma, smoking, etc. (Aydemir and Uluşu 2020a, b; Cerri et al. 2019; Evlice and Uluşu 2017; Howard et al. 2016; Simon et al. 2020; Stephenson et al. 2018; Tran and Ha-Duong 2015).

These neurodegenerative diseases are complex syndromes. All of them have more than one specific biomarker protein for the biochemical/medical discrimination of the disease; for example, AD A β 1–42, total tau (t-tau), and phosphorylated tau –181 (p-tau) are the key proteins. In AD, the cerebrospinal fluid (CSF) of the patients' reduced levels of A β , 1–42, and increased levels of t-tau and p-tau are indicators of the disease. This disease can be classified as young (end of the thirties and mid-sixties) and old onset (begins at the end of sixties, and the risk of the disease increases with aging). Amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) proteins are important for the early onset of Alzheimer's disease (EOAD), and for the late onset of Alzheimer's disease (LOAD), APOE is the most strongly associated with LOAD (Khan 2016a, b; Borges et al. 2019). The transfer mechanism of the accumulated β -amyloid peptide 1–42 is unclear (Jiang et al. 2019). Tau mutations cause alternative splicing and phosphorylation state, interaction with tubulin, and self-association and cause various forms of tau, which are found in the human brain. The mutant tau proteins can cause synaptic dysfunction, memory loss, and neuropathology, and change the protein content of the tau exosome as well as their transfer mechanism. Since the hyperphosphorylated tau protein (p-tau), which is related to frontotemporal dementia, familial dementia, AD, and PD, is very complex, the transport of these cytotoxic proteins has begun to be solved with new research (Ferreira and Afreen 2017; Lee et al. 2001; Podvin et al. 2020). The mutations or modifications of tau, such as hyperphosphorylations and truncation can cause aggregation of the tau protein and cause neuronal cell death (Chun 2007; Rankovic and Zweckstetter 2019). Engineered extracellular vesicles can be used against AD patients' therapies (Yin et al. 2023).

ALS disease is one of the rare and lethal neurodegenerative diseases causing upper and lower neuron loss and the effects of disease seen in aging (Broussalis et al. 2019; Wijesekera and Nigel Leigh 2009). In ALS disease, age-related muscle fatigue, decline, loss of muscle mass, strength, and endurance can be seen in both sexes (Tartaglia et al. 2007). It is possible to detect EVs in ALS patients' body fluids, such as blood and cerebrospinal fluids, that give some important ideas about the patient's health status. Superoxide dismutase 1 (SOD1) enzyme is detected in primary astrocyte cultures, and animal models of ALS, and the SOD1 enzyme is an antioxidant enzyme that has a protective role against ROS (Basso et al. 2013; Gagliardi et al. 2021; Gomes et al. 2007; Liu et al. 2019a). EVs could open doors to novel therapies for ALS disease (Bonafede and Mariotti 2017).

There are many factors affecting healthy aging, and one of the key factors is oxidative stress. Oxidative stress has significant effects on all neurodegenerative diseases, including diabetes, chronic kidney diseases, renal damage, liver diseases, viral infections, etc. (Aydemir and Uluşu 2021; Bulbul et al. 2018; Can et al. 2005; Divo et al. 2014; Gokturk et al. 2014; Uluşu 2015a, b, c). EVs are carrying cargoes that contain signaling molecules and have crucial roles in the maintenance of cellular

homeostasis and physiopathological change accepted as very important particles (VIPs) in age-related diseases (Mas-Bargues and Alique 2023).

8 Multiple Organ Dysfunction or Diseases and Extracellular Vesicles

There are many complex cellular mechanisms involved in the background of the diseases and the progression of the diseases, for instance, cancer (Anand et al. 2023). Cancer metastasis has sequential multiple physiological and biochemical sub-processes. Metastasis changes according to the invasiveness of the cancer type. It is not a linear cascade of biochemical and physiological reactions. Cancer metastasis must be evaluated via multidisciplinary systems based on the tumor heterogeneity, key factors, and critical features of the molecular signaling background of metastasis. Different cancer types have distinct responses to similar stress situations or the same gene perturbations. Different cancer cell line properties characterize the Cancer Dependency Map Database. Crosstalk between different genes and DNA damage, cell cycle regulations, and various other interactions is gaining too much importance for understanding the exact underlying molecular mechanisms and progression of the disease (Clarke et al. 2020; Jung et al. 2021; Suhail et al. 2019). On the other hand, multiple types of cancer cause metastasis to bone. Therefore, it is important to understand the crosstalk between the osteoblasts, osteoclasts, and bone metastatic cells to overcome the ongoing problem of cancer metastasis (Rossi et al. 2018; Shupp et al. 2018). There are many factors affecting cancer cell proliferation, progression, invasion, metastasis, and drug resistance. Forkhead box protein M1 protein (FoxM1) has critical roles in various types of cancer, such as gastric and breast cancers. Gastric cancers are the second-leading type of cancer in the world, and breast cancer is the second-leading cause of cancer death in the world. FoxM1 is one of the biomarkers of breast cancers and affects all the steps of cancer cell proliferation, and metastasis. However, there are also many factors present that are upregulating and downregulating the FoxM1 expression in breast cancer cells, and it is investigated that the FoxM1 protein. On the other hand, FoxM1 is identified in circulating exosomes (Zhang et al. 2021a, b).

9 The Impact of Extracellular Vesicles (EVs) on Cancer Treatment as Promising Tools

The International Society for Extracellular Vesicles (ISEV) describes EVs as lipid-enveloped vesicles released from cells that do not contain a nucleus and cannot replicate themselves. EVs are divided into three groups: microvesicles, exosomes, apoptotic bodies, and others. EVs are released from prokaryotic and eukaryotic cells, such as epithelial cells, mesenchymal stem cells, neurons, and endothelial cells, to regulate intracellular regulation and communication through blood circulation. Therefore, EVs are considered potential and promising biomarkers, drug delivery

tools, and therapeutic approaches (L. Wang et al. 2023). Current techniques used for cancer diagnosis are imaging, biomarkers, and symptoms; however, some cancers, such as prostate and colon, do not exert symptoms until they become metastatic. On the other hand, imaging or biochemical evaluation is not enough to determine tumor type, whether malign or benign, for thyroid and lung nodules requiring invasive biopsies (Zhou et al. 2023).

Since delayed diagnosis and treatment lead to poor prognosis and increased risk for mortality in cancer patients, novel cancer biomarkers are urgently required for early prognosis, disease progression monitoring, and personalized therapies since the incidence of cancer is increasing every year worldwide (Krzyzszczuk et al. 2018). Liquid biopsy and biological fluids, especially blood, are considered the most important strategies for cancer detection, treatment, screening, and management. Since cell-cell interactions between tumor cells and cancer cell communication with stromal and immune cells occur via EVs, they are considered promising biomarkers for early detection of cancer since they are secreted from cells and circulated through blood (Lone et al. 2022). On the other hand, chemotherapeutic drugs have limitations for cancer treatment since they have poor bioavailability, uncontrolled cytotoxicity in the peripheral tissues, systemic side effects, and poor targeting ability. However, EVs have superior properties to overcome the indicated limitations because they have extended circulation time, excellent tumor-targeting capabilities, lower immunogenicity, higher biocompatibility, and reduced systemic toxicity in the peripheral tissues (Saari et al. 2015). In this chapter, we discussed the impact of different types of EVs on cancer treatment.

9.1 Mesenchymal Stem Cell-Derived EVs (MSC-EVs)

Mesenchymal stem cells (MSCs) are found in different tissues, including bone marrow, umbilical cord, placental tissue, umbilical cord blood, and menstrual blood, as the main stem cell source and have differentiation potential (Kou et al. 2022). MSCs have renewal ability and differentiate into chondrocytes, osteoblasts, and adipocytes due to their pluripotency. Since cancer cells are under the control of the immune system, they need to develop strategies for immune evasion and immunosuppression. Thus, cancer cells use the immunosuppressive and angiogenic effects of MSCs to escape the immune system and to supply nutrients, respectively (Boutillier and Elswa 2021).

Mesenchymal stem cell-derived EVs (MSC-EVs) are widely used for drug delivery tools due to their immunomodulatory, regenerative, and immunosuppressive effects; for instance, paclitaxel containing MSC-EVs can inhibit cell proliferation of pancreatic cancer cells (Pascucci et al. 2014). miR-193a downregulated focal adhesion kinase (FAK) containing MSC-EVs inhibited proliferation, migration, and invasion of the colon cancer cells. In liver cancer, miRNA-20a-3p carrying MSC-EVs induces apoptosis via targeting cFLIP (Deng et al. 2021). microRNA-379 containing EVs reduced tumor growth and lymph node metastases in breast cancer, and inhibited proliferation, invasion, and migration in various cancers,

including colorectal, hepatocellular cancer, vascular smooth muscle, mesothelioma, and osteosarcoma cell lines (O'Brien et al. 2018). However, the application of mesenchymal stem cells in therapy has been limited due to their potential tumorigenicity (Kou et al. 2022).

Needle or surgical biopsies are the most common materials collected from cancer patients to evaluate cancer progression, type, and metastasis; however, biopsies have risks and complications due to invasiveness. Thus, liquid biopsies have gained attention in cancer prognosis and monitoring which uses blood samples to evaluate EVs, proteins, DNA, and circulating cancer cells (Lone et al. 2022). Liquid biopsies are easy to obtain and handle without complications such as infection, hemorrhage, and pain. However, possible contamination with normal cells, short half-life, and isolation are major drawbacks of liquid biopsies. Among liquid biopsy samples, EVs have gained attention since they contain MSCs and cancer cell-derived miRNAs. EVs containing miRNAs are found in most of the biological fluids, including bile, saliva, plasma, serum, breast milk, and cerebrospinal fluid (Handa et al. 2021; Lone et al. 2022; Zhou et al. 2023). An increased number of circulated exosomes are found in cancers compared to healthy individuals; therefore, MSC-derived exosomal RNAs are used for the prognosis and diagnosis of various types of cancers, including brain, prostate, head and neck, ovarian, breast, colon, lung, liver, prostate and bladder. For instance, exosomal miR-214, miR-221, and miR-222 are correlated with gastric cancer progression and development. Overexpression of miR-214 is associated with angiogenesis and poor prognosis in hepatocellular cancer (Lone et al. 2022; Wang et al. 2023; Wang and Zhou 2013).

9.2 Immune Cell-Derived Extracellular Vesicles (EVs)

Immune cell-derived extracellular vehicles (EVs) are excreted from innate and adaptive immune cells having superior properties, including accessibility, storage, and crossing blood-brain and blood-tumor barriers. Innate immune cells (macrophages, natural killer (NK) cells, dendritic cells (DCs), and neutrophils) are the first line of defense system against pathogen-infected and tumor cells via secretion of inflammatory molecules (Eppler and Jewell 2020). CD4 + T, CD8 + T, and B cells are adaptive immune cells that complete the function of innate immune cells. Also, T and B cells have long-term memory and can kill the tumor cells with high specificity by preventing recurrence. Cancer immunotherapy approaches have been developed by using T cell and DCs functions such as immune checkpoint antibodies, vaccinations, monoclonal antibodies, and chimeric antigen receptor (CAR)-T cell therapies (S. Cheng et al. 2020). On the other hand, indicated therapeutic approaches have limitations because of the escape strategies and heterogeneity of the tumor. Furthermore, (CAR)-T cell is one of the most promising approaches among others; however, it induces side effects such as hypoxia, multi-organ toxicity, high fever, encephalopathy, and cytokine release syndrome in the patients. Immune cell-derived extracellular vesicles (EVs) are promising options to overcome the major drawbacks of immunotherapy (Cheng et al. 2020; Neelapu et al. 2018).

NK-derived EVs carry small antimicrobial peptides, and TNF-related apoptosis-inducing ligands (TRAIL/CD253), including perforin, granzymes, granulysin, and FasL/CD178. Indicated molecules can kill various types of cancer cells, such as melanoma, breast, and blood, via a direct killing mechanism. miRNA-186 carrying NK-derived EVs inhibits expression of different oncogenes, including the v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN), transforming growth factor-beta (TGF- β) receptor type 1 (TGFR1), TGFR2 and Aurora kinase (AURKA) in neuroblastoma patients (Neviani et al. 2019). Macrophage-derived EVs exert different opposite effects on the cancer via regulating tumor initiation and progress. M2 macrophages induce tumor growth and metastasis, whereas M1 macrophages kill tumor cells via phagocytosis. Macrophage-derived EVs contain adhesion molecules and surface ligands, including tetraspanins, intercellular adhesion molecule 1 ICAM-1, and phosphatidylserine to bind T cells for activation (Ismail et al. 2013). miRNA-16-5p containing M1-macrophage-derived EVs inhibits PD-L1 leading to the inhibition of in vivo and in vitro gastric cancer formation (Li et al. 2020b). Long noncoding RNAs (*lncRNA*) containing macrophage derived EVs regulate tumor pathogenesis and microenvironment (Wu et al. 2021a).

DCs are antigen-presenting cells to activate T-cells which regulate innate and immune responses. DCs secreted EVs carry proteins, RNAs, miRNAs, and metabolic enzymes, including pyruvate, peroxidases, lipid kinases, and enolase 1. The release of DCs secreted EVs depends on various factors, such as DNA damaging treatment, the maturation stage of DCs, and signals produced by T-cells. DCs-derived EVs play a vital role in cancer immunotherapy since they activate both T and B cells inducing antitumor immunity (Théry et al. 1999). Other immune cell-derived EVs are secreted from neutrophils, CD4+ cells, CD8+, and B cells involved in tumor immunity, immunotherapy, and drug carrier systems (Yang et al. 2021). On the other hand, immune cell derived EVs are engineered to produce antitumor vaccines or adjuvant candidates via reprogramming genetic, membrane, and cargo functions (Yang et al. 2021). IDO, FasL, or IL-4 can be delivered into DCs via genetic modifications to produce overexpressing DC-derived EVs targeting tumors. Chemical crosslinking regulates membrane composition and inserts specific targets on the surface. Moreover, cancer drugs, antibodies, miRNAs, and siRNAs can be carried via EVs by using cargo delivery engineering. Antigen or adjuvant-containing DCs-derived EVs can directly trigger CD4+ and CD8+ T cells as vaccines. Also, immune cell-derived EVs containing chemotherapeutic drugs inhibit tumor proliferation and metastasis. However, detailed, and advanced studies are required to understand complex interactions between immune cell-derived EVs and tumor microenvironment (Cheng et al. 2018; Li et al. 2021).

9.3 Tumor-Derived Extracellular Vesicles (TD-EVs)

Tumor-derived extracellular vesicles (TD-EVs) carry specific labels such as antigens, oncogenic proteins, and nucleic acids involved in the tumor progression, that are used

for biomarker and drug carriers. Chemotherapy-loaded TD-EVs decreased tumor size and increased survival time compared to traditional chemotherapy, for instance, platinum-loaded TD-EVs derived from A549 cell lines decreased tumor size in lung cancer patients having platinum resistance (Walker et al. 2019). Pancreatic cancer cell derived EVs decreased pancreatic tumor growth with minimum damage to the peripheral tissues and enhanced the survival time of mice. Thus, EVs have a superiority for targeted drug delivery tools for targeted therapy to treat cancer (Li et al. 2020a). On the other hand, several studies reported that chemoresistance is associated with TD-EVs, and it is hypothesized that eliminating TD-EVs of interest can increase tumor vulnerability to chemotherapeutics. For instance, removing Her-2 positive EVs from the tumor site enhanced the anti-proliferative effects of trastuzumab (Saleem et al. 2022). Paclitaxel (PTX) containing EVs derived from pancreatic cancer cells, including PC-3 and LNCap, significantly enhanced in vitro cytotoxicity of PTX (Challagundla et al. 2015). TD-EVs can trigger tumor growth and metastasis via activating oncogenic pathways and exerting immunosuppressive effects. TD-EVs can enhance the migration ability of tumor cells by promoting epithelial-to-mesenchymal transition (EMT) and extracellular matrix degeneration. They promote angiogenesis by activating macrophages indirectly and by noncoding RNA and proteins directly (Bai et al. 2022). On the other hand, miRNAs containing TD-EVs are also involved in drug resistance and efficacy in cancer patients, for instance, miR-222 and miR-100 were found in breast cancer patients having chemoresistance (Saari et al. 2015).

10 Challenges in EVs-Related Techniques and Applications

10.1 Isolation and Quantification of EVs

EVs play a crucial role in cellular communications; thus, they are considered promising tools for biomarkers, drug delivery, and therapeutic approaches. On the other hand, storage, isolation, purification, biodistribution, thrombosis, alloimmune responses, elimination, and pharmacokinetic calculations are the major drawbacks of EV-related applications. Urine, blood, plasma, and serum samples from EV sources that can be contaminated with virions and infectious particles are used. Ultracentrifugation up to 1,000,000 g is the most used and effective technique to collect EVs from biological samples; however, this technique has several drawbacks, including time-consuming, low reproducibility, low RNA yield, mechanical damage, and uniform methodology (Zeng et al. 2022). Commercial kits are also used for EV purification but are time-consuming, expensive, and offer low purity. Immunoaffinity capture, polymer precipitation, and microfluidic techniques are alternative techniques used for EV purification, and several studies have suggested using different techniques in combination to overcome centrifugation-related limitations. Since there is no uniform and common technique to collect EVs, each study needs to develop an effective method for EVs of interest. Furthermore, low EV yield obtained from human samples leads to limitations in the clinical translation (Herrmann et al. 2021).

EVs are collected from cell cultures besides biological samples such as blood, urine, plasma, serum, saliva, and cerebrospinal fluid. Therefore, cell culture conditions, including temperature, CO₂, humidity, passaging, cell type, density, collection frequency, and stress, affect collected EVs' quality, yield, and activity. For instance, before collecting EVs from culture, cells are exposed to different methods to increase EV yield, such as hypoxia, increasing intracellular calcium levels, heat stress, exogenous agents, and low nutrient medium that leads to different behavior of cells than average conditions. On the other hand, the origin of EVs plays a critical role in the clinical translation. Thus, EVs collected from cell culture should be thoroughly characterized and tested to confirm suitability for clinical applications (Herrmann et al. 2021; Vida et al. 2017; Zeng et al. 2022). For therapeutic application, the effective dose of EVs must be precisely calculated. Since EVs contain nucleic acids, lipids, and protein, total RNA, lipid, protein, particle number, or specific molecule content can be used to quantify EVs. Based on the separation technique, e protein content can be miscalculated due to protein calculation and aggregation. On the other hand, some EVs are not suitable for total lipid calculation, and RNA quantification cannot be applied for EV quantification since non-EV RNA is abundant in the cells and biological fluids. Thus, effective techniques should be optimized for precise EV quantification and clinical applications (Gupta et al. 2021; Thone and Kwon 2020; Wiklander et al. 2019).

10.2 Storage of EVs

EVs storage is another critical parameter following collection and handling associated with EV stability and reproducibility. Generally, EVs are stored at -80°C for the long term, and EVs are suspended in the PBS storing at 4°C for the short term. Freezing temperature, additions including DMSO, protease, or phosphate inhibitors, freeze-thaw cycles, and post-thaw centrifugation steps should be thoroughly evaluated to handle the storage conditions of EVs. The most effective long-term storage condition is in PBS at -80°C for up to 24 months without any alterations in size, morphology, concentration, protein, and transcriptome markers. However, protein and transcriptome markers are degraded when EVs are stored at -20°C (Barreiro et al. 2021; Wu et al. 2021b).

10.3 Drug Delivery

EVs have superior properties for drug delivery applications, including extended circulation time, excellent targeting capabilities, lower immunogenicity, crossing biological barriers, higher biocompatibility, and reduced systemic toxicity in the peripheral tissues. However, the major drawbacks of EVs-related drug delivery systems are targeting, dose evaluation, drug clearance, contamination, and biodistribution (Gupta et al. 2021; Wiklander et al. 2019). Firstly, dose determination of EVs has limits since there are no optimized methods for EVs quantification

leading to miscalculation of drug loaded EVs, as discussed above. On the other hand, the profile of cell culture-derived EVs is affected by culture conditions such as seeding density, cross-contamination with other molecules, antibiotic usage, glucose concentration of cell media, and passage numbers. The pharmacokinetic profile of EVs varies between cell culture and *in vivo* applications; thus, dosages of EVs should be carefully determined for clinical applications. Furthermore, drug-loading capacity and drug-loading methods EVs cause decreased efficacy and targeting abilities of EVs. EVs primarily target the liver, kidney, and spleen regardless of carrying targeting molecules affecting the biodistribution properties of EVs. A detailed understanding of the mechanism of EV-related applications in specific diseases should be studied for precise prediction of the biodistribution and therapeutic effects of EVs (Gupta et al. 2021; Herrmann et al. 2021; Thone and Kwon 2020; Wiklander et al. 2019; Wu et al. 2021b).

11 Conclusion

Recent technological advances have shown that EVs have critically important cellular roles and functions in drug delivery, potential biomarkers, and therapeutic approaches. In intercellular communication via EVs, the biochemical content of the vesicles changes according to the (Herrmann et al. 2021; Thone and Kwon 2020; Wiklander et al. 2019) physiological status of the individuals/organisms. EVs contain various messengers to transmit the signals from cells to the target cells. EVs have enormous roles in multiple physiological and biochemical processes and intercellular communications in health and disease and solving all the details of the extracellular vesicles is crucial to identifying unknown cellular mechanisms and disorders. Age and life expectancy may be calculated from the detailed EV analysis with the help of machine learning. From the EVs that can be analyzed from blood and other body fluids, no secret can be kept, such as cancer type, age, metastatic status, the disease that the patient has, or bone fracture. The critical point is controlling all the EV components and understanding what best predicts the patient's condition. Since cell-cell interactions between tumor cells and cancer cell communication with stromal and immune cells occur via EVs, they are considered promising biomarkers for the early detection of cancers since they are secreted from cells circulating in the blood and are easy to collect. EVs have superior properties to overcome chemotherapy-related limitations because they have extended circulation time, excellent tumor-targeting capabilities, lower immunogenicity, higher biocompatibility, and reduced systemic toxicity in the peripheral tissues. In this chapter, we discussed the biogenesis, role, and functions of various types of EVs in aging-related diseases and cancer treatment. Therefore, EVs are expected to be used as biomarkers in personalized medicine to diagnose diseases in the future.

References

- Abels ER, Breakefield XO (2016) Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. *Cell Mol Neurobiol* 36(3):301–312. <https://doi.org/10.1007/s10571-016-0366-z>. Epub 2016 Apr 6. PMID: 27053351; PMCID: PMC5546313
- Abreu H, Canciani E, Raineri D, Cappellano G, Rimondini L, Chiocchetti A (2021) Extracellular vesicles in musculoskeletal regeneration: modulating the therapy of the future. *Cells* 11(1):43. <https://doi.org/10.3390/cells11010043>
- Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, De Falco V, Upadhyay A, Kandimalla R, Chaudhary A, Dhanjal JK, Dewanjee S, Vallamkondu J, Pérez de la Lastra JM (2023) Cancer chemotherapy and beyond: current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis* 10(4):1367–1401. <https://doi.org/10.1016/j.gendis.2022.02.007>
- Aunan JR, Watson MM, Hagland HR, Søreide K (2016) Molecular and biological hallmarks of ageing. *Br J Surg* 103(2):e29–e46. <https://doi.org/10.1002/bjs.10053>
- Aunan JR, Cho WC, Søreide K (2017) The biology of aging and cancer: a brief overview of shared and divergent molecular hallmarks. *Aging Dis* 8(5):628. <https://doi.org/10.14336/AD.2017.0103>
- Aydemir D, Uluşu NN (2020a) Comment on the: molecular mechanism of CAT and SOD activity change under MPA-CdTe quantum dots induced oxidative stress in the mouse primary hepatocytes (*Spectrochim Acta A Mol Biomol Spectrosc*. 2019 Sep 5; 220:117104). *Spectrochim Acta A Mol Biomol Spectrosc* 229:117792. <https://doi.org/10.1016/j.saa.2019.117792>
- Aydemir D, Uluşu NN (2020b) Importance of the serum biochemical parameters as potential biomarkers for rapid diagnosis and evaluating preclinical stage of ALS. *Med Hypotheses* 141: 109736. <https://doi.org/10.1016/j.mehy.2020.109736>
- Aydemir D, Uluşu NN (2021) People with blood disorders can be more vulnerable during COVID-19 pandemic: a hypothesis paper. *Transfus Apher Sci* 60(3):103080. <https://doi.org/10.1016/j.transci.2021.103080>
- Aydemir D, Karabulut G, Şimşek G, Gök M, Barlas N, Uluşu NN (2018) Impact of the Di (2-Ethylhexyl) phthalate administration on trace element and mineral levels in relation of kidney and liver damage in rats. *Biol Trace Elem Res* 186(2):474–488. <https://doi.org/10.1007/s12011-018-1331-0>
- Aydemir D, Hashemkhani M, Acar HY, Uluşu NN (2019) In vitro interaction of glutathione S-transferase-pi enzyme with glutathione-coated silver sulfide quantum dots: A novel method for biodetection of glutathione S-transferase enzyme. *Chem Biol Drug Des* 94(6):2094–2102. <https://doi.org/10.1111/cbdd.13614>
- Aydemir D, Salman N, Karimzadehkhoei M, Alaca BE, Turan B, Uluşu NN (2021) Evaluation of the effects of aging on the aorta stiffness in relation with mineral and trace element levels: an optimized method via custom-built stretcher device. *Biol Trace Elem Res* 199(7):2644–2652. <https://doi.org/10.1007/s12011-020-02380-9>
- Bai S, Wang Z, Wang M, Li J, Wei Y, Xu R, Du J (2022) Tumor-derived exosomes modulate primary site tumor metastasis. *Front Cell Dev Biol* 10. <https://doi.org/10.3389/fcell.2022.752818>
- Barreiro K, Dwivedi OP, Valkonen S, Groop P, Tuomi T, Holthofer H, Rannikko A, Yliperttula M, Siljander P, Laitinen S, Serkkola E, Af Hållström T, Forsblom C, Groop L, Puhka M (2021) Urinary extracellular vesicles: assessment of pre-analytical variables and development of a quality control with focus on transcriptomic biomarker research. *J Extracell Vesicles* 10(12). <https://doi.org/10.1002/jev2.12158>
- Basso M, Pozzi S, Tortarolo M, Fiordaliso F, Bisighini C, Pasetto L, Spaltro G, Lidonnici D, Gensano F, Battaglia E, Bendotti C, Bonetto V (2013) Mutant copper-zinc superoxide dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes. *J Biol Chem* 288(22):15699–15711. <https://doi.org/10.1074/jbc.M112.425066>

- Bebelman MP, Smit MJ, Pegtel DM, Baglio SR (2018) Biogenesis and function of extracellular vesicles in cancer. *Pharmacol Ther* 188:1–11. <https://doi.org/10.1016/j.pharmthera.2018.02.013>
- Becker T, Haferkamp S (2013) Molecular mechanisms of cellular senescence. In: *Senescence and senescence-related disorders*. InTech. <https://doi.org/10.5772/54120>
- Berger F (2004) The new life of a centenarian: signalling functions of NAD(P). *Trends Biochem Sci* 29(3):111–118. <https://doi.org/10.1016/j.tibs.2004.01.007>
- Bernardi S, Balbi C (2020) Extracellular vesicles: from biomarkers to therapeutic tools. *Biology (Basel)* 9(9):258. <https://doi.org/10.3390/biology9090258>. PMID: 32878063; PMCID: PMC7564466
- Bonafede R, Mariotti R (2017) ALS pathogenesis and therapeutic approaches: the role of mesenchymal stem cells and extracellular vesicles. *Front Cell Neurosci* 11. <https://doi.org/10.3389/fncel.2017.00080>
- Borges CR, Poyares D, Piovezan R et al (2019) Alzheimer's disease and sleep disturbances: a review. *Arq Neuropsiquiatr* 77:815–824. <https://doi.org/10.1590/0004-282x20190149>
- Boutillier AJ, ElSawa SF (2021) Macrophage polarization states in the tumor microenvironment. *Int J Mol Sci* 22(13):6995. <https://doi.org/10.3390/ijms22136995>
- Broussalis E, Griessenauer C, Mutzenbach S et al (2019) Reduction of cerebral DWI lesion burden after carotid artery stenting using the CASPER stent system. *J Neurointerv Surg* 11:62–67
- Bulbul MC, Dagele T, Afsar B, Ulusu NN, Kuwabara M, Covic A, Kanbay M (2018) Disorders of lipid metabolism in chronic kidney disease. *Blood Purif* 46(2):144–152. <https://doi.org/10.1159/000488816>
- Can B, Ulusu NN, Kiliç K, Leyla Acan N, Saran Y, Turan B (2005) Selenium treatment protects diabetes-induced biochemical and ultrastructural alterations in liver tissue. *Biol Trace Elem Res* 105(1–3):135–150. <https://doi.org/10.1385/BTER:105:1-3:135>
- Cappellesso R, Tinazzi A, Giurici T, Simonato F, Guzzardo V, Ventura L, Crescenzi M, Chiarelli S, Fassina A (2014) Programmed cell death 4 and microRNA 21 inverse expression is maintained in cells and exosomes from ovarian serous carcinoma effusions. *Cancer Cytopathol* 122(9):685–693. <https://doi.org/10.1002/cncy.21442>
- Cerri S, Mus L, Blandini F (2019) Parkinson's disease in women and men: what's the difference? *J Parkinsons Dis* 9(3):501–515. <https://doi.org/10.3233/JPD-191683>
- Challagundla KB, Wise PM, Neviani P, Chava H, Murtadha M, Xu T, Kennedy R, Ivan C, Zhang X, Vannini I, Fanini F, Amadori D, Calin GA, Hadjidanil M, Shimada H, Jong A, Seeger RC, Asgharzadeh S, Goldkorn A, Fabbri M (2015) Exosome-mediated transfer of microRNAs within the tumor microenvironment and neuroblastoma resistance to chemotherapy. *JNCI J Natl Cancer Inst* 107(7). <https://doi.org/10.1093/jnci/djv135>
- Chang W-H, Cerione RA, Antonyak MA (2021) Extracellular vesicles and their roles in cancer progression, pp 143–170. https://doi.org/10.1007/978-1-0716-0759-6_10
- Chatterjee V, Yang X, Ma Y, Wu MH, Yuan SY (2020) Extracellular vesicles: new players in regulating vascular barrier function. *Am J Phys Heart Circ Phys* 319(6):H1181–H1196. <https://doi.org/10.1152/ajpheart.00579.2020>
- Cheng Q, Shi X, Han M, Smbatyan G, Lenz H-J, Zhang Y (2018) Reprogramming exosomes as nanoscale controllers of cellular immunity. *J Am Chem Soc* 140(48):16413–16417. <https://doi.org/10.1021/jacs.8b10047>
- Cheng S, Xu C, Jin Y, Li Y, Zhong C, Ma J, Yang J, Zhang N, Li Y, Wang C, Yang Z, Wang Y (2020) Artificial mini dendritic cells boost T cell-based immunotherapy for ovarian cancer. *Adv Sci* 7(7). <https://doi.org/10.1002/adv.201903301>
- Chun W (2007) The role of tau phosphorylation and cleavage in neuronal cell death. *Front Biosci* 12(1):733. <https://doi.org/10.2741/2097>
- Claridge B, Lozano J, Poh QH, Greening DW (2021) Development of extracellular vesicle therapeutics: challenges, considerations, and opportunities. *Front Cell Dev Biol* 9. <https://doi.org/10.3389/fcell.2021.734720>

- Clarke R, Kraikivski P, Jones BC, Sevigny CM, Sengupta S, Wang Y (2020) A systems biology approach to discovering pathway signaling dysregulation in metastasis. *Cancer Metastasis Rev* 39(3):903–918. <https://doi.org/10.1007/s10555-020-09921-7>
- Cocucci E, Meldolesi J (2015) Ectosomes and exosomes: shedding the confusion between extracellular vesicles. *Trends Cell Biol* 25(6):364–372. <https://doi.org/10.1016/j.tcb.2015.01.004>
- Colombo M, Raposo G, Théry C (2014) Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 30:255–289. <https://doi.org/10.1146/annurev-cellbio-101512-122326>. Epub 2014 Aug 21. PMID: 25288114
- Cuesta CM, Guerri C, Ureña J, Pascual M (2021) Role of microbiota-derived extracellular vesicles in gut-brain communication. *Int J Mol Sci* 22(8):4235. <https://doi.org/10.3390/ijms22084235>
- Davis C, Dukes A, Drewry M, Helwa I, Johnson MH, Isales CM, Hill WD, Liu Y, Shi X, Fulzele S, Hamrick MW (2017) MicroRNA-183-5p increases with age in bone-derived extracellular vesicles, suppresses bone marrow stromal (stem) cell proliferation, and induces stem cell senescence. *Tissue Eng A* 23(21–22):1231–1240. <https://doi.org/10.1089/ten.tea.2016.0525>
- de Freitas RCC, Hirata RDC, Hirata MH, Aikawa E (2021) Circulating extracellular vesicles as biomarkers and drug delivery vehicles in cardiovascular diseases. *Biomol Ther* 11(3):388. <https://doi.org/10.3390/biom11030388>
- Deng L, Wang C, He C, Chen L (2021) Bone mesenchymal stem cells derived extracellular vesicles promote TRAIL-related apoptosis of hepatocellular carcinoma cells via the delivery of microRNA-20a-3p. *Cancer Biomark* 30(2):223–235. <https://doi.org/10.3233/CBM-201633>
- Di Bella MA (2022) Overview and update on extracellular vesicles: considerations on exosomes and their application in modern medicine. *Biology* 11(6):804. <https://doi.org/10.3390/biology11060804>
- Divo MJ, Martinez CH, Mannino DM (2014) Ageing and the epidemiology of multimorbidity. *Eur Respir J* 44(4):1055–1068. <https://doi.org/10.1183/09031936.00059814>
- Eppler HB, Jewell CM (2020) Biomaterials as tools to decode immunity. *Adv Mater* 32(13). <https://doi.org/10.1002/adma.201903367>
- Erbayraktar Z, Evlice AT, Yilmaz G, Yazici C, Yener G, Uluşu NN (2017) Evaluation of renal function in Alzheimer's disease and geriatric patients: results from a Turkish two-center study. *J Med Biochem* 36(1):54–61. <https://doi.org/10.1515/jomb-2016-0028>
- Erbayraktar Z, Evlice A, Yener G, Uluşu NN (2018) Effects of donepezil on liver and kidney functions for the treatment of Alzheimer's disease. *J Integr Neurosci* 16(3):335–346. <https://doi.org/10.3233/JIN-170020>
- Evlice A, Uluşu NN (2017) Glucose-6-phosphate dehydrogenase a novel hope on a blood-based diagnosis of Alzheimer's disease. *Acta Neurol Belg* 117(1):229–234. <https://doi.org/10.1007/s13760-016-0666-6>
- Fafián-Labora JA, Rodríguez-Navarro JA, O'Loughlin A (2020) Small extracellular vesicles have GST activity and ameliorate senescence-related tissue damage. *Cell Metab* 32(1):71–86.e5. <https://doi.org/10.1016/j.cmet.2020.06.004>
- Ferreira A, Afreen S (2017) Methods related to studying tau fragmentation, pp 245–258. <https://doi.org/10.1016/bs.mcb.2017.06.004>
- Flohé L (2020) Looking back at the early stages of redox biology. *Antioxidants* 9(12):1254. <https://doi.org/10.3390/antiox9121254>
- Fujita K, Kubota Y, Ishida H, Sasaki Y (2015) Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer. *World J Gastroenterol* 21(43):12234–12248. <https://doi.org/10.3748/wjg.v21.i43.12234>. PMID: 26604633; PMCID: PMC4649109
- Gagliardi D, Bresolin N, Comi G, Pietro, Corti S (2021) Extracellular vesicles and amyotrophic lateral sclerosis: from misfolded protein vehicles to promising clinical biomarkers. *Cell Mol Life Sci* 78(2):561–572. <https://doi.org/10.1007/s00018-020-03619-3>
- Garten A, Schuster S, Penke M, Gorski T, de Giorgis T, Kiess W (2015) Physiological and pathophysiological roles of NAMPT and NAD metabolism. *Nat Rev Endocrinol* 11(9):535–546. <https://doi.org/10.1038/nrendo.2015.117>

- Gaurav I, Thakur A, Iyaswamy A, Wang X, Chen X, Yang Z (2021) Factors affecting extracellular vesicles based drug delivery systems. *Molecules* 26(6):1544. <https://doi.org/10.3390/molecules26061544>
- Gokturk H, Ulusu NN, Gok M, Tuncay E, Can B, Turan B (2014) Long-term treatment with a beta-blocker timolol attenuates renal-damage in diabetic rats via enhancing kidney antioxidant-defense system. *Mol Cell Biochem* 395(1–2):177–186. <https://doi.org/10.1007/s11010-014-2123-2>
- Gołębiewska JE, Wardowska A, Pietrowska M, Wojakowska A, Dębska-Ślizień A (2021) Small extracellular vesicles in transplant rejection. *Cells* 10(11):2989. <https://doi.org/10.3390/cells10112989>. PMID: 34831212; PMCID: PMC8616261
- Gomes C, Keller S, Altevogt P, Costa J (2007) Evidence for secretion of Cu, Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. *Neurosci Lett* 428(1):43–46. <https://doi.org/10.1016/j.neulet.2007.09.024>
- Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M (2014) Long noncoding RNAs (lncRNAs) and the molecular hallmarks of aging. *Aging* 6(12):992–1009. <https://doi.org/10.18632/aging.100710>
- Gu D, Liu F, Meng M, Zhang L, Gordon ML, Wang Y, Cai L, Zhang N (2020) Elevated matrix metalloproteinase-9 levels in neuronal extracellular vesicles in Alzheimer's disease. *Ann Clin Transl Neurol* 7(9):1681–1691. <https://doi.org/10.1002/acn3.51155>
- Gupta D, Zickler AM, El Andaloussi S (2021) Dosing extracellular vesicles. *Adv Drug Deliv Rev* 178:113961. <https://doi.org/10.1016/j.addr.2021.113961>
- Guz G, Demirogullari B, Ulusu NN, Dogu C, Demirtola A, Kavutcu M, Omeroglu S, Stefek M, Karasu C (2007) Stobadine protects rat kidney against Ischaemia/reperfusion injury. *Clin Exp Pharmacol Physiol* 34(3):210–216. <https://doi.org/10.1111/j.1440-1681.2007.04574.x>
- Handa T, Kuroha M, Nagai H, Shimoyama Y, Naito T, Moroi R, Kanazawa Y, Shiga H, Kakuta Y, Kinouchi Y, Masamune A (2021) Liquid biopsy for colorectal adenoma: is the exosomal miRNA derived from organoid a potential diagnostic biomarker? *Clin Transl Gastroenterol* 12(5):e00356. <https://doi.org/10.14309/ctg.0000000000000356>
- Harishkumar M, Radha M, Yuichi N, Muthukalianan GK, Kaoru O, Shiomori K, Sakai K, Nozomi W (2021) Designer exosomes: smart nano-communication tools for translational medicine. *Bioengineering* 8(11):158. <https://doi.org/10.3390/bioengineering8110158>
- Hassanpour M, Rezaie J, Nouri M, Panahi Y (2020) The role of extracellular vesicles in COVID-19 virus infection. *Infect Genet Evol* 85:104422. <https://doi.org/10.1016/j.meegid.2020.104422>. Epub 2020 Jun 13. PMID: 32544615; PMCID: PMC7293471
- He J, Ren W, Wang W, Han W, Jiang L, Zhang D, Guo M (2021) Exosomal targeting and its potential clinical application. *Drug Deliv Transl Res*. <https://doi.org/10.1007/s13346-021-01087-1>
- Herrmann IK, Wood MJA, Fuhrmann G (2021) Extracellular vesicles as a next-generation drug delivery platform. *Nat Nanotechnol* 16(7):748–759. <https://doi.org/10.1038/s41565-021-00931-2>
- Howard J, Trevick S, Younger DS (2016) Epidemiology of multiple sclerosis. *Neurol Clin* 34(4): 919–939. <https://doi.org/10.1016/j.ncl.2016.06.016>
- Hu T, Wolfram J, Srivastava S (2021) Extracellular vesicles in cancer detection: hopes and hypes. *Trends Cancer* 7(2):122–133. <https://doi.org/10.1016/j.trecan.2020.09.003>
- Huang T (2019) The role of NAD⁺ in anti-aging therapies. *Am J Biomed Sci Res* 6(5):446–453. <https://doi.org/10.34297/AJBSR.2019.06.001080>
- Ismail N, Wang Y, Dakhllallah D, Moldovan L, Agarwal K, Batte K, Shah P, Wisler J, Eubank TD, Tridandapani S, Paulaitis ME, Piper MG, Marsh CB (2013) Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. *Blood* 121(6):984–995. <https://doi.org/10.1182/blood-2011-08-374793>
- Jiang L, Dong H, Cao H, Ji X, Luan S, Liu J (2019) Exosomes in pathogenesis, diagnosis, and treatment of Alzheimer's disease. *Med Sci Monit* 25:3329–3335. <https://doi.org/10.12659/MSM.914027>

- Jiang C, Fu Y, Liu G, Shu B, Davis J, Tofaris GK (2022) Multiplexed profiling of extracellular vesicles for biomarker development. *Nano-Micro Letters* 14(1):3. <https://doi.org/10.1007/s40820-021-00753-w>
- Jung Y, Kraikivski P, Shafiekhani S, Terhune SS, Dash RK (2021) Crosstalk between Plk1, p53, cell cycle, and G2/M DNA damage checkpoint regulation in cancer: computational modeling and analysis. *Npj Syst Biol Appl* 7(1):46. <https://doi.org/10.1038/s41540-021-00203-8>
- Kalluri R, LeBleu VS (2020) The biology, function, and biomedical applications of exosomes. *Science* 367(6478):eaau6977. <https://doi.org/10.1126/science.aau6977>. PMID: 32029601; PMCID: PMC7717626
- Karn V, Ahmed S, Tsai L-W, Dubey R, Ojha S, Singh H, Kumar M, Gupta P, Sadhu S, Jha N, Kumar A, Pandit S, Kumar S (2021) Extracellular vesicle-based therapy for COVID-19: promises, challenges and future prospects. *Biomedicines* 9(10):1373. <https://doi.org/10.3390/biomedicines9101373>
- Katsyuba E, Auwerx J (2017) Modulating NAD⁺ metabolism, from bench to bedside. *EMBO J* 36(18):2670–2683. <https://doi.org/10.15252/embj.201797135>
- Keller S, Ridinger J, Rupp A-K, Janssen JW, Altevogt P (2011) Body fluid derived exosomes as a novel template for clinical diagnostics. *J Transl Med* 9(1):86. <https://doi.org/10.1186/1479-5876-9-86>
- Kenneweg F, Bang C, Xiao K, Boulanger CM, Loyer X, Mazlan S, Schroen B, Hermans-Beijnsberger S, Foinquinos A, Hirt MN, Eschenhagen T, Funcke S, Stojanovic S, Genschel C, Schimmel K, Just A, Pfanne A, Scherf K, Dehmel S et al (2019) Long noncoding RNA-enriched vesicles secreted by hypoxic cardiomyocytes drive cardiac fibrosis. *Mol Ther Nucleic Acids* 18:363–374. <https://doi.org/10.1016/j.omtn.2019.09.003>
- Khan TK (2016a) Genetic biomarkers in Alzheimer's disease. In: *Biomarkers in Alzheimer's disease*. Elsevier, pp 103–135. <https://doi.org/10.1016/B978-0-12-804832-0.00004-3>
- Khan TK (2016b) Introduction to Alzheimer's disease biomarkers. In: *Biomarkers in Alzheimer's disease*. Elsevier, pp 3–23. <https://doi.org/10.1016/B978-0-12-804832-0.00001-8>
- Kim KM, Abdelmohsen K, Mustapic M, Kapogiannis D, Gorospe M (2017) RNA in extracellular vesicles. *Wiley Interdiscip Rev RNA* 8(4). <https://doi.org/10.1002/wrna.1413>. Epub 2017 Jan 28. PMID: 28130830; PMCID: PMC5474163
- Kitdumrongthum S, Methetrairut C, Charoensawan V, Ounjai P, Janpipatkul K, Panvongsa W, Weerachayaphorn J, Piyachaturawat P, Chairoungdua A (2018) Dysregulated microRNA expression profiles in cholangiocarcinoma cell-derived exosomes. *Life Sci* 210:65–75. <https://doi.org/10.1016/j.lfs.2018.08.058>
- Koju N, Qin Z, Sheng R (2022) Reduced nicotinamide adenine dinucleotide phosphate in redox balance and diseases: a friend or foe? *Acta Pharmacol Sin*. <https://doi.org/10.1038/s41401-021-00838-7>
- Kou M, Huang L, Yang J, Chiang Z, Chen S, Liu J, Guo L, Zhang X, Zhou X, Xu X, Yan X, Wang Y, Zhang J, Xu A, Tse H, Lian Q (2022) Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: a next generation therapeutic tool? *Cell Death Dis* 13(7):580. <https://doi.org/10.1038/s41419-022-05034-x>
- Kovalenko TF, Larionova TD, Antipova NV, Shakhparonov MI, Pavlyukov MS (2021) The role of non-coding RNAs in the pathogenesis of glial tumors. *Acta Nat* 13(3):38–51. <https://doi.org/10.32607/actanaturae.11270>
- Krzyszczak P, Acevedo A, Davidoff EJ, Timmins LM, Marrero-Berrios I, Patel M, White C, Lowe C, Sherba JJ, Hartmanshenn C, O'Neill KM, Balter ML, Fritz ZR, Androulakis IP, Schloss RS, Yarmush ML (2018) The growing role of precision and personalized medicine for cancer treatment. *Technology* 6(03n04):79–100. <https://doi.org/10.1142/S2339547818300020>
- Kumari R, Jat P (2021) Mechanisms of cellular senescence: cell cycle arrest and senescence associated secretory phenotype. *Front Cell Dev Biol* 9. <https://doi.org/10.3389/fcell.2021.645593>

- Kusindarta DL, Wihadmadyatami H (2018) The role of extracellular matrix in tissue regeneration. In: Tissue Regeneration. InTech
- Kwok ZH, Wang C, Jin Y (2021) Extracellular vesicle transportation and uptake by recipient cells: a critical process to regulate human diseases. *PRO* 9(2):273. <https://doi.org/10.3390/pr9020273>
- Lananna BV, Imai S (2021) Friends and foes: extracellular vesicles in aging and rejuvenation. *FASEB BioAdv* 3(10):787–801. <https://doi.org/10.1096/fba.2021-00077>
- Lazo S, Noren Hooten N, Green J, Eitan E, Mode NA, Liu Q, Zonderman AB, Ezike N, Mattson MP, Ghosh P, Evans MK (2021) Mitochondrial DNA in extracellular vesicles declines with age. *Aging Cell* 20(1). <https://doi.org/10.1111/ace.13283>
- Lee VM-Y, Goedert M, Trojanowski JQ (2001) Neurodegenerative Tauopathies. *Annu Rev Neurosci* 24(1):1121–1159. <https://doi.org/10.1146/annurev.neuro.24.1.1121>
- Lee JC, Zhao J-T, Gundara J, Serpell J, Bach LA, Sidhu S (2015) Papillary thyroid cancer-derived exosomes contain miRNA-146b and miRNA-222. *J Surg Res* 196(1):39–48. <https://doi.org/10.1016/j.jss.2015.02.027>
- Lewin S, Hunt S, Lambert DW (2020) Extracellular vesicles and the extracellular matrix: a new paradigm or old news? *Biochem Soc Trans* 48(5):2335–2345. <https://doi.org/10.1042/BST20200717>
- Li Y-J, Wu J-Y, Wang J-M, Hu X-B, Cai J-X, Xiang D-X (2020a) Gemcitabine loaded autologous exosomes for effective and safe chemotherapy of pancreatic cancer. *Acta Biomater* 101:519–530. <https://doi.org/10.1016/j.actbio.2019.10.022>
- Li Z, Suo B, Long G, Gao Y, Song J, Zhang M, Feng B, Shang C, Wang D (2020b) Exosomal miRNA-16-5p derived from M1 macrophages enhances T cell-dependent immune response by regulating PD-L1 in gastric cancer. *Front Cell Dev Biol* 8. <https://doi.org/10.3389/fcell.2020.572689>
- Li Y-J, Wu J-Y, Liu J, Xu W, Qiu X, Huang S, Hu X-B, Xiang D-X (2021) Artificial exosomes for translational nanomedicine. *J Nanobiotechnol* 19(1):242. <https://doi.org/10.1186/s12951-021-00986-2>
- Liebana-Jordan M, Brotons B, Falcon-Perez JM, Gonzalez E (2021) Extracellular Vesicles in the Fungi Kingdom. *Int J Mol Sci* 22(13):7221. <https://doi.org/10.3390/ijms22137221>
- Liu W, Bai X, Zhang A, Huang J, Xu S, Zhang J (2019a) Role of exosomes in central nervous system diseases. *Front Mol Neurosci* 12. <https://doi.org/10.3389/fnmol.2019.00240>
- Liu Y, Li Q, Hosen MR, Zietzer A, Flender A, Levermann P, Schmitz T, Frühwald D, Goody P, Nickenig G, Werner N, Jansen F (2019b) Atherosclerotic conditions promote the packaging of functional MicroRNA-92a-3p into endothelial microvesicles. *Circ Res* 124(4):575–587. <https://doi.org/10.1161/CIRCRESAHA.118.314010>
- Liu C, Bayado N, He D, Li J, Chen H, Li L, Li J, Long X, Du T, Tang J, Dang Y, Fan Z, Wang L, Yang PC (2021a) Therapeutic applications of extracellular vesicles for myocardial repair. *Front Cardiovasc Med* 8. <https://doi.org/10.3389/fcvm.2021.758050>
- Liu Y, Cheng Y, Wang S, Su Y, Liu Y, Li C, Jin L, Wan Q, Sang X, Wang Z (2021b) Therapeutic effects and perspective of stem cell extracellular vesicles in aging and cancer. *J Cell Physiol* 236(7):4783–4796. <https://doi.org/10.1002/jcp.30212>
- Lo Cicero A, Stahl PD, Raposo G (2015) Extracellular vesicles shuffling intercellular messages: for good or for bad. *Curr Opin Cell Biol* 35:69–77. <https://doi.org/10.1016/j.ceb.2015.04.013>
- Lone SN, Nisar S, Masoodi T, Singh M, Rizwan A, Hashem S, El-Rifai W, Bedognetti D, Batra SK, Haris M, Bhat AA, Macha MA (2022) Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments. *Mol Cancer* 21(1):79. <https://doi.org/10.1186/s12943-022-01543-7>
- López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. *Cell* 153(6):1194–1217. <https://doi.org/10.1016/j.cell.2013.05.039>
- Lu L, Chen XM, Tao HM, Xiong W, Jie SH, Li HY (2015) Regulation of the expression of zinc finger protein genes by microRNAs enriched within acute lymphoblastic leukemia-derived microvesicles. *Genet Mol Res* 14(4):11884–11895. <https://doi.org/10.4238/2015.October.5.2>

- Ludwig N, Whiteside TL, Reichert TE (2019) Challenges in exosome isolation and analysis in health and disease. *Int J Mol Sci* 20(19):4684. <https://doi.org/10.3390/ijms20194684>
- Martinez-Dominguez MV, Zottel A, Šamec N, Jovčevska I, Dincer C, Kahlert UD, Nickel A-C (2021) Current technologies for RNA-directed liquid diagnostics. *Cancers* 13(20):5060. <https://doi.org/10.3390/cancers13205060>
- Mas-Bargues C, Alique M (2023) Extracellular vesicles as “very important particles” (VIPs) in aging. *Int J Mol Sci* 24(4):4250. <https://doi.org/10.3390/ijms24044250>
- Matsumoto S, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, Kitamura T, Hamasaki T, Nanto S, Kawahara Y, Komuro I (2013) Circulating p53-responsive MicroRNAs are predictive indicators of heart failure after acute myocardial infarction. *Circ Res* 113(3):322–326. <https://doi.org/10.1161/CIRCRESAHA.113.301209>
- McGinley M, Ontaneda D (2019) MS progression is predominantly driven by age-related mechanisms – NO. *Mult Scler J* 25(7):904–906. <https://doi.org/10.1177/1352458518819712>
- McHugh D, Gil J (2018) Senescence and aging: causes, consequences, and therapeutic avenues. *J Cell Biol* 217(1):65–77. <https://doi.org/10.1083/jcb.201708092>
- Melidoni A (2020) Small extracellular vesicles combat senescence. *Nat Rev Mol Cell Biol* 21(9):498–499. <https://doi.org/10.1038/s41580-020-0271-7>
- Melnikov I, Kozlov S, Saburova O, Zubkova E, Guseva O, Domogatsky S, Arefieva T, Radyukhina N, Zvereva M, Avtaeva Y, Buryachkovskaya L, Gabbasov Z (2020) CRP is transported by monocytes and monocyte-derived exosomes in the blood of patients with coronary artery disease. *Biomedicines* 8(10):435. <https://doi.org/10.3390/biomedicines8100435>
- Meng W, He C, Hao Y, Wang L, Li L, Zhu G (2020) Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source. *Drug Deliv* 27(1):585–598. <https://doi.org/10.1080/10717544.2020.1748758>
- Merchant ML, Rood IM, Deegens JKJ, Klein JB (2017) Isolation and characterization of urinary extracellular vesicles: implications for biomarker discovery. *Nat Rev Nephrol* 13(12):731–749. <https://doi.org/10.1038/nrneph.2017.148>
- Morales J, Li L, Fattah FJ, Dong Y, Bey EA, Patel M, Gao J, Boothman DA (2014) Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. *Crit Rev Eukaryot Gene Expr* 24(1):15–28. <https://doi.org/10.1615/CritRevEukaryotGeneExpr.2013006875>
- Nawaz M, Shah N, Zanetti B, Maugeri M, Silvestre R, Fatima F, Neder L, Valadi H (2018) Extracellular vesicles and matrix remodeling enzymes: the emerging roles in extracellular matrix remodeling, progression of diseases and tissue repair. *Cells* 7(10):167. <https://doi.org/10.3390/cells7100167>
- Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, Komanduri KV, Lin Y, Jain N, Daver N, Westin J, Gulbis AM, Loghin ME, de Groot JF, Adkins S, Davis SE, Rezvani K, Hwu P, Shpall EJ (2018) Chimeric antigen receptor T-cell therapy – assessment and management of toxicities. *Nat Rev Clin Oncol* 15(1):47–62. <https://doi.org/10.1038/nrclinonc.2017.148>
- Neviani P, Wise PM, Murtadha M, Liu CW, Wu C-H, Jong AY, Seeger RC, Fabbri M (2019) Natural killer–derived Exosomal miR-186 Inhibits Neuroblastoma growth and immune escape mechanisms. *Cancer Res* 79(6):1151–1164. <https://doi.org/10.1158/0008-5472.CAN-18-0779>
- Nguyen M-A, Karunakaran D, Geoffrion M, Cheng HS, Tandoc K, Perisic Matic L, Hedin U, Maegdefessel L, Fish JE, Rayner KJ (2018) Extracellular vesicles secreted by atherogenic macrophages transfer MicroRNA to inhibit cell migration. *Arterioscler Thromb Vasc Biol* 38(1):49–63. <https://doi.org/10.1161/ATVBAHA.117.309795>
- O’Brien KP, Khan S, Gilligan KE, Zafar H, Lalor P, Glynn C, O’Flatharta C, Ingoldsby H, Dockery P, De Bhulbh A, Schweber JR, St John K, Leahy M, Murphy JM, Gallagher WM, O’Brien T, Kerin MJ, Dwyer RM (2018) Employing mesenchymal stem cells to support tumor-targeted delivery of extracellular vesicle (EV)-encapsulated microRNA-379. *Oncogene* 37(16):2137–2149. <https://doi.org/10.1038/s41388-017-0116-9>

- O'Neill C, Gilligan K, Dwyer R (2019) Role of extracellular vesicles (EVs) in cell stress response and resistance to cancer therapy. *Cancers* 11(2):136. <https://doi.org/10.3390/cancers11020136>
- Pascucci L, Coccè V, Bonomi A, Ami D, Ceccarelli P, Ciusani E, Viganò L, Locatelli A, Sisto F, Doglia SM, Parati E, Bernardo ME, Muraca M, Alessandri G, Bondiolotti G, Pessina A (2014) Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: A new approach for drug delivery. *J Control Release* 192:262–270. <https://doi.org/10.1016/j.jconrel.2014.07.042>
- Patel NJ, Ashraf A, Chung EJ (2023) Extracellular vesicles as regulators of the extracellular matrix. *Bioengineering* 10(2):136. <https://doi.org/10.3390/bioengineering10020136>
- Pavlyukov MS, Yu H, Bastola S, Minata M, Shender VO, Lee Y, Zhang S, Wang J, Komarova S, Wang J, Yamaguchi S, Alsheikh HA, Shi J, Chen D, Mohyeldin A, Kim S-H, Shin YJ, Anufrieva K, Evtushenko EG et al (2018) Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors. *Cancer Cell* 34(1): 119–135.e10. <https://doi.org/10.1016/j.ccell.2018.05.012>
- Pegtel DM, Gould SJ (2019) Exosomes. *Annu Rev Biochem* 88(1):487–514. <https://doi.org/10.1146/annurev-biochem-013118-111902>
- Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem* 30(1):11–26. <https://doi.org/10.1007/s12291-014-0446-0>
- Podvin S, Jones A, Liu Q, Aulston B, Ransom L, Ames J, Shen G, Lietz CB, Jiang Z, O'Donoghue AJ, Winston C, Ikezu T, Rissman RA, Yuan S, Hook V (2020) Dysregulation of exosome cargo by mutant tau expressed in human-induced pluripotent stem cell (iPSC) neurons revealed by proteomics analyses. *Mol Cell Proteomics* 19(6):1017–1034. <https://doi.org/10.1074/mcp.RA120.002079>
- Prattichizzo F, Bonafè M, Olivieri F, Franceschi C (2016) Senescence associated macrophages and “macroph-aging”: are they pieces of the same puzzle? *Aging* 8(12):3159–3160. <https://doi.org/10.18632/aging.101133>
- Pusic AD, Kraig RP (2014) Youth and environmental enrichment generate serum exosomes containing miR-219 that promote CNS myelination. *Glia* 62(2):284–299. <https://doi.org/10.1002/glia.22606>
- Rankovic M, Zweckstetter M (2019) Upregulated levels and pathological aggregation of abnormally phosphorylated Tau-protein in children with neurodevelopmental disorders. *Neurosci Biobehav Rev* 98:1–9. <https://doi.org/10.1016/j.neubiorev.2018.12.014>
- Raposo G, Stoorvogel W (2013) Extracellular vesicles: Exosomes, microvesicles, and friends. *J Cell Biol* 200(4):373–383. <https://doi.org/10.1083/jcb.201211138>
- Robbins PD, Morelli AE (2014) Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol* 14(3):195–208. <https://doi.org/10.1038/nri3622>. PMID: 24566916; PMCID: PMC4350779
- Rogers MA, Buffolo F, Schlotter F, Atkins SK, Lee LH, Halu A, Blaser MC, Tsolaki E, Higashi H, Luther K, Daaboul G, Bouten CVC, Body SC, Singh SA, Bertazzo S, Libby P, Aikawa M, Aikawa E (2020) Annexin A1-dependent tethering promotes extracellular vesicle aggregation revealed with single-extracellular vesicle analysis. *Sci Adv* 6(38). <https://doi.org/10.1126/sciadv.abb1244>
- Rossi M, Battafarano G, D'Agostini M, del Fattore A (2018) The role of extracellular vesicles in bone metastasis. *Int J Mol Sci* 19(4):1136. <https://doi.org/10.3390/ijms19041136>
- Ryu JK, Rafalski VA, Meyer-Franke A, Adams RA, Poda SB, Rios Coronado PE, Pedersen LØ, Menon V, Baeten KM, Sikorski SL, Bedard C, Hanspers K, Bardehle S, Mendiola AS, Davalos D, Machado MR, Chan JP, Plastira I, Petersen MA, Pfaff SJ, Ang KK, Hallenbeck KK, Syme C, Hakoziaki H, Ellisman MH, Swanson RA, Zamvil SS, Arkin MR, Zorn SH, Pico AR, Mucke L, Freedman SB, Stavenhagen JB, Nelson RB, Akassoglou K (2018) Fibrin-targeting immunotherapy protects against neuroinflammation and neurodegeneration. *Nat Immunol* 19(11):1212–1223. <https://doi.org/10.1038/s41590-018-0232-x>. Epub 2018 Oct 15. PMID: 30323343; PMCID: PMC6317891

- Saari H, Lázaro-Ibáñez E, Viitala T, Vuorimaa-Laukkanen E, Siljander P, Yliperttula M (2015) Microvesicle- and exosome-mediated drug delivery enhances the cytotoxicity of Paclitaxel in autologous prostate cancer cells. *J Control Release* 220:727–737. <https://doi.org/10.1016/j.jconrel.2015.09.031>
- Saleem T, Sumrin A, Bilal M, Bashir H, Khawar MB (2022) Tumor-derived extracellular vesicles: Potential tool for cancer diagnosis, prognosis, and therapy. *Saudi J Biol Sci* 29(4):2063–2071. <https://doi.org/10.1016/j.sjbs.2022.01.012>
- Sanai SA, Saini V, Benedict RH, Zivadinov R, Teter BE, Ramanathan M, Weinstock-Guttman B (2016) Aging and multiple sclerosis. *Mult Scler J* 22(6):717–725. <https://doi.org/10.1177/1352458516634871>
- Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, El Beyrouthy M, Polito L, Iriti M, Martins N, Martorell M, Docea AO et al (2020) Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Front Physiol* 11. <https://doi.org/10.3389/fphys.2020.00694>
- Shimoda M (2019) Extracellular vesicle-associated MMPs: a modulator of the tissue microenvironment, pp 35–66. <https://doi.org/10.1016/bs.acc.2018.10.006>
- Shimoda M, Khokha R (2017) Metalloproteinases in extracellular vesicles. *Biochim Biophys Acta Mol Cell Res* 1864(11):1989–2000. <https://doi.org/10.1016/j.bbamcr.2017.05.027>
- Shupp A, Kolb A, Mukhopadhyay D, Bussard K (2018) Cancer metastases to bone: concepts, mechanisms, and interactions with bone osteoblasts. *Cancers* 10(6):182. <https://doi.org/10.3390/cancers10060182>
- Simon DK, Tanner CM, Brundin P (2020) Parkinson disease epidemiology, pathology, genetics, and pathophysiology. *Clin Geriatr Med* 36(1):1–12. <https://doi.org/10.1016/j.cger.2019.08.002>
- Sinning J-M, Losch J, Walenta K, Bohm M, Nickenig G, Werner N (2011) Circulating CD31+/Annexin V+ microparticles correlate with cardiovascular outcomes. *Eur Heart J* 32(16):2034–2041. <https://doi.org/10.1093/eurheartj/ehq478>
- Skotland T, Sagini K, Sandvig K, Llorente A (2020) An emerging focus on lipids in extracellular vesicles. *Adv Drug Deliv Rev* 159:308–321. <https://doi.org/10.1016/j.addr.2020.03.002>
- Squier TC (2001) Oxidative stress and protein aggregation during biological aging. *Exp Gerontol* 36(9):1539–1550. [https://doi.org/10.1016/S0531-5565\(01\)00139-5](https://doi.org/10.1016/S0531-5565(01)00139-5)
- Stephenson J, Nutma E, van der Valk P, Amor S (2018) Inflammation in CNS neurodegenerative diseases. *Immunology* 154(2):204–219. <https://doi.org/10.1111/imm.12922>
- Su L-J, Zhang J-H, Gomez H, Murugan R, Hong X, Xu D, Jiang F, Peng Z-Y (2019) Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxidative Med Cell Longev* 2019:1–13. <https://doi.org/10.1155/2019/5080843>
- Suhail Y, Cain MP, Vanaja K, Kurywach PA, Levchenko A, Kalluri R, Kshitiz. (2019) Systems biology of cancer metastasis. *Cell Syst* 9(2):109–127. <https://doi.org/10.1016/j.cels.2019.07.003>
- Takahashi A, Okada R, Nagao K, Kawamata Y, Hanyu A, Yoshimoto S, Takasugi M, Watanabe S, Kanemaki MT, Obuse C, Hara E (2017) Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. *Nat Commun* 8(1):15287. <https://doi.org/10.1038/ncomms15287>
- Takasugi M (2018) Emerging roles of extracellular vesicles in cellular senescence and aging. *Aging Cell* 17(2):e12734. <https://doi.org/10.1111/acel.12734>
- Tandogan B, Uluş NN (2010) A comparative study with colchicine on glutathione reductase. *Protein J* 29(5):380–385. <https://doi.org/10.1007/s10930-010-9263-3>
- Tang Z, Li D, Hou S, Zhu X (2020) The cancer exosomes: clinical implications, applications and challenges. *Int J Cancer* 146(11):2946–2959. <https://doi.org/10.1002/ijc.32762>
- Tartaglia MC, Rowe A, Findlater K, Orange JB, Grace G, Strong MJ (2007) Differentiation between primary lateral sclerosis and amyotrophic lateral sclerosis. *Arch Neurol* 64(2):232. <https://doi.org/10.1001/archneur.64.2.232>

- Théry C, Regnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castagnoli P, Raposo G, Amigorena S (1999) Molecular characterization of dendritic cell-derived exosomes. *J Cell Biol* 147(3): 599–610. <https://doi.org/10.1083/jcb.147.3.599>
- Thone MN, Kwon YJ (2020) Extracellular blebs: artificially-induced extracellular vesicles for facile production and clinical translation. *Methods* 177:135–145. <https://doi.org/10.1016/j.jmeth.2019.11.007>
- Tran L, Ha-Duong T (2015) Exploring the Alzheimer amyloid- β peptide conformational ensemble: A review of molecular dynamics approaches. *Peptides* 69:86–91. <https://doi.org/10.1016/j.peptides.2015.04.009>
- Turan B, Acan NL, Ulusu NN, Tezcan EF (2001) A comparative study on effect of dietary selenium and vitamin e on some antioxidant enzyme activities of liver and brain tissues. *Biol Trace Elem Res* 81(2):141–152. <https://doi.org/10.1385/BTER:81:2:141>
- Ulusu NN (2015a) Curious cases of the enzymes/Neobiča Istorija Enzima. *J Med Biochem* 34(3): 271–281. <https://doi.org/10.2478/jomb-2014-0045>
- Ulusu NN (2015b) Evolution of Enzyme Kinetic Mechanisms. *J Mol Evol* 80(5–6):251–257. <https://doi.org/10.1007/s00239-015-9681-0>
- Ulusu NN (2015c) Glucose-6-phosphate dehydrogenase deficiency and Alzheimer's disease: partners in crime? The hypothesis. *Med Hypotheses* 85(2):219–223. <https://doi.org/10.1016/j.mehy.2015.05.006>
- Ulusu NN, Tandogan B (2006) Purification and kinetics of sheep kidney cortex glucose-6-phosphate dehydrogenase. *Comp Biochem Physiol B: Biochem Mol Biol* 143(2):249–255. <https://doi.org/10.1016/j.cbpb.2005.11.018>
- Ulusu NN, Acan NL, Turan B, Tezcan EF (2003) Inhibition of glutathione reductase by cadmium ion in some rabbit tissues and the protective role of dietary selenium. *Biol Trace Elem Res* 91(2): 151–156. <https://doi.org/10.1385/BTER:91:2:151>
- Ulusu NN, Tandogan B, Tezcan FE (2005) Kinetic properties of glucose-6-phosphate dehydrogenase from lamb kidney cortex. *Biochimie* 87(2):187–190. <https://doi.org/10.1016/j.biochi.2004.11.002>
- Ulusu NN, Yilmaz G, Erbayraktar Z, Evlice AT, Aras S, Yener G, Avci A (2015) A Turkish 3-center study evaluation of serum folic acid and vitamin B12 levels in Alzheimer disease. *Turk J Med Sci* 45:1159–1166. <https://doi.org/10.3906/sag-1406-136>
- Ulusu NN, Gök M, Sayin Şakul AA, Ari N, Stefek M, Karasu Ç (2017) Antioxidant SMe1EC2 modulates pentose phosphate pathway and glutathione-dependent enzyme activities in tissues of aged diabetic rats. *Interdiscip Toxicol* 10(4):148–154. <https://doi.org/10.1515/intox-2017-0021>
- Vader P, Mol EA, Pasterkamp G, Schiffelers RM (2016) Extracellular vesicles for drug delivery. *Adv Drug Deliv Rev* 106:148–156. <https://doi.org/10.1016/j.addr.2016.02.006>
- van Niel G, D'Angelo G, Raposo G (2018) Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol* 19(4):213–228. <https://doi.org/10.1038/nrm.2017.125>
- Vida A, Márton J, Mikó E, Bai P (2017) Metabolic roles of poly(ADP-ribose) polymerases. *Semin Cell Dev Biol* 63:135–143. <https://doi.org/10.1016/j.semcdb.2016.12.009>
- Vinceti M, Dennert G, Crespi CM, Zwahlen M, Brinkman M, Zeegers MP, Horneber M, D'Amico R, del Giovane C (2014) Selenium for preventing cancer. *Cochrane Database Syst Rev*. <https://doi.org/10.1002/14651858.CD005195.pub3>
- Walker S, Busatto S, Pham A, Tian M, Suh A, Carson K, Quintero A, Lafrence M, Malik H, Santana MX, Wolfram J (2019) Extracellular vesicle-based drug delivery systems for cancer treatment. *Theranostics* 9(26):8001–8017. <https://doi.org/10.7150/thno.37097>
- Wallis R, Mizen H, Bishop CL (2020) The bright and dark side of extracellular vesicles in the senescence-associated secretory phenotype. *Mech Ageing Dev* 189:111263. <https://doi.org/10.1016/j.mad.2020.111263>
- Wang Y, Zhou BP (2013) Epithelial-mesenchymal transition – a hallmark of breast cancer metastasis. *Cancer Hallmarks* 1(1):38–49. <https://doi.org/10.1166/ch.2013.1004>

- Wang Z, Deng Z, Dahmane N, Tsai K, Wang P, Williams DR, Kossenkova AV, Showe LC, Zhang R, Huang Q, Conejo-Garcia JR, Lieberman PM (2015) Telomeric repeat-containing RNA (TERRA) constitutes a nucleoprotein component of extracellular inflammatory exosomes. *Proc Natl Acad Sci* 112(46):E6293–E6300. <https://doi.org/10.1073/pnas.1505962112>
- Wang Y, Jiang L, He J, Hu M, Zeng F, Li Y, Tian H, Luo X (2018) The adverse effects of se toxicity on inflammatory and immune responses in chicken spleens. *Biol Trace Elem Res* 185(1): 170–176. <https://doi.org/10.1007/s12011-017-1224-7>
- Wang L, Yu X, Zhou J, Su C (2023) Extracellular vesicles for drug delivery in cancer treatment. *Biol Proced Online* 25(1):28. <https://doi.org/10.1186/s12575-023-00220-3>
- Weilner S, Keider V, Winter M, Harreither E, Salzer B, Weiss F, Schraml E, Messner P, Pietschmann P, Hildner F, Gabriel C, Redl H, Grillari-Voglauer R, Grillari J (2016) Vesicular Galectin-3 levels decrease with donor age and contribute to the reduced osteo-inductive potential of human plasma derived extracellular vesicles. *Aging* 8(1):16–30. <https://doi.org/10.18632/aging.100865>
- Whiteside SK, Grant FM, Gyori DS, Conti AG, Imianowski CJ, Kuo P, Nasrallah R, Sadiyah F, Lira SA, Tacke F, Eil RL, Burton OT, Dooley J, Liston A, Okkenhaug K, Yang J, Roychoudhuri R (2021) CCR8 marks highly suppressive Treg cells within tumours but is dispensable for their accumulation and suppressive function. *Immunology* 163(4):512–520
- Wijesekera LC, Nigel Leigh P (2009) Amyotrophic lateral sclerosis. *Orphanet J Rare Dis* 4:3. <https://doi.org/10.1186/1750-1172-4-3>. PMID: 19192301; PMCID: PMC2656493
- Wiklander OPB, Brennan MÅ, Lötvall J, Breakefield XO, EL Andaloussi S (2019) Advances in therapeutic applications of extracellular vesicles. *Sci Transl Med* 11(492). <https://doi.org/10.1126/scitranslmed.aav8521>
- Wood KA, Youle RJ (2006) Apoptosis and free radicals. *Ann N Y Acad Sci* 738(1):400–407. <https://doi.org/10.1111/j.1749-6632.1994.tb21829.x>
- Wu J, Gao W, Tang Q, Yu Y, You W, Wu Z, Fan Y, Zhang L, Wu C, Han G, Zuo X, Zhang Y, Chen Z, Ding W, Li X, Lin F, Shen H, Tang J, Zhang Y, Wang X (2021a) Retracted: M2 macrophage-derived exosomes facilitate HCC metastasis by transferring α M β 2 integrin to tumor cells. *Hepatology* 73(4):1365–1380. <https://doi.org/10.1002/hep.31432>
- Wu J-Y, Li Y-J, Hu X-B, Huang S, Xiang D-X (2021b) Preservation of small extracellular vesicles for functional analysis and therapeutic applications: a comparative evaluation of storage conditions. *Drug Deliv* 28(1):162–170. <https://doi.org/10.1080/10717544.2020.1869866>
- Xavier CPR, Caires HR, Barbosa MAG, Bergantim R, Guimarães JE, Vasconcelos MH (2020) The role of extracellular vesicles in the hallmarks of cancer and drug resistance. *Cells* 9(5):1141. <https://doi.org/10.3390/cells9051141>
- Yáñez-Mó M, Siljander PR-M, Andreu Z, Bedina Zavec A, Borràs FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, Colás E, Cordeiro-da Silva A, Fais S, Falcon-Perez JM, Ghoobrial IM, Giebel B, Gimona M, Graner M, Gursel I et al (2015) Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 4(1):27066. <https://doi.org/10.3402/jev.v4.27066>
- Yang P, Peng Y, Feng Y, Xu Z, Feng P, Cao J, Chen Y, Chen X, Cao X, Yang Y, Jie J (2021) Immune cell-derived extracellular vesicles – new strategies in cancer immunotherapy. *Front Immunol* 12. <https://doi.org/10.3389/fimmu.2021.771551>
- Yin T, Liu Y, Ji W, Zhuang J, Chen X, Gong B, Chu J, Liang W, Gao J, Yin Y (2023) Engineered mesenchymal stem cell-derived extracellular vesicles: a state-of-the-art multifunctional weapon against Alzheimer's disease. *Theranostics* 13(4):1264–1285. <https://doi.org/10.7150/thno.81860>
- Yoshida M, Satoh A, Lin JB, Mills KF, Sasaki Y, Rensing N, Wong M, Apte RS, Imai S (2019) Extracellular vesicle-contained eNAMPT delays aging and extends lifespan in mice. *Cell Metab* 30(2):329–342.e5. <https://doi.org/10.1016/j.cmet.2019.05.015>
- Zeng Y, Qiu Y, Jiang W, Shen J, Yao X, He X, Li L, Fu B, Liu X (2022) Biological features of extracellular vesicles and challenges. *Front Cell Dev Biol* 10. <https://doi.org/10.3389/fcell.2022.816698>

- Zhang L, Yu D (2019) Exosomes in cancer development, metastasis, and immunity. *Biochim Biophys Acta Rev Cancer* 1871(2):455–468. <https://doi.org/10.1016/j.bbcan.2019.04.004>
- Zhang Y, Chen L, Ye X, Wu Z, Zhang Z, Sun B, Fu H, Fu C, Liang X, Jiang H (2021a) Expression and mechanism of exosome-mediated A FOXM1 related long noncoding RNA in gastric cancer. *J Nanobiotechnol* 19(1):133. <https://doi.org/10.1186/s12951-021-00873-w>
- Zhang Y-L, Ma Y, Zeng Y-Q, Liu Y, He E-P, Liu Y-T, Qiao F-L, Yu R, Wang Y-S, Wu X-Y, Leng P (2021b) A narrative review of research progress on FoxM1 in breast cancer carcinogenesis and therapeutics. *Ann Transl Med* 9(22):1704–1704. <https://doi.org/10.21037/atm-21-5271>
- Zhang Y, Xin L, Xiang M, Shang C, Wang Y, Wang Y, Cui X, Lu Y (2022) The molecular mechanisms of ferroptosis and its role in cardiovascular disease. *Biomed Pharmacother* 145: 112423. <https://doi.org/10.1016/j.biopha.2021.112423>
- Zhou Y, Dong Y, Zhang A, Wu J, Sun Q (2023) The role of mesenchymal stem cells derived exosomes as a novel nanobiotechnology target in the diagnosis and treatment of cancer. *Front Bioeng Biotechnol* 11. <https://doi.org/10.3389/fbioe.2023.1214190>



Versatile Component of Extracellular Matrix: Hyaluronan with Dual Application in Understanding Cancer Progression Initiation and Cancer Inhibition

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Abstract

This chapter comprehensively examines hyaluronic acid (HA), an intrinsic biopolymer in the human extracellular matrix. It traces HA's evolution from discovery to commercial applications, beginning with a historical overview, emphasizing its structural complexity and physiological significance in connective tissues. The narrative delves into the intricate interplay between HA and the cell-surface receptor CD44, detailing their involvement in diverse biological processes, including inflammation and cancer progression. HA synthases and hyaluronidases' regulatory roles in orchestrating extracellular matrix interactions, particularly in cancer contexts, are highlighted. Besides, the exploration of HA's therapeutic potential, specifically in drug delivery systems, is also illuminated. Various methodologies, such as HA-conjugated and encapsulated drugs, are examined, showcasing HA's capacity to enhance the biocompatibility and solubility of anticancer medications. The versatility of HA is underscored as a crucial element in multifunctional nanoparticles (NPs), leveraging its targeting capabilities for cancer treatment. Tumors with elevated HA concentrations are associated with malignancy, prompting the exploration of strategies involving hyaluronidases, HA oligosaccharides (oHA), and HA-based medications to

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address these challenges. The chapter elucidates the complex interplay between HA, CD44, and cancer progression. Different HA variants, including high molecular weight HA (HMW-HA) and small oligo-HA, are explored, elucidating their diverse impacts on cancer cell behavior. Small oligo-hyaluronic acids (oHA) emerge as promising tumor inhibitors, contrasting larger HA molecules. The role of low molecular weight HA (LMW-HA) is detailed as a dynamic regulator influencing cancer cell processes through matrix metalloproteinases (MMPs). This chapter lays the foundation for understanding HA's multifaceted role in cancer treatment, offering insights into potential therapeutic targets. The intricate relationships between HA, CD44, and cancer progression open avenues for future research and innovative therapeutic options. The integration of HA in cancer treatment strategies underscores its potential as a versatile and promising element for effective cancer therapies.

Keywords

Biological processes · Cancer progression · Cancer treatment strategies · CD44 receptor · Drug delivery systems · HA synthases · HA-conjugated drugs · Hyaluronic acid · Hyaluronidases · Nanoparticles · Therapeutic targets

1 Introduction

Hyaluronan is a frequently occurring biopolymer in extracellular matrix inside our body. The word “hyaluronan” was used in 1986 for polysaccharides, which aligns with the accepted names under an internationally recognized classification system. It was initially created to assign the several forms that the molecule can acquire, as to its acid form (hyaluronic acid), and salts like (sodium hyaluronate), which form at physiological pH (Balazs et al. 1986; Laurent 1989). Numerous laboratories investigated HA's biological function, physicochemical structure, and characteristics after it was separated from various sources (Kreil 1995; Necas et al. 2008). French scientist Portes identified a distinct type of mucus in vitreous bodies that differed from mucoids found in corneas and cartilage and named it “hyalomucine” in 1880, marking the first study on HA that can be found (Boeriu et al. 2013). The discovery of a novel polysaccharide called HA—the abbreviation for “hyaloid” (vitreous) and “uronic acid”—was made by Meyer and Palmer in 1934 after they extracted it from bovine vitreous humor. The compound had an amino sugar and a uronic acid (Kendall et al. 1937; Boas 1949; Fallacara et al. 2018). They were among the researchers who isolated HA from rooster comb, streptococci, and human umbilical cord in the 1930s and 1950s. The first commercial use of HA occurred in 1942 when Endre Balazs sought a patent to replace egg whites in baked goods (Necas et al. 2008). The structure, physical properties, physiological activity, and metabolism of hyaluronan (HA) have been studied for nearly a century. It is abundant in vertebrate connective tissues and forms a significant component of the extracellular matrix

(ECM) (Meyer and Palmer 1934; Weissmann and Meyer 1954; Kobayashi et al. 2020).

HA comprises β glucuronic acid and β -N-acetyl glucosamine units that repeat linearly. HA is involved in many developmental processes and is a significant part of the extracellular matrix in both standard and malignant cells (Aruffo et al. 1990). Different enzymes synthesize different molecular weights of HA and they are synthesized by HA synthases. The β -(1–3)-link allows the disaccharides unit of “N-acetyl-D-glucosamine” and “D-glucuronic acid” to polymerize (Vigetti et al. 2014; Chaudhry et al. 2021). The linear polymer is produced by repeatedly adding this disaccharide component thousands of times, and its molecular mass can range from 5×10^1 to $4\text{--}5 \times 10^4$ Da and beyond. The highly moist extracellular matrix provides the perfect environment for cell migration and proliferation because of hyaluronan’s hydrophilic characteristics. The presence of hyaluronan in the skin, the joints, and the vitreous humor makes it a crucial space-filling molecule (Day and Prestwich 2002; Vigetti et al. 2009, 2010, 2011, 2014). The interactive behavior of hyaluronan modulates its cellular functions, hyaluronan modulates its function inside the body, which include (i) developmental process, (ii) morphogenesis, (iii) tumorigenesis, (iv) migration, (v) apoptosis, (vi) cell survival, and (vii) inflammation. Having interactive behavior in response to receptor proteins, hyaladerins “TSG6”, “CD44”, “RHAMM”, “HARE”, and “toll-like receptor” (TLR) 4/2 are considered as interactive proteins in the regulation of hyaluronan functionality. The synthesis of hyaluronan with molecular masses up to 1 MDa takes place at the inner side of plasma membranes, in contrast to all glycans proteins (i.e., glycosaminoglycans) formed in the Golgi apparatus. Then, it makes its way to the cell’s surface via structures resembling pores (Prehm 1984). Hyaluronan synthases (HASs) are glycosyltransferases linked to cell membranes and responsible for producing hyaluronan. Three distinct HASs have been identified, which has improved our knowledge of hyaluronan biosynthesis. The human chromosome 19q13.3 encodes the *has1* gene, which encodes the HAS-1 protein synthesizing high molecular weight hyaluronan. The production of HA in response to “shock”, “inflammation”, and tissue repair is regulated by the *has2* gene, which is located at chromosome 8q24.12; molecules with masses up to 100 kDa are produced by *has3*, which is located on chromosome 16q22.1 (Gaffney et al. 2010; Itano and Kimata 2008; Karbownik and Nowak 2011, 2013). To further understand how the HA matrix shapes HA-specific effects, it is helpful to think of it as a canvas constantly being knitted, unraveled, and embellished with patterns of hyaladherins.

Several ECM proteins directly interact with HA, which significantly increases the variety of HA responses by increasing the repertoire of HA interactions with ECM components (Stober et al. 2017; Garantziotis and Savani 2019). Because of its unique hydrodynamic properties, hyaluronan—present in all human tissues—plays a significant role in regulating the architecture of these tissues. (Toole 2004) and Tammi et al. (2008) found that HA is either overexpressed or highly concentrated in tumor cells and the extracellular matrix (ECM) around many malignant tumors. Additionally, several types of cancer patients have demonstrated a correlation between tumoral and stromal HA accumulation and a bad prognosis. Specifically,

in gastric and colorectal cancer patients, HA expression in tumor cells is linked to a poor prognosis (Ropponen et al. 1998; Setälä et al. 1999). Additional research has linked elevated stromal HA levels to worse prognoses for patients with ovarian, breast, and prostate malignancies (Auvinen et al. 2000; Anttilä et al. 2000). These results imply that tumors with a high HA content are more aggressive than tumors with a low HA content, at least in specific cancer types (Sato et al. 2016). The hygroscopicity of hyaluronan is thought to have a significant role in regulating the osmotic balance and tissue hydration (Dechert et al. 2006). In addition to its role as a passive structural molecule, hyaluronan controls cell motility, proliferation, and differentiation by interacting with receptors on cell surfaces. Kosaki et al. (1999), Camenisch et al. (2000) and Necas et al. (2008) all found that hyaluronan is essential for embryogenesis, even though it may have a role in cancer.

2 Potential Companion of HA in Cancer Regulation: CD44

Among the several cellular hyaladherins, CD44 is the most studied and widespread of the non-kinase type 1 receptor; its many functions have been well-documented. There are numerous processes in which CD44 is implicated. These include (i) inflammation, (ii) lymphocyte homing, (iii) cell proliferation (including carcinogenesis), (iv) HA clearance, and (v) damage resolution, to name a few. The CD44 variants generated via spliced exons and nine alternatively spliced exons generate various CD44 isoforms, each with its unique and as-of-yet-unknown function. A type I transmembrane glycoprotein, CD44, is generally considered the primary cell-surface HA binding protein (Aruffo et al. 1990). It is promiscuous in the proteins it binds to, including HA, osteopontin, collagen, and fibronectin (Chen et al. 2018; Garantziotis and Savani 2019). Interactions between CD44 and HA have been extensively explored in various situations due to their significant roles in tumor metastasis, lymphocyte adhesion, T cell signaling, angiogenesis, and inflammation (DeGrendele et al. 1996, 1997; Cuff et al. 2001; Clark et al. 1996; Petrey and de la Motte 2014). The molecular weight of CD44 ranges from “85 to 200” kDa, and it is expressed everywhere in the body (Basakran 2015). According to research by Rudzki and Jothy (1997) and Rall and Rustgi (1995), the conserved form of CD44, known as standard CD44s, is a protein with a molecular weight of around 85–90 kDa. It is composed of spliced-together transcription of exons 1–5 and 16–20.

The CD44 primarily consists of three domains: the ectodomain, which is located outside of cells; the “transmembrane domain”; and the “intracellular domain”, also known as the cytoplasmic domain (Gul-e-Saba et al. 2010, 2014; Iczkowski 2010; Gul-e-Saba and Abdullah 2015). As shown in Fig. 1, the extracellular domain is responsible for interacting with and sensing the external environment. The transmembrane domain facilitates lymphocyte homing and interaction with adaptor proteins and co-factors (Underhill 1992; Gul-e-Saba et al. 2010; Williams et al. 2013). Nuclear localization and transcription mediation are two of the many roles played by the CD44 intracellular domain (CD44-ICD), which can take either a short-tail or long-tail shape (Williams et al. 2013; Okamoto et al. 2001; Senbanjo and

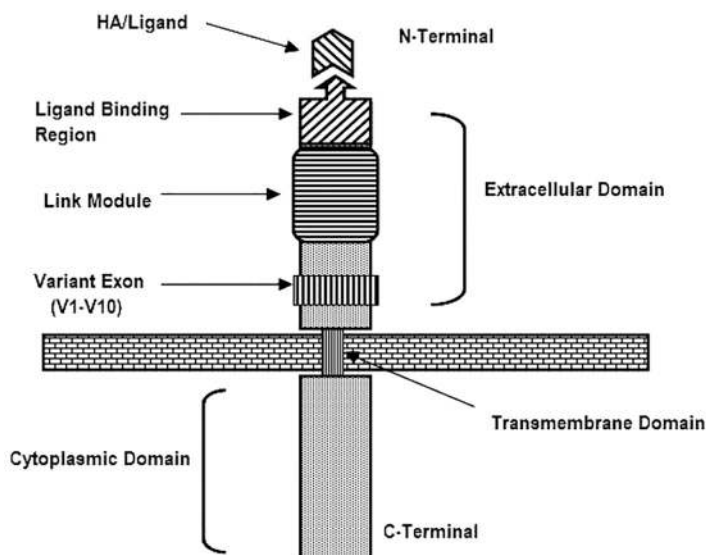


Fig. 1 Schematic structure of CD44 receptor-HA interaction

Chellaiah 2017). The alternate splicing shows much structural variety; there are more than 800 possible isoforms, but only a subset is expressed. Variant CD44 and CD44v refer to the variants that contain alternatively spliced exons, whereas standard CD44 and CD44s refer to the variants that do not. It is possible that reversible posttranslational glycosylation can further boost CD44 diversity. For cytoskeleton attachment, CD44's short cytoplasmic tail binds to “ankyrin and ezrin-radixin-moesin”; merlin, on the other hand, prevents this binding. Many possible states of CD44 exist, including inactivity and either constitutively active, inducible (binding HA upon interaction with inducers), or incapable of binding HA. Karbownik and Nowak (2011), Misra et al. (2011) and Karbownik and Nowak (2013) found that CD44 variants had an exceptionally high affinity for HA.

CD44 needs activation before binding to HA, while it is expressed (endogenously) at low levels in normal tissues on different cell types (Cichy and Pure 2003). The smallest HA fragments that bind to CD44 are HA6 units, which are six monosaccharide units. According to Orian and Sleeman (2014), even after being broken down by HYALs into tiny pieces, HMW HA in the ECM can still bind to CD44. Upregulation of integrins that enhance stem cell attachment is induced by CD44 adherence to HA (Lundell et al. 1997; Misra et al. 2015). Evidence linking tumor growth to overexpression of various CD44 variant isoforms raises the possibility that these variants' signaling characteristics are distinct. According to research conducted by Kuniyasu et al. (2001) and Kuhn et al. (2007), CD44 v3 enhances invasion and resistance to apoptosis in colon cancer, while CD44 v6 is linked to metastasis and a shorter disease-free lifespan. The v5 and v6 variants seem to increase metastasis in squamous cell carcinoma and bronchioalveolar carcinoma,

the two subtypes of lung cancer that exhibit preferential expression of CD44 variants (Pirinen et al. 2000; Mizera-Nyczak et al. 2001). The link between breast cancer metastasis and CD44 v3-containing isoforms is just one of many publications demonstrating that CD44 variations accelerate the spread of breast cancer (Kalish et al. 1999; Wang and Bourguignon 2011). The CD44 receptor may control migratory and invasion processes in cancer progression by integrating adhesive and signaling functions (Lokeshwar et al. 1995). Many studies have investigated how CD44 receptors mediate HA-mediated signaling and tumor cells' migration, proliferation, and survival (Bourguignon et al. 1998, 2001; Kuniyasu et al. 2001; Wang and Bourguignon 2006a, b; Wang et al. 2009). The actin cytoskeleton is dynamically controlled to control cellular shape changes and the production of sticky structures. According to multiple studies (Chellaiah et al. 2000; Linder and Aeppelbacher 2003; Desai et al. 2008), the actin cytoskeleton and migratory structures are dynamically regulated by the spatial and temporal locations of actin-binding proteins. Desai et al. (2007, 2008), Gupta et al. (2013), and Senbanjo and Chellaiah (2017) found that PC3 cells secrete active matrix metalloproteinase 9, migrate, and invade when CD44 is expressed on the cell surface and interacts with matrix metalloproteinase 9. The RhoGTPases, Ras-MAPK, and PI3K/AKT pathways are critical signaling pathways that CD44 activates. Due to the lack of intrinsic kinase activity in CD44, the actin cytoskeleton is attached to its cytoplasmic tail through adaptor proteins and intracellular kinases (Okamoto et al. 2001). CD44 can enhance signaling through its interactions with growth factors, enzymes, and cytokines linked with cell surfaces (Marhaba and Zoller 2004; Ouhtit et al. 2018).

3 Understanding HA-CD44 Interaction in Cancer Progression Initiation and Development

A large body of evidence indicates that CD44 and HA are highly expressed at tumor attachment sites. Additionally, it has been found that binding HA to the extracellular domain of CD44 triggers certain behaviors specific to tumor cells. These findings suggest that CD44 activation is crucial for tumor progression. According to Li et al. (2006), Toole and Slomiany (2008), Toole (2004), and Xu et al. (2016), CD44 mediates the activity of signaling receptors such as EGF, EGF-2, platelet-derived growth factor beta, and transforming growth factor-beta receptor type 1. This process is triggered by HA binding. As a result, this can activate Rho family GTPases and interact with a non-receptor kinase of the Src family (Nam et al. 2015). In order to improve cell proliferation, survival, migration, invasiveness, and chemoresistance, HA binding may cause changes in the location and conformation of the CD44 membrane, which in turn activates a signaling cascades. Zhu and Bourguignon (1998) and Zhu and Bourguignon (2000) found that CD44 interacts with "ankyrin" and other "membrane-associated cytoskeletal proteins" expressed in many biological systems. CD44 binds directly to ankyrin's conserved "N-terminal ankyrin repeat domain" (ARD) (Guo et al. 2022). According to various studies (Zhu and Bourguignon 1998, 2000; Bourguignon et al. 1993; Lokeshwar et al. 1994, 1996), this

interaction between CD44 and ankyrin causes the activation of the cytoskeleton. This interaction mediates key HA-mediated processes, including cell adhesion, proliferation, and migration. After HA binds to CD44, the “cytoskeletal protein ankyrin” and “inositol-1,4,5-triphosphate” (IP3) receptors are more easily recruited into lipid rafts that contain cholesterol. Several biological processes, such as cell adhesion and proliferation, are initiated when the ankyrin ARD binds the “IP3” receptor to CD44 at lipid rafts, as described by Singleton and Bourguignon (2004). According to Bourguignon (2008), when cholesterol-containing lipid rafts interact with ankyrin and IP3 receptors, CD44 regulates HA-mediated Ca^{2+} signaling and several critical cellular processes. In a head and neck squamous cancer cell line, CD44v3 promotes migration via activating the actomyosin contractile complex through ankyrin (Franzmann et al. 2001; Anand and Sharma 2017).

The CD44 receptor protein is an essential activator of survival pathways to prevent cancer cells from undergoing cell death. CD44's function in cell survival impacts several molecules that control pre- or anti-apoptotic processes. Lakshman et al. (2004), Yasuda et al. (2001), Park et al. (2012) identified several of these changes, including the expression of Fas, caspase 3/9, “Bcl-xl/Bak”, phosphorylation of “AKT”, “pRb”, and increased anti-apoptotic protein “Bcl-2”. According to Park et al. (2012), when CD44 is knocked down in colon cancer cells, apoptotic molecules like Bax and caspase-3/8/9 are upregulated, while anti-apoptotic molecules like Bcl-2 and Bcl-xL decrease. Following treatment with the anticancer reagent etoposide, CD44-transfected cancer cells showed a downregulation of AKT phosphorylation, p21, and pRb. According to Lakshman et al. (2004), CD44 expression may influence the cell cycle regulators “pRb” and “p21” and the “pro-survival protein” AKT. In lung cancer cells, CD44 regulates “Fas”, which adds to cell survival. According to Yasuda et al. (2001), and Chen et al. (2018), HA binding to CD44 decreased Fas expression and apoptosis mediated by Fas. The role that CD44 plays in preventing cell death is crucial when discussing carcinogenesis. The most well-studied underlying mechanisms include the activation of TGF β 1, HB-EGF, and OPN by CD44v6 isoforms and the suppression of Fas signaling by CD44 (Ponta et al. 2003; Yu and Stamenkovic 2004; Mielgo et al. 2006). On the other hand, according to Jung et al. CD44v6 isoforms are responsible for establishing a pre-metastatic niche, which produces chemoresistance and enhances tumor cell survival (Jung et al. 2009, 2011). The inhibitor of apoptosis-stimulating protein of p53 (IASPP), a new CD44 interacting protein identified by the proteomic technique detailed in Skandalis et al., may also play a role in this process (Skandalis et al. 2010; Karousou et al. 2017).

4 HA Oligosaccharides Synthesis and Degradation Regulatory Machineries

Hyaluronic acid (HA) plays a vital role in mammals, particularly in embryonic development, and the expression of three HA synthases is both tissue and time-specific (Vigetti et al. 2003; Viola et al. 2015). The external microenvironment of a

cell significantly influences its basic growth regulation. Changes in cell growth can induce swift growth and migration, leading to metastasis (Jiang et al. 2015). HA, as a biopolymer and a principal constituent of the extracellular matrix (ECM), contributes a hydrophilic element to the matrix, offering cell support. However, its significance becomes particularly noteworthy regarding cell survival, growth, and differentiation (Laurent and Fraser 1992; Chaudhry et al. 2021). Hyaluronic acid (HA) diseases result from an imbalanced interplay between production and degradation, leading to HA concentration and size alterations. In arthritis, HA is diminished in synovial fluid, a condition addressed by articular visco supplementation, where an HA solution is injected into the articular space to restore the appropriate HA concentration (Balazs and Denlinger 1993). Skin exposure to UV light induces a gradual loss of HA, contributing to a persistent inflammatory state. This continual HA reduction in the dermis and epidermis is implicated in the formation of wrinkles. The development of wrinkles primarily stems from tissue dehydration, and in aged skin, efforts to reinstate the correct HA content are made using gel fillers. The decline of HA is prevalent in aging, manifesting in symptoms akin to those observed in articular degeneration, where a decreased HA concentration adversely impacts the lubricating properties of synovial fluid (Balazs and Denlinger 1993; Viola et al. 2015).

Indeed, the elimination of the expression of HAS2, the primary enzyme responsible for hyaluronic acid (HA) synthesis, is fatal (Camenisch et al. 2000). The results in naked mole rats corroborate these discoveries because they have an extraordinary survival rate and cancer resistance due to their high levels of large-sized HA (Tian et al. 2013). Excessive or incomplete HA synthesis can result from interference with HA synthases or hyaluronidases during transcription or translation. HAS and HYAL regulate the production of HA in various cancers; its production is associated with tumor progression and metastasis. Overproduction of HA contributes to tumor growth, invasion, and metastasis; HYAL-fragmented HA is one component of this process; ROS-fragmented HA is another (Stern and Jedrzejewski 2006). To increase HA overproduction, which drives the production of sarcomas and other melanoma cells, synthases HAS2 and HAS3 are overexpressed (Kosaki et al. 1999; Liu et al. 2001; Chaudhry et al. 2021). Inflammation is commonly associated with HA overproduction (Jiang et al. 2011) and interactions between inflammatory cells and HA via their membrane receptors (CD44, TLR). The formation of new collagen and the healing of damaged tissues typically follows the presence of HA in inflammation. Because fibrosis can result from either an overabundance of fibroblast stimulation by HA oligosaccharides or an overabundance of inflammation caused by excessive amounts of HA oligosaccharides, it is essential to fine-tune this phenomenon (Evanko et al. 2015). Chronic inflammation and HA production go hand in hand in inflammatory bowel diseases, including ulcerative colitis and Crohn's disease.

The ECM is altered due to the overproduction of HA by smooth muscle cells in these diseases. Through CD44 receptors, inflammatory cells in this milieu can interact with HA. Inflammatory cells often target structures called cables, which are formed by super molecularly organized HA chains, and their existence is visible in this case (Hascall et al. 2004; de La Motte et al. 1999; Viola et al. 2015). Vascular

disorders such as atherosclerosis and diabetes were also found to have an overproduction of HA (Merrilees et al. 2011). Even when produced by smooth muscle cells, the quantity of HA is extraordinary in these diseases. In vascular degeneration, HA is associated with increased cell migration, thickening of the vessel wall, and neointima development (Merrilees et al. 2011). Evidence of increased HA synthesis has been shown in both in vivo and in vitro diabetes studies, specifically in kidney cells cultivated in hyperglycemic conditions (Heickendorff et al. 1994; McDonald et al. 2007) and in the kidneys of diabetic mice (Wang and Hascall 2009). At some point, HA production has been increased in inflammatory lung illnesses, asthma, pneumonia, and other lung conditions (Swaiani et al. 2013; Lauer et al. 2013). During inflammation of the lungs, tumor necrosis factor-stimulated gene-6 (TSG-6) enhances HA synthesis by airway smooth muscle cells and helps stabilize the HA polymer structure, which facilitates the transfer of chondroitin sulfate chains from Bakunin to HA chains (Lauer et al. 2013; Tolg et al. 2014; Lord et al. 2013; Viola et al. 2015). The reparative mechanisms in various tissues commonly involve a comprehensive extracellular matrix (ECM) transformation. The initial phase of the healing process is marked by the synthesis of hyaluronic acid (HA), promoting the migration of inflammatory cells, proper macrophage and fibroblast migration, new collagen synthesis, and neo-angiogenesis (Motolese et al. 2013; Evanko et al. 2015; Tolg et al. 2006). The presence or absence of HA influences wound healing and the natural creation of scars, and HA is essential in all these processes (Tolg et al. 2014). Due to the crucial role of HA content in the microenvironment, it should be no surprise that most solid tumors include HA. Most malignancies are associated with an inflammatory microenvironment (Toole 2004; Vigetti and Passi 2014). Like the other inflammations mentioned before, HMWHA and low molecular weight HA play crucial roles in cancer progression (Iijima et al. 2011). Cancers of the breast, prostate, lung, and glioma are among those for which HA has been suggested as a potential diagnostic marker (Toole 2004; Tammi et al. 2008). There has been no definitive answer as to whether cells in the stroma or cancer cells can produce the HA that encases tumors. On the other hand, evidence suggests that cancer cells' increased motility is associated with increased HA receptors CD44 on most cancer cells (Toole 2004; Viola et al. 2015).

5 HA Formulation Systems in Cancer Therapeutics

HA, as a versatile biopolymer, is hypothesized to significantly augment the solubility of anticancer medications characterized by low solubility. This multifunctional attribute positions HA as a promising agent for enhancing the therapeutic efficacy of anticancer drugs. (Chaudhry et al. 2021). According to the studies conducted by Choi et al. (2012) and Kim et al. (2018), it is evident that hyaluronic acid (HA) nanoparticles offer a myriad of advantages in the field of medical applications. The versatility of hyaluronic acid (HA), attributed to its numerous functional groups, facilitates a broad spectrum of conjugations and modifications. These benefits encompass minimal to no immunogenicity, non-inflammatory responses,

biodegradability, biocompatibility, and enhanced bioavailability. This adaptability renders HA a pivotal component of multifunctional nanoparticles (NPs) that offer complementary approaches to cancer treatment (Lokeshwar et al. 2014). To harness the targeting capabilities inherent in HA, several methodologies have been devised to formulate HA NP systems. These methodologies encompass: (i) HA-conjugated drugs, (ii) HA-encapsulated drugs, (iii) gagomers, (iv) micelles, (v) nanocarriers, (vi) hydrogels, and (vii) inorganic NP systems (Mattheolabakis et al. 2015; Lapcik Jr et al. 1998; Cai et al. 2017; Zhang et al. 2016; Zamboni et al. 2017; Souness et al. 2018; Kim et al. 2018; Luo et al. 2000a; Rosato et al. 2006; Chaudhry et al. 2021). The capacity of HA to render anticancer medications more biocompatible and augment their solubility contributes to an overall enhancement in their therapeutic effectiveness (Chaudhry et al. 2021). The appeal of HA as a polymer in drug delivery stems from its ability to enhance the efficacy of HA-conjugated nanocarriers delivering anticancer medications, such as HA-epirubicin, HA-DOX, HA-PTX, HA-MMC, HA-siRNA, and more. Similarly, the combination therapy of HA-CUR/DOX-NPs has proven effective in treating drug-resistant tumor cells. Tumors and metastatic malignancies with a high concentration of HA featuring specific oligosaccharide lengths promote cell growth, invasion, angiogenesis, metastasis, and resistance to anticancer drugs. Approaches involving hyaluronidases in conjunction with chemotherapeutics, HA oligosaccharides (oHA) for apoptotic induction, HA inhibitors, and HA-based medications have been explored to address these challenges (Chaudhry et al. 2021). Paclitaxel was found to be an effective anticancer drug; however, despite its efficacy in inhibiting cell development, the potent antimitotic agent paclitaxel (PTX) faces limitations in pharmacological applications due to its poor water solubility. The conjugation of HA with PTX, as exemplified by the medicine HYTAD1-p20, demonstrates enhanced solubility and biocompatibility, particularly in the treatment of bladder and other malignancies (Luo et al. 2000b; Rosato et al. 2006). Furthermore, compounds such as PTX-NHS, HA-PTX and HA-SN-38, derived from irinotecan and HA, respectively, exhibit inhibitory effects on tumor cell growth and metastasis, especially when administered to CD44-expressing cell lines found in gastric, colon, and breast cancers (Luo and Prestwich 1999; Luo et al. 2000a; Serafino et al. 2011; Bassi et al. 2011; Montanger et al. 2013; Chaudhry et al. 2020, 2021). Nanocarriers, including dots, graphene, carbon nanotubes, gold, iron oxide, and silica nanoparticles, have shown promise in delivering therapeutic agents to targeted sites (Pellegrino et al. 2005; Geim 2009; Baker and Baker 2010; Tenne 2006; Lee et al. 2012; Kumar et al. 2007; Lu et al. 2010). Some of these nanoparticles have exhibited novel properties upon conjugation with HA (Cho et al. 2011). Hyaluronic acid (HA) polymers resulting from controlled chemical modification of the carboxylic group exhibit significant potential applications in biomedicine, particularly in developing prodrugs (Prestwich et al. 1998). Utilizing HA micelles enhances the efficient transport of hydrophobic drugs to cancer cells, thereby augmenting the bioavailability and half-life of these medications (Choi et al. 2012). The solubilization of hydrophobic drugs plays a crucial role in mitigating the risks associated with drug agglomeration and embolism formation following intravenous administration. Hydrogels at the micro- or

nano-scale, owing to their relatively large pore size, facilitate the rapid release of drug molecules not bound by covalent bonds or specific interactions (Xu et al. 2012). HA micelles, as a self-organizing structure consisting of a carboxyl group on one polymer and an amino group on another, such as polyethylene glycol (PEG), have been investigated for their cytotoxic efficacy. Studies conducted by Liu et al. (2011), Qiu et al. (2014) and Zhang et al. (2012) demonstrated the effective cytotoxicity of CD44-expressing cells exposed to HA-micelles containing paclitaxel (HA-PTX), doxorubicin (HA-DOX), and salinomycin. HA-encapsulated medications are currently undergoing clinical trials, demonstrating reasonable cytotoxic efficacy in both in vitro and in vivo contexts (Rosenthal et al. 2005; Gibbs et al. 2009). The polymersomes, capable of encapsulating water-soluble and lipophilic drugs, contribute to this process (Bermudez et al. 2002; Discher et al. 1999). These polymersomes offer advantages over conventional bilayer vesicles, including biocompatibility, robust mechanical properties, and a high-water content. Similarly, biocompatible polymersomes, predominantly composed of polyethylene glycols (PEG), exhibit inert characteristics, refraining from adverse reactions with blood components or unintended tissue damage. An additional challenge in the utilization of promising substances for drug delivery in medicine is the potential side effects, particularly concerning cancer-related implications. Nevertheless, HA-doxorubicin (5-FU) and HA-5-fluorouracil (HyFIVETM) represent self-assembling lipid molecules coated with high molecular weight hyaluronan (HMW) as liposomes or lipoplexes have targeted various cancers, including head and neck cancer, carrying plasmid DNA and small interfering RNA (siRNA). These molecules exhibit expression of the CD44 receptor (Peer and Margalit 2004a, b; Surace et al. 2009; Eliaz and Szoka Jr 2001; Ruhela et al. 2006; Dufay Wojcicki et al. 2012). However, it is crucial to acknowledge that inorganic NPs lack cell specificity and may pose risks to cells. To address this, combining biopolymers, particularly polysaccharides, with inorganic NPs has been investigated to enhance stability and biocompatibility and reduce toxicity (Cai et al. 2017; Kim et al. 2018). Inorganic matrices such as graphene oxide (GO), quantum dots (QD), silica nanoparticles (NPs), gold NPs, and iron oxide NPs have been extensively explored for their potential applications in cancer imaging, drug delivery, and other theranostic purposes, as noted by Arpicco et al. (2014).

6 HA Polymeric Length Dynamics in Cancer Regulation

The majority of malignancies exhibit elevated levels of hyaluronic acid (HA). Integral membrane proteins identified as hyaluronan synthases (HAS) are pivotal in collagen synthesis. Three distinct isoforms of these synthases, namely (i) HAS1, (ii) HAS2, and (iii) HAS3, contribute to the elongation of hyaluronic acid (HA) by sequentially incorporating N-acetyl-D-glucosamine and D-glucuronic acid into the resulting polysaccharide (Itano and Kimata 2002). As elucidated by Schulz et al. (2007), HA is extruded from the cell into the extracellular space through the action of the ABC transporter. The functionality of these enzymes is determined by

posttranslational modifications, including alternative splicing and epigenetic mechanisms, operating at both the transcriptional and translational levels. While HA typically manifests as lengthy, unbranched molecules, the enzymes governing its synthesis can generate polymers of diverse sizes.

Interestingly, high molecular weight hyaluronic acid (HMW-HA) protects several human physiological contexts, including inflammation, embryogenesis, wound healing, and cancer (Aya and Stern 2014; Price et al. 2018). The high molecular weight HA (HMW-HA) reverses MU's inhibitory and non-metastatic effects on MIA PaCa-2 cells, with the inhibition being molecular weight (MW) dependent. However, research indicates that a deficiency in these synthase enzymes leads to heart and artery abnormalities during pregnancy, resulting in embryonic demise (Camenisch et al. 2000; Michalczyk et al. 2022). In a lung injury model induced by lipopolysaccharide, Liu et al. (2008) demonstrated that intraperitoneal therapy with HMW-HA effectively prevented the invasion of monocytes and neutrophils. HMW-HA has exhibited anticancer properties, including suppression of cell migration and regrowth, inhibition of inflammatory mediator production, and other beneficial effects in various tumor models (Berdiaki et al. 2009; Mueller et al. 2010; Nikitovic et al. 2015). Research by Cuff et al. (2001) revealed that HMW-HA possesses antiangiogenic properties, inhibiting the proliferation of vascular smooth muscle cells by maintaining them in the G1 phase. Moreover, when HMW-HA binds to CD44, it mainly inhibits Rac GTP-loading and Rac-dependent signaling to cyclin D1, suppressing cell cycle progression and cell proliferation (Pure and Assoian 2009). Investigations by Chang et al. (2012) and Michalczyk et al. (2022) suggest that HMW-HA can mitigate pro-inflammatory effects by reducing COX2 and MMP synthesis and blocking MAPKs and NF- κ B activation. The inhibitors for inhibiting hyaluronan synthesis play a role in its functional dynamics. The 4-methylumbelliferone (MU) inhibits HA formation, reduces uridine diphosphate glucuronic acid levels, and downregulates hyaluronan synthase (HAS) in human skin fibroblasts. Like MU, 4-methylumbelliferone demonstrates anticancer effects in mouse models of various cancers and inhibits HA production in pancreatic cancer cells (KP1-NL), as well as liver metastases in mice injected with pancreatic cancer cells (Provenzano et al. 2012). Researchers have identified two mechanisms through which MU impedes HA production. Firstly, MU depletes UDP-GlcUA, a precursor to HA (Nagase et al. 2017). Secondly, as mentioned by Kakizaki et al. (2004), MU diminishes HAS mRNA expression. However, the precise mechanism by which MU reduces HAS mRNA expression lacks detailed exploration in existing research. Conflicting evidence exists regarding the effects of MU on HAS mRNA expression. While some studies suggest that MU inhibits the gene (Kultti et al. 2009; Lokeshwar et al. 2010; Urakawa et al. 2012a, b), others have reported the opposite (Arai et al. 2011). It is noteworthy, however, that different cell types exhibit varied responses to MU in terms of expression, with some experiencing upregulation and others downregulation (Chaudhry et al. 2021). Further investigation is necessary to delineate the specific role of MU in suppressing total HAS mRNA and its impact on CD44 expression.

Hyaluronidases play a crucial role in reducing bodily fluids' viscosity and enhancing connective tissue's permeability. Termed as the "spreading factor", hyaluronidases facilitate the entry of pathogens and toxins into the body through the skin and organs during illness. The expression of hyaluronidases is ubiquitous across various tissues. In humans, hyaluronidases can be classified into six different types: (i) HYAL-1, (ii) HYAL-2, (iii) HYAL-3, (iv) HYAL-4, (v) PH-20/SPAM1, and (vi) PHYAL134. Stern and Jedrzejewski (2006) and Michalczyk et al. (2022) have identified that most members of the hyaluronidase family are implicated in tumor-related activities. HYALs, well-known enzymes in this process, facilitate hyaluronic acid (HA) degradation. Studies conducted by Jan and Chaudhry (2019) and others reveal that the expression of wild-type HYAL-1 (HYAL-1-v1) induces apoptosis in bladder cancer cells through G2-M arrest. However, the clinical application of HYALs is constrained by their adhesive impact on normal tissue, leading to inherent limitations and side effects (Lokeshwar et al. 2006). An intriguing discovery suggests that chemosensitivity in EMT-6 tumor spheroids can be improved by enhancing accessibility using commercial bovine testicular or bacterial HYAL, acting as an anti-adhesive agent (Croix et al. 1998). Multiple researchers have associated the expression of HYAL-1 with tumor growth and unfavorable clinical outcomes. Notably, head and neck cancers resulting from tobacco exposure exhibited an increased risk of carcinogenesis when HYAL-1 activity was reduced (Geim 2009). Studies conducted by Liu et al. (2011) reveal that overexpression of HYAL-1 accelerates breast tumor formation and induces significant angiogenesis within the tumor. In xenograft models of breast, bladder, and colon cancer, HYAL-1 has been found to enhance tumor mobility and penetration (Qiu et al. 2014; Zhang et al. 2012; Pellegrino et al. 2005). Baker and Baker (2010) and Chaudhry et al. (2021) postulate that these findings suggest a potential oncogenic function for HYAL-1. Hyaluronidase (HYAL) and reactive oxygen species (ROS) generate breakdown products of HA, such as low molecular weight HA (LMW-HA) and oligo-HA, known to stimulate tumor growth and increase cancer cell invasion (Stern et al. 2006; Karbownik and Nowak 2013). Conversely, HMW-HA is proven to maintain tissue homeostasis. Scientific evidence suggests that oligo-HA promotes inflammation, influencing tumor development and metastasis.

The therapeutic application of tiny oligo-hyaluronic acids (HA) (3–9 disaccharide units) starkly contrasts their tumor-promoting effects. Synthesized small oligo-HAs can selectively target and induce cell death in specific tumor cells while concurrently triggering apoptosis in adjacent healthy tissues. Interestingly, HA oligosaccharides (oHA) have been found to inhibit downstream cancer survival pathways, inducing apoptosis in lymphoma cell types. According to Chaudhry et al. (2021), oligosaccharides (oHA) exhibit no effect on NF- κ B activation in lymphoma cells but restrict PIP3 synthesis, activate PI3-K, and deactivate Akt via reduced phosphorylation. In vivo experiments demonstrate that the injection of HA oligomers, promoting migration, invasion, and metastasis, significantly inhibits melanoma growth.

Interestingly, small HA oligomers (6–10 disaccharides) have been shown to reverse cancer cell adhesion, migration, and invasion in certain ovarian cell lines, as reported by Slomiany et al. in 2009. These shorter bioactive HA fragments exhibit

distinct interactions with cancer cells and can modulate their activity in comparison to high molecular weight HA (HMW-HA) (Bourguignon et al. 2011; Urakawa et al. 2012a, b; Michalczyk et al. 2022). According to Dang et al. (2013), oligo-HA has been found to enhance the *in vivo* proliferation of papillary thyroid cancer cells through a signaling pathway dependent on toll-like receptor (TLR) 4. Studies demonstrate that LMW-HA enhances cancer cell adherence, proliferation, and remodeling by secreting matrix metalloproteinases (MMPs). Monslow et al. (2015) reported that LMW-HA exhibits pro-inflammatory and pro-angiogenic capabilities, potentially facilitating cancer cell invasion. The significance of LMW-HA as a cancer diagnostic marker may be attributed to its association with breast cancer metastasis (Wu et al. 2015; Michalczyk et al. 2022). The abundance of hyaluronic acid (HA) adversely affects the survival rate in pancreatic ductal adenocarcinoma. The interaction between HA and the CD44 receptor results in cell proliferation, invasion, and metastasis, making various forms of the CD44 receptor, the HA ligand, and the HA-CD44 interaction potential therapeutic targets in the battle against cancer (Chaudhry et al. 2021).

7 Conclusion and Future Prospective

Finally, the manuscript delves further into the complex relationship between hyaluronic acid (HA) and CD44 in cancer start, progression, and development and how HA-CD may be used against cancer. The results here emphasize the critical function of HA-induced CD44 activation in regulating tumor cell-specific behaviors, which in turn impact a variety of signaling pathways, cytoskeletal dynamics, and survival mechanisms. An imbalance in HA production can lead to several disorders, including cancer, and this highlights the importance of HA synthases, which are expressed differently depending on the tissue and the time of day. More importantly, the research highlights the ever-changing function of HA polymeric length in cancer control. High molecular weight HA (HMW-HA) can guard against cancer and has protective effects in physiological settings, in contrast to low molecular weight HA (LMW-HA), which promote tumor growth. However, a potential new direction for cancer treatment is the use of tiny HA oligomers as a therapeutic agent to induce apoptosis in particular tumor cells while avoiding healthy tissues. Additionally, the chapter digs into the potential uses of HA in cancer treatments, namely in drug delivery systems. Anticancer medications can improve their solubility and bioavailability by utilizing HA's adaptability as a biopolymer. We highlight the significance of HA in increasing therapeutic efficacy and minimizing side effects. We address several formulations for targeted drug administration, including nanoparticles, micelles, hydrogels, and inorganic NP systems. Further investigation into the molecular processes controlling the HA-CD44 interaction and its consequences in various cancer types is suggested for future research in this publication. Further, optimizing the efficacy of HA-based formulations for targeted drug administration and studying their potential in clinical situations is critical. Research into the therapeutic potential of specific HA lengths and the role of HA polymeric length dynamics in cancer

regulation could yield valuable insights for developing targeted therapeutics. The chapter lays the groundwork for future research to convert these discoveries into creative therapeutic options for cancer treatment and thoroughly explains the HA-CD44 relationship in cancer progression.

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References

- Anand V, Sharma A (2017) CD44: a multifaceted molecule in cancer progression. *J Cancer Prev Curr Res* 8:00279
- Anttila MA, Tammi RH, Tammi MI, Syrjänen KJ, Saarikoski SV, Kosma VM (2000) High levels of stromal hyaluronan predict poor disease outcome in epithelial ovarian cancer. *Cancer Res* 60(1): 150–155
- Arai E, Nishida Y, Wasa J, Urakawa H, Zhuo L, Kimata K, Kozawa E, Futamura N, Ishiguro N (2011) Inhibition of hyaluronan retention by 4-methylumbelliferone suppresses osteosarcoma cells *in vitro* and lung metastasis *in vivo*. *Br J Cancer* 105(12):1839–1849. <https://doi.org/10.1038/bjc.2011.459>
- Arpicco S, Milla P, Stella B, Dosio F (2014) Hyaluronic acid conjugates as vectors for the active targeting of drugs, genes and nanocomposites in cancer treatment. *Molecules* 19(3):3193–3230. <https://doi.org/10.3390/molecules19033193>
- Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B (1990) CD44 is the principal cell surface receptor for hyaluronate. *Cell* 61(7):1303–1313. [https://doi.org/10.1016/0092-8674\(90\)90694-a](https://doi.org/10.1016/0092-8674(90)90694-a)
- Auvinen P, Tammi R, Parkkinen J, Tammi M, Agren U, Johansson R, Hirvikoski P, Eskelinen M, Kosma VM (2000) Hyaluronan in peritumoral stroma and malignant cells associates with breast cancer spreading and predicts survival. *Am J Pathol* 156(2):529–536. [https://doi.org/10.1016/S0002-9440\(10\)64757-8](https://doi.org/10.1016/S0002-9440(10)64757-8)
- Aya KL, Stern R (2014) Hyaluronan in wound healing: rediscovering a major player. *Wound Repair Regen* 22(5):579–593. <https://doi.org/10.1111/wrr.12214>
- Baker SN, Baker GA (2010) Luminescent carbon nanodots: emergent nanolights. *Angew Chem Int Ed* 49(38):6726–6744. <https://doi.org/10.1002/anie.200906623>
- Balazs EA, Denlinger JL (1993) Viscosupplementation: a new concept in the treatment of osteoarthritis. *J Rheumatol Suppl* 39:3–9
- Balazs EA, Laurent TC, Jeanloz RW (1986) Nomenclature of hyaluronic acid. *Biochem J* 235(3): 903. <https://doi.org/10.1042/bj2350903>
- Basakran NS (2015) CD44 as a potential diagnostic tumor marker. *Saudi Med J* 36(3):273–279. <https://doi.org/10.15537/smj.2015.3.9622>
- Bassi PF, Volpe A, D’Agostino D, Palermo G, Renier D, Franchini S, Rosato A, Racioppi M (2011) Paclitaxel-hyaluronic acid for intravesical therapy of bacillus Calmette-Guérin refractory carcinoma in situ of the bladder: results of a phase I study. *J Urol* 185(2):445–449. <https://doi.org/10.1016/j.juro.2010.09.073>
- Berdiaki A, Nikitovic D, Tsatsakis A, Katonis P, Karamanos NK, Tzanakakis GN (2009) bFGF induces changes in hyaluronan synthase and hyaluronidase isoform expression and modulates the migration capacity of fibrosarcoma cells. *Biochim Biophys Acta* 1790(10):1258–1265. <https://doi.org/10.1016/j.bbagen.2009.06.013>
- Bermudez H, Brannan AK, Hammer DA, Bates FS, Discher DE (2002) Molecular weight dependence of polymersome membrane structure, elasticity, and stability. *Macromolecules* 35(21): 8203–8208

- Boas NF (1949) Isolation of hyaluronic acid from the cock's comb. *J Biol Chem* 181(2):573–575. [https://doi.org/10.1016/S0021-9258\(18\)56578-9](https://doi.org/10.1016/S0021-9258(18)56578-9)
- Boeriu CG, Springer J, Kooy FK, van den Broek LA, Eggink G (2013) Production methods for hyaluronan. *Int J Carbohydr Chem* 2013. <https://doi.org/10.1155/2013/624967>
- Bourguignon LY (2008) Hyaluronan-mediated CD44 activation of RhoGTPase signaling and cytoskeleton function promotes tumor progression. *Semin Cancer Biol* 18(4):251–259. <https://doi.org/10.1016/j.semcancer.2008.03.007>
- Bourguignon LY, Lokeshwar VB, Chen X, Kerrick WG (1993) Hyaluronic acid-induced lymphocyte signal transduction and HA receptor (GP85/CD44)-cytoskeleton interaction. *J Immunol* 151(12):6634–6644
- Bourguignon LY, Gunja-Smith Z, Iida N, Zhu HB, Young LJ, Muller WJ, Cardiff RD (1998) CD44v(3,8-10) is involved in cytoskeleton-mediated tumor cell migration and matrix metalloproteinase (MMP-9) association in metastatic breast cancer cells. *J Cell Physiol* 176(1):206–215. [https://doi.org/10.1002/\(SICI\)1097-4652\(199807\)176:1<206::AID-JCP22>3.0.CO;2-3](https://doi.org/10.1002/(SICI)1097-4652(199807)176:1<206::AID-JCP22>3.0.CO;2-3)
- Bourguignon LY, Zhu H, Shao L, Chen YW (2001) CD44 interaction with c-Src kinase promotes cortactin-mediated cytoskeleton function and hyaluronic acid-dependent ovarian tumor cell migration. *J Biol Chem* 276(10):7327–7336. <https://doi.org/10.1074/jbc.M006498200>
- Bourguignon LY, Wong G, Earle CA, Xia W (2011) Interaction of low molecular weight hyaluronan with CD44 and toll-like receptors promotes the actin filament-associated protein 110-actin binding and MyD88-NFκB signaling leading to proinflammatory cytokine/chemokine production and breast tumor invasion. *Cytoskeleton* 68(12):671–693. <https://doi.org/10.1002/cm.20544>
- Cai Z, Zhang H, Wei Y, Cong F (2017) Hyaluronan-inorganic nanohybrid materials for biomedical applications. *Biomacromolecules* 18(6):1677–1696. <https://doi.org/10.1021/acs.biomac.7b00424>
- Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro A Jr, Kubalak S, Klewer SE, McDonald JA (2000) Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *J Clin Invest* 106(3):349–360. <https://doi.org/10.1172/JCI10272>
- Chang CC, Hsieh MS, Liao ST, Chen YH, Cheng CW, Huang PT, Lin YF, Chen CH (2012) Hyaluronan regulates PPARγ and inflammatory responses in IL-1β-stimulated human chondrosarcoma cells, a model for osteoarthritis. *Carbohydr Polym* 90(2):1168–1175. <https://doi.org/10.1016/j.carbpol.2012.06.071>
- Chaudhry GS, Akim A, Zafar MN, Abdullah MA, Sung YY, Muhammad TST (2020) Induction of apoptosis and role of PTX loaded HA-crosslinked nanoparticle in the regulation of AKT and RhoA. *J Adv Pharm Technol Res* 11(3):101–106. https://doi.org/10.4103/japtr.JAPTR_26_20
- Chaudhry GE, Akim A, Naveed Zafar M, Safdar N, Sung YY, Muhammad TST (2021) Understanding hyaluronan receptor (CD44) interaction, HA-CD44 activated potential targets in cancer therapeutics. *Adv Pharm Bull* 11(3):426–438. <https://doi.org/10.34172/apb.2021.050>
- Chellaiiah M, Kizer N, Silva M, Alvarez U, Kwiatkowski D, Hruska KA (2000) Gelsolin deficiency blocks podosome assembly and produces increased bone mass and strength. *J Cell Biol* 148(4):665–678. <https://doi.org/10.1083/jcb.148.4.665>
- Chen C, Zhao S, Karnad S, Freeman JW (2018) The biology and role of CD44 in cancer progression: therapeutic implications. *J Hematol Oncol* 11:64. <https://doi.org/10.1186/s13045-018-0605-5>
- Cho HJ, Yoon HY, Koo H, Ko SH, Shim JS, Lee JH, Kim K, Kwon IC, Kim DD (2011) Self-assembled nanoparticles based on hyaluronic acid-ceramide (HA-CE) and Pluronic® for tumor-targeted delivery of docetaxel. *Biomaterials* 32(29):7181–7190. <https://doi.org/10.1016/j.biomaterials.2011.06.028>
- Choi KY, Saravanakumar G, Park JH, Park K (2012) Hyaluronic acid-based nanocarriers for intracellular targeting: interfacial interactions with proteins in cancer. *Colloids Surf B Biointerfaces* 99:82–94. <https://doi.org/10.1016/j.colsurfb.2011.10.029>

- Cichy J, Pure E (2003) The liberation of CD44. *J Cell Biol* 161(5):839–843. <https://doi.org/10.1083/jcb.200302098>
- Clark RA, Alon R, Springer TA (1996) CD44 and hyaluronan-dependent rolling interactions of lymphocytes on tonsillar stroma. *J Cell Biol* 134(4):1075–1087. <https://doi.org/10.1083/jcb.134.4.1075>
- Croix BS, Man S, Kerbel RS (1998) Reversal of intrinsic and acquired forms of drug resistance by hyaluronidase treatment of solid tumors. *Cancer Lett* 131(1):35–44. [https://doi.org/10.1016/S0304-3835\(98\)00199-2](https://doi.org/10.1016/S0304-3835(98)00199-2)
- Cuff CA, Kothapalli D, Azonobi I, Chun S, Zhang Y, Belkin R, Yeh C, Secreto A, Assoian RK, Rader DJ, Pure E (2001) The adhesion receptor CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. *J Clin Invest* 108(7):1031–1040. <https://doi.org/10.1172/JCI12455>
- Dang S, Peng Y, Ye L, Wang Y, Qian Z, Chen Y, Wang X, Lin Y, Zhang X, Sun X, Wu Q, Cheng Y, Nie H, Jin M, Xu H (2013) Stimulation of TLR4 by LMW-HA induces metastasis in human papillary thyroid carcinoma through CXCR7. *Clin Dev Immunol* 2013:712561. <https://doi.org/10.1155/2013/712561>
- Day AJ, Prestwich GD (2002) Hyaluronan-binding proteins: tying up the giant. *J Biol Chem* 277(7):4585–4588. <https://doi.org/10.1074/jbc.R100036200>
- de La Motte CA, Hascall VC, Calabro A, Yen-Lieberman B, Strong SA (1999) Mononuclear leukocytes preferentially bind via CD44 to hyaluronan on human intestinal mucosal smooth muscle cells after virus infection or treatment with poly (I:C). *J Biol Chem* 274(43):30747–30755. <https://doi.org/10.1074/jbc.274.43.30747>
- Dechert TA, Ducale AE, Ward SI, Yager DR (2006) Hyaluronan in human acute and chronic dermal wounds. *Wound Repair Regen* 14(3):252–258
- DeGrendele HC, Estess P, Picker LJ, Siegelman MH (1996) CD44 and its ligand hyaluronate mediate rolling under physiologic flow: a novel lymphocyte-endothelial cell primary adhesion pathway. *J Exp Med* 183(3):1119–1130. <https://doi.org/10.1084/jem.183.3.1119>
- DeGrendele HC, Estess P, Siegelman MH (1997) Requirement for CD44 in activated T cell extravasation into an inflammatory site. *Science* 278(5338):672–675. <https://doi.org/10.1126/science.278.5338.672>
- Desai B, Rogers MJ, Chellaiah MA (2007) Mechanisms of osteopontin and CD44 as metastatic principles in prostate cancer cells. *Mol Cancer* 6:18. <https://doi.org/10.1186/1476-4598-6-18>
- Desai B, Ma T, Chellaiah MA (2008) Invadopodia and matrix degradation, a new property of prostate cancer cells during migration and invasion. *J Biol Chem* 283(20):13856–13866. <https://doi.org/10.1074/jbc.M709401200>
- Discher BM, Won YY, Ege DS, Lee JC, Bates FS, Discher DE, Hammer DA (1999) Polymersomes: tough vesicles made from diblock copolymers. *Science* 284(5417):1143–1146. <https://doi.org/10.1126/science.284.5417.1143>
- Dufay Wojcicki A, Hillaireau H, Nascimento TL, Arpicco S, Taverna M, Ribes S, Bourge M, Nicolas V, Bochot A, Vauthier C, Tsapis N, Fattal E (2012) Hyaluronic acid-bearing lipoplexes: Physico-chemical characterization and *in vitro* targeting of the CD44 receptor. *J Control Release* 162(3):545–552. <https://doi.org/10.1016/j.jconrel.2012.07.015>
- Eliaz RE, Szoka FC Jr (2001) Liposome-encapsulated doxorubicin targeted to CD44: a strategy to kill CD44-overexpressing tumor cells. *Cancer Res* 61(6):2592–2601
- Evanko SP, Potter-Perigo S, Petty LJ, Workman GA, Wight TN (2015) Hyaluronan controls the deposition of fibronectin and collagen and modulates TGF- β 1 induction of lung myofibroblasts. *Matrix Biol* 42:74–92. <https://doi.org/10.1016/j.matbio.2014.12.001>
- Fallacara A, Baldini E, Manfredini S, Vertuani S (2018) Hyaluronic acid in the third millennium. *Polymers* 10(7):701. <https://doi.org/10.3390/polym10070701>
- Franzmann EJ, Weed DT, Civantos FJ, Goodwin WJ, Bourguignon LY (2001) A novel CD44 v3 isoform is involved in head and neck squamous cell carcinoma progression. *Otolaryngol Head Neck Surg* 124(4):426–432. <https://doi.org/10.1067/mhn.2001.114674>

- Gaffney J, Matou-Nasri S, Grau-Olivares M, Slevin M (2010) Therapeutic applications of hyaluronan. *Mol BioSyst* 6(3):437–443. <https://doi.org/10.1039/b910552m>
- Garantziotis S, Savani RC (2019) Hyaluronan biology: a complex balancing act of structure, function, location and context. *Matrix Biol* 78–79:1–10. <https://doi.org/10.1016/j.matbio.2019.02.002>
- Geim AK (2009) Graphene: status and prospects. *Science* 324(5934):1530–1534. <https://doi.org/10.1126/science.1158877>
- Gibbs P, Brown TJ, Ng R, Jennens R, Cinc E, Pho M, Michael M, Fox RM (2009) A pilot human evaluation of a formulation of irinotecan and hyaluronic acid in 5-fluorouracil-refractory metastatic colorectal cancer patients. *Chemotherapy* 55(1):49–59. <https://doi.org/10.1159/000180339>
- Gul-e-Saba, Abdullah MA (2015) Polymeric nanoparticle mediated targeted drug delivery to cancer cells. In: Thangadurai D, Sangeetha J (eds) *Biotechnology and bioinformatics: advances and applications for bioenergy, bioremediation, and biopharmaceutical research*, pp 1–34. Apple Academic Press, Waretown. ISBN: 9781771880015
- Gul-e-Saba, Akim A, Abdullah A (2010) Hyaluronan-mediated CD44 receptor cancer cells progression and the application of controlled drug-delivery system. *Int J Curr Chem* 1(4):245–265
- Gul-e-Saba, Akim A, Abdullah MA (2014) Cytotoxic effects of drug-loaded hyaluronan glutaraldehyde cross-linked nanoparticles and the release kinetics modeling. *J Adv Chem Eng* 1:104. <https://doi.org/10.4172/2090-4568.1000104>
- Guo Q, Yang C, Gao F (2022) The state of CD44 activation in cancer progression and therapeutic targeting. *FEBS J* 289(24):7970–7986. <https://doi.org/10.1111/febs.16179>
- Gupta A, Cao W, Sadashivaiah K, Chen W, Schneider A, Chellaiah MA (2013) Promising noninvasive cellular phenotype in prostate cancer cells knockdown of matrix metalloproteinase 9. *Sci World J* 2013:493689. <https://doi.org/10.1155/2013/493689>
- Hascall VC, Majors AK, De La Motte CA, Evanko SP, Wang A, Drazba JA, Strong SA, Wight TN (2004) Intracellular hyaluronan: a new frontier for inflammation? *Biochim Biophys Acta* 1673(1–2):3–12. <https://doi.org/10.1016/j.bbagen.2004.02.013>
- Heickendorff L, Ledet T, Rasmussen LM (1994) Glycosaminoglycans in the human aorta in diabetes mellitus: a study of tunica media from areas with and without atherosclerotic plaque. *Diabetologia* 37:286–292. <https://doi.org/10.1007/BF00398056>
- Iczkowski KA (2010) Cell adhesion molecule CD44: its functional roles in prostate cancer. *Am J Transl Res* 3(1):1–7
- Iijima J, Konno K, Itano N (2011) Inflammatory alterations of the extracellular matrix in the tumor microenvironment. *Cancers* 3(3):3189–3205. <https://doi.org/10.3390/cancers3033189>
- Itano N, Kimata K (2002) Mammalian hyaluronan synthases. *IUBMB Life* 54(4):195–199. <https://doi.org/10.1080/15216540214929>
- Itano N, Kimata K (2008) Altered hyaluronan biosynthesis in cancer progression. *Semin Cancer Biol* 18(4):268–274. <https://doi.org/10.1016/j.semcancer.2008.03.006>
- Jan R, Chaudhry GE (2019) Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. *Adv Pharm Bull* 9(2):205–218. <https://doi.org/10.15171/apb.2019.024>
- Jiang D, Liang J, Noble PW (2011) Hyaluronan as an immune regulator in human diseases. *Physiol Rev* 91(1):221–264. <https://doi.org/10.1152/physrev.00052.2009>
- Jiang WG, Sanders AJ, Katoh M, Ungefroren H, Gieseler F, Prince M, Thompson SK, Zollo M, Spano D et al (2015) Tissue invasion and metastasis: molecular, biological and clinical perspectives. *Semin Cancer Biol* 35:S244–S275. <https://doi.org/10.1016/j.semcancer.2015.03.008>
- Jung T, Castellana D, Klingbeil P, Cuesta Hernandez I, Vitacolonna M, Orlicky DJ, Roffler SR, Brodt P, Zoller M (2009) CD44v6 dependence of premetastatic niche preparation by exosomes. *Neoplasia* 11(10):1093–1105. <https://doi.org/10.1593/neo.09822>
- Jung T, Gross W, Zoller M (2011) CD44v6 coordinates tumor matrix-triggered motility and apoptosis resistance. *J Biol Chem* 286(18):15862–15874. <https://doi.org/10.1074/jbc.M110.208421>
- Kakizaki I, Kojima K, Takagaki K, Endo M, Kannagi R, Ito M, Maruo Y, Sato H, Yasuda T, Mita S, Kimata K, Itano N (2004) A novel mechanism for the inhibition of hyaluronan biosynthesis by 4-methylumbelliferone. *J Biol Chem* 279(32):33281–33289. <https://doi.org/10.1074/jbc.M405918200>

- Kalish ED, Iida N, Moffat FL, Bourguignon LY (1999) A new CD44V3-containing isoform is involved in tumor cell growth and migration during human breast carcinoma progression. *Front Biosci* 4:A1–A8. <https://doi.org/10.2741/kalish>
- Karbownik MS, Nowak JZ (2011) Hyaluronan in cancer–pathophysiology and pharmacotherapy perspectives. *J Oncol* 61:380–395
- Karbownik MS, Nowak JZ (2013) Hyaluronan: towards novel anti-cancer therapeutics. *Pharmacol Rep* 65(5):1056–1074. [https://doi.org/10.1016/s1734-1140\(13\)71465-8](https://doi.org/10.1016/s1734-1140(13)71465-8)
- Karousou E, Misra S, Ghatak S, Dobra K, Gotte M, Vigetti D, Passi A, Karamanos NK, Skandalis SS (2017) Roles and targeting of the HAS/hyaluronan/CD44 molecular system in cancer. *Matrix Biol* 59:3–22. <https://doi.org/10.1016/j.matbio.2016.10.001>
- Kendall FE, Heidelberger M, Dawson MH (1937) A serologically inactive polysaccharide elaborated by mucoid strains of group A hemolytic streptococcus. *J Biol Chem* 118(1):61–69. [https://doi.org/10.1016/S0021-9258\(18\)74517-1](https://doi.org/10.1016/S0021-9258(18)74517-1)
- Kim JH, Moon MJ, Kim DY, Heo SH, Jeong YY (2018) Hyaluronic acid-based nanomaterials for cancer therapy. *Polymers* 10(10):1133. <https://doi.org/10.3390/polym10101133>
- Kobayashi T, Chanmee T, Itano N (2020) Hyaluronan: metabolism and function. *Biomol Ther* 10: 1525. <https://doi.org/10.3390/biom10111525>
- Kosaki R, Watanabe K, Yamaguchi Y (1999) Overproduction of hyaluronan by expression of the hyaluronan synthase Has2 enhances anchorage-independent growth and tumorigenicity. *Cancer Res* 59(5):1141–1145
- Kreil G (1995) Hyaluronidases—a group pf neglected enzymes. *Protein Sci* 4(9):1666–1669. <https://doi.org/10.1002/pro.5560040902>
- Kuhn S, Koch M, Nubel T, Ladwein M, Antolovic D, Klingbeil P, Hildebrand D, Moldenhauer G, Langbein L, Franke WW, Weitz J, Zoller M (2007) A complex of EpCAM, claudin-7, CD44 variant isoforms, and tetraspanins promotes colorectal cancer progression. *Mol Cancer Res* 5(6): 553–567. <https://doi.org/10.1158/1541-7786.MCR-06-0384>
- Kultti A, Pasonen-Seppänen S, Jauhainen M, Rilla KJ, Kärnä R, Pyörä E, Tammi RH, Tammi MI (2009) 4-Methylumbelliferone inhibits hyaluronan synthesis by depletion of cellular UDP-glucuronic acid and downregulation of hyaluronan synthase 2 and 3. *Exp Cell Res* 315(11):1914–1923. <https://doi.org/10.1016/j.yexcr.2009.03.002>
- Kumar A, Sahoo B, Montpetit A, Behera S, Lockey RF, Mohapatra SS (2007) Development of hyaluronic acid-Fe₂O₃ hybrid magnetic nanoparticles for targeted delivery of peptides. *Nanomedicine* 3(2):132–137. <https://doi.org/10.1016/j.nano.2007.03.001>
- Kuniyasu H, Oue N, Tsutsumi M, Tahara E, Yasui W (2001) Heparan sulfate enhances invasion by human colon carcinoma cell lines through expression of CD44 variant exon 3. *Clin Cancer Res* 7(12):4067–4072
- Lakshman M, Subramaniam V, Rubenthiran U, Jothy S (2004) CD44 promotes resistance to apoptosis in human colon cancer cells. *Exp Mol Pathol* 77(1):18–25. <https://doi.org/10.1016/j.yexmp.2004.03.002>
- Lapcik L Jr, Lapcik L, De Smedt S, Demeester J, Chabreck P (1998) Hyaluronan: preparation, structure, properties, and applications. *Chem Rev* 98(8):2663–2684. <https://doi.org/10.1021/cr941199z>
- Lauer ME, Cheng G, Swaidani S, Aronica MA, Weigel PH, Hascall VC (2013) Tumor necrosis factor-stimulated gene-6 (TSG-6) amplifies hyaluronan synthesis by airway smooth muscle cells. *J Biol Chem* 288(1):423–431. <https://doi.org/10.1074/jbc.M112.389882>
- Laurent T (1989) The biology of hyaluronan. Introduction. *Ciba Found Symp* 143:1–20
- Laurent TC, Fraser JR (1992) Hyaluronan. *FASEB J* 6:2397–2404. <https://doi.org/10.1096/fasbj.6.7.1563592>
- Lee MY, Yang JA, Jung HS, Beack S, Choi JE, Hur W, Koo H, Kim K, Yoon SK, Hahn SK (2012) Hyaluronic acid-gold nanoparticle/interferon α complex for targeted treatment of hepatitis C virus infection. *ACS Nano* 6(11):9522–9531. <https://doi.org/10.1021/nn302538y>
- Li L, Heldin CH, Heldin P (2006) Inhibition of platelet-derived growth factor-BB-induced receptor activation and fibroblast migration by hyaluronan activation of CD44. *J Biol Chem* 281(36): 26512–26519. <https://doi.org/10.1074/jbc.M60567200>

- Linder S, Aepfelbacher M (2003) Podosomes: adhesion hot-spots of invasive cells. *Trends Cell Biol* 13(7):376–385. [https://doi.org/10.1016/s0962-8924\(03\)00128-4](https://doi.org/10.1016/s0962-8924(03)00128-4)
- Liu N, Gao F, Han Z, Xu X, Underhill CB, Zhang L (2001) Hyaluronan synthase 3 overexpression promotes the growth of TSU prostate cancer cells. *Cancer Res* 61(13):5207–5214
- Liu YY, Lee CH, Dedaj R, Zhao H, Mrabat H, Sheidlin A, Syrkina O, Huang PM, Garg HG, Hales CA, Quinn DA (2008) High-molecular-weight hyaluronan—a possible new treatment for sepsis-induced lung injury: a preclinical study in mechanically ventilated rats. *Crit Care* 12: R102. <https://doi.org/10.1186/cc6982>
- Liu Y, Sun J, Cao W, Yang J, Lian H, Li X, Sun Y, Wang Y, Wang S, He Z (2011) Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery. *Int J Pharm* 421(1): 160–169. <https://doi.org/10.1016/j.ijpharm.2011.09.006>
- Lokeshwar VB, Fregien N, Bourguignon LY (1994) Ankyrin-binding domain of CD44(GP85) is required for the expression of hyaluronic acid-mediated adhesion function. *J Cell Biol* 126(4): 1099–1109. <https://doi.org/10.1083/jcb.126.4.1099>
- Lokeshwar BL, Lokeshwar VB, Block NL (1995) Expression of CD44 in prostate cancer cells: association with cell proliferation and invasive potential. *Anticancer Res* 15(4):1191–1198
- Lokeshwar VB, Iida N, Bourguignon LY (1996) The cell adhesion molecule, GP116, is a new CD44 variant (ex14/v10) involved in hyaluronic acid binding and endothelial cell proliferation. *J Biol Chem* 271(39):23853–23864. <https://doi.org/10.1074/jbc.271.39.23853>
- Lokeshwar VB, Estrella V, Lopez L, Kramer M, Gomez P, Soloway MS, Lokeshwar BL (2006) HYAL1-v1, an alternatively spliced variant of HYAL1 hyaluronidase: a negative regulator of bladder cancer. *Cancer Res* 66(23):11219–11227. <https://doi.org/10.1158/0008-5472.CAN-06-1121>
- Lokeshwar VB, Lopez LE, Munoz D, Chi A, Shiroadkar SP, Lokeshwar SD, Escudero DO, Dhir N, Altman N (2010) Antitumor activity of hyaluronic acid synthesis inhibitor 4-methylumbelliferone in prostate cancer cells. *Cancer Res* 70(7):2613–2623. <https://doi.org/10.1158/0008-5472.CAN-09-3185>
- Lokeshwar VB, Mirza S, Jordan A (2014) Targeting hyaluronic acid family for cancer chemoprevention and therapy. *Adv Cancer Res* 123:35–65. <https://doi.org/10.1016/B978-0-12-800092-2.00002-2>
- Lord MS, Day AJ, Youssef P, Zhuo L, Watanabe H, Caterson B, Whitelock JM (2013) Sulfation of the bikunin chondroitin sulfate chain determines heavy chain hyaluronan complex formation. *J Biol Chem* 288(32):22930–22941. <https://doi.org/10.1074/jbc.M112.404186>
- Lu J, Liong M, Li Z, Zink JJ, Tamanoi F (2010) Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals. *Small* 6(16):1794–1805. <https://doi.org/10.1002/smll.201000538>
- Lundell BI, McCarthy JB, Kovach NL, Verfaillie CM (1997) Activation of beta1 integrins on CML progenitors reveals cooperation between beta1 integrins and CD44 in the regulation of adhesion and proliferation. *Leukemia* 11(6):822–829. <https://doi.org/10.1038/sj.leu.2400653>
- Luo Y, Prestwich GD (1999) Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate. *Bioconjug Chem* 10(5):755–763. <https://doi.org/10.1021/bc9900338>
- Luo Y, Kirker KR, Prestwich GD (2000a) Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery. *J Control Release* 69(1):169–184. [https://doi.org/10.1016/s0168-3659\(00\)00300-x](https://doi.org/10.1016/s0168-3659(00)00300-x)
- Luo Y, Ziebell MR, Prestwich GD (2000b) A hyaluronic acid-taxol antitumor bioconjugate targeted to cancer cells. *Biomacromolecules* 1(2):208–218. <https://doi.org/10.1021/bm000283n>
- Marhaba R, Zoller M (2004) CD44 in cancer progression: adhesion, migration and growth regulation. *J Mol Histol* 35:211–231. <https://doi.org/10.1023/b:hijo.0000032354.94213.69>
- Mattheolabakis G, Milane L, Singh A, Amiji MM (2015) Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. *J Drug Target* 23(7–8):605–618. <https://doi.org/10.3109/1061186X.2015.1052072>
- McDonald T, Gerrity RG, Jen C, Chen HJ, Wark K, Wight TN, Chait A, O'Brien KD (2007) Diabetes and arterial extracellular matrix changes in a porcine model of atherosclerosis. *J Histochem Cytochem* 55(11):1149–1157. <https://doi.org/10.1369/jhc.7A7221.2007>

- Merrilees MJ, Beaumont BW, Braun KR, Thomas AC, Kang I, Hinek A, Passi A, Wight TN (2011) Neointima formed by arterial smooth muscle cells expressing versican variant V3 is resistant to lipid and macrophage accumulation. *Arterioscler Thromb Vasc Biol* 31(6):1309–1316. <https://doi.org/10.1161/ATVBAHA.111.225573>
- Meyer K, Palmer JW (1934) The polysaccharide of the vitreous humor. *J Biol Chem* 107(3):75338–75336
- Michalczyk M, Humeniuk E, Adamczuk G, Korga-Plewko A (2022) Hyaluronic acid as a modern approach in anticancer therapy-review. *Int J Mol Sci* 24(1):103. <https://doi.org/10.3390/ijms24010103>
- Mielgo A, van Driel M, Bloem A, Landmann L, Günthert U (2006) A novel antiapoptotic mechanism based on interference of Fas signaling by CD44 variant isoforms. *Cell Death Differ* 13(3):465–477. <https://doi.org/10.1038/sj.cdd.4401763>
- Misra S, Heldin P, Hascall VC, Karamanos NK, Skandalis SS, Markwald RR, Ghatak S (2011) Hyaluronan-CD44 interactions as potential targets for cancer therapy. *FEBS J* 278(9):1429–1443. <https://doi.org/10.1111/j.1742-4658.2011.08071.x>
- Misra S, Hascall VC, Markwald RR, Ghatak S (2015) Interactions between hyaluronan and its receptors (CD44, RHAMM) regulate the activities of inflammation and cancer. *Front Immunol* 6:201. <https://doi.org/10.3389/fimmu.2015.00201>
- Mizera-Nyczak E, Dyszkiewicz W, Heider KH, Zeromski J (2001) Isoform expression of CD44 adhesion molecules, Bcl-2, p53 and Ki-67 proteins in lung cancer. *Tumour Biol* 22(1):45–53. <https://doi.org/10.1159/000030154>
- Monslow J, Govindaraju P, Puré E (2015) Hyaluronan—a functional and structural sweet spot in the tissue microenvironment. *Front Immunol* 6:231. <https://doi.org/10.3389/fimmu.2015.00231>
- Montagner IM, Banzato A, Zuccolotto G, Renier D, Campisi M, Bassi P, Zanovello P, Rosato A (2013) Paclitaxel-hyaluronan hydrosoluble bioconjugate: MECHANISM of action in human bladder cancer cell lines. *Urol Oncol* 31(7):1261–1269. <https://doi.org/10.1016/j.urolonc.2012.01.005>
- Motolese A, Vignati F, Brambilla R, Cerati M, Passi A (2013) Interaction between a regenerative matrix and wound bed in nonhealing ulcers: results with 16 cases. *Biomed Res Int* 2013:849321. <https://doi.org/10.1155/2013/849321>
- Mueller BM, Schraufstatter IU, Goncharova V, Povaliy T, DiScipio R, Khaldoyanidi SK (2010) Hyaluronan inhibits postchemotherapy tumor regrowth in a colon carcinoma xenograft model. *Mol Cancer Ther* 9(11):3024–3032. <https://doi.org/10.1158/1535-7163.MCT-10-0529>
- Nagase H, Kudo D, Suto A, Yoshida E, Suto S, Negishi M, Kakizaki I, Hakamada K (2017) 4-Methylumbelliferone suppresses hyaluronan synthesis and tumor progression in SCID mice intra-abdominally inoculated with pancreatic cancer cells. *Pancreas* 46(2):190–197. <https://doi.org/10.1097/MPA.0000000000000741>
- Nam K, Oh S, Lee KM, Yoo SA, Shin I (2015) CD44 regulates cell proliferation, migration, and invasion via modulation of c-Src transcription in human breast cancer cells. *Cell Signal* 27(9):1882–1894. <https://doi.org/10.1016/j.cellsig.2015.05.002>
- Necas JBLBP, Bartosikova L, Brauner P, Kolar JJVM (2008) Hyaluronic acid (hyaluronan): a review. *Vet Med* 53(8):397–411. <https://doi.org/10.17221/1930-VETMED>
- Nikitovic D, Tzardi M, Berdiaki A, Tsatsakis A, Tzanakakis GN (2015) Cancer microenvironment and inflammation: role of hyaluronan. *Front Immunol* 6:169. <https://doi.org/10.3389/fimmu.2015.00169>
- Okamoto I, Kawano Y, Murakami D, Sasayama T, Araki N, Miki T, Wong AJ, Saya H (2001) Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. *J Cell Biol* 155(5):755–762. <https://doi.org/10.1083/jcb.200108159>
- Orian-Rousseau V, Sleeman J (2014) CD44 is a multidomain signaling platform that integrates extracellular matrix cues with growth factor and cytokine signals. *Adv Cancer Res* 123:231–254. <https://doi.org/10.1016/B978-0-12-800092-2.00009-5>

- Ouhitit A, Rizeq B, Saleh HA, Rahman MM, Zayed H (2018) Novel CD44-downstream signaling pathways mediating breast tumor invasion. *Int J Biol Sci* 14(13):1782–1790. <https://doi.org/10.7150/ijbs.23586>
- Park YS, Huh JW, Lee JH, Kim HR (2012) shRNA against CD44 inhibits cell proliferation, invasion and migration, and promotes apoptosis of colon carcinoma cells. *Oncol Rep* 27(2): 339–346. <https://doi.org/10.3892/or.2011.1532>
- Peer D, Margalit R (2004a) Loading mitomycin C inside long circulating hyaluronan targeted nanoliposomes increases its antitumor activity in three mice tumor models. *Int J Cancer* 108(5): 780–789. <https://doi.org/10.1002/ijc.11615>
- Peer D, Margalit R (2004b) Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal doxorubicin in syngeneic and human xenograft mouse tumor models. *Neoplasia* 6(4):343–353. <https://doi.org/10.1593/neo.03460>
- Pellegrino T, Kuder S, Liedl T, Muñoz Javier A, Manna L, Parak WJ (2005) On the development of colloidal nanoparticles towards multifunctional structures and their possible use for biological applications. *Small* 1(1):48–63. <https://doi.org/10.1002/smll.200400071>
- Petrey AC, de la Motte CA (2014) Hyaluronan, a crucial regulator of inflammation. *Front Immunol* 5:101. <https://doi.org/10.3389/fimmu.2014.00101>
- Pirinen R, Hirvikoski P, Böhm J, Kellokoski J, Moisio K, Viren M, Johansson R, Hollmén S, Kosma VM (2000) Reduced expression of CD44v3 variant isoform is associated with unfavorable outcome in non-small cell lung carcinoma. *Hum Pathol* 31(9):1088–1095. <https://doi.org/10.1053/hupa.2000.16277>
- Ponta H, Sherman L, Herrlich PA (2003) CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 4(1):33–45. <https://doi.org/10.1038/nrm1004>
- Prehm P (1984) Hyaluronate is synthesized at plasma membranes. *Biochem J* 220(2):597–600. <https://doi.org/10.1042/bj2200597>
- Prestwich GD, Marecak DM, Marecek JF, Vercruysse KP, Ziebell MR (1998) Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives. *J Control Release* 53(1–3):93–103. [https://doi.org/10.1016/s0168-3659\(97\)00242-3](https://doi.org/10.1016/s0168-3659(97)00242-3)
- Price ZK, Lokman NA, Ricciardelli C (2018) Differing roles of hyaluronan molecular weight on cancer cell behavior and chemotherapy resistance. *Cancers* 10(12):482. <https://doi.org/10.3390/cancers10120482>
- Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR (2012) Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 21(3):418–429. <https://doi.org/10.1016/j.ccr.2012.01.007>
- Puré E, Assoian RK (2009) Rheostatic signaling by CD44 and hyaluronan. *Cell Signal* 21(5): 651–655. <https://doi.org/10.1016/j.cellsig.2009.01.024>
- Qiu L, Li Z, Qiao M, Long M, Wang M, Zhang X, Tian C, Chen D (2014) Self-assembled pH-responsive hyaluronic acid-g-poly((L)-histidine) copolymer micelles for targeted intracellular delivery of doxorubicin. *Acta Biomater* 10(5):2024–2035. <https://doi.org/10.1016/j.actbio.2013.12.025>
- Rall CJ, Rustgi AK (1995) CD44 isoform expression in primary and metastatic pancreatic adenocarcinoma. *Cancer Res* 55:1831–1835
- Ropponen K, Tammi M, Parkkinen J, Eskelinen M, Tammi R, Lipponen P, Agren U, Alhava E, Kosma VM (1998) Tumor cell-associated hyaluronan as an unfavorable prognostic factor in colorectal cancer. *Cancer Res* 58(2):342–347
- Rosato A, Banzato A, De Luca G, Renier D, Bettella F, Pagano C, Esposito G, Zanovello P, Bassi P (2006) HYTAD1-p20: a new paclitaxel-hyaluronic acid hydrosoluble bioconjugate for treatment of superficial bladder cancer. *Urol Oncol* 24(3):207–215. <https://doi.org/10.1016/j.urolonc.2005.08.020>
- Rosenthal MA, Gibbs P, Brown TJ, Wong S, Uren S, Ellis A, Li L, Helden P, Fox RM (2005) Phase I and pharmacokinetic evaluation of intravenous hyaluronic acid in combination with doxorubicin or 5-fluorouracil. *Chemotherapy* 51(2–3):132–141. <https://doi.org/10.1159/000085621>

- Rudzki Z, Jothy S (1997) CD44 and the adhesion of neoplastic cells. *Mol Pathol* 50(2):57–71. <https://doi.org/10.1136/mp.50.2.57>
- Ruhela D, Riviere K, Szoka FC (2006) Efficient synthesis of an aldehyde functionalized hyaluronic acid and its application in the preparation of hyaluronan-lipid conjugates. *Bioconjug Chem* 17(5):1360–1363. <https://doi.org/10.1021/bc0600721>
- Sato N, Kohi S, Hirata K, Goggins M (2016) Role of hyaluronan in pancreatic cancer biology and therapy: once again in the spotlight. *Cancer Sci* 107(5):569–575. <https://doi.org/10.1111/cas.12913>
- Schulz T, Schumacher U, Prehm P (2007) Hyaluronan export by the ABC transporter MRP5 and its modulation by intracellular cGMP. *J Biol Chem* 282(29):20999–21004. <https://doi.org/10.1074/jbc.M700915200>
- Senbanjo LT, Chellaiah MA (2017) CD44: a multifunctional cell surface adhesion receptor is a regulator of progression and metastasis of cancer cells. *Front Cell Dev Biol* 5:18. <https://doi.org/10.3389/fcell.2017.00018>
- Serafino A, Zonfrillo M, Andreola F, Psaila R, Mercuri L, Moroni N, Renier D, Campisi M, Secchieri C, Pierimarchi P (2011) CD44-targeting for antitumor drug delivery: a new SN-38-hyaluronan bioconjugate for locoregional treatment of peritoneal carcinomatosis. *Curr Cancer Drug Targets* 11(5):572–585. <https://doi.org/10.2174/156800911795655976>
- Setälä LP, Tammi MI, Tammi RH, Eskelinen MJ, Lipponen PK, Agren UM, Parkkinen J, Alhava EM, Kosma VM (1999) Hyaluronan expression in gastric cancer cells is associated with local and nodal spread and reduced survival rate. *Br J Cancer* 79(7):1133–1138. <https://doi.org/10.1038/sj.bjc.6690180>
- Singleton PA, Bourguignon LY (2004) CD44 interaction with ankyrin and IP3 receptor in lipid rafts promotes hyaluronan-mediated Ca²⁺ signaling leading to nitric oxide production and endothelial cell adhesion and proliferation. *Exp Cell Res* 295(1):102–118. <https://doi.org/10.1016/j.yexcr.2003.12.025>
- Skandalis SS, Kozlova I, Engstrom U, Hellman U, Heldin P (2010) Proteomic identification of CD44 interacting proteins. *IUBMB Life* 62(11):833–840. <https://doi.org/10.1002/iub.392>
- Slomiany MG, Dai L, Tolliver LB, Grass GD, Zeng Y, Toole BP (2009) Inhibition of functional hyaluronan-CD44 interactions in CD133-positive primary human ovarian carcinoma cells by small hyaluronan oligosaccharides. *Clin Cancer Res* 15(24):7593–7601. <https://doi.org/10.1158/1078-0432.CCR-09-2317>
- Souness A, Zamboni F, Walker GM, Collins MN (2018) Influence of scaffold design on 3D printed cell constructs. *J Biomed Mater Res B Appl Biomater* 106(2):533–545. <https://doi.org/10.1002/jbm.b.33863>
- Stern R, Jedrzejewski MJ (2006) Hyaluronidases: their genomics, structures, and mechanisms of action. *Chem Rev* 106(3):818–839. <https://doi.org/10.1021/cr050247k>
- Stern R, Asari AA, Sugahara KN (2006) Hyaluronan fragments: an information-rich system. *Eur J Cell Biol* 85(8):699–715. <https://doi.org/10.1016/j.ejcb.2006.05.009>
- Stober VP, Johnson CG, Majors A, Lauer ME, Cali V, Midura RJ, Wisniewski HG, Aronica MA, Garantziotis S (2017) TNF-stimulated gene 6 promotes formation of hyaluronan-inter- α -inhibitor heavy chain complexes necessary for ozone-induced airway hyperresponsiveness. *J Biol Chem* 292(51):20845–20858. <https://doi.org/10.1074/jbc.M116.756627>
- Surace C, Arpicco S, Dufay-Wojcicki A, Marsaud V, Bouclier C, Clay D, Cattel L, Renoir JM, Fattal E (2009) Lipoplexes targeting the CD44 hyaluronic acid receptor for efficient transfection of breast cancer cells. *Mol Pharm* 6(4):1062–1073. <https://doi.org/10.1021/mp800215d>
- Swaidani S, Cheng G, Lauer ME, Sharma M, Mikecz K, Hascall VC, Aronica MA (2013) TSG-6 protein is crucial for the development of pulmonary hyaluronan deposition, eosinophilia, and airway hyperresponsiveness in a murine model of asthma. *J Biol Chem* 288(1):412–422. <https://doi.org/10.1074/jbc.M112.389874>
- Tammi RH, Kultti A, Kosma VM, Pirinen R, Auvinen P, Tammi MI (2008) Hyaluronan in human tumors: Pathobiological and prognostic messages from cell-associated and stromal hyaluronan. *Semin Cancer Biol* 18(4):288–295. <https://doi.org/10.1016/j.semcancer.2008.03.005>

- Tenne R (2006) Inorganic nanotubes and fullerene-like nanoparticles. *Nat Nanotechnol* 1(2): 103–111. <https://doi.org/10.1038/nnano.2006.62>
- Tian X, Azpurua J, Hine C, Vaidya A, Myakishev-Rempel M, Ablaeva J, Mao Z, Nevo E, Gorbunova V, Seluanov A (2013) High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* 499(7458):346–349. <https://doi.org/10.1038/nature12234>
- Tolg C, Hamilton SR, Nakrieko KA, Kooshesh F, Walton P, McCarthy JB, Bissell MJ, Turley EA (2006) Rhamm—/— fibroblasts are defective in CD44-mediated ERK1,2 mitogenic signaling, leading to defective skin wound repair. *J Cell Biol* 175:1017–1028. <https://doi.org/10.1083/jcb.200511027>
- Tolg C, Telmer P, Turley E (2014) Specific sizes of hyaluronan oligosaccharides stimulate fibroblast migration and excisional wound repair. *PLoS One* 9(2):e88479. <https://doi.org/10.1371/journal.pone.0088479>
- Toole BP (2004) Hyaluronan: from extracellular glue to pericellular cue. *Nat Rev Cancer* 4(7): 528–539. <https://doi.org/10.1038/nrc1391>
- Toole BP, Slomiany MG (2008) Hyaluronan, CD44 and emmprin: partners in cancer cell chemoresistance. *Drug Resist Updat* 11(3):110–121. <https://doi.org/10.1016/j.drup.2008.04.002>
- Underhill C (1992) CD44: the hyaluronan receptor. *J Cell Sci* 103(2):293–298. <https://doi.org/10.1242/jcs.103.2.293>
- Urakawa H, Nishida Y, Knudson W, Knudson CB, Arai E, Kozawa E, Futamura N, Wasa J, Ishiguro N (2012a) Therapeutic potential of hyaluronan oligosaccharides for bone metastasis of breast cancer. *J Orthop Res* 30(4):662–672. <https://doi.org/10.1002/jor.21557>
- Urakawa H, Nishida Y, Wasa J, Arai E, Zhuo L, Kimata K, Kozawa E, Futamura N, Ishiguro N (2012b) Inhibition of hyaluronan synthesis in breast cancer cells by 4-methylumbelliferone suppresses tumorigenicity in vitro and metastatic lesions of bone *in vivo*. *Int J Cancer* 130(2): 454–466. <https://doi.org/10.1002/ijc.26014>
- Vigetti D, Passi A (2014) Hyaluronan synthases posttranslational regulation in cancer. *Adv Cancer Res* 123:95–119. <https://doi.org/10.1016/B978-0-12-800092-2.00004-6>
- Vigetti D, Viola M, Gornati R, Ori M, Nardi I, Passi A, De Luca G, Bernardini G (2003) Molecular cloning, genomic organization and developmental expression of the *Xenopus laevis* hyaluronan synthase 3. *Matrix Biol* 22(6):511–517. [https://doi.org/10.1016/s0945-053x\(03\)00082-9](https://doi.org/10.1016/s0945-053x(03)00082-9)
- Vigetti D, Rizzi M, Viola M, Karousou E, Genasetti A, Clerici M, Bartolini B, Hascall VC, De Luca G, Passi A (2009) The effects of 4-methylumbelliferone on hyaluronan synthesis, MMP2 activity, proliferation, and motility of human aortic smooth muscle cells. *Glycobiology* 19(5): 537–546. <https://doi.org/10.1093/glycob/cwp022>
- Vigetti D, Genasetti A, Karousou E, Viola M, Moretto P, Clerici M, Deleonibus S, De Luca G, Hascall VC, Passi A (2010) Proinflammatory cytokines induce hyaluronan synthesis and monocyte adhesion in human endothelial cells through hyaluronan synthase 2 (HAS2) and the nuclear factor-kappaB (NF-kappaB) pathway. *J Biol Chem* 285(32):24639–24645. <https://doi.org/10.1074/jbc.M110.134536>
- Vigetti D, Rizzi M, Moretto P, Deleonibus S, Dreyfuss JM, Karousou E, Viola M, Clerici M, Hascall VC, Ramoni MF, De Luca G, Passi A (2011) Glycosaminoglycans and glucose prevent apoptosis in 4-methylumbelliferone-treated human aortic smooth muscle cells. *J Biol Chem* 286(40):34497–34503. <https://doi.org/10.1074/jbc.M111.266312>
- Vigetti D, Viola M, Karousou E, De Luca G, Passi A (2014) Metabolic control of hyaluronan synthases. *Matrix Biol* 35:8–13. <https://doi.org/10.1016/j.matbio.2013.10.002>
- Viola M, Vigetti D, Karousou E, D'Angelo ML, Caon I, Moretto P, De Luca G, Passi A (2015) Biology and biotechnology of hyaluronan. *Glycoconj J* 32:93–103. <https://doi.org/10.1007/s10719-015-9586-6>
- Wang SJ, Bourguignon LY (2006a) Hyaluronan and the interaction between CD44 and epidermal growth factor receptor in oncogenic signaling and chemotherapy resistance in head and neck cancer. *Archiv Otolaryngol Head Neck Surg* 132(7):771–778. <https://doi.org/10.1001/archotol.132.7.771>

- Wang SJ, Bourguignon LY (2006b) Hyaluronan-CD44 promotes phospholipase C-mediated Ca²⁺ signaling and cisplatin resistance in head and neck cancer. *Archiv Otolaryngol Head Neck Surg* 132(1):19–24. <https://doi.org/10.1001/archotol.132.1.19>
- Wang SJ, Bourguignon LY (2011) Role of hyaluronan-mediated CD44 signaling in head and neck squamous cell carcinoma progression and chemoresistance. *Am J Pathol* 178(3):956–963. <https://doi.org/10.1016/j.ajpath.2010.11.077>
- Wang A, Hascall VC (2009) Hyperglycemia, intracellular hyaluronan synthesis, cyclin D3 and autophagy. *Autophagy* 5(6):864–865. <https://doi.org/10.4161/auto.9041>
- Wang SJ, Wong G, de Heer AM, Xia W, Bourguignon LY (2009) CD44 variant isoforms in head and neck squamous cell carcinoma progression. *Laryngoscope* 119(8):1518–1530. <https://doi.org/10.1002/lary.20506>
- Weissmann B, Meyer K (1954) The structure of hyalobiuronic acid and of hyaluronic acid from umbilical cord. *J Am Chem Soc* 76(7):1753–1757. <https://doi.org/10.1021/ja01636a010>
- Williams K, Motiani K, Giridhar PV, Kasper S (2013) CD44 integrates signaling in normal stem cell, cancer stem cell and (pre)metastatic niches. *Exp Biol Med* 238(3):324–338. <https://doi.org/10.1177/1535370213480714>
- Wu M, Cao M, He Y, Liu Y, Yang C, Du Y, Wang W, Gao F (2015) A novel role of low molecular weight hyaluronan in breast cancer metastasis. *FASEB J* 29(4):1290–1298. <https://doi.org/10.1096/fj.14-259978>
- Xu X, Jha AK, Harrington DA, Farach-Carson MC, Jia X (2012) Hyaluronic acid-based hydrogels: from a natural polysaccharide to complex networks. *Soft Matter* 8(12):3280–3294. <https://doi.org/10.1039/C2SM06463D>
- Xu H, Wu K, Tian Y, Liu Q, Han N, Yuan X, Zhang L, Wu GS, Wu K (2016) CD44 correlates with clinicopathological characteristics and is upregulated by EGFR in breast cancer. *Int J Oncol* 49(4):1343–1350
- Yasuda M, Tanaka Y, Fujii K, Yasumoto K (2001) CD44 stimulation down-regulates Fas expression and Fas-mediated apoptosis of lung cancer cells. *Int Immunol* 13(10):1309–1319. <https://doi.org/10.1093/intimm/13.10.1309>
- Yu Q, Stamenkovic I (2004) Transforming growth factor-beta facilitates breast carcinoma metastasis by promoting tumor cell survival. *Clin Exp Metastasis* 21:235–242. <https://doi.org/10.1023/b:clin.0000037705.25256.d3>
- Zamboni F, Keays M, Hayes S, Albadarin AB, Walker GM, Kiely PA, Collins MN (2017) Enhanced cell viability in hyaluronic acid coated poly(lactic-co-glycolic acid) porous scaffolds within microfluidic channels. *Int J Pharm* 532(1):595–602. <https://doi.org/10.1016/j.ijpharm.2017.09.053>
- Zhang Y, Zhang H, Wang X, Wang J, Zhang X, Zhang Q (2012) The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles. *Biomaterials* 33(2):679–691. <https://doi.org/10.1016/j.biomaterials.2011.09.072>
- Zhang M, Xu C, Wen L, Han MK, Xiao B, Zhou J, Zhang Y, Zhang Z, Viennois E, Merlin D (2016) A hyaluronidase-responsive nanoparticle-based drug delivery system for targeting colon cancer cells. *Cancer Res* 76(24):7208–7218. <https://doi.org/10.1158/0008-5472.CAN-16-1681>
- Zhu D, Bourguignon LY (1998) The ankyrin-binding domain of CD44s is involved in regulating hyaluronic acid-mediated functions and prostate tumor cell transformation. *Cell Motil Cytoskeleton* 39(3):209–222. [https://doi.org/10.1002/\(SICI\)1097-0169\(1998\)39:33.O.CO](https://doi.org/10.1002/(SICI)1097-0169(1998)39:33.O.CO)
- Zhu D, Bourguignon LY (2000) Interaction between CD44 and the repeat domain of ankyrin promotes hyaluronic acid-mediated ovarian tumor cell migration. *J Cell Physiol* 183(2):182–195. [https://doi.org/10.1002/\(SICI\)1097-4652\(200005\)183:2<182::AID-JCP5>3.0.CO;2-O](https://doi.org/10.1002/(SICI)1097-4652(200005)183:2<182::AID-JCP5>3.0.CO;2-O)

Molecular Markers in Circulating Tumour Cells: Advancing Clinical Precision

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Abstract

Circulating tumour cells (CTCs) represent a unique and dynamic aspect of cancer progression, offering valuable insights into diagnosis, prognosis, disease monitoring, drug selection, and treatment response assessment. While conventional methods rely on physical markers such as size and density to isolate CTCs, the journey of CTC detection has evolved. Early techniques involving cell lysis and mRNA expression have evolved into approaches that leverage surface markers, enabling the capture of intact CTCs. For instance, EpCAM (epithelial cell adhesion molecule) is a well-established marker expressed on CTCs, widely adopted in the FDA-approved CellSearch[®] system to detect and capture CTCs. Notably, CTCs are closely linked with the metastatic spread of cancer. Studying the phenotypes of CTCs, such as clustering, epithelial-to-mesenchymal

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transition, and stemness, enhances the understanding of their behaviours. This chapter aims to provide a comprehensive knowledge of CTC, underscoring its potential as a non-invasive cancer biomarker. We address the challenges in isolating the entire CTC population, the limited sensitivity of current detection and isolation approaches, as well as the ongoing efforts to unlock their full clinical potentials. Additionally, the promising role of CTCs in advancing precision medicine is highlighted, wherein identifying specific molecular characteristics could guide personalised treatment strategies in the future.

Keywords

Circulating tumour cells · Liquid biopsy · Molecular marker · Precision medicine

1 Introduction

Circulating tumour cells (CTCs) are rare cells that spread from primary tumour to secondary sites via the bloodstream (Ashworth 1869; Ferreira et al. 2016; Dive and Brady 2017; Micalizzi et al. 2017; Marcuello et al. 2019). CTCs can be detected in the blood, enabling their non-invasive collection through simple blood draws (Pantel and Speicher 2016). As a result, analysing CTCs can provide comprehensive diagnostic and predictive information directly from the blood, eliminating the need for invasive conventional biopsies (Silva et al. 2020). Furthermore, CTCs have the potential to offer direct insight into every stage of cancer development and act as the leading carriers for cancer spreading and the return of cancer after a period of improvement or remission (Freeman et al. 2015; Tsai et al. 2019).

Figure 1 illustrates the process by which cancer cells from the primary site (colon) are released into the bloodstream, leading to the formation of a new tumour at the secondary site (liver) (Tieng et al. 2020).

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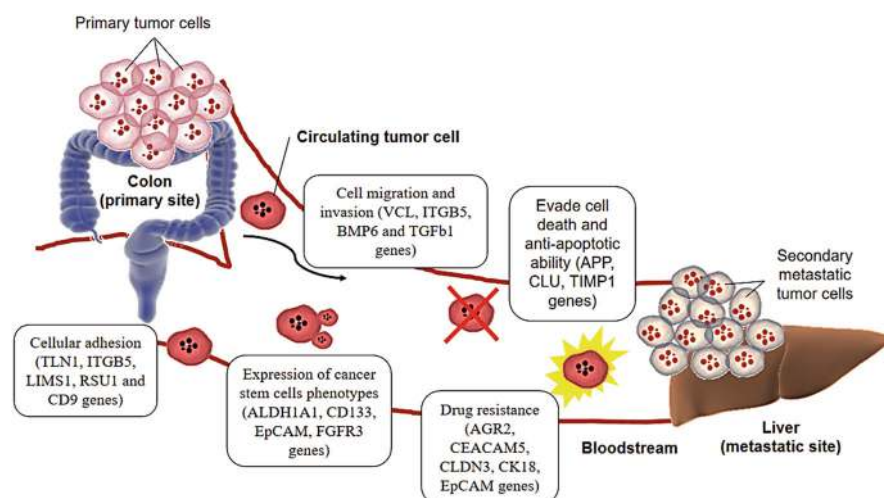


Fig. 1 The release of CTCs from the primary site (colon) and the mechanisms involved in establishing the secondary site (liver) in colorectal cancer. (Reproduced from Tieng et al. 2020)

2 Formation of Circulating Tumour Cells

As cancer metastasises, cells will detach from the primary tumour and enter the bloodstream, where the cells eventually transform into CTCs as they travel through the blood (Chang et al. 2000; Micalizzi et al. 2017). Most of the CTCs die off following the events, yet a few survive and initiate the formation of small clusters called micrometastases (Koyanagi et al. 2008; Oakman et al. 2010; Tamimi 2015), where only a fraction of these clusters develop into visible tumours (Molnar et al. 2008; de Wit et al. 2014), which eventually fuse together to form new tumour sites and release more CTCs into the bloodstream. At times, a tumour might recur at the original site, also known as local relapse, and CTCs have been reported to be associated with early metastatic relapse (Ferreira et al. 2016; They et al. 2019; Tieng et al. 2020).

The progression of secondary tumour formation is underpinned by two key concepts. The first concept, known as the ‘seed and soil’ hypothesis, was first proposed by Stephen Paget in 1889. According to this idea, CTCs act like seeds that can only grow into new tumours in organs that are suitable for the process, such as the liver or lungs (Paget 1989). The second theory is the ‘mechanistic theory’ by James Ewing, which suggests that the potential for secondary tumour formation depends on the outflow of blood from the original tumour (Ewing 1922).

Figure 2 shows the formation of primary and secondary CTCs during cancer development.

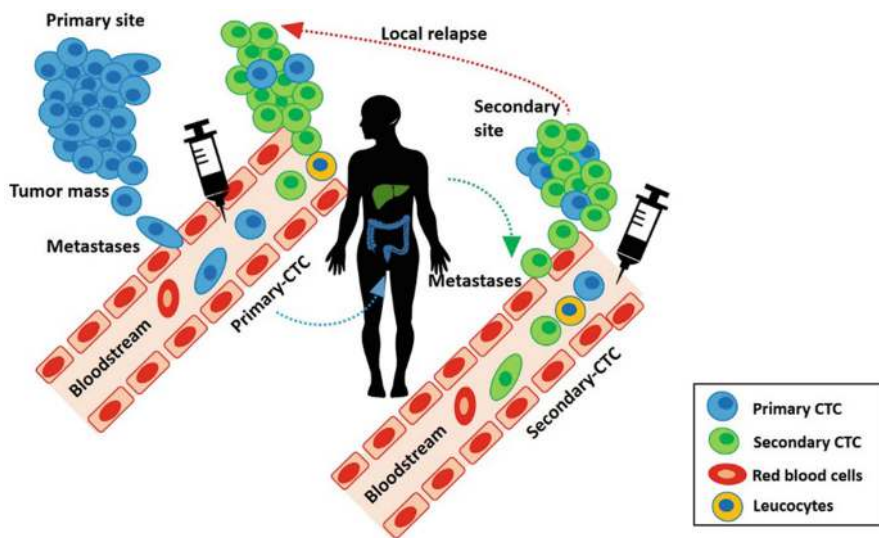


Fig. 2 Formation of primary and secondary CTCs. (Adopted from Tieng et al. 2021)

3 Early CTC Detection and Molecular Markers

The examination of CTCs has garnered interest due to their possession of both physical and genetic traits from the primary tumours, potentially serving as initiators of cancer spread (Lim et al. 2019). This makes CTCs a promising candidate for identifying cancer markers without the need for invasive procedures (Freeman et al. 2015). Following this, various molecular markers have been introduced to detect CTCs (Table 1). In the early attempts, a technique called reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was employed to measure the expression levels of messenger ribonucleic acid (mRNA) (Wong et al. 1997; Guadagni et al. 2001).

Briefly, whole blood collected from cancer patients undergoes a separation process by spinning at high speed (centrifugation), leading to the formation of different layers. The layer containing peripheral blood mononucleated cells (PBMCs) is collected, as it is presumed to contain CTCs, as proposed by Castells et al. (1998). Next, CTCs are isolated based on size or density, followed by subsequent steps involving cell lysis to obtain mRNA for quantification. However, analytical specificity is limited due to potential issues such as low CTC purity and the presence of haematological contamination, specifically white blood cells (Alix-Panabières and Pantel 2016; Merker et al. 2018; Stewart et al. 2018; Fiala and Diamandis 2019).

Table 1 Molecular markers available for CTCs

CTC technique	Molecular marker	Citation
<i>Early attempts of CTC detection via RNA expression</i>		
Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)	<i>CK-19/20, CEA, GCC, TEM-8, CD44</i>	Jonas et al. (1996), Wong et al. (1997), Soeth et al. (1997), Castells et al. (1998), Noh et al. (1999), Guadagni et al. (2001), Fava et al. (2001), Silva et al. (2002), Hampton et al. (2002), Katsumata et al. (2006), and Raeisossadati et al. (2011)
<i>CTC visualisation via molecular markers</i>		
Flow cytometry (FCM)	Epithelial: CK; Intracellular: CK7/20; Hematopoietic: CD3, CD8, CD45	Bahnassy et al. (2019) and Muşină et al. (2021)
Nucleic acid staining	Epithelial: <i>CK8/18/19</i> ; Hematopoietic: <i>CD45</i>	Denève et al. (2013)
RNA-immunocytochemistry (ICC)	Epithelial: <i>CK8/18/19, EPCAM</i> ; Mesenchymal: <i>VIM, PRL-3</i>	Yokobori et al. (2013) and Hinz et al. (2017)
RNA-immunofluorescence (IF)	Epithelial: <i>EPCAM, pan-CK</i> ; Metastasis/EMT: <i>CD44</i> ; Stem cell: <i>CD133</i>	Bahnassy et al. (2019) and Dizdar et al. (2019)
RNA-in situ hybridisation (ISH)	Epithelial: <i>EPCAM, CK8/18/19</i> ; Mesenchymal: <i>VIM, TWIST1, AKT2, SNAI1, LGR5</i> ; Hematopoietic: <i>CD45</i>	Wu et al. (2015, 2017), Zhao et al. (2017), and Wang et al. (2018)
EPISPOT assay (protein expression)	Epithelial: CK19	Denève et al. (2013)
Immunohistochemistry (IHC)	Mesenchymal: PRL-3	Su et al. (2020)
<i>CTC validation</i>		
RT-qPCR	Epithelial: <i>CEA, GA733-2 (EPCAM), CK20, MUC1, CK8/18/19</i> ; Mesenchymal/EMT: <i>PI3Kα, AKT2, TWIST1, survivin, CD44, VIL1, CLU, TIMP1, LOXL3, ZEB2</i> ; Stem cell: <i>ALDH1, CD133</i> ; Metastasis/EMT: <i>CD44</i> ; Chemoresistance: <i>KRAS, EGFR, HER2</i>	Raimondi et al. (2014), Ning et al. (2015, 2018), Hinz et al. (2017), and Bahnassy et al. (2019)

AKT2 RAC-beta serine/threonine-protein kinase, *ALDH1* aldehyde dehydrogenase 1, *CEA* carcinoembryonic antigen, *CD* cluster of differentiation, *CK* cytokeratin, *CLU* clusterin, *EGFR* epidermal growth factor receptor, *EMT* epithelial-mesenchymal transition, *EPCAM* gene encoding EpCAM protein, *EpCAM* epithelial cell adhesion molecule, *GCC* guanylyl cyclase C, *HER2* human epidermal growth factor receptor 2, *KRAS* Kirsten rat sarcoma virus, *LOXL3* lysyl oxidase-like 3, *LGR5* leucine-rich repeat-containing G-protein coupled receptor 5, *PRL-3* protein tyrosine phosphatase type IVA 3, *PI3Kα* phosphoinositide 3-kinase isoform alpha, *SNAI1* snail family transcriptional repressor 1, *TEM-8* tumour endothelial marker 8, *TIMP1* TIMP metalloproteinase inhibitor 1, *TWIST1* twist family bHLH transcription factor 1, *VIL1* villin-2, *VIM* vimentin, *ZEB3* zinc-finger E-box-binding homeobox 3

3.1 Evolution of Molecular-Based CTC Detection

The initial method for detecting CTCs involves breaking down cells with a high risk of contamination. Advances in imaging and sorting technologies have facilitated a transition towards membrane molecular-based CTC detection, which now allows for the isolation of intact CTCs. By employing techniques such as cytomorphology, flow cytometry, fluorescence in situ hybridisation (FISH), immunofluorescence (IF), or immunocytochemistry (ICC), it becomes possible to visually examine and quantify CTCs (Yokobori et al. 2013; Hinz et al. 2017; Dizdar et al. 2019).

Based on the molecular characteristics of cell membranes, this method forms the basis for current CTC detection systems, exploiting the unique cell surface markers expressed on CTCs without being confounded by other non-target cells such as white blood cells and red blood cells. In simple terms, specific markers on CTCs are stained, making them visible under a microscope. Next, images of the stained CTCs are captured, followed by enumeration of CTC counts. This process yields valuable information for cancer prognosis, deepening our understanding of the underlying mechanisms of cancer progression and metastasis.

Although CTCs have the potential to contribute to cancer diagnosis and prognosis, their isolation poses challenges due to low frequency of CTCs in blood (approximately 1 CTC per 10^7 to 10^9 blood cells/mL), short lifespan outside the human body, the absence of a single universal CTC-specific marker, and technical limitations related to low cell separation and recovery (Hayes and Smerage 2010; Miller et al. 2010; Krebs et al. 2010; Andree et al. 2016; van der Toom et al. 2016; Zou and Cui 2018).

4 CTC Detection and Isolation Technologies

The first stage in applying CTCs as cancer biomarkers involves the detection and capture of CTCs from blood samples, as outlined by Tian et al. (2020). Generally, methods for CTC enrichment are based on the physical and biological properties of the targeted cells (Shen et al. 2017).

4.1 CTC Isolation via Physical Markers

Physical markers provide a dual advantage by distinguishing between CTCs and non-targeted cells, while also playing a crucial role in generating forces for effective cell separation. The identified physical characteristics serving as markers for CTC isolation encompass cell size, density, deformability, or electrical properties (Williams et al. 2012).

Separating CTCs from PBMCs can be challenging due to their similar size and density. For instance, the technique called isolation-by-size-of-epithelial-tumour-cells (ISET), which uses an 8- μ m diameter membrane, has a low capture efficiency as observed by Vona et al. (2000). Despite efforts to enhance its capture efficiency,

the inconsistency in CTC counts between different experiments limit its widespread use (Harouaka et al. 2014).

Ficoll is another example of a method that relies on density to separate cells. However, it has low specificity and cannot effectively distinguish between different cell types (Rosenberg et al. 2002). An improved version of this method called APOSTREAM has been developed, where a special technique called dielectric electrophoresis within a microfluidic chamber has been utilised to enhance the capture of CTCs (Gupta et al. 2012).

The development of microfluidics and microchip technologies has facilitated the isolation of CTCs without the requirement for molecular markers. One approach involves cells adhering to specific surfaces (Kobayashi et al. 2022), whereas another employs specialised systems to enhance CTC capture efficiency (Feng et al. 2020b).

While physical property-based methods for CTC isolation may appear straightforward, they often require larger sample volumes, tend to be inefficient, yield impure results, and lack specificity. Besides, the recovery efficiency is also limited due to concerns such as filtration resistance, the formation of CTC aggregates, and membrane clogging (Gabriel et al. 2016; Habli et al. 2020).

4.2 CTC Isolation via Molecular Markers

The isolation of CTCs typically involves the use of antibody-antigen interaction, known as immunoaffinity (Bankó et al. 2019; Vafaei et al. 2020). This strategy is further categorised into two types: positive and negative enrichment strategies, which rely on the presence of specific molecular markers on the targeted cells. Positive enrichment aims to capture CTCs expressing specific molecular markers, while negative enrichment involves binding unwanted cells by markers on their surface, and subsequently eluting CTCs. The selective isolation of CTCs is highly dependent on these molecular markers, which contribute to the accuracy of the process.

4.2.1 Positive Enrichment via CTC Molecular Markers

Currently, the epithelial cell adhesion molecule (EpCAM) stands out as the most well-known molecular marker for the positive enrichment of CTCs. The CellSearch[®] system, developed by Veridex, is the first and only technique approved by the United States Food and Drug Administration (FDA) that is specifically designed to detect, capture, and enumerate CTCs in patients with metastatic breast, colorectal, or prostate cancer (Kowalik et al. 2017; Mamdouhi et al. 2019). The system identifies CTCs in whole blood by detecting molecular markers of epithelial origin, such as EpCAM⁺, and cytokeratins 8⁺, 18⁺, and/or 19⁺. Once these markers are identified, the CTCs are quantified, aiding in the assessment of prognosis and survival (Cristofanilli et al. 2004; Cohen et al. 2008, 2009; Millner et al. 2013; Rothé et al. 2019). In a nutshell, the CellSearch[®] system is designed to identify specific molecular markers expressed by CTCs, and the cells are then counted to provide insight into prognosis.

Following this, many systems utilising EpCAM have been developed to capture CTCs in the blood. One example is the CellMax CTC detection assay produced by CellMax Life, which can accurately isolate CTCs using an EpCAM-coated microfluidic chip with a minimum of 80% sensitivity, specificity, and recovery rate (Gupta et al. 2019; Tsai et al. 2019). The IsoFlux method (Fluxion Biosciences) is another approach that utilises magnetic beads coated with EpCAM to capture CTCs, as described by Toh and colleagues (2020). Magnetic-activated cell sorting (MACS), on the other hand, involves the use of magnetic nanoparticles to label EpCAM-positive CTCs, which are then separated from other cells using a magnetic field (Feng et al. 2020a).

Using a positive enrichment method that specifically targets EpCAM has proven to be an effective means of distinguishing CTCs from normal blood cells (Mamdouhi et al. 2019). However, this method has a limitation as it tends to selectively capture certain types of CTCs that express EpCAM exclusively (Eslami-S et al. 2020). Consequently, it might overlook the detection of other CTCs that have different markers since CTCs can change their characteristics during their journey through the bloodstream by transitioning between epithelial and mesenchymal states (Alix-Panabières et al. 2017; Dongre and Weinberg 2019).

The illustration presented in Fig. 3 depicts the process of CTCs transitioning between epithelial and mesenchymal states. This process plays a significant role in the cancer progression, recurrence of cancer, and metastasis (Thompson and Haviv 2011).

Moreover, incorporating multiple markers to capture various types of CTCs is not a straightforward task. The markers related to these changes are found either within the cell (cytoplasmic) or in the nucleus (Barbazán et al. 2014; Zhao et al. 2017; Wang et al. 2018). Simply put, the positive enrichment approach may not effectively

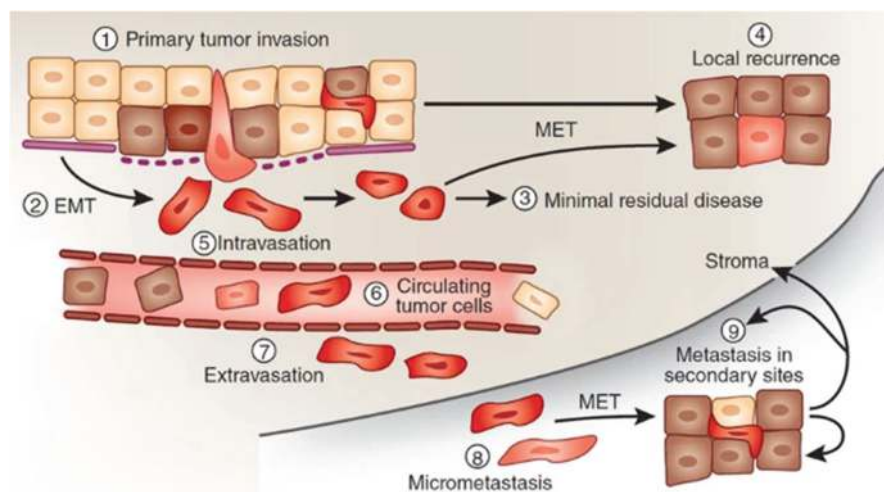


Fig. 3 Process of epithelial-to-mesenchymal transition in CTCs during cancer progression that leads to cancer progression and metastasis. (Reproduced from Thompson and Haviv 2011)

detect all types of CTCs, especially those undergoing epithelial-to-mesenchymal transition (EMT).

4.2.2 Negative Enrichment via CTC Molecular Markers

Negative enrichment is introduced to overcome the limitation of positive enrichment. This particular approach involves the capturing of blood cells by targeting on the markers that present on their surfaces, resulting in the elution of CTCs as explained in the study by Bankó et al. (2019). With negative enrichment, all forms of CTCs can be extracted as they do not depend on the molecular markers that are found on the CTC membrane (Kang et al. 2019).

Generally, all negative enrichment techniques share a common principle regardless of the method, including CellSearch[®] system (Veridex) (Bahnassy et al. 2019), Cytel method (Wang et al. 2019), RosetteSep[™] system (StemCell Technologies) (Soler et al. 2017), subtraction enrichment, and immunostaining-fluorescence in situ hybridisation (SE-iFISH) (Cytelligen) (Huang et al. 2020) or EasySep[™] (StemCell Technologies) (Hamid et al. 2020b). These methods all involve the removal of white blood cells using the lymphocyte common antigen (CD45) as the primary marker. CD45 is a surface marker commonly present on all nucleated hematopoietic cells, especially white blood cells. After that, specific molecular markers are employed to validate the presence of captured CTCs.

Detecting CTCs in the presence of contaminating background cells poses a challenge with the negative enrichment approach, leading to low purity. Additionally, this method is associated with a significant loss of CTCs, resulting in lower specificity compared to positive enrichment methods.

In an attempt to tackle this issue, Soler and his colleagues made an effort to modify the RosetteSep[™] System by incorporating multiple CTC surface markers, including CD2, CD16, CD19, CD36, CD38, CD45, CD66b, and glycophorin A. Their primary goal was to enhance the recovery of various CTC types while also ensuring high viability by using a combination of these markers in the negative enrichment process. However, it is crucial to note that such cases are very limited (Wilson et al. 2020).

The techniques for enriching CTCs that utilise molecular markers for both detection and validation tests are summarised in Table 2.

5 Clinical Applications of CTCs

Following advancements in CTC identification and isolation techniques, there have been numerous studies investigating the role of CTCs as biomarkers in cancers, including breast (Poonia et al. 2023), lung (Ruan et al. 2020), colorectal (Kozuka et al. 2021), hepatocellular (Chen et al. 2022), and gastric (Negishi et al. 2022) cancers.

Potential clinical applications of CTCs include early diagnosis, prognosis, prediction, recognition of drug resistance, molecular phenotyping, an indication of

Table 2 CTC enrichment techniques using molecular markers for detection and validation tests

Type	Principal	Molecular marker	CTC validation marker	CTC enrichment technique	Citation
Physical property	Size	N/A	EpCAM, CK8/18/19	Ficoll-Hypaque density cushion	Hinz et al. (2017)
	Density	N/A	EpCAM, CK8/18/19, VIM, TWIST1, AKT2, SNAI1, LGR5	CanPatrol™, 8 µm membrane	Wu et al. (2015, 2017), Zhao et al. (2017), Wang et al. (2018), and Su et al. (2020)
	Dielectric electrophoresis	N/A	EpCAM	Isolation-by-Size-of-Epithelial-Tumour-cell (iSET) method	Ried et al. (2017)
Immunoaffinity	Positive enrichment	EpCAM	CK8, CK18, CK19, HER2, CD45	CellSearch® system (Veridex)	Rothé et al. (2019)
		EpCAM	CK20, CD45	CellMax platform (CellMax Life)	Gupta et al. (2019)
		EpCAM	CK20, CD45	CellMax platform (CellMax Life)	Tsai et al. (2019)
		EpCAM	CK8, CK18, CD45	Polymeric CTC chip	Kure et al. (2020)
		EpCAM	CK7/8/18/19, CD45	IsoFlux (Fluxion Biosciences)	Toh et al. (2020)
		EpCAM or KRAS	CK20, CD45	MACS/microemulsion method	Feng et al. (2020a)
		EpCAM	EpCAM, pan-CK, CD45	GILUPI CellCollector	Dizdar et al. (2019)
		EpCAM	CK8, CK18, CK19, CD44v6, CD45	CellSearch® CXC kit (Menarini Silicon Biosystems)	Nicolazzo et al. (2020)

Negative enrichment	CD45	N/A	Cytel method	Wang et al. (2019)
	CD45	pan-CK, EPCAM, VIM	RosetteSep™ System (StemCell Technologies)	Lopresti et al. (2019)
	CD45	EpCAM, CK	EasySep™ (StemCell Technologies)	Hamid et al. (2020b)
	CD45	CK18, CEP8	SE-iFISH (Cytelligen)	Huang et al. (2020)
	CD45	CK3	CellSearch® system (Veridex)	Bahnassy et al. (2019)
	CD2, CD16, CD19, CD36, CD38, CD45, CD66b, and glycophorin A	CK19, VEGF	RosetteSep™ System (StemCell Technologies)	Soler et al. (2017)

ALDH1 aldehyde dehydrogenase 1, *CCSC* circulating cancer stem cell, *CD* cluster of differentiation, *CEP8* centromeric probe for chromosome 8, *CRC* colorectal cancer, *CTC* circulating tumour cells, *CK* cytokeratin, *DAPI* 4',6-diamidino-2-phenylindole, *EMT* epithelial–mesenchymal transition, *EpCAM* epithelial cell adhesion molecule, *FACS* fluorescent-activated cell sorting, *FCM* flow cytometry, *MACS* magnetic-activated cell sorting, *MUC1* mucin 1, *RT-qPCR* reverse transcriptase quantitative polymerase chain reaction, *SE-iFISH* subtraction enrichment and immunostaining-fluorescence in situ hybridisation, *VIM* vimentin

tumour invasiveness and recurrence monitoring, as well as serving as a marker for precision cancer treatment.

5.1 Early Cancer Diagnosis

The detection of CTCs provides a promising approach for early cancer diagnosis, enabling timely intervention and treatment. The non-invasive nature of CTCs makes them an attractive option for early cancer diagnosis. Furthermore, current imaging protocols are ineffective for detecting tumours with fewer than 10^9 cells (Alix-Panabières and Pantel 2014). In contrast, CTCs have the potential to be identified as early as stage I in breast cancer, even when primary tumours remain undetectable by imaging devices (Barrière et al. 2012b; Thery et al. 2019).

The variations in CTC counts between colorectal polyps and primary tumours suggest the potential utility of CTCs in both cancer screening and diagnosing malignant growths (Yang et al. 2018b).

A prospective clinical study was conducted to compare CellMax platform-based screening for colorectal cancer (CRC) with conventional diagnostic techniques such as colonoscopy and biopsy. The study analysed 620 samples, including 182 healthy controls, 111 patients with precancerous lesions, and 327 patients with stage I-IV CRC. The study concluded that the overall accuracy achieved was 88% (Tsai et al. 2018).

Although it appears to be highly accurate, this approach fails to consider the molecular characteristics of CTCs, which impairs our ability to achieve a complete understanding and precise diagnosis of cancer. The clinical application of this method is complicated by several challenges, including the scarcity of CTCs, lack of standardised isolation methods, and limited sensitivity (Zhao et al. 2017).

In summary, while CTC detection is widely used for cancer screening and prognosis, its adequacy for diagnosis remains a topic of ongoing research (Zhong et al. 2021; Lin et al. 2021).

5.2 Cancer Prognosis and Prediction

The presence and characteristics of CTCs can provide insights into the prognosis of the disease, allowing clinicians to anticipate the course of cancer. The clinical use of the CellSearch system enables the identification of CTCs as an independent prognostic marker for early cancer detection (Miller et al. 2010).

In France, a phase II trial (NCT01442935) was conducted to identify baseline CTCs in stage IV CRC patients with liver metastases using the CellSearch system. The study revealed that 19% of the patients had a CTC count of ≥ 3 at the beginning of the study. Furthermore, it was found that patients who had a CTC count ≥ 3 both at baseline and 4 weeks after therapy had a shorter overall survival. This suggests that the CellSearch system can be used as a prognostic tool, which can aid clinicians in

predicting the likely outcome of the disease and tailor treatment strategies accordingly (Bidard et al. 2019).

Examining mRNA markers in CTCs is an alternative approach to assessing cancer prognosis. A comparative study by Bahnassy et al. compared counting CTCs through flow cytometry and examining cytomorphology with specific CTC markers (CK3, CK19, MUC1, CD44, CD133, ALDH1) to the CellSearch system, and found that the results were similar in terms of sensitivity and specificity. In short, mRNA markers in CTCs were identified as independent prognostic factors for both progression-free and overall survival in CRC (Bahnassy et al. 2019).

A previous investigation also found that CTCs with *CEACAM5* mRNA marker can be regarded as a dynamic adverse prognostic CTC biomarker in metastatic CRC patients (Messaritakis et al. 2019). Additionally, a study on *CK20* using RT-qPCR demonstrated that CTCs function as an independent and highly specific prognostic marker in individuals with CRC (Hinz et al. 2017).

Collectively, these studies support the clinical value of CTC in predicting the prognosis of cancer.

5.3 Recognition of Drug Resistance and Therapeutic Targets

The mutations found in CTCs present in the blood differ from those found in the primary tumour sites, suggesting that detecting the expressions of mutated genes in CTCs can aid in identifying the mechanisms behind acquired drug resistance during treatment. This knowledge can then be used to make necessary adjustments to cancer treatment strategies (Hamid et al. 2020a). Characterising CTCs also provides insight into cell-cell interactions, and can help to identify potential therapeutic targets in determining which patients may benefit from a specific cancer treatment (Hardingham et al. 2015).

For example, the identification of *KRAS* (Kirsten rat sarcoma viral oncogene homolog)-mutated CTCs allows the exclusion of CRC patients with *KRAS* mutations, enabling the selection of cancer treatment not related to the EGFR (epidermal growth factor receptor) pathway (Feng et al. 2020a). This is because *KRAS* gene mutation is associated with resistance to therapies that target the EGFR pathway. Therefore, by identifying CTCs with *KRAS* mutation, healthcare providers can avoid prescribing ineffective treatments against EGFR, preventing the development of drug resistance, and ensuring more targeted and successful cancer therapy.

On the other hand, the presence of the nuclear androgen receptor splice variant 7 (AR-V7) on CTCs is linked to the resistance of androgen receptor-targeted drug in metastatic prostate cancer (Antonarakis et al. 2014; Sobhani et al. 2021), whereas the expression of multidrug-resistance-related proteins (MRPs) and aldehyde dehydrogenase 1 (ALDH1) is a predictive of response to chemotherapy in breast cancer (Gradilone et al. 2011). The expression of both stemness and partial epithelial-mesenchymal transition (EMT) characteristics on CTCs is also associated with worse responses to breast cancer therapy (Papadaki et al. 2019).

Thus, examining CTCs can contribute to the prevention of drug resistance development and guide adjustments in treatment strategies.

5.4 Molecular Phenotyping

The prediction of cancer outcomes can be achieved by studying the molecular characteristics of CTCs. By identifying different CTC phenotypes, such as epithelial, mesenchymal, and those associated with EMT and stemness, we can categorise cancer patients for improved prognosis and treatment decisions.

The focus of clinical studies often revolves around EMT and stemness in CTCs due to their association with poorer survival outcomes. Table 3 highlights several markers that are associated with EMT in CTCs, including *PI3K α* , *AKT2*, *survivin*, *VIM*, *TWIST*, and *MUC1* (Ning et al. 2015; Qi et al. 2018; Su et al. 2020), while *ALDH1* and *CD133* are two markers linked to stemness in CTCs (Ning et al. 2018; Bahnassy et al. 2019). High levels of these markers in CTCs are found to be correlated with worse prognosis in cancer patients.

In 2017, a cohort study included 1203 CRC patients of all ages and discovered that epithelial and mesenchymal phenotypes in CTCs offer valuable information for tumour staging and evaluating metastasis (Zhao et al. 2017). The study also revealed that mesenchymal phenotypes are associated with tumour size, staging, and vascular invasion, which can lead to cancer metastasis and recurrence (Zhong et al. 2021). Moreover, in hepatocellular carcinoma patients, the presence of CTCs expressing EMT has been reported to be a diagnostic and prognostic indicator (Qi et al. 2018).

Pre-clinical models have demonstrated that the clustering characteristic of CTCs can promote cell-cell adhesion and interaction, enable the cells to adapt to shear stress (Maeshiro et al. 2021), assist in the formation of secondary tumours (Gkoutela et al. 2019), and increase the expression of stemness signatures (Liu et al. 2019) during cancer progression.

Therefore, by analysing molecular features of CTCs, we can attain a more detailed understanding of the specific characteristics of the tumour.

5.5 Indicator of Tumour Invasiveness and Recurrence Monitoring

CTCs are not only beneficial for diagnosing and predicting cancer but also provide valuable insights into the invasiveness and metastatic potential of tumours. Research on breast and pancreatic cancer models has revealed that CTCs play a crucial role in initiating metastasis (Bacelli et al. 2013; Rhim et al. 2014; Hosseini et al. 2016; Yamaguchi et al. 2021). Furthermore, the presence of CTCs is strongly associated with a high degree of aggressiveness, metastasis, and a greater risk of cancer recurrence (Chaffer and Weinberg 2011). For example, the presence of *HER2* (human epidermal growth factor receptor 2)-positive CTCs is an indicator of *HER2*-positive breast cancer, which is associated with an increased risk of recurrence and death. This information is crucial for determining the most appropriate

Table 3 Clinical applications of CTCs in various cancers

CTC utility	CTC type	Type of study	CTC molecular marker	Finding	Citation
Diagnosis	Breast cancer (stage IV)	Cohort study (350 patients)	IHC: EpCAM, CD44, CD47, MET	Metastatic-initiating CTCs and their associated markers may aid the design of better tools to diagnose and treat metastatic breast cancer	Bacelli et al. (2013)
	CRC (stage I–IV)	Cohort study (1203 patients)	RNA-ISH: <i>EPCAM</i> , <i>CK8/18/19</i> , <i>VIM</i> , <i> Twist1</i> , <i>AKT2</i> , <i>SNAIL</i>	Assessment of both epithelial, and mesenchymal markers in CTCs may offer valuable assistance for tumour staging and metastasis evaluation	Zhao et al. (2017)
Diagnosis & prognosis	Breast cancer (stage I–III)	Pooled analysis (3173 patients)	IHC & FISH: HER2	The presence of CTCs as an independent predictor of poor disease-free, overall, breast cancer-specific, and distant disease-free survival	Janni et al. (2016)
	Hepatocellular carcinoma (stage I–IV)	Research study (1112 patients)	RNA-ISH: <i>EPCAM</i> , <i>CK8/18/19</i> , <i>VIM</i> , <i> Twist1</i>	CTC undergoing EMT provides a metric for diagnosis and prognosis of patients with hepatocellular carcinoma	Qi et al. (2018)
	CRC (stage I–IV)	Prospective cohort study (149 patients)	IHC: CK19	Monitoring CTCs may have value in evaluating clinical staging and estimating clinical prognosis	Pan et al. (2021)
	CRC (stage I–IV)	Prospective, open-labelled, cohort-controlled trial (74 patients)	CanPatrol [®] Technique, followed by RNA-ISH: <i>EPCAM</i> , <i>VIM</i>	Presence of mesenchymal CTC is linked with tumour size, T stage, TNM stage, vascular invasion, and CEA. Increased staging results in increment of mesenchymal CTCs and lower survival	Zhong et al. (2021)

(continued)

Table 3 (continued)

CTC utility	CTC type	Type of study	CTC molecular marker	Finding	Citation
Prognosis	CRC (stage IV)	Case-control (88 mCRC patients and 20 healthy control)	RT-qPCR: <i>CK20</i> , <i>survivin</i>	CK20 and survivin-expressing CTCs predict survival in mCRC patients by a combined immunomagnetic RT-qPCR approach	Ning et al. (2015)
	CRC (stage I–IV)	Prospective study (299 patients)	RT-qPCR: <i>CK20</i>	Detection of CTC with CK20 RT-PCR is a highly specific and independent prognostic marker in CRC patients	Hinz et al. (2017)
	CRC (stage IV)	Research study (78 patients)	RT-qPCR: <i>ALDH1</i> , <i>P13Kα</i> , <i>AKT2</i>	CTC may serve as a clinically useful prognostic marker in mCRC patients and warrants further evaluation in prospective trials	Ning et al. (2018)
	CRC (stage I–IV)	Research study (66 patients)	CanPatrol [®] Technique, followed by RNA-ISH: <i>EPCAM</i> , <i>CK8/18/19</i> , <i>VIM</i> , <i> Twist1</i> , <i>AKT2</i> , <i>SNAIL</i> , <i>LGR5</i>	Mesenchymal marker and LGR5 expression levels in circulating tumour cells correlate CRC prognosis; the ratio of mesenchymal phenotype CTCs to total CTCs for evaluation of CRC prognosis and metastasis	Wang et al. (2018)
	CRC (stage IV)	Prospective ancillary study to CT (153 CRC with liver metastasis)	CellSearch: EpcAM, CK	Baseline CTCs ≥ 3 was detected in 19% of the patients. CTC ≥ 3 at baseline and 4 weeks after therapy showed shorter overall survival; phase II trial (NCT01442935)	Bidard et al. (2019)

	CRC (stage I-III)	Comparative study (63 CRC patients, 40 benign bowel diseases and 40 normal controls)	Flow cytometry, cytomorphology, CellSearch & RT-qPCR: <i>CK19</i> , <i>MUC1</i> , <i>CD44</i> , <i>CD133</i> , <i>ALDH1</i>	CTCs mRNA markers are independent prognostic factors for progression-free and overall survival	Bahnassy et al. (2019)
	CRC (stage IV)	Research study (198 patients)	RT-qPCR: <i>CEACAM5</i>	CTCs with <i>CEACAM5</i> mRNA marker is a dynamic adverse prognostic CTC biomarker in mCRC patients	Messariakis et al. (2019)
	CRC (stage I-IV)	Cohort study (156 patients)	IHC: VIM, TWIST, PRL-3	Mesenchymal and PRL-3 status in CTCs may serve as a crucial prognostic marker for assessing relapse or metastasis in postoperative patients	Su et al. (2020)
	CRC (stage IV)	Research study (198 patients)	RT-qPCR: <i>CEACAM5</i>	CTCs with <i>CEACAM5</i> mRNA marker is a dynamic adverse prognostic CTC biomarker in mCRC patients	Messariakis et al. (2019)
	Prostate, breast, ovarian, lung and renal cancer (stage I-IV)	Observational study (277 cancer patients and 132 asymptomatic individuals)	N/A; Isolation-by-Size-of-Epithelial-Tumour (ISET) technique (Rarecells, France)	CTC screening permits early detection of cancer, and higher CTC counts are associated with higher risk of malignancy; CTC monitoring over time indicated treatment effectiveness	Ried et al. (2017)
Prognosis & therapeutic monitoring					
Prognosis & treatment selection	Prostate cancer (stage IV)	Phase III clinical trial (118 patients)	CellSearch: AR-V7; AdnaTest mRNA: AR-V7; Epic Sciences protein-based assay: AR-V7	Validation of the prognostic significance of baseline CTC AR-V7 in patients treated with endocrine therapy (using CellSearch [®] method coupled with AdnaTest CTC AR-V7 mRNA and the Epic Sciences protein-based assay)	NCT02269982 (Armstrong et al. 2019)

(continued)

Table 3 (continued)

CTC utility	CTC type	Type of study	CTC molecular marker	Finding	Citation
Therapeutic monitoring	Breast cancer (stage I–IV)	Research study (114 patients)	ICC; ER; CK	CTC detection may be useful to monitor the efficacy of treatment in advanced or metastatic cancer	Pierga et al. (2004)
Therapeutic monitoring & treatment selection	Breast cancer (stage I–III)	Research study (431 patients)	RT-qPCR: <i>HER2</i> , <i>ER</i> , <i>PR</i>	Primary tumour seems to direct the spread of CTCs; distinct expression profiles between CTCs and the primary tumour suggest re-evaluation of the adjuvant treatment selection	Fehm et al. (2009)
Therapeutic monitoring & drug selection	Breast cancer (stage I–III)	Clinical trial (61 patients)	RT-qPCR: <i>PI3Kα</i> , <i>Akt-2</i> , <i>TWIST1</i> , <i>ALDH1</i> , <i>BMI-1</i> , <i>CD44</i>	Analysis of CTCs with mesenchymal or stemness characteristics facilitates assessment of new drugs in clinical trials	Barrière et al. (2012a)
Breast cancer (stage I)	Breast cancer (stage I)	Phase II clinical trial (recruiting)	CellSearch; PDL-1; CellSieve; CAMLS	Evaluation of the impact on progression-free survival (PFS) of the combination pembrolizumab—carboplatin in HER-2 negative patients previously treated with anthracyclines and taxanes in primary setting	NCT03213041 (Northwestern University 2021)
		Phase II clinical trial (17 patients)	IF: A45-B/B3, CD45, EGFR	A pilot feasibility study to evaluate the efficacy of ZD1839 (IRESSA) in eliminating chemo- and hormone-resistant cytokeratin-positive CTCs in the blood from female breast cancer patients	NCT00428896 (Kalykaki et al. 2014)

Breast cancer (stage IIIB & IV)	Phase II clinical trial (22 patients)	IF: CK8, CK18, CK19, HER2	A pilot feasibility study to evaluate the efficacy of lapatinib in eliminating cytokeratin-positive CTCs in the blood from female breast cancer patients	NCT00694252 (Georgoulas 2015)
CRC (stage IV)	Phase III clinical trial (349 patients)	CellSearch: EpCAM	Comparison of the efficacy, in terms of PFS, of first-line triplet chemotherapy (FOLFOXIRI-bevacizumab) and doublet chemotherapy (FOLFOX-bevacizumab) in metastatic CRC patients with elevated baseline CTC count (≥ 7.5 mL)	NCT01640405 (Aranda et al. 2020)

AKT2 RAC-beta serine/threonine-protein kinase, *ALDH1* aldehyde dehydrogenase 1, *AR-V7* androgen receptor splice variant 7, *BMI-1* B-cell-specific Moloney murine leukaemia virus integration site 1, *CAMLs* cancer-associated macrophage-like cells, *CEA* carcinoembryonic antigen, *CD* cluster of differentiation, *CRC* colorectal cancer, *CTC* circulating tumour cells, *CK* cytokeratin, *CLU* clusterin, *EGFR* epidermal growth factor receptor, *EMT* epithelial–mesenchymal transition, *EPCAM* gene encoding EpCAM protein, *EpCAM* epithelial cell adhesion molecule, *GCC* guanylyl cyclase C, *HER2* human epidermal growth factor receptor 2, *ICC* immunocytochemistry, *IF* immunofluorescence, *ISH* in situ hybridisation, *KRAS* Kirsten rat sarcoma virus, *LOXL3* lysyl oxidase like 3, *LGR5* leucine-rich repeat-containing G-protein coupled receptor 5, *PDL-1* programmed death ligand 1, *PRL-3* protein tyrosine phosphatase type IVA 3, *PI3K α* phosphoinositide 3-kinase isoform alpha, *RT-qPCR* reverse transcriptase quantitative polymerase chain reaction, *SNAIL* snail family transcriptional repressor 1, *TEM-8* tumour endothelial marker 8, *TIMP1* TIMP metalloproteinase inhibitor 1, *Twist* family bHLH transcription factor 1, *VIL1* villin-2, *VIM* vimentin

course of treatment, as drugs targeting HER2 can effectively control the growth of HER2-positive cancer cells (Fehm et al. 2009; Janni et al. 2016).

Arrazubi and colleagues conducted a study, which found that the presence of CTCs before and after surgery in patients with resected liver metastasis can be predictive of metastasis, relapse, disease-free survival, and overall survival (Arrazubi et al. 2019). The ratio of mesenchymal phenotype CTCs to total CTCs can also be used to evaluate prognosis and metastasis (Wang et al. 2018). Further investigations on the tumour TNM staging system demonstrated a favourable relationship between the CTC number and primary tumour size, depth of invasion, lymph node invasion, and distant metastasis, indicating that CTCs are valuable indicators for evaluating cancer infiltration, invasiveness and metastasis (Szczerba et al. 2019; Zhong et al. 2021).

In short, the surveillance of CTC can offer valuable insights into the invasiveness of the tumour and identify indications of cancer recurrence, allowing for early intervention.

5.6 Marker for Precision Cancer Treatment

The most commonly used standard for evaluating therapeutic response in solid tumours is the RECIST (Response Evaluation Criteria in Solid Tumours) guideline (Eisenhauer et al. 2009; Schwartz et al. 2016). However, it does not include the assessment of CTCs.

During a phase II clinical trial, researchers discovered that an intensive 4-drug regimen is particularly beneficial for stage IV CRC patients with a high CTC count. This regimen includes irinotecan, oxaliplatin, tegafur-uracil with leucovorin, and cetuximab, which was discovered to help prevent high toxicity in patients with low CTC numbers (Krebs et al. 2015).

A study conducted by Chou et al. in 2018 demonstrated that the development of a prognostic model based on CTCs could reliably predict clinical outcomes for patients with metastatic CRC who have been treated with chemotherapy (Chou et al. 2018). Meanwhile, in a prospective cohort study focusing on size-isolated CTCs, the presence of CTCs after surgery was identified as an independent indicator of CRC relapse and low survival rates (Yang et al. 2018a).

In essence, these studies imply the potential role of CTC enumeration as a biomarker for precision medications, providing valuable insights into cancer treatment responses.

Table 3 summarises the clinical applications of CTCs in cancer diagnosis, prognosis, treatment selection, and therapeutic monitoring.

6 Conclusion

The fatality of cancer is primarily caused by metastatic diseases, accounting for 90% of cases. Although studying CTCs can be challenging, they are of great significance in providing precise information for diagnosis, prognosis, drug selection, recurrence

monitoring, and assessing drug response. While the detection and isolation of CTCs still require better methods, enumerating CTCs has proven to be prognostically significant. The heterogeneity of CTCs, similar to that of primary tumours, complicates the identification of molecular markers for CTC subpopulations. To improve the clinical applications of CTCs, advancements in isolation techniques, standardised protocol and studies targeting the phenotypic characteristics of the entire CTC population are essential. Without these improvements, the full clinical potential of CTC analyses remains undemonstrated.

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References

- Alix-Panabières C, Pantel K (2014) Challenges in circulating tumour cell research. *Nat Rev Cancer* 14:623–631. <https://doi.org/10.1038/nrc3820>
- Alix-Panabières C, Pantel K (2016) Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. *Cancer Discov* 6:479–491. <https://doi.org/10.1158/2159-8290.CD-15-1483>
- Alix-Panabières C, Cayrefourcq L, Mazard T et al (2017) Molecular portrait of metastasis-competent circulating tumor cells in colon cancer reveals the crucial role of genes regulating energy metabolism and DNA repair. *Clin Chem* 63:700–713. <https://doi.org/10.1373/clinchem.2016.263582>
- Andree KC, van Dalum G, Terstappen LWM (2016) Challenges in circulating tumor cell detection by the CellSearch system. *Mol Oncol* 10:395–407. <https://doi.org/10.1016/j.molonc.2015.12.002>
- Antonarakis ES, Lu C, Wang H et al (2014) AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 371:1028–1038. <https://doi.org/10.1056/NEJMoa1315815>
- Aranda E, Viéitez JM, Gómez-España A et al (2020) FOLFOXIRI plus bevacizumab versus FOLFOX plus bevacizumab for patients with metastatic colorectal cancer and ≥ 3 circulating tumour cells: the randomised phase III VISNÚ-1 trial. *ESMO Open* 5. <https://doi.org/10.1136/esmoopen-2020-000944>
- Armstrong AJ, Halabi S, Luo J et al (2019) Prospective multicenter validation of androgen receptor splice variant 7 and hormone therapy resistance in high-risk castration-resistant prostate cancer: the PROPHECY study. *JCO* 37:1120–1129. <https://doi.org/10.1200/JCO.18.01731>
- Arrazubi V, Mata E, Antelo ML et al (2019) Circulating tumor cells in patients undergoing resection of colorectal cancer liver metastases. Clinical utility for long-term outcome: a prospective trial. *Ann Surg Oncol* 26:2805–2811. <https://doi.org/10.1245/s10434-019-07503-8>
- Ashworth TR (1869) A case of cancer in which cells similar to those in the tumors were seen in the blood after death. *Aust Med J* 14:146–149
- Baccelli I, Schneeweiss A, Riethdorf S et al (2013) Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nat Biotechnol* 31:539–544. <https://doi.org/10.1038/nbt.2576>

- Bahnassy AA, Salem SE, Mohanad M et al (2019) Prognostic significance of circulating tumor cells (CTCs) in Egyptian non-metastatic colorectal cancer patients: a comparative study for four different techniques of detection (flowcytometry, CellSearch, quantitative real-time PCR and cytomorphology). *Exp Mol Pathol* 106:90–101. <https://doi.org/10.1016/j.yexmp.2018.12.006>
- Bankó P, Lee SY, Nagygyörgy V et al (2019) Technologies for circulating tumor cell separation from whole blood. *J Hematol Oncol* 12:48. <https://doi.org/10.1186/s13045-019-0735-4>
- Barbázán J, Muínelo-Romay L, Vieito M et al (2014) A multimarker panel for circulating tumor cells detection predicts patient outcome and therapy response in metastatic colorectal cancer. *Int J Cancer* 135:2633–2643. <https://doi.org/10.1002/ijc.28910>
- Barrière G, Riouallon A, Renaudie J et al (2012a) Mesenchymal and stemness circulating tumor cells in early breast cancer diagnosis. *BMC Cancer* 12:114. <https://doi.org/10.1186/1471-2407-12-114>
- Barrière G, Tartary M, Rigaud M (2012b) Epithelial mesenchymal transition: a new insight into the detection of circulating tumor cells. In: *ISRN oncology*. <https://www.hindawi.com/journals/isrn/2012/382010/>. Accessed 21 Feb 2021
- Bidard F-C, Kiavue N, Ychou M et al (2019) Circulating tumor cells and circulating tumor DNA detection in potentially resectable metastatic colorectal cancer: a prospective ancillary study to the unicancer prodige-14 trial. *Cells* 8:516. <https://doi.org/10.3390/cells8060516>
- Castells A, Boix L, Bessa X et al (1998) Detection of colonic cells in peripheral blood of colorectal cancer patients by means of reverse transcriptase and polymerase chain reaction. *Br J Cancer* 78: 1368–1372. <https://doi.org/10.1038/bjc.1998.686>
- Chaffer CL, Weinberg RA (2011) A perspective on cancer cell metastasis. *Science* 331:1559–1564. <https://doi.org/10.1126/science.1203543>
- Chang YS, di Tomaso E, McDonald DM et al (2000) Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. *Proc Natl Acad Sci USA* 97:14608–14613
- Chen L, Zhou W, Ye Z et al (2022) Predictive value of circulating tumor cells based on subtraction enrichment for recurrence risk in stage II colorectal cancer. *ACS Appl Mater Interfaces* 14: 35389–35399. <https://doi.org/10.1021/acsami.2c08560>
- Chou W-C, Wu M-H, Chang P-H et al (2018) A prognostic model based on circulating tumour cells is useful for identifying the poorest survival outcome in patients with metastatic colorectal cancer. *Int J Biol Sci* 14:137–146. <https://doi.org/10.7150/ijbs.23182>
- Cohen SJ, Punt CJ, Iannotti N et al (2008) Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 26:3213–3221
- Cohen SJ, Punt CJA, Iannotti N et al (2009) Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol* 20:1223–1229. <https://doi.org/10.1093/annonc/mdn786>
- Cristofanilli M, Budd GT, Ellis MJ et al (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 351:781–791. <https://doi.org/10.1056/NEJMoa040766>
- de Wit S, van Dalum G, Terstappen LWMM (2014) Detection of circulating tumor cells. *Scientifica* 2014:Article ID 819362. <https://doi.org/10.1155/2014/819362>
- Denève E, Riethdorf S, Ramos J et al (2013) Capture of viable circulating tumor cells in the liver of colorectal cancer patients. *Clin Chem* 59:1384–1392. <https://doi.org/10.1373/clinchem.2013.202846>
- Dive C, Brady G (2017) SnapShot: circulating tumor cells. *Cell* 168:742–742.e1. <https://doi.org/10.1016/j.cell.2017.01.026>
- Dizdar L, Fluegen G, van Dalum G et al (2019) Detection of circulating tumor cells in colorectal cancer patients using the GILUPI CellCollector: results from a prospective, single-center study. *Mol Oncol* 13:1548–1558. <https://doi.org/10.1002/1878-0261.12507>
- Dongre A, Weinberg RA (2019) New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol* 20:69–84. <https://doi.org/10.1038/s41580-018-0080-4>

- Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 45:228–247. <https://doi.org/10.1016/j.ejca.2008.10.026>
- Eslami-S Z, Cortés-Hernández LE, Alix-Panabières C (2020) Epithelial cell adhesion molecule: an anchor to isolate clinically relevant circulating tumor cells. *Cells* 9. <https://doi.org/10.3390/cells9081836>
- Ewing J (1922) *Neoplastic disease*. Wb Saunders, Philadelphia
- Fava TA, Desnoyers R, Schulz S et al (2001) Ectopic expression of guanylyl cyclase C in CD34+ progenitor cells in peripheral blood. *J Clin Oncol Off J Am Soc Clin Oncol* 19. <https://doi.org/10.1200/JCO.2001.19.19.3951>
- Fehm T, Hoffmann O, Aktas B et al (2009) Detection and characterization of circulating tumor cells in blood of primary breast cancer patients by RT-PCR and comparison to status of bone marrow disseminated cells. *Breast Cancer Res* 11:R59. <https://doi.org/10.1186/bcr2349>
- Feng C, Wang J, Yang X et al (2020a) Construction and characterization of KRAS immune lipid magnetic balls for colorectal cancer circulating tumor cells. *Cancer Manag Res* 12:10067–10075. <https://doi.org/10.2147/CMAR.S258565>
- Feng J, Mo J, Zhang A et al (2020b) Antibody-free isolation and regulation of adherent cancer cells via hybrid branched microtube-sandwiched hydrodynamic system. *Nanoscale* 12:5103–5113. <https://doi.org/10.1039/D0NR00153H>
- Ferreira MM, Ramani VC, Jeffrey SS (2016) Circulating tumor cell technologies. *Mol Oncol* 10:374–394. <https://doi.org/10.1016/j.molonc.2016.01.007>
- Fiala C, Diamandis EP (2019) New approaches for detecting cancer with circulating cell-free DNA. *BMC Med* 17:159. <https://doi.org/10.1186/s12916-019-1400-z>
- Freeman J, Gray ES, Ziman M (2015) Circulating tumor cells as biomarkers in cancer. In: *Biomarkers in cancer*. Springer, Dordrecht, pp 31–51. https://doi.org/10.1007/978-94-007-7681-4_21
- Gabriel MT, Calleja LR, Chalopin A et al (2016) Circulating tumor cells: a review of non-EpCAM-based approaches for cell enrichment and isolation. *Clin Chem* 62:571–581. <https://doi.org/10.1373/clinchem.2015.249706>
- Georgoulas V (2015) A pilot feasibility study to evaluate the efficacy of lapatinib in eliminating cytokeratin-positive tumour cells circulating in the blood of women with breast cancer. clinicaltrials.gov
- Gkoutela S, Castro-Giner F, Szczerba BM et al (2019) Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. *Cell* 176:98–112.e14. <https://doi.org/10.1016/j.cell.2018.11.046>
- Gradilone A, Naso G, Raimondi C et al (2011) Circulating tumor cells (CTCs) in metastatic breast cancer (MBC): prognosis, drug resistance and phenotypic characterization. *Ann Oncol* 22:86–92. <https://doi.org/10.1093/annonc/mdq323>
- Guadagni F, Kantor J, Aloe S et al (2001) Detection of blood-borne cells in colorectal cancer patients by nested reverse transcription-polymerase chain reaction for carcinoembryonic antigen messenger Rna: longitudinal analyses and demonstration of its potential importance as an adjunct to multiple serum markers. *Cancer Res* 61:2523–2532
- Gupta V, Jafferji I, Garza M et al (2012) ApoStream™, a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood. *Biomicrofluidics* 6:024133. <https://doi.org/10.1063/1.4731647>
- Gupta P, Gulzar Z, Hsieh B et al (2019) Analytical validation of the CellMax platform for early detection of cancer by enumeration of rare circulating tumor cells. *J Circ Biomark* 8:1849454419899214. <https://doi.org/10.1177/1849454419899214>
- Habli Z, AlChamaa W, Saab R et al (2020) Circulating tumor cell detection technologies and clinical utility: challenges and opportunities. *Cancers* 12:1930. <https://doi.org/10.3390/cancers12071930>
- Hamid FB, Gopalan V, Matos M et al (2020a) Genetic heterogeneity of single circulating tumour cells in colorectal carcinoma. *Int J Mol Sci* 21:7766. <https://doi.org/10.3390/ijms21207766>

- Hamid F-B, Islam F, Lu C-T et al (2020b) Abstract 5367: identification and clinical value of the Circulating Tumor Cells (CTCs) in the colorectal cancer. *Cancer Res* 80:5367–5367. <https://doi.org/10.1158/1538-7445.AM2020-5367>
- Hampton R, Walker M, Marshall J, Juhl H (2002) Differential expression of carcinoembryonic antigen (CEA) splice variants in whole blood of colon cancer patients and healthy volunteers: implication for the detection of circulating colon cancer cells. *Oncogene* 21:7817–7823. <https://doi.org/10.1038/sj.onc.1205906>
- Hardingham JE, Grover P, Winter M et al (2015) Detection and clinical significance of circulating tumor cells in colorectal cancer—20 years of progress. *Mol Med* 21:S25–S31. <https://doi.org/10.2119/molmed.2015.00149>
- Harouaka RA, Zhou M-D, Yeh Y-T et al (2014) Flexible micro spring array device for high-throughput enrichment of viable circulating tumor cells. *Clin Chem* 60:323–333. <https://doi.org/10.1373/clinchem.2013.206805>
- Hayes DF, Smerage JB (2010) Circulating tumor cells. In: *Progress in molecular biology and translational science*. Elsevier, pp 95–112. <https://doi.org/10.1016/B978-0-12-385071-3.00005-8>
- Hinz S, Hendricks A, Wittig A et al (2017) Detection of circulating tumor cells with CK20 RT-PCR is an independent negative prognostic marker in colon cancer patients—a prospective study. *BMC Cancer* 17:53. <https://doi.org/10.1186/s12885-016-3035-1>
- Hosseini H, Obradović MMS, Hoffmann M et al (2016) Early dissemination seeds metastasis in breast cancer. *Nature* 540:552–558. <https://doi.org/10.1038/nature20785>
- Huang T, Xu C, Xiao J et al (2020) Determination of the optimal detection time of circulating tumor cells for the postoperative monitoring of colorectal cancer. *Oncol Lett* 19:2996–3002. <https://doi.org/10.3892/ol.2020.11388>
- Janni WJ, Rack B, Terstappen LWM et al (2016) Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. *Clin Cancer Res* 22:2583–2593. <https://doi.org/10.1158/1078-0432.CCR-15-1603>
- Jonas S, Windeatt S, O-Boateng A et al (1996) Identification of carcinoembryonic antigen-producing cells circulating in the blood of patients with colorectal carcinoma by reverse transcriptase polymerase chain reaction. *Gut* 39:717–721. <https://doi.org/10.1136/gut.39.5.717>
- Kalykaki A, Agelaki S, Kallergi G et al (2014) Elimination of EGFR-expressing circulating tumor cells in patients with metastatic breast cancer treated with gefitinib. *Cancer Chemother Pharmacol* 73:685–693. <https://doi.org/10.1007/s00280-014-2387-y>
- Kang H, Kim J, Cho H, Han K-H (2019) Evaluation of positive and negative methods for isolation of circulating tumor cells by lateral magnetophoresis. *Micromachines (Basel)* 10. <https://doi.org/10.3390/mi10060386>
- Katsumata K, Sumi T, Mori Y et al (2006) Detection and evaluation of epithelial cells in the blood of colon cancer patients using RT-PCR. *Int J Clin Oncol* 11:385–389. <https://doi.org/10.1007/s10147-006-0590-5>
- Kobayashi S, Sugasaki A, Yamamoto Y et al (2022) Enrichment of cancer cells based on antibody-free selective cell adhesion. *ACS Biomater Sci Eng* 8:4547. <https://doi.org/10.1021/acsbiomaterials.2c00662>
- Kowalik A, Kowalewska M, Gózdź S (2017) Current approaches for avoiding the limitations of circulating tumor cells detection methods—implications for diagnosis and treatment of patients with solid tumors. *Transl Res* 185:58–84.e15. <https://doi.org/10.1016/j.trsl.2017.04.002>
- Koyanagi K, Bilchik AJ, Saha S et al (2008) Prognostic relevance of occult nodal micrometastases and circulating tumor cells in colorectal cancer in a prospective multicenter trial. *Clin Cancer Res* 14:7391–7396. <https://doi.org/10.1158/1078-0432.CCR-08-0290>
- Kozuka M, Battaglin F, Jayachandran P et al (2021) Clinical significance of circulating tumor cell induced epithelial-mesenchymal transition in patients with metastatic colorectal cancer by single-cell RNA-sequencing. *Cancers (Basel)* 13:4862. <https://doi.org/10.3390/cancers13194862>
- Krebs MG, Hou J-M, Ward TH et al (2010) Circulating tumour cells: their utility in cancer management and predicting outcomes. *Ther Adv Med Oncol* 2:351–365. <https://doi.org/10.1177/1758834010378414>

- Krebs MG, Renahan AG, Backen A et al (2015) Circulating tumor cell enumeration in a phase II trial of a four-drug regimen in advanced colorectal cancer. *Clin Colorectal Cancer* 14:115–122. e2. <https://doi.org/10.1016/j.clcc.2014.12.006>
- Kure K, Hosoya M, Ueyama T et al (2020) Using the polymeric circulating tumor cell chip to capture circulating tumor cells in blood samples of patients with colorectal cancer. *Oncol Lett* 19:2286–2294. <https://doi.org/10.3892/ol.2020.11335>
- Lim SB, Di Lee W, Vasudevan J et al (2019) Liquid biopsy: one cell at a time. *NPJ Precis Oncol* 3: 23. <https://doi.org/10.1038/s41698-019-0095-0>
- Lin D, Shen L, Luo M et al (2021) Circulating tumor cells: biology and clinical significance. *Signal Transduct Target Ther* 6:1–24. <https://doi.org/10.1038/s41392-021-00817-8>
- Liu X, Taftaf R, Kawaguchi M et al (2019) Homophilic CD44 interactions mediate tumor cell aggregation and polyclonal metastasis in patient-derived breast cancer models. *Cancer Discov* 9: 96–113. <https://doi.org/10.1158/2159-8290.CD-18-0065>
- Lopresti A, Malergue F, Bertucci F et al (2019) Sensitive and easy screening for circulating tumor cells by flow cytometry. *JCI Insight* 4:e128180. <https://doi.org/10.1172/jci.insight.128180>
- Maeshiro M, Shinriki S, Liu R et al (2021) Colonization of distant organs by tumor cells generating circulating homotypic clusters adaptive to fluid shear stress. *Sci Rep* 11:6150. <https://doi.org/10.1038/s41598-021-85743-z>
- Mamdouhi T, Twomey JD, McSweeney KM, Zhang B (2019) Fugitives on the run: circulating tumor cells (CTCs) in metastatic diseases. *Cancer Metastasis Rev* 38:297–305. <https://doi.org/10.1007/s10555-019-09795-4>
- Marcuello M, Vymetalkova V, Neves RPL et al (2019) Circulating biomarkers for early detection and clinical management of colorectal cancer. *Mol Asp Med* 69:107. <https://doi.org/10.1016/j.mam.2019.06.002>
- Merker JD, Oxnard GR, Compton C et al (2018) Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. *J Clin Oncol* 36:1631–1641. <https://doi.org/10.1200/JCO.2017.76.8671>
- Messaritakis I, Sfakianaki M, Vogiatzoglou K et al (2019) P-079Circulating tumor cell detection and microsatellite instability status in predicting outcomes of advanced CRC patients. *Ann Oncol* 30. <https://doi.org/10.1093/annonc/mdz155.078>
- Micalizzi DS, Maheswaran S, Haber DA (2017) A conduit to metastasis: circulating tumor cell biology. *Genes Dev* 31:1827. <https://doi.org/10.1101/gad.305805.117>
- Miller MC, Doyle GV, Terstappen LWMM (2010) Significance of circulating tumor cells detected by the CellSearch system in patients with metastatic breast colorectal and prostate cancer. *J Oncol* 2010. <https://doi.org/10.1155/2010/617421>
- Millner LM, Linder MW, Valdes R (2013) Circulating tumor cells: a review of present methods and the need to identify heterogeneous phenotypes. *Ann Clin Lab Sci* 43:295–304
- Molnar B, Floro L, Sipos F et al (2008) Elevation in peripheral blood circulating tumor cell number correlates with macroscopic progression in UICC stage IV colorectal cancer patients. In: *Disease markers*. <https://www.hindawi.com/journals/dm/2008/941509/>. Accessed 1 Oct 2020
- Muşină A-M, Zlei M, Mentel M et al (2021) Evaluation of circulating tumor cells in colorectal cancer using flow cytometry. *J Int Med Res* 49:0300060520980215. <https://doi.org/10.1177/0300060520980215>
- Negishi R, Yamakawa H, Kobayashi T et al (2022) Transcriptomic profiling of single circulating tumor cells provides insight into human metastatic gastric cancer. *Commun Biol* 5:20. <https://doi.org/10.1038/s42003-021-02937-x>
- Nicolazzo C, Loreni F, Caponnetto S et al (2020) Baseline CD44v6-positive circulating tumor cells to predict first-line treatment failure in patients with metastatic colorectal cancer. *Oncotarget* 11: 4115–4122. <https://doi.org/10.18632/oncotarget.27794>
- Ning Y, Hanna DL, Zhang W et al (2015) Cytokeratin-20 and Survivin-expressing circulating tumor cells predict survival in metastatic colorectal cancer patients by a combined immunomagnetic qRT-PCR approach. *Mol Cancer Ther* 14:2401–2408. <https://doi.org/10.1158/1535-7163.MCT-15-0359>

- Ning Y, Zhang W, Hanna DL et al (2018) Clinical relevance of EMT and stem-like gene expression in circulating tumor cells of metastatic colorectal cancer patients. *Pharmacogenomics J* 18:29–34. <https://doi.org/10.1038/tpj.2016.62>
- Noh YH, Im G, Ku JH et al (1999) Detection of tumor cell contamination in peripheral blood by RT-PCR in gastrointestinal cancer patients. *J Korean Med Sci* 14:623–628. <https://doi.org/10.3346/jkms.1999.14.6.623>
- Northwestern University (2021) I-CURE-1: a phase II, single arm study of pembrolizumab combined with carboplatin in patients with Circulating Tumor Cells (CTCs) Positive Her-2 negative Metastatic Breast Cancer (MBC). clinicaltrials.gov
- Oakman C, Pestrin M, Bessi S et al (2010) Significance of micrometastases: circulating tumor cells and disseminated tumor cells in early breast cancer. *Cancers (Basel)* 2:1221–1235. <https://doi.org/10.3390/cancers2021221>
- Paget S (1989) The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* 8:98–101
- Pan R-J, Hong H-J, Sun J et al (2021) Detection and clinical value of circulating tumor cells as an assisted prognostic marker in colorectal cancer patients. *Cancer Manag Res* 13:4567–4578. <https://doi.org/10.2147/CMAR.S300554>
- Pantel K, Speicher MR (2016) The biology of circulating tumor cells. *Oncogene* 35:1216–1224. <https://doi.org/10.1038/onc.2015.192>
- Papadaki MA, Stoupis G, Theodoropoulos PA et al (2019) Circulating tumor cells with stemness and epithelial-to-mesenchymal transition features are chemoresistant and predictive of poor outcome in metastatic breast cancer. *Mol Cancer Ther* 18:437–447. <https://doi.org/10.1158/1535-7163.MCT-18-0584>
- Pierga J-Y, Bonneton C, Vincent-Salomon A et al (2004) Clinical significance of immunocytochemical detection of tumor cells using digital microscopy in peripheral blood and bone marrow of breast cancer patients. *Clin Cancer Res* 10:1392–1400. <https://doi.org/10.1158/1078-0432.ccr-0102-03>
- Poonia S, Goel A, Chawla S et al (2023) Marker-free characterization of full-length transcriptomes of single live circulating tumor cells. *Genome Res* 33:80–95. <https://doi.org/10.1101/gr.276600.122>
- Qi L-N, Xiang B-D, Wu F-X et al (2018) Circulating tumor cells undergoing EMT provide a metric for diagnosis and prognosis of patients with hepatocellular carcinoma. *Cancer Res* 78:4731–4744. <https://doi.org/10.1158/0008-5472.CAN-17-2459>
- Raeisossadati R, Farshchian M, Ganji A et al (2011) Quantitative analysis of TEM-8 and CEA tumor markers indicating free tumor cells in the peripheral blood of colorectal cancer patients. *Int J Color Dis* 26:1265–1270. <https://doi.org/10.1007/s00384-011-1230-8>
- Raimondi C, Nicolazzo C, Gradilone A et al (2014) Circulating tumor cells. *Cancer Biol Ther* 15:496–503. <https://doi.org/10.4161/cbt.28020>
- Rhim AD, Thege FI, Santana SM et al (2014) Detection of circulating pancreas epithelial cells in patients with pancreatic cystic lesions. *Gastroenterology* 146:647–651. <https://doi.org/10.1053/j.gastro.2013.12.007>
- Ried K, Eng P, Sali A (2017) Screening for circulating tumour cells allows early detection of cancer and monitoring of treatment effectiveness: an observational study. *Asian Pac J Cancer Prev* 18:2275–2285. <https://doi.org/10.22034/APJCP.2017.18.8.2275>
- Rosenberg R, Gertler R, Friederichs J et al (2002) Comparison of two density gradient centrifugation systems for the enrichment of disseminated tumor cells in blood. *Cytometry* 49:150–158. <https://doi.org/10.1002/cyto.10161>
- Rothé F, Maetens M, Rouas G et al (2019) CTCs as a prognostic and predictive biomarker for stage II/III colon cancer: a companion study to the PePiTA trial. *BMC Cancer* 19:304. <https://doi.org/10.1186/s12885-019-5528-1>
- Ruan H, Zhou Y, Shen J et al (2020) Circulating tumor cell characterization of lung cancer brain metastases in the cerebrospinal fluid through single-cell transcriptome analysis. *Clin Transl Med* 10:e246. <https://doi.org/10.1002/ctm2.246>

- Schwartz LH, Litière S, de Vries E et al (2016) RECIST 1.1—update and clarification: from the RECIST committee. *Eur J Cancer* 62:132–137. <https://doi.org/10.1016/j.ejca.2016.03.081>
- Shen Z, Wu A, Chen X (2017) Current detection technologies for circulating tumor cells. *Chem Soc Rev* 46:2038–2056. <https://doi.org/10.1039/c6cs00803h>
- Silva JM, Rodriguez R, Garcia JM et al (2002) Detection of epithelial tumour RNA in the plasma of colon cancer patients is associated with advanced stages and circulating tumour cells. *Gut* 50: 530–534. <https://doi.org/10.1136/gut.50.4.530>
- Silva VSE, Abdallah EA, de Mello CAL et al (2020) Prospective study with circulating tumor cells as potential prognosis biomarker in metastatic colorectal cancer. *JCO* 38:203–203. https://doi.org/10.1200/JCO.2020.38.4_suppl.203
- Sobhani N, Neeli PK, D'Angelo A et al (2021) AR-V7 in metastatic prostate cancer: a strategy beyond redemption. *Int J Mol Sci* 22:5515. <https://doi.org/10.3390/ijms22115515>
- Soeth E, Vogel I, Röder C et al (1997) Comparative analysis of bone marrow and venous blood isolates from gastrointestinal cancer patients for the detection of disseminated tumor cells using reverse transcription PCR. *Cancer Res* 57:3106–3110
- Soler A, Cayrefourcq L, Mazel M, Alix-Panabières C (2017) EpCAM-independent enrichment and detection of viable circulating tumor cells using the EPISPOT assay. In: Magbanua MMJ, Park JW (eds) *Circulating tumor cells: methods and protocols*. Springer, New York, pp 263–276
- Stewart CM, Kothari PD, Mouliere F et al (2018) The value of cell-free DNA for molecular pathology. *J Pathol* 244:616–627. <https://doi.org/10.1002/path.5048>
- Su P, Lai W, Liu L et al (2020) Mesenchymal and phosphatase of regenerating liver-3 status in circulating tumor cells may serve as a crucial prognostic marker for assessing relapse or metastasis in postoperative patients with colorectal cancer. *Clin Transl Gastroenterol* 11: e00265. <https://doi.org/10.14309/ctg.0000000000000265>
- Szczerba BM, Castro-Giner F, Vetter M et al (2019) Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* 566:553–557. <https://doi.org/10.1038/s41586-019-0915-y>
- Tamimi Y (2015) Micrometastatic circulating tumor cells; a challenge for an early detection and better survival rates. *J Carcinog Mutagen* 6:9
- Thery L, Meddis A, Cabel L et al (2019) Circulating tumor cells in early breast cancer. *JNCI Cancer Spectr* 3. <https://doi.org/10.1093/jncics/pkz026>
- Thompson EW, Haviv I (2011) The social aspects of EMT-MET plasticity. *Nat Med* 17:1048–1049. <https://doi.org/10.1038/nm.2437>
- Tian C, Xu X, Wang Y et al (2020) Development and clinical prospects of techniques to separate circulating tumor cells from peripheral blood. *Cancer Manag Res* 12:7263–7275. <https://doi.org/10.2147/CMAR.S248380>
- Tieng FYF, Baharudin R, Abu N et al (2020) Single cell transcriptome in colorectal cancer—current updates on its application in metastasis, chemoresistance and the roles of circulating tumor cells. *Front Pharmacol* 11:135. <https://doi.org/10.3389/fphar.2020.00135>
- Tieng FYF, Abu N, Nasir SN et al (2021) Liquid biopsy-based colorectal cancer screening via surface markers of circulating tumor cells. *Diagnostics (Basel)* 11:2136. <https://doi.org/10.3390/diagnostics11112136>
- Toh JWT, Lim SH, MacKenzie S et al (2020) Association between microsatellite instability status and peri-operative release of circulating tumour cells in colorectal cancer. *Cells* 9:425. <https://doi.org/10.3390/cells9020425>
- Tsai W-S, Nimgaonkar A, Segurado O et al (2018) Prospective clinical study of circulating tumor cells for colorectal cancer screening. *JCO* 36:556–556. https://doi.org/10.1200/JCO.2018.36.4_suppl.556
- Tsai W-S, You J-F, Hung H-Y et al (2019) Novel circulating tumor cell assay for detection of colorectal adenomas and cancer. *Clin Transl Gastroenterol* 10. <https://doi.org/10.14309/ctg.0000000000000088>
- Vafaei S, Roudi R, Madjd Z et al (2020) Potential theranostics of circulating tumor cells and tumor-derived exosomes application in colorectal cancer. *Cancer Cell Int* 20:288. <https://doi.org/10.1186/s12935-020-01389-3>

- van der Toom EE, Verdone JE, Gorin MA, Pienta KJ (2016) Technical challenges in the isolation and analysis of circulating tumor cells. *Oncotarget* 7:62754–62766. <https://doi.org/10.18632/oncotarget.11191>
- Vona G, Sabile A, Louha M et al (2000) Isolation by size of epithelial tumor cells. *Am J Pathol* 156: 57–63
- Wang W, Wan L, Wu S et al (2018) Mesenchymal marker and LGR5 expression levels in circulating tumor cells correlate with colorectal cancer prognosis. *Cell Oncol* 41:495–504. <https://doi.org/10.1007/s13402-018-0386-4>
- Wang L, Zhou S, Zhang W et al (2019) Circulating tumor cells as an independent prognostic factor in advanced colorectal cancer: a retrospective study in 121 patients. *Int J Color Dis* 34:589–597. <https://doi.org/10.1007/s00384-018-03223-9>
- Williams A, Balic M, Datar R, Cote R (2012) Size-based enrichment technologies for CTC detection and characterization. *Recent Results Cancer Res* 195:87–95. https://doi.org/10.1007/978-3-642-28160-0_8
- Wilson RE, O'Connor R, Gallops CE et al (2020) Immunomagnetic capture and multiplexed surface marker detection of circulating tumor cells with magnetic multicolor surface-enhanced Raman scattering nanotags. *ACS Appl Mater Interfaces* 12:47220–47232. <https://doi.org/10.1021/acsami.0c12395>
- Wong LS, Cantrill JE, Odogwu S et al (1997) Detection of circulating tumour cells and nodal metastasis by reverse transcriptase-polymerase chain reaction technique. *Br J Surg* 84:834–839
- Wu S, Liu S, Liu Z et al (2015) Classification of circulating tumor cells by epithelial-mesenchymal transition markers. *PLoS One* 10:e0123976. <https://doi.org/10.1371/journal.pone.0123976>
- Wu F, Zhu J, Mao Y et al (2017) Associations between the epithelial-mesenchymal transition phenotypes of circulating tumor cells and the clinicopathological features of patients with colorectal cancer. *Dis Markers* 2017:9474532. <https://doi.org/10.1155/2017/9474532>
- Yamaguchi J, Kokuryo T, Yokoyama Y et al (2021) Premalignant pancreatic cells seed stealth metastasis in distant organs in mice. *Oncogene* 40:2273–2284. <https://doi.org/10.1038/s41388-021-01706-8>
- Yang C, Shi D, Wang S et al (2018a) Prognostic value of pre- and post-operative circulating tumor cells detection in colorectal cancer patients treated with curative resection: a prospective cohort study based on Iset device. *Cancer Manag Res* 10:4135–4144. <https://doi.org/10.2147/CMAR.S176575>
- Yang C, Zhuang W, Hu Y, Zhu L (2018b) Clinical significance of peripheral circulating tumor cell counts in colorectal polyps and non-metastatic colorectal cancer. *World J Surg Oncol* 16:13. <https://doi.org/10.1186/s12957-017-1305-2>
- Yokobori T, Iinuma H, Shimamura T et al (2013) Platin3 is a novel marker for circulating tumor cells undergoing the epithelial-mesenchymal transition and is associated with colorectal cancer prognosis. *Cancer Res* 73:2059–2069. <https://doi.org/10.1158/0008-5472.CAN-12-0326>
- Zhao R, Cai Z, Li S et al (2017) Expression and clinical relevance of epithelial and mesenchymal markers in circulating tumor cells from colorectal cancer. *Oncotarget* 8:9293–9302. <https://doi.org/10.18632/oncotarget.14065>
- Zhong Y, Ma T, Qiao T et al (2021) Role of phenotypes of circulating tumor cells in the diagnosis and treatment of colorectal cancer. *Cancer Manag Res* 13:7077–7085. <https://doi.org/10.2147/CMAR.S316544>
- Zou D, Cui D (2018) Advances in isolation and detection of circulating tumor cells based on microfluidics. *Cancer Biol Med* 15:335–353. <https://doi.org/10.20892/j.issn.2095-3941.2018.0256>



Cancer-Associated Programmed Cell Death Mechanisms: Extended with Biochemical Markers and Experimental Approaches

Goksemin F. Sengul , Fatma Secer-Celik, and Gaye Pisiren

Abstract

Cell death is a fundamental mechanism of life that is directly associated with organismal growth, development, aging, and diseases and is highly studied life science topic from 1960s to the present day. Cell death can be classified as accidental cell death (ACD) and programmed cell death (PCD). ACD is a form of passive, sudden, and uncontrollable cell death induced by extreme physical, chemical, or mechanical changes in pressure, temperature, and/or osmotic pressure. In contrast to ACD, PCD is an active kind of cell death happening under physiological conditions regulated by genes to maintain hemostasis in healthy as well as pathological cells. There are unique biochemical and molecular processes enabling the pharmacological or genetic intervention of PCD. Advanced research in this field paved the way for the discovery of novel mechanisms in different types of PCD pathways. This chapter summarizes and compares biological and morphological characteristics, molecular mechanisms, and biochemical markers of various PCD pathways including apoptosis, autophagy, necroptosis, pyroptosis, ferroptosis, and cuproptosis. Additionally, it also tries to explain the involvement of distinct PCD mechanisms in the diagnosis, pathological progression, and treatment of multiple organismal disorders, particularly in malignancies.

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Keywords

Accidental cell death · Cell death · Malignancies · Programmed cell death

1 Introduction

1.1 Apoptosis

Apoptosis can be defined as a programmed and active cellular death mechanism which is controlled by particular genes or pathways (Kerr et al. 1972). Apoptotic cell death contains sequences of events where not only morphological but also biochemical changes can take place. Apoptosis is marked by the number of typical morphological characteristics including dispersal of cell junctions, nuclear chromatin condensation, thereby decreasing in cell volume, nuclear lysis, cytoplasmic contraction, endoplasmic reticulum (ER) dilation, cell membrane blebbing, and formation of membrane-bound apoptotic bodies. The fragments of chromatin and organelles retain their structural integrity. Moreover, the morphology of the mitochondria also does not alter. Apoptosis takes place during the growth and developmental processes, thereby controlling the type and numbers of the cells (Chen et al. 2024).

The standard process of apoptosis entails a series of hydrolysis, caspase activation, and transmission of signals through cascades. Classical apoptosis is categorized as intrinsic apoptosis, also known as mitochondrial apoptosis, and extrinsic apoptosis, also called as death receptor-mediated pathway (Maiuri et al. 2007; Chen et al. 2024). Disruptions in internal microenvironment by deficiency of growth factor, DNA damage, ER stress, accumulation of prooxidants, microbundle alterations, and mitotic abnormalities trigger intrinsic apoptosis (Brumatti et al. 2010; Chen et al. 2024). The crucial stage in this apoptotic pathway is the permeabilization of outer mitochondrial membrane, mostly regulated by BCL2 family members. Cytochrome C (CYCS) and apoptosis-inducing factor (AIF) are two major proapoptotic signals located on the inner and outer mitochondrial membranes, respectively. Different apoptotic signals lead to the release of CYCS from mitochondria to cytoplasm where it can interact with apoptotic protease activating factor 1 (APAF1). The binding of these two proteins initiates cascades of events which eventually stimulate the activation of deoxyribonucleases for the hydrolysis of DNA and cytoskeleton proteins. This process is known as intrinsic apoptosis or mitochondrial apoptotic pathway due to key initiatory mitochondrial proapoptotic proteins (Brumatti et al. 2010; Chen et al. 2024). Disruptions in external microenvironment lead to the activation of particular death receptors on the cell membrane, which finally triggers the extrinsic apoptotic pathway. Thus, this programmed cell death (PCD) pathway is also called as the death receptor pathway of apoptosis (Tuckermann et al. 2005; Chen et al. 2024). Death receptors such as apoptosis antigen 1 (APO1 or FAS), APO2, APO3, and tumor necrosis factor receptor (TNFR) have in common cytoplasmic death domain consisting of about 80 amino acids which is essential for the transmission of apoptotic signal from the cell membrane to the intracellular signaling

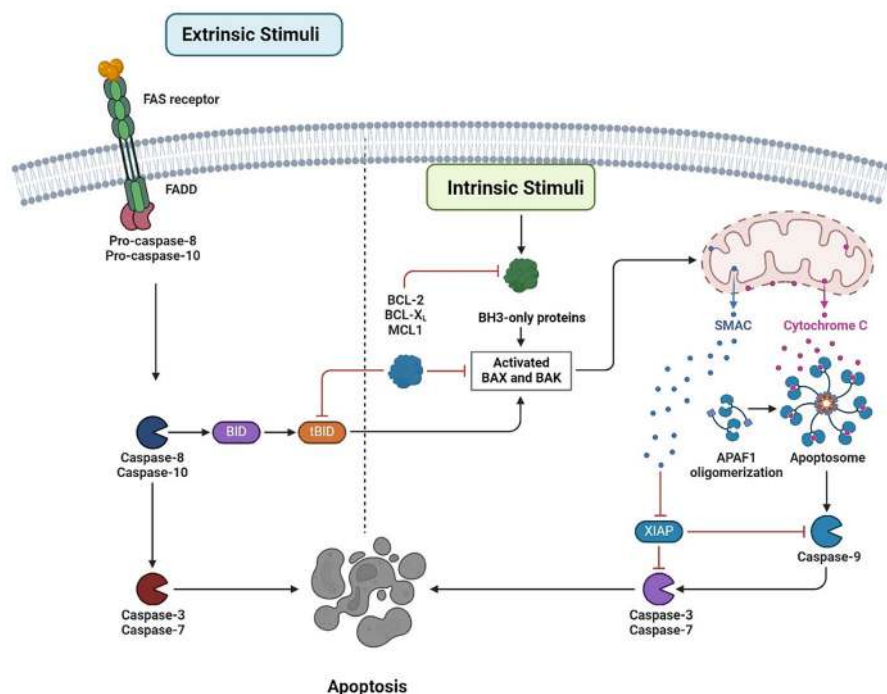


Fig. 1 Depiction of apoptotic cell death mechanism. (The image was drawn using BioRender's scientific illustration software (included in publication license) and modified from Park et al. 2023)

cascades (Wilson et al. 2009; Wang et al. 2010). Moreover, the induction of extrinsic apoptotic pathway also requires the activation of caspases (cysteiny-l-aspartic acid protease, CASPs). Activated FAS binds to FAS ligand (FASL) and FAS-associated death domain (FADD) where pro-CASP8 interacts and together forms the death-inducing signaling complex (DISC). Upon the cleavage of pro-CASP8, the formation of cleaved CASP8 (active CASP8) further activates downstream caspases and, ultimately, induces the apoptosis (Hengartner 2000; Gajate and Mollinedo 2021; Chen et al. 2024; Fig. 1; Table 1). Caspases can be classified into two main subfamilies, namely proapoptotic (CASP2,3,6,7,8,9,10) and inflammatory (CASP4,5,11) caspases. The former ones are responsible for the proteolytic cleavage of intracellular proteins and DNA fragmentation while the latter ones are involved in the maturation and secretion of cytokines, thereby controlling the inflammatory responses (Earnshaw et al. 1999; Chen et al. 2024). Apoptotic caspases also segregate into two groups: initiator caspases (CASP2,8,9,10) and executioner caspases (CASP3,6,7). The inactive forms of all these procaspases are activated via proteolytic cleave as described above for CASP8. The activation of master regulator of apoptosis, cleaved (activated) CASP3, irreversibly directs cells to death. Furthermore, phosphatidylserine (PS), located at the inner layer of the phospholipid bilayer under normal circumstances, is translocated to the outer layer of the cell membrane

Table 1 Biochemical markers of six reviewed programmed cell death mechanisms

Programmed cell death mechanism	Biochemical markers used in studies
Apoptosis	AIF1; CYCS; APAF1; FAS; FADD; DISC; CASP3,8,9; BCL2; BAX
Autophagic cell death	ATGs; PI3K-I,III; ULK1; FIP200; Beclin1; LC3; p62; AMBRA1
Pyroptosis	PRRs; CASP1,4,5,11; ASC; GSDMD; PFD; IL-1 β ; IL-18
Necroptosis	DAMPs; RIK1; RIPK3; MLKL
Ferroptosis	Ferrous iron (Fe ²⁺); NCOA4; DHODH; FSP1; ROS; GSH; GPX; SLC7A11
Cuproptosis	Copper (Cu ²⁺); FDX1; DLAT; LIAS

during apoptosis. Thus, externalized PS can be accepted as a definitive biochemical marker of apoptosis (Earnshaw et al. 1999; Chen et al. 2024). In addition to these reactions, when PSs are flipped out and presented on the cell surface, they are recognized by phagocytic cells for engulfment and elimination of apoptotic bodies, followed just after by several execution reactions. It is well known that after all phases of apoptosis is completed, immunological reactions are not stimulated due to the following reasons: (1) particles of cells are not discharged to the cellular environment, (2) the final phase phagocytosis happens rapidly, and (3) molecules having a potential to induce the immune system are not secreted at the end of the engulfing process. Thus, apoptosis is accepted as a unique mode of “programmed” cell death mechanism. Because apoptosis serves as a primary cell death mechanism, it is generally targeted in tumor tissues to eliminate cancerous cells. Therefore, anticancer drugs with apoptotic effects not only enable precise and rapid cure for cancer patients but also prolong their lifespan (Chen et al. 2024).

Annexin V/propidium iodide (PI) staining is commonly applied to distinguish flipped-out PS by the ability of annexin V to interact with PS while PI co-stains fragmented DNA of cells with impaired membrane integrity (Guo et al. 2021). DNA fragmentation might also be visualized by DNA ladder methodology on agarose gel electrophoresis for tissues or cell cultures with high numbers of apoptotic cells. However, this technique contains many disadvantages. First, it is not appropriate for tissues or cell cultures with low numbers of apoptotic cells. Second, DNA fragmentation takes place during the later stages of apoptosis. Therefore, the absence of DNA ladder on the gel does not rule out the possibility that cells are going through early apoptosis. Furthermore, DNA degradation process is not only specific to apoptosis, but it is also encountered in cellular necrosis, for example. In contrast, the presence of DNA ladder does not show apoptosis; it might be the indicator of DNA repairment or mRNA transcription. Thus, it should be coupled with other techniques as in the case of annexin V/PI assay (Wyllie 1980; Elmore 2007; Galluzzi et al. 2007). Additionally, the protein levels of intrinsic apoptotic markers such as BCL2, BCL2-associated X protein (BAX), CYCS, AIF, and APAF1 and extrinsic apoptotic markers including FAS, FADD, TNFR, pro-/cleaved CASP8, and pro-/cleaved CASP3 are mostly investigated via western blotting or immunoprecipitation to

decide whether these two pathways are activated or not upon anticancer or anti-tumorigenic drug treatment of cancerous cells. Immunohistochemistry assays are also applied to image and detect those of apoptotic markers under light or fluorescence or electron microscopies (Grabarek et al. 2002; White and Cinti 2004; Elmore 2007). The changes in protein levels cannot be always confirmed by alterations in mRNA transcript amounts. Therefore, the gene expression levels of aforementioned proteins can also be measured via quantitative reverse transcription-polymerase chain reaction (qRT-PCR) method in various research papers to check the activation or inhibition of apoptosis particularly in cancer cases (Hofmann et al. 2001; Vallat et al. 2003; Elmore 2007). Not but not least, mitochondrial assays including mitochondrial permeability transition, inner mitochondrial membrane depolarization, assessment of mitochondrial redox status, and CYCS release enable to check the alterations in the initial stages of intrinsic apoptosis. The biggest drawback is that similar changes in mitochondrial parameters can also be monitored during necrosis. For this reason, the obtained data should also be verified by other apoptosis assays (Poot and Pierce 1999; Elmore 2007).

Apoptosis plays a crucial role in maintaining physiological conditions for survival, and therefore, perturbations in this mechanism can lead to the development of various diseases such as cancer. According to studies, it has been shown that the mechanism can be disrupted due to several abnormalities occurring at any phases of apoptotic cell death. Furthermore, while some sicknesses are caused by excessive activation of apoptosis such as in degenerative diseases, others are induced by reduced/blocked apoptotic action, as in the case of cancer.

Cancer cells can evade from apoptosis via utilizing diverse mechanisms and maintain their survival with an uncontrolled cell proliferation character. The evasion mechanisms can be attributed, but not restricted, to the dysregulation of Bcl-2 family members, CASP function, and the action of death receptor signaling. The early study of antiapoptotic Bcl-2 indicated that the overexpression of this protein promotes metastasis not only in breast cancer cell lines but also in *in vivo* mouse models (Del Bufalo et al. 1997). Following this study, many other researches also revealed that the upregulation of antiapoptotic proteins lead to the inhibition of apoptosis while enhancing the cancer progression in various neoplasms including prostate cancer, hepatocellular carcinoma and myeloid malignancies (Kajiwara et al. 1999; Hosseini-khah et al. 2021). In addition, it has been demonstrated that the dysregulation of Bcl-2 family members might cause development of resistance against chemotherapeutic drugs (Miyake et al. 1998). Though abnormal activation of antiapoptotic Bcl-2 proteins can directly hinder the functions of CASPs, their upregulation and downregulation were shown to be associated with tumor development as well. In a study employing pancreatic cancer tissues and chronic pancreatitis samples acquired from patients, CASP1 was shown to be highly expressed which even promotes proliferation of tumor cells (Ramadani et al. 2001). However, the downregulation of CASP1 has also been found in various cancers. Research proposed that the insufficient expression of CASP1 might enhance the tumorigenic behavior of breast cancer cells (Sun and Guo 2018). Furthermore, decreased expression of well-characterized caspases such as CASP3 and CASP9 was also reported in certain cancer types

including breast cancer and colorectal cancer, which were correlated with increased metastasis, hence poor prognosis (Devarajan et al. 2002; Shen et al. 2010). Regarding the extrinsic apoptotic pathway, several studies have shown that alterations in the expression levels of death receptors, which are the key regulators of the pathway, triggered both tumorigenesis and drug resistance. Previous research has indicated the lower expression of Fas death receptor in ovarian epithelial tumors compared to benign lesions postulating that this form of carcinogenesis might be controlled by reduced Fas-mediated apoptosis (Reed et al. 2005). Similarly, the expression level of Fas receptor was significantly downregulated in CD133⁺CD24^{lo} colon cancer cells, causing a limitation in apoptotic cell death. They have also described the possible link between the high metastatic potential of colon cancer cells and their immune escape strategy (Xiao et al. 2019). Given the significant impact of evasion mechanism on cancer progression, apoptotic (pro-/anti-) proteins and pathways are considered as one of the promising therapeutic targets to fight against cancer. Recent research has proven that the role of antiapoptotic proteins can be depressed by using targeted inhibitor molecules (e.g., Bcl-2, Mcl-1, and IAP inhibitors) or by using antagonists (e.g., MDM2 antagonist) (Kapoor et al. 2020; Carter et al. 2022; Cerna et al. 2021).

1.2 Autophagy-Dependent Cell Death

One another mode of PCD, namely autophagy-dependent cell death (ADCD), is exerted by the activation of autophagy during cell death process (Schweichel and Merker 1973; Nevalainen 1975; Shen and Codogno 2011; Jung et al. 2020; Park et al. 2023; Chen et al. 2024). Autophagy was described as an evolutionarily conserved catabolic process by Christian de Duve in his lysosomal function–related paper published in 1963 (Ohsumi 2014; Ktistakis 2017; Park et al. 2023). However, the biochemical mechanisms behind the autophagy were first identified with the discovery of autophagy-related genes by Yoshinori Ohsumi and his colleagues in 1992 (Ohsumi 2014; Park et al. 2023). Autophagy involves the formation of bilayer membrane–bound vesicular organelle, autophagosome, which encloses cytoplasmic fragmented and/or degenerating organelles (such as mitochondria, ER, and peroxisomes), cytosol, and macromolecules (such as ubiquitinated proteins) or pathogenic microorganisms. Autophagic vesicles are eventually fuses with lysosomes where their cargos are degraded by the lysosomal hydrolases (Yang and Klionsky 2009; Zhang and Baehrecke 2015; Jung et al. 2020; Park et al. 2023; Chen et al. 2024). Autophagy is stimulated by starvation, pathogen infection, oxygen deprivation, cytotoxic agents, or ER stress and ensures the maintenance of cellular hemostasis and recycle of older or defected organelles, but excessive levels of autophagy induce cellular death (Chen et al. 2024). The regulatory mechanism behind autophagy is extremely complicated because it is controlled by different biochemical pathways and their components. Recent studies have indicated that autophagy is essentially modulated by phosphatidylinositol 3-phosphate kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling

pathway (Pattingre et al. 2008; Yang and Klionsky 2009; Zhang and Baehrecke 2015; Chen et al. 2024).

Activation of PI3K type I (PI3K-I) stimulates the conversion of phosphatidylinositol 3,4-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-triphosphate (PIP3). AKT interacts with PIP3 at the plasma membrane, which enables 3-phosphatidylinositol-dependent kinase 1 (PDK1) to access and phosphorylate AKT. Activated AKT (pAKT) can further phosphorylate, thereby activating mammalian target of rapamycin complex 1 (mTORC1) which inhibits autophagy by phosphorylating autophagy-related proteins including unc-51-like kinase 1 (ULK1), ATG13, AMBRA1, and ATG14L (Jung et al. 2010; Ayala et al. 2014; Chen et al. 2024; Fig. 2; Table 1) under high-nutrient conditions. However, the inhibition of ULK complex phosphorylation under starvation liberates ULK1 from mTORC1 complex, which then drives ULK1 to phosphorylate ATG13, FIP200, and itself to induce autophagy. Therefore, mTOR pathway is accepted as gatekeeper of autophagy (Jung et al. 2010; Ayala et al. 2014; Li et al. 2015; Chen et al. 2024; Fig. 2; Table 1). ATG family members also interact with other proteins to control autophagy. Beclin1, mammalian ortholog of yeast ATG6, is one of the firstly discovered positive regulators of autophagy, which takes role in the phagophore formation. The binding of Beclin1 with PI3K-III initiates the formation of autophagosome and eventually triggers the induction of ADCD process (Maejima et al. 2016; Chen et al. 2024; Fig. 2; Table 1). However, the interaction of Beclin1 with intrinsic apoptosis marker BCL2 suppresses the autophagy. Moreover, two ubiquitin-like systems, namely ATG5-ATG12-ATG16L and ATG8/LC3, also contribute important involvement in the biogenesis of autophagosome. Thus, LC3 lipidation and increased LC3-II/LC3-I ratio are commonly used as biochemical markers of autophagy (Table 1). Autosis is a non-apoptotic and non-necrotic form of ADCD, which is mediated by an integral plasma membrane protein Na^+/K^+

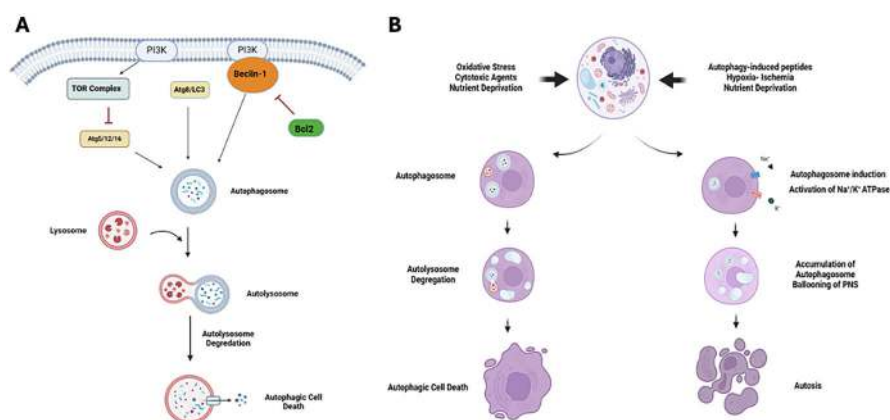


Fig. 2 The illustration of autophagy-dependent cell death (a) and its comparison with autosis (b). (The image was drawn using BioRender's scientific illustration software (included in publication license) and modified from Chen et al. 2024 and Park et al. 2023)

adenosine triphosphatase (ATPase) (Liu et al. 2013; Chen et al. 2024; Fig. 2). The binding of Na^+/K^+ ATPase with Tat-Beclin1 during starvation stress activates autosis, while the inhibition of Na^+/K^+ ATPase blocks this cell death mechanism (Liu et al. 2013; Chen et al. 2024; Fig. 2).

ADCD has been characterized in various pathophysiological processes such as cancer, neurodegenerative diseases (NDs), ischemia-reperfusion injury, and cardiovascular diseases (CVDs) under cytotoxic, neurotoxic, or hypoxic conditions. ADCD is mainly observed in cancer types when tumor cells are resistant against apoptosis-driven chemotherapy. It has been shown that the activation of ADCD slowed down the tumor progression as well as extended the life span in mouse model of glioma, while silencing of essential autophagy-activating enzyme, ATG7, attenuated the effects of chemotherapeutic drugs on tumor survival and growth (Shchors et al. 2015; Bialik et al. 2018). Moreover, key autophagy-related proteins as well as their interacting partners were shown to fulfill tumor suppressive functions (Sinha and Levine 2008; Cianfanelli et al. 2015; Bialik et al. 2018). Monoallelic deletions in Beclin1 have been detected in breast, ovarian, and prostate cancer (Sinha and Levine 2008). Additionally, monoallelic mutation of its binding partner, UV irradiation resistance-associated gene (UVRAG), was encountered in patients with colon cancers (Liang et al. 2006). Another Beclin1-interacting protein, endophilin B1 (also referred as Bif1, Bax-interacting factor 1), exclaimed the association of autophagy with tumor suppression because the knockout of this gene in mouse model resulted in the formation of spontaneous tumors (Takahashi et al. 2007). Furthermore, the activator of Beclin1, AMBRA1 (autophagy and Beclin1 regulator 1), deficiency caused uncontrolled cell proliferation, again suggesting the tumor-suppressive action of autophagy (Fimia et al. 2007).

Autophagy is not only involved in cancer treatment and tumor suppression but also takes part in tumorigenesis and tumor progression. It is well known that autophagic pathway is activated to support the survival of the cells by cleaning the defected organelles and/or macromolecules. On the contrary, the promotion of this mechanism was also shown to facilitate cancer progression by advancing the survival ability of tumor cells (Vanzo et al. 2019; Linder and Kögel 2019). A recent study by Nassour et al. suggested that the suppression of ADCD led to continuous growth and increased genomic instability of tumor cells demonstrating the role of this cell death pathway on oncogenic transformation (Nassour et al. 2019; Linder and Kögel 2019). The complexity of multifaced roles of autophagic proteins and pathway in survival and death of tumor cells displayed the necessity of future studies to declare them as therapeutic targets for different types of cancers.

As it was mentioned earlier, autophagy also plays key regulatory roles in the pathogenesis of NDs. Aggregation of misfolded proteins and/or loss of neuronal cells are commonly observed in many NDs including Alzheimer's, Parkinson's, and Huntington's Diseases and amyotrophic lateral sclerosis. Impairment in the autophagy process during late onset of these diseases causes accumulation of damaged proteins and organelles, eventually triggering neuronal death (Nixon and Yang 2012; Guo et al. 2018). Overall, ADCD is responsible for restoring cellular

hemostasis, and dysfunctional autophagy explains the etiology behind many diseases.

To uncover cellular and biochemical secrets of autophagy in mammalian cells, immunoblotting, immunohistochemical staining, ELISA, and gene expression analysis can be performed (Zhang et al. 2016). p62 protein interacts with ubiquitin and LC3 and directs ubiquitinated proteins into autophagosomes destined for clearance by autophagy. Therefore, p62 is prevalently used as a reporter of autophagic activity (Rusten and Stenmark 2010; Liu et al. 2016a). The accumulation of its protein product, which can be shown via western blotting or ELISA techniques or visualized through immunostaining under the microscope, denotes the defect in autophagic cell death (Zhang et al. 2016). Moreover, changes in p62 gene expression profile might also provide information about autophagic activation at the transcript level (Jain et al. 2010). Additionally, similar experimental analysis can also be performed with other autophagy markers such as LC3, Beclin1, ULK1, and ATG family members in different types of cultured cells and animal models to assess autophagic flux (Zhang et al. 2016). Furthermore, related PI3K and mTOR signaling pathway components might be also checked to investigate the modulation of the autophagic activity (Jung et al. 2010; Ayala et al. 2014; Chen et al. 2024). The novel interactome can also be demonstrated via co-immunoprecipitation assays by using well-characterized autophagic proteins as baits (Zhang et al. 2016). Therefore, functional association of autophagy with different cellular pathways can be identified, which enables further elucidation of the regulatory mechanisms governing autophagy and autophagy-related diseases. Ultimately, all these studies pave the way for the discovery of new therapeutics against many incurable NDs and cancers.

1.3 Pyroptosis

Pyroptosis is one of the novel PCD mechanisms in which several proteins work together to stimulate innate immune-regulated killing pathway. Although pyroptosis shares some common morphological features with necrosis and apoptosis, the main morphological changes are the formation of membrane holes followed by cell swelling and disruption of cell membrane causing the release of inflammatory molecules (Wei et al. 2022).

Pyroptotic cell death can take place in various cell types, mainly in innate immune cells such as monocytes, macrophages, and dendritic cells to fight against pathogens (Vande Walle and Lamkanfi 2016). This mechanism can be divided into two main pathways, namely canonical and noncanonical pathways. In canonical pyroptosis, the pathway can be activated by particles belonging to the damaged cells and/or pathogens recognized via pattern recognition receptors (PRRs). Thereupon, the multiprotein complex inflammasome starts to assemble once its component PRRs come together with pro-CASP1 and adaptor proteins (ASC) (Zitvogel et al. 2012; Latz et al. 2013). This assembly process stimulates the activation of CASP1, which then activates Gasdermin D (GSDMD) and is one of the proteins that belongs to the Gasdermin superfamily (He et al. 2015). This protein family includes two domains;

one of them is responsible for pore formation, called as N-terminal pore-forming domain (PFD), and the other one is the repressor domain found at the C-terminal. The activation of GSDMD is mediated by the cleavage of its N- and C-terminal domains, which results in the formation of GSDM-N (also known as GSDM-PFD) and GSDMD-C fragments (Sborgi et al. 2016; Shi et al. 2015). This process lets the GSDM-PFD to self-oligomerize and then transfer to the cell membrane, where it forms ring-shaped oligomeric structures for the formation of pores. This causes cell rupture and the release of biomolecules, especially cytoplasmic lactate dehydrogenase (LDH), interleukin-1 β (IL-1 β), and interleukin-18 (IL-18) as inflammatory molecules, ultimately leading to cell swelling and cell death (Sborgi et al. 2016; Liu et al. 2016a, b; Fig. 3). The noncanonical pyroptosis is generally mediated by CASP4 and CASP5 or by murine CASP11 (which is the homologous of human CASP4 and CASP5), instead of canonical pathway inflammatory caspase, CASP1 (Shi et al. 2014; Chan et al. 2023; Fig. 3). In this type of pyroptosis, the nonactive form of caspases is directly activated by lipopolysaccharide (LPS), which is found in gram-negative bacteria infected cells, binds, and promotes their oligomerization

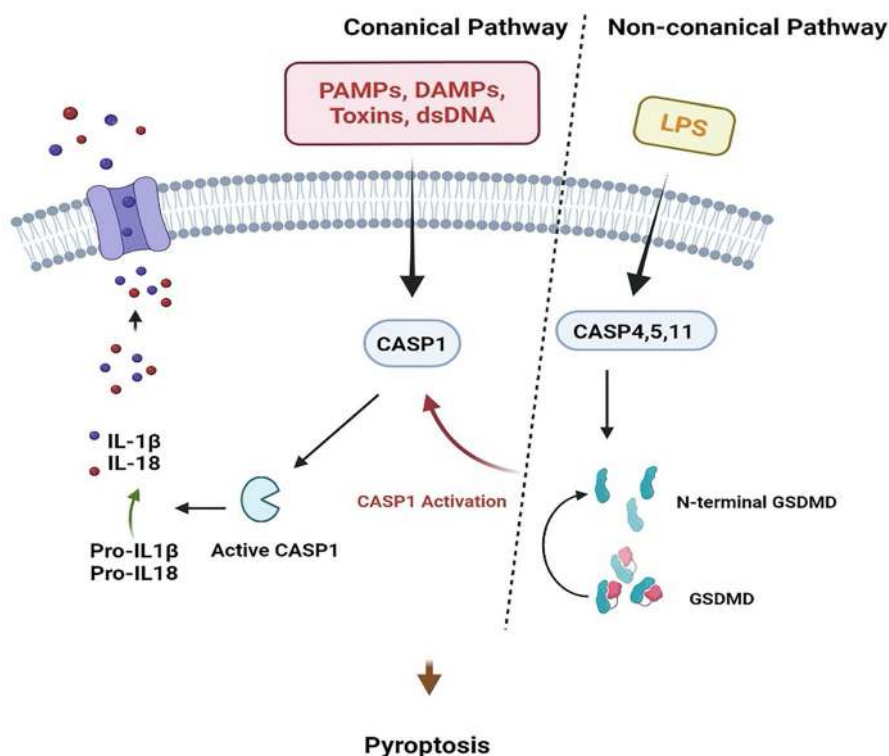


Fig. 3 Depiction of canonical and noncanonical pyroptotic pathways. (The image was drawn using BioRender's scientific illustration software (included in publication license) and modified from Chen et al. 2024)

(Rathinam et al. 2019; Fig. 3). Just like the canonical pathway, GSDMD is cleaved into two fragments. GSDM-PFD then gets responsible for pore formation, which culminates with the movement of cytosolic molecules and proinflammatory cytokines to the outside of the cells to induce pyroptotic cell death (Downs et al. 2020; Fig. 3).

Pyroptosis has an important impact for the maintenance of physiological conditions like other cell death mechanisms, and therefore, any perturbations happened in this pathway can cause the development of various types of diseases such as CVDs, NDs, and several types of cancers (Jia et al. 2019; Rao et al. 2022; Han et al. 2023). Although the relation between pyroptotic cell death and tumorigenesis is not yet fully elucidated, extensive studies have been recently focused on uncovering it. For instance, the low level of GSDMD has been observed in gastric cancer cells compared to adjacent healthy gastric cells which are also correlated with high proliferation characteristics of cancer cells. It is shown that GSDMD has an inhibitory effect on ERK1/2, STAT3, and PI3K/AKT pathways, which in return reduce the expression level of CyclinA2 and cyclin-dependent kinase (CDK2) proteins in gastric cancer cells. Hence, in the absence/low level of GSDMD, pyroptosis cannot take place while proliferation of cancer cells is promoted due to the increased expression of CyclinA2/CDK2 (Wang et al. 2018). The role of pyroptosis is also investigated in breast cancer. It was observed that the progression of breast cancer cells can be inhibited via pyroptosis as in the case of other cell death mechanisms. However, there were some studies proposing that cancer cells can also utilize this pathway for their survival (Xia et al. 2022; Lei et al. 2023). It was reported that breast cancer cells enhance their proliferation and metastatic behavior upon increased IL-1 β and IL-18 secretion. According to the results of one in vivo study conducted by Kaplanov et al. 2019, it was detected that IL-1 β plays a crucial role in the development of breast cancer, and this process can be hindered via the inhibition of cytokine secretion while it can be enhanced in the presence of checkpoint inhibitor (Kaplanov et al. 2019). A study which investigated the link between IL-18 and tight junction protein, Claudin, by using breast cancer cell line showed that the tumorigenic behavior of the cancerous cells is decreased when the expression of IL-18 is rendered (Yang et al. 2015). Therefore, the multifaced roles of pyroptosis in different cancer types justify requirement of further studies.

The involvement of pyroptosis in the development and progression of various diseases makes it a promising target to find an effective treatment against them. NLRP3, GSDMs, and CASPs are widely used in many studies because of their profound role in pyroptosis. Research showed that atherosclerosis can be rendered by targeting pyroptosis in macrophages, which was achieved by using MCC950, NLRP3 inhibitory molecule (Zeng et al. 2021). In a study of breast cancer, it was understood that metformin has also the ability to stimulate pyroptosis by leading to mitochondrial dysfunction, which then negatively affects cancer cell proliferation (Zheng et al. 2020). Research studying the brain death-induced kidney injury in rats showed that after using CASP1 inhibitory molecule, Z-YVAD-FMK, pyroptotic cell death was inhibited with decreased GSDMD, IL-1 β , and IL-18 levels, resulting in

attenuated kidney injury, thereby leading to enhanced renal function in rats (Liu et al. 2021).

Although there are still so many difficulties in monitoring pyroptosis, advanced experimental methods allowed us to achieve rapid progress on the understanding of its mechanism of action and its relationships with various types of diseases, especially with cancers (Wang et al. 2021a, b). To visualize pyroptosis-related morphological changes including cell swelling, membrane blebbing, and rapture bubble-like protrusions, images under light-, fluorescence-, confocal-, scanning electron-, and transmission electron microscopies are commonly applied in research (Chen et al. 2016; Wang et al. 2017; Sarhan et al. 2018; Wang et al. 2020; Li et al. 2021). Moreover, automated live cell imagers enable to distinguish pyroptotic cell death from other PCD mechanisms by recording the entire process of GSDM-mediated pore formation (Wang et al. 2022; Yang et al. 2020). Similarly, MTT or XTT cell viability and LDH or G6PD cytotoxicity assays might be performed to detect pyroptotic cell death in experimental setup (Liu et al. 2018; Du et al. 2019; Wang et al. 2021a, b). Moreover, TUNEL assay might be used for monitoring DNA fragmentation in pyroptosis (Jiang and Poon 2019; Wang et al. 2021a, b). As in the case of apoptosis, PS on the inner side of the cell membrane can be labeled with annexin V while PI freely penetrates into the cell and co-stains the fragmented DNA upon membrane disruption induced by pyroptosis (Wang et al. 2017; Wang et al. 2021a, b). Additionally, other low-molecular weight dyes including ethidium bromide (EtBr) and 7-aminoactinomycin are also able to diffuse into the cell and stain the fragmented DNA in pyroptotic cells (Fink and Cookson 2006; Wang et al. 2021a, b). Furthermore, the cleavage of GSDM family members and activation of CASP1,3,4,5,11 can also be checked via western blotting and immunohistochemistry analysis at the protein level and via qPCR analysis at the mRNA transcript level (Wang et al. 2017; Zhou et al. 2020; Zhang et al. 2020a, b; Volchuk et al. 2020; Russo et al. 2021; Wang et al. 2022). Those proteins can also be used as a bait to identify their novel interaction partners as preys via co-immunoprecipitation (co-IP) experiments. The levels of IL-1 β , IL-18, and LDH should also be measured by ELISA technique to determine induced inflammation in pyroptotic cell death mechanism (Mirshafiee et al. 2018; Du et al. 2019; Wang et al. 2020; Wang et al. 2022). Overall, all these promising experimental approaches and tools targeting pyroptosis not only provide insights for the better grasping of its role in the development and progression of different cancer types but also might enable the discovery of novel diagnostic markers and/or therapeutic implications against these diseases.

1.4 Necroptosis

The traditional definition of necrosis is an uncontrolled type of cell death brought on by a severe chemical or physical injury. The following external factors including high pressure, temperature, chemical stress, or osmotic shock could cause this unintentional cell death. Necrosis is characterized by cell rupture, which allows the contents of the cell to seep into the extracellular space. As a consequence, damage-

associated molecular patterns (DAMPs), which can be detected by immune cells and initiate an inflammatory response, are released. Although necrosis was previously believed to be inert and unprogrammed, recent studies have found CASP-independent cellular event mimicking necrosis. Therefore, necroptosis is defined as a type of planned necrosis that can be triggered either by intracellular cues (foreign microbial nucleic acids) or by extracellular signals (DR-ligand interaction), but it is actively repressed by CASP activity.

There are also more forms of necrotic cell death that are programmed, including ferroptosis and pyroptosis. Necrotic cell death in its various forms, especially the coordinated ones, is momentous, because it has been linked to the pathophysiology of numerous illnesses, such as fibrosis, inflammatory autoimmune diseases, and neurodegenerative disorders, like ALS, multiple sclerosis (MS), and Alzheimer's disease (AD).

Necroptosis is an inflammatory and lytic type of PCD that occurs when death receptors, such as tumor necrosis factor (TNF) receptor, or pattern recognition receptors, such as Toll-like receptors (TLR)-3 and 4, are activated (Kaiser et al. 2013). It is believed that necroptotic death originated as an inherent immune defense against pathogenic diseases (Pearson et al. 2017; Fletcher-Etherington et al. 2020; Zhang et al. 2020a, b). The optimal understanding of necroptotic signaling occurs after TNF-receptor activation. When the family members of cellular inhibitor of apoptosis proteins (cIAPs) including apoptotic effector proteases, caspases, and E3 ligases are disarmed or depleted, TNF signaling can encourage the autophosphorylation of receptor-interacting serine/threonine protein kinase-1 (RIPK1). The necrosome, a megadalton cytoplasmic complex, is formed upon RIPK1 phosphorylation, signaling for the recruitment of RIPK3 kinase (Mompéán et al. 2018). RIPK3 is activated by autophosphorylation and then phosphorylates the terminal effector protein of necroptosis, mixed lineage kinase domain-like (MLKL) pseudokinase (Chen et al. 2013; Meng et al. 2022) within the necrosome (Sun et al. 2012; Murphy et al. 2013).

Necroptosis is initiated by ligand binding to death receptors, including tumor necrosis factor receptor 1 (TNFR1). Although many proteins are involved in the necroptotic pathway, the most reliable method to detect necroptosis is by measuring the phosphorylation status of MLKL and specific inhibition of the necroptotic pathway. MLKL is present in the cytoplasm as an inactive form at baseline conditions (Meng et al. 2021). The phosphorylation of MLKL by RIPK3 induces structural changes leading to MLKL oligomerization and plasma membrane translocation. There, they form sizable membrane-scaffolded assemblies that have the ability to permeabilize the membranes (Samson et al. 2020; Garnish et al. 2021). The innate immune system recognizes the intracellular contents as damage-associated molecular patterns (DAMPs) in the extracellular milieu due to this fatal membrane disruption (Murai et al. 2018), which is accepted as the inflammatory hallmark of necroptosis.

Effectors of the pathway are thought to be attractive therapeutic targets, and the inflammatory character of necroptosis links this death mode to a variety of human disorders. Although the inhibition of RIPK1 and RIPK3 might cause harmful crosstalk with other signaling pathways according to some evidence (Shi et al.

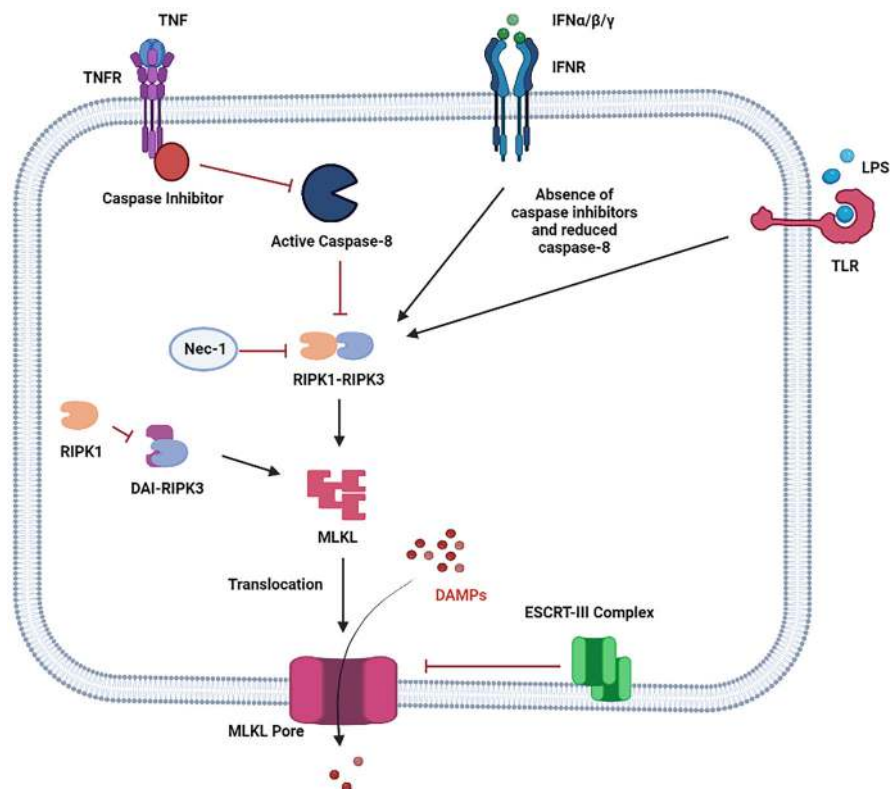


Fig. 4 Necroptotic cell death mechanism was depicted. (The image was drawn using BioRender's scientific illustration software (included in publication license) and modified from Dai et al. 2021)

2024), several RIPK3 inhibitors have been demonstrated to cause apoptotic cell death. Here, we highlight the unsolved parts of MLKL's molecular activity and evaluate the existing structural understanding of its metamorphosis into a cellular executioner (summarized in Fig. 4).

Many cancer cell lines had either no RIPK3 expression at all or very little of it (Koo et al. 2015; Geserick et al. 2015). Of the more than 60 cancer cell lines that were evaluated, two thirds had no RIPK3 protein expression at all (Koo et al. 2015). Additionally, samples from human cancer patients with colorectal, breast, acute myeloid leukemia (AML), melanoma, and colorectal cancer were shown to have decreased RIPK3 expression (Nugues et al. 2014; Feng et al. 2015; Moriwaki et al. 2015; Höckendorf et al. 2016). Based on data presented by McCormick et al., there was also downregulation of RIPK1 expression in head and neck squamous cell carcinoma, which has been associated with disease progression. According to scientists, tumor cells can avoid anoikis due to the lower levels of RIPK1 expression modulated by epigenetic modifications during tumor formation. This may enhance tumorigenesis by improving the tumor cells' capacity for metastasis (McCormick

et al. 2016). Therefore, escape from necroptosis is observed as a characteristic of several cancers which makes this PCD mechanism a promising target against cancer therapies. However, further scientific evidence is warranted to unveil the biochemical involvement of necroptosis in carcinogenesis.

1.5 Ferroptosis

Ferroptosis is a recently identified type of controlled cell death with unique morphological and biochemical properties. It neither leads to the loss of plasma membrane integrity that happens during necrosis nor the chromatin condensation seen during apoptosis. Rather, condensed mitochondria, a decrease in mitochondrial cristae, and higher membrane densities are the physical characteristics defining ferroptosis.

Increased lipid peroxides are the outcome of ferroptosis so that it is an iron-dependent kind of cell death. Consequently, it is primarily controlled by mechanisms that are involved in oxidative stress and iron homeostasis. Ferritin (FTH) is a big protein complex that plays a role in controlling iron homeostasis by keeping iron in a soluble and nontoxic state. Ferritinophagy is a selective autophagy mechanism that targets ferritin and controls its levels. Nuclear receptor coactivator 4 (NCOA4), a selective ferritin cargo receptor, mediates this route. Additionally, certain common iron chelators such as deferoxamine have been proven to suppress the process of ferroptosis.

A trend in the direction of stimulating systems eventually results in the deadly accumulation of lipid peroxides, which induces ferroptosis. Ferroptosis has been associated with hemochromatosis, cancers, ischemia/reperfusion injury, neurological illnesses (including PD and AD), cystic fibrosis, chronic obstructive pulmonary disease, and cardiomyopathy related to doxorubicin (Yan et al. 2021). Ferroptosis has emerged as a novel target for anticancer efficacy against drug resistance, cancer stemness, and epithelial-mesenchymal transition in many recent studies (Lei et al. 2022; Zhang et al. 2022a, b; Fig. 5).

Ferroptosis has been linked to the overexpression of marker proteins, such as prostaglandin-endoperoxide synthase 2 (PTGS2/COX2), acyl-CoA synthetase long-chain family member 4 (ACSL4), and others that aid in the synthesis of lipid hydroperoxides (Tang et al. 2021). Downregulation of ferroptosis-inhibiting proteins, including dihydroorotate dehydrogenase (DHODH), ferroptosis suppressor protein 1 (FSP1), and glutathione peroxidase 4 (GPX4), also facilitates ferroptosis (Tkachenko et al. 2023).

Particularly, the opening of the mitochondrial permeability transition pore (MPTP) can be observed both in necroptosis and in ferroptosis. Moreover, it has been discovered that heat shock protein (HSP90) and cysteine mediate the crosstalk between two programmed cell death mechanisms (Zhou et al. 2021). Ferroptosis is characterized morphologically by changes in the mitochondrial structure, including fragmentation, cristae distortion, and shrinking of the mitochondria (Oh et al. 2022). In addition, mitochondrial ROS play a significant role in controlling RIPK1, which is required for the recruitment of RIPK3 (Zhang et al. 2017). Thus, there are several

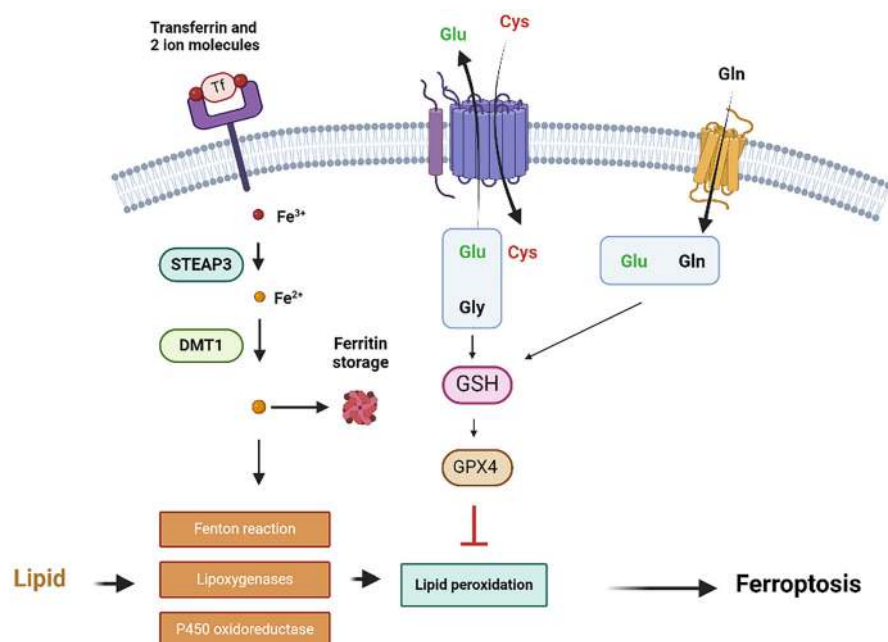


Fig. 5 Depiction of ferroptotic cell death mechanism. (The image was drawn using BioRender's scientific illustration software (included in publication license) and modified from Sun et al. 2022)

levels of interaction between necroptosis and ferroptosis, which highlight how intricate the mechanism governing cell death signaling is.

Higher antioxidant capability is seen in tumor cells as an adaptive response to elevated ROS levels brought on by aberrations in metabolism and signaling (Gorrini et al. 2013). To inhibit ferroptosis and promote tumor growth, tumor cells have acquired critical properties including stabilization and overexpression of anti-ferroptotic systems. One important evasion strategy developed by tumor cells is the activation of the SLC7A11/GSH/GPX4 axis, a crucial defense mechanism against ferroptosis (Koppula et al. 2018; Badgley et al. 2020; Koppula et al. 2021). SLC7A11 might be overexpressed due to the inactivation of tumor suppressors such as TP53, BAP1, and ARF, in numerous malignancies, which also explains the mechanism behind tumor development and growth in cells escaping from ferroptosis (Jiang et al. 2015; Chen et al. 2017; Zhang et al. 2018).

To prevent ferroptosis, tumor cells also use mechanisms that restrict pro-ferroptotic systems. Ferroptosis evasion and tumor development have been linked to reduced peroxidized polyunsaturated fatty acids-phospholipids (PUFA-PLs) and labile iron pool (LIP) levels within cancer cells (Sun et al. 2021). By secreting oleic acid, mammary adipocytes protect triple-negative breast cancer (TNBC) cells from ferroptosis and create a special microenvironment that supports cancer cell survival (Xie et al. 2022). As previously published studies have

suggested, ferroptosis suppresses tumor development and growth as well as regulates the immune response (Jin et al. 2024). It was also documented that sex hormones might play a regulatory role in ferroptosis surveillance (Liang et al. 2023). Today, the physiological of ferroptosis in carcinogenesis remains incompletely understood. Further research can offer the biochemical or immunological induction of ferroptosis as therapeutic strategy against different types of cancers.

1.6 Cuproptosis

Copper is one of the essential transition metals that has multifaced roles in a cell. By contributing to or receiving electrons, copper acted as a co-factor for numerous enzymes (Festa and Thiele 2011), but an excess of this metal can cause various metabolic malfunctions, even cell death (Cobine et al. 2021). Humans mostly get copper via their diets from organ meats and shellfish, often having the highest copper content. To maintain systemic copper homeostasis, people should currently consume 0.8–2.4 mg of copper per day (Bost et al. 2016).

Human body contains the trace element copper, which has a strong correlation with several signaling pathways and biological processes connected to tumors (Festa and Thiele 2011). However, too much copper can result in cell death, in which the exact mechanism and types of copper-induced cell death have long been unknown. A recent study revealed that cuproptosis, which was found to be strongly associated with mitochondrial respiration and the lipoic acid (LA) pathway, is an independent form of cell death (Tsvetkov et al. 2022; Fig. 6). Moreover, cuproptosis primarily takes places in cells producing their energy mainly via aerobic glycolysis, thereby having a high metabolic state as typically seen in several cancer forms including melanoma, breast cancer, leukemia, and other malignancies with tumor stem cells (Buccarelli et al. 2021; Raggi et al. 2021), as well as some drug-resistant tumors (Denise et al. 2015; Cruz-Bermúdez et al. 2019).

Studies on malignancies of the thyroid, breast, prostate, lung, and other organs have found a statistically significant rise in serum copper ion levels in tumor patients as compared to normal individuals (Kosova et al. 2012; Pavithra et al. 2015; Saleh et al. 2020; Wang et al. 2021a, b). Furthermore, serum copper ion concentrations were higher in lung cancer patients with a worse clinical stage, which were likewise linked to a worse clinical prognosis. Therefore, the control of copper homeostasis and the interplay of many proteins taking role in this pathway are necessary for the proper function of copper ions in cancer cells.

The discovery of cuproptosis was significantly assisted by copper ionophores, lipid-soluble molecules that bind copper ions reversibly. These molecules may also be used as anticancer medicines in clinical settings (Oliveri 2022). Copper ionophores have the ability to transfer copper ions across the plasma or mitochondrial membrane. After Tsvetkov et al. discovered the process of copper-induced cell death, known as cuproptosis, studies on this subject have advanced to a new stage. Interestingly, the expression levels of cleaved CASPs were not increased, and no inhibitors for apoptosis, necroptosis, ROS-induced cell death, or ferroptosis could

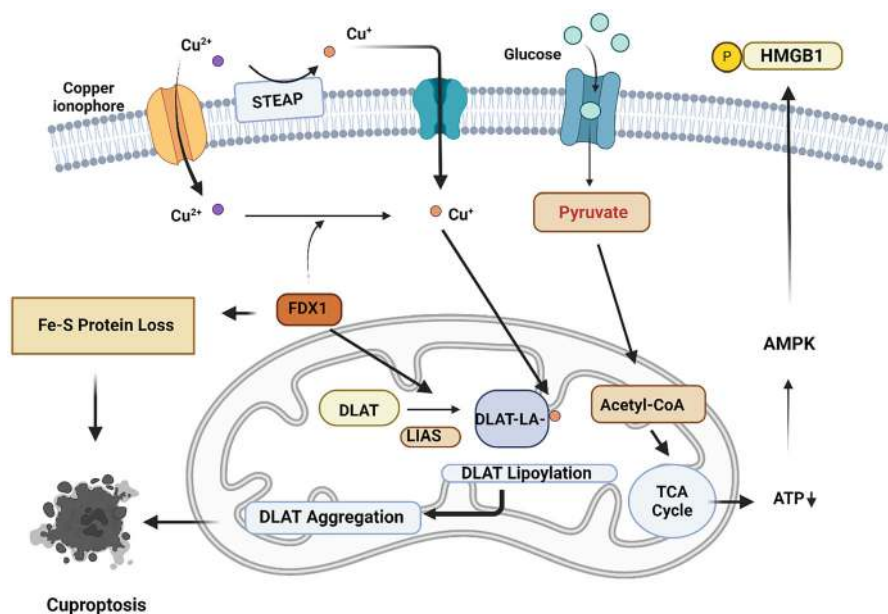


Fig. 6 Illustration of cuproptotic cell death mechanism. (The image was drawn using BioRender's scientific illustration software (included in publication license) and modified from Wang et al. 2023)

save the cells from copper-induced cell death (Tsvetkov et al. 2022). However, the copper chelator was the only solution that could. Therefore, further biochemical and cell viability analysis are needed to determine which cell death mechanisms are ultimately converging on cuproptosis.

Ferredoxin1, an iron-sulfur protein involved in several redox processes, is encoded by the *FDX1* gene. FDX1 was involved in steroidogenesis by functioning as cytochrome P450's monooxygenase (Sheftel et al. 2010). Previous research has demonstrated that FDX1 was essential for the formation of the iron-sulfur clusters (Cai et al. 2017). Moreover, it was shown that FDX1 has increased cytotoxic effects and improved mitochondrial function by reducing copper ions from divalent to monovalent states (Tsvetkov et al. 2019). Furthermore, FDX1 was a key player in cuproptosis, controlling the process of protein lipoylation as the pathway upstream of the LA (Tsvetkov et al. 2022).

FDX1 expression levels were downregulated in solid tumors such as hepatocellular carcinoma and lung adenocarcinoma, while its expression is increased in glioblastoma and female genital cancers as compared to corresponding normal tissues (Zhang et al. 2022a, b). High levels of FDX1 were thought to be linked to a poor prognosis for patients with low-grade glioma and with head and neck squamous cell carcinoma. On the other hand, clear cell renal cell carcinoma and cervical squamous cell carcinoma patients have better prognoses when FDX1 expression is high (Yang et al. 2022). Therefore, decreased expression levels of FDX1 in clear cell renal cell carcinoma and colorectal cancer patients were thought

to have more aggressive and metastatic malignancies along with poor overall survival (OS) and disease-free survival (DFS) (Huang et al. 2022; Wang et al. 2022).

Current research on the process of copper-induced cell death has reached a new milestone through the theory of cuproptosis, which forms enough basement for further mechanistic studies on cuproptosis. Unfortunately, a lot of research has just indicated the indirect relationship between cuproptosis and cancer types, which motivates scientists for future investigations to reveal the direct relationship between them. Furthermore, the therapeutic potentials of cuproptosis-inducing small molecule drugs might be also studied for targeted cancer treatments.

2 Conclusion

Several PCD mechanisms have been identified over the years. One of the earliest known PCD pathways, apoptosis, has distinct morphological properties including membrane integrity, cell shrinkage, and the formation of the apoptotic bodies, and it does not induce inflammatory response. However, other cell death pathways, namely, necroptosis, pyroptosis, and ferroptosis exhibit necrosis-like morphological characteristics including cell swelling, membrane disruption, collapse of organelles, and activation of inflammatory response. Although all these PCD mechanisms are described separately with their unique activators and components, there are so much evidence suggesting crosstalk among these PCD pathways for their regulation (Chen et al. 2021). The loss or over-activation of one or multiple modes of cell death pathways causes severe organismal diseases, particularly cancer. Nowadays, it is quite popular to target these pathways to cure different cancer types. Therefore, researchers try to identify the biochemical mechanisms and markers (Table 1) of these PCD pathways to discover antitumor therapeutics. Ultimately, we have explained biological and morphological aspects, molecular processes, and biochemical markers (Table 1) of numerous PCD mechanisms in detail. Furthermore, the associations of these PCD pathways with carcinogenesis have also been elaborately discussed. Our current work entitled as “overview of PCD mechanisms with extended biochemical markers and experimental approaches” can be used by academic community to boost their knowledge and to develop new extensive and intricate research questions.

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Compliance with Ethical Standards Authors have no disclosure of potential conflicts of interest.

References

- Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Med Cell Longev* 2014:360438
- Badgley MA, Kremer DM, Maurer HC, DelGiorno KE, Lee HJ, Purohit V, Olive KP (2020) Cysteine depletion induces pancreatic tumor ferroptosis in mice. *Science* 368(6486):85–89
- Bialik S, Dasari SK, Kimchi A (2018) Autophagy-dependent cell death—where, how and why a cell eats itself to death. *J Cell Sci* 131(18):jcs215152. <https://doi.org/10.1242/jcs.215152>
- Bost M, Houdart S, Oberli M, Kalonji E, Huneau J-F, Margaritis I (2016) Dietary copper and human health: current evidence and unresolved issues. *J Trace Elem Med Biol Germany* 35: 107–115
- Brumatti G, Salmanidis M, Ekert PG (2010) Crossing paths: interactions between the cell death machinery and growth factor survival signals. *Cell Mol Life Sci* 67:1619–1630
- Buccarelli M, D'Alessandris QG, Matarrese P, Mollinari C, Signore M, Cappannini A et al (2021) Elesclomol-induced increase of mitochondrial reactive oxygen species impairs glioblastoma stem-like cell survival and tumor growth. *J Exp Clin Cancer Res* 40:228
- Cai K, Tonelli M, Frederick RO, Markley JL (2017) Human mitochondrial ferredoxin 1 (FDX1) and ferredoxin 2 (FDX2) both bind cysteine Desulfurase and donate electrons for iron-sulfur cluster biosynthesis. *Biochemistry* 56:487–499
- Carter BZ, Mak PY, Tao W, Warmoes M, Lorenzi PL, Mak D et al (2022) Targeting MCL-1 dysregulates cell metabolism and leukemia-stroma interactions and resensitizes acute myeloid leukemia to BCL-2 inhibition. *Haematologica* 107(1):58–76. <https://doi.org/10.3324/haematol.2020.260331>
- Cerna D, Lim B, Adelabu Y, Yoo S, Carter D, Fahim A et al (2021) SMAC mimetic/IAP inhibitor Birinapant enhances radiosensitivity of glioblastoma Multiforme. *Radiat Res* 195(6):549–560. <https://doi.org/10.1667/rade-20-00171.1>
- Chan AH, Burgener SS, Vezirgiannis K, Wang X, Acklam J, Von Pein JB et al (2023) Caspase-4 dimerisation and D289 auto-processing elicit an interleukin-1 β -converting enzyme. *Life Sci Alliance* 6(10). <https://doi.org/10.26508/lsa.202301908>
- Chen W, Zhou Z, Li L, Zhong CQ, Zheng X, Wu X, Zhang Y, Ma H, Huang D, Li W et al (2013) Diverse sequence determinants control human and mouse receptor interacting protein 3 (RIP3) and mixed lineage kinase domain-like (MLKL) interaction in necroptotic signaling. *J Biol Chem* 288:16247–16261
- Chen X, He W, Hu L, Li J, Fang Y, Wang X et al (2016) Pyroptosis is driven by non-selective gasdermin-D pore and its morphology is different from MLKL channel-mediated necroptosis. *Cell Res* 26:1007–1020
- Chen D, Tavana O, Chu B, Erber L, Chen Y, Baer R, Gu W (2017) NRF2 is a major target of ARF in p53-independent tumor suppression. *Mol Cell* 68(1):224–232
- Chen X, Kang R, Kroemer G, Tang D (2021) Broadening horizons: the role of ferroptosis in cancer. *Nat Rev Clin Oncol* 18:280–296
- Chen Y, Li X, Yang M et al (2024) Research progress on morphology and mechanism of programmed cell death. *Cell Death Dis* 15:327. <https://doi.org/10.1038/s41419-024-06712-8>
- Cianfanelli V, Fuoco C, Lorente M, Salazar M, Quondamatteo F, Gherardini PF, De Zio D, Nazio F, Antonioli M, D'Orazio M et al (2015) AMBRA1 links autophagy to cell proliferation and tumorigenesis by promoting c-Myc dephosphorylation and degradation. *Nat Cell Biol* 17:706. <https://doi.org/10.1038/ncb3171>
- Cobine PA, Moore SA, Leary SC (2021) Getting out what you put in: copper in mitochondria and its impacts on human disease. *Biochim Biophys Acta, Mol Cell Res* 1868:118867
- Cruz-Bermúdez A, Laza-Briviesca R, Vicente-Blanco RJ, García-Grande A, Coronado MJ, Laine-Méndez S et al (2019) Cisplatin resistance involves a metabolic reprogramming through ROS and PGC-1 α in NSCLC which can be overcome by OXPHOS inhibition. *Free Radic Biol Med United States* 135:167–181

- Dai W, Cheng J, Leng X, Hu X, Ao Y (2021) The potential role of necroptosis in clinical diseases. *Int J Mol Med* 47(5):1–16
- Del Bufalo D, Biroccio A, Leonetti C, Zupi G (1997) Bcl-2 overexpression enhances the metastatic potential of a human breast cancer line. *FASEB J* 11(12):947–953. <https://doi.org/10.1096/fasebj.11.12.9337147>
- Denise C, Paoli P, Calvani M, Taddei ML, Giannoni E, Kopetz S et al (2015) 5-fluorouracil resistant colon cancer cells are addicted to OXPHOS to survive and enhance stem-like traits. *Oncotarget* 6:41706–41721
- Devarajan E, Sahin AA, Chen JS, Krishnamurthy RR, Aggarwal N, Brun AM et al (2002) Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. *Oncogene* 21(57):8843–8851. <https://doi.org/10.1038/sj.onc.1206044>
- Downs KP, Nguyen H, Dorfleutner A, Stehlik C (2020) An overview of the non-canonical inflammasome. *Mol Asp Med* 76:100924. <https://doi.org/10.1016/j.mam.2020.100924>
- Du Q, Ge D, Mirshafiee V, Chen C, Li M, Xue C et al (2019) Assessment of neurotoxicity induced by different-sized stober silica nanoparticles: induction of pyroptosis in microglia. *Nanoscale* 11:12965–12972
- Earnshaw WC, Martins LM, Kaufmann SH (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 68:383–424
- Elmore S (2007) Apoptosis: a review of programmed cell death. *Toxicol Pathol* 35(4):495–516. <https://doi.org/10.1080/01926230701320337>
- Feng X, Song Q, Yu A, Tang H, Peng Z, Wang X (2015) Receptor-interacting protein kinase 3 is a predictor of survival and plays a tumor suppressive role in colorectal cancer. *Neoplasma* 62(4):592–601
- Festa RA, Thiele DJ (2011) Copper: an essential metal in biology. *Curr Biol* 21:R877–R883
- Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, Corazzari M, Fuoco C, Ucar A, Schwartz P et al (2007) Ambra1 regulates autophagy and development of the nervous system. *Nature* 447:1121–1125. <https://doi.org/10.1038/nature05925>
- Fink SL, Cookson BT (2006) Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell Microbiol* 8:1812–1825. <https://doi.org/10.1111/j.1462-5822.2006.00751.x>
- Fletcher-Etherington A, Nobre L, Nightingale K, Antrobus R, Nichols J, Davison AJ, Stanton RJ, Weekes MP (2020) Human cytomegalovirus protein pUL36: a dual cell death pathway inhibitor. *Proc Natl Acad Sci USA* 117:18771–18779
- Gajate C, Mollinedo F (2021) Edelfosine and perifosine induce selective apoptosis in multiple myeloma by recruitment of death receptors and downstream signaling molecules into lipid rafts. *Blood* 109:711–719
- Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L et al (2007) Cell death modalities: classification and pathophysiological implications. *Cell Death Differ* 14(7):1237–1243. <https://doi.org/10.1038/sj.cdd.4402148>
- Garnish SE, Meng Y, Koide A, Sandow JJ, Denbaum E, Jacobsen AV, Yeung W, Samson AL, Horne CR, Fitzgibbon C et al (2021) Conformational interconversion of MLKL and disengagement from RIPK3 precede cell death by necroptosis. *Nat Commun* 12:2211
- Geserick P, Wang J, Schilling R, Horn S, Harris PA, Bertin J et al (2015) Absence of RIPK3 predicts necroptosis resistance in malignant melanoma. *Cell Death Dis* 6(9):e1884–e1812
- Gorrini C, Harris IS, Mak TW (2013) Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discov* 12(12):931–947
- Grabarek J, Amstad P, Darzynkiewicz Z (2002) Use of fluorescently labeled caspase inhibitors as affinity labels to detect activated caspases. *Hum Cell* 15:1–12
- Guo F, Liu X, Cai H, Le W (2018) Autophagy in neurodegenerative diseases: pathogenesis and therapy. *Brain Pathol* 28(1):3–13. <https://doi.org/10.1111/bpa.12545>. Epub 2017 Aug 6. PMID: 28703923; PMCID: PMC5739982

- Guo M, Lu B, Gan J, Wang S, Jiang X, Li H (2021) Apoptosis detection: a purpose-dependent approach selection. *Cell Cycle* 20(11):1033–1040. <https://doi.org/10.1080/15384101.2021.1919830>
- Han YH, Liu XD, Jin MH, Sun HN, Kwon T (2023) Role of NLRP3 inflammasome-mediated neuronal pyroptosis and neuroinflammation in neurodegenerative diseases. *Inflamm Res* 72(9):1839–1859. <https://doi.org/10.1007/s00011-023-01790-4>
- He WT, Wan H, Hu L, Chen P, Wang X, Huang Z et al (2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1 β secretion. *Cell Res* 25(12):1285–1298. <https://doi.org/10.1038/cr.2015.139>
- Hengartner MO (2000) The biochemistry of apoptosis. *Nature* 407:770–776
- Höckendorf U, Yabal M, Herold T, Munkhbaatar E, Rott S, Jilg S et al (2016) RIPK3 restricts myeloid leukemogenesis by promoting cell death and differentiation of leukemia initiating cells. *Cancer Cell* 30(1):75–91
- Hofmann WK, de Vos S, Tsukasaki K, Wachsman W, Pinkus GS, Said JW, Koeffler HP (2001) Altered apoptosis pathways in mantle cell lymphoma detected by oligonucleotide microarray. *Blood* 98:787–794
- Hosseini-khah Z, Babaei MR, Tehrani M, Cucchiariini M, Madry H, Ajami A et al (2021) SOX2 and Bcl-2 as a novel prognostic value in hepatocellular carcinoma progression. *Curr Oncol* 28(4):3015–3029
- Huang X, Wang T, Ye J, Feng H, Zhang X, Ma X et al (2022) FDX1 expression predicts favourable prognosis in clear cell renal cell carcinoma identified by bioinformatics and tissue microarray analysis. *Front Genet* 13:994741
- Jain A, Lamark T, Sjøttem E, Bowitz Larsen K, Atesoh Awuh J, Øvervatn A, McMahon M, Hayes JD, Johansen T (2010) P62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J Biol Chem* 285(29):22576–22591. <https://doi.org/10.1074/jbc.M110.118976>
- Jia C, Chen H, Zhang J, Zhou K, Zhuge Y, Niu C et al (2019) Role of pyroptosis in cardiovascular diseases. *Int Immunopharmacol* 67:311–318. <https://doi.org/10.1016/j.intimp.2018.12.028>
- Jiang L, Poon I (2019) Methods for monitoring the progression of cell death, cell disassembly and cell clearance. *Apoptosis* 24:208–220
- Jiang L, Kon N, Li T, Wang SJ, Su T, Hibshoosh H, Gu W (2015) Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520(7545):57–62
- Jin X, Tang J, Qiu X, Nie X, Ou S, Wu G, Zhang R, Zhu J (2024) Ferroptosis: emerging mechanisms, biological function, and therapeutic potential in cancer and inflammation. *Cell Death Discov* 10(1):45. <https://doi.org/10.1038/s41420-024-01825-7>
- Jung CH, Ro SH, Cao J, Otto NM, Kim DH (2010) mTOR regulation of autophagy. *FEBS Lett* 584(7):1287–95. <https://doi.org/10.1016/j.febslet.2010.01.017>. Epub 2010 Jan 18. PMID: 20083114; PMCID: PMC2846630
- Jung S, Jeong H, Yu SW (2020) Autophagy as a decisive process for cell death. *Exp Mol Med* 52:921–930
- Kaiser WJ, Sridharan H, Huang C, Mandal P, Upton JW, Gough PJ, Sehon CA, Marquis RW, Bertin J, Mocarski ES (2013) Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. *J Biol Chem* 288:31268–31279
- Kajiwara T, Takeuchi T, Ueki T, Moriyama N, Ueki K, Kakizoe T et al (1999) Effect of Bcl-2 overexpression in human prostate cancer cells in vitro and in vivo. *Intl J Urol* 6(10):520–525. <https://doi.org/10.1046/j.1442-2042.1999.00102.x>
- Kaplanov I, Carmi Y, Kornetsky R, Shemesh A, Shurin GV, Shurin MR et al (2019) Blocking IL-1 β reverses the immunosuppression in mouse breast cancer and synergizes with anti-PD-1 for tumor abrogation. *Proc Natl Acad Sci USA* 116(4):1361–1369. <https://doi.org/10.1073/pnas.1812266115>
- Kapoor I, Bodo J, Hill BT, Hsi ED, Almasan A (2020) Targeting BCL-2 in B-cell malignancies and overcoming therapeutic resistance. *Cell Death Dis* 11(11):941. <https://doi.org/10.1038/s41419-020-03144-y>

- Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239–257
- Koo GB, Morgan MJ, Lee DG, Kim WJ, Yoon JH, Koo JS et al (2015) Methylation dependent loss of RIP3 expression in cancer represses programmed necrosis in response to chemotherapeutics. *Cell Res* 25(6):707–725
- Koppula P, Zhang Y, Zhuang L, Gan B (2018) Amino acid transporter SLC7A11/xCT at the crossroads of regulating redox homeostasis and nutrient dependency of cancer. *Cancer Commun* 38:1–13
- Koppula P, Zhuang L, Gan B (2021) Cystine transporter SLC7A11/xCT in cancer: ferroptosis, nutrient dependency, and cancer therapy. *Protein Cell* 12(8):599–620
- Kosova F, Cetin B, Akinci M, Aslan S, Seki A, Pirhan Y et al (2012) Serum copper levels in benign and malignant thyroid diseases. *Bratisl Lek Listy Slovakia* 113:718–720
- Ktistakis NT (2017) In praise of M. Anselmier who first used the term “autophagie” in 1859. *Autophagy* 13:2015–2017
- Latz E, Xiao TS, Stutz A (2013) Activation and regulation of the inflammasomes. *Nat Rev Immunol* 13(6):397–411. <https://doi.org/10.1038/nri3452>
- Lei G, Zhuang L, Gan B (2022) Targeting ferroptosis as a vulnerability in cancer. *Nat Rev Cancer* 22(7):381–396. <https://doi.org/10.1038/s41568-022-00459-0>
- Lei S, Li S, Xiao W, Jiang Q, Yan S, Xiao W et al (2023) Azurocidin 1 inhibits the aberrant proliferation of triple-negative breast cancer through the regulation of pyroptosis. *Oncol Rep* 50(4). <https://doi.org/10.3892/or.2023.8625>
- Li P, Shi J, He Q, Hu Q, Wang YY, Zhang LJ et al (2015) *Streptococcus pneumoniae* induces autophagy through the inhibition of the PI3K-I/Akt/mTOR pathway and ROS hypergeneration in A549 cells. *PLoS One* 10:e0122753
- Li Z, Huang Z, Zhang H, Lu J, Tian Y, Wei Y et al (2021) P2X7 receptor induces pyroptotic inflammation and cartilage degradation in osteoarthritis *via* NF- κ B/NLRP3 crosstalk. *Oxidative Med Cell Longev* 2021:8868361
- Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, Jung JU (2006) Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* 8:688–699. <https://doi.org/10.1038/ncb1426>
- Liang D, Feng Y, Zandkarimi F, Wang H, Zhang Z, Kim J, Jiang X (2023) Ferroptosis surveillance independent of GPX4 and differentially regulated by sex hormones. *Cell* 186(13):2748–2764
- Linder B, Kögel D (2019) Autophagy in cancer cell death. *Biology (Basel)* 8(4):82. <https://doi.org/10.3390/biology8040082>. PMID: 31671879; PMCID: PMC6956186
- Liu Y, Shoji-Kawata S, Sumpter RM Jr, Wei Y, Ginet V, Zhang L, et al. (2013). Autosis is a Na⁺,K⁺-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia-ischemia. *Proc Natl Acad Sci USA*, 110:20364–20371
- Liu WJ, Ye L, Huang WF et al (2016a) p62 links the autophagy pathway and the ubiquitin–proteasome system upon ubiquitinated protein degradation. *Cell Mol Biol Lett* 21(29):1–14. <https://doi.org/10.1186/s11658-016-0031-z>
- Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H et al (2016b) Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* 535(7610):153–158. <https://doi.org/10.1038/nature18629>
- Liu L, Sha R, Yang L, Zhao X, Zhu Y, Gao J et al (2018) Impact of morphology on iron oxide nanoparticles-induced inflammasome activation in macrophages. *ACS Appl Mater Inter* 10: 41197–41206
- Liu W, Yang D, Shi J, Wen P, Zhang J, Wang Z et al (2021) Caspase-1 inhibitor reduces Pyroptosis induced by brain death in kidney [Original Research]. *Front Surg* 8. <https://doi.org/10.3389/fsurg.2021.760989>
- Maejima Y, Isobe M, Sadoshima J (2016) Regulation of autophagy by Beclin 1 in the heart. *J Mol Cell Cardiol* 95:19–25
- Maiuri MC, Zalckvar E, Kimchi A, Kroemer G (2007) Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 8:741–752

- McCormick KD, Ghosh A, Trivedi S, Wang L, Coyne CB, Ferris RL et al (2016) Innate immune signaling through differential RIPK1 expression promote tumor progression in head and neck squamous cell carcinoma. *Carcinogenesis* 37(5):522–529
- Meng Y, Davies KA, Fitzgibbon C, Young SN, Garnish SE, Horne CR, Luo C, Garnier JM, Liang LY, Cowan AD et al (2021) Human RIPK3 maintains MLKL in an inactive conformation prior to cell death by necroptosis. *Nat Commun* 12:6783
- Meng Y, Horne CR, Samson AL, Dagley LF, Young SN, Sandow JJ, Czabotar PE, Murphy JM (2022) Human RIPK3 C-lobe phosphorylation is essential for necroptotic signaling. *Cell Death Dis* 13:565
- Mirshafiee V, Sun B, Chang C, Liao Y, Jiang W, Jiang J et al (2018) Toxicological profiling of metal oxide nanoparticles in liver context reveals pyroptosis in Kupffer cells and macrophages versus apoptosis in hepatocytes. *ACS Nano* 12:3836–3852
- Miyake H, Hanada N, Nakamura H, Kagawa S, Fujiwara T, Hara I et al (1998) Overexpression of Bcl-2 in bladder cancer cells inhibits apoptosis induced by cisplatin and adenoviral-mediated p53 gene transfer. *Oncogene* 16(7):933–943. <https://doi.org/10.1038/sj.onc.1201602>
- Mompeán M, Li W, Li J, Laage S, Siemer AB, Bozkurt G, Wu H, McDermott AE (2018) The structure of the necrosome RIPK1-RIPK3 core, a human hetero-amyloid signaling complex. *Cell* 173:1244–1253.e1210
- Moriwaki K, Bertin J, Gough PJ, Orlowski GM, Chan FKM (2015) Differential roles of RIPK1 and RIPK3 in TNF-induced necroptosis and chemotherapeutic agent-induced cell death. *Cell Death Dis* 6:e1636
- Murai S, Yamaguchi Y, Shirasaki Y, Yamagishi M, Shindo R, Hildebrand JM, Miura R, Nakabayashi O, Totsuka M, Tomida T et al (2018) A FRET biosensor for necroptosis uncovers two different modes of the release of DAMPs. *Nat Commun* 9:4457
- Murphy J, Czabotar P, Hildebrand J, Lucet Isabelle S, Zhang J, Alvarez-Diaz S, Lewis R, Lalaoui N, Metcalf D, Webb A et al (2013) The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity* 39:443–453
- Nassour J, Radford R, Correia A, Fuste JM, Schoell B, Jauch A, Shaw RJ, Karlseder J (2019) Autophagic cell death restricts chromosomal instability during replicative crisis. *Nature* 2019(565):659–663. <https://doi.org/10.1038/s41586-019-0885-0>
- Nevalainen TJ (1975) Cytotoxicity of vinblastine and vincristine to pancreatic acinar cells. *Virchows Arch B Cell Pathol* 18:119–127
- Nixon RA, Yang DS (2012) Autophagy and neuronal cell death in neurological disorders. *Cold Spring Harb Perspect Biol* 4(10):a008839. <https://doi.org/10.1101/cshperspect.a008839>. PMID: 22983160; PMCID: PMC3475163
- Nugues A-L, El Bouazzati H, Hetuin D, Berthon C, Loyens A, Bertrand E et al (2014) RIP3 is downregulated in human myeloid leukemia cells and modulates apoptosis and caspase-mediated p65/RelA cleavage. *Cell Death Dis* 5(8):e1384
- Oh S-J, Ikeda M, Ide T, Hur KY, Lee M-S (2022) Mitochondrial event as an ultimate step in ferroptosis. *Cell Death Discov* 8(1):414. <https://doi.org/10.1038/s41420-022-01199-8>
- Ohsumi Y (2014) Historical landmarks of autophagy research. *Cell Res* 24:9–23
- Oliveri V (2022) Selective targeting of cancer cells by copper ionophores: an overview. *Front Mol Biosci* 9:841814
- Park W, Wei S, Kim BS et al (2023) Diversity and complexity of cell death: a historical review. *Exp Mol Med* 55:1573–1594. <https://doi.org/10.1038/s12276-023-01078-x>
- Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P (2008) Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie* 90:313–323
- Pavithra V, Sathisha TG, Kasturi K, Mallika DS, Amos SJ, Ragunatha S. (2015). Serum levels of metal ions in female patients with breast cancer. *J Clin Diagn Res*, 9:BC25-c27
- Pearson JS, Giogha C, Muhlen S, Nachbur U, Pham CL, Zhang Y, Hildebrand JM, Oates CV, Lung TW, Ingle D et al (2017) EspL is a bacterial cysteine protease effector that cleaves RHIM proteins to block necroptosis and inflammation. *Nat Microbiol* 2:16258

- Poot M, Pierce RH (1999) Detection of changes in mitochondrial function during apoptosis by simultaneous staining with multiple fluorescent dyes and correlated multiparameter flow cytometry. *Cytometry* 35:311–317
- Raggi C, Taddei ML, Sacco E, Navari N, Correnti M, Piombanti B et al (2021) Mitochondrial oxidative metabolism contributes to a cancer stemcell phenotype in cholangiocarcinoma. *J Hepatol Netherlands* 74:1373–1385
- Ramadani M, Gansauge F, Schlosser S, Yang Y, Beger HG, Gansauge S (2001) Overexpression of caspase-1 in pancreatic disorders: implications for a function besides apoptosis. *J Gastrointest Surg* 5(4):352–358. [https://doi.org/10.1016/S1091-255X\(01\)80061-5](https://doi.org/10.1016/S1091-255X(01)80061-5)
- Rao Z, Zhu Y, Yang P, Chen Z, Xia Y, Qiao C et al (2022) Pyroptosis in inflammatory diseases and cancer. *Theranostics* 12(9):4310–4329. <https://doi.org/10.7150/thno.71086>
- Rathinam VAK, Zhao Y, Shao F (2019) Innate immunity to intracellular LPS. *Nat Immunol* 20(5): 527–533. <https://doi.org/10.1038/s41590-019-0368-3>
- Reed, J, Hakam, A, Nicosia, SV, & Coppola, D. (2005) Significance of Fas receptor protein expression in epithelial ovarian cancer. *Hum Pathol* 36(9):971–976. <https://doi.org/10.1016/j.humpath.2005.06.015>
- Russo AJ, Vasudevan SO, Mendez-Huergo SP, Kumari P, Menoret A, Duduskar S et al (2021) Intracellular immune sensing promotes inflammation *via* gasdermin D-driven release of a lectin alarmin. *Nat Immunol* 22:154–165
- Rusten T, Stenmark H (2010) p62, an autophagy hero or culprit? *Nat Cell Biol* 12:207–209. <https://doi.org/10.1038/ncb0310-207>
- Saleh SAK, Adly HM, Abdelkhalik AA, Nassir AM (2020) Serum levels of selenium, zinc, copper, manganese, and iron in prostate cancer patients. *Curr Urol* 14:44–49
- Samson AL, Zhang Y, Geoghegan ND, Gavin XJ, Davies KA, Mlodzianoski MJ, Whitehead LW, Frank D, Garnish SE, Fitzgibbon C et al (2020) MLKL trafficking and accumulation at the plasma membrane control the kinetics and threshold for necroptosis. *Nat Commun* 11: 3151–3151
- Sarhan J, Liu BC, Muendlein HI, Li P, Nilson R, Tang AY et al (2018) Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during *Yersinia* infection. *Proc Natl Acad Sci USA* 115: E10888–E10897
- Sborgi L, Rühl S, Mulvihill E, Pipercevic J, Heilig R, Stahlberg H et al (2016) GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J* 35(16):1766–1778. <https://doi.org/10.15252/embj.201694696>
- Schweichel JU, Merker HJ (1973) The morphology of various types of cell death in preliminary issues. *Teratology* 7:253–266
- Shchors K, Massaras A, Hanahan D (2015) Dual targeting of the Autophagic regulatory circuitry in gliomas with repurposed drugs elicits cell-lethal autophagy and therapeutic benefit. *Cancer Cell* 28(4):456–471. <https://doi.org/10.1016/j.ccell.2015.08.012>
- Sheftel AD, Stehling O, Pierik AJ, Elsässer HP, Mühlenhoff U, Webert H et al (2010) Humans possess two mitochondrial ferredoxins, Fdx1 and Fdx2, with distinct roles in steroidogenesis, heme, and Fe/S cluster biosynthesis. *Proc Natl Acad Sci USA* 107:11775–11780
- Shen HM, Codogno P (2011) Autophagic cell death: loch ness monster or endangered species? *Autophagy* 7:457–465
- Shen XG, Wang C, Li Y, Wang L, Zhou B, Xu B et al (2010) Downregulation of caspase-9 is a frequent event in patients with stage II colorectal cancer and correlates with poor clinical outcome. *Color Dis* 12(12):1213–1218. <https://doi.org/10.1111/j.1463-1318.2009.02009.x>
- Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P et al (2014) Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* 514(7521):187–192. <https://doi.org/10.1038/nature13683>
- Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H et al (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 526(7575):660–665. <https://doi.org/10.1038/nature15514>

- Shi Y, Wu C, Shi J, Gao T, Ma H, Li L, Zhao Y (2024) Protein phosphorylation and kinases: potential therapeutic targets in necroptosis. *Eur J Pharmacol* 176508
- Sinha S, Levine B (2008) The autophagy effector Beclin 1: a novel BH3-only protein. *Oncogene* 27 (Suppl 1):S137–S148. <https://doi.org/10.1038/onc.2009.51>
- Sun Y, Guo Y (2018) Expression of Caspase-1 in breast cancer tissues and its effects on cell proliferation, apoptosis and invasion. *Oncol Lett* 15(5):6431–6435. <https://doi.org/10.3892/ol.2018.8176>
- Sun L, Wang H, Wang Z, He S, Chen S, Liao D, Wang L, Yan J, Liu W, Lei X, Wang X (2012) Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell* 148:213–227
- Sun WY, Tyurin VA, Mikulska-Ruminska K, Shrivastava IH, Anthonymuthu TS, Zhai YJ, Kagan VE (2021) Phospholipase iPLA2 β averts ferroptosis by eliminating a redox lipid death signal. *Nat Chem Biol* 17(4):465–476
- Sun Y, Xue Z, Huang T, Che X, Wu G (2022) Lipid metabolism in ferroptosis and ferroptosis-based cancer therapy. *Front Oncol* 12:941618
- Takahashi Y, Coppola D, Matsushita N, Cuaing HD, Sun M, Sato Y, Liang C, Jung JU, Cheng JQ, Mule JJ et al (2007) Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* 9:1142–1151. <https://doi.org/10.1038/ncb1634>
- Tang D, Chen X, Kang R, Kroemer G (2021) Ferroptosis: molecular mechanisms and health implications. *Cell Res* 31(2):107–125. <https://doi.org/10.1038/s41422-020-00441-1>
- Tkachenko A, Onishchenko A, Myasoedov V, Yefimova S, Havranek O (2023) Assessing regulated cell death modalities as an efficient tool for in vitro nanotoxicity screening: a review. *Nanotoxicology* 17(3):218–248. <https://doi.org/10.1080/17435390.2023.2203239>
- Tsvetkov P, Detappe A, Cai K, Keys HR, Brune Z, Ying W et al (2019) Mitochondrial metabolism promotes adaptation to proteotoxic stress. *Nat Chem Biol* 15:681–689
- Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M et al (2022) Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science* 375:1254–1261
- Tuckermann JP, Kleiman A, McPherson KG, Reichardt HM (2005) Molecular mechanisms of glucocorticoids in the control of inflammation and lymphocyte apoptosis. *Crit Rev Clin Lab Sci* 42:71–104
- Vallat L, Magdelenat H, Merle-Beral H, Masdehors P, Potocki de Montalk G, Davi F, Kruhoffer M, Sabatier L, Orntoft TF, Delic J (2003) The resistance of B-CLL cells to DNA damage-induced apoptosis defined by DNA microarrays. *Blood* 101:4598–4606
- Vande Walle L, Lamkanfi M (2016) Pyroptosis. *Curr Biol* 26(13):R568–r572. <https://doi.org/10.1016/j.cub.2016.02.019>
- Vanzo R, Bartkova J, Merchut-Maya JM, Hall A, Bouchal J, Dyrskjot L, Frankel LB, Gorgoulis V, Maya-Mendoza A, Jaattela M et al (2019) Autophagy role(s) in response to oncogenes and DNA replication stress. *Cell Death Differ*. <https://doi.org/10.1038/s41418-019-0403-9>
- Volchuk A, Ye A, Chi L, Steinberg BE, Goldenberg NM (2020) Indirect regulation of HMGB1 release by gasdermin D. *Nat Commun* 11:4561
- Wang L, Yang JK, Kabaleeswaran V, Rice AJ, Cruz AC, Park AY, et al. (2010) The Fas-FADD death domain complex structure reveals the basis of DISC assembly and disease mutations. *Nat Struct Mol Biol* 17:1324
- Wang Y, Gao W, Shi X, Ding J, Liu W, He H et al (2017) Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature* 547:99–103
- Wang WJ, Chen D, Jiang MZ, XU B, Li XW, Chu Y et al (2018) Downregulation of gasdermin D promotes gastric cancer proliferation by regulating cell cycle-related proteins. *J Dig Dis* 19(2): 74–83. <https://doi.org/10.1111/1751-2980.12576>
- Wang Q, Wang Y, Ding J, Wang C, Zhou X, Gao W et al (2020) A bioorthogonal system reveals antitumour immune function of pyroptosis. *Nature* 579:421–426
- Wang S, Liu Y, Zhang L, Sun Z (2021a) Methods for monitoring cancer cell pyroptosis. *Cancer Biol Med* 19(4):398–414. <https://doi.org/10.20892/j.issn.2095-3941.2021.0504>. Epub ahead of print. PMID: 34931767; PMCID: PMC9088190

- Wang W, Wang X, Luo J, Chen X, Ma K, He H et al (2021b) Serum copper level and the copper-to-zinc ratio could be useful in the prediction of Lung cancer and its prognosis: a case-control study in Northeast China. *Nutr Cancer United States* 73:1908–1915
- Wang L, Cao Y, Guo W, Xu J. (2022) High expression of cuproptosis-related gene FDX1 in relation to good prognosis and immune cells infiltration in colon adenocarcinoma (COAD). Germany: *J Cancer Res Clin Oncol*:2022
- Wang Y, Chen Y, Zhang J, Yang Y, Fleishman JS, Wang Y, Wang H (2023) Cuproptosis: a novel therapeutic target for overcoming cancer drug resistance. *Drug Resist Updat* 101018
- Wei S, Feng M, Zhang S (2022) Molecular characteristics of cell Pyroptosis and its inhibitors: a review of activation, regulation, and inhibitors. *Int J Mol Sci* 23(24):16115
- White MK, Cinti C (2004) A morphologic approach to detect apoptosis based on electron microscopy. *Methods Mol Biol* 285:105–111
- Wilson NS, Dixit V, Ashkenazi A (2009) Death receptor signal transducers: nodes of coordination in immune signaling networks. *Nat Immunol* 10:348–355
- Wyllie AH (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284:555–556
- Xia J, Chu C, Li W, Chen H, Xie W, Cheng R et al (2022) Mitochondrial protein UCP1 inhibits the malignant behaviors of triple-negative breast cancer through activation of mitophagy and pyroptosis. *Int J Biol Sci* 18(7):2949–2961. <https://doi.org/10.7150/ijbs.68438>
- Xiao, W, Ibrahim, ML, Redd, PS, Klement, JD, Lu, C, Yang, D, et al. (2019). Loss of FAS expression and function is coupled with colon cancer resistance to immune checkpoint inhibitor immunotherapy. *Mol Cancer Res* 17(2):420–430. <https://doi.org/10.1158/1541-7786.Mcr-18-0455>
- Xie Y, Wang B, Zhao Y, Tao Z, Wang Y, Chen G, Hu X (2022) Mammary adipocytes protect triple-negative breast cancer cells from ferroptosis. *J Hematol Oncol* 15(1):72
- Yan HF, Zou T, Tuo QZ, Xu S, Li H, Belaidi AA, Lei P (2021) Ferroptosis: mechanisms and links with diseases. *Signal Transduct Targeted Ther* 6(1):49. <https://doi.org/10.1038/s41392-020-00428-9>
- Yang Z, Klionsky DJ (2009) An overview of the molecular mechanism of autophagy. *Curr Top Microbiol Immunol* 335:1–32
- Yang Y, Cheon S, Jung MK, Song SB, Kim D, Kim HJ et al (2015) Interleukin-18 enhances breast cancer cell migration via down-regulation of claudin-12 and induction of the p38 MAPK pathway. *Biochem Biophys Res Commun* 459(3):379–386. <https://doi.org/10.1016/j.bbrc.2015.02.108>
- Yang W, Liu S, Li Y, Wang Y, Deng Y, Sun W et al (2020) Pyridoxine induces monocyte-macrophages death as specific treatment of acute myeloid leukemia. *Cancer Lett* 492:96–105
- Yang L, Zhang Y, Wang Y, Jiang P, Liu F, Feng N (2022) Ferredoxin 1 is a cuproptosis- key gene responsible for tumor immunity and drug sensitivity: a pan-cancer analysis. *Front Pharmacol* 13: 938134
- Zeng W, Wu D, Sun Y, Suo Y, Yu Q, Zeng M et al (2021) The selective NLRP3 inhibitor MCC950 hinders atherosclerosis development by attenuating inflammation and pyroptosis in macrophages. *Sci Rep* 11(1):19305. <https://doi.org/10.1038/s41598-021-98437-3>
- Zhang H, Baehrecke EH (2015) Eaten alive: novel insights into autophagy from multicellular model systems. *Trends Cell Biol* 25:376–387
- Zhang Z, Singh R, Aschner M (2016) Methods for the detection of autophagy in mammalian cells. *Curr Protoc Toxicol* 69:20.12.1–20.12.26. <https://doi.org/10.1002/cptx.11>. PMID: 27479363; PMCID: PMC4982470
- Zhang Y, Su SS, Zhao S, Yang Z, Zhong C-Q, Chen X, Cai Q, Yang Z-H, Huang D, Wu R, Han J (2017) RIP1 autophosphorylation is promoted by mitochondrial ROS and is essential for RIP3 recruitment into necrosome. *Nat Commun* 8(1):14329. <https://doi.org/10.1038/ncomms14329>
- Zhang Y, Shi J, Liu X, Feng L, Gong Z, Koppula P, Gan B (2018) BAP1 links metabolic regulation of ferroptosis to tumour suppression. *Nat Cell Biol* 20(10):1181–1192

- Zhang Z, Zhang Y, Xia S, Kong Q, Li S, Liu X et al (2020a) Gasdermin e suppresses tumour growth by activating anti-tumour immunity. *Nature* 579:415–420
- Zhang T, Yin C, Boyd DF, Quarato G, Ingram JP, Shubina M, Ragan KB, Ishizuka T, Crawford JC, Tummers B et al (2020b) Influenza virus Z-RNAs induce ZBP1-mediated necroptosis. *Cell* 180: 1115–1129 e1113
- Zhang C, Liu X, Jin S, Chen Y, Guo R (2022a) Ferroptosis in cancer therapy: a novel approach to reversing drug resistance. *Mol Cancer* 21(1):47. <https://doi.org/10.1186/s12943-022-01530-y>
- Zhang C, Zeng Y, Guo X, Shen H, Zhang J, Wang K et al (2022b) Pan-cancer analyses confirmed the cuproptosis-related gene FDX1 as an immunotherapy predictor and prognostic biomarker. *Front Genet* 13:923737
- Zheng Z, Bian Y, Zhang Y, Ren G, Li G (2020) Metformin activates AMPK/SIRT1/NF- κ B pathway and induces mitochondrial dysfunction to drive caspase3/GSDME-mediated cancer cell pyroptosis. *Cell Cycle* 19(10):1089–1104. <https://doi.org/10.1080/15384101.2020.1743911>
- Zhou Z, He H, Wang K, Shi X, Wang Y, Su Y et al (2020) Granzyme a from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. *Science* 368:eaaz754
- Zhou Y, Liao J, Mei Z, Liu X, Ge J. (2021). Insight into crosstalk between ferroptosis and necroptosis: novel therapeutics in ischemic stroke. *Oxid Med Cell Longev* 2021:9991001. <https://doi.org/10.1155/2021/9991001>
- Zitvogel L, Kepp O, Galluzzi L, Kroemer G (2012) Inflammasomes in carcinogenesis and anticancer immune responses. *Nat Immunol* 13(4):343–351. <https://doi.org/10.1038/ni.2224>



Clinical Implications of Epithelial-to-Mesenchymal Transition (EMT) in Cancer

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Abstract

Epithelial-to-mesenchymal transition (EMT) plays a critical role in the transformation of epithelial cells into a mesenchymal state. This process is significant in various biological contexts, including organ development, wound healing, and cancer progression. The aim of this chapter is to elucidate the role of EMT in gastrointestinal and gynecological cancers, its contribution to cancer progression and metastasis, and its implications for therapeutic strategies.

EMT is characterized by the loss of epithelial markers and the acquisition of mesenchymal markers, leading to increased cell motility and invasion. In gastrointestinal cancers, EMT is associated with poor prognosis and resistance to chemotherapy. Subtypes of gastric and colorectal cancers demonstrate varying EMT characteristics, influencing their clinical outcomes. In gynecological cancers, EMT contributes to tumor progression, metastasis, and chemoresistance.

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Key transcription factors like Snail, Twist, and Zeb1 regulate EMT, while the tumor microenvironment, including hormones and hypoxia, significantly influences this process. EMT also plays a pivotal role in cancer stem cell properties and therapy resistance, with implications for targeted therapeutic approaches.

EMT is a fundamental process in cancer progression, influencing metastasis, therapy resistance, and cancer stem cell properties. Understanding the molecular mechanisms and regulatory networks of EMT can inform the development of targeted therapies. Therapeutic strategies that inhibit EMT or reverse mesenchymal traits to epithelial characteristics hold promise in improving treatment efficacy and patient outcomes. However, careful consideration is required to balance the impact on normal physiological processes and avoid adverse effects.

Keywords

Epithelial-to-mesenchymal transition · Gastrointestinal cancers · Gynecological cancers

1 Introduction

The phenomenon known as the epithelial-to-mesenchymal transition (EMT) plays a pivotal role in the conversion of epithelial cells into a mesenchymal state. This profound transformation is marked by the loss of essential epithelial markers including E-cadherin, β -catenin, occludin, claudin, plakophilin, cytokeratin, and desmoplakin, alongside the acquisition of mesenchymal markers such as N-cadherin, vimentin, and fibronectin (Mirantes et al. 2013). Through this transition, epithelial cells not only lose their polarity and detachment from adjacent cells and the basement membrane but also acquire augmented migration and invasion abilities. Stimulated by a range of environmental factors, including hypoxia and oxidative stress, EMT manifests in three distinct biological contexts: organ implantation and growth; wound healing and tissue regeneration; and cancer progression. In the context of cancer, EMT facilitates the detachment, migration, and metastasis formation of tumor cells (Iwatsuki et al. 2010). Moreover, EMT plays a crucial role in fostering cancer stem cell traits, inhibiting apoptosis and senescence, and promoting resistance to chemotherapy treatments.

2 Gastrointestinal Cancers

The Cancer Genome Atlas (TCGA) identified four genomic gastric cancer subtypes: Epstein-Barr virus-associated (EBV), microsatellite instable (MSI), genomically stable (GS), and chromosomal instability (CIN) groups (Cancer Genome Atlas Research Network 2014). The Asian Cancer Research Group (ACRG) focused on gene expression profiles and divided gastric cancer into four subtypes with distinct

clinical outcomes: MSI, EMT, MSS/p53 active, and MSS/p53 inactive (Cristescu et al. 2015). The GS group (20% in TCGA cohort) presents diffuse histology and CDH1, RHOA mutation, and CLDN18-ARHGAP fusion. Mutation in CDH1 (the gene encoding E-cadherin) leads to the process of EMT whereby the cell loses its cell–cell adhesion capabilities and apical polarity, being a major predictor of poor prognosis after radical surgery for gastric cancer. This mutation has also been reported in the MSS/EMT group (15% in the ACRG cohort), which shows a more advanced pT stage, lymph node metastasis, perineural invasion, and a particular propensity to spread to the peritoneum. Recently, genomic and proteomic analyses have identified two distinct molecular subtypes, the mesenchymal phenotype and the epithelial phenotype. The mesenchymal phenotype shows high genomic integrity, characterized by low mutation rates and microsatellite stability, with markedly poor survival and resistance to standard chemotherapy (overlapping with GS and MSS/EMT groups). On the other hand, epithelial phenotype tumors show low genomic integrity (high mutation, MSI, and copy number alteration) with better survival rates and sensitivity to chemotherapy (Oh et al. 2018).

The pathophysiology of colorectal cancer has been shown to involve three distinct pathways: CIN, MSI, and CpG island methylation phenotype (CIMP). However, it is well known that cancer cells acquire properties associated with a more aggressive phenotype through EMT (Lu et al. 2023). To resolve inconsistencies between reported gene expression-based colorectal cancer classifications and to facilitate clinical translation, the Colorectal Cancer Subtyping Consortium (CRCSC) identifies four molecular subtypes (CMSs). Hypermutation, MSI, and a strong immune response are features of CMS1 (MSI Immune). CMS2 (canonical) shows epithelial characteristics and strong stimulation of WNT and MYC signaling. CMS3 (metabolic) displays epithelial characteristics and metabolic abnormalities. A mesenchymal phenotype with strong transforming TGF- β activation, enhanced angiogenesis, stromal activation, and inflammatory infiltration defines CMS4 (mesenchymal). Samples with mixed characteristics may represent a transitional phenotype or intra-tumoral heterogeneity. Notably, the CMS4 categorization was associated with an EMT signature and accounted for 23% of the total. CMS4 tumors tended to be diagnosed at more advanced stages and showed worse overall and relapse-free survival (Guinney et al. 2015).

3 Gynecological Cancers

Recent research is uncovering the mechanisms behind the initiation and progression of gynecological tumors, highlighting EMT as a critical factor. These studies indicate that disruptions in EMT processes within epithelial cells could be crucial for the onset, progression, and therapeutic resistance of these cancers. In ovarian cancer, the intratumoral diversity can be attributed to reversible EMT changes. Significant phenotypic variability is also observed in cases of endometrial carcinoma. A robust case in the scientific literature posits that a combination of genetic mutations, anomalies in signaling pathways, and the tumor microenvironment may

collectively drive EMT in these tumors. EMT's significant role extends to cervical cancers and, although less common, vulvar and vaginal cancers, where its role is less understood (Meacham and Morrison 2013).

Numerous signaling pathways converge to facilitate EMT, crucial for cancer progression and metastasis. EMT plays a vital role in evolving serous borderline ovarian tumors into low-grade serous ovarian carcinomas and is pivotal in promoting metastasis and chemoresistance in high-grade serous ovarian carcinoma. In gynecological cancers, a complex web of molecular mechanisms governs EMT, with transcription factors like Twist, Slug, ZEB1, ZEB2, and Snail at the forefront, leading to reduced E-cadherin expression (Yoshida et al. 2009). Alterations in the regulation of tumor suppressors and oncogenes also activate EMT, with metabolic and epigenetic changes further modulating this process, influenced by specific miRNAs (Dong et al. 2011).

The EMT induction in gynecological tumors stems from complex interactions within the tumor microenvironment. Hormones such as 17 β -estradiol (E2), follicle-stimulating hormone (FSH), and progesterone play key roles in both endometrial and ovarian carcinomas (Park et al. 2008). This environment also includes a diverse network of inflammatory cytokines and growth factors, essential in modulating EMT and fostering interactions between the tumor and surrounding stroma. Additionally, tumor hypoxia, the activation of hypoxia-inducible factor-1 (HIF-1), and oxidative stress are major contributors to the transition (Höckel and Vaupel 2001).

Further evidence suggests that EMT significantly aids in the development of chemoresistance in ovarian carcinoma. Moreover, recent findings also establish a link between EMT and the presence of circulating tumor cells in both ovarian and endometrial cancers.

4 Cancer Progression

During EMT, epithelial cells lose tight cell–cell connections and polarity, which confers migratory and invasive properties. In the reverse process referred to as mesenchymal-epithelial transition (MET), cells lose migratory freedom, begin expressing junction complexes, and adopt apicobasal polarity. Therefore, EMT and MET contribute to cancer progression and metastasis (Yang and Weinberg 2008).

In detail, the EMT confers properties that result in dissociation of tumor cells from the primary tumor mass, invasion through the extracellular matrix, intravasation into blood vessels, and colonization of distant organs, which is pivotal in cancer progression. Moreover, the re-activation of epithelial properties at the secondary site via the MET causes metastases, the primary cause of death in cancer patients (Yao et al. 2011).

Among MET-TFs that promote cancer progression and metastasis, SNAIL and Twist are the most investigated. Snail (SNAIL) was identified as the first EMT-TF that directly repressed transcription of the epithelial cell–cell adhesion molecule, E-cadherin. Overexpression of SNAIL in MDCK and many carcinoma cell lines led

to the loss of cell–cell adhesion mediated by E-cadherin, transformed the morphology of cells from epithelial to spindle-like mesenchymal, and enhanced their migratory and invasive traits *in vitro*. *SNAIL* was also shown to induce the expression of mesenchymal markers fibronectin and *Zeb1*, the latter of which is an EMT-TF that can promote tumor invasiveness *in vitro* and is correlated with tumor cell differentiation *in vivo*. Later, *Twist* was identified as yet another EMT-TF that inhibited E-cadherin as well as regulated other components of EMT in breast cancer cell lines. Silencing *Twist* suppressed the number of lung metastases significantly, however, did not completely inhibit them, still leaving open the possibility that *Twist*, and potentially other EMT-TFs, may act more as catalysts rather than drivers of metastasis. Therefore, the adoption of an EMT-like program enables tumor cells to invade extracellular matrix through mesenchymal invasion mode (Jolly et al. 2017).

5 Frontiers in Therapeutics

EMT causes the loss of cell–cell adhesions and disrupts apicobasal polarity, leading to an elongated cell shape that can penetrate the extracellular matrix. At the molecular level, EMT involves downregulating epithelial-specific proteins and acquiring mesenchymal markers (Nieto 2011). Genetic mutations, such as the loss of *P53* and *PTEN* or mutations in *KRAS*, can promote EMT (Yamasaki et al. 2022). EMT contributes to metastasis, with transcription factors like *Twist* and *Zeb1* playing crucial roles in various cancer types. During metastasis, the MET program helps migratory cancer stem cells (CSCs) settle and regain epithelial characteristics. Reversible EMT-MET programs are common in secondary tumor establishment. Furthermore, EMT is linked to therapy resistance, with cancer cells acquiring a mesenchymal phenotype that upregulates drug efflux transporters, contributing to multidrug resistance. For example, P-glycoprotein levels are higher in mesenchymal, therapy-resistant ovarian cancer cells (Lambert et al. 2017). EMT also promotes “stemness” in CSCs, which protect themselves from chemotherapy by expressing high levels of drug efflux pumps. EMT enhances DNA repair, counteracting chemotherapy-induced damage. Proteins like *SIRT6* and transcription factors like *Slug* regulate DNA repair genes, furthering resistance to DNA-damaging agents (Shibue and Weinberg 2017).

Studies show a strong correlation between mesenchymal markers and treatment resistance. In ovarian cancer, inhibiting EMT can eliminate cisplatin resistance (Zhang et al. 2019a). A mesenchymal gene signature is associated with resistance to specific inhibitors in non-small-cell lung cancer (NSCLC) (Byers et al. 2013). Despite advancements in therapies, drug resistance remains a major challenge, causing up to 90% of cancer-related deaths. This resistance is often transient and reversible, influenced by epigenetic modifications, transcription factors, signaling pathways, and the tumor microenvironment. In ovarian cancer, EMT plays a role in tumor initiation, supported by evidence that EMT leads to precursor lesions. *TGF- β* , found in follicular fluid, can induce EMT and promote these lesions. Standard chemotherapy for epithelial ovarian cancer involves carboplatin and paclitaxel, but

recurrence rates are high, underscoring the need to understand therapy resistance mechanisms (Shaffer et al. 2017).

EMT contributes to resistance by facilitating drug efflux, enhancing DNA repair, and promoting stemness, leading to the survival of therapy-resistant cancer cells. EMT also impairs immunosurveillance and promotes immunotherapy resistance by upregulating immune checkpoint markers like PD-L1 (Chen et al. 2014). Addressing these challenges involves targeting EMT-promoting pathways and molecular drivers of EMT (Jonckheere et al. 2022).

Targeting interactions between EMT-TFs (epithelial-mesenchymal transition transcription factors) and important cofactors, as well as multiple EMT-TFs, can be an effective strategy. Additionally, targeting mesenchymal cells, the extracellular matrix (ECM), or inhibiting MET (mesenchymal-epithelial transition) are potential therapeutic approaches.

Addressing quasi-mesenchymal cancer cells in the EMT process may prevent immunosuppression and enhance sensitivity to checkpoint immunotherapy. Another approach involves targeting the molecular drivers of EMT. While EMT-TFs are primary regulators, directly targeting them is challenging due to their complex interactions and overlapping functions (Pattabiraman et al. 2016). Precision control of epigenetic processes can offer reversible and enduring reprogramming. Modified synthetic miRNAs, such as liposomal miR-34 (MRX34), have been tested in clinical trials for their impact on tumor growth and metastasis (Zhang et al. 2019a, b). Targeting mesenchymal cells and the ECM or inhibiting MET involves re-differentiation by impeding mesenchymal cell proteins, reducing cellular plasticity, and re-sensitizing tumor cells to treatments. Biologically active compounds or monoclonal antibodies against proteins like vimentin, fibronectin, and N-cadherin can be used. A distinct group of quasi-mesenchymal (qM) cells within the EMT process tends to metastasize and resist immune checkpoint therapy. Disrupting factors targeting qM cells can prevent immunosuppression and enhance response to immunotherapy (Dongre et al. 2021). Targeting interactions between cancer cells and the ECM, particularly through integrins, is another promising strategy. Inhibiting integrins has shown efficacy in reducing tumor metastasis and maintaining stable disease states (Li et al. 2021). EMT activation involves ECM degradation by proteases from the MMP family, aiding cancer cell entry into the circulatory system. Research on MMP inhibitors and other potential anti-cancer drugs is ongoing, but EMT-targeted therapy poses significant challenges due to potential adverse effects (Alba et al. 2022). EMT-targeted therapy may inhibit tumor cell migration and invasion but must prevent early cancer cell dissemination. Combining EMT-targeted therapy with conventional chemotherapy may enhance drug sensitivity and confine cancer cells to the primary site (Shen et al. 2019). In later stages, EMT inhibitors may reduce CSCs (cancer stem cells) and deter secondary tumor formation. In fact, in triple-negative breast cancer, the GSK-3 β inhibitor BIO effectively inhibited EMT and CSC characteristics. Reducing CSC properties decreases ABC protein expression, reducing drug efflux and improving chemotherapy efficacy (Vijay et al. 2019). The optimal timing for EMT-targeted therapy, whether in early or advanced disease stages, remains uncertain.

Targeting EMT may have dual effects, potentially inducing MET, which could promote tumor proliferation and metastasis. Given EMT's role in physiological processes like wound healing, targeting it could affect normal cells and repair mechanisms. Therefore, EMT-targeted therapy requires careful consideration of its intended impact and potential side effects.

Data Availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Compliance with Ethical Standards

Funding No.

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References

- Alba J, Barcia R, Gutiérrez-Berzal J, Ramos-Martínez JI (2022) Could inhibition of metalloproteinases be used to block the process of metastasis? *Cell Biochem Funct* 40(6): 600–607
- Byers LA, Diao L, Wang J, Saintigny P, Girard L, Peyton M, Shen L, Fan Y, Giri U, Tumula PK, Nilsson MB, Gudikote J, Tran H, Cardnell RJ, Bearss DJ, Warner SL, Foulks JM, Kanner SB, Gandhi V, Krett N, Rosen ST, Kim ES, Herbst RS, Blumenschein GR, Lee JJ, Lippman SM, Ang KK, Mills GB, Hong WK, Weinstein JN, Wistuba II, Coombes KR, Minna JD, Heymach JV (2013) An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. *Clin Cancer Res* 19(1):279–290
- Cancer Genome Atlas Research Network (2014) Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 513(7517):202–209
- Chen L, Gibbons DL, Goswami S, Cortez MA, Ahn YH, Byers LA, Zhang X, Yi X, Dwyer D, Lin W, Diao L, Wang J, Roybal J, Patel M, Ungewiss C, Peng D, Antonia S, Mediavilla-Varela M, Robertson G, Suraokar M, Welsh JW, Erez B, Wistuba II, Chen L, Peng D, Wang S, Ullrich SE, Heymach JV, Kurie JM, Qin FX (2014) Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. *Nat Commun* 28(5):5241
- Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A (2015) Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* 21(5):449–456
- Dong P, Kaneuchi M, Watari H, Hamada J, Sudo S, Ju J, Sakuragi N (2011) MicroRNA-194 inhibits epithelial to mesenchymal transition of endometrial cancer cells by targeting oncogene BMI-1. *Mol Cancer* 18(10):99
- Dongre A, Rashidian M, Eaton EN, Reinhardt F, Thiru P, Zagorulya M, Nepal S, Banaz T, Martner A, Spranger S, Weinberg RA (2021) Direct and indirect regulators of epithelial-mesenchymal transition-mediated immunosuppression in breast carcinomas. *Cancer Discov* 11(5):1286–1305
- Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa E, Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L,

- Delorenzi M, Kopetz S, Vermeulen L, Tejpar S (2015) The consensus molecular subtypes of colorectal cancer. *Nat Med* 21(11):1350–1356
- Höckel M, Vaupel P (2001) Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 93(4):266–276
- Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S, Baba H, Mori M (2010) Epithelial-mesenchymal transition in cancer development and its clinical significance. *Cancer Sci* 101(2):293–299
- Jolly MK, Ware KE, Gilja S, Somarelli JA, Levine H (2017) EMT and MET: necessary or permissive for metastasis? *Mol Oncol* 11(7):755–769
- Jonckheere S, Adams J, De Groote D, Campbell K, Bex G, Goossens S (2022) Epithelial-Mesenchymal Transition (EMT) as a therapeutic target. *Cells Tissues Organs* 211(2):157–182
- Lambert AW, Pattabiraman DR, Weinberg RA (2017) Emerging biological principles of metastasis. *Cell* 168(4):670–691
- Li M, Wang Y, Li M, Wu X, Setrerrahmane S, Xu H (2021) Integrins as attractive targets for cancer therapeutics. *Acta Pharm Sin B* 11(9):2726–2737. <https://doi.org/10.1016/j.apsb.2021.01.004>
- Lu J, Kornmann M, Traub B (2023) Role of epithelial to mesenchymal transition in colorectal cancer. *Int J Mol Sci* 24(19):14815
- Meacham CE, Morrison SJ (2013) Tumour heterogeneity and cancer cell plasticity. *Nature* 501(7467):328–337
- Mirantes C, Espinosa I, Ferrer I, Dolcet X, Prat J, Matias-Guiu X (2013) Epithelial-to-mesenchymal transition and stem cells in endometrial cancer. *Hum Pathol* 44(10):1973–1981
- Nieto MA (2011) The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annu Rev Cell Dev Biol* 27:347–376
- Oh SC, Sohn BH, Cheong JH, Kim SB, Lee JE, Park KC, Lee SH, Park JL, Park YY, Lee HS, Jang HJ, Park ES, Kim SC, Heo J, Chu IS, Jang YJ, Mok YJ, Jung W, Kim BH, Kim A, Cho JY, Lim JY, Hayashi Y, Song S, Elimova E, Estralla JS, Lee JH, Bhutani MS, Lu Y, Liu W, Lee J, Kang WK, Kim S, Noh SH, Mills GB, Kim SY, Ajani JA, Lee JS (2018) Clinical and genomic landscape of gastric cancer with a mesenchymal phenotype. *Nat Commun* 9(1):1777
- Park SH, Cheung LW, Wong AS, Leung PC (2008) Estrogen regulates Snail and Slug in the down-regulation of E-cadherin and induces metastatic potential of ovarian cancer cells through estrogen receptor alpha. *Mol Endocrinol* 22(9):2085–2098
- Pattabiraman DR, Brier B, Kober KI, Thiru P, Krall JA, Zill C, Reinhardt F, Tam WL, Weinberg RA (2016) Activation of PKA leads to mesenchymal-to-epithelial transition and loss of tumor-initiating ability. *Science* 351(6277):aad3680
- Shaffer SM, Dunagin MC, Torborg SR, Torre EA, Emert B, Krepler C, Beqiri M, Sproesser K, Brafford PA, Xiao M, Egan E, Anastopoulos IN, Vargas-Garcia CA, Singh A, Nathanson KL, Herlyn M, Raj A (2017) Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. *Nature* 546(7658):431–435
- Shen M, Xu Z, Xu W, Jiang K, Zhang F, Ding Q, Xu Z, Chen Y (2019) Inhibition of ATM reverses EMT and decreases metastatic potential of cisplatin-resistant lung cancer cells through JAK/STAT3/PD-L1 pathway. *J Exp Clin Cancer Res* 38(1):149
- Shibue T, Weinberg RA (2017) EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol* 14(10):611–629
- Vijay GV, Zhao N, Den Hollander P, Toneff MJ, Joseph R, Pietila M, Taube JH, Sarkar TR, Ramirez-Pena E, Werden SJ, Shariati M, Gao R, Sobieski M, Stephan CC, Sphyrin N, Miura N, Davies P, Chang JT, Soundararajan R, Rosen JM, Mani SA (2019) GSK3 β regulates epithelial-mesenchymal transition and cancer stem cell properties in triple-negative breast cancer. *Breast Cancer Res* 21(1):37
- Yamasaki J, Hirata Y, Otsuki Y, Suina K, Saito Y, Masuda K, Okazaki S, Ishimoto T, Saya H, Nagano O (2022) MEK inhibition suppresses metastatic progression of KRAS-mutated gastric cancer. *Cancer Sci* 113(3):916–925
- Yang J, Weinberg RA (2008) Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell* 14(6):818–829

- Yao D, Dai C, Peng S (2011) Mechanism of the mesenchymal-epithelial transition and its relationship with metastatic tumor formation. *Mol Cancer Res* 9(12):1608–1620
- Yoshida J, Horiuchi A, Kikuchi N, Hayashi A, Osada R, Ohira S, Shiozawa T, Konishi I (2009) Changes in the expression of E-cadherin repressors, Snail, Slug, SIP1, and Twist, in the development and progression of ovarian carcinoma: the important role of Snail in ovarian tumorigenesis and progression. *Med Mol Morphol* 42(2):82–91
- Zhang M, Cong Q, Zhang XY, Zhang MX, Lu YY, Xu CJ (2019a) Pyruvate dehydrogenase kinase 1 contributes to cisplatin resistance of ovarian cancer through EGFR activation. *J Cell Physiol* 234(5):6361–6370
- Zhang L, Liao Y, Tang L (2019b) MicroRNA-34 family: a potential tumor suppressor and therapeutic candidate in cancer. *J Exp Clin Cancer Res* 38(1):53

The Unfolded Protein Response Role in Cancer

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Abstract

The endoplasmic reticulum (ER) produces, folds, and modifies proteins before they reach their final destinations. The unfolded protein response (UPR) is initiated when there is, in the ER, an increase of misfolded or unfolded proteins.

Cancer cells are subjected to oxidative stress, glucose deprivation, hypoxia, insufficient amino acid supply, and a lack of growth signals, all of which impair protein processing in the ER. These circumstances can lead to the accumulation of altered proteins, causing ER stress and activating UPR.

The homeostatic UPR induces at gene protein level modifications that help cellular adaptability and growth. If these activities are unsuccessful to solve ER stress, a fatal UPR program starts, leading to cellular death.

This chapter condenses the emerging data that tumor cells are susceptible to ER stress and responsive to therapies that target UPR pathways.

Keywords

Cancer · Chemotherapy resistance · Endoplasmic reticulum · Endoplasmic reticulum stress · Unfolded protein response

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1 Introduction

Prior to being sent to their final locations either inside or outside of the cell, proteins must first be synthesized, folded, and modified by the endoplasmic reticulum (ER). An accumulation of misfolded or unfolded proteins in the ER of cells triggers the unfolded protein response (UPR), a cellular response system to stress.

Proteins that are misfolded or unfolded might accumulate when the ER's folding capacity is exceeded, possibly as a result of several stressors such as nutritional deficiency, variations in calcium levels, or viral infection. If this buildup is not controlled, it can impair cellular activity and result in damage or even death of the cells.

In order to help cells deal with this stress, the UPR stops protein synthesis, increases the synthesis of chaperone proteins, which aid in folding proteins, and encourages the breakdown of misfolded proteins. In addition, if the stress is not addressed, the UPR might signal for programmed cell death.

The significance of the UPR in preserving cellular homeostasis is highlighted by the fact that dysregulation of the UPR has been linked to a number of illnesses, including cancer, diabetes, and neurological disorders (Bonsignore et al. 2023).

Binding immunoglobulin protein (BiP), often referred to as glucose-regulated protein 78 (GRP78), is an essential molecular chaperone that aids in protein folding in the ER.

When newly generated proteins enter the ER under normal physiological conditions, GRP78 attaches to them to aid in folding and avoid aggregation. Nevertheless, GRP78 separates from its client proteins to attach to the exposed hydrophobic areas of the unfolded/misfolded proteins when unfolded proteins build up in the ER. This stops the unfolded proteins from aggregating and promotes their refolding.

Three important ER transmembrane proteins—ATF6 (activating transcription factor 6), PERK (protein kinase RNA-like endoplasmic reticulum kinase), and IRE1 (inositol-requiring enzyme 1)—are released and activated upon GRP78's dissociation from its client proteins (Kim 2024).

This process initiates the UPR. Subsequently, these proteins start signaling cascades that eventually restore protein-folding capacity, increase the expression of chaperone proteins (including GRP78 itself), decrease the new proteins amount entering the ER, and promote the breakdown of misfolded proteins in order to restore ER homeostasis.

In order to preserve ER function and cell survival, GRP78 acts as a sentinel molecule that detects ER stress and coordinates the cellular response.

2 Key Sensors in the UPR

One of the major transmembrane proteins involved in the UPR is PERK, or protein kinase RNA-like endoplasmic reticulum kinase.

It is situated within the ER membrane. As a component of the UPR signaling, PERK is activated when unfolded or misfolded proteins build up in the ER. Upon activation, PERK adds phosphate groups to the protein known as eIF2 α , or eukaryotic translation initiation factor 2 alpha. The process of phosphorylating eIF2 α prevents it from starting the mRNA translation into proteins, which lowers the protein synthesis total rate.

By decreasing the amount of new proteins entering the organelle, giving already-existing proteins more time to fold correctly, and halting the continued buildup of unfolded proteins, this slowing in protein synthesis helps lessen the load on the ER (Gregersen and Bross 2010).

Dysregulation of PERK signaling has been implicated in various diseases, including neurodegenerative disorders, diabetes, and cancer, highlighting its importance in cellular physiology and pathology (Bu and Diehl 2016).

Moreover, PERK activation can trigger the production of genes linked to programmed cell death and antioxidant responses, which improves the cell's capacity to withstand ER stress and preserve cellular homeostasis.

A second transmembrane protein implicated in the UPR is inositol-requiring enzyme 1 (IRE1). Mammals have two isoforms of IRE1, IRE1 α and IRE1 β , which are located in the ER membrane. Of these, IRE1 α has been researched the most.

After activation, IRE1 cleaves RNA molecules in its capacity as an endoribonuclease. A short intron is removed during the splicing process of a particular mRNA known as XBP1 (X-box binding protein 1), producing a spliced variant of XBP1 (XBP1s). The XBP1 protein, once spliced, is a strong transcription factor that increases the expression of genes related to many aspects of ER function, such as misfolded protein degradation, protein folding, and ER-associated degradation (ERAD) (Zhang et al. 2020).

Furthermore, under situations of extended or extreme ER stress, IRE1 can also start an alternate pathway of apoptosis, or programmed cell death. c-Jun N-terminal kinase (JNK) and Apoptosis signal-regulating kinase 1 (ASK1) are two downstream effectors that are activated by this pathway, which eventually results in cell death (Zheng et al. 2015).

Another important part of the UPR is played by the activating transcription factor 6 (ATF6). ATF6 is liberated from the ER membrane and moves to the Golgi apparatus in response to UPR. There, it is subjected to proteolytic cleavage by site-1 protease (S1P) and site-2 protease (S2P). This breakage releases ATF6's transcription-factory N-terminal cytoplasmic fragment (Macke et al. 2023).

Following cleavage, the ATF6 fragment translocates to the nucleus, where it binds to specific DNA sequences in target gene promoters known as ER stress response elements (ERSE) or ER stress response element-like sequences (ERSE-II). ATF6 binds to these areas, stimulating the expression of genes involved in ER-associated degradation (ERAD), lipid metabolism, and protein folding. ATF6 activates a set of genes that include chaperone proteins that help fold proteins, like GRP78, and elements of the ERAD machinery that break down misfolded proteins (Benedetti et al. 2022).

3 UPR and Chemotherapy Resistance

Tumor cells have developed a number of drug resistance mechanisms in response to chemotherapeutic drugs in order to withstand chemotherapy. The reduction of drug accumulation, the improvement of DNA injury restoration, the insensitivity of drug-induced cell death, drug inactivation induction, and other processes have all been linked to chemotherapy resistance. Recent data indicates that the UPR and the aforementioned mechanisms are strongly related.

Diffusion, active transport, and endocytosis are three ways that drugs can enter tumor cells (Tzanova et al. 2021). Tumor cells, however, restrict this entrance by either decreasing drug uptake or boosting drug efflux. ATP-binding cassette (ABC) transporters, which include multidrug resistance protein 1 (MDR1), MDR-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP), are the proteins that cause drug efflux (Nobili et al. 2020).

Numerous investigations have indicated that the upregulation of ABC transporter expression by the UPR facilitates drug efflux. Daunorubicin activates the ATF4-mediated stress response-like transcriptional pathway, which promotes drug efflux, induces MDR1 expression, and results in resistance to chemotherapeutic medicines. Analogously, IRE1 α -XBP1 pathway, which is activated by 5-fluorouracil, enhances drug efflux in colon tumor cells by upregulating the expression of ABC transporters (Gao et al. 2020). Apart from the IRE1 α -XBP1 signaling, the GRP78 overexpression enhances the ABC transporter's efflux activity, which in turn gives pancreatic cancer cells resistance to chemotherapeutic treatments (Dauer et al. 2018).

UPR sensors or downstream transcription factors may be intimately linked to DNA damage repair (DDR), according to a growing amount of evidence. For instance, mammalian IRE1 α -XBP1 regulates H4 acetylation, which is crucial to the nonhomologous end-joining (NHEJ) repair pathway (Tao et al. 2011). Similar research has demonstrated that certain DDR gene transcriptions are directly upregulated by XBP1s in human hepatic cells (Argemí et al. 2017).

Tumor cellular resistance to chemotherapeutic drug-induced cell death may, at least partially, be explained by UPR-mediated increase of expression of some antioxidant genes and the removal of reactive oxygen species (ROS). PERK directly phosphorylates transcription factor NRF2, controlling the production of antioxidant genes in response to chemotherapeutic drugs (Shin et al. 2018).

4 UPR and Cancer Progression

Although the main function of the UPR is to preserve cells by restoring ER homeostasis and encouraging cell survival, UPR dysregulation has been linked to a number of illnesses, including cancer.

Depending on the circumstances and stage of carcinogenesis, the UPR in cancer can have both positive and negative effects.

Increased levels of ER stress are normally detected in tumor cells as a result of oncogene or tumor suppressor gene alterations, hypoxia, fast cell division, and

nutritional deprivation. By decreasing protein synthesis, increasing antioxidant defenses, and improving protein folding capability, UPR activation in cancer cells can increase their ability to survive.

All UPR branches (ATF6, IRE1a, and PERK) have been shown in multiple studies to be highly activated in a broad series of human cancers, such as glioblastoma, and hematopoietic neoplasms (leukemia, lymphoma, and myeloma) and some carcinomas such as stomach, colon, esophageal, lung, prostate, pancreatic, and liver. Furthermore, a number of cancer types show overexpression of the ER chaperone GRP78, which support adaptability to ER stress. Hepatitis C virus and human papillomavirus are two examples of oncogenic viruses that also upregulate GRP78 (Lieberman et al. 1999).

Even though targeted therapies and cytotoxic chemotherapy have significantly improved in efficacy, most eventually fail due to resistant clone emergence. Depending on the type of cancer and the therapy used, there are many different mechanisms underlying chemotherapy resistance. Nonetheless, common mechanisms include enhanced DNA repair, upregulation of efflux pumps drug transporters downregulation, pro-survival proteins upregulation, and drug inactivation through detoxification pathways.

UPR is a major factor in cancer patients' resistance to hormone treatment, chemotherapy, and specific treatments. When the activation of UPR was recognized to be associated with resistance in solid cancers, notably breast and colorectal neoplasms, the first link was made. Tamoxifen resistance is related to decreased recurrence time, shorter survival, and higher expression of UPR elements and downstream proteins (such as XBP1s) (Lee et al. 2006).

Data from tumors have shown that the activation of UPR can improve resistance to chemotherapeutic drugs. Numerous evidence has shown that overexpressing GRP78 can reduce apoptosis induced by drugs, in part by activating caspase-7 and inhibiting the formation of pro-apoptotic Bcl-2. It has been documented that PERK increases chemoresistance in HT29 colon cancer cells by pharmacologically or genetically inhibiting PERK, which makes human colon tumor cells more sensitive to chemotherapy with 5-fluorouracil (Shi et al. 2019).

Solid tumor growth is limited by the ability to give enough glucose oxygen, and other nutrients to the increasing cell mass, particularly those in the tumor's midpoint. In response to glucose and hypoxia deficiency, tumor cells increase the release of vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), and other pro-angiogenic signals, which induce neo-angiogenesis.

The UPR, which controls regulation at gene and protein level of multiple pro-angiogenic factors, is activated by these same conditions that also induce ER stress.

It has been demonstrated that all three UPR arms significantly upregulate VEGF-A, which promotes angiogenesis to shield hastily from hypoxia proliferating cancer cells. The VEGF-A promoter is directly bound by the UPR transcription factor ATF4, and XBP1 upregulates at gene level the expression of IL-6 and VEGF-A, potentially via a mechanism that is dependent on hypoxia-inducible factor-1a (HIF1a) (X. Chen et al. 2014b). PERK can increase autophagy and thus support

the survival of tumor cells in hypoxic environments. The upregulation of tumor angiogenesis is one way that the ER chaperone GRP78 facilitates the growth and chemoresistance of cancer cells (Dong et al. 2008).

5 UPR and Immune System

Tumorigenesis, tumor progression, metastasis, and recurrence all depend on immune-suppression. In the cancer microenvironment, the development of an immunosuppressive system resulting from interactions between host stromal cells (such as macrophages, B cells, T cells, fibroblasts, and dendritic cells) cancer cells and may stimulate tumor growth, shield the tumor from recognition of immune system, and reduce the effectiveness of interventions of immunotherapy (Tie et al. 2022).

Immune cells' ER homeostasis can be disrupted by external variables in the tumor microenvironment (TME), such as metabolites, ROS, hypoxia and acidosis, and nutritional deprivation. This can result in abnormal innate and adaptive immunity.

The UPR can be triggered when immune cells are exposed to molecules originating from tumors. Immunosuppressive effect can be promoted by tumor lipids via inducing IRE1 α in myeloid cells. Expression of IRE1 α in dendritic cells causes abnormal lipid metabolism, which leads to increased production of lipid droplets and poor antigen presentation. Moreover, the effector activity of T lymphocytes may be suppressed by the release of glucocorticoids into the microenvironment. Additionally, T-cell activation of IRE1 α disrupted mitochondrial respiration and N-linked glycosylation, which produced less IFN- γ . In T cells, PERK expression was linked to decreased glycolysis and transcription factor T-bet inhibition, the master transcription factor driving Th1 differentiation (Cubillos-Ruiz et al. 2017). Reduced glycolysis and increased generation of mtROS (mitochondrial ROS) were linked to PERK expression in T cells, as well as the suppression of T-bet. IRE1 α suppresses phagocytosis in macrophages while promoting angiogenesis and tolerogenic characteristics. PERK activation in tumor-associated macrophages (TAMs) improves mitochondrial performance and epigenetic reprogramming, which increases immunosuppressive function, however the tumor-derived mechanisms that cause this activation are still unknown.

Genetic changes or mutations can also cause ER stress, which triggers the UPR. In order to maintain cell survival in melanoma, it has been proposed that oncogenic BRAF mutation continuously generates ER stress (Corazzari et al. 2015). It would therefore be interesting to investigate if oncogenic transformation might affect UPR activity and whether this can lead to modifications in factors released or other environmental signals that affect immune cell effector function for immune evasion. Moreover, it's interesting to note that IRE1 α and PERK signaling of the UPR may be activated in tumor cell aneuploidy (Xian et al. 2021).

T-cell function deteriorates in response to inflammatory activation or chronic antigen exposure. T-cell exhaustion is the term for this kind of decline, which is frequently linked to a loss of tumor control (Sharpe and Pauken 2018). High expression of immunological checkpoint molecules, including PD-1 and CTLA-4,

was seen in exhausted CD8+ T cells (Xia et al. 2023). Numerous investigations have demonstrated that T-cell exhaustion is caused by both prolonged UPR activation and ER stress (Costa et al. 2018). High cholesterol causes XBP1s to be expressed in melanoma, which in turn causes immune checkpoint molecules to be expressed more frequently and CD8+ T-cell antitumor immunity to be inhibited (Ma et al. 2014).

The UPR role in macrophage-associated immune elusion has been shown in a growing number of papers recently. IRE1 α -XBP1 pathway activation in melanoma increases macrophage PD-L1 expression and encourages tumor immune evasion (Batista et al. 2020). Furthermore, it has been demonstrated that ER stress increases the macrophage PD-L1 expression via the miR-23a-PTEN-AKT pathway, which in turn promotes the growth of tumors (Liu et al. 2019). These findings demonstrate how PD-L1 production in macrophages controlled by ER stress can suppress T-cell activity and encourage tumor cell evasion of antitumor immunity.

6 Mitochondrial Unfolded Protein Response (UPR^{mt})

Unchecked cell growth in cancer causes these cells to become vulnerable in several ways, which means important support systems need to shield them (Vyas et al. 2016). Growth and survival during the different phases of cancer are primarily facilitated by mitochondria (Murphy 2009). Nevertheless, genetic changes in mitochondria can also lead to an abnormal electron transport chain, which produces an excess of mtROS (Liou and Storz 2010). Moderate amounts of mtROS are produced by mitochondria under healthy settings and in the early stages of illness, which are advantageous for cellular development and survival. Tumor cells may become fatally affected by mtROS levels that are beyond the acceptable threshold as mitochondrial dysfunction intensifies (Liou and Storz 2010). In order to leave mitochondria in a more delicate and dysfunctional state, the mtROS encourages the unfolding and aggregation of mitochondrial proteins.

By preserving mitochondrial integrity and stimulating tumor growth, UPR^{mt}, a mitochondrial stress response seen in *C. elegans* and the mammalian system (Inigo and Chandra 2022), functions as a crucial support mechanism in cancer. A number of chaperones and proteases that are activated by UPR^{mt} decrease the harmful effects of mtROS. A growing body of research indicates that mammals and *C. elegans* share the UPR^{mt} (Inigo and Chandra 2022).

Proteotoxic stress, which is triggered by the unfolding, misfolding, and aggregation of mitochondrial proteins, is brought on by innate mitochondrial malfunction and persistent oxidative stress. If unchecked, protein aggregation buildup can intensify oxidative stress and create a vicious cycle that feeds back into mitochondrial malfunction.

Strong conservation in the UPR^{mt} response was later found between *C. elegans*, mice, and humans by transcriptome and proteome studies. Mammalian UPR^{mt} response has been identified to activate over 400 genes (Nargund et al. 2012), which are classified by different activities such as metabolism, ROS detoxification,

protein folding quality control, and mitochondrial biogenesis (Schulz and Haynes 2015).

UPR^{mt} signaling is important in cancer (Deng and Haynes 2017) and needs more research to show exactly how it affects the development and spread of tumors. The existence and function of UPR^{mt} during mitochondrial stress have been established by numerous studies, mostly using *C. elegans* and mammalian cells; nevertheless, there has not been much research on the UPR^{mt} system as a whole in cancer. Nonetheless, specific UPR^{mt} components have demonstrated functions in tumor development and survival. Given the frequency of mitochondrial malfunction and oxidative stress throughout the genesis and progression of cancer, this is not surprising.

7 MicroRNA and UPR

microRNAs (miRNA), which are short non-coding RNA molecules, play an important role in the regulation of gene expression post-transcriptionally. Usually, when they attach to the 3' untranslated region (UTR) of a target messenger RNA (mRNA), translational suppression or mRNA destruction results.

miRNAs have been discovered to alter the expression of several genes involved in ER stress signaling pathways within the framework of the UPR. These miRNAs have the ability to either enhance or inhibit the UPR. So, miRNAs that promote aberrant protein folding and modification may be a major contributor to ER stress. While it is known that a group of miRNAs targets and dysregulates ER-associated enzymes, their roles in the ER stress remain to be fully elucidated (Soczewski et al. 2023).

Nevertheless, by focusing on genes involved in protein folding and modification, a number of miRNAs have been shown to be significant regulators in the ER stress.

In vascular cells, miR-204 induces ER stress (Kassan et al. 2017). By accumulating the improperly acetylated proteins, miR-204 overexpression increases ER stress. In the animal model, endothelial dysfunction caused by ER stress is actually encouraged by the overexpression of vascular miR-204.

Further miRNAs are linked to ER stress through aberrant protein folding and modification including miR-224 and miR-520c (Kelleher et al. 2003; Jeon et al. 2018).

ER chaperone GRP78 interacts with UPR sensors to act as a door lock on ER stress signaling pathways. The release of GRP78 from the sensor proteins causes the ERAD and UPR to become active. It has been shown that a number of miRNAs target GRP78 mRNA, including the miR-181 family, the miR-30 family, the miR-378-5p, the miR-378-3p, the miR-495, and the miR-199a-5p.

The miR-30 family directly targets GRP78 in cancer and muscle cells to increase ER stress signals and UPR (Chen et al. 2014a). It has also been demonstrated that miR-199a-5p targets GRP78 in hepatocytes; this apparatus guards against ER-stressed hepatocytes brought on by liver damage (Dai et al. 2013).

ER stress-induced UPR is centered on three main UPR sensors, namely IRE1 α , PERK, and ATF6 α , but miRNAs linked to UPR sensors, on the other hand, are essential participants in ER stress reactions.

Under ER stress, miRNAs are the major controllers of the defective cell death systems in UPR-activated cancer cells. Numerous studies have consistently demonstrated that miRNA activities prevent ER and UPR-activated tumor cells from switching from a survival to a death state. The PERK-eIF2 α CHOP pathway has been linked to the majority of miRNAs associated with ER stress-induced cell death.

In hepatocellular carcinoma (HCC), miR-663 generated by ER stress stimulates cellular division and prevents cellular death [196]. Conversely, the ER stress adversely modulates the miR-199a/214 cluster, which reduces hepatocellular carcinoma survival and progression (Duan et al. 2012). In colorectal cancer, miR-451a that is triggered by ER stress promotes cell proliferation while suppressing cell death (Xu et al. 2019).

According to recent reports, ER stress encourages the exosomal-mediated release of certain miRNAs from cancer cells. Exosomes containing miR-23a-3p are secreted by hepatocellular carcinoma cells when they are under ER stress (Liu et al. 2019). The amount of miR-27a-3p in breast cancer exosomes is likewise elevated by ER stress (Yao et al. 2020). The activation of PD-L1 in macrophages by cancer-derived exo-miRs generated by ER stress is noteworthy as it facilitates the immunological escape of cancer cells. The association between ER stress and cancer has been further illuminated by the identification of a new functional mechanism of ER stress-associated miRNAs in cancer formation.

Drugs that target miRNAs linked to ER stress have not been tested in clinical trials to treat cancer. Given the importance of miRNAs in cancer cells with ER stress, the therapeutic potential of miRNAs and exosomal miRNAs implicated in the ER stress system should be considered for cancer therapy.

8 UPR and EMT Induction

The genetic and biological pathway that promotes cell motility is known as epithelial-to-mesenchymal transition (EMT). EMT has emerged as a key player in tissue and organ development throughout morphogenesis, as well as wound healing in adulthood.

Pathological EMT is connected to organ fibrosis and cancer. In normal and pathological EMT, the expression of EMT transcription factors (EMT-TFs) initiates a sophisticated cellular program that leads to apical-basal epithelial polarity loss and more mesenchymal-like behavior and morphology (Santamaría et al. 2019).

The majority of carcinoma cancers use the EMT program to proceed to malignancy. Environmental cues and signal exchange between tumor cells and their microenvironment in carcinomas have a significant impact on EMT implementation, mesenchymal feature acquisition, and malignant development (Cubillos-Ruiz et al. 2017).

In some cancer cases, ER stress and UPR activation have been linked to malignancy. An EMT signature is strongly related to ATF4 expression in stomach, lung, breast, and metastatic tumor tissues (Feng et al. 2014). A small cohort of tumor tissue samples showed GP78 and nuclear β -catenin staining near the invasive front, indicating ER stress and EMT connection in colorectal cancer. A decreased overall survival rate is related to IRE1-induced EMT. Furthermore, in hepatocellular carcinoma, XBP1 levels correlate positively with vimentin and negatively with E-cadherin [49]. Furthermore, in hepatocellular carcinoma, XBP1 levels correlate negatively with E-cadherin and positively with vimentin (Wu et al. 2018).

Higher activation of the IRE1-XBP1 axis in glioblastoma is related to shorter patient life, significant tumor immune cell infiltration, and enhanced invasiveness and angiogenesis.

The most important aspect of breast tumor is an EMT signature in cells that express PERK activation. In adenocarcinoma tumors, ER stress proteins such as PERK and IRE1 co-express with EMT protein markers, as compared to comparable normal neighboring tissues (Lhomond et al. 2018).

9 Therapeutic Targeting of UPR

Two approaches to targeting UPR components in cancer are to induce misfolded protein accumulation in the ER, which overloads the UPR, and to inhibit UPR adaptive and anti-apoptotic signaling.

Bortezomib was the first successful development of a proteasome inhibitor for cancer therapy (Obeng et al. 2006). Bortezomib is highly effective against several cancer cell types in which the ER is already susceptible for a significant unfolded or misfolded protein presence, such as multiple myeloma (Sterz et al. 2008). Different types of cancer, such as colon, ovarian, breast, pancreatic, oral, prostate, and lung cancer, are cytotoxically affected by bortezomib treatment. The FDA has approved it for relapsed multiple myeloma treatment and lately also for relapsed mantle cell lymphoma (Robak and Robak 2019). Moreover, combination chemotherapy regimens containing Bortezomib have been developed. (Edelman 2005).

ER misfolded proteins accumulation is driven by ERAD defects, which triggers ER stress and UPR (Hoseki et al. 2010). There is a pharmacological inhibitor that can impede ERAD showing also cytotoxic effect against cancer cells. Eeyarestatin I (EerI) inhibits the deubiquitination of p97-associated ERAD substrates (Wang et al. 2010).

The protein disulfide isomerase (PDI) is one of the most prevalent ER chaperone, serving as a custodian for proper protein folding. PDI is an important protein family able to catalyze protein folding (Kranz et al. 2017). In human ovarian and leukemia tumor cells treated with O(2)-[2,4-dinitro-5-(N-methyl-N-4-carboxyphenylamino)phenyl]1-(N,N-methylamino)diazen-1-ium-1,2-diolate (PABA/NO), UPR was activated by S-glutathionylation of PDI, causing translational reduction and PERK and its effector eIF2 activation (Saavedra et al. 2006).

Levels of GPR78 are commonly raised in cancer cell lines and solid tumors (Lu et al. 2020). Epigallocatechin gallate (EGCG) counteracts GRP78 by targeting its ATP-binding region of and disrupts protective function from UPR, sensitizing glioma cancer cells to the chemotherapeutic drug temozolomide. (Xipell et al. 2016). In mesothelioma cells, EGCG sensitizes cells to cell death (Ranzato et al. 2012), working also in combination with classic chemotherapeutic drugs (Volta et al. 2013).

There is extensive preclinical (Ranzato et al. 2022) and clinical data (Baier et al. 2023) indicating that BOLD-100 (sodium trans-[tetrachlorobis(1H indazole) ruthenate(III)]), which alters UPR through selective GRP78 inhibition, could improve the efficacy of a wide range of existing cancer therapies, and significantly improve patient outcomes.

10 Conclusion

Protein folding and quality control are emerging as important targets for cancer treatments. UPR aids cancer cells in adapting and surviving in an unfavorable microenvironment by activating stress-response signaling and upregulating chaperones. Targeting the UPR pathway is a novel approach to cancer treatment that has shown effectiveness in clinical trials.

It is not assured that specific therapy for UPR or new aspects of protein quality control are specific for tumor cells. Protein quality regulation remains poorly understood in relation to organismal evolution, organ function, and fitness. There is an urgent need to understand the core mechanism of unfolded protein response and to identify critical new actors in ER protein quality control in order to fight cancer cells.

References

- Argemí J, Kress TR, Chang HCY, Ferrero R, Bértolo C, Moreno H et al (2017) X-box binding protein 1 regulates unfolded protein, acute-phase, and DNA damage responses during regeneration of mouse liver. *Gastroenterology* 152(5):1203–1216.e15. <https://doi.org/10.1053/j.gastro.2016.12.040>
- Baier D, Mendrina T, Schoenhacker-Alte B, Pirker C, Mohr T, Rusz M et al (2023) The lipid metabolism as target and modulator of BOLD-100 anticancer activity: crosstalk with histone acetylation. *Adv Sci (Weinh)* 10(32):e2301939. <https://doi.org/10.1002/advs.202301939>
- Batista A, Rodvold JJ, Xian S, Searles SC, Lew A, Iwawaki T et al (2020) IRE1 α regulates macrophage polarization, PD-L1 expression, and tumor survival. *PLoS Biol* 18(6):e3000687. <https://doi.org/10.1371/journal.pbio.3000687>
- Benedetti R, Romeo MA, Arena A, Gilardini Montani MS, Di Renzo L, D'Orazi G et al (2022) ATF6 prevents DNA damage and cell death in colon cancer cells undergoing ER stress. *Cell Death Discov* 8(1):295. <https://doi.org/10.1038/s41420-022-01085-3>
- Bonsignore G, Martinotti S, Ranzato E (2023) Endoplasmic reticulum stress and cancer: could unfolded protein response be a druggable target for cancer therapy? *Int J Mol Sci* 24(2). <https://doi.org/10.3390/ijms24021566>
- Bu Y, Diehl JA (2016) PERK integrates oncogenic signaling and cell survival during cancer development. *J Cell Physiol* 231(10):2088–2096. <https://doi.org/10.1002/jcp.25336>

- Chen M, Ma G, Yue Y, Wei Y, Li Q, Tong Z et al (2014a) Downregulation of the miR-30 family microRNAs contributes to endoplasmic reticulum stress in cardiac muscle and vascular smooth muscle cells. *Int J Cardiol* 173(1):65–73. <https://doi.org/10.1016/j.ijcard.2014.02.007>
- Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziaepostolou M et al (2014b) XBP1 promotes triple-negative breast cancer by controlling the HIF1 α pathway. *Nature* 508(7494): 103–107. <https://doi.org/10.1038/nature13119>
- Corazzari M, Rapino F, Ciccocanti F, Giglio P, Antonioli M, Conti B et al (2015) Oncogenic BRAF induces chronic ER stress condition resulting in increased basal autophagy and apoptotic resistance of cutaneous melanoma. *Cell Death Differ* 22(6):946–958. <https://doi.org/10.1038/cdd.2014.183>
- Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M et al (2018) Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. *Cancer Cell* 33(3): 463–479.e10. <https://doi.org/10.1016/j.ccell.2018.01.011>
- Cubillos-Ruiz JR, Bettigole SE, Glimcher LH (2017) Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. *Cell* 168(4):692–706. <https://doi.org/10.1016/j.cell.2016.12.004>
- Dai BH, Geng L, Wang Y, Sui CJ, Xie F, Shen RX et al (2013) microRNA-199a-5p protects hepatocytes from bile acid-induced sustained endoplasmic reticulum stress. *Cell Death Dis* 4(4): e604. <https://doi.org/10.1038/cddis.2013.134>
- Dauer P, Sharma NS, Gupta VK, Nomura A, Dudeja V, Saluja A et al (2018) GRP78-mediated antioxidant response and ABC transporter activity confers chemoresistance to pancreatic cancer cells. *Mol Oncol* 12(9):1498–1512. <https://doi.org/10.1002/1878-0261.12322>
- Deng P, Haynes CM (2017) Mitochondrial dysfunction in cancer: potential roles of ATF5 and the mitochondrial UPR. *Semin Cancer Biol* 47:43–49. <https://doi.org/10.1016/j.semcancer.2017.05.002>
- Dong D, Ni M, Li J, Xiong S, Ye W, Virrey JJ et al (2008) Critical role of the stress chaperone GRP78/BiP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development. *Cancer Res* 68(2):498–505. <https://doi.org/10.1158/0008-5472.CAN-07-2950>
- Duan Q, Wang X, Gong W, Ni L, Chen C, He X et al (2012) ER stress negatively modulates the expression of the miR-199a/214 cluster to regulates tumor survival and progression in human hepatocellular cancer. *PLoS One* 7(2):e31518. <https://doi.org/10.1371/journal.pone.0031518>
- Edelman MJ (2005) The potential role of bortezomib in combination with chemotherapy and radiation in non-small-cell lung cancer. *Clin Lung Cancer* 7(Suppl 2):S64–S66. <https://doi.org/10.3816/clc.2005.s.011>
- Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA et al (2014) Epithelial-to-mesenchymal transition activates PERK-eIF2 α and sensitizes cells to endoplasmic reticulum stress. *Cancer Discov* 4(6):702–715. <https://doi.org/10.1158/2159-8290.CD-13-0945>
- Gao Q, Li XX, Xu YM, Zhang JZ, Rong SD, Qin YQ et al (2020) IRE1 α -targeting downregulates ABC transporters and overcomes drug resistance of colon cancer cells. *Cancer Lett* 476:67–74. <https://doi.org/10.1016/j.canlet.2020.02.007>
- Gregersen N, Bross P (2010) Protein misfolding and cellular stress: an overview. *Methods Mol Biol* 648:3–23. https://doi.org/10.1007/978-1-60761-756-3_1
- Hoseki J, Ushioda R, Nagata K (2010) Mechanism and components of endoplasmic reticulum-associated degradation. *J Biochem* 147(1):19–25. <https://doi.org/10.1093/jb/mvp194>
- Inigo JR, Chandra D (2022) The mitochondrial unfolded protein response (UPR). *J Hematol Oncol* 15(1):98. <https://doi.org/10.1186/s13045-022-01317-0>
- Jeon YJ, Kim T, Park D, Nuovo GJ, Rhee S, Joshi P et al (2018) miRNA-mediated TUSC3 deficiency enhances UPR and ERAD to promote metastatic potential of NSCLC. *Nat Commun* 9(1):5110. <https://doi.org/10.1038/s41467-018-07561-8>
- Kassan M, Vikram A, Li Q, Kim YR, Kumar S, Gabani M et al (2017) MicroRNA-204 promotes vascular endoplasmic reticulum stress and endothelial dysfunction by targeting Sirtuin1. *Sci Rep* 7(1):9308. <https://doi.org/10.1038/s41598-017-06721-y>

- Kelleher DJ, Karaoglu D, Mandon EC, Gilmore R (2003) Oligosaccharyltransferase isoforms that contain different catalytic STT3 subunits have distinct enzymatic properties. *Mol Cell* 12(1): 101–111. [https://doi.org/10.1016/s1097-2765\(03\)00243-0](https://doi.org/10.1016/s1097-2765(03)00243-0)
- Kim P (2024) Understanding the unfolded protein response (UPR) pathway: insights into neuropsychiatric disorders and therapeutic potentials. *Biomol Ther (Seoul)* 32(2):183–191. <https://doi.org/10.4062/biomolther.2023.181>
- Kranz P, Neumann F, Wolf A, Classen F, Pomsch M, Ocklenburg T et al (2017) PDI is an essential redox-sensitive activator of PERK during the unfolded protein response (UPR). *Cell Death Dis* 8(8):e2986. <https://doi.org/10.1038/cddis.2017.369>
- Lee E, Nichols P, Spicer D, Groshen S, Yu MC, Lee AS (2006) GRP78 as a novel predictor of responsiveness to chemotherapy in breast cancer. *Cancer Res* 66(16):7849–7853. <https://doi.org/10.1158/0008-5472.CAN-06-1660>
- Lhomond S, Avril T, Dejeans N, Voutetakis K, Doultsinos D, McMahon M et al (2018) Dual IRE1 RNase functions dictate glioblastoma development. *EMBO Mol Med* 10(3). <https://doi.org/10.15252/emmm.201707929>
- Lieberman E, Fong YL, Selby MJ, Choo QL, Cousens L, Houghton M et al (1999) Activation of the grp78 and grp94 promoters by hepatitis C virus E2 envelope protein. *J Virol* 73(5):3718–3722. <https://doi.org/10.1128/JVI.73.5.3718-3722.1999>
- Liou GY, Storz P (2010) Reactive oxygen species in cancer. *Free Radic Res* 44(5):479–496. <https://doi.org/10.3109/10715761003667554>
- Liu J, Fan L, Yu H, Zhang J, He Y, Feng D et al (2019) Endoplasmic reticulum stress causes liver cancer cells to release exosomal miR-23a-3p and Up-regulate programmed death ligand 1 expression in macrophages. *Hepatology* 70(1):241–258. <https://doi.org/10.1002/hep.30607>
- Lu G, Luo H, Zhu X (2020) Targeting the GRP78 pathway for cancer therapy. *Front Med (Lausanne)* 7:351. <https://doi.org/10.3389/fmed.2020.00351>
- Ma XH, Piao SF, Dey S, McAfee Q, Karakousis G, Villanueva J et al (2014) Targeting ER stress-induced autophagy overcomes BRAF inhibitor resistance in melanoma. *J Clin Invest* 124(3): 1406–1417. <https://doi.org/10.1172/JCI70454>
- Macke AJ, Pachikov AN, Divita TE, Morris ME, LaGrange CA, Holzapfel MS et al (2023) Targeting the ATF6-mediated ER stress response and autophagy blocks integrin-driven prostate cancer progression. *Mol Cancer Res* 21(9):958–974. <https://doi.org/10.1158/1541-7786.MCR-23-0108>
- Murphy MP (2009) How mitochondria produce reactive oxygen species. *Biochem J* 417(1):1–13. <https://doi.org/10.1042/BJ20081386>
- Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM (2012) Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science* 337(6094):587–590. <https://doi.org/10.1126/science.1223560>
- Nobili S, Lapucci A, Landini I, Coronello M, Roviello G, Mini E (2020) Role of ATP-binding cassette transporters in cancer initiation and progression. *Semin Cancer Biol* 60:72–95. <https://doi.org/10.1016/j.semcancer.2019.08.006>
- Obeng EA, Carlson LM, Gutman DM, Harrington WJ, Lee KP, Boise LH (2006) Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. *Blood* 107(12):4907–4916. <https://doi.org/10.1182/blood-2005-08-3531>
- Ranzato E, Martinotti S, Magnelli V, Murer B, Biffo S, Mutti L et al (2012) Epigallocatechin-3-gallate induces mesothelioma cell death via H₂O₂-dependent T-type Ca²⁺ channel opening. *J Cell Mol Med* 16(11):2667–2678. <https://doi.org/10.1111/j.1582-4934.2012.01584.x>
- Ranzato E, Bonsignore G, Martinotti S (2022) ER stress response and induction of apoptosis in malignant pleural mesothelioma: the achilles heel targeted by the anticancer ruthenium drug BOLD-100. *Cancers (Basel)* 14(17). <https://doi.org/10.3390/cancers14174126>
- Robak P, Robak T (2019) Bortezomib for the treatment of hematologic malignancies: 15 years later. *Drugs R D* 19(2):73–92. <https://doi.org/10.1007/s40268-019-0269-9>

- Saavedra JE, Srinivasan A, Buzard GS, Davies KM, Waterhouse DJ, Inami K et al (2006) PABA/NO as an anticancer lead: analogue synthesis, structure revision, solution chemistry, reactivity toward glutathione, and in vitro activity. *J Med Chem* 49(3):1157–1164. <https://doi.org/10.1021/jm050700k>
- Santamaría PG, Mazón MJ, Eraso P, Portillo F (2019) UPR: an upstream signal to EMT induction in cancer. *J Clin Med* 8(5). <https://doi.org/10.3390/jcm8050624>
- Schulz AM, Haynes CM (2015) UPR(mt)-mediated cytoprotection and organismal aging. *Biochim Biophys Acta* 1847(11):1448–1456. <https://doi.org/10.1016/j.bbabbio.2015.03.008>
- Sharpe AH, Pauken KE (2018) The diverse functions of the PD1 inhibitory pathway. *Nat Rev Immunol* 18(3):153–167. <https://doi.org/10.1038/nri.2017.108>
- Shi Z, Yu X, Yuan M, Lv W, Feng T, Bai R et al (2019) Activation of the PERK-ATF4 pathway promotes chemo-resistance in colon cancer cells. *Sci Rep* 9(1):3210. <https://doi.org/10.1038/s41598-019-39547-x>
- Shin D, Kim EH, Lee J, Roh JL (2018) Nrf2 inhibition reverses resistance to GPX4 inhibitor-induced ferroptosis in head and neck cancer. *Free Radic Biol Med* 129:454–462. <https://doi.org/10.1016/j.freeradbiomed.2018.10.426>
- Soczewski E, Murrieta-Coxca JM, Miranda L, Fuentes-Zacarías P, Gutiérrez-Samudio R, Grasso E et al (2023) miRNAs associated with endoplasmic reticulum stress and unfolded protein response during decidualization. *Reprod Biomed Online* 47(5):103289. <https://doi.org/10.1016/j.rbmo.2023.103289>
- Sterz J, von Metzler I, Hahne JC, Lamottke B, Rademacher J, Heider U et al (2008) The potential of proteasome inhibitors in cancer therapy. *Expert Opin Investig Drugs* 17(6):879–895. <https://doi.org/10.1517/13543784.17.6.879>
- Tao R, Chen H, Gao C, Xue P, Yang F, Han JD et al (2011) Xbp1-mediated histone H4 deacetylation contributes to DNA double-strand break repair in yeast. *Cell Res* 21(11):1619–1633. <https://doi.org/10.1038/cr.2011.58>
- Tie Y, Tang F, Wei YQ, Wei XW (2022) Immunosuppressive cells in cancer: mechanisms and potential therapeutic targets. *J Hematol Oncol* 15(1):61. <https://doi.org/10.1186/s13045-022-01282-8>
- Tzanova MM, Randelov E, Stein PC, Hiorth M, di Cagno MP (2021) Towards a better mechanistic comprehension of drug permeation and absorption: introducing the diffusion-partitioning interplay. *Int J Pharm* 608:121116. <https://doi.org/10.1016/j.ijpharm.2021.121116>
- Volta V, Ranzato E, Martinotti S, Gallo S, Russo MV, Mutti L et al (2013) Preclinical demonstration of synergistic active nutrients/drug (AND) combination as a potential treatment for malignant pleural mesothelioma. *PLoS One* 8(3):e58051. <https://doi.org/10.1371/journal.pone.0058051>
- Vyas S, Zaganjor E, Haigis MC (2016) Mitochondria and cancer. *Cell* 166(3):555–566. <https://doi.org/10.1016/j.cell.2016.07.002>
- Wang Q, Shinkre BA, Lee JG, Weniger MA, Liu Y, Chen W et al (2010) The ERAD inhibitor eeyarestatin I is a bifunctional compound with a membrane-binding domain and a p97/VCP inhibitory group. *PLoS One* 5(11):e15479. <https://doi.org/10.1371/journal.pone.0015479>
- Wu S, Du R, Gao C, Kang J, Wen J, Sun T (2018) The role of XBP1s in the metastasis and prognosis of hepatocellular carcinoma. *Biochem Biophys Res Commun* 500(3):530–537. <https://doi.org/10.1016/j.bbrc.2018.04.033>
- Xia ZA, Lu C, Pan C, Li J, Mao Y, Sun L et al (2023) The expression profiles of signature genes from CD103. *BMC Med* 21(1):268. <https://doi.org/10.1186/s12916-023-02960-1>
- Xian S, Dosset M, Almanza G, Searles S, Sahani P, Waller TC et al (2021) The unfolded protein response links tumor aneuploidy to local immune dysregulation. *EMBO Rep* 22(12):e52509. <https://doi.org/10.15252/embr.202152509>
- Xipell E, Aragón T, Martínez-Velez N, Vera B, Idoate MA, Martínez-Irujo JJ et al (2016) Endoplasmic reticulum stress-inducing drugs sensitize glioma cells to temozolomide through downregulation of MGMT, MPG, and Rad51. *Neuro-Oncology* 18(8):1109–1119. <https://doi.org/10.1093/neuonc/now022>

- Xu K, Han B, Bai Y, Ma XY, Ji ZN, Xiong Y et al (2019) MiR-451a suppressing BAP31 can inhibit proliferation and increase apoptosis through inducing ER stress in colorectal cancer. *Cell Death Dis* 10(3):152. <https://doi.org/10.1038/s41419-019-1403-x>
- Yao X, Tu Y, Xu Y, Guo Y, Yao F, Zhang X (2020) Endoplasmic reticulum stress-induced exosomal miR-27a-3p promotes immune escape in breast cancer via regulating PD-L1 expression in macrophages. *J Cell Mol Med* 24(17):9560–9573. <https://doi.org/10.1111/jcmm.15367>
- Zhang K, Liu H, Song Z, Jiang Y, Kim H, Samavati L et al (2020) The UPR transducer IRE1 promotes breast cancer malignancy by degrading tumor suppressor microRNAs. *iScience* 23(9): 101503. <https://doi.org/10.1016/j.isci.2020.101503>
- Zheng GF, Cai Z, Meng XK, Zhang Y, Zhu W, Pang XY et al (2015) Unfolded protein response mediated JNK/AP-1 signal transduction, a target for ovarian cancer treatment. *Int J Clin Exp Pathol* 8(6):6505–6511



PRDM2 in Cancer: Deciphering the Molecular Orchestra of a Multifunctional Regulator

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Abstract

PRDM2/RIZ is a histone methyltransferase that belongs to the Positive Regulatory Domain I-binding factor 1 (PRDM) superfamily, identified by a PR domain with methyltransferase activity and by zinc finger domains. Among PRDM members, PRDM2 represents a relevant member that has emerged as a dynamic player in the intricate cancer biology landscape. As observed for other PRDMs, two main proteins differing by the PR domain, RIZ1 and RIZ2, are encoded by the PRDM2 locus. The imbalance in the RIZ1/RIZ2 amounts could be responsible for several malignancies. Different reports, indeed, highlight a yin-yang behavior, supporting a tumor suppressor and an oncogene role for RIZ1 and RIZ2, respectively. Moreover, a tight connection among PRDM2 and estrogen receptor α exists. Interestingly, RIZ1 represents an important downstream target of estradiol/estrogen receptor action and RIZ2 is an estrogen-responsive gene. Recently, novel insights have been added on the putative oncogene role of RIZ2; however, additional studies are strongly needed to elucidate the subtending molecular mechanisms. The RIZ2 overexpression, indeed, increased oncogenicity of both HEK-293 and DLD1 cell lines, by regulating several crucial genes

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involved in many cancer-related pathways, as revealed by transcriptome studies. Besides, RIZ2 induced EGF pathway deregulation in colon cancer cells. This comprehensive chapter delves into the multifaceted PRDM2 roles in several cancer types, exploring its impact on crucial cellular processes, its intricate interactions with signaling pathways, and its potential as a diagnostic and therapeutic target.

Keywords

Cancer · PRDM2 · RIZ

1 Introduction

The PRDM [PRDI-BF1 (Positive Regulatory Domain I-binding factor 1)] family is composed by 19 genes. This gene family is defined by a PR domain, homolog of the SET domain of histone methyltransferase, and multiple zinc-fingers that mediate protein-protein interaction, nuclear import, and sequence-specific DNA binding (Di Zazzo et al. 2013; Mzoughi et al. 2016; Casamassimi et al. 2020). Although the PR domain shows lysine methyltransferases (KMTs) properties, enzymatic activity has been demonstrated for few PRDM proteins (PRDMs). PRDMs directly regulate the chromatin architecture or indirectly recruit histone-modifying enzymes, thus modulating the epigenetic modifications subtending gene expression (Di Zazzo et al. 2013; Mzoughi et al. 2016; Casamassimi et al. 2020). Of note, PRDMs show a strong cell-context dependency, and participate in many developmental processes as well as in the transduction of many signals controlling cell fate. Several studies suggest that the modification of the structure, sequence, or expression level of PRDMs could be involved in many human diseases (Di Zazzo et al. 2013; Mzoughi et al. 2016; Casamassimi et al. 2020). The PRDMs have been implicated in the complex interplay between cell proliferation and differentiation and increasing evidence suggests their role in several cancer types. Through pan cancer bioinformatics analyses performed on The Cancer Genome Atlas (TCGA) database, we identified several PRDMs that are deregulated across human tumors, findings further confirmed in a cohort of several primary tumors (Sorrentino et al. 2018a, b; Casamassimi et al. 2020). However, the molecular mechanisms by which PRDMs exert their role and the signaling pathways involved still need to be fully elucidated.

PRDM2, also known as Positive Regulatory Domain containing 2, is a relevant member of the PRDMs family. *PRDM2* gene products show the ability to bind DNA and transcription factors. PRDM2, indeed, has been identified as a DNA-binding protein (MTB-Zf), a retinoblastoma-binding protein (RIZ) (Buyse et al. 1995), and as a GATA3 transcription factor binding protein (G3B) (Shapiro et al. 1995). MTB-Zf (identical to PRDM2/RIZ2) binds to the MTE DNA element of hemeoxygenase-1 gene to weakly activate transcription (Muraosa et al. 1996). G3B binds the transcription factor GATA-3 and regulates the gene expression of numerous factors pivotal for T-cells function and development (Zhu et al. 2006; Di

Zazzo et al. 2023). Several reports showed that PRDM2 plays a critical role in the regulation of various cellular processes, such as gene expression, cell cycle control, and differentiation (Di Zazzo et al. 2013, 2023; Casamassimi et al. 2020; Sorrentino et al. 2018a, b; Rienzo et al. 2021). Accordingly, emerging studies have highlighted the significance of PRDM2 in cancer, as alterations in its expression and function have been associated with the development and progression of various malignancies.

Two main molecular variants, with (PR+) and without (PR−) the PR domain, are commonly encoded by PRDM genes (Fig. 1). Moreover, some PRDMs display a typical “yin-yang” expression where the use of alternative promoters or alternative splicing can generate two molecular variants playing opposite roles, particularly in cancer: full-length proteins, with tumor suppressor activities, or shorter ones, lacking the PR domain, endowed of oncogenic function. In several cancer types, silencing of the PR+ product and/or increased expression of the PR− one induces the imbalance in their expression levels (Di Zazzo et al. 2013; Mzoughi et al. 2016; Casamassimi et al. 2020).

RIZ1 (PR+ product) and RIZ2 (PR− product) represent the main molecular variants encoded by the PRDM2 locus (Fig. 1). In cancers, epigenetic or genetic alterations result in the silencing of RIZ1 with an imbalance in the RIZ1/RIZ2 expression level in favor of RIZ2 that could be responsible of tumorigenesis (Sorrentino et al. 2018a, b).

This chapter aims to provide a comprehensive overview of PRDM2 in cancer, synthesizing current knowledge and highlighting the open questions that could pave the way for future investigations into the diverse functions and therapeutic potential of this intriguing regulatory protein.

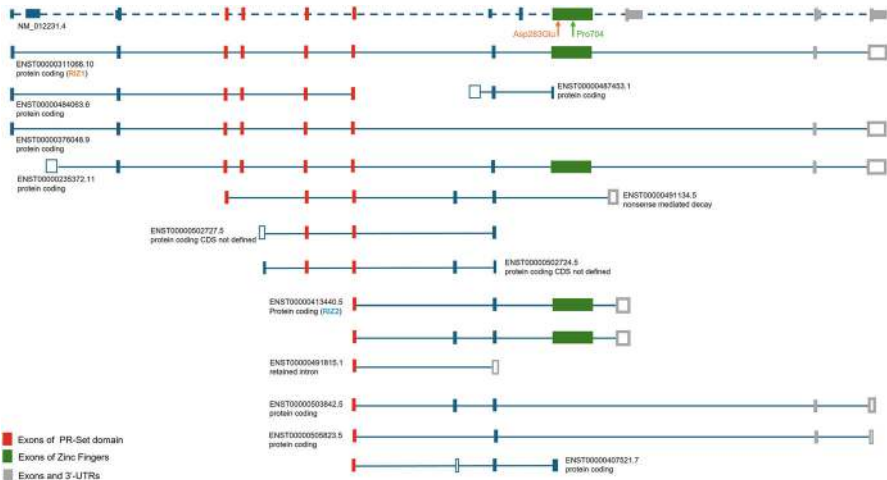


Fig. 1 Schematic illustration of *PRDM2* gene and related transcripts. Different *PRDM2* transcripts from Ensembl database (release 112—May 2024) are shown (Harrison et al. 2024). Transcripts encoding for RIZ1 and RIZ2 proteins are reported in the figure with orange and blue colors respectively. Exons containing sequences encoding for PR domain and zinc fingers are highlighted with different colors. The most described mutations are also represented

1.1 PRDM2 in Cancer

The chromosome region (1p36) containing the *PRDM2* gene locus is frequently characterized by genetic alterations, as deletions, loss-of-heterozygosity or rearrangements in several cancer types (Sorrentino et al. 2018a, b). Our TCGA analysis revealed that *PRDM2* gene is often mutated in stomach, colon, and endometrial cancers, with a frequency of at least 5% (Fig. 2) (Sorrentino et al. 2018a, b). In addition, the data analysis of a polycystic ovary syndrome (PCOS) genome-wide association study revealed that *PRDM2* alleles were associated with the reproductive PCOS subtype showing high luteinizing hormone (LH) and sex hormone binding globulin (SHBG) levels with low body mass index (BMI) and insulin levels (Dapas et al. 2020). Germline variants and somatic uniparental disomy of *PRDM2* were detected by whole-exome sequencing of DNA extracted from the paraffin-embedded sections in rare cases of malignant struma ovarii with peritoneal dissemination (Yamashita et al. 2023).

Additionally, the *PRDM2* gene polymorphisms have been related to tumor onset (Sorrentino et al. 2018a, b; Sasaki et al. 2002; Fang et al. 2000, 2001; Mir et al. 2017). For example, the Pro704 deletion and the T1704A substitution polymorphisms of *PRDM2* gene have been observed in hepatocellular carcinoma (HCC) and in leukemia, respectively. Moreover, the Pro704 deletion polymorphism of *PRDM2* gene exhibits a high degree of heterozygosity in different human cancers, such as colon, breast, and stomach. Notably, the Pro704(+) allele loss was detected in HCC specimens with loss of heterozygosity. Moreover, the Pro704-/- genotype has been linked to chronic myeloid leukemia (CML) increased risk and is significantly associated with advanced disease stage and resistance to therapy, thus suggesting that the Pro704 -/- genotype could play a pivotal role in CML progression (Mir et al. 2017). These findings imply a more potent tumor suppressor function for the Pro704(+) allele in contrast to the polymorphic Pro704(-) allele. Consequently, it is plausible that individuals homozygous for the Pro704(-) allele are more prone to tumor development compared to subjects homozygous or heterozygous for the Pro704(+) allele (Fang et al. 2001; Mir et al. 2017). In human leukemia, the T1704A mutation results in the Asp283Glu substitution between complement receptor (CR) 1- and CR2-E1A-related motifs of PRDM2 proteins, in the region near the conserved Rb-binding motif LXCXE (Sasaki et al. 2002). This Asp283Glu substitution might influence the affinity interaction between Rb and PRDM2 and could potentially contribute to leukemia onset (Sasaki et al. 2002). This accumulated evidence suggests that the chromosomal region 1p36 contains tumor suppressor genes implicated in several human cancer types (Mitelman et al. 1997). Hence, PRDM2 has been proposed to play a role as a tumor suppressor (Huang et al. 1998; Abbondanza et al. 2000). However, the PRDM2 role is even more intriguing and complex. Two main protein variants, namely RIZ1 (PR+) and RIZ2 (PR-), are produced via two alternative promoters, each with or without the PR domain (Liu et al. 1997). Normal tissues abundantly express *RIZ1* and *RIZ2* transcripts with a 1:1 ratio (Liu et al. 1997), while numerous cancer cell lines and tissues show a notable *RIZ1* decrease or absence by both genetic inactivation and epigenetic silencing

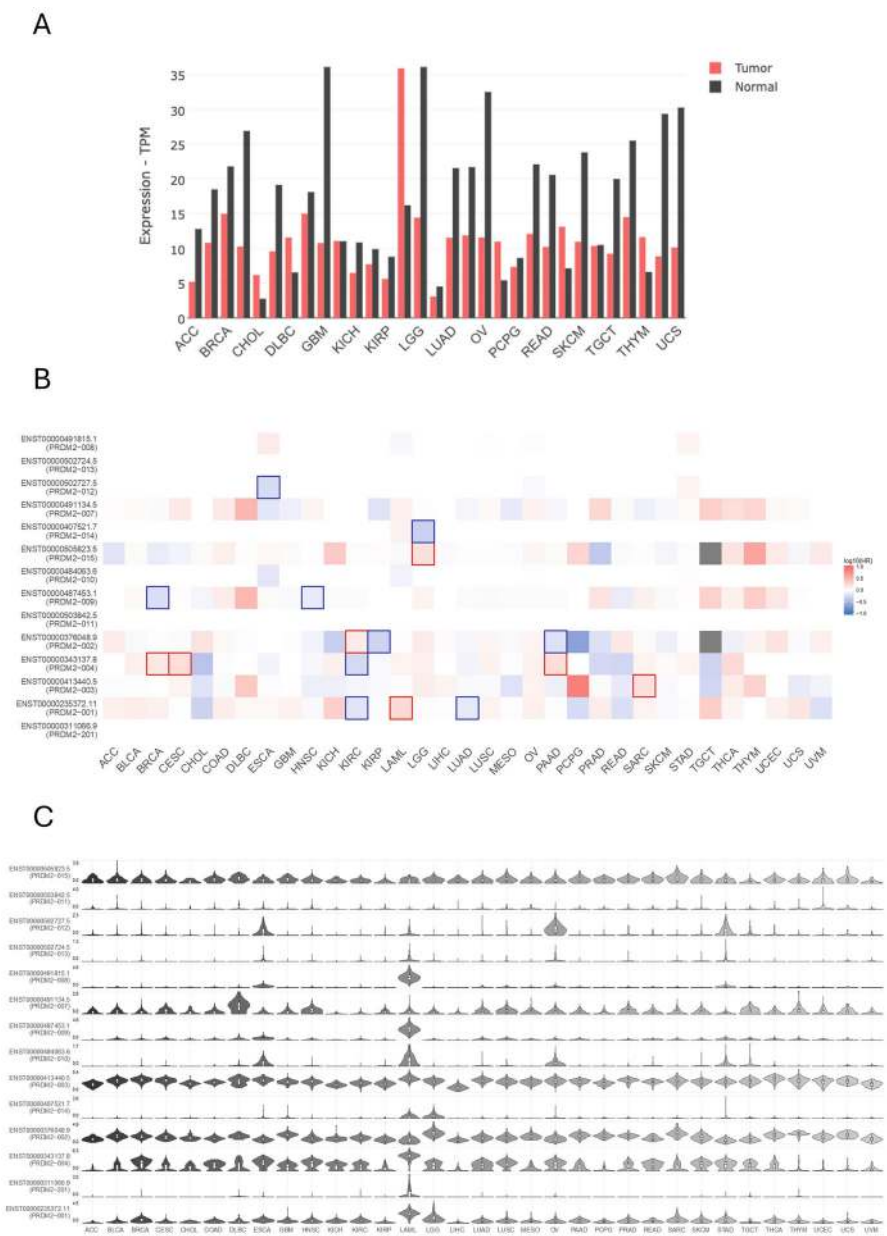


Fig. 2 *PRDM2* analysis in cancer through GEPIA2 platform (Tang et al. 2019). (a) Expression profiles of *PRDM2* in normal (black) and tumor tissues (red); (b) Survival map of hazardous ratio of all *PRDM2* transcripts; (c) *PRDM2* isoform-based analyses

and/or an increase in *RIZ2* expression levels (Abbondanza et al. 2000; He et al. 1998).

This evidence suggests a *RIZ1* negative regulatory effect on cell growth and tumorigenesis thus being a tumor-suppressor, whereas *RIZ2* could function as an oncogene by prompting cell proliferation (He et al. 1998; Congdon et al. 2014). *Riz1* knockout mice, which retain normal *Riz2*, indeed, exhibit a tumor predisposition in both wild-type and mutant *p53* genetic backgrounds. Notably, *Riz1* deficiency (*Riz1* $-/-$) on the *p53* $+/ -$ background is associated with increased tumorigenesis (Steele-Perkins et al. 2001).

Similarly to many tumor suppressor genes, the *PRDM2/RIZ1* promoter shows a CpG island that is commonly methylated in cancer (Buyse et al. 1995; Liu et al. 1997; Du et al. 2001). Consistently, the methylation of *PRDM2/RIZ1* promoter CpG island is frequently observed in breast and liver cancers, pituitary adenomas, nasopharyngeal carcinomas as well as in colon and lung cancer cell lines (Sorrentino et al. 2018a, b; Casamassimi et al. 2020). In addition, *PRDM2/RIZ1* is frequently hypermethylated in HCC tissues and cell lines (Zhao et al. 2017; Zhang et al. 2016). It is well established that the hepatitis B virus (HBV)-X protein (HBx) plays a pivotal role in the molecular pathogenesis of HBV-related HCC, although the underlying mechanism remains to be fully elucidated. An intriguing link was established between HBx action and the epigenetic *PRDM2/RIZ1* inactivation in HCC cells (Zhao et al. 2017). HBx transfection led to a miR-152 expression decrease, resulting in the DNA methyltransferase 1 (*DNMT1*) upregulation, a direct target gene of miR-152 across several cancer types. Subsequently, it has been demonstrated that *DNMT1* binds to the *PRDM2/RIZ1* promoter, thereby modulating its methylation and contributing to its epigenetic inactivation (Zhao et al. 2017).

1.2 PRDM2 in Gastrointestinal Cancers

Several evidence suggested a not negligible role of *PRDM2* in cancers pertaining to the gastrointestinal tract. Interestingly, in a cohort of sessile serrated lesions (SSLs), a premalignant subtype of serrated polyps able to progress in CRCs, *PRDM2* gene promoter methylation correlates with the expression status of *MLH1* a tumor suppressor involved in DNA mismatch repair system. *PRDM2* gene promoter was hypermethylated in *MLH1*-deficient dysplastic SSLs and hypomethylated in *MLH1*-proficient dysplastic SSLs (van Toledo et al. 2024). These data strongly suggest that *PRDM2* promoter hypermethylation, reducing mainly the PR+ product (*PRDM2/RIZ1*) expression, is an early event in dysplasia progression to CRC and reinforces our hypothesis of a tight correlation between the *RIZ1/RIZ2* imbalance and CRC molecular signature.

Recently, we added novel insights on the *PRDM2* role in CRC. Interestingly, we observed that *PRDM2/RIZ2* overexpression increased cell growth and viability, formation of colonies, migration, and organoid size in DLD1, a cell line carrying the CRC-CMS1 (Consensus Molecular Subtype 1) molecular signature and expressing low *PRDM2* levels. Additionally, RNA-seq revealed that the overexpression of

PRDM2/RIZ2 modified CRC cell transcriptome by deregulation of EGF pathway. Indeed, high EGF concentration levels were secreted by *PRDM2/RIZ2* over-expressing DLD1 cells, revealing that *RIZ2* could participate in the EGF autocrine modulation of DLD1 cell behavior that fosters tumorigenesis (Di Donato et al. 2023). Our finding concerning the *RIZ2*-induced increase in EGF secretion represents a very intriguing aspect that could be responsible for CRC pathogenesis and progression. The EGF secretion by CRC cells augments the tumor micro-environment cancer-driving effects by the M2-macrophage polarization induction (Lian et al. 2019), thus encouraging immune suppression, neoangiogenesis, activation, and stroma remodeling. Furthermore, EGF inhibits *LGR5* expression, a marker of normal and cancer intestinal stem cells which serves as Wnt/B-catenin signaling co-receptor. This regulation is finely tuned during the progression from adenoma to carcinoma and in CRC metastasis (Morgan et al. 2018; de Sousa e Melo et al. 2017).

Multiple studies have confirmed *PRDM2* involvement in tumors characterized by chromosomal instability (CIN). Notably, colorectal, gastric, and pancreatic cancers positive for microsatellite instability (MIN) frequently exhibit frameshift mutations, prevalently 1- or 2-bp deletions in two poly-adenosine tracts of microsatellite repeats located in *PRDM2* C-terminal coding region (Casamassimi et al. 2020). Recent analysis from TCGA identified a somatic frameshift mutation in the (A)₉ tract as a prominent microsatellite indel driver hotspot in 48% of stomach tumors, marking the first indication of *PRDM2* role as a cancer driver gene (Maruvka et al. 2017). Notably, these frameshift mutations are biallelic or occur in homozygous/hemizygous manner, aligning *PRDM2* with the two-hit model of tumor suppressor genes.

Moreover, it has been hypothesized that the mutations/deletions in the region encoding for the PR domain or frameshift mutations occurring in the 3' end prevent the interactions between the N-terminal PR domain and the C-terminal PR-binding motif of *RIZ1* protein, thus impairing its function (Chadwick et al. 2000). Despite their prevalence, the functional implications of the *PRDM2* molecular variants with a truncated C-terminal sequence induced by frameshift mutations in tumorigenesis remain unclear and warrant investigation. Intriguingly, genome editing to restore the canonical *PRDM2* gene sequence of c.4467delA allele in CRC cells reinstated H3K9me2 activity, impairing cell growth, reducing anchorage-independent growth, decreasing colony-forming ability, cellular migration, and tumor growth in a mouse xenograft model. Moreover, the restoration of H3K9me2 activity downregulated numerous genes associated with cancer pathways, primarily those involved in epithelial-to-mesenchymal transition (EMT), contributing to a less aggressive cancer phenotype (Pandzic et al. 2017).

1.3 PRDM2 in Hormone-Dependent Cancers

PRDM2/RIZ1 mediates estrogen proliferation induction in estrogen-responsive cells and tissues (Di Zazzo et al. 2013, 2016; Sorrentino et al. 2018a, b; Abbondanza et al. 2000, 2012; Medici et al. 1999; Gazzerri et al. 2006). The proliferation rate increase was associated with a *RIZ1* expression downregulation, and a total *PRDM2/RIZ*

mRNA increase resulting in an alteration in the balance of *RIZ1* and *RIZ2* intracellular concentrations due to *PRDM2/RIZ2* transcript accumulation. Consequently, the 1:1 ratio of *RIZ2* to *RIZ1*, observed in hormone-deprived cells, upon estradiol treatment was approximately 3:1 (Abbondanza et al. 2000, 2012; Medici et al. 1999; Gazzero et al. 2006; Di Zazzo et al. 2016). Considering that *PRDM2/RIZ1* gene has no estrogen response elements (EREs), and no changes in estrogen receptor (ER) binding was observed in its promoter, the impact of estrogen on its expression was indirect (Gazzero et al. 2006). Conversely, our research team revealed that the *PRDM2/RIZ2* promoter shows an ERE regulated by ER α , through the AF1 and AF2 domains, and an increased ER binding has been described upon estradiol treatment. Of note, the pattern of ER α binding, histone H3 methylation and histone H4 acetylation, was similar to other estrogen-regulated genes. The transcriptional activation induced by estrogen was confirmed through the association of topoisomerase II β with the promoter of *PRDM2/RIZ2*. Hence, the transcriptional activation of *PRDM2/RIZ2* could be a direct effect mediated by ERs upon estradiol treatment (Gazzero et al. 2006; Abbondanza et al. 2012).

Significantly, we showed that estradiol triggers a remodeling of the chromatin architecture at the *PRDM2* gene locus to induce the *PRDM2/RIZ2* mRNA expression, as revealed by DNA-picked chromatin (DPC) assays. The 17 β -estradiol facilitates the creation of a loop, favoring the production of transcripts containing exon 9a while diminishing transcripts with exons 9b and 10 (Fig. 1). This process leads to the generation of oncogenic variants (exon 9a) (Abbondanza et al. 2011). The significance and potential functions of these RNA tails remain unclear and warrant further investigation. Estradiol may influence *RIZ2/RIZ1* ratio by mechanisms unrelated to direct gene expression regulation by ER. It could be hypothesized that miRNAs recognize consensus sequences within exon 9, as uncovered through bioinformatics analysis. This finding is notable because various miRNAs are recognized for their role in regulating the estradiol response in breast cancer cells (Bhat-Nakshatri et al. 2009). Interestingly, in breast cancer cells several miRNAs govern estradiol response. Indeed, the miR-let-7, miR98, and miR203 recognize consensus sequences within exon 9 of *PRDM2/RIZ* gene as revealed by bioinformatics analysis (<http://mirdb.org/cgi-bin/search.cgi>). Additionally, the *PRDM2/RIZ2* promoter contains a canonical binding site for c-Myc and estradiol might increase *PRDM2/RIZ2* expression levels by inducing *MYC* gene expression. This induction occurs through a decrease in *PRDM2/RIZ1* expression and ER/Sp1 binding to the c-Myc promoter (Gazzero et al. 2006). We hypothesize that *RIZ2*, by negatively modulating *RIZ1* function, mediates the estrogen proliferative effect by regulating the expression of survival and differentiation genes.

As mentioned previously, it has been suggested that *RIZ2* could function as an oncogene. The *RIZ2* oncogenic properties are associated with the first Zn-finger domain clusters (aa 359–507), that are shown by both *RIZ1* and *RIZ2*. Stable transfection of Zn-finger domains into MCF-7 cells, under estrogen stimulation or deprivation conditions, increased the proliferation rate compared to control cells. These engineered MCF-7 cells also exhibited high cyclin D1 and A expression levels and were less responsive to the anti-estrogens inducing growth inhibitory

effects, probably because of the modified expression of proteins involved in cell proliferation and differentiation (Rossi et al. 2004; Chambery et al. 2006). Moreover, a mass spectrometry proteomic analysis revealed that the forced expression of *PRDM2/RIZ* gene Zn-finger domains in MCF-7 cells correlated with the expression of proteins related to various cancer types or controlling cell proliferation/differentiation, including acid protease cathepsin D, keratin K8, nucleoside diphosphate kinase A, and glycolytic enzyme α -enolase (Chambery et al. 2006).

Moreover, *PRDM2/RIZ1* was implicated in mediating the effects of both estrogen and androgen, even in non-canonical estradiol target tissues such as the prostate (Rossi et al. 2009). However, in non-primary estrogen target tissues, 17 β -estradiol exerts an opposing effect, inducing a shift in *RIZ1/RIZ2* molar ratio in favor of *PRDM2/RIZ1*. Specifically, hormone stimulation did not affect *PRDM2/RIZ1* expression in certain cell types, such as the EPN cell line (derived from normal human prostate), while it either remained unaffected or increased in others, such as SAOS2 (osteosarcoma) cells (Abbondanza et al. 2008; Rossi et al. 2009). Additionally, in germ cell lines the expression level of *PRDM2* is regulated by estradiol whose exposure in intra-uterine life may represent a relevant risk factor for the testicular germ cell tumors (TGCT) onset in adulthood. Moreover, *PRDM2* binds to ER α and exerts influence over proliferation, survival, and apoptosis in both normal and cancerous germ cell lines. This suggests a *PRDM2* putative tumor-suppressor role in TGCT (Di Zazzo et al. 2016). Furthermore, loss of *RIZ1* protein or its switch in nuclear-cytoplasmic localization has been observed in prostate and endometrial cancer cells and tissues. This is accompanied by a decrease in *RIZ1* staining in high and poor differentiated tumors (Rossi et al. 2009; Yang et al. 2017).

PRDM2 may also mediate androgen effects. Upon 5 α -dihydrotestosterone (DHT) treatment of EPN cells a modest increase in cell growth, correlating with a significant rise in *PRDM2/RIZ1* mRNA and protein levels, has been observed. Noteworthy, it has been identified as an androgen-responsive element (ARE) at -361 bp in the regulatory region upstream of *PRDM2* gene promoter 1 (Rossi et al. 2009). However, further studies are warranted to demonstrate that *PRDM2* is a gene responsive to androgens.

Furthermore, retinoic acid led to the *RIZ1* expression and its nuclear redistribution, which correlated with granulocytic differentiation in a human promyelocytic leukemia cell line (HL60) (Gazzerro et al. 2001).

1.4 PRDM2 in Other Cancers and in Tumor Metabolism Re-writers

We recently uncovered the *RIZ2* tumor-promoting function by examining cell viability, cell death, proliferation, migration, invasion, and organoid growth in *RIZ2*-overexpressing HEK293 cells (Rienzo et al. 2021). The forced *PRDM2/RIZ2* expression increased cell viability and growth, facilitated the G2-to-M phase transition and promoted organoid formation in the estrogen-unresponsive HEK-293 cell line, expressing both *RIZ1* and *RIZ2*. Additionally, our microarray analysis demonstrated that *RIZ2* is able to modify the expression of several genes involved

in mitosis. Accordingly, silencing of total *PRDM2/RIZ* (*RIZ1* and *RIZ2*) resulted in reduced cell proliferation and increased apoptosis rates in both MCF-7 (estrogen-responsive) and MDA-MB-231 (estrogen-unresponsive) cells (Rienzo et al. 2021).

It has been observed that in primary meningioma cells *PRDM2* prevented cell proliferation and prompted apoptosis. In addition, *PRDM2* restrained the growth of high-grade meningioma xenografted in nude mice by its methyltransferase activity (Ding et al. 2015). Indeed, the expression of numerous genes involved in critical cellular processes, as the *MYC* oncogene and the tumor suppressor thioredoxin binding protein (*TXNIP*) are respectively down- and up-regulated by the PR domain (Ding et al. 2015). Moreover, *PRDM2/RIZ1* induced G2/M arrest of meningioma cells by influencing the checkpoint protein *UBCH10* expression, a member of the ubiquitin-conjugating enzymes family, by c-Myc degradation (Cai et al. 2017). The *PRDM2* expression levels were lower in drug-refractory prolactinomas than drug-responsive prolactinomas (Cunha et al. 2015; Gao et al. 2015). Interestingly, the *PRDM2* expression restoration increased D(2) dopamine receptor (*D2DR*) levels and synergistically acted with bromocriptine in the reduction of prolactin secretion and MMQ prolactinoma cell line viability.

Cell metabolism alterations are among the most consistent hallmarks of cancer. Cancer cells alter their metabolism to sustain survival, proliferation, invasion, and metastasis. Interestingly, several data suggest that *PRDM2* can be involved in retailoring metabolism pathways. For example, *PRDM2/RIZ1* is involved in insulin-like growth factor (*IGF-1*) and Akt signaling pathways (Pastural et al. 2007). Several cancer types exhibit heightened activation of the *IGF-1* signaling pathway, and *PRDM2/RIZ1* opposes the *IGF-1* receptor and signaling cascade downstream components, such as *AKT* and *ERK1/2*. Indeed, *PRDM2/RIZ1* negatively regulates the expression of *IGF1* gene and upregulates insulin-like growth factor binding protein-2 (*IGFBP-2*) and secreted protein acidic and cysteine-rich (*SPARC*) gene expression (Pastural et al. 2007). Additionally, *PRDM2/RIZ1* knock-out (KO) mice gained more weight on a high fat diet and showed higher *Akt3* mRNA levels and activated *PI3K/Akt/mTOR* pathway than wild-type ones (Xie et al. 2016). Recently, a study demonstrated that *PRDM2/RIZ1* can bind to the *Akt3* promoter and repress its expression by inducing H3K9 methylation (Liu et al. 2018). Moreover, data from our RNA-Seq analysis suggested that *PRDM2/RIZ2* overexpression induced profound modulation of several metabolic drivers (such as regulators of mTOR and lipid metabolism) (Di Donato et al. 2023). Overall, these interesting findings suggest a possible role of *PRDM2* proteins also in cancer cell metabolism that needs to be further investigated.

2 Conclusion

In this chapter we explored the current knowledge on the *PRDM2* role in cancer. *PRDM2* has emerged as a key player in various biological processes and cellular pathways. However, despite significant research efforts to understand its functions in both healthy and diseased states have been done, numerous questions remain

unanswered. The detailed structure of PRDM2 and its post-translational modifications are poorly understood. Moreover, the specific activities of different PRDM2 protein isoforms remain unclear. In this scenario, proteomic studies hold promise in elucidating these aspects by revealing interactions and partnerships with other factors. In addition, while several evidence suggest that PRDM2 forms molecular dimers, the known intra- and inter-chain interactions are insufficient to define the complete composition of these complexes. In addition, the possibility of multimer assembly within the nucleus also needs investigation. The presence of transcripts with varying untranslated regions (UTRs) hints at potential post-transcriptional regulation of *PRDM2* (Fig. 1). Intriguingly, PRDM2 ability to bind estrogen and progesterone receptors suggests a role in regulating genes responsive to these hormones. Again, the significance of interactions with Rb and GATA-3 requires further exploration. Several studies indicate that the PR-positive isoform of PRDM2 controls lipid metabolism through the AKT-mTOR pathway. Additionally, this isoform might act as a sensor for S-adenosylmethionine, influencing gene expression responsiveness to methyl donors. Several reports suggest tumor suppressor activity for RIZ1, while RIZ2 might have growth-promoting properties. Over the past decades, RIZ2 research has been limited due to challenges related to the RIZ1 and RIZ2 sequence identity. RT-PCR analysis in fact cannot distinguish between RIZ1 and RIZ2 expression. Commercially available antibodies target RIZ1 or both molecular variants, hindering specific RIZ2 detection. Overcoming these limitations requires identifying unique features of RIZ2 and developing tools for its specific identification. Interestingly, in a recent study on lymphocytes, we observed that the short tail amplicon (containing exon 9a) was prevalently correlated to *RIZ2* transcript (Di Zazzo et al. 2023). Noteworthy, differences in the 3'-tails of *RIZ1* and *RIZ2* transcripts could be useful to selectively target *RIZ1* and *RIZ2* transcripts and should be further characterized. Numerous issues remain regarding the underlying mechanisms and involved cellular pathways in PRDM2 function. Chromatin immunoprecipitation (ChIP) studies followed by next-generation sequencing on RIZ2 overexpressing cells might provide us the profile of its downstream targets to further depict its function. Understanding these mechanisms could have significant implications for disease management. PRDM2, indeed, shows the potential as a diagnostic marker and therapeutic target for various malignancies. Investigating the mechanisms by which PRDM2 influences tumor promotion or suppression can provide valuable insights into the molecular events underlying cancer onset and progression. New efforts are needed for understanding the intricate involvement of PRDM2 in cancer biology for the development of targeted therapeutic strategies. Elucidating the role of PRDM2 in the pathogenesis and progression of cancer represents an interesting interdisciplinary challenge today. First of all, clarifying the interplay between PRDM2 and the molecular pathways it regulates and/or the networks able to modulate its expression is a priority that would shed light on the role not only of PRDM2 but of an extended family of genes, such as PRDMs, which are evolutionarily conserved and indisputably related to cell differentiation and fate. Among other PRDMs, PRDM2, directly or through its interactors, seems to be able not only to determine the fate of preneoplastic lesions and of the primary tumors, but

also to rewrite the features of the tumor microenvironment by acting at least at two levels such as cellular metabolism and the activation and differentiation of cells responsible for the antitumor immune response. In conclusion, we are convinced that clarifying the role of RIZ1 and RIZ2 could allow to identify new markers and features useful in the diagnosis and molecular stratification of tumors. The identification of small molecules or drugs, currently unknown, able of selectively counteracting or modulating PRDM2 and its interactors, is a challenge that could reserve interesting pharmacological and clinical applications.

References

- Abbondanza C, Medici N, Nigro V, Rossi V, Gallo L, Piluso G, Belsito A, Roscigno A, Bontempo P, Puca AA, Molinari AM, Moncharmont B, Puca GA (2000) The retinoblastoma-interacting zinc-finger protein RIZ is a downstream effector of estrogen action. *Proc Natl Acad Sci USA* 97(7):3130–3135. <https://doi.org/10.1073/pnas.97.7.3130>
- Abbondanza C, de Nigris F, De Rosa C, Rossiello R, Puca GA, Napoli C (2008) Silencing of YY1 downregulates RIZ1 promoter in human osteosarcoma. *Oncol Res* 17(1):33–41. <https://doi.org/10.3727/096504008784046081>
- Abbondanza C, De Rosa C, Ombra MN, Aceto F, Medici N, Altucci L, Moncharmont B, Puca GA, Porcellini A, Avvedimento EV, Perillo B (2011) Highlighting chromosome loops in DNA-picked chromatin (DPC). *Epigenetics* 6(8):979–986. <https://doi.org/10.4161/epi.6.8.16060>
- Abbondanza C, De Rosa C, D’Arcangelo A, Pacifico M, Spizuoco C, Piluso G, Di Zazzo E, Gazzerri P, Medici N, Moncharmont B, Puca GA (2012) Identification of a functional estrogen-responsive enhancer element in the promoter 2 of PRDM2 gene in breast cancer cell lines. *J Cell Physiol* 227(3):964–975. <https://doi.org/10.1002/jcp.22803>
- Bhat-Nakshatri P, Wang G, Collins NR, Thomson MJ, Geistlinger TR, Carroll JS, Brown M, Hammond S, Srour EF, Liu Y, Nakshatri H (2009) Estradiol-regulated microRNAs control estradiol response in breast cancer cells. *Nucleic Acids Res* 37(14):4850–4861. <https://doi.org/10.1093/nar/gkp500>
- Buyse IM, Shao G, Huang S (1995) The retinoblastoma protein binds to RIZ, a zinc-finger protein that shares an epitope with the adenovirus E1A protein. *Proc Natl Acad Sci USA* 92(10):4467–4471. <https://doi.org/10.1073/pnas.92.10.4467>
- Cai Z, Zou Y, Hu H, Lu C, Sun W, Jiang L, Hu G (2017) RIZ1 negatively regulates ubiquitin-conjugating enzyme E2C/UbcH10 via targeting c-Myc in meningioma. *Am J Transl Res* 9(5):2645–2655
- Casamassimi A, Rienzo M, Di Zazzo E, Sorrentino A, Fiore D, Proto MC, Moncharmont B, Gazzerri P, Bifulco M, Abbondanza C (2020) Multifaceted role of PRDM proteins in human cancer. *Int J Mol Sci* 21(7):2648. <https://doi.org/10.3390/ijms21072648>
- Chadwick RB, Jiang GL, Bennington GA, Yuan B, Johnson CK, Stevens MW, Niemann TH, Peltomaki P, Huang S, de la Chapelle A (2000) Candidate tumor suppressor RIZ is frequently involved in colorectal carcinogenesis. *Proc Natl Acad Sci USA* 97(6):2662–2667. <https://doi.org/10.1073/pnas.040579497>
- Chambery A, Farina A, Di Maro A, Rossi M, Abbondanza C, Moncharmont B, Malorni L, Cacace G, Pocsfalvi G, Malorni A, Parente A (2006) Proteomic analysis of MCF-7 cell lines expressing the zinc-finger or the proline-rich domain of retinoblastoma-interacting-zinc-finger protein. *J Proteome Res* 5(5):1176–1185. <https://doi.org/10.1021/pr0504743>
- Congdon LM, Sims JK, Tuzon CT, Rice JC (2014) The PR-Set7 binding domain of Riz1 is required for the H4K20me1-H3K9me1 trans-tail ‘histone code’ and Riz1 tumor suppressor function. *Nucleic Acids Res* 42(6):3580–3589. <https://doi.org/10.1093/nar/gkt1377>

- Cunha FS, Domenice S, Câmara VL, Sircili MH, Gooren LJ, Mendonça BB, Costa EM (2015) Diagnosis of prolactinoma in two male-to-female transsexual subjects following high-dose cross-sex hormone therapy. *Andrologia* 47(6):680–684. <https://doi.org/10.1111/and.12317>
- Dapas M, Lin FTJ, Nadkarni GN, Sisk R, Legro RS, Urbanek M, Hayes MG, Dunaif A (2020) Distinct subtypes of polycystic ovary syndrome with novel genetic associations: an unsupervised, phenotypic clustering analysis. *PLoS Med* 17(6):e1003132. <https://doi.org/10.1371/journal.pmed.1003132>
- de Sousa e Melo F, Kurtova AV, Harnoss JM, Kljavin N, Hoeck JD, Hung J, Anderson JE, Storm EE, Modrusan Z, Koepfen H, Dijkgraaf GJ, Piskol R, de Sauvage FJ (2017) A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. *Nature* 543(7647):676–680. <https://doi.org/10.1038/nature21713>
- Di Donato M, Di Zazzo E, Salvati A, Sorrentino C, Giurato G, Fiore D, Proto MC, Rienzo M, Casamassimi A, Gazzero P, Bifulco M, Castoria G, Weisz A, Nassa G, Abbondanza C (2023) RIZ2 at the crossroad of the EGF/EGFR signaling in colorectal cancer. *J Transl Med* 21(1):736. <https://doi.org/10.1186/s12967-023-04621-6>
- Di Zazzo E, De Rosa C, Abbondanza C, Monchamont B (2013) PRDM proteins: molecular mechanisms in signal transduction and transcriptional regulation. *Biology (Basel)*. 2(1): 107–141. <https://doi.org/10.3390/biology2010107>
- Di Zazzo E, Porcile C, Bartollino S, Monchamont B (2016) Critical function of PRDM2 in the neoplastic growth of testicular germ cell tumors. *Biology (Basel)* 5(4):54. <https://doi.org/10.3390/biology5040054>
- Di Zazzo E, Rienzo M, Casamassimi A, De Rosa C, Medici N, Gazzero P, Bifulco M, Abbondanza C (2023) Exploring the putative role of PRDM1 and PRDM2 transcripts as mediators of T lymphocyte activation. *J Transl Med* 21(1):217. <https://doi.org/10.1186/s12967-023-04066-x>
- Ding MH, Wang Z, Jiang L, Fu HL, Gao J, Lin XB, Zhang CL, Liu ZY, Shi YF, Qiu GZ, Ma Y, Cui DX, Hu GH, Jin WL (2015) The transducible TAT-RIZ1-PR protein exerts histone methyltransferase activity and tumor-suppressive functions in human malignant meningiomas. *Biomaterials* 56:165–178. <https://doi.org/10.1016/j.biomaterials.2015.03.058>
- Du Y, Carling T, Fang W, Piao Z, Sheu JC, Huang S (2001) Hypermethylation in human cancers of the RIZ1 tumor suppressor gene, a member of a histone/protein methyltransferase superfamily. *Cancer Res* 61(22):8094–8099
- Fang W, Piao Z, Simon D, Sheu JC, Huang S (2000) Mapping of a minimal deleted region in human hepatocellular carcinoma to 1p36.13-p36.23 and mutational analysis of the RIZ (PRDM2) gene localized to the region. *Genes Chromosomes Cancer* 28(3):269–275
- Fang W, Piao Z, Buyse IM, Simon D, Sheu JC, Perucho M, Huang S (2001) Preferential loss of a polymorphic RIZ allele in human hepatocellular carcinoma. *Br J Cancer* 84(6):743–747. <https://doi.org/10.1054/bjoc.2000.1667>
- Gao H, Wang F, Lan X, Li C, Feng J, Bai J, Cao L, Gui S, Hong L, Zhang Y (2015) Lower PRDM2 expression is associated with dopamine-agonist resistance and tumor recurrence in prolactinomas. *BMC Cancer* 15:272. <https://doi.org/10.1186/s12885-015-1267-0>
- Gazzero P, Bontempo P, Schiavone EM, Abbondanza C, Monchamont B, Armetta I, Medici N, De Simone M, Nola E, Puca GA, Molinari AM (2001) Differentiation of myeloid cell lines correlates with a selective expression of RIZ protein. *Mol Med* 7(8):552–560
- Gazzero P, Abbondanza C, D'Arcangelo A, Rossi M, Medici N, Monchamont B, Puca GA (2006) Modulation of RIZ gene expression is associated to estradiol control of MCF-7 breast cancer cell proliferation. *Exp Cell Res* 312(3):340–349. <https://doi.org/10.1016/j.yexcr.2005.11.002>
- Harrison PW, Amode MR, Austine-Orimoloye O, Azov AG, Barba M, Barnes I, Becker A, Bennett R, Berry A, Bhai J, Bhurji SK, Boddu S et al (2024) Ensembl 2024. *Nucleic Acids Res* 52(D1):D891–D899. <https://doi.org/10.1093/nar/gkad1049>
- He L, Yu JX, Liu L, Buyse IM, Wang MS, Yang QC, Nakagawara A, Brodeur GM, Shi YE, Huang S (1998) RIZ1, but not the alternative RIZ2 product of the same gene, is underexpressed in breast cancer, and forced RIZ1 expression causes G2-M cell cycle arrest and/or apoptosis. *Cancer Res* 58(19):4238–4244

- Huang S, Shao G, Liu L (1998) The PR domain of the Rb-binding zinc finger protein RIZ1 is a protein binding interface and is related to the SET domain functioning in chromatin-mediated gene expression. *J Biol Chem* 273(26):15933–15939. <https://doi.org/10.1074/jbc.273.26.15933>
- Lian G, Chen S, Ouyang M, Li F, Chen L, Yang J (2019) Colon cancer cell secretes EGF to promote M2 polarization of TAM through EGFR/PI3K/AKT/mTOR pathway. *Technol Cancer Res Treat* 18:1533033819849068. <https://doi.org/10.1177/1533033819849068>. Erratum in: *Technol Cancer Res Treat* 2023 Jan-Dec;22:15330338231188082
- Liu L, Shao G, Steele-Perkins G, Huang S (1997) The retinoblastoma interacting zinc finger gene RIZ produces a PR domain-lacking product through an internal promoter. *J Biol Chem* 272(5): 2984–2991. <https://doi.org/10.1074/jbc.272.5.2984>
- Liu Q, Qu X, Xie X, He P, Huang S (2018) Repression of Akt3 gene transcription by the tumor suppressor RIZ1. *Sci Rep* 8(1):1528. <https://doi.org/10.1038/s41598-018-19943-5>
- Maruvka YE, Mouw KW, Karlic R, Parasuraman P, Kamburov A, Polak P, Haradhvala NJ, Hess JM, Rheinbay E, Brody Y, Koren A, Braunstein LZ, D'Andrea A, Lawrence MS, Bass A, Bernards A, Michor F, Getz G (2017) Analysis of somatic microsatellite indels identifies driver events in human tumors. *Nat Biotechnol* 35(10):951–959. <https://doi.org/10.1038/nbt.3966>
- Medici N, Abbondanza C, Nigro V, Rossi V, Piluso G, Belsito A, Gallo L, Roscigno A, Bontempo P, Puca AA, Molinari AM, Moncharmont B, Puca GA (1999) Identification of a DNA binding protein cooperating with estrogen receptor as RIZ (retinoblastoma interacting zinc finger protein). *Biochem Biophys Res Commun* 264(3):983–989. <https://doi.org/10.1006/bbrc.1999.1604>
- Mir R, Najar IA, Guru S, Javaid J, Yadav P, Masroor M, Zuberi M, Farooq S, Bhat M, Gupta N, Ray PC, Saxena A (2017) A deletion polymorphism in the RIZ gene is associated with increased progression of imatinib treated chronic myeloid leukemia patients. *Leuk Lymphoma* 58(7): 1694–1701. <https://doi.org/10.1080/10428194.2016.1251589>
- Mitelman F, Mertens F, Johansson B (1997) Spec No:417–74) A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nat Genet.* <https://doi.org/10.1038/ng0497supp-417>
- Morgan RG, Mortenstson E, Legge DN, Gupta B, Collard TJ, Greenhough A, Williams AC (2018) LGR5 expression is regulated by EGF in early colorectal adenomas and governs EGFR inhibitor sensitivity. *Br J Cancer* 118(4):558–565. <https://doi.org/10.1038/bjc.2017.412>
- Muraosa Y, Takahashi K, Yoshizawa M, Shibahara S (1996) cDNA cloning of a novel protein containing two zinc-finger domains that may function as a transcription factor for the human heme-oxygenase-1 gene. *Eur J Biochem* 235(3):471–479. <https://doi.org/10.1111/j.1432-1033.1996.00471.x>
- Mzoughi S, Tan YX, Low D, Guccione E (2016) The role of PRDMs in cancer: one family, two sides. *Curr Opin Genet Dev* 36:83–91. <https://doi.org/10.1016/j.gde.2016.03.009>
- Pandzic T, Rendo V, Lim J, Larsson C, Larsson J, Stoimenov I, Kundu S, Ali MA, Hellström M, He L, Lindroth AM, Sjöblom T (2017) Somatic PRDM2 c.4467delA mutations in colorectal cancers control histone methylation and tumor growth. *Oncotarget* 8(58):98646–98659. <https://doi.org/10.18632/oncotarget.21713>
- Pastural E, Takahashi N, Dong WF, Bainbridge M, Hull A, Pearson D, Huang S, Lowsky R, DeCoteau JF, Geyer CR (2007) RIZ1 repression is associated with insulin-like growth factor-1 signaling activation in chronic myeloid leukemia cell lines. *Oncogene* 26(11):1586–1594. <https://doi.org/10.1038/sj.onc.1209959>
- Rienzo M, Sorrentino A, Di Zazzo E, Di Donato M, Carafa V, Marino MM, De Rosa C, Gazzzerro P, Castoria G, Altucci L, Casamassimi A, Abbondanza C (2021) Searching for a putative mechanism of RIZ2 tumor-promoting function in cancer models. *Front Oncol* 10:583533. <https://doi.org/10.3389/fonc.2020.583533>
- Rossi M, Abbondanza C, D'Arcangelo A, Gazzzerro P, Medici N, Moncharmont B, Puca GA (2004) The Zn-finger domain of RIZ protein promotes MCF-7 cell proliferation. *Cancer Lett* 215(2): 229–237. <https://doi.org/10.1016/j.canlet.2004.05.014>

- Rossi V, Staibano S, Abbondanza C, Pasquali D, De Rosa C, Mascolo M, Bellastella G, Visconti D, De Bellis A, Moncharmont B, De Rosa G, Puca GA, Bellastella A, Sinisi AA (2009) Expression of RIZ1 protein (retinoblastoma-interacting zinc-finger protein 1) in prostate cancer epithelial cells changes with cancer grade progression and is modulated in vitro by DHT and E2. *J Cell Physiol* 221(3):771–777. <https://doi.org/10.1002/jcp.21920>
- Sasaki O, Meguro K, Tohmiya Y, Funato T, Shibahara S, Sasaki T (2002) Nucleotide alteration of retinoblastoma protein-interacting zinc finger gene, RIZ, in human leukemia. *Tohoku J Exp Med* 196(3):193–201. <https://doi.org/10.1620/tjem.196.193>
- Shapiro VS, Lee P, Winoto A (1995) Identification and cloning of the G3B cDNA encoding a 3' segment of a protein binding to GATA-3. *Gene* 163(2):329–330. [https://doi.org/10.1016/0378-1119\(95\)00420-b](https://doi.org/10.1016/0378-1119(95)00420-b)
- Sorrentino A, Federico A, Rienzo M, Gazzero P, Bifulco M, Ciccodicola A, Casamassimi A, Abbondanza C (2018a) PR/SET domain family and cancer: novel insights from the cancer genome atlas. *Int J Mol Sci* 19(10):3250. <https://doi.org/10.3390/ijms19103250>
- Sorrentino A, Rienzo M, Ciccodicola A, Casamassimi A, Abbondanza C (2018b) Human PRDM2: structure, function and pathophysiology. *Biochim Biophys Acta Gene Regul Mech* S1874-9399 (18):30071–30073. <https://doi.org/10.1016/j.bbarm.2018.06.002>
- Steele-Perkins G, Fang W, Yang XH, Van Gele M, Carling T, Gu J, Buyse IM, Fletcher JA, Liu J, Bronson R, Chadwick RB, de la Chapelle A, Zhang X, Speleman F, Huang S (2001) Tumor formation and inactivation of RIZ1, an Rb-binding member of a nuclear protein-methyltransferase superfamily. *Genes Dev* 15(17):2250–2262. <https://doi.org/10.1101/gad.870101>
- Tang Z, Kang B, Li C, Chen T, Zhang Z (2019) GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res* 47(W1):W556–W560. <https://doi.org/10.1093/nar/gkz430>
- van Toledo DE, Bleijenberg AG, Venema A, de Wit MJ, van Eeden S, Meijer GA, Carvalho B, Dekker E, Henneman P, JE IJ, van Noesel CJ (2024) Aberrant PRDM2 methylation as an early event in serrated lesions destined to evolve into microsatellite-unstable colorectal cancers. *J Pathol Clin Res* 10(2):e348. <https://doi.org/10.1002/cjp2.348>
- Xie X, Man X, Zhu Z, Yuan D, Huang S (2016) Tumor suppressor RIZ1 in obesity and the PI3K/AKT/mTOR pathway. *Obesity (Silver Spring)* 24(2):389–397. <https://doi.org/10.1002/oby.21364>
- Yamashita H, Nakayama K, Kanno K, Ishibashi T, Ishikawa M, Sato S, Iida K, Razia S, Kyo S (2023) Identifying the carcinogenic mechanism of malignant Struma Ovarii using whole-exome sequencing and DNA methylation analysis. *Curr Issues Mol Biol* 45(3):1843–1851. <https://doi.org/10.3390/cimb45030118>
- Yang T, Ren C, Jiang A, Yu Z, Li G, Wang G, Zhang Q (2017) RIZ1 is regulated by estrogen and suppresses tumor progression in endometrial cancer. *Biochem Biophys Res Commun* 489(2):96–102. <https://doi.org/10.1016/j.bbrc.2017.05.095>
- Zhang C, Li J, Huang T, Duan S, Dai D, Jiang D, Sui X, Li D, Chen Y, Ding F, Huang C, Chen G, Wang K (2016) Meta-analysis of DNA methylation biomarkers in hepatocellular carcinoma. *Oncotarget* 7(49):81255–81267. <https://doi.org/10.18632/oncotarget.13221>
- Zhao Z, Hu Y, Shen X, Lao Y, Zhang L, Qiu X, Hu J, Gong P, Cui H, Lu S, Zheng Y, Zhou M, Fan H (2017) HBx represses RIZ1 expression by DNA methyltransferase 1 involvement in decreased miR-152 in hepatocellular carcinoma. *Oncol Rep* 37(5):2811–2818. <https://doi.org/10.3892/or.2017.5518>
- Zhu J, Yamane H, Cote-Sierra J, Guo L, Paul WE (2006) GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. *Cell Res* 16(1):3–10. <https://doi.org/10.1038/sj.cr.7310002>



Importance of Selected Antioxidants in Carcinogenesis and Cancer Therapy

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Abstract

Oxidative stress is a key driver of carcinogenesis, and antioxidants play a critical role in maintaining redox homeostasis and preserving cellular integrity. While antioxidants hold promise as chemopreventive agents and adjunctive therapies in cancer treatment, their dualistic nature complicates their use in clinical practice. Personalised approaches are needed to optimise the efficacy and safety of antioxidant-based interventions, taking into account such factors as the tumour type, stage, genetic background, and redox environment.

In this chapter, we aim to elucidate the importance of selected antioxidants in carcinogenesis and cancer therapy by synthesising evidence from epidemiological studies, preclinical research, and clinical trials. By delineating the multiple roles of antioxidants in modulating key pathways involved in cancer development and treatment response, we aim to provide insights into the rational use of personalised antioxidant-based interventions aimed at reducing cancer incidence and improving therapeutic outcomes.

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Preclinical research has elucidated the molecular mechanisms by which antioxidants modulate key pathways involved in carcinogenesis, including DNA damage repair, inflammation, angiogenesis, and metastasis. In addition, clinical trials have investigated the efficacy of antioxidant supplementation in cancer prevention and therapy, with mixed results depending on such factors as the antioxidant type, dosage, timing, and patient population. Controversies remain regarding the optimal use of antioxidants in cancer therapy, as high doses may potentially interfere with the cytotoxic effects of anti-cancer treatments and promote tumour growth in certain contexts. In addition, emerging antioxidant compounds with potential anti-cancer properties are being actively investigated as novel therapeutic agents.

This chapter provides an overview of the importance of selected antioxidants in carcinogenesis and cancer therapy. Oxidative stress is a key driver of carcinogenesis, and antioxidants play a critical role in maintaining redox homeostasis and preserving cellular integrity. While antioxidants hold promise as chemopreventive agents and adjunctive therapies in cancer treatment, their dualistic nature complicates their use in clinical practice.

Keywords

Antioxidants · Oncopathologies · Oxidative stress · Prevention · Reactive oxygen species · Treatment · Tumours

Abbreviations

AOE	antioxidant enzymes;
AP-1	activator protein-1 (a transcription factor);
FRs	free radicals;
GPx	glutathione peroxidase;
GR	glutathione reductase;
GSH	glutathione reduced;
GSSG	glutathione oxidised;
GST	glutathione S-transferase;
H ₂ O ₂	hydrogen peroxide;
HIF-1	hypoxia-inducible factor 1;
HO-1	heme oxygenase 1;
LDH	lactate dehydrogenase;
LINEs	long interspersed nuclear elements;
LPO	lipid peroxidation;
MT1 and MT2	melatonin receptors;
NAC	N-acetylcysteine;
NOX	NADPH oxidases;
Nrf2	nuclear factor-erythroid 2 related factor 2;

O ₂ ^{•−}	superoxide anion radical;
OS	oxidative stress;
p53	tumour protein p53;
PKD1	3-phosphoinositide-dependent protein kinase-1;
RAC1	Rac Family Small GTPase 1;
RhoA	small guanosine triphosphatase (GTPase);
RNS	reactive nitrogen species;
ROS	reactive oxygen species;
RUNX3	runt-related transcription factor 3;
SOD	superoxide dismutase;
Trx	thioredoxin;
VEGF	vascular endothelial growth factor.

1 Introduction

The mechanisms of carcinogenesis and the processes of transformation of normal cells into tumour cells are one of the most pressing problems in oncology. Currently, the processes of proliferation and apoptosis of tumour cells involve extremely active agents capable of interacting with organic compounds and initiating a chain of chemical reactions, i.e. free radicals (FRs) and their variants, mainly reactive oxygen species (ROS) (Mitra et al. 2019; Checa and Aran 2020). In recent years, the question of the link between FRs and carcinogenic processes has gained momentum and become one of the most controversial (Lobo et al. 2010). New studies are underway in pancreatic cancer (Yu and Kim 2014), colorectal cancer (Kundaktepe et al. 2021), ovarian cancer pathogenesis and neoangiogenesis (Ding et al. 2021), mechanisms of lung carcinogenesis (Filaire et al. 2013), and cervical cancer (Silva et al. 2018). This is largely because there is still a relative lack of consistency in the results despite the large number of studies conducted in this area.

Particular attention should be paid to studies which, based on the study of the kinetic patterns of tumour growth, have shown that free radical reactions play a key role not only in the primary process of cell malignancy but also in further progression (Liou and Storz 2010; Sainz et al. 2012; Marengo et al. 2016). Many oncogenic electrophilic agents have been shown to undergo oxidative stress (OS), producing highly reactive products that bind tightly to membrane and nuclear DNA, i.e. the process is considered to be metabolic activation of oncogenes. In this case, excess fatty acid oxidation products play the role of co-carcinogens or tumour promoters (Koundouros and Poulogiannis 2020). Significantly, Fe²⁺-catalysed free radical systems are more effective in activating carcinogens than microsomal oxidase systems (Romero et al. 2014). There is evidence that FRs are involved in the mechanisms of DNA methylation, damage to p53, and disruption of its tumour suppressor (apoptotic) function during carcinogenesis (Budanov 2014; Chen 2016).

ROS production in cancer cells also commonly occurs after exposure to chemotherapeutic drugs, such as doxorubicin, vinblastine, paclitaxel, and platinum compounds, resulting in multiple cellular responses to OS (Bułdak et al. 2014). Tumour cells have higher levels of ROS than normal cells. Moderate increases in ROS production in cancer cells can promote cell proliferation and differentiation (Zhou et al. 2014). On the other hand, excessive levels of ROS can cause oxidative damage to proteins, DNA, and lipids and act as toxic agents (Juan et al. 2021). Cancer cells can also adapt to survive certain levels of ROS, mainly by increasing the activity of antioxidant enzymes (AOEs) (Bułdak et al. 2014; Arfin et al. 2021). Cancer cells in advanced tumours often exhibit multiple genetic alterations and high levels of OS, suggesting that it may be possible to preferentially eliminate these cells by pharmacological ROS insults. However, upregulation of antioxidant capacity as an adaptation to intrinsic OS in cancer cells may confer drug resistance (Trachootham et al. 2009).

The opinions of researchers and clinicians are divided and often contradictory regarding both the role of FRs in cancer and the associated defence mechanisms—cellular enzyme systems and the use of antioxidants in malignant neoplasms. The ratio of pro- and antioxidant status of the body plays an important role in the pathogenesis of tumour diseases and complications of their treatment (Rahal et al. 2014). It should be noted that FRs play an important role in carcinogenesis (Sainz et al. 2012). Therefore, maintaining a balance between oxidants and antioxidants through appropriate therapy may help in the treatment of precancerous conditions (Poljsak et al. 2013; Fuchs-Tarlovsky 2013; Athreya and Xavier 2017). Proponents of the increased free radical theory in tumours insist on the need to test and include antioxidants, antihypoxants, and membrane protectors in the standard treatment regimen for cancer patients (Calaf et al. 2018; Sur et al. 2022). Proponents of the enhanced antioxidant defence theory argue that the use of antioxidants during cancer treatment may worsen the outcomes (Block et al. 2007; Poljsak and Milisav 2018). Therefore, the question of the role of FRs in tumours remains unresolved and holds great promise for the development of new methods and tactics for the treatment of cancer patients based on free radical processes or the use of antioxidant defence. The role of FRs in the tumour itself remains a subject of debate and further research.

Carcinogenesis, the complex process of cancer development, involves a myriad of molecular pathways and cellular interactions. AOE play a dual role—protecting cells from oxidative damage while potentially contributing to cancer progression (Hawk et al. 2016; Marengo et al. 2016; Ito et al. 2019). This review examines the nuanced functions of selected AOE in carcinogenesis, their interplay with cellular redox signalling pathways, their impact on tumour initiation, progression, and metastasis, and their potential as therapeutic targets. By elucidating the intricate balance between antioxidant defence mechanisms and cancer development, this review aims to shed light on new avenues for understanding and potentially intervening in the carcinogenic process.

2 Free Radicals and Their Role in Normal Cells in Physiological Conditions

In recent decades, there has been increasing interest in the molecular aspects of changes in the gene structure and expression in the pathogenesis of tumour development (Hanahan and Weinberg 2011). This is largely due to the development of knowledge of DNA and changes that occur therein under the influence of various factors. Particular attention has been paid to the effect of FRs on DNA and their role in the tumourigenic transformation of cells (Diaz-Cano 2012). FRs differ from normal molecules in that they have an unpaired (single) electron in their outer electron shell. This makes the radicals chemically overactive (Phaniendra et al. 2015).

The oxygen molecule itself is a radical, and because it has two unpaired electrons, it is called a biradical. Examples of radicals include superoxide ($O_2^{\cdot-}$), oxygen radical ($O_2^{\cdot}$), hydroxyl ($OH^{\cdot}$), alkoxyl radical ($RO^{\cdot}$), peroxy radical ($ROO^{\cdot}$), nitric oxide (nitrogen monoxide) ($NO^{\cdot}$), and nitrogen dioxide ($NO_2^{\cdot}$) (Halliwell 2001) (Fig. 1). The high reactivity of these radicals is related to the presence of an unpaired electron, which they tend to donate or gain another electron to achieve stability. Non-radical species include hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), hypobromous acid ($HOBr$), ozone (O_3), singlet oxygen ($^1O_2$), nitrous acid (HNO_2), nitrosyl cation (NO^+), nitroxyl anion (NO^-), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), nitronium (nitryl) cation (NO_2^+), organic peroxides ($ROOH$), aldehydes ($HCOR$), and peroxyxynitrite ($ONOOH$) (Kohen and Nyska 2002; Phaniendra et al. 2015) (Fig. 1). These non-radical species are not free radicals but can easily lead to free radical reactions in living organisms (Genestra 2007).

ROS are formed by the oxidation of oxygen with one, two, or three electrons. They are the first in the chain of free radical oxidation reactions and give rise to other radicals, i.e. reactive forms of chlorine, nitrogen (RNS), etc. Both ROS and RNS together form FRs and other non-radical reactive species (Pham-Huy et al. 2008). They play a dual role as both beneficial and toxic compounds to the living system (Phaniendra et al. 2015). At moderate or low levels, ROS and RNS have beneficial effects and are involved in various physiological functions, such as immune function (i.e. defence against pathogenic microorganisms), a number of cellular signalling pathways, mitogenic response, and redox regulation (Nordberg and Arnér 2001). However, at higher concentrations, both ROS and RNS generate oxidative and nitrosative stress, respectively, causing potential damage to biomolecules (Phaniendra et al. 2015). Oxidative and nitrosative stress develop when there is an excess production of ROS and RNS and a deficiency of enzymatic and non-enzymatic antioxidants (Rahal et al. 2014). Most importantly, excess ROS can damage the integrity of various biomolecules, including lipid peroxidation (LPO) of polyunsaturated fatty acids (such as membrane phospholipids) (Ito et al. 2019), oxidation of proteins (Hawkins and Davies 2019), and damage to DNA or RNA (Yan and Zaher 2019), leading to increased OS in various human diseases (Phaniendra et al. 2015). They cause irreversible damage to DNA, lipids, and

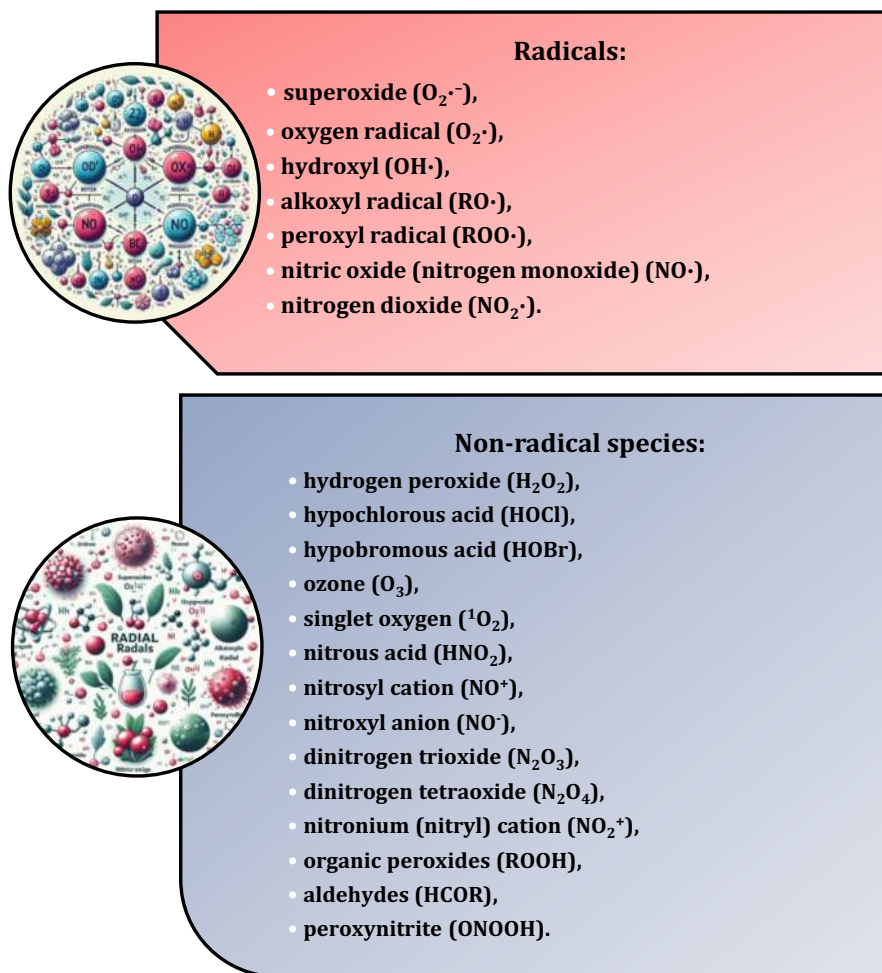


Fig. 1 Examples of radical and non-radical species. (According to Halliwell 2001; Kohen and Nyska 2002; Genestra 2007; Phaniendra et al. 2015)

enzymes in the cell cytosol by oxidising and modifying cellular components, preventing them from performing their original functions (Juan et al. 2021).

Systems and mechanisms that increase ROS production include (a) disruption of electron transport in the mitochondrial respiratory chain (Zhao et al. 2019); (b) increased catecholamine synthesis and oxidation (Bindoli et al. 1992); (c) activation of the xanthine-xanthine oxidase system (Medow et al. 2011); (d) activation of phagocytes (Dupré-Crochet et al. 2013); (e) appearance of a pool of catalytically active metal ions of variable valence (Poljsak 2011).

It should be emphasised that FRs normally play an important role in the life-supporting processes of cells in various biological systems, participating in reactions

of oxidative phosphorylation, biosynthesis of prostaglandins and nucleic acids, regulation of lipid metabolism, and processes of mitosis (Di Meo and Venditti 2020). Superoxide radicals, organic peroxides, and other ROS are involved in the synthesis of eicosanoids, metabolism of xenobiotics, and induction of the transcription of certain genes and are mediators in the formation of cellular responses (Su et al. 2019; Checa and Aran 2020; Fujii et al. 2022). In addition, a number of studies have recently been published on the role of ROS and RNS in the regulation of cell proliferation (Zarkovic 2020; Di Meo and Venditti 2020). For example, researchers have found that the superoxide radical and hydrogen peroxide stimulate cell division at low concentrations (Burdon and Rice-Evans 1989; Dumitrescu 1992).

Products of LPO can inhibit DNA synthesis and cell division (Gentile et al. 2017). For example, the free radical oxidation rates in the liver are significantly lower during periods of high cell proliferative activity, e.g. in the foetal and neonatal liver (Torres-Cuevas et al. 2017). FRs play an important role in the mechanisms of apoptosis (Redza-Dutordoir and Averill-Bates 2016). Accordingly, chemical and physical factors that act on cells to induce OS are its inducers (Pizzino et al. 2017). It can therefore be concluded that, in physiological conditions, FRs act as regulators of cell division in normal cells (Phaniendra et al. 2015). However, their role in biological systems is highly dynamic, as excessive production of FRs can rapidly lead to the accumulation of toxic oxidation products and damage to biological cell membranes and vital cell structures (Pizzino et al. 2017; Checa and Aran 2020). This process is referred to as oxidative stress.

3 Oxidative Stress and Carcinogenesis

The role of OS in the body is of particular interest in the pathogenesis of proliferative diseases, including benign and malignant hyperplastic processes. It is currently considered to be a universal non-specific mechanism for initiating tumour growth (Li et al. 2024). Particularly noteworthy are studies that, based on the study of the kinetic patterns of tumour growth, show that free radical reactions play a key role not only in the primary process of cell malignancy but also in further progression (Sainz et al. 2012). A malignant tumour is characterised by a significant disruption of oxidative processes (Arfin et al. 2021). In 1984, Cheeseman and co-workers reported low levels of LPO in Novikoff hepatoma cells. Similar processes were observed in Ehrlich tumour cells by Shuster (1955). They also suggested that cancer cells were somehow relieved of the effects of FRs, which inhibit cell division. This was perhaps the first time that it was proposed that free radical oxidation products could be a regulator of cell division.

It has been shown that many oncogenic electrophilic agents are subjected to OS, which produces highly reactive products that bind tightly to membrane and nuclear DNA, i.e. the process is considered as metabolic activation of oncogenes (Fig. 2). In this case, excess fatty acid oxidation products play the role of co-carcinogens or tumour promoters (Barrera 2012; Sakanyan 2018). Li and Spector (1997) showed

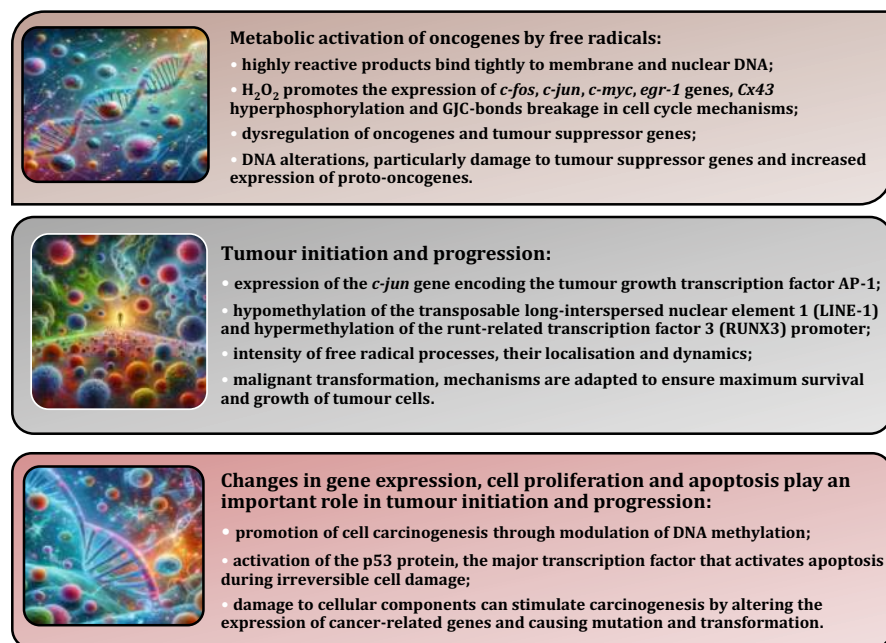


Fig. 2 Oxidative stress and carcinogenesis

that H_2O_2 promotes the expression of *c-fos*, *c-jun*, *c-myc*, *egr-1* genes, *Cx43* hyperphosphorylation, and GJC-bond breakage in cell cycle mechanisms. The involvement of oxygen radicals and H_2O_2 as secondary intracellular messengers in the expression of the *c-jun* gene encoding the tumour growth transcription factor AP-1 in response to ionising radiation has been demonstrated (Munshi and Ramesh 2013; Marinho et al. 2014). OS causes changes in gene expression, cell proliferation, and apoptosis and plays an important role in tumour initiation and progression (Arfin et al. 2021). OS also promotes urothelial cell carcinogenesis through the modulation of DNA methylation. Hypomethylation of the transposable long-interspersed nuclear element 1 (LINE-1) and hypermethylation of the runt-related transcription factor 3 (RUNX3) promoter in a bladder cancer cell line were experimentally induced by ROS (Wongpaiboonwattana et al. 2013).

Studies of FRs in tumours have confirmed the hypothesis of increased peroxide responses in the early stages of tumour formation and growth (Phaniendra et al. 2015). If the amount of cellular FRs produced is sufficient to overcome the endogenous antioxidant response, the irreversible oxidative damage to nucleic acids, lipids, and proteins can cause genetic and/or epigenetic changes leading to dysregulation of oncogenes and tumour suppressor genes (Lobo et al. 2010). OS and chronic inflammatory processes are closely linked, and failure to block these processes may result in genetic or epigenetic changes leading to the initiation of carcinogenesis (Jelic et al. 2021). OS affects several signalling pathways associated with cell proliferation, such

as the epidermal growth factor receptor signalling pathway, and key signalling proteins, such as nuclear factor erythroid 2-related factor 2 (Nrf2), RAS/RAF, mitogen-activated protein kinases ERK1/2 and MEK, phosphatidylinositol 3-kinase, phospholipase C, and protein kinase C (Sosa et al. 2013; Mileo and Miccadei 2016).

One of the important functions of ROS is their involvement in the initiation and execution of programmed cell death—apoptosis. FRs act as one of the activators of the p53 protein, the major transcription factor that activates apoptosis during irreversible cell damage (Watson 2013). It has also been shown that one of the targets of p53 is the PIG gene family, whose products enhance the generation of ROS, leading to their accumulation, mitochondrial membrane disruption, and release of cytochrome C protein into the cytosol, triggering a further cascade of apoptotic responses (Srinivasula et al. 1998). Approximately 2/3 of DNA damage is caused by short-lived (nanoseconds, microseconds) high-energy primary and secondary FRs (Greenberg 2016). FRs can cause DNA alterations, particularly damage to tumour suppressor genes and increased expression of proto-oncogenes (Wei 1992; Bohr and Dianov 1999). There are potentially hundreds of different types of chemical changes in DNA caused by FRs that could cause cancer (Weitzman and Gordon 1990; Waris and Ahsan 2006).

The contrasting findings regarding the ability of FRs to either induce or inhibit tumour formation appear to be associated with changes in radical function depending on specific conditions. Such conditions may include the intensity of free radical processes as well as their localisation and dynamics. For example, there is evidence that the influence of FRs is dose-dependent. It has been shown that high concentrations of FRs inhibit proliferation, whereas low concentrations have a growth-stimulating effect on tumour cells (Kondakova et al. 2005; Sainz et al. 2012). Relatively high levels of H_2O_2 promote the growth and proliferation of breast cancer cells. However, excessive intracellular levels of H_2O_2 lead to the death of breast cancer cells (Wang et al. 2017). Under acute OS, the growth of tumour cell lines is suppressed. Opposite processes are observed under chronic stress: an increase in cell growth and survival (Barrera 2012; Mahalingaiah and Singh 2014). When acute OS causes damage to cellular components, chronic OS can stimulate carcinogenesis through alterations in the expression of cancer-related genes and induction of mutation and transformation (Kruk and Aboul-Enein 2017).

In subsequent years, the data on the low intensity of LPO in malignant tumours have been confirmed and extended (Ayala et al. 2014). Many authors believe the low content of FRs to be caused by: (a) reduced levels of phospholipids and low levels of polyunsaturated fatty acids in tumour tissue (Corn et al. 2020); (b) low levels of cytochrome P-450 enzymes, which can induce OS, usually contained in tumour cells (Elfaki et al. 2018); (c) high levels of antioxidant compounds in tumour tissue (Hawk et al. 2016). It is possible that, during malignant transformation, mechanisms are adapted to ensure maximum survival and growth of tumour cells through their ability to degrade exogenous hydroperoxides extracellularly and to regulate intracellular levels of FRs with antioxidant enzymes (Liou and Storz 2010; Sainz et al. 2012). AOE can act as regulators of proliferation by controlling the concentration of FRs

(Kurutas 2016). However, there are also conflicting data. Some researchers note the presence of a large amount of FRs in tumours and a decrease in antioxidants in tumour tissue (Valko et al. 2006; Ríos-Arrabal et al. 2013). This is most likely related to the need for an increased concentration of FRs to stimulate cell division and growth and the depletion of AOE s.

4 Antioxidant Defence Systems in Malignant Cells

The body has a well-balanced system of antioxidant regulation of the rate of free radical oxidation, consisting of a water- and lipid-soluble pool of radical scavengers, chelators of metal ions of variable valence, and other substances that protect cells from OS during normal metabolism. Their importance increases with increasing levels of FRs (Pham-Huy et al. 2008; Chaudhary et al. 2023). Since free radical reactions cause many pathological changes, there must be a system to prevent excessive radical formation. This multi-level antioxidant defence system controls the intensity of free radical reactions in the intracellular and extracellular spaces of the body (Sharifi-Rad et al. 2020). The leading role in this system is played by AOE s, including superoxide dismutase (SOD), which catalyses the dismutation of O_2^- to hydrogen peroxide and molecular oxygen, catalase, which reduces H_2O_2 , and glutathione-dependent enzymes such as glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione reductase (GR), which remove organic peroxides (Snezhkina et al. 2019).

Many authors provided evidence that AOE s can inhibit cell proliferation, which served as the basis for the first experiments with antioxidants as anti-tumour agents. The experiments showed regression of tumour cultures (Lubos et al. 2011; Bułdak et al. 2014; Marengo et al. 2016; Mut-Salud et al. 2016; George and Abrahamse 2020). Researchers have directly demonstrated that the well-known antioxidant vitamin C has an inhibitory effect on tumour growth in gastric cancer (Zhang et al. 2002; Didier et al. 2023). On the other hand, it should be noted that the high activity of AOE s in tumour cells may be one of the causes of their resistance to OS (Bułdak et al. 2014; Aboelella et al. 2021) and may serve as a factor in the development of tumour resistance to therapeutic interventions based on the influence on free radical processes (Hayes et al. 2020; Gupta et al. 2020). For example, in a randomised trial conducted in the USA in a group of head and neck cancer patients receiving radiotherapy, α -tocopherol and β -carotene were added to reduce side effects. Compared with a group that did not receive antioxidants, the tumour recurrence rates were generally higher (Bairati et al. 2005). It has been shown that high doses of antioxidants used as adjuvant therapy may reduce the effectiveness of treatment by stimulating proliferation in tumour tissue (Luo et al. 2022). The role of antioxidant defence systems in malignant cells is diverse, involving both adaptive cytoprotective mechanisms and potential vulnerabilities that can be targeted for therapeutic benefit (Fig. 3).

The widespread involvement of superoxide radicals in the enzymatic reactions of prostaglandin synthesis and xenobiotic metabolism, cell proliferation, and the



Fig. 3 Role of antioxidant defence systems in malignant cells

expression of certain genes allows consideration of SOD as an enzyme with not only protective but also regulatory functions, a key link in the regulation of steady-state ROS levels (Buettner 2011). In addition, in the presence of reduced glutathione, SOD is able to catalyse the degradation of a type of nitrogen oxide depot—S-nitrosothiols—thereby inducing the long-term generation of NO (Singh et al. 1996). It is also thought that the removal of $\cdot\text{O}_2^-$ is necessary to protect against the oxidation of intracellular glutathione, which in its reduced state acts as a potent radical scavenger. The combined action of SOD and catalase protects cells from OS much more effectively than either enzyme alone (Shields et al. 2021). Thus, the antioxidant defence system is characterised by 1) high specificity of action directed against specific forms of FRs, 2) specificity of cellular and organ localisation, which often overlap in a complementary manner, 3) specificity of the use of metals as catalysts, which include copper, zinc, manganese, iron (often in the composition of haem), nickel, and selenium (Nordberg and Arnér 2001; Kohen and Nyska 2002; Genestra 2007; Chaudhary et al. 2023).

The level of intracellular enzymatic antioxidants is under genetic control, the principle being that when the concentration of ROS in the cell increases, the transcription of genes that trigger the synthesis of catalase, GR, GPx, SOD, etc. is activated (Rahal et al. 2014; Ito et al. 2019). Exposures that increase ROS production induce the synthesis of enzymatic antioxidants. In humans, the level of AOE is constant in normal conditions and largely independent of sex, age, weight, and other physiological parameters, but their concentration and activity in cells and tissues changes in pathological conditions (Sharifi-Rad et al. 2020). A significant decrease in AOE levels in tumour tissues suggests that disruption of their synthesis may

underlie the malignant transformation of cells (Aggarwal et al. 2019). The reduced level of MnSOD in tumour cells indicates inhibition of intracellular $\cdot\text{O}_2^-$ production (Miriyyala et al. 2012). MnSOD may be a tumour suppressor gene in a variety of human tumours. Reduced proliferation of cancer cells with high activity of the MnSOD isoenzyme has been observed in U118 and U118-9 human glioma cells (Zhong et al. 1997) and MIA PaCa-2 pancreatic cell carcinoma (Weydert et al. 2003). Upregulation of SOD in human cancer cell lines increases H_2O_2 production and reduces tumour growth in the absence of anti-cancer agents (Zhong et al. 1996). These results contrast with other data presented by Palazzotti and co-workers (1999). This research group showed that the overexpression of MnSOD had marginal effects on the growth of the human cervical carcinoma HeLa cell line in standard medium, but significantly protected the cells from growth suppression and cell death in serum starvation conditions. These observations may be related to the fact that HeLa cells express high levels of SOD and CAT enzymes and are therefore able to counteract the cytotoxic effects of peroxide. The finding of increased SOD activity more likely reflects the ability of SOD to reduce superoxide anion levels (Palazzotti et al. 1999).

MnSOD levels were increased in diffuse hyperplasia, adenomatous goitre, and follicular adenoma. It was significantly higher in papillary carcinoma than in adjacent normal thyroid tissue (Nishida et al. 1993). Follicular carcinoma also showed significantly higher levels of MnSOD. In the immunohistochemical study, adjacent normal thyroid tissue showed granular positive staining for MnSOD in the cytoplasm. An increase in MnSOD was observed in the papillary proliferative lesion of diffuse hyperplasia and in follicles adjacent to lymphoid tissue in chronic thyroiditis with hypothyroidism. Strong positive staining for MnSOD was observed in papillary and follicular carcinomas, whereas the staining was significantly less intense in anaplastic carcinoma. These results suggest that MnSOD levels vary according to the degree of differentiation of thyroid carcinomas (Nishida et al. 1993; Jelic et al. 2021).

Increased expression of MnSOD and decreased expression of Cu,Zn-SOD have been found in malignant gastric tissue compared to the corresponding normal mucosa (Janssen et al. 2000). In particular, the MnSOD ratio (levels in normal and malignant tissue) has been shown to be an independent prognostic parameter in gastric cancer patients and appears to be clinically relevant to patient survival (the higher the ratio, the worse the overall survival) (Janssen et al. 2000). In a study comparing gastric cancer patients with and without metastases, MnSOD was upregulated in primary tumours with lymph node metastases, supporting the involvement of MnSOD and possibly ROS in metastasis (Malafa et al. 2000; Jelic et al. 2021).

Since mitochondria are one of the main sources of superoxide anions in the cell, it can be argued that tumour transformation is accompanied not only by inhibition of oxidative phosphorylation and energy shift to glycolysis, but also by a decrease in O_2^- production by mitochondria (Zorov et al. 2014). The fact that the production of the proliferation-enhancing superoxide anion by isolated mitochondria is preserved and the generation of H_2O_2 , which inhibits cell proliferation, is significantly reduced indicates that mitochondria in tumour cells are biased towards producing a signal for

cell proliferation (Wong et al. 2017). The imbalance in the production of $\cdot\text{O}_2^-$ and H_2O_2 in transformed cells is exacerbated by increased levels of glutathione-dependent enzymes (GPx, GST, GR) involved in the degradation of peroxides. It is thought that, in the developmental (promotion) stage, transformed cells have increased levels of AOE protecting cells from death (Liou and Storz 2010). However, established tumours are characterised by low levels of AOE, which may be due to the regulatory effect of antioxidants on the proliferation process (Lubos et al. 2011; George and Abrahamse 2020).

The importance of AOE activity in cancer is controversial. One view is that, when normal cells degenerate into cancer cells, intense metabolic pathways of proliferation are triggered (Chae and Hong 2022). These same pathways lead to excessive production of FRs, which are cytotoxic. Cellular OS initiates the oxidation of proteins, lipids, and DNA, thereby interfering with tumourigenesis and cell survival (Arfin et al. 2021). In response to increased oxidation, AOE, particularly glutathione peroxidase, begin to function intensively in tumour cells to protect the cells (Chang et al. 2020). In an experiment in mice with blocked hepatic lipogenesis (by knocking out acetyl-CoA carboxylase genes), administration of diethylnitrosamine led to the development of hepatocellular carcinoma. The lipogenesis blocking in mice resulted in a twofold increase in tumours. Hepatocyte antioxidant metabolism, including NADPH and reduced glutathione, was found to be significantly increased, which appears to increase cell survival (Nelson et al. 2017). There is also evidence of high catalase activity, which makes tumour cells resistant to oxidation. It can be used in cancer therapy through inhibition of glutathione homeostasis (Sznarkowska et al. 2017). However, it has also been shown that cells with low catalase activity are more resistant to toxic pro-oxidants (Nandi et al. 2019). These tumour cells are more resistant than cells with high enzyme activity. Thus, no direct correlation has been found between the catalase activity and sensitivity to pro-oxidants (Kang et al. 2013).

The ability to generate and metabolise ROS varies widely between breast cancer cell lines, suggesting that redox balance is finely regulated during carcinogenesis. Breast cancer is characterised by increased expression of SOD and decreased activity of catalase with greater resistance to H_2O_2 (Goh et al. 2011; Wang et al. 2017). In contrast, studies conducted by Hecht and co-workers (2016) observed higher catalase activity in MDA-MB-231 breast cancer epithelial cells. Defects in AOE systems are themselves a prerequisite for tumourigenesis. In particular, an imbalance in AOE leads to overproduction of ROS and DNA damage in cervical cells. This makes the cells more susceptible to the human papillomavirus and, consequently, to the development of cervical cancer (Jiang et al. 2013).

5 Glutathione Redox System in Carcinogenesis

In recent years, researchers investigating the mechanisms of carcinogenesis have paid particular attention to thiol-containing compounds—molecules containing sulfhydryl (SH) groups, which are very abundant in the cell in the form of glutathione tripeptide and numerous proteins. Thiols are present in the cell in both reduced

(-SH-) and oxidised (-SS-) states. The concentration of SH groups is many times higher than that of SS groups because thiol proteins are active in the reduced state. GSH is the main component of the cell redox buffer system, maintaining a reducing environment within the cell (Dalzoppo et al. 2017). Thiol compounds are involved in all major biochemical processes: energy and ion exchange, nerve impulse conduction, muscle contraction, reception, etc. (Zeida et al. 2014). The glutathione redox system plays a multifaceted role in carcinogenesis, influencing oxidative stress, detoxification, cell signalling, chemoresistance, and metabolic reprogramming (Fig. 4).

Numerous experimental and clinical studies have shown that a wide variety of diseases cause the same response—a decrease in the concentration of SH groups in the blood serum of patients, and the more severe the clinically expressed disease, the lower the level of SH groups in the blood. Similar changes have been found in the body exposed to cold, magnetic fields, physical activity, and emotional stress (Deneke 2000; Sen 2000). Since thiols exist in both reduced and oxidised forms in the cell, they form a single thiol-disulphide system, and the interconversion of these forms is of greater importance in a non-specific adaptive response. The thiol-disulphide system responds to any internal or external influence by changing its redox state, which can be characterised by the ratio of the concentration of -SH and

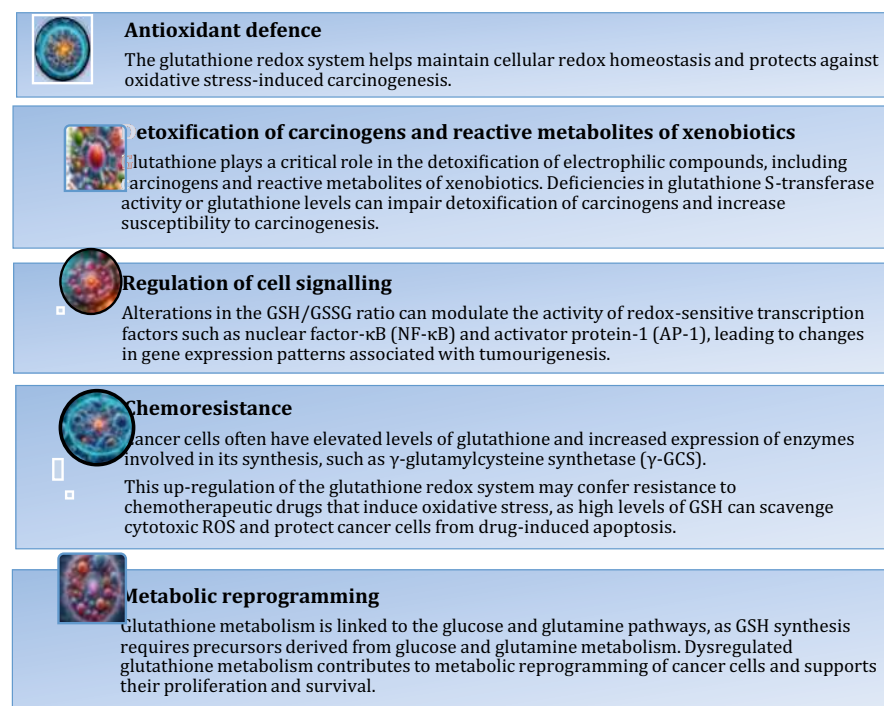


Fig. 4 Glutathione redox system in carcinogenesis

-SS groups (SH/SS) (Messens and Collet 2013). Changes in the SH/SS redox equilibrium are phase-dependent and depend on the strength and duration of the acting factor. The initial increase in SH- (shift towards reduction) is replaced by a decrease in SH- (shift towards oxidation), which is considered a sign of activation and exhaustion in the dynamics of the adaptive process (Harris and Hansen 2012; Messens and Collet 2013). The higher the initial SH/SS value, i.e. the greater the buffering capacity of the system, the higher the level of resistance of the body. The SH/SS level can serve as an integral indicator of the adaptive capacity or non-specific resistance of the body and is particularly valuable for assessing the mechanisms of OS in pathologies, including tumours (Mitchell et al. 1989).

The increasing understanding of the molecular mechanisms of the pathogenesis of a wide range of diseases in recent years has confirmed the concept of the important role of thiol-disulphide metabolism in these processes. The biochemical basis of redox signalling is the maintenance of oxidising conditions by stabilising disulphides (-SS-) on the external cell surface, while the intracellular environment is maintained in a reduced state (SH) by free sulfhydryl groups (Harris and Hansen 2012; Messens and Collet 2013). The major disulphide reductase, thioredoxin (Trx), is a small redox-active protein with two cysteine residues in the active centre. It is required for the redox regulation of protein function and the transduction of metabolic control signals. Thioredoxins are reduced by NADP-dependent thioredoxin reductase (Arner and Holmgren 2000). The main components of the glutathione redox system are GSH, GSSG, Se-containing GPx, GST, and GR (Vašková et al. 2023). The non-protein thiol GSH acts as a cofactor for such enzymes as GPx and GST in the detoxification of lipid peroxides, thus protecting cells from cytotoxic and carcinogenic chemical reactions during OS. GPx simultaneously catalyses GSH and GSSG when they react with FRs. In addition, GSSG is returned to its reduced form (GSH) by GR; at the same time, this reaction maintains a high GSH/GSSG ratio within the cellular system (Flohé 2016).

The inactivation of H_2O_2 in cells is catalysed by GPx, whose each molecule contains 4 atoms of selenium (Se). At selenium deficiency in the diet, the level of GPx decreases, which reduces the body's resistance to OS and may lead to the development of pathology (Lubos et al. 2011). A decrease in GPx activity as a result of Se deficiency has been reported in cardiovascular disease and cancer (Short and Williams 2017; Chang et al. 2020). In the assessment of its antioxidant potential, the GPx enzyme has been shown to catalyse the reduction of unstable organic hydroperoxides, including hydroperoxides of polyunsaturated fatty acids, to stable hydroxy acids by glutathione. Selenium-containing GPx also exhibits peroxynitrite reductase activity, reducing ONOO^- to the nitrite anion NO_2^- , thereby preventing dangerous oxidation and nitrosylation reactions in which peroxynitrite is actively involved (Storkey et al. 2015).

In cancer patients with metastatic disease, low Se levels and plasma GPx activity have been associated with increased LPO, suggesting that loss of GPx contributes to systemic OS. Erythrocyte SOD activity and both plasma and erythrocyte copper levels were significantly higher in patients (Güven et al. 1999). Decreased plasma GPx expression in cancer patients has been reported in several tumour types,

including uterine and colorectal cancer (Kumar et al. 1995; Psathakis et al. 1998). The GPx expression was also lower in both plasma and tumour tissue of patients with non-small cell lung cancer, glioblastoma, and hepatocellular carcinoma (Sreekanthreddy et al. 2010; Qi et al. 2014; Oh et al. 2014). In papillary serous ovarian cancer, systemic levels of GPx were decreased in a tumour stage-dependent manner, with late-stage patients showing a more pronounced decrease in plasma GPx levels (Agnani et al. 2011). However, high GPx expression in serous ovarian adenocarcinoma tumour tissue was inversely associated with poor prognosis and higher tumour stage (Worley et al. 2019). In adenocarcinoma and fibroadenoma of the breast, the level of malonic dialdehyde (MDA) and the activity of Cu,Zn-SOD, catalase, GPx, and glucose-6-phosphate dehydrogenase in the blood were significantly higher. At the same time, the shifts in Cu,Zn-SOD and GPx were more pronounced in cancerous than benign mammary tumours (Valko et al. 2006).

Increased GPx activity also enables cells to survive with high levels of ROS and maintain cellular viability. Increased activity of GPx or CAT in cancer cells may render tumour cells less susceptible to the effects of anti-cancer drugs. It has been shown that the addition of radical scavengers and compounds with peroxide activity can reduce the cytotoxic effect of anti-cancer drugs in vitro (Doroshov 1986). The increase in GSH during the adaptation process may enhance the export of anti-cancer drugs and their inactivation. This altered drug metabolism, together with increased cell survival, may make cancer cells more resistant to chemotherapeutic agents (Trachootham et al. 2009). Gromadzinska and co-workers (2008) showed that the levels of glutathione, GST, and GPx were higher in breast cancer tumour tissue than in normal tissue in 68–85% of patients. At the same time, Kumaraguruparan and co-workers (2002) observed a decrease in the activity of SOD, catalase, GSH, GPx, and GST in women with the same tumours, against a background of an increase in LPO products. According to the authors, this indicates instability of the redox balance in patients with fibroadenoma and adenocarcinoma. Other authors have reported a similar decrease in blood AOE activity in breast cancer (Rajneesh et al. 2008), invasive cervical and endometrial cancer (Kim et al. 2004; Gök et al. 2023), uterine fibroids (Pejić et al. 2015), ovarian cancer (Caglayan et al. 2017), and colorectal cancer (Janion et al. 2020).

A decrease in the level of GPx activity in the blood may be an unfavourable prognostic sign for metastasis in tumour diseases (Minato et al. 2021). The progressive growth of a malignant tumour is accompanied by accumulation of oxidised glutathione in the blood and a decrease in the GSH/GSSG ratio (Navarro et al. 1999). Two reasons may explain the increase in blood GSSG: (a) the increase in peroxide production by the tumour, which, in addition to alterations in glutathione-related and AOE activities, may lead to GSH oxidation within red blood cells and (b) an increase in the release of GSSG from various tissues into the blood (Navarro et al. 1999). The redox balance of glutathione also changes in cells during the development of the proliferation, differentiation, and apoptosis processes. In fibrosarcoma, it decreases, although an increase in cell proliferation is observed (Traverso et al. 2013). During apoptosis, GSH is released from dying cells and the reducing part of the GSH/GSSG potential increases (Circu and Aw 2008). At the same time, cysteine (Cys) deficiency

is associated with an increase in oxidative potential (Circu and Aw 2008). GSH levels in the tumour/blood system can be used as an index of carcinogenicity and help identify radioresistant tumours (Kennedy et al. 2020).

6 Chemotherapy and Antioxidants in Cancer Therapy

Antioxidants are substances with both low and high molecular weight that are able to react with high levels of ROS, thereby protecting cellular structures from free radical damage (Lobo et al. 2010). Currently, antioxidants are divided into enzymatic (catalase, superoxide dismutase, enzymes of glutathione metabolism) and non-enzymatic (glutathione, thioredoxin, ceruloplasmin, tocopherol, retinol, ascorbic acid, flavonoids, carotenoids, ubiquinone, etc.) (Sies 1997). Antioxidants are widely used to prevent a number of diseases associated with ageing, such as cardiovascular diseases, age-related immunosuppression, cognitive impairment, cataracts, or thrombosis (Hajhashemi et al. 2010; Obrenovich et al. 2011; Rusu et al. 2022). It is known that they are often used as a means of prevention and complex therapy of cancer (Fuchs-Tarlovsky 2013; Athreya and Xavier 2017; Luo et al. 2022). However, there is increasing information in the world literature showing an increased risk of developing neoplasia with their uncontrolled use (Goodman et al. 2011).

The dual role of FRs in carcinogenesis is the basis for a discussion of conflicting strategies to regulate oxidative processes (Gupta et al. 2020). This is particularly true for the use of anti-tumour drugs. During anti-tumour therapy, an increased amount of FRs is generated and AOE systems are inactivated, leading to intoxication and damage to cells, tissues, and organs (Phaniendra et al. 2015). Furthermore, the toxic side effects of anti-cancer drugs are directly explained by their pro-oxidant properties (Jiang et al. 2023). In this context, a pressing issue is the neutralisation of FRs during the action of anti-tumour drugs with antioxidants (modification of chemotherapy with antioxidant therapy). Experimental and clinical studies are being conducted on the effectiveness of prescribing chemotherapeutic drugs together with antioxidants of various chemical bases (Sak 2012; Wieland et al. 2021). Improved tolerability of chemotherapy with antioxidants has been reported in a number of studies (Conklin 2000; Lawenda et al. 2008; Singh et al. 2018). While antioxidants have the potential to modulate chemotherapy-induced oxidative stress and reduce treatment-related side effects, their concurrent use with chemotherapy may also compromise treatment efficacy (Fig. 5). The decision to use antioxidants during cancer therapy should be individualised and based on careful consideration of the available evidence, patient preferences, and potential risks and benefits.

Recently, there has been increasing evidence that intravenous vitamin C can improve the quality of life in cancer patients both with and without adjuvant chemotherapy and radiotherapy (Yeom et al. 2007; Vollbracht et al. 2011; Stephenson et al. 2013). Two early trials of high-dose vitamin C in patients with advanced cancer showed that many patients experienced some subjective improvement in quality of life, including reduced pain and need for analgesics (Cameron and Campbell 1974; Murata et al. 1982). It has also been shown in the hepatocellular

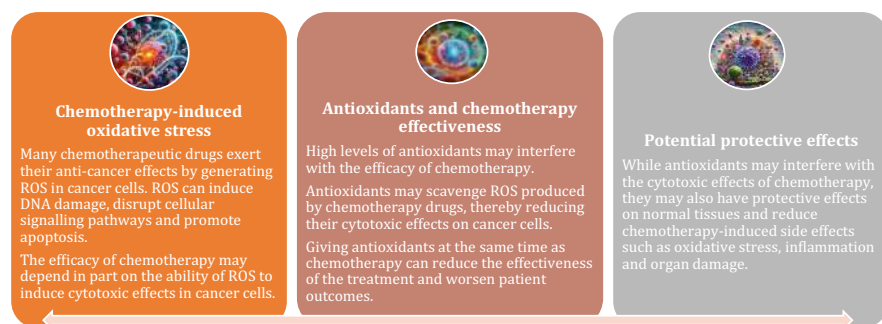


Fig. 5 Effects of antioxidants on chemotherapy efficacy and toxicity

carcinoma cell line HepG2 that treatment with hydrogen peroxide leads to OS and a decrease in antioxidant activity. H_2O_2 -treated HepG2 cells showed increased migration and invasiveness. Combined application of the antioxidants tocopherol acetate (TA) and S-adenosylmethionine (SAM) effectively attenuated the expression of Nrf2 and 8-hydroxydeoxyguanosine (8-OHdG) in HepG2 cells. Thus, the invasiveness and migration of these cells is significantly reduced in the presence of antioxidants (Ma-On et al. 2017).

A few studies have confirmed the neuroprotective role of vitamin E against platinum-induced neurotoxicity in humans, showing that patients taking vitamin E had a lower incidence and severity of neurotoxicity compared with those receiving placebo (Pace et al. 2005; Salehi and Roayaei 2015). Argyriou and co-workers (2005) also demonstrated a 50% reduction in the incidence of peripheral neuropathy in patients undergoing cisplatin chemotherapy who received 600 mg of oral vitamin E per day compared with those who received placebo. However, some studies have failed to demonstrate a meaningful clinical benefit of vitamin E for the above indications (Kidwell et al. 2012).

Vitamin E and selenium are known to be synergists, increasing each other's antioxidant effects (Amraoui et al. 2018). However, the combined intake thereof may increase the risk of prostate cancer by 91% in men with high selenium status compared with men with low selenium status (Kristal et al. 2014). The combined use of vitamin E (at an average dose of 50 mg/day) and the antioxidant β -carotene (20 mg/day) was also found to increase lung cancer mortality by 18% in male smokers aged 50–69 years compared with placebo. There was an increased incidence of prostate adenocarcinoma and bladder cancer in these men (Albanes et al. 1996). The use of α -tocopherol, retinol, and β -carotene was found to increase cancer mortality by 4% in study participants who took these antioxidants compared with those who did not (Bjelakovic et al. 2013).

Modern research shows the role of antioxidants in preventing and inhibiting oral cancer (Choudhari et al. 2014). The use of nutraceuticals to eliminate cancer cells is being considered (Salami et al. 2013; Maiuolo et al. 2021). Recently, antioxidant therapy and dietary antioxidant supplementation have received considerable interest

in the treatment of cervical cancer (Ono et al. 2020; Gholamalizadeh et al. 2023). Another view is based on the fact that the administration of antioxidants inhibits the anti-tumour effect of chemotherapy, and in this case the antioxidants themselves can only provoke cancer (Sayin et al. 2014; Le Gal et al. 2015; Diao et al. 2016). This suggests that, in cancer, there is a shift in the normal oxidative state towards the predominance of antioxidants in tumour cells. Antioxidants may not have a positive anti-tumour effect. In particular, vitamin and antioxidant supplements did not prevent bladder cancer (Park et al. 2017). In addition, antioxidants are not always safe to use: the excessive intake of these substances may cause serious health problems (Lobo et al. 2010). When antioxidants block OS, the mechanism of tumour cell apoptosis mediated by FR formation is abolished (Sainz et al. 2012). In this case, protecting healthy tissue with antioxidants is an obstacle to destroying cancerous tissue. This raises the question of developing new drugs with antioxidant activity.

High levels of ROS can prevent the development of tumours (Huang et al. 2021). This makes antioxidant cancer therapy a promising area of research. Due to their highly cytotoxic nature, ROS can also be used to kill cancer cells if their generation can be selectively modulated in cancer (Fang et al. 2009; Luo et al. 2022). The controversial role of ROS suggests the development of therapeutic methods aimed at modulating the levels of oxidative processes (Glasauer and Chandel 2014). Several therapeutic strategies have been proposed in this direction. In particular, a unique therapeutic strategy, termed “oxidation therapy”, has been developed by delivering cytotoxic ROS directly to the solid tumour or, alternatively, by inhibiting the antioxidant enzyme system, such as heme oxygenase 1 (HO-1), in the tumour. This anti-cancer strategy was investigated by using $O_2^{\cdot -}$ or H_2O_2 -generating enzymes (i.e. xanthine oxidase and d-amino acid oxidase, respectively) and by discovering the inhibitor of HO-1 (i.e. zinc protoporphyrin and its polymeric derivatives). These macromolecular drugs also showed superior pharmacokinetics, including a much longer in vivo half-life, particularly tumour-targeted accumulation, and thus remarkable anti-tumour effects (Fang et al. 2009).

Some anti-cancer drugs, such as paclitaxel, can attack cancer cells by generating or interfering with the production of ROS. Alexandre and co-workers (2007) showed that paclitaxel promotes ROS generation by increasing the activity of plasma membrane-associated NADPH oxidase (NOX). Treatment of breast cancer cells resulted in increased translocation of Rac1, a positive regulatory protein of NOX, to the membrane fraction. Importantly, the increase in extracellular ROS caused lethal damage to the bystander cancer cells not exposed to paclitaxel, as shown by two different methods using co-culture systems in which the bystander cells were differentiated from the paclitaxel-treated cells by fluorescent or radioactive labelling. This cytotoxic bystander effect was also observed with the other microtubule-targeting drugs, vincristine and taxotere, but not with 5-fluorouracil or doxorubicin (Alexandre et al. 2007).

Some anti-cancer agents, such as piperlongumine, bind to the active sites of several key cellular antioxidants, including GST and carbonyl reductase 1, only in cancer cells. Although natural antioxidants, alone or in combination with diet, can provide some benefits for chemoprevention, their position in cancer therapy,

especially in the early stages of carcinogenesis, is declining. On the other hand, antioxidants can promote the survival of detached cells from the extracellular medium, playing dual activities in terms of tumourigenesis, inhibiting tumourigenesis by preventing oxidative damage to DNA and otherwise maintaining the tumour by promoting cell survival through metabolic rescue (Saeidnia and Abdollahi 2013).

Apoptosis can be inhibited with the help of antioxidant defence systems, which have evolved in all living organisms and prevent both the initiation of programmed cell death and its execution upon activation of the p53 protein (Budanov 2014). These systems include various antioxidant proteins: GSH, GPx, GR, catalase, SOD, ceruloplasmin, thioredoxin, etc. (Wojcik et al. 2010). The KEAP1/NRF2/ARE signalling pathway is thought to be of great importance in regulating the expression of antioxidant proteins, with the *NRF2* gene, located on chromosome 2, playing the role of the master transcription factor controlling the activity of the entire molecular pathway (Lee and Hu 2020; Liu et al. 2022). The activity of *NRF2* has been shown to be under the control of the *B-Raf* and *K-Ras* oncogenes (DeNicola et al. 2011). High expression of *NRF2* has been observed in pancreatic cancer, which is the cause of its increased resistance to OS induced by radiotherapy (Lister et al. 2011). The *NRF2* expression can also be affected by certain drugs. The antidiabetic drugs saxagliptin, sitagliptin (DPP4 inhibitors), and α -lipoic acid, which have strong antioxidant properties, have been shown to increase the metastasis of tumour cells without affecting the proliferation of the primary tumour. A correlation was found between the activity of *NRF2* and some genes associated with cancer metastasis: *HIF-1 α* (hypoxia-inducible factor 1 α), its target *VEGF-A*, which encodes vascular endothelial growth factor, and the *COX-2* gene, whose product is cyclooxygenase 2 (Wang et al. 2016). According to the authors, saxagliptin, sitagliptin, and α -lipoic acid inhibited KEAP1, thereby preventing the ubiquitination of *NRF2* and prolonging its expression (Wang et al. 2016). This information is of practical importance, as accumulated epidemiological data indicate that diabetes mellitus significantly increases the risk of developing cancer, most commonly colon, liver, bladder, pancreatic, and breast cancer (Giovannucci et al. 2010).

7 Glutathione Redox System in Cancer Therapy

The glutathione system is considered to be the main defence against the damaging effects of ROS (Deponte 2017). The ratio of reduced to oxidised glutathione indicates the level of OS in tissues. Some drugs can be metabolised to glutathione. One such drug is the widely used mucolytic agent N-acetylcysteine (NAC) (Hou et al. 2015). NAC is a membrane-permeable cysteine precursor capable of increasing the intracellular cysteine pool and enhancing cellular glutathione (GSH) synthesis, thus potentiating the endogenous antioxidant mechanism (Zavala-Valencia et al. 2024). Due to the presence of a free sulfhydryl group in the molecule, acetylcysteine breaks the disulphide bonds of acidic sputum mucopolysaccharides, reducing their polymerisation and the viscosity of bronchial secretions and accelerating

mucociliary clearance (Rochat et al. 2004). In addition, due to the presence of a free sulfhydryl group, NAC has a pronounced antioxidant, antitoxic, and immunomodulatory effect, neutralising FRs formed in the body even during normal metabolic activity (Aldini et al. 2018). NAC protects cells from the effects of FRs, both by reacting directly with them and by providing cysteine for the synthesis of glutathione (Tenório et al. 2021). A number of researchers believe that this latter mechanism of action is the most important (Shattuck et al. 1998).

A powerful natural antioxidant is the fat-soluble vitamin E— α -tocopherol, which is present in the membrane of all cells and protects them from LPO (Rizvi et al. 2014). There is evidence that NAC and vitamin E supplementation increased the proliferation and invasive properties of tumour cells in mice with K-Ras and B-Raf-induced lung cancer in vivo and in oncogene-expressing fibroblasts in vitro (Sayin et al. 2014). It was found that, in tumour cells, administration of NAC and vitamin E significantly reduced the amount of DNA damage caused by the action of ROS; inactivation of the p53 protein was also detected in these cells, which contributed to their stimulation (Sayin et al. 2014). As NAC is often used in clinical practice as a mucolytic agent (Huang et al. 2023), the authors believe that this drug should be prescribed with caution to people at risk—smokers and patients with chronic obstructive pulmonary disease who use NAC to reduce the viscosity of mucus (Sayin et al. 2014).

The ability of NAC and a water-soluble analogue of vitamin E (Trolox, i.e. a standard for antioxidant activity) to enhance melanoma migration in vivo has been noted by Le Gal and co-workers (2015). Administration of NAC increased lymph node metastases in an endogenous mouse model of malignant melanoma, but had no effect on the number and size of primary tumours. Similarly, NAC and the soluble vitamin E analogue Trolox significantly increased the migration and invasive properties of human malignant melanoma cells, but did not affect their proliferation. Both antioxidants increased the ratio of reduced to oxidised glutathione in melanoma cells and in lymph node metastases, and the increased migration depended on the synthesis of new glutathione. Furthermore, both NAC and Trolox increased the activation of the small guanosine triphosphatase (GTPase) RhoA, and blocking downstream RhoA signalling abolished antioxidant-induced migration (Le Gal et al. 2015). The above information suggests that, when prescribing antioxidant drugs to patients with malignant neoplasia (as one of the means of complex treatment) and therapeutic drugs with pronounced antioxidant activity to patients at risk (smokers, patients with diabetes), it is necessary to take into account their possible influence on the growth and metastatic activity of malignant tumours.

8 Anticarcinogenic Properties of Melatonin

As a rhythm regulator, melatonin exerts a wide range of effects: circadian regulation, thermoregulation, sleep induction, and antioxidant, immunomodulatory, and anti-stress activity (Kurhaluk 2021). Exogenous melatonin appears to be a promising hepatoprotector and immunoprotector due to its antioxidant and anti-inflammatory

properties, making it useful in combination with other drugs to prevent alcoholic organ damage (Kurhaluk et al. 2017, 2020, 2021a; Kurhaluk 2021) and low-dose lipopolysaccharide-induced OS (Kurhaluk et al. 2018a,b). Naturally produced melatonin and exogenous dietary sources can participate in free radical reactions and activate the endogenous defence system (Kurhaluk and Tkachenko 2020). Our studies have shown that melatonin levels are related to the redox properties of antioxidant defences against LPO and protein damage (Kurhaluk et al. 2021b).

The question of the oncostatic and antiproliferative properties of melatonin has been of considerable interest to researchers (Huether 1993). A large number of studies and meta-analyses of published work have been carried out in this area. Prior to the discovery of melatonin and its isolation as a separate molecule, several studies conducted in the 20–40s of the twentieth century showed that removal of the pineal gland increased the likelihood of growth of various tumours. In addition, in 1957, extensive clinical and epidemiological work confirmed the link between the formation of pineal calcifications and the incidence of breast cancer (Drexler et al. 1957). The anticarcinogenic action of melatonin is mediated by several mechanisms, both with and without membrane receptors for this hormone (Kopustinskiene and Bernatoniene 2021) (Fig. 6).



Fig. 6 Promising anti-cancer properties of melatonin

Melatonin affects all the main characteristics of cancer: it participates in maintaining the integrity of the genome, reduces the level of proliferation and induces apoptosis of tumour cells of various origins, inhibits angiogenesis, influences metabolism, prevents epithelial-mesenchymal transition and metastasis of tumour cells, enhances the anti-tumour immune response, etc. (Bonmati-Carrion and Tomas-Loba 2021). The combined effects of the hormone melatonin and metformin have shown positive results in oncological practice. The comparative synergistic effect of oral antidiabetic metformin and melatonin administered alone and in combination in two different rat models of mammary tumour proliferation *in vivo* was investigated in our previous study (Kurhaluk and Tkachenko 2022). Melatonin reduced the level of OS in heart tissue induced by N-methyl-N-nitrosourea (NMU) carcinogenesis and a high-fat diet significantly more efficiently than metformin (Kurhaluk et al. 2018c). Long-term administration of metformin and melatonin exerted antioxidant properties in the liver, especially in combination (Kurhaluk et al. 2018d).

Melatonin suppresses the proliferation of tumour cells of various origins. Several mechanisms of action have been identified. First, melatonin affects the progression of a cell through the stages of the cell cycle. Melatonin has been shown to inhibit some cyclins and cyclin-dependent kinases (Talib et al. 2021). Several studies have shown that melatonin inhibits the PI3K/AKT/mTOR signalling cascade in cancer cells (Chen et al. 2021). The combination of melatonin and sorafenib has also been shown to reduce the expression of the *Myc* oncogene in hepatocarcinoma cells (Long et al. 2017). An increase in the expression of anti-oncogenes, i.e. factors that inhibit tumour growth, including the *p53*, *Brca1* and *Brca2* genes, has been shown in breast cancer cell lines after treatment with melatonin (Hill et al. 2009; Marvalim et al. 2023). Further analysis of the effects of melatonin revealed the ability of this hormone to induce phosphorylation of p53 at the Ser-15 residue, resulting in inhibition of proliferation and prevention of accumulation of DNA damage (Santoro et al. 2012).

Melatonin may affect the resistance of cancer cells to apoptosis. Treatment of cancer cells with melatonin has been shown to alter the balance of pro- and anti-apoptotic proteins, specifically increasing the levels of caspase-3 and Bax and decreasing the levels of Bcl-2 and Birc5/survivin (Kim and Yoo 2010; Xu et al. 2013). In addition, melatonin induces p53 expression in prostate cancer cells (Kim and Yoo 2010). The exact mechanisms by which melatonin induces apoptosis in cancer cells are not fully understood, but it has been shown that the membrane receptors MT1 and MT2 are not necessarily required (Rubio et al. 2007).

The effect of melatonin on telomerase activity in MCF-7 cells transplanted into athymic mice has been studied. A dose-dependent decrease in this activity was observed *in vivo* and *in vitro* (Leon-Blanco et al. 2003). In nude mice implanted with melanoma, melatonin administration enhanced the effect of the BRAF inhibitor vemurafenib by reducing the nuclear translocation of NF- κ B (a transcription factor whose target is the telomerase catalytic subunit gene). As a result, a decrease in telomerase expression and an increase in apoptosis and tumour cell migration were observed (Hao et al. 2019). The synergistic anti-tumour effect of melatonin and a

chemotherapy drug (cisplatin) was also shown in a model of cultured ovarian cancer cell lines. In addition, a decrease in telomerase activity was observed in one of the cell lines studied in this work (Futagami et al. 2001).

Many studies have investigated the effects of melatonin on endothelial cell migration, invasion, and angiogenesis. Melatonin reduces the expression of vascular endothelial growth factor (VEGF) by tumour cells (Dai et al. 2008) as well as the activation of the VEGF receptor in the presence of this growth factor and inhibits the migration of human umbilical vein endothelial cells HUVEC (Cerezo et al. 2017). Treatment of HUVEC cells with melatonin resulted in a 32% reduction in migration and a 50% reduction in vessel formation (Alvarez-García et al. 2013). Nude mice transplanted with MDA-MB-231 human breast cancer cells and treated with melatonin showed a decrease in the expression level of VEGFR-2, and a decrease in capillary density in the tumours was also observed (Jardim-Perassi et al. 2014). Impaired tumour vascularisation with melatonin has also been shown in a mouse model of renal adenocarcinoma (Kim et al. 2013). Melatonin has been shown to reduce the invasive potential of cancer cells by affecting tight junction and adhesion proteins, the expression of which is reduced in invasive tumours. A study of MCF-7 breast cancer cells showed that treatment with melatonin significantly reduced their invasive potential, mainly by increasing the expression of E-cadherin (a protein that forms tight intercellular junctions) and the cell adhesion molecule integrin β -1 (Cos et al. 1998). There is also evidence that melatonin inhibits epithelial-mesenchymal transition by regulating the NF κ B pathway (Jardim-Perassi et al. 2014).

Melatonin has been suggested to protect DNA from adverse effects through its antioxidant properties (Galano et al. 2018). Prophylactic administration of melatonin to rats prior to irradiation has been shown to reduce oxidative damage caused by ionising radiation (Mihandoost et al. 2014). The use of melatonin in mice has also been shown to reduce the number of chromosomal aberrations and abnormal sperm heads caused by various cytostatic drugs (Musatov et al. 1997). A similar protective effect of melatonin has been observed in vivo against DNA damage caused by bisphenol A (Wu et al. 2013) and phenytoin (Erenberk et al. 2014). In vitro studies have shown that melatonin increases DNA repair capacity in MCF-7 (breast cancer) and HCT-15 (colon cancer) cell lines by acting on genes involved in DNA repair systems (Liu et al. 2013). Activation of DNA repair in vitro has also been observed after exposure to melatonin following H₂O₂-induced DNA damage (Sliwinski et al. 2007).

Melatonin has an inhibitory effect on the transcription factor HIF1, which regulates the expression of most glycolytic enzymes and glucose transporters (Dai et al. 2008; Park et al. 2010). The *MYC* oncogene is another regulator of cancer cell metabolism. Overexpression of *MYC* in cancer cells increases the expression of many genes, including those encoding glycolytic enzymes, 3-phosphoinositide-dependent protein kinase-1 (PDK1), lactate dehydrogenase (LDH), and glucose transporters (Altman et al. 2015). In tumour cells, inhibition of LDH and a decrease in lactate levels are observed upon melatonin treatment (Sanchez-Sanchez et al. 2015). Melatonin has been shown to activate the anti-tumour immune response in several ways. The use of melatonin reduces the number of regulatory T cells

(a subpopulation of lymphocytes that suppress the anti-tumour response) in a mouse model of transplantable gastric tumour; in addition, this work showed a reduction in tumour volume in melatonin-treated mice compared to controls (Liu et al. 2011). In a mouse model of transplantable mammary tumour treated with melatonin, a decrease in tumour volume was accompanied by an increase in interferon- γ in blood serum (Talib and Saleh 2015).

Thus, melatonin affects several key features of cancer through a variety of mechanisms. *In vivo* inhibition of tumour growth in laboratory animals has been demonstrated in a variety of models, including spontaneous mammary tumours (Subramanian and Kothari 1991), chemically induced tumours of the ovary (Chuffa et al. 2016), skin (Kumar and Das 2000), and breast (Tamarkin et al. 1981), transplanted syngeneic breast tumours (Talib and Saleh 2015), and liver (Cini et al. 1998), kidney (Kim et al. 2013), stomach (Liu et al. 2011), and xenograft tumours (Han et al. 2011; Dauchy et al. 2014; Jardim-Perassi et al. 2014).

9 Conclusion

It is clear that the paradoxical nature of free radicals, which can both perform beneficial physiological functions and cause damage, is also reflected in the processes of carcinogenesis. The question of the relationship between radicals and cancer is far from being answered. Only further experimental analysis will make it possible to clarify, specify, and formulate systematic ideas on this subject. It is particularly important to clarify the conditions under which the role of radicals can be reversed (from anti-tumour to tumour-inducing and vice versa). Dysregulation of redox homeostasis is emerging as a hallmark of cancer, driving malignant transformation and conferring resistance to therapy. Through targeted interventions aimed at restoring redox balance, antioxidants are emerging as indispensable allies in cancer treatment, able to sensitise tumour cells to therapeutic intervention while protecting normal tissues from OS-induced damage.

A large number of comprehensive studies underline the central role of selected antioxidants in attenuating carcinogenesis. Their ability to neutralise free radicals and inhibit oxidative damage to cellular components highlights their potential as preventive agents against cancer initiation and progression. Antioxidants can protect not only normal cells from the damaging effects of free radicals, but also tumour cells, supporting their survival and growth. There is evidence that the levels of endogenous antioxidant proteins are increased in the cells of some malignant tumours. The synthesis of these proteins is controlled by the KEAP1/NRF2/ARE cellular signalling pathway. It has been shown that oncogenes are involved in the regulation of this pathway. The p53 protein, which activates apoptosis in pathologically altered cells, can be inactivated by antioxidants, which in turn can stimulate the survival and growth of malignant tumour cells.

By elucidating the intricate mechanisms by which antioxidants modulate signalling pathways involved in cancer development, many studies underscore the promise of combinatorial approaches that integrate antioxidants with conventional cancer

therapies. Such synergistic interactions hold immense therapeutic potential, offering new ways to enhance treatment efficacy and minimise side effects. There is currently sufficient information on the effect of certain drugs from different pharmacological groups with antioxidant properties on tumour growth. These include the widely used mucolytic agent N-acetylcysteine, anti-diabetic drugs, alpha-lipoic acid used in the treatment of neuropathies of various origins, and vitamins A and E. Their uncontrolled use has been shown to increase the risk of developing malignant neoplasms in high-risk patients (smokers, patients with chronic obstructive pulmonary disease and diabetes mellitus) and to complicate the course of existing oncological diseases.

In the future, the translation of preclinical findings into clinical practice will require rigorous evaluation of antioxidant interventions in well-designed clinical trials. Furthermore, the identification of biomarkers predictive of treatment response will facilitate patient stratification and optimise therapeutic outcomes. In addition, the exploration of novel antioxidant formulations and delivery strategies holds promise for overcoming existing challenges related to bioavailability and specificity.

References

- Aboelella NS, Brandle C, Kim T, Ding ZC, Zhou G (2021) Oxidative stress in the tumor microenvironment and its relevance to cancer immunotherapy. *Cancers (Basel)* 13(5):986. <https://doi.org/10.3390/cancers13050986>
- Aggarwal V, Tuli HS, Varol A et al (2019) Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. *Biomol Ther* 9(11):735. <https://doi.org/10.3390/biom9110735>
- Agnani D, Camacho-Vanegas O, Camacho C et al (2011) Decreased levels of serum glutathione peroxidase 3 are associated with papillary serous ovarian cancer and disease progression. *J Ovarian Res* 4:18. <https://doi.org/10.1186/1757-2215-4-18>
- Albanes D, Heinonen OP, Taylor PR et al (1996) Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* 88(21):1560–1570. <https://doi.org/10.1093/jnci/88.21.1560>
- Aldini G, Altomare A, Baron G et al (2018) N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic Res* 52(7):751–762. <https://doi.org/10.1080/10715762.2018.1468564>
- Alexandre J, Hu Y, Lu W, Pelicano H, Huang P (2007) Novel action of paclitaxel against cancer cells: bystander effect mediated by reactive oxygen species. *Cancer Res* 67(8):3512–3517. <https://doi.org/10.1158/0008-5472.CAN-06-3914>
- Altman BJ, Hsieh AL, Sengupta A et al (2015) MYC disrupts the circadian clock and metabolism in cancer cells. *Cell Metab* 22(6):1009–1019. <https://doi.org/10.1016/j.cmet.2015.09.003>
- Alvarez-García V, González A, Alonso-González C, Martínez-Campa C, Cos S (2013) Antiangiogenic effects of melatonin in endothelial cell cultures. *Microvasc Res* 87:25–33. <https://doi.org/10.1016/j.mvr.2013.02.008>
- Amraoui W, Adjabi N, Bououza F et al (2018) Modulatory role of selenium and vitamin E, natural antioxidants, against bisphenol A-induced oxidative stress in Wistar albinos rats. *Toxicol Res* 34(3):231–239. <https://doi.org/10.5487/TR.2018.34.3.231>
- Arfin S, Jha NK, Jha SK et al (2021) Oxidative stress in cancer cell metabolism. *Antioxidants (Basel)* 10(5):642. <https://doi.org/10.3390/antiox10050642>

- Argyriou AA, Chroni E, Koutras A et al (2005) Vitamin E for prophylaxis against chemotherapy-induced neuropathy: a randomized controlled trial. *Neurology* 64(1):26–31. <https://doi.org/10.1212/01.WNL.0000148609.35718.7D>
- Arner ES, Holmgren A (2000) Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 267(20):6102–6109. <https://doi.org/10.1046/j.1432-1327.2000.01701.x>
- Athreya K, Xavier MF (2017) Antioxidants in the treatment of cancer. *Nutr Cancer* 69(8): 1099–1104. <https://doi.org/10.1080/01635581.2017.1362445>
- Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Med Cell Longev* 2014:360438. <https://doi.org/10.1155/2014/360438>
- Bairati I, Meyer F, Gélinais M et al (2005) Randomized trial of antioxidant vitamins to prevent acute adverse effects of radiation therapy in head and neck cancer patients. *J Clin Oncol* 23(24): 5805–5813. <https://doi.org/10.1200/JCO.2005.05.514>
- Barrera G (2012) Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncol* 2012:137289. <https://doi.org/10.5402/2012/137289>
- Bindoli A, Rigobello MP, Deeb DJ (1992) Biochemical and toxicological properties of the oxidation products of catecholamines. *Free Radic Biol Med* 13(4):391–405. [https://doi.org/10.1016/0891-5849\(92\)90182-g](https://doi.org/10.1016/0891-5849(92)90182-g)
- Bjelakovic G, Nikolova D, Gluud C (2013) Antioxidant supplements to prevent mortality. *JAMA* 310(11):1178–1179. <https://doi.org/10.1001/jama.2013.277028>
- Block KI, Koch AC, Mead MN, Tothy PK, Newman RA, Gyllenhaal C (2007) Impact of antioxidant supplementation on chemotherapeutic efficacy: a systematic review of the evidence from randomized controlled trials. *Cancer Treat Rev* 33(5):407–418. <https://doi.org/10.1016/j.ctrv.2007.01.005>
- Bohr VA, Dianov GL (1999) Oxidative DNA damage processing in nuclear and mitochondrial DNA. *Biochimie* 81(1–2):155–160. [https://doi.org/10.1016/s0300-9084\(99\)80048-0](https://doi.org/10.1016/s0300-9084(99)80048-0)
- Bonmati-Carrión MA, Tomas-Loba A (2021) Melatonin and cancer: a polyhedral network where the source matters. *Antioxidants (Basel)* 10(2):210. <https://doi.org/10.3390/antiox10020210>
- Budanov AV (2014) The role of tumor suppressor p53 in the antioxidant defense and metabolism. *Subcell Biochem* 85:337–358. https://doi.org/10.1007/978-94-017-9211-0_18
- Buettner GR (2011) Superoxide dismutase in redox biology: the roles of superoxide and hydrogen peroxide. *Anti Cancer Agents Med Chem* 11(4):341–346. <https://doi.org/10.2174/187152011795677544>
- Buđak RJ, Buđak Ł, Kukla M, Gabriel A, Zwirska-Korczala K (2014) Significance of selected antioxidant enzymes in cancer cell progression. *Pol J Pathol* 65(3):167–175. <https://doi.org/10.5114/pjp.2014.45779>
- Burdon RH, Rice-Evans C (1989) Free radicals and the regulation of mammalian cell proliferation. *Free Radic Res Commun* 6(6):345–358. <https://doi.org/10.3109/10715768909087918>
- Caglayan A, Katlan DC, Selçuk Tuncer Z et al (2017) Impaired antioxidant enzyme functions with increased lipid peroxidation in epithelial ovarian cancer. *IUBMB Life* 69(10):802–813. <https://doi.org/10.1002/iub.1675>
- Calaf GM, Aguayo F, Sergi CM, Juarraz A, Roy D (2018) Antioxidants and cancer: theories, techniques, and trials in preventing cancer. *Oxidative Med Cell Longev* 2018:5363064. <https://doi.org/10.1155/2018/5363064>
- Cameron E, Campbell A (1974) The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. *Chem Biol Interact* 9(4):285–315. [https://doi.org/10.1016/0009-2797\(74\)90019-2](https://doi.org/10.1016/0009-2797(74)90019-2)
- Cerezo AB, Hornedo-Ortega R, Álvarez-Fernández MA, Troncoso AM, García-Parrilla MC (2017) Inhibition of VEGF-induced VEGFR-2 activation and HUVEC migration by melatonin and other bioactive indolic compounds. *Nutrients* 9(3):249. <https://doi.org/10.3390/nu9030249>
- Chae HS, Hong ST (2022) Overview of cancer metabolism and signaling transduction. *Int J Mol Sci* 24(1):12. <https://doi.org/10.3390/ijms24010012>

- Chang C, Worley BL, Phaëton R, Hempel N (2020) Extracellular glutathione peroxidase GPx3 and its role in cancer. *Cancers (Basel)* 12(8):2197. <https://doi.org/10.3390/cancers12082197>
- Chaudhary P, Janmeda P, Docea AO et al (2023) Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Front Chem* 11:1158198. <https://doi.org/10.3389/fchem.2023.1158198>
- Checa J, Aran JM (2020) Reactive oxygen species: drivers of physiological and pathological processes. *J Inflamm Res* 13:1057–1073. <https://doi.org/10.2147/JIR.S275595>
- Cheeseman KH, Burton GW, Ingold KU, Slater TF (1984) Lipid peroxidation and lipid antioxidants in normal and tumor cells. *Toxicol Pathol* 12(3):235–239. <https://doi.org/10.1177/019262338401200305>
- Chen J (2016) The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. *Cold Spring Harb Perspect Med* 6(3):a026104. <https://doi.org/10.1101/cshperspect.a026104>
- Chen K, Zhu P, Chen W, Luo K, Shi XJ, Zhai W (2021) Melatonin inhibits proliferation, migration, and invasion by inducing ROS-mediated apoptosis via suppression of the PI3K/Akt/mTOR signaling pathway in gallbladder cancer cells. *Aging (Albany NY)* 13(18):22502–22515. <https://doi.org/10.18632/aging.203561>
- Choudhari SK, Chaudhary M, Gadbail AR, Sharma A, Tekade S (2014) Oxidative and antioxidative mechanisms in oral cancer and precancer: a review. *Oral Oncol* 50(1):10–18. <https://doi.org/10.1016/j.oraloncology.2013.09.011>
- Chuffa LG, Alves MS, Martinez M et al (2016) Apoptosis is triggered by melatonin in an *in vivo* model of ovarian carcinoma. *Endocr Relat Cancer* 23(2):65–76. <https://doi.org/10.1530/ERC-15-0463>
- Cini G, Coronello M, Mini E, Neri B (1998) Melatonin's growth-inhibitory effect on hepatoma AH 130 in the rat. *Cancer Lett* 125(1–2):51–59. [https://doi.org/10.1016/s0304-3835\(97\)00480-1](https://doi.org/10.1016/s0304-3835(97)00480-1)
- Circu ML, Aw TY (2008) Glutathione and apoptosis. *Free Radic Res* 42(8):689–706. <https://doi.org/10.1080/10715760802317663>
- Conklin KA (2000) Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. *Nutr Cancer* 37(1):1–18. https://doi.org/10.1207/S15327914NC3701_1
- Corn KC, Windham MA, Rafat M (2020) Lipids in the tumor microenvironment: from cancer progression to treatment. *Prog Lipid Res* 80:101055. <https://doi.org/10.1016/j.plipres.2020.101055>
- Cos S, Fernández R, Güézmés A, Sánchez-Barceló EJ (1998) Influence of melatonin on invasive and metastatic properties of MCF-7 human breast cancer cells. *Cancer Res* 58(19):4383–4390
- Dai M, Cui P, Yu M, Han J, Li H, Xiu R (2008) Melatonin modulates the expression of VEGF and HIF-1 alpha induced by CoCl₂ in cultured cancer cells. *J Pineal Res* 44(2):121–126. <https://doi.org/10.1111/j.1600-079X.2007.00498.x>
- Dalzoppo D, Di Paolo V, Calderan L et al (2017) Thiol-activated anticancer agents: the state of the art. *Anti Cancer Agents Med Chem* 17(1):4–20
- Dauchy RT, Xiang S, Mao L et al (2014) Circadian and melatonin disruption by exposure to light at night drives intrinsic resistance to tamoxifen therapy in breast cancer. *Cancer Res* 74(15):4099–4110. <https://doi.org/10.1158/0008-5472.CAN-13-3156>
- Deneke SM (2000) Thiol-based antioxidants. *Curr Top Cell Regul* 36:151–180. [https://doi.org/10.1016/s0070-2137\(01\)80007-8](https://doi.org/10.1016/s0070-2137(01)80007-8)
- DeNicola GM, Karreth FA, Humpton TJ et al (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475(7354):106–109. <https://doi.org/10.1038/nature10189>
- Deponce M (2017) The incomplete glutathione puzzle: just guessing at numbers and figures? *Antioxid Redox Signal* 27(15):1130–1161. <https://doi.org/10.1089/ars.2017.7123>
- Di Meo S, Venditti P (2020) Evolution of the knowledge of free radicals and other oxidants. *Oxidative Med Cell Longev* 2020:9829176. <https://doi.org/10.1155/2020/9829176>

- Diao QX, Zhang JZ, Zhao T et al (2016) Vitamin E promotes breast cancer cell proliferation by reducing ROS production and p53 expression. *Eur Rev Med Pharmacol Sci* 20(12):2710–2717
- Diaz-Cano SJ (2012) Tumor heterogeneity: mechanisms and bases for a reliable application of molecular marker design. *Int J Mol Sci* 13(2):1951–2011. <https://doi.org/10.3390/ijms13021951>
- Didier AJ, Stiene J, Fang L, Watkins D, Dworkin LD, Creeden JF (2023) Antioxidant and anti-tumor effects of dietary vitamins A, C, and E. *Antioxidants (Basel)* 12(3):632. <https://doi.org/10.3390/antiox12030632>
- Ding DN, Xie LZ, Shen Y et al (2021) Insights into the role of oxidative stress in ovarian cancer. *Oxidative Med Cell Longev* 2021:8388258. <https://doi.org/10.1155/2021/8388258>
- Doroshov JH (1986) Prevention of doxorubicin-induced killing of MCF-7 human breast cancer cells by oxygen radical scavengers and iron chelating agents. *Biochem Biophys Res Commun* 135(1):330–335. [https://doi.org/10.1016/0006-291x\(86\)90981-2](https://doi.org/10.1016/0006-291x(86)90981-2)
- Drexler J, Meaney TF, McCormack LJ (1957) The calcified pineal body and carcinoma. *Cleve Clin Q* 24(4):242–247. <https://doi.org/10.3949/ccjm.24.4.242>
- Dumitrescu C (1992) The role of free radicals in normal and pathologic cell proliferation. *Rom J Endocrinol* 30(1–2):45–62
- Dupré-Crochet S, Erard M, Nüße O (2013) ROS production in phagocytes: why, when, and where? *J Leukoc Biol* 94(4):657–670. <https://doi.org/10.1189/jlb.1012544>
- Elfaki I, Mir R, Almutairi FM, Duhier FMA (2018) Cytochrome P450: polymorphisms and roles in cancer, diabetes and atherosclerosis. *Asian Pac J Cancer Prev* 19(8):2057–2070. <https://doi.org/10.22034/APJCP.2018.19.8.2057>
- Erenberk U, Dundaroz R, Gok O et al (2014) Melatonin attenuates phenytoin sodium-induced DNA damage. *Drug Chem Toxicol* 37(2):233–239. <https://doi.org/10.3109/01480545.2013.838777>
- Fang J, Seki T, Maeda H (2009) Therapeutic strategies by modulating oxygen stress in cancer and inflammation. *Adv Drug Deliv Rev* 61(4):290–302. <https://doi.org/10.1016/j.addr.2009.02.005>
- Filaire E, Dupuis C, Galvaing G et al (2013) Lung cancer: what are the links with oxidative stress, physical activity and nutrition. *Lung Cancer* 82(3):383–389. <https://doi.org/10.1016/j.lungcan.2013.09.009>
- Flohé L (2016) The impact of thiol peroxidases on redox regulation. *Free Radic Res* 50(2):126–142. <https://doi.org/10.3109/10715762.2015.1046858>
- Fuchs-Tarlovsky V (2013) Role of antioxidants in cancer therapy. *Nutrition* 29(1):15–21. <https://doi.org/10.1016/j.nut.2012.02.014>
- Fujii J, Homma T, Osaki T (2022) Superoxide radicals in the execution of cell death. *Antioxidants (Basel)* 11(3):501. <https://doi.org/10.3390/antiox11030501>
- Futagami M, Sato S, Sakamoto T, Yokoyama Y, Saito Y (2001) Effects of melatonin on the proliferation and cis-diamminedichloroplatinum (CDDP) sensitivity of cultured human ovarian cancer cells. *Gynecol Oncol* 82(3):544–549. <https://doi.org/10.1006/gyno.2001.6330>
- Galano A, Tan DX, Reiter RJ (2018) Melatonin: a versatile protector against oxidative DNA damage. *Molecules* 23(3):530. <https://doi.org/10.3390/molecules23030530>
- Genestra M (2007) Oxy radicals, redox-sensitive signalling cascades and antioxidants. *Cell Signal* 19(9):1807–1819. <https://doi.org/10.1016/j.cellsig.2007.04.009>
- Gentile F, Arcaro A, Pizzimenti S et al (2017) DNA damage by lipid peroxidation products: implications in cancer, inflammation and autoimmunity. *AIMS Genet* 4(2):103–137. <https://doi.org/10.3934/genet.2017.2.103>
- George S, Abrahamse H (2020) Redox potential of antioxidants in cancer progression and prevention. *Antioxidants (Basel)* 9(11):1156. <https://doi.org/10.3390/antiox9111156>
- Gholamalizadeh M, Ardekanizadeh NH, Aghakhaninejad Z et al (2023) The effects of dietary supplements in patients with cervical cancer: a comprehensive systematic review. *Eur J Obstet Gynecol Reprod Biol* 19:100217. <https://doi.org/10.1016/j.eurox.2023.100217>
- Giovannucci E, Harlan DM, Archer MC et al (2010) Diabetes and cancer: a consensus report. *CA Cancer J Clin* 60(4):207–221. <https://doi.org/10.3322/caac.20078>

- Glasauer A, Chandel NS (2014) Targeting antioxidants for cancer therapy. *Biochem Pharmacol* 92(1):90–101. <https://doi.org/10.1016/j.bcp.2014.07.017>
- Goh J, Enns L, Fatemie S et al (2011) Mitochondrial targeted catalase suppresses invasive breast cancer in mice. *BMC Cancer* 11:191. <https://doi.org/10.1186/1471-2407-11-191>
- Gök F, Ekin S, Karaman E, Erten R, Yıldız D, Bakır A (2023) Total sialic acid, antioxidant enzyme activities, trace elements, and vitamin status before and after surgery in women with uterine myoma and endometrial cancer. *Reprod Sci* 30(9):2743–2757. <https://doi.org/10.1007/s43032-023-01241-9>
- Goodman M, Bostick RM, Kucuk O, Jones DP (2011) Clinical trials of antioxidants as cancer prevention agents: past, present, and future. *Free Radic Biol Med* 51(5):1068–1084. <https://doi.org/10.1016/j.freeradbiomed.2011.05.018>
- Greenberg MM (2016) Reactivity of nucleic acid radicals. *Adv Phys Org Chem* 50:119–202. <https://doi.org/10.1016/bs.apoc.2016.02.001>
- Gromadzińska J, Reszka E, Bruzelius K, Wasowicz W, Akesson B (2008) Selenium and cancer: biomarkers of selenium status and molecular action of selenium supplements. *Eur J Nutr* 47 (Suppl 2):29–50. <https://doi.org/10.1007/s00394-008-2005-z>
- Gupta N, Verma K, Nalla S, Kulshreshtha A, Lall R, Prasad S (2020) Free radicals as a double-edged sword: the cancer preventive and therapeutic roles of curcumin. *Molecules* 25(22):5390. <https://doi.org/10.3390/molecules25225390>
- Güven M, Öztürk B, Sayal A, Özetürk A, Ulutin T (1999) Lipid peroxidation and antioxidant system in the blood of cancerous patients with metastasis. *Cancer Biochem Biophys* 17(1–2): 155–162
- Hajhashemi V, Vaseghi G, Pourfarzam M, Abdollahi A (2010) Are antioxidants helpful for disease prevention? *Res Pharm Sci* 5(1):1–8
- Halliwell B (2001) Free radicals and other reactive species in disease. In: *Nature Encyclopedia of life sciences*, pp 1–7
- Han Y, Demorrow S, Invernizzi P et al (2011) Melatonin exerts by an autocrine loop antiproliferative effects in cholangiocarcinoma: its synthesis is reduced favoring cholangiocarcinoma growth. *Am J Physiol Gastrointest Liver Physiol* 301(4):G623–G633. <https://doi.org/10.1152/ajpgi.00118.2011>
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- Hao J, Fan W, Li Y et al (2019) Melatonin synergizes BRAF-targeting agent vemurafenib in melanoma treatment by inhibiting iNOS/hTERT signaling and cancer-stem cell traits. *J Exp Clin Cancer Res* 38(1):48. <https://doi.org/10.1186/s13046-019-1036-z>
- Harris C, Hansen JM (2012) Oxidative stress, thiols, and redox profiles. *Methods Mol Biol* 889: 325–346. https://doi.org/10.1007/978-1-61779-867-2_21
- Hawk MA, McCallister C, Schafer ZT (2016) Antioxidant activity during tumor progression: a necessity for the survival of cancer cells? *Cancers (Basel)* 8(10):92. <https://doi.org/10.3390/cancers8100092>
- Hawkins CL, Davies MJ (2019) Detection, identification, and quantification of oxidative protein modifications. *J Biol Chem* 294(51):19683–19708. <https://doi.org/10.1074/jbc.REV119.006217>
- Hayes JD, Dinkova-Kostova AT, Tew KD (2020) Oxidative stress in cancer. *Cancer Cell* 38(2): 167–197. <https://doi.org/10.1016/j.ccell.2020.06.001>
- Hecht F, Cazarin JM, Lima CE et al (2016) Redox homeostasis of breast cancer lineages contributes to differential cell death response to exogenous hydrogen peroxide. *Life Sci* 158:7–13. <https://doi.org/10.1016/j.lfs.2016.06.016>
- Hill SM, Frasch T, Xiang S, Yuan L, Duplessis T, Mao L (2009) Molecular mechanisms of melatonin anticancer effects. *Integr Cancer Ther* 8(4):337–346. <https://doi.org/10.1177/1534735409353332>
- Hou Y, Wang L, Yi D, Wu G (2015) N-acetylcysteine and intestinal health: a focus on its mechanism of action. *Front Biosci (Landmark Ed)* 20(5):872–891. <https://doi.org/10.2741/4342>

- Huang R, Chen H, Liang J et al (2021) Dual role of reactive oxygen species and their application in cancer therapy. *J Cancer* 12(18):5543–5561. <https://doi.org/10.7150/jca.54699>
- Huang C, Kuo S, Lin L, Yang Y (2023) The efficacy of N-acetylcysteine in chronic obstructive pulmonary disease patients: a meta-analysis. *Ther Adv Respir Dis* 17:17534666231158563. <https://doi.org/10.1177/17534666231158563>
- Huether G (1993) The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. *Experientia* 49(8):665–670. <https://doi.org/10.1007/BF01923948>
- Ito F, Sono Y, Ito T (2019) Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants (Basel)* 8(3):72. <https://doi.org/10.3390/antiox8030072>
- Janion K, Szczepańska E, Nowakowska-Zajdel E, Walkiewicz K, Strzelczyk J (2020) Lipid peroxidation and total oxidant/antioxidant status in colorectal cancer patients. *J Biol Regul Homeost Agents* 34(1):239–244. <https://doi.org/10.23812/19-386-L>
- Janssen AM, Bosman CB, van Duijn W et al (2000) Superoxide dismutases in gastric and esophageal cancer and the prognostic impact in gastric cancer. *Clin Cancer Res* 6(8):3183–3192
- Jardim-Perassi BV, Arbab AS, Ferreira LC et al (2014) Effect of melatonin on tumor growth and angiogenesis in xenograft model of breast cancer. *PLoS One* 9(1):e85311. <https://doi.org/10.1371/journal.pone.0085311>
- Jelic MD, Mandic AD, Maricic SM, Srdjenovic BU (2021) Oxidative stress and its role in cancer. *J Cancer Res Ther* 17(1):22–28. https://doi.org/10.4103/jcrt.JCRT_862_16
- Jiang B, Xiao S, Khan MA, Xue M (2013) Defective antioxidant systems in cervical cancer. *Tumour Biol* 34(4):2003–2009. <https://doi.org/10.1007/s13277-013-0804-1>
- Jiang H, Zuo J, Li B et al (2023) Drug-induced oxidative stress in cancer treatments: angel or devil? *Redox Biol* 63:102754. <https://doi.org/10.1016/j.redox.2023.102754>
- Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E (2021) The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int J Mol Sci* 22(9):4642. <https://doi.org/10.3390/ijms22094642>
- Kang MY, Kim HB, Piao C et al (2013) The critical role of catalase in prooxidant and antioxidant function of p53. *Cell Death Differ* 20(1):117–129. <https://doi.org/10.1038/cdd.2012.102>
- Kennedy L, Sandhu JK, Harper ME, Cuperlovic-Culf M (2020) Role of glutathione in cancer: from mechanisms to therapies. *Biomol Ther* 10(10):1429. <https://doi.org/10.3390/biom10101429>
- Kidwell KM, Yothers G, Ganz PA et al (2012) Long-term neurotoxicity effects of oxaliplatin added to fluorouracil and leucovorin as adjuvant therapy for colon cancer: results from National Surgical Adjuvant Breast and Bowel Project trials C-07 and LTS-01. *Cancer* 118(22):5614–5622. <https://doi.org/10.1002/cncr.27593>
- Kim CH, Yoo YM (2010) Melatonin induces apoptotic cell death via p53 in LNCaP cells. *Korean J Physiol Pharmacol* 14(6):365–369. <https://doi.org/10.4196/kjpp.2010.14.6.365>
- Kim YT, Kim JW, Choi JS, Kim SH, Choi EK, Cho NH (2004) Relation between deranged antioxidant system and cervical neoplasia. *Int J Gynecol Cancer* 14(5):889–895. <https://doi.org/10.1111/j.1048-891X.2004.14526.x>
- Kim KJ, Choi JS, Kang I, Kim KW, Jeong CH, Jeong JW (2013) Melatonin suppresses tumor progression by reducing angiogenesis stimulated by HIF-1 in a mouse tumor model. *J Pineal Res* 54(3):264–270. <https://doi.org/10.1111/j.1600-079X.2012.01030.x>
- Kohen R, Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 30(6):620–650. <https://doi.org/10.1080/01926230290166724>
- Kondakova IV, Kakurina GV, Smirnova LP, Borunov EV (2005) Regulation of tumor cell proliferation and apoptosis by free radicals. *Siberian J Oncol* 1:58–61. (in Russ)
- Kopustinskiene DM, Bernatoniene J (2021) Molecular mechanisms of melatonin-mediated cell protection and signaling in health and disease. *Pharmaceutics* 13(2):129. <https://doi.org/10.3390/pharmaceutics13020129>

- Koundouros N, Pouligiannis G (2020) Reprogramming of fatty acid metabolism in cancer. *Br J Cancer* 122(1):4–22. <https://doi.org/10.1038/s41416-019-0650-z>
- Kristal AR, Darke AK, Morris JS et al (2014) Baseline selenium status and effects of selenium and vitamin e supplementation on prostate cancer risk. *J Natl Cancer Inst* 106(3):djt456. <https://doi.org/10.1093/jnci/djt456>
- Kruk J, Aboul-Enein HY (2017) Reactive oxygen and nitrogen species in carcinogenesis: implications of oxidative stress on the progression and development of several cancer types. *Mini Rev Med Chem* 17(11):904–919. <https://doi.org/10.2174/1389557517666170228115324>
- Kumar CA, Das UN (2000) Effect of melatonin on two stage skin carcinogenesis in Swiss mice. *Med Sci Monit* 6(3):471–475
- Kumar A, Sharma S, Pundir CS, Sharma A (1995) Decreased plasma glutathione in cancer of the uterine cervix. *Cancer Lett* 94(1):107–111. [https://doi.org/10.1016/0304-3835\(95\)03832-h](https://doi.org/10.1016/0304-3835(95)03832-h)
- Kumaraguruparan R, Subapriya R, Viswanathan P, Nagini S (2002) Tissue lipid peroxidation and antioxidant status in patients with adenocarcinoma of the breast. *Clin Chim Acta* 325(1–2): 165–170. [https://doi.org/10.1016/s0009-8981\(02\)00292-9](https://doi.org/10.1016/s0009-8981(02)00292-9)
- Kundaktepe BP, Sozer V, Durmus S et al (2021) The evaluation of oxidative stress parameters in breast and colon cancer. *Medicine (Baltimore)* 100(11):e25104. <https://doi.org/10.1097/MD.00000000000025104>
- Kurhaluk N (2021) Alcohol and melatonin. *Chronobiol Int* 38(6):785–800. <https://doi.org/10.1080/07420528.2021.1899198>
- Kurhaluk N, Tkachenko H (2020) Melatonin and alcohol-related disorders. *Chronobiol Int* 37(6): 781–803. <https://doi.org/10.1080/07420528.2020.1761372>
- Kurhaluk N, Tkachenko H (2022) Effects of melatonin and metformin in preventing lysosome-induced autophagy and oxidative stress in rat models of carcinogenesis and the impact of high-fat diet. *Sci Rep* 12(1):4998. <https://doi.org/10.1038/s41598-022-08778-w>
- Kurhaluk N, Sliuta A, Kyriienko S, Winklewski PJ (2017) Melatonin restores white blood cell count, diminishes glycated haemoglobin level and prevents liver, kidney and muscle oxidative stress in mice exposed to acute ethanol intoxication. *Alcohol Alcohol* 52(5):521–528. <https://doi.org/10.1093/alcalc/agx045>
- Kurhaluk N, Szarmach A, Zaitseva OV, Sliuta A, Kyriienko S, Winklewski PJ (2018a) Effects of melatonin on low-dose lipopolysaccharide-induced oxidative stress in mouse liver, muscle, and kidney. *Can J Physiol Pharmacol* 96(11):1153–1160. <https://doi.org/10.1139/cjpp-2018-0011>
- Kurhaluk N, Zaitseva OV, Sliuta A, Kyriienko S, Winklewski PJ (2018b) Melatonin diminishes oxidative stress in plasma, retains erythrocyte resistance and restores white blood cell count after low dose lipopolysaccharide exposure in mice. *Gen Physiol Biophys* 37(5):571–580. https://doi.org/10.4149/gpb_2018010
- Kurhaluk N, Bojkova B, Radkowski M et al (2018c) Melatonin and metformin diminish oxidative stress in heart tissue in a rat model of high fat diet and mammary carcinogenesis. *Adv Exp Med Biol* 1047:7–19. https://doi.org/10.1007/5584_2017_128
- Kurhaluk N, Bojková B, Winklewski PJ (2018d) Liver antioxidant and aerobic status improves after metformin and melatonin administration in a rat model of high-fat diet and mammary carcinogenesis. *Can J Physiol Pharmacol* 96(8):790–797. <https://doi.org/10.1139/cjpp-2018-0001>
- Kurhaluk N, Tkachenko H, Lukash O (2020) Melatonin modulates oxidative phosphorylation, hepatic and kidney autophagy-caused subclinical endotoxemia and acute ethanol-induced oxidative stress. *Chronobiol Int* 37(12):1709–1724. <https://doi.org/10.1080/07420528.2020.1830792>
- Kurhaluk N, Tkachenko H, Lukash O, Winklewski PJ, Wszedybyl-Winklewska M (2021a) Melatonin maintains the function of the blood redox system at combined ethanol-induced toxicity and subclinical inflammation in mice. *Sleep Breath* 25(2):1045–1054. <https://doi.org/10.1007/s11325-020-02191-1>
- Kurhaluk N, Tkachenko H, Lukash O (2021b) Photoperiod-induced alterations in biomarkers of oxidative stress and biochemical pathways in rats of different ages: focus on individual

- physiological reactivity. *Chronobiol Int* 38(12):1673–1691. <https://doi.org/10.1080/07420528.2021.1939364>
- Kurutas EB (2016) The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J* 15(1):71. <https://doi.org/10.1186/s12937-016-0186-5>
- Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, Blumberg JB (2008) Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? *J Natl Cancer Inst* 100(11):773–783. <https://doi.org/10.1093/jnci/djn148>
- Le Gal K, Ibrahim MX, Wiel C et al (2015) Antioxidants can increase melanoma metastasis in mice. *Sci Transl Med* 7(308):308re8. <https://doi.org/10.1126/scitranslmed.aad3740>
- Lee S, Hu L (2020) Nrf2 activation through the inhibition of Keap1-Nrf2 protein-protein interaction. *Med Chem Res* 29(5):846–867. <https://doi.org/10.1007/s00044-020-02539-y>
- Leon-Blanco MM, Guerrero JM, Reiter RJ, Calvo JR, Pozo D (2003) Melatonin inhibits telomerase activity in the MCF-7 tumor cell line both *in vivo* and *in vitro*. *J Pineal Res* 35(3):204–211. <https://doi.org/10.1034/j.1600-079x.2003.00077.x>
- Li DW, Spector A (1997) Hydrogen peroxide-induced expression of the proto-oncogenes, *c-jun*, *c-fos* and *c-myc* in rabbit lens epithelial cells. *Mol Cell Biochem* 173(1–2):59–69. <https://doi.org/10.1023/a:1006828402225>
- Li K, Deng Z, Lei C, Ding X, Li J, Wang C (2024) The role of oxidative stress in tumorigenesis and progression. *Cells* 13(5):441. <https://doi.org/10.3390/cells13050441>
- Liou GY, Storz P (2010) Reactive oxygen species in cancer. *Free Radic Res* 44(5):479–496. <https://doi.org/10.3109/10715761003667554>
- Lister A, Nedjadi T, Kitteringham NR et al (2011) Nrf2 is overexpressed in pancreatic cancer: implications for cell proliferation and therapy. *Mol Cancer* 10:37. <https://doi.org/10.1186/1476-4598-10-37>
- Liu H, Xu L, Wei JE, Xie MR, Wang SE, Zhou RX (2011) Role of CD4+ CD25+ regulatory T cells in melatonin-mediated inhibition of murine gastric cancer cell growth *in vivo* and *in vitro*. *Anat Rec (Hoboken)* 294(5):781–788. <https://doi.org/10.1002/ar.21361>
- Liu R, Fu A, Hoffman AE, Zheng T, Zhu Y (2013) Melatonin enhances DNA repair capacity possibly by affecting genes involved in DNA damage responsive pathways. *BMC Cell Biol* 14:1. <https://doi.org/10.1186/1471-2121-14-1>
- Liu S, Pi J, Zhang Q (2022) Signal amplification in the KEAP1-NRF2-ARE antioxidant response pathway. *Redox Biol* 54:102389. <https://doi.org/10.1016/j.redox.2022.102389>
- Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn Rev* 4(8):118–126. <https://doi.org/10.4103/0973-7847.70902>
- Long F, Dong C, Jiang K et al (2017) Melatonin enhances the anti-tumor effect of sorafenib via AKT/p27-mediated cell cycle arrest in hepatocarcinoma cell lines. *RSC Adv* 7:21342–21351. <https://doi.org/10.1039/C7RA02113E>
- Lubos E, Loscalzo J, Handy DE (2011) Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 15(7):1957–1997. <https://doi.org/10.1089/ars.2010.3586>
- Luo M, Zhou L, Huang Z et al (2022) Antioxidant therapy in cancer: rationale and progress. *Antioxidants (Basel)* 11(6):1128. <https://doi.org/10.3390/antiox11061128>
- Mahalingaiah PK, Singh KP (2014) Chronic oxidative stress increases growth and tumorigenic potential of MCF-7 breast cancer cells. *PLoS One* 9(1):e87371. <https://doi.org/10.1371/journal.pone.0087371>
- Maiuolo J, Gliozzi M, Carresi C et al (2021) Nutraceuticals and cancer: potential for natural polyphenols. *Nutrients* 13(11):3834. <https://doi.org/10.3390/nu13113834>
- Malafa M, Margenthaler J, Webb B, Neitzel L, Christophersen M (2000) MnSOD expression is increased in metastatic gastric cancer. *J Surg Res* 88(2):130–134. <https://doi.org/10.1006/jsre.1999.5773>

- Ma-On C, Sanpavat A, Whongsiri P et al (2017) Oxidative stress indicated by elevated expression of Nrf2 and 8-OHdG promotes hepatocellular carcinoma progression. *Med Oncol* 34(4):57. <https://doi.org/10.1007/s12032-017-0914-5>
- Marengo B, Nitti M, Furfaro AL et al (2016) Redox homeostasis and cellular antioxidant systems: crucial players in cancer growth and therapy. *Oxidative Med Cell Longev* 2016:6235641. <https://doi.org/10.1155/2016/6235641>
- Marinho HS, Real C, Cyrne L, Soares H, Antunes F (2014) Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biol* 2:535–562. <https://doi.org/10.1016/j.redox.2014.02.006>
- Marvalim C, Datta A, Lee SC (2023) Role of p53 in breast cancer progression: an insight into p53 targeted therapy. *Theranostics* 13(4):1421–1442. <https://doi.org/10.7150/thno.81847>
- Medow MS, Bamji N, Clarke D, Ocon AJ, Stewart JM (2011) Reactive oxygen species (ROS) from NADPH and xanthine oxidase modulate the cutaneous local heating response in healthy humans. *J Appl Physiol* (1985) 111(1):20–26. <https://doi.org/10.1152/japplphysiol.01448.2010>
- Messens J, Collet JF (2013) Thiol-disulfide exchange in signaling: disulfide bonds as a switch. *Antioxid Redox Signal* 18(13):1594–1596. <https://doi.org/10.1089/ars.2012.5156>
- Mihandoost E, Shirazi A, Mahdavi SR, Aliasgharzadeh A (2014) Can melatonin help us in radiation oncology treatments? *Biomed Res Int* 2014:578137. <https://doi.org/10.1155/2014/578137>
- Mileo AM, Miccadei S (2016) Polyphenols as modulator of oxidative stress in cancer disease: new therapeutic strategies. *Oxidative Med Cell Longev* 2016:6475624. <https://doi.org/10.1155/2016/6475624>
- Minato A, Noguchi H, Ohnishi R, Tomisaki I, Nakayama T, Fujimoto N (2021) Reduced expression level of GPX2 in T1 bladder cancer and its role in early-phase invasion of bladder cancer. *In Vivo* 35(2):753–759. <https://doi.org/10.21873/in vivo.12316>
- Miriyala S, Spasojevic I, Tovmasyan A et al (2012) Manganese superoxide dismutase, MnSOD and its mimics. *Biochim Biophys Acta* 1822(5):794–814. <https://doi.org/10.1016/j.bbadis.2011.12.002>
- Mitchell JB, Cook JA, DeGraff W, Glatstein E, Russo A (1989) Glutathione modulation in cancer treatment: will it work? *Int J Radiat Oncol Biol Phys* 16(5):1289–1295. [https://doi.org/10.1016/0360-3016\(89\)90301-5](https://doi.org/10.1016/0360-3016(89)90301-5)
- Mitra S, Nguyen LN, Akter M, Park G, Choi EH, Kaushik NK (2019) Impact of ROS generated by chemical, physical, and plasma techniques on cancer attenuation. *Cancers (Basel)* 11(7):1030. <https://doi.org/10.3390/cancers11071030>
- Munshi A, Ramesh R (2013) Mitogen-activated protein kinases and their role in radiation response. *Genes Cancer* 4(9–10):401–408. <https://doi.org/10.1177/1947601913485414>
- Murata A, Morishige F, Yamaguchi H (1982) Prolongation of survival times of terminal cancer patients by administration of large doses of ascorbate. *Int J Vitam Nutr Res Suppl* 23:103–113
- Musatov SA, Rosenfeld SV, Togo EF, Mikheev VS, Anisimov VN (1997) Vliianie melatonina na mutagennost' i protivopukhlevyĭ éffekt tsitostatikov u mysheĭ [The influence of melatonin on mutagenicity and antitumor action of cytostatic drugs in mice]. *Vopr Onkol* 43(6):623–627
- Mut-Salud N, Álvarez PJ, Garrido JM, Carrasco E, Aránega A, Rodríguez-Serrano F (2016) Antioxidant intake and antitumor therapy: toward nutritional recommendations for optimal results. *Oxidative Med Cell Longev* 2016:6719534. <https://doi.org/10.1155/2016/6719534>
- Nandi A, Yan LJ, Jana CK, Das N (2019) Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxidative Med Cell Longev* 2019:9613090. <https://doi.org/10.1155/2019/9613090>
- Navarro J, Obrador E, Carretero J et al (1999) Changes in glutathione status and the antioxidant system in blood and in cancer cells associate with tumour growth *in vivo*. *Free Radic Biol Med* 26(3–4):410–418. [https://doi.org/10.1016/s0891-5849\(98\)00213-5](https://doi.org/10.1016/s0891-5849(98)00213-5)
- Nelson ME, Lahiri S, Chow JD et al (2017) Inhibition of hepatic lipogenesis enhances liver tumorigenesis by increasing antioxidant defence and promoting cell survival. *Nat Commun* 8:14689. <https://doi.org/10.1038/ncomms14689>

- Nishida S, Akai F, Iwasaki H et al (1993) Manganese superoxide dismutase content and localization in human thyroid tumours. *J Pathol* 169(3):341–345. <https://doi.org/10.1002/path.1711690311>
- Nordberg J, Arnér ES (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31(11):1287–1312. [https://doi.org/10.1016/s0891-5849\(01\)00724-9](https://doi.org/10.1016/s0891-5849(01)00724-9)
- Obrenovich ME, Li Y, Parvathaneni K et al (2011) Antioxidants in health, disease and aging. *CNS Neurol Disord Drug Targets* 10(2):192–207. <https://doi.org/10.2174/187152711794480375>
- Oh IJ, Kim HE, Song SY et al (2014) Diagnostic value of serum glutathione peroxidase 3 levels in patients with lung cancer. *Thorac Cancer* 5(5):425–430. <https://doi.org/10.1111/1759-7714.12113>
- Ono A, Koshiyama M, Nakagawa M et al (2020) The preventive effect of dietary antioxidants on cervical cancer development. *Medicina (Kaunas)* 56(11):604. <https://doi.org/10.3390/medicina56110604>
- Pace A, Bove L, Jandolo B (2005) Vitamin E for prophylaxis against chemotherapy-induced neuropathy: a randomized controlled trial. *Neurology* 65(3):501–502. <https://doi.org/10.1212/wnl.65.3.501>
- Palazzotti B, Pani G, Colavitti R et al (1999) Increased growth capacity of cervical-carcinoma cells over-expressing manganous superoxide dismutase. *Int J Cancer* 82(1):145–150. [https://doi.org/10.1002/\(sici\)1097-0215\(19990702\)82:1<145::aid-ijc24>3.0.co;2-b](https://doi.org/10.1002/(sici)1097-0215(19990702)82:1<145::aid-ijc24>3.0.co;2-b)
- Park SY, Jang WJ, Yi EY et al (2010) Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. *J Pineal Res* 48(2):178–184. <https://doi.org/10.1111/j.1600-079x.2009.00742.x>
- Park SJ, Myung SK, Lee Y, Lee YJ (2017) Effects of vitamin and antioxidant supplements in prevention of bladder cancer: a meta-analysis of randomized controlled trials. *J Korean Med Sci* 32(4):628–635. <https://doi.org/10.3346/jkms.2017.32.4.628>
- Pejić S, Todorović A, Stojiljković V, Gavrilović L, Popović N, Pajović SB (2015) Antioxidant status in women with uterine leiomyoma: relation with sex hormones. *An Acad Bras Cienc* 87(3):1771–1782. <https://doi.org/10.1590/0001-3765201520130416>
- Pham-Huy LA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health. *Int J Biomed Sci* 4(2):89–96
- Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem* 30(1):11–26. <https://doi.org/10.1007/s12291-014-0446-0>
- Pizzino G, Irrera N, Cucinotta M et al (2017) Oxidative stress: harms and benefits for human health. *Oxidative Med Cell Longev* 2017:8416763. <https://doi.org/10.1155/2017/8416763>
- Poljsak B (2011) Strategies for reducing or preventing the generation of oxidative stress. *Oxidative Med Cell Longev* 2011:194586. <https://doi.org/10.1155/2011/194586>
- Poljsak B, Milisav I (2018) The role of antioxidants in cancer, friends or foes? *Curr Pharm Des* 24(44):5234–5244. <https://doi.org/10.2174/1381612825666190123112647>
- Poljsak B, Šuput D, Milisav I (2013) Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. *Oxidative Med Cell Longev* 2013:956792. <https://doi.org/10.1155/2013/956792>
- Psathakis D, Wedemeyer N, Oevermann E, Krug F, Siegers CP, Bruch HP (1998) Blood selenium and glutathione peroxidase status in patients with colorectal cancer. *Dis Colon Rectum* 41(3):328–335. <https://doi.org/10.1007/BF02237487>
- Qi X, Ng KT, Lian QZ et al (2014) Clinical significance and therapeutic value of glutathione peroxidase 3 (GPx3) in hepatocellular carcinoma. *Oncotarget* 5(22):11103–11120. <https://doi.org/10.18632/oncotarget.2549>
- Rahal A, Kumar A, Singh V et al (2014) Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomed Res Int* 2014:761264. <https://doi.org/10.1155/2014/761264>
- Rajneesh CP, Manimaran A, Sasikala KR, Adaikappan P (2008) Lipid peroxidation and antioxidant status in patients with breast cancer. *Singapore Med J* 49(8):640–643

- Redza-Dutordoir M, Averill-Bates DA (2016) Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta* 1863(12):2977–2992. <https://doi.org/10.1016/j.bbamcr.2016.09.012>
- Ríos-Arrabal S, Artacho-Cordón F, León J et al (2013) Involvement of free radicals in breast cancer. *Springerplus* 2:404. <https://doi.org/10.1186/2193-1801-2-404>
- Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F (2014) The role of vitamin e in human health and some diseases. *Sultan Qaboos Univ Med J* 14(2):e157–e165
- Rochat T, Lacroix JS, Jornot L (2004) N-acetylcysteine inhibits Na⁺ absorption across human nasal epithelial cells. *J Cell Physiol* 201(1):106–116. <https://doi.org/10.1002/jcp.20066>
- Romero A, Ramos E, de Los RC, Egea J, Del Pino J, Reiter RJ (2014) A review of metal-catalyzed molecular damage: protection by melatonin. *J Pineal Res* 56(4):343–370. <https://doi.org/10.1111/jpi.12132>
- Rubio S, Estévez F, Cabrera J, Reiter RJ, Loro J, Quintana J (2007) Inhibition of proliferation and induction of apoptosis by melatonin in human myeloid HL-60 cells. *J Pineal Res* 42(2): 131–138. <https://doi.org/10.1111/j.1600-079X.2006.00392.x>
- Rusu ME, Fizeşan I, Vlase L, Popa DS (2022) Antioxidants in age-related diseases and anti-aging strategies. *Antioxidants (Basel)* 11(10):1868. <https://doi.org/10.3390/antiox11101868>
- Saeidnia S, Abdollahi M (2013) Antioxidants: friends or foe in prevention or treatment of cancer: the debate of the century. *Toxicol Appl Pharmacol* 271(1):49–63. <https://doi.org/10.1016/j.taap.2013.05.004>
- Sainz RM, Lombo F, Mayo JC (2012) Radical decisions in cancer: redox control of cell growth and death. *Cancers (Basel)* 4(2):442–474. <https://doi.org/10.3390/cancers4020442>
- Sak K (2012) Chemotherapy and dietary phytochemical agents. *Chemother Res Pract* 2012:282570. <https://doi.org/10.1155/2012/282570>
- Sakanyan V (2018) Reactive chemicals and electrophilic stress in cancer: a minireview. *High Throughput* 7(2):12. <https://doi.org/10.3390/ht7020012>
- Salami A, Seydi E, Pourahmad J (2013) Use of nutraceuticals for prevention and treatment of cancer. *Iran J Pharm Res* 12(3):219–220
- Salehi Z, Roayaei M (2015) Effect of vitamin E on oxaliplatin-induced peripheral neuropathy prevention: a randomized controlled trial. *Int J Prev Med* 6:104. <https://doi.org/10.4103/2008-7802.169021>
- Sanchez-Sanchez AM, Antolin I, Puente-Moncada N et al (2015) Melatonin cytotoxicity is associated to Warburg effect inhibition in Ewing sarcoma cells. *PLoS One* 10(8):e0135420. <https://doi.org/10.1371/journal.pone.0135420>
- Santoro R, Marani M, Blandino G, Muti P, Strano S (2012) Melatonin triggers p53Ser phosphorylation and prevents DNA damage accumulation. *Oncogene* 31(24):2931–2942. <https://doi.org/10.1038/onc.2011.469>
- Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P, Bergo MO (2014) Antioxidants accelerate lung cancer progression in mice. *Sci Transl Med* 6(221):221ra15. <https://doi.org/10.1126/scitranslmed.3007653>
- Sen CK (2000) Cellular thiols and redox-regulated signal transduction. *Curr Top Cell Regul* 36: 1–30. [https://doi.org/10.1016/s0070-2137\(01\)80001-7](https://doi.org/10.1016/s0070-2137(01)80001-7)
- Sharifi-Rad M, Anil Kumar NV, Zucca P et al (2020) Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Front Physiol* 11:694. <https://doi.org/10.3389/fphys.2020.00694>
- Shattuck KE, Rassín DK, Grinnell CD (1998) N-acetylcysteine protects from glutathione depletion in rats exposed to hyperoxia. *JPEN J Parenter Enteral Nutr* 22(4):228–233. <https://doi.org/10.1177/0148607198022004228>
- Shields HJ, Traa A, Van Raamsdonk JM (2021) Beneficial and detrimental effects of reactive oxygen species on lifespan: a comprehensive review of comparative and experimental studies. *Front Cell Dev Biol* 9:628157. <https://doi.org/10.3389/fcell.2021.628157>
- Short SP, Williams CS (2017) Selenoproteins in tumorigenesis and cancer progression. *Adv Cancer Res* 136:49–83. <https://doi.org/10.1016/bs.acr.2017.08.002>

- Shuster CW (1955) Effects of oxidized fatty acids on ascites tumor metabolism. *Proc Soc Exp Biol Med* 90(2):423–426. <https://doi.org/10.3181/00379727-90-22054>
- Sies H (1997) Oxidative stress: oxidants and antioxidants. *Exp Physiol* 82(2):291–295. <https://doi.org/10.1113/expphysiol.1997.sp004024>
- Silva GÁF, Nunes RAL, Morale MG, Boccardo E, Aguayo F, Termini L (2018) Oxidative stress: therapeutic approaches for cervical cancer treatment. *Clinics (Sao Paulo)* 73(suppl 1):e548s. <https://doi.org/10.6061/clinics/2018/e548s>
- Singh RJ, Hogg N, Joseph J, Kalyanaraman B (1996) Mechanism of nitric oxide release from S-nitrosothiols. *J Biol Chem* 271(31):18596–18603. <https://doi.org/10.1074/jbc.271.31.18596>
- Singh K, Bhoori M, Kasu YA, Bhat G, Marar T (2018) Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity—exploring the armoury of obscurity. *Saudi Pharm J* 26(2):177–190. <https://doi.org/10.1016/j.jsps.2017.12.013>
- Sliwinski T, Rozej W, Morawiec-Bajda A, Morawiec Z, Reiter R, Blasiak J (2007) Protective action of melatonin against oxidative DNA damage: chemical inactivation versus base-excision repair. *Mutat Res* 634(1–2):220–227. <https://doi.org/10.1016/j.mrgentox.2007.07.013>
- Snezhkina AV, Kudryavtseva AV, Kardymon OL et al (2019) ROS generation and antioxidant defense systems in normal and malignant cells. *Oxidative Med Cell Longev* 2019:6175804. <https://doi.org/10.1155/2019/6175804>
- Sosa V, Moliné T, Somoza R, Paciucci R, Kondoh H, Lleonart ME (2013) Oxidative stress and cancer: an overview. *Ageing Res Rev* 12(1):376–390. <https://doi.org/10.1016/j.arr.2012.10.004>
- Sreekanthreddy P, Srinivasan H, Kumar DM et al (2010) Identification of potential serum biomarkers of glioblastoma: serum osteopontin levels correlate with poor prognosis. *Cancer Epidemiol Biomarkers Prev* 19(6):1409–1422. <https://doi.org/10.1158/1055-9965.EPI-09-1077>
- Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES (1998) Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol Cell* 1(7):949–957. [https://doi.org/10.1016/s1097-2765\(00\)80095-7](https://doi.org/10.1016/s1097-2765(00)80095-7)
- Stephenson CM, Levin RD, Spector T, Lis CG (2013) Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. *Cancer Chemother Pharmacol* 72(1):139–146. <https://doi.org/10.1007/s00280-013-2179-9>
- Storkey C, Pattison DI, Ignasiak MT, Schiesser CH, Davies MJ (2015) Kinetics of reaction of peroxynitrite with selenium- and sulfur-containing compounds: absolute rate constants and assessment of biological significance. *Free Radic Biol Med* 89:1049–1056. <https://doi.org/10.1016/j.freeradbiomed.2015.10.424>
- Su LJ, Zhang JH, Gomez H et al (2019) Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxidative Med Cell Longev* 2019:5080843. <https://doi.org/10.1155/2019/5080843>
- Subramanian A, Kothari L (1991) Melatonin, a suppressor of spontaneous murine mammary tumors. *J Pineal Res* 10(3):136–140. <https://doi.org/10.1111/j.1600-079x.1991.tb00830.x>
- Sur D, Gorzo A, Sabarimurugan S et al (2022) A comprehensive review of the use of antioxidants and natural products in cancer patients receiving anticancer therapy. *Anti Cancer Agents Med Chem* 22(8):1511–1522. <https://doi.org/10.2174/1871520621666210901100827>
- Sznarkowska A, Kostecka A, Meller K, Bielawski KP (2017) Inhibition of cancer antioxidant defense by natural compounds. *Oncotarget* 8(9):15996–16016. <https://doi.org/10.18632/oncotarget.13723>
- Talib WH, Saleh S (2015) Propionibacterium acnes augments antitumor, anti-angiogenesis and immunomodulatory effects of melatonin on breast cancer implanted in mice. *PLoS One* 10(4):e0124384. <https://doi.org/10.1371/journal.pone.0124384>
- Talib WH, Alsayed AR, Abuawad A, Daoud S, Mahmod AI (2021) Melatonin in cancer treatment: current knowledge and future opportunities. *Molecules* 26(9):2506. <https://doi.org/10.3390/molecules26092506>

- Tamarkin L, Cohen M, Roselle D, Reichert C, Lippman M, Chabner B (1981) Melatonin inhibition and pinealectomy enhancement of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in the rat. *Cancer Res* 41(11 Pt 1):4432–4436
- Tenório MCDS, Graciliano NG, Moura FA, Oliveira ACM, Goulart MOF (2021) N-acetylcysteine (NAC): impacts on human health. *Antioxidants* (Basel) 10(6):967. <https://doi.org/10.3390/antiox10060967>
- Torres-Cuevas I, Parra-Llorca A, Sánchez-Illana A et al (2017) Oxygen and oxidative stress in the perinatal period. *Redox Biol* 12:674–681. <https://doi.org/10.1016/j.redox.2017.03.011>
- Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov* 8(7):579–591. <https://doi.org/10.1038/nrd2803>
- Traverso N, Ricciarelli R, Nitti M et al (2013) Role of glutathione in cancer progression and chemoresistance. *Oxidative Med Cell Longev* 2013:972913. <https://doi.org/10.1155/2013/972913>
- Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160(1):1–40. <https://doi.org/10.1016/j.cbi.2005.12.009>
- Vašková J, Kočan L, Vaško L, Perjési P (2023) Glutathione-related enzymes and proteins: a review. *Molecules* 28(3):1447. <https://doi.org/10.3390/molecules28031447>
- Vollbracht C, Schneider B, Leendert V, Weiss G, Auerbach L, Beuth J (2011) Intravenous vitamin C administration improves quality of life in breast cancer patients during chemo-/radiotherapy and aftercare: results of a retrospective, multicentre, epidemiological cohort study in Germany. *In Vivo* 25(6):983–990
- Wang H, Liu X, Long M et al (2016) NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. *Sci Transl Med* 8(334):334ra51. <https://doi.org/10.1126/scitranslmed.aad6095>
- Wang L, Luo X, Li C et al (2017) Triethylenetetramine synergizes with pharmacologic ascorbic acid in hydrogen peroxide mediated selective toxicity to breast cancer cell. *Oxidative Med Cell Longev* 2017:3481710. <https://doi.org/10.1155/2017/3481710>
- Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog* 5:14. <https://doi.org/10.1186/1477-3163-5-14>
- Watson J (2013) Oxidants, antioxidants and the current incurability of metastatic cancers. *Open Biol* 3(1):120144. <https://doi.org/10.1098/rsob.120144>
- Wei H (1992) Activation of oncogenes and/or inactivation of anti-oncogenes by reactive oxygen species. *Med Hypotheses* 39(3):267–270. [https://doi.org/10.1016/0306-9877\(92\)90120-2](https://doi.org/10.1016/0306-9877(92)90120-2)
- Weitzman SA, Gordon LI (1990) Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood* 76(4):655–663
- Weydert C, Roling B, Liu J et al (2003) Suppression of the malignant phenotype in human pancreatic cancer cells by the overexpression of manganese superoxide dismutase. *Mol Cancer Ther* 2(4):361–369
- Wieland LS, Moffet I, Shade S et al (2021) Risks and benefits of antioxidant dietary supplement use during cancer treatment: protocol for a scoping review. *BMJ Open* 11(4):e047200. <https://doi.org/10.1136/bmjopen-2020-047200>
- Wojcik M, Burzynska-Pedziwiatr I, Wozniak LA (2010) A review of natural and synthetic antioxidants important for health and longevity. *Curr Med Chem* 17(28):3262–3288. <https://doi.org/10.2174/092986710792231950>
- Wong HS, Dighe PA, Mezera V, Monternier PA, Brand MD (2017) Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions. *J Biol Chem* 292(41):16804–16809. <https://doi.org/10.1074/jbc.R117.789271>
- Wongpaiboonwattana W, Tosukhowong P, Dissayabutra T, Mutirangura A, Boonla C (2013) Oxidative stress induces hypomethylation of LINE-1 and hypermethylation of the RUNX3 promoter in a bladder cancer cell line. *Asian Pac J Cancer Prev* 14(6):3773–3778. <https://doi.org/10.7314/apjcp.2013.14.6.3773>

- Worley BL, Kim YS, Mardini J et al (2019) GPx3 supports ovarian cancer progression by manipulating the extracellular redox environment. *Redox Biol* 25:101051. <https://doi.org/10.1016/j.redox.2018.11.009>
- Wu HJ, Liu C, Duan WX et al (2013) Melatonin ameliorates bisphenol A-induced DNA damage in the germ cells of adult male rats. *Mutat Res* 752(1–2):57–67. <https://doi.org/10.1016/j.mrgentox.2013.01.005>
- Xu C, Wu A, Zhu H et al (2013) Melatonin is involved in the apoptosis and necrosis of pancreatic cancer cell line SW-1990 via modulating of Bcl-2/Bax balance. *Biomed Pharmacother* 67(2): 133–139. <https://doi.org/10.1016/j.biopha.2012.10.005>
- Yan LL, Zaher HS (2019) How do cells cope with RNA damage and its consequences? *J Biol Chem* 294(41):15158–15171. <https://doi.org/10.1074/jbc.REV119.006513>
- Yeom CH, Jung GC, Song KJ (2007) Changes of terminal cancer patients' health-related quality of life after high dose vitamin C administration. *J Korean Med Sci* 22(1):7–11. <https://doi.org/10.3346/jkms.2007.22.1.7>
- Yu JH, Kim H (2014) Oxidative stress and cytokines in the pathogenesis of pancreatic cancer. *J Cancer Prev* 19(2):97–102. <https://doi.org/10.15430/JCP.2014.19.2.97>
- Zarkovic N (2020) Roles and functions of ROS and RNS in cellular physiology and pathology. *Cells* 9(3):767. <https://doi.org/10.3390/cells9030767>
- Zavala-Valencia AC, Velasco-Hidalgo L, Martínez-Avalos A, Castillejos-López M, Torres-Espíndola LM (2024) Effect of N-Acetylcysteine on cisplatin toxicity: a review of the literature. *Biologics* 18:7–19. <https://doi.org/10.2147/BTT.S438150>
- Zeida A, Guardia CM, Lichtig P et al (2014) Thiol redox biochemistry: insights from computer simulations. *Biophys Rev* 6(1):27–46. <https://doi.org/10.1007/s12551-013-0127-x>
- Zhang ZW, Abdullahi M, Farthing MJ (2002) Effect of physiological concentrations of vitamin C on gastric cancer cells and *Helicobacter pylori*. *Gut* 50(2):165–169. <https://doi.org/10.1136/gut.50.2.165>
- Zhao RZ, Jiang S, Zhang L, Yu ZB (2019) Mitochondrial electron transport chain, ROS generation and uncoupling (review). *Int J Mol Med* 44(1):3–15. <https://doi.org/10.3892/ijmm.2019.4188>
- Zhong W, Oberley LW, Oberley TD, Yan T, Domann FE, St Clair DK (1996) Inhibition of cell growth and sensitization to oxidative damage by overexpression of manganese superoxide dismutase in rat glioma cells. *Cell Growth Differ* 7(9):1175–1186
- Zhong W, Oberley LW, Oberley TD, St Clair DK (1997) Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. *Oncogene* 14(4): 481–490. <https://doi.org/10.1038/sj.onc.1200852>
- Zhou D, Shao L, Spitz DR (2014) Reactive oxygen species in normal and tumor stem cells. *Adv Cancer Res* 122:1–67. <https://doi.org/10.1016/B978-0-12-420117-0.00001-3>
- Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 94(3):909–950. <https://doi.org/10.1152/physrev.00026.2013>



Decoding Metabolic Changes in Cancer Cells Resistant to Therapy

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Abstract

Metabolic pathways play a significant role in cancer, and their disturbances have been shown to result in therapy resistance. Upregulation of glycolysis, involving key players like GLUT1, HK2, and LDH, promotes an enhanced metabolism of glucose, as well as anabolic processes within resistant cancer cells. Additionally, the resistance of cells involves how they rewire mitochondrial metabolism and engage in dynamic cycles of fission and fusion in response to therapeutic stress. Important contributions come from mitochondrial function, particularly the components of oxidative phosphorylation (OXPHOS). Glutamine dependency and serine-glycine metabolic modification were some of the mechanisms put in place that facilitate the proliferation of tumor cells even in stressful conditions. Lipid metabolism, particularly the role of fatty acid oxidation in meeting energy demands during treatment, is crucial. Additionally, lipogenesis serves as an adaptive process to counteract stress induced by treatment. The processes that contribute to metabolic reprogramming involve hypoxia-induced adaptation through hypoxia-inducible factors, oncogenic signaling networks like PI3K/AKT/mTOR, and epigenetic regulation of metabolic gene expression. In this chapter, the possibility of using metabolic biomarkers in predicting resistance to therapy is also covered, along with their application in clinical use for tailoring treatment to individual needs. It primarily emphasizes therapeutic targeting of metabolic vulnerabilities, such as glycolysis inhibitors, OXPHOS, inhibitors of

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amino acid metabolism, and lipid metabolism inhibitors. This chapter also covers the new and emerging combination therapies combining metabolic inhibitors with standard therapy and considers dietary intervention as an adjunct strategy. This chapter underlines future precision oncology through the integration of multi-omics technologies and artificial intelligence to identify metabolic resistance in cancer therapy.

Keywords

Glutamine · Hypoxia-inducible factors · Lipid metabolism inhibitors · Mitochondrial metabolism · Tumor cells

1 Introduction

In oncology, the complex association between the metabolism of cancer and its resistance to treatment has gained immense attention in the recent past. Among the newly established characteristics of cancer is the energy metabolism reprogramming in tumor cells (Hanahan 2022), and one new and significant mechanism of adaptive resistance is thought to be metabolic rewiring (Zaal and Berkers 2018). Therapy resistance is caused by a variety of factors. The majority of them have been identified as being either tied to the characteristics of cancer or linked to interactions between the tumor cells and the TME. They are frequently also engaged in the growth of tumors (Assaraf et al. 2019). For cancer to begin and spread, it is believed that cancer cells must rewire their anabolic and catabolic metabolisms to acquire energy and synthesize biomass for cell survival and development, particularly in harsh microenvironments (Pavlova and Thompson 2016).

Some tumor microenvironment (TME) characteristics, or “niches,” are frequently linked to therapy resistance because they can influence adaptive stem-like tumor cell morphologies that are more likely to contribute to long-term clinical recurrence and minimal residual illness (Boumahdi and de Sauvage 2020). Harsh conditions in the TME, particularly hypoxia, lead cancer cells to switch from oxidative phosphorylation (OXPHOS) to glycolysis, which raises proton pump expression, causes TME acidification, and selects cancer cells with high cell death resistance (Ciccarese 2022).

Malignant cells have a distinct microenvironment than healthy cells. Cancer cells must rewire pathways of metabolism to meet their energy, biosynthesis, and redox requirements to respond to hypoxia and nutritional deficiencies (Xia et al. 2021). The “Warburg effect” during carcinogenesis is currently one of the most studied reprogramming processes of cell metabolism. Even in the presence of enough oxygen, cancerous cells primarily use glycolysis to produce adenosine triphosphate (ATP), and they modify their exact metabolic mode in response to variations in the quantity supplied by outside nutrients and stressors (Lue et al. 2017; Sun et al. 2018). Gene and protein levels, as well as connections among tumor cells and host cells, can cause metabolic reprogramming that can lead to alterations like enhanced glucose

absorption in cancer. It is extensively dispersed throughout various metabolic pathways like the metabolism of amino acids, lipids, and glucose. By producing various biological macromolecules to support proliferation, tumor cells generate ATP through aerobic glycolysis, the pentose phosphate pathway (PPP), glutaminase (GLS) breakdown, one-carbon metabolism, and de novo fatty acid (FA) synthesis. (Nong et al. 2023). Metabolic reprogramming plays a complex function in promoting resistance to chemotherapy, targeted therapy, and immunotherapy (Zhao et al. 2022). The induction of glycolysis makes it possible for cancer cells to maintain ATP levels despite the presence of cytotoxic agents. Moreover, lactate accumulation can create an acidic microenvironment that protects tumor cells from apoptosis by therapeutic drugs (Pérez-Tomás and Pérez-Guillén 2020). Altered metabolic pathways will have consequences on the signal cascades that allow for survival despite targeted inhibition; in particular, upregulation of alternative pathways can circumvent blocked oncogenic signals. Metabolic alterations that tumors impose on the local microenvironment can also impede immune cell function in such an environment; excessive concentrations of lactate and other metabolites can prevent the activation and functioning of T cells, thereby impeding immunotherapeutic potency (Zheng et al. 2024). The process of reprogramming metabolism is the main feature of cancer progression that results in resistance to various anticancer treatments. So targeting the cancer metabolic vulnerability could help to combat cancer due to the overcoming resistance in cancer therapy (Galluzzi et al. 2013). Inhibitors of glycolytic and OXPHOS, and metabolism of lipids and amino acids are primary areas that can be targeted to overcome resistance to various treatments (Luengo et al. 2017).

To meet these distinctive growth demands of tumor cells, the chapter approaches metabolic processes in four directions: glucose metabolism, mitochondrial metabolism, lipid metabolism, and amino acid metabolism. Further, it provides an elaborate review of factors that are driving the reprogramming of tumor metabolism, specific conditions for changes in the three pathways, and potential therapeutic targets for the reprogramming of tumor metabolism.

2 Core Metabolic Pathways in Cancer and Their Alterations in Therapy Resistance

2.1 Glycolysis and Glucose Metabolism

Currently, glycolysis and TCA cycle, involving most mitochondrial metabolism, could not but be ineluctably associated with researching glucose metabolic reprogramming. Pyruvate proved to be an essential molecule in the process of sugar metabolism and also connected glycolysis with the TCA cycle so that conversion might be made from one process toward the other process (Tang et al. 2024). Besides glucose, cancer cells can also metabolize lactate, alanine, and glutamine to pyruvate. Using the gluconeogenic enzyme PCK, surplus oxaloacetate can be readily converted to pyruvate. In summary, some of these compounds like lactate and

pyruvate are crucial for the development and proliferation of cancer cells while also being important for rewiring glucose metabolism (Nong et al. 2023).

Glucose is transported into the cells through glucose transporters and later converted by hexokinase to form glucose 6-phosphate, which then is converted to fructose 6-phosphate (Wolf et al. 2011). After a number of complicated events, pyruvate is produced. In reduced oxygen concentration environments, some pyruvate transforms into lactate and yields scant amounts of ATP. While in normal oxygen concentration environments, the pyruvate is ferried into the mitochondria. In such conversion, more ATP molecules are produced when pyruvate is converted into acetyl coenzyme A, more commonly known as acetyl-CoA because this is the step by which PDH catalyzes the TCA cycle oxidative phosphorylation. The transformation of pyruvate into lactate in blood is further allowed by the cooperative role played by monocarboxylate transporters (MCTs) and lactate dehydrogenase (LDH) (Tu et al. 2021; Patgiri et al. 2020). There are several isoforms of LDH; some of them are lactate dehydrogenase A (LDHA), lactate dehydrogenase B (LDHB), and lactate dehydrogenase D (LDHD). However, the most prominent is LDHA, which supports the reconstitution of NAD⁺ while performing glycolysis (Tu et al. 2021). LDHA has been associated with the cause of many cancers, including pancreatic cancer, stomach cancer, bladder cancer, and endometrial cancer, through dysregulation (Mishra and Banerjee 2019). Neoplastic cells can initiate the process of gluconeogenesis and simultaneously use lactate instead of glucose to initiate more energy and help glucose uptake through GLUT with nonconventional activation. Researchers have found the key gluconeogenic enzyme as phosphoenolpyruvate carboxykinase 1 (PCK1). After PCK1 has initiated retrograde carbon flux created through gluconeogenesis due to hypoxia, it helps in creating substrates needed for the growth of tumor cells (Raimundo et al. 2011; Tang et al. 2021). Glycolysis and the TCA cycle constitute the main part of glucose metabolism. The most important new feature of cancers is aerobic glycolysis, often termed glucose metabolism reprogramming, which interfaces with the TCA cycle (Fig. 1).

2.2 Mitochondrial Function and OXPHOS

Mitochondria are important for metabolism and for cell survival. It is the “powerhouse of the cell” due to the oxidative phosphorylation (OXPHOS) reaction that occurs in the mitochondria. OXPHOS, sometimes referred to as the electron transport chain (ETC), is a protein-based system found in the membrane on the inside of the mitochondria that is arranged based on its redox capacity to transfer electrons and produce ATP. The solid-state model states that electron slippage is reduced by the respiratory complex’s close connection. Superoxide, a precursor to reactive oxygen species (ROS), is created when electrons mix with oxygen when there is minimal electron leakage (Vakifahmetoglu-Norberg et al. 2017). Within the mitochondrial matrix, one important metabolic process is the tricarboxylic acid cycle (TCA). The biosynthetic mediators for the anabolic digestion of proteins, amino acids, lipids, carbohydrates, and nucleotides are produced by this cycle (Eniafe and Jiang 2021).

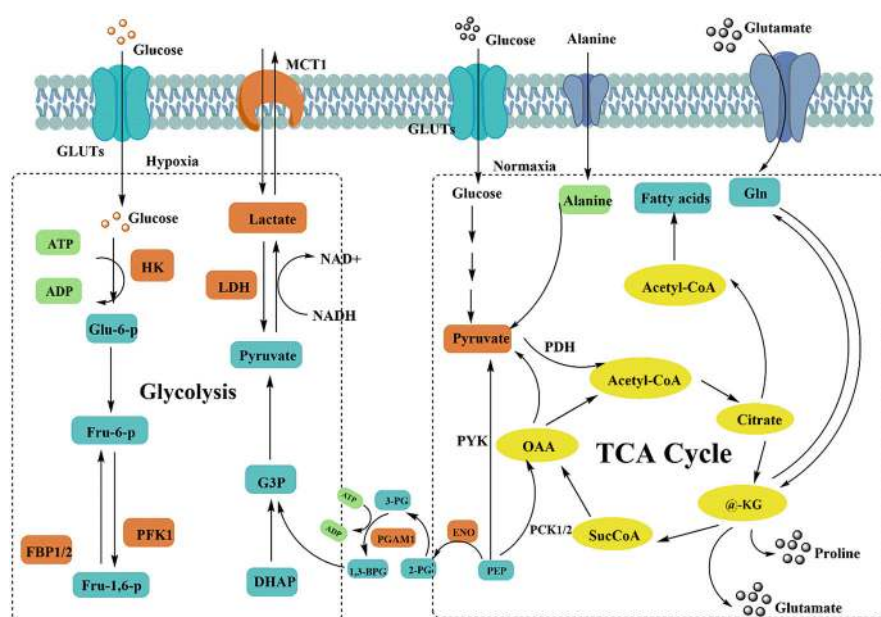


Fig. 1 Reprogramming the cycle of tricarboxylic acid (TCA) and glucose metabolism in cancer cells is shown graphically. Glycolysis and the tricarboxylic acid cycle are the two main components of glucose metabolism. The TCA cycle is shown in the right section, while the glycolysis process is shown in the left

The onset and recurrence of cancer are correlated with metabolic reprogramming caused by mutations in the TCA cycle's enzymes.

As was already mentioned, mitochondria plays a function in more than only cellular metabolism. By controlling cell death through apoptosis and cell survival through autophagy, mitochondria are also shown to play a direct role in preserving cell viability. Cytochrome c, a protein found in the mitochondrial membrane, is translocated from the mitochondria to the cytosol, where it initiates the caspase cascade, which eventually results in cell death (Wang and Youle 2009). The ability of cancer cells to evade programmed cell death is widely known, and this helps to create chemoresistance (Soengas and Lowe 2003).

Autophagy also contributes to cell survival. It is a biological mechanism necessary to preserve cellular homeostasis under stressful circumstances. Mitophagy, or mitochondrial specialized autophagy, is essential to the mitochondrial quality management system. By triggering the signaling of cell death, autophagy prevents the progression of cancer in its early stages. However, in the latter phases, it supplies the necessary metabolites for the growth of cancer cells (Yun and Lee 2018). The development of chemoresistance is facilitated by the activation of autophagy, which also stimulates signaling pathways such as p53, vascular endothelial growth factor also known as VEGF, microRNAs (miRNAs), and the epidermal growth factor receptor (EGFR) (Sui et al. 2013).

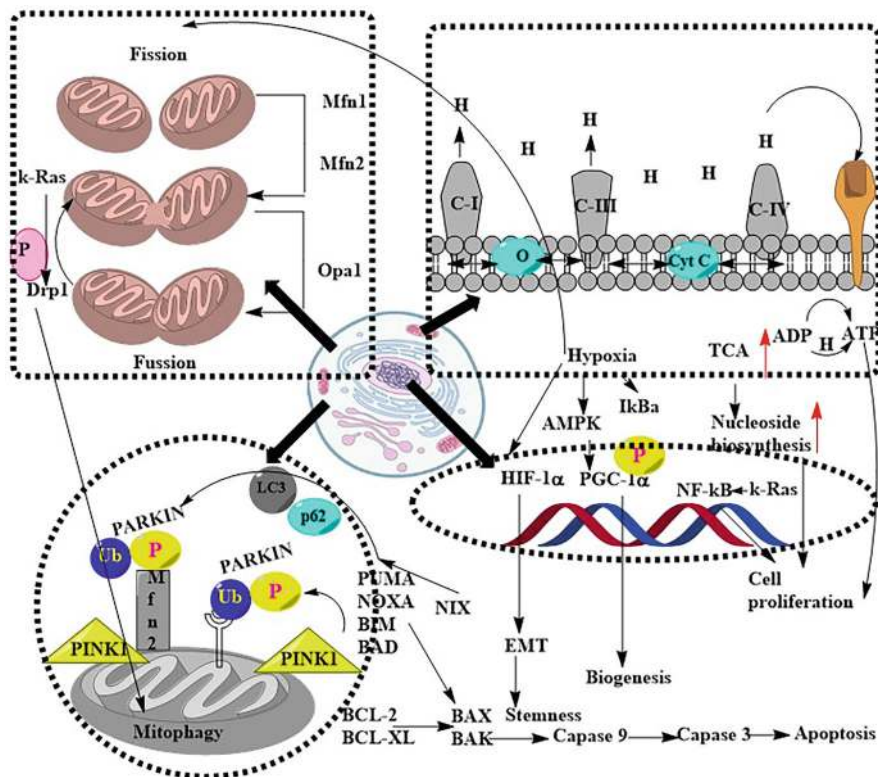


Fig. 2 An overview of the role of mitochondria in the development of cancer is presented. The production of ROS in the mitochondrial ETC initiates oncogenic signaling pathways, including k-Ras, and potentiates cellular proliferation pathways that involve AMPK and NF- κ B. The AMPK signaling pathway also promotes mitochondrial biogenesis through the phosphorylation of PGC-1 α . Hypoxia induces the expression of HIF-1 α , which activates the EMT pathway, facilitating the progression of cancer stem cells (CSCs). Moreover, HIF-1 α induces mitophagy through the proapoptotic protein Nix. In this process, the PINK-Parkin pathway recruits LC3 protein for the formation of an autophagosome. This mitophagy process eliminates the dysfunctional mitochondria from healthy cells in order to produce building blocks of cancer cells. The process of mitofission is further facilitated by k-Ras, an oncogenic protein that phosphorylates Drp1. k-Ras also promotes mitophagy. Proapoptotic as well as antiapoptotic signals interact in healthy cells. When the antiapoptotic signaling overpowers the proapoptotic signaling in the cells, then cancer cells achieve immortality. Moreover, hypoxia favors an elevated level of calcium signaling, leading to the induction of the TCA cycle. TCA cycles enhance ATP synthesis and assist in nucleoside synthesis, both of which facilitate cell growth

The mitochondria, a semiautonomous organelle, also undergo periodic turnover through mitophagy and are constantly undergoing biogenesis (Fig. 2). Fission is the process by which mitochondria are typically created from preexisting mitochondria under normal physiological conditions. But to meet their energy and metabolic needs, cancer cells accelerate the de novo production of mitochondria (Martinez-

Outschoorn et al. 2011). The biosynthesis of new mitochondria is controlled by an essential protein designated as the peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, or PGC-1 α (Xu et al. 2021). New research has shown that keeping mitochondrial fusion and fission in balance is vital for cellular viability (Mouli et al. 2009). Hyperactive fusion may cause mitochondrial entanglement that lessens its susceptibility to the autophagy process, whereas hyperactive fission has been shown to result in accumulated mitochondrial division (Maycotte et al. 2017).

The process by which mitochondria fuse and fission to determine their mass, shape, and number is known as mitochondrial dynamics. Numerous highly conserved big guanosine triphosphatases (GTPases) control mitochondrial dynamics (Giacomello et al. 2020). Ocular atrophy 1 (OPA1) regulates the inner membrane of the mitochondria (IMM) fusion, whereas mitofusins 1 and 2 (Mfn1 and Mfn2) regulate the outer membrane of the mitochondria (OMM) fusion. DRP1 (dynamin-related protein 1) regulates mitochondrial fission (Fig. 2) (Chen et al. 2023). Cancer cells' needs for bioenergetics and biosynthesis can be changed by oncogenic signals that promote hyper proliferation, raise intracellular stress, and restrict nutrition delivery. (Chen et al. 2023; Trotta and Chipuk 2017). Therefore, mitochondrial structure and function quickly adjust to these unfavorable circumstances to promote the growth of cancer cells and prevent the cell death pathway from being activated (Trotta and Chipuk 2017).

2.3 Lipid Metabolism and Fatty Acid Oxidation

Another significant metabolic characteristic of tumors that is intimately linked to the metabolism of glycolysis is the enhancement of fatty acid production (Currie et al. 2013; Menendez and Lupu 2007). Important lipid molecules found in cells and fatty acids play several biological roles in tumor cells. Tumor cells undergo lipid metabolism reprogramming, which makes them more reliant on exogenous fatty acid uptake and de novo fatty acid synthesis to sustain their fast growth and supply essential energy sources during metabolic stress.

2.4 De Novo Synthesis of Fatty Acids

Fatty acids come from two primary sources: endogenous synthesis (also known as de novo synthesis of fatty acids) and external ingestion. Fatty acids in tumor cells are typically endogenously produced de novo, and those important enzymes in the synthesis route can be controlled, whereas the majority of normal cells get FA from exogenous mechanisms. Once within the bioactive pool, FA might trigger fatty acyl-CoA synthetase to modify CoA. Lipid droplets (LDs) are where sterol esters (SEs) or triglycerides (TGs) are produced and stored. FAs can be utilized in cells for a variety of functions, including signaling molecules, oxidizing carbon dioxide as an energy source, and serving as a raw material for cell membrane activity. This suggests that FA is involved in the development and carcinogenesis

of tumors. Tumor cells produce *de novo* fatty acids through several methods. A significant amount of pyruvate is produced by the ongoing activation of glycolysis. After entering the mitochondria, this pyruvate is broken down by PDH to make acetyl-CoA. In the presence of CS, acetyl-CoA and oxaloacetate (OAA) are subsequently produced to produce citric acid (Nong et al. 2023). Some citric acids join the TCA cycle, whereas others can cross the membrane of the mitochondria and enter the cytoplasm. Acetyl-CoA and the oxaloacetic acid reactions are catalyzed by ATP-citrate lyase (ACLY). Acetyl-CoA is a necessary building ingredient for the cytoplasmic synthesis of fatty acids. ACC transforms partial acetyl-CoA into malonyl-CoA. Then, under the effect of FASN, one acetyl-CoA unit and seven malonyl-CoA molecules continuously condense to form palmitic acid. Desaturating and extending palmitic acid, a saturated fatty acid with 16 carbons, can produce molecules of fatty acids with different lengths and saturated levels (Currie et al. 2013).

Tumors can upregulate metabolic pathways that assist in the accumulation of certain lipids during tumor growth. For instance, there was an upregulation of ACC, FASN, and ACLY, among other important lipid metabolic enzymes, in malignancies. It is possible to inhibit tumor development and metastasis by blocking these enzymes involved in FA production. Inhibition of ACC results in decreased adipose production and apoptosis. The enzyme that limits the rate at which FA is synthesized, ACC, is highly expressed in cancers of the breast and prostate (Ohashi-Fukuda et al. 2015). FASN is an essential enzyme for FA anabolism, and many tumor cells exhibit noticeably elevated FASN expression levels. Citrate is converted to OAA by a cytosolic enzyme known as ACLY, which is elevated or active in a variety of tumor forms. Tumor cell proliferation can be considerably reduced by inhibiting ACLY activity. A class of membrane-binding transcription factors in the ER is called SREBP. It is crucial in the regulation of lipid metabolism (Qiu et al. 2021). The PI3K/AKT/mTOR signaling pathway plays a crucial role in lipid production. It stimulates the expression of genes that are associated with lipid synthesis through the activation of SREBP1. It further promotes *de novo* FA synthesis by upregulating FASN and ACLY (Yi et al. 2020). FA is an alternative source of energy for tumor cells, and it is broken down during Fatty acid oxidation (FAO). In glucose-deficient melanoma cells, in the study of Shen et al. (2020), it was found that inhibition resulted in ROS growth and eventual cell death (Shen et al. 2020). Additionally, it is demonstrated that it is required for melanoma cell survival, progression, as well as metastasis maintaining redox balance under stress conditions (Fig. 3).

Normal cells can *de novo* synthesize fatty acids, but the lactation cycle limits this phenomenon strictly to the liver, adipose tissues, and mammary glands. *De novo* synthesis of fatty acids is an indispensable characteristic of metabolisms that clearly distinguishes neoplastic cells from nonneoplastic ones. The elaboration of how fatty acid syntheses are controlled and its potential role in tumorigenesis should be considered while understanding thoroughly the pathogenesis of cancer and therapeutic responses (Nong et al. 2023).

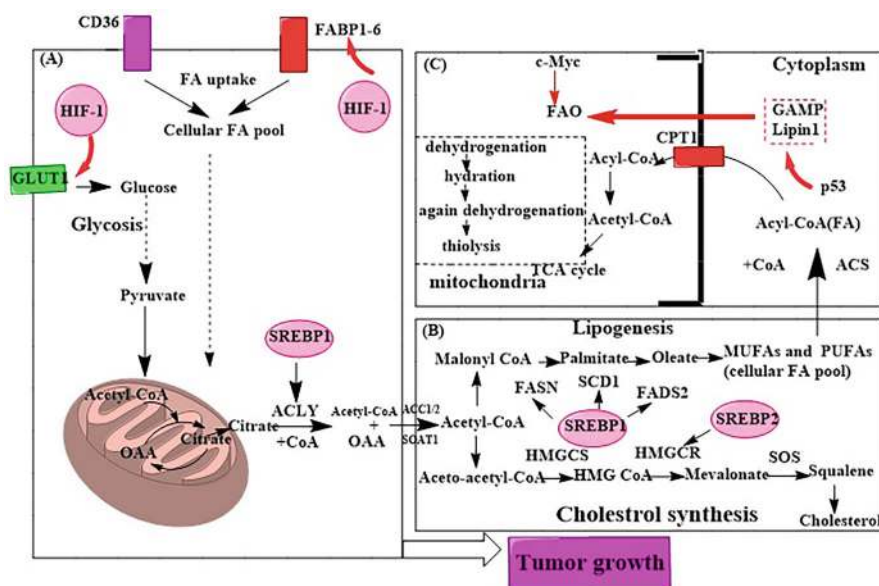


Fig. 3 Cancer involves lipid metabolic reprogramming. (a) Two primary suppliers of fatty acids (FAs) are exogenous ingestion and endogenous production. Tumor cells can absorb FA through a variety of carriers, particularly CD36 and FA binding proteins 1–6 (FABP1–6). These FAs contribute to the formation of the intracellular FA pool as well as lipid formation. For de novo synthesis, continuous glycolysis generates an enormous quantity of a compound which hits the mitochondria and is converted to acetyl-CoA via pyruvate dehydrogenase (PDH). Citrate synthase (CS) converts OAA into citric acid. The citric acid then undergoes the cycle of tricarboxylic acid, or TCA, while others pass through the membrane of the mitochondria and into the cytoplasm. Acetyl-CoA in the cytoplasm is a vital ingredient for fatty acid production. (b) SREBP activation stimulates lipogenesis and cholesterol production via regulating gene expression. Acetyl-CoA in the cytoplasm contributes to lipogenesis and cholesterol production. (c) C-MYC can promote the oxidation of fatty acids (FAO), which may activate FA and provide as an alternate energy source for tumor cells. Meanwhile, p53 can increase FA catabolism by stimulating GAMP and lipin1

3 Amino Acid Metabolism in Tumors

3.1 Glutamine Metabolism

Glutamine (Gln) is the most abundant amino acid in human plasma and has a preference for utilization by tumor cells (Zhu et al. 2022). During lipid synthesis, acetyl-coA can be produced from citrate derived from glutamine (Mullen et al. 2012). The main transporter for the efficient uptake of Gln by tumor cells is the alanine-serine-cysteine transporter 2, or SLC1A5. Its overexpression occurred in tumor cells (Bröer et al. 2018; Fuchs and Bode 2005). When transferring to cells, Gln is converted into glutamic acid by glutaminase (GLS), which then converts into α -ketoglutaric acid, and this goes into TCA and promotes the growth of

malignancies (Son et al. 2013; Zhang et al. 2017). Glutamine (Gln) metabolites, which are taken up by neoplastic cells and become part of the TCA cycle, play roles beyond merely fueling neoplastic proliferation (Altman et al. 2016). They also support the biosynthesis of UDP-GlcNAc, an essential intermediate in the HBP, which is necessary for UPR and protein glycosylation in tumor cells. Simultaneously, the Gln metabolic pathway supports the generation of GSH and NADPH, both of which are important for controlling the levels of ROS in cells, thereby maintaining redox balance and supporting the survival of tumor cells (Diehn et al. 2009). In addition, the mechanism of leucine transport supports the activation and regulation of rapamycin complex 1 (mTORC1) through glutamine (Gln) for cell proliferation (Mahmood and Emadi 2021). Gln metabolism has the interesting property that it can arrest the autophagic response in tumor cells by activating the mTOR pathway of signaling (Nicklin et al. 2009). Furthermore, where mitochondrial dysfunction prevails, Gln is used for reductive carboxylation rather than being oxidatively metabolized which makes survival of tumor cells.

Recent investigations on the expression process of related metabolizing enzymes have shown that various transcription factors, such as MYC and RAS, can precisely regulate the mechanism of Gln usage and metabolism by cancerous cells, which includes crucial enzymes like GLS1. This ensures the biology of tumor cells and the Gln metabolism process (Yang et al. 2021). In numerous studies, MYC has been demonstrated to increase the expression of Gln transporters in a variety of tumor cell types. These include the following: c-Myc promotes the production of GLS1, carbamoyl phosphate synthase 2 (CAD), and phosphopyrophosphate synthase (PRPS2), which facilitates Gln absorption, increases cell proliferation, and displays glutamine addiction. It also triggers SN2 and ASCT2 transcription (Eberhardy and Farnham 2001; Gao et al. 2009; Tambay et al. 2021); glutamate transaminase (ALT, AST) is upregulated, and glutamate dehydrogenase is inhibited to increase endocytosis due to the overactivation of the oncogene RAS in pancreatic cancer. After that, the tumor cells remove the extracellular protein and convert it into amino acids, such as Gln, which eventually give the tumor cells nourishment (Son et al. 2013); Additionally, GLS2 can be upregulated by mutant P53 to prevent tumor cells from becoming tolerant of Gln, which is advantageous for tumor cell survival (Tran et al. 2017). Lukey et al. (2019) consistently discovered that GLS2 expression may encourage the growth of breast cancer's luminal subtypes (Lukey et al. 2019), suggesting a role for GLS2 in the development of breast cancer. Surprisingly, previous research has shown that the tumor suppressor gene *GLS2* can control the growth of a variety of tumor forms. Furthermore, Suzuki et al. (2022) discovered that GLS2 may promote ferroptosis through the regulation of glutaminolysis, which aided in its tumor-suppressive properties (Suzuki et al. 2022). Regarding the contentious phenomenon, we suggest that it may be a reflection of the intricacy of GLS2's function in various cancers, which may be connected to tumor heterogeneity and need further clarification in the future.

Drugs that target certain key enzymes or intermediates in the Gln metabolism route can be employed in clinical trials to slow the disease's progression. In acute myeloid leukemia (AML), glutaminase C (GAC) is a particularly common GA

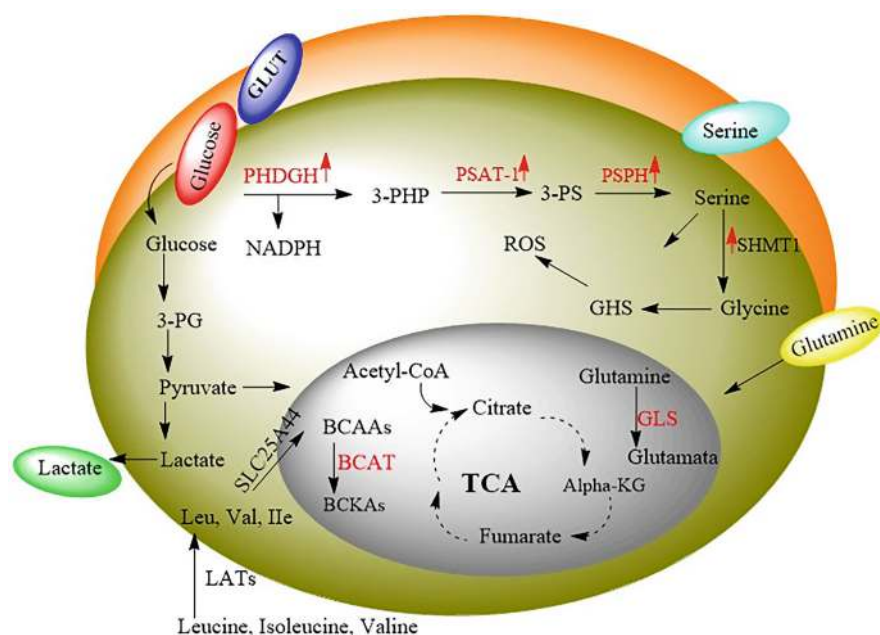


Fig. 4 Tumor cell metabolism of amino acids and how it interacts with other metabolic pathways. The tumor cells' metabolism of serine, glutamine, and branched chain. An outline of the main metabolic pathways for amino acids and the related essential compounds found in tumor cells. Important enzymes in the metabolic pathway are shown by red arrows when their expression is upregulated and indicated by a cross when it is blocked. The text provides more information

subtype (Jacque et al. 2015). Additionally, the medication azacytidine in conjunction with CB-839 can block GAC activity, which causes leukemia cells to be inhibited and to undergo apoptosis in vivo without having harmful effects on healthy CD34⁺ progenitor cells. The combined findings of the previously described studies indicate that cancers experience reprogramming of Gln metabolism, leading to changes in the expression of distinct metabolic enzyme profiles. An innovative method of treating cancers could be to target Gln metabolism (Fig. 4).

3.2 Serine and Glycine Metabolism

Both glycine (Gly) and serine (Ser) are nonessential amino acids that are involved in lipid biosynthesis, nucleotide production, and redox equilibrium. Phosphoglycerate dehydrogenase (PHGDH) and NAD(P)H catalyze the glycolysis of 3-phosphoglycerate (3-PGA) in normal cells to yield 3-hydroxypyruvate (3-HP), a precursor of serine (Qiu et al. 2023). After phosphoserine transferase (PSAT1) catalyzes the transamination process between 3-HP and Glu, α -KG and phosphoserine are produced. Phosphoserine phosphatase (PSPH) then

dephosphorylates these products to produce Ser. Gly and 5,10-methylene tetrahydrofolic acid (5,10-CH₂-THF) are produced from the newly created Ser by the cytoplasmic serine hydroxymethyltransferase 1 and 2 (SHMT1, SHMT2) (Geeraerts et al. 2021). Furthermore, Ser can participate in the production of phosphatidylserine (PS), sphingosine, cysteine (Cys), and GSH (Locasale 2013). Remarkably, c-Myc directly targets the SHMT enzyme, the primary source of one-carbon units needed for folic acid metabolism, nucleotide, and amino acid production, when cells develop in hypoxia (Nikiforov et al. 2002). Choline, betaine, dimethylglycine, and sarcosine can undergo a sequence of demethylation processes to produce Gly, or it can be transformed via threonine aldol cleavage in certain cell types (Amelio et al. 2014).

It has been noted that Ser, rather than Gly, mostly consumes the tumor cells. In addition to several metabolic alterations such as P53 activation and nucleotide synthesis inhibition, the overgrowth of tumor cells causes the uptake of Ser to surpass the requirement of protein production, frequently leading to a Ser deficit scenario (Mattaini et al. 2016). Cancerous melanoma (MM) cells and colorectal cancer (CRC) cells, for example, use a small amount of serine (Ser) for vital serine-dependent processes to sustain ongoing cell development (Maddocks et al. 2013). Regarding the mechanism of metabolic changes, it was recently found that the serine synthesis route in tumor cells differently upregulated a number of synthetases, including PHGDH (Tsun and Possemato 2015), demonstrating the alteration in metabolic programming. For instance, the PHGDH protein level is increased in almost 70% of ER-negative breast cancer cases. The serine downstream pathway, which ensures that tumor cells can continue to grow even in the absence of serine, is intimately associated with serine production and the upregulation of crucial enzymes such as PSAT1, PSPH, SHMT2, and methylenetetrahydrofolate dehydrogenase (MTHFD1) (Possemato et al. 2011); PSAT1 is also significantly upregulated in NSCLC, which promotes cell cycle progression, cell proliferation, and carcinogenesis. Its expression level is favorably connected with phosphorylated GSK-3 β , cyclin D1, and Rb (Yang et al. 2015). According to this set of research, focusing on the Ser metabolic pathway may be a viable anticancer approach. However, numerous scientific issues still need to be resolved by additional research, for example, the mechanism of changes under hypoxia, the expression variations of important enzymes of the Ser metabolic pathway in tumor cells, and so forth (Fig. 5) (Qiu et al. 2023).

4 Mechanisms Driving Metabolic Reprogramming in Resistant Cancer Cells

4.1 Hypoxia-Induced Adaptations and Therapy Resistance

In solid tumors, hypoxia—which is mainly characterized by a low oxygen level—occurs frequently. It adds to cancer cell therapy resistance and is crucial for the metabolic reprogramming of cancer cells (Jing et al. 2019). Hypoxia-inducible

of chemotherapeutic drugs, decreasing their efficacy (Ortmann 2024). HIFs also increase resistance to treatment by promoting survival signals that prevent apoptosis (Jing et al. 2019; Karakashev and Reginato 2015).

5 Hypoxia-Driven Shifts in Glycolysis, Glutamine Metabolism, and Lipid Oxidation Under Treatment Pressure

5.1 Glycolysis Under Hypoxia

Cancer cells under hypoxic conditions are mostly dependent on glycolysis for the generation of energy because their mitochondrial respiration is very low. Upregulation of glycolytic enzymes enables the cells to metabolize glucose into pyruvate fastly, which is further converted into lactate and not fed into TCA cycle. It not only produces ATP but also sustains redox balance by regenerating NAD⁺, which is required for continuous glycolytic activity (Ortmann 2024; Quenneville et al. 2024).

5.2 Glutamine Metabolism

Glutamine is used as a reserve energy source when cells are exposed to hypoxia. HIFs increase glutamine uptake and metabolism by inducing glutamine transporters and enzymes responsible for its conversion into α -ketoglutarate, thus fueling both energy and biosynthetic pathways required for cell proliferation (Kao et al. 2023; Ortmann 2024). Furthermore, glutamine metabolism contributes to alleviating oxidative stress through the availability of precursors for antioxidant synthesis.

5.3 Lipid Oxidation

Lipid oxidation is a survival strategy that cancer cells may use when placed under therapeutic stress, primarily in nutrient-poor conditions. When glucose availability is low, HIFs can stimulate the production of fatty acid transporters and enzymes that catalyze lipid metabolism, allowing cancer cells to use fatty acids as fuel. In addition to energy acquisition, this adaptation facilitates membrane biogenesis and the development of signal molecules that are essential for tumor growth (Kao et al. 2023; Karakashev and Reginato 2015).

5.4 Oncogenic Signaling Pathways

Oncogenic signaling pathways are key contributors in progression and therapy resistance in cancer. They include PI3K/AKT/mTOR signaling cascade, MYC,

RAS pathways, and interactions with enzymes that regulate metabolism and survival pathways.

5.5 PI3K/AKT/mTOR Signaling Governs Metabolic Plasticity in Drug Resistance

Typically hyperactivated in most cancers, this pathway regulates cell growth, metabolism, and survival mechanisms through the PI3K/AKT/mTOR signaling pathway, contributing to drug resistance. It causes enhancement in the survival mechanisms through the pathways, which enhances glycolysis and inhibits apoptosis by bringing about metabolic plasticity, which helps in maintaining the survival of cancer cells against drug pressure. For instance, it upregulates the multidrug resistance proteins, like P-glycoprotein, which enhances drug efflux and decreases chemotherapy efficacy (Rascio et al. 2021). More often, this results in a constitutive signaling from mutations in factors like PI3K or loss of the tumor suppressor PTEN, thus aiding tumor growth and survival following therapy (Dong et al. 2021; Glaviano et al. 2023).

5.6 MYC, RAS, and Metabolic Enzymes: Downstream Regulation Contributing to Resistance

In cancer, oncogenes such as *MYC* and *RAS* play a critical role in regulating the expression of metabolic enzymes involved in cell survival and drug resistance. *MYC* accelerates rapid cell proliferation through increased expression of genes associated with ribosome biogenesis, glycolysis, and glutaminolysis. Similarly, activation of the PI3K/AKT and MAPK pathways are among the downstream effectors activated by RAS signaling. This oncogenic signaling leads to the overproduction of metabolites, promoting the biosynthesis needed for cell division (Rascio et al. 2021). The tight web formed by these oncogenes and the metabolic enzymes facilitate the adaptability of cancer cells to the many demands made by treatments to preserve their proliferation capability (Liu et al. 2020; Skolariki et al. 2022).

5.7 Crosstalk Between Metabolic and Survival Pathways: Feedback Loops Driving Resistance

Metabolic pathways interact with survival signaling pathways that produce feed-forward loops reinforcing resistance mechanisms for medication. Such a phenomenon is observed in which the therapy activates PI3K/AKT, where the resultant increase in glycolytic metabolism inhibits apoptotic signaling pathways of the cells, leading them to survive. Metabolic intermediates can also influence the activity of oncogenic signaling pathways; for example, high levels of specific metabolites may activate additional survival signals transmitted by signaling molecule cascades

(Dong et al. 2021). Because of this complex interplay, cancer cells can adapt their metabolism to changes in their environment and treatment regimens, remaining alive even after treatment (Glaviano et al. 2023; Liu et al. 2020).

5.8 Epigenetic Regulation of Cancer Metabolism

The important mechanisms such as epigenetic modification, chromatin remodeling, and the participation of noncoding RNAs in the regulation of gene expression and metabolism involved in cancer make them resistant to environmental changes as well as therapeutic pressures.

5.9 Epigenetic Modifications (Histone Marks, DNA Methylation) That Enable Metabolic Flexibility

Histone modifications and DNA methylation are the two main epigenetic changes that regulate the expression of genes that take part in metabolic pathways. To deal with stressors such as hypoxia or lack of food, cancer cells may use histone marks both to activate and to inhibit the transcription of metabolic genes. For example, the overexpression of glycolytic genes is often associated with higher histone acetylation, which enables the Warburg effect to occur (Al Tameemi et al. 2019). DNA methylation that suppresses metabolic regulators or tumor suppressor genes can lead to a metabolic phenotype favorable for tumor development and survival in treatment settings. Such alterations make them more resilient to treatment due to their ease of jumping between metabolic pathways by cancer cells (Wang et al. 2023a).

5.10 Role of Chromatin Remodeling in Metabolic Gene Expression During Therapy Resistance

Dynamic regulation of gene expression relies mainly on chromatin remodeling in cancer cells. Chromatin shapes may sometimes be better and sometimes worse for accessibility of the transcriptional machinery for some gene loci. Therapeutic resistance could induce a particular set of chromatin remodeling mechanisms that activate survival-encoding and metabolic adaptation-factor-encoding genes. For example, certain histone variations and remodeling complexes enhance the expression of genes linked to autophagy, and cancer cells use this system to lessen stress brought on by chemotherapy. This remodeling keeps the growth alive by conserving metabolisms and eliminating apoptosis in cancer cells that manage to survive and resist treatment (Kao et al. 2023; Oh et al. 2024).

5.11 Noncoding RNAs (miRNAs, lncRNAs) Influencing Metabolic Pathways in Resistant Cancer Cells

It has been proved that ncRNAs, including lncRNAs and miRNAs, play important roles in regulating metabolic pathways within resistant cancer cells. Through post-transcriptional control, miRNAs might regulate the expression of many enzymes and signaling molecules that facilitate metabolism. For example, some miRNAs could act to suppress glucose transporters or enzymes for glycolysis, affecting metabolic adaptability. Through their interactions with transcription factors or chromatin remodeling complexes, lncRNAs can also modify metabolic gene expression patterns that promote tumor resistance and survival (Chetta and Zadra 2021). The metabolic reprogramming seen in resistant cancer morphologies is caused by the loss of such noncoding RNAs, which also makes treatment results considerably more complicated (Wang et al. 2023a) (Table 1).

6 Techniques for Identifying Metabolic Biomarkers: Metabolomics, Fluxomics, and Isotope Labeling

Finding metabolic biomarkers is important for researching cancer metabolism and treatment resistance. The three main methods that have been employed are isotope labeling, fluxomics, and metabolomics.

6.1 Metabolomics

A study of metabolites within the biological sample is a holistic study of the metabolome, giving insight into the metabolic status of cells (Bhardwaj et al. 2024). This method utilizes mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy for wide-array, accurate metabolite identification and quantification in tissues, biofluids, and cells (Grooms et al. 2024; Zhao et al. 2023). So the variations in the progression of cancer and the efficacy of treatment can be determined by the analysis of metabolomic profiles. Hence, it helps in determining all possible biomarkers for resistance to treatment (Emwas et al. 2022).

6.2 Fluxomics

A new field of study termed fluxomics came into the limelight, and fluxes through metabolism were measured within biological systems (Mishra and Kumar 2024). Fluxomics could be derived with the determination of metabolic fluxes or the rate in which the metabolite goes through different forms of conversions (Mirveis et al. 2023). This approach typically applies stable isotopic labeling to measure the kinetics of metabolites as they flow in metabolic networks in real time, a case in point being with molecules such as ^{13}C -labeled (Emwas et al. 2022). As fluxomics

Table 1 Therapy-resistant malignancies and related metabolism-interfering treatment techniques include glucose metabolism, OXPHOS dependency, amino acid metabolism, and lipid metabolism

Cancer	Biological model	Therapy resistance	Metabolic rewiring	Metabolism-interfering therapeutic modalities	References
Ovarian cancer	Cell lines such as OVCAR3 and SKOV3	Glycolysis-induced chemoresistance	Enhanced absorption of glucose	GLUT inhibitors, 2-DG	Cunha et al. (2023), Lin et al. (2019)
Lung cancer	Cell lines (H1975 and A549)	Resistance to cisplatin	Changing the metabolism of pyruvate	Inhibitors of pyruvate dehydrogenase	Lin et al. (2020), Zhao et al. (2013)
Pancreatic cancer	Cell lines as MiaPaCa-2 and Panc-1	Resistance to gemcitabine	Elevated synthesis of lactate	Agents that reprogram metabolism	Cunha et al. (2023), Lin et al. (2019)
Glioblastoma	Cell lines (U87MG and T98G)	Resistance to temozolomide	Increased glycolysis in aerobic processes	Glucose transporter inhibitors	Lin et al. (2020), Zhao et al. (2013)
Prostate cancer	Cell lines (PC-3, LNCaP)	Resistance to androgen deprivation treatment	Increased glycolysis	Treatments in combination with metabolic inhibitors	Cunha et al. (2023), Varghese et al. (2020)
Breast cancer	Cell line such as Py2T obtained From MMTV - PyMT transgenic mouse	Antiangiogenics (nintedanib)	Metabolic symbiosis based on lactate (increased expression of MCT4 and GLUT1 in hypoxic tumor regions)	Glycolysis inhibition (PFKFB3 inhibition, 3PO)-MCT4 gene deletion	Pisarsky et al. (2016)
Breast cancer	Xenografts and cell lines from human	Treatments that target PI3K/mTOR	ERR α -dependent metabolism of lactate (with glutamine metabolism)	ERR α inhibitors (XCT790 + cpd 29) Metformin (complex I inhib) and MPC inhib (UK5099)	Park et al. (2016)

Pancreatic cancer	PanNET cell lines from the mouse, RIP1Tag2 Transgenic mice,	Antiangiogenic (axitinib and sunitinib)	Lactate-based metabolic symbiosis: Differentiated expression of MCT1 in normoxic tumor cells and MCT4 and GLUT1 in hypoxic tumor cells Increased signaling of mTOR	The inhibitor of lactate absorption (CHC, 7ACC2) Alanine aminotransferase and glutaminase inhibitors (DON, AOA) Rapamycin (mTOR signaling inhibitor)	Allen et al. (2016)
Ovarian cancer	Cancer cell lines from ovarian cancer in humans, xenografts	Salts of platinum and taxane	High FAO- and glutamine-fueled OXPHOS and chronic oxidative stress	Metformin (ETC complex I inhibitor)	Gentric et al. (2019)
NSCLC	Cell lines, xenografts and tumor samples from patient	The EGFR inhibitor (erlotinib); MET inhibitor (JNJ-605, crizotinib)	Lactate-based symbiosis between CAFs (secretion of HGF) and tumor cells (increased GLUT1 and MCT4)	Genetic and pharmacological suppression of LDH (NHI-Glc-2) and MCT1 (AZD3965)	Apicella et al. (2018)
Melanoma	Xenografts, human cell lines from human, specimens of prime human melanoma	BRAF inhib such as vemurafenib and PLX4720	A higher OXPHOS gene signature—a higher number of mitos (mitobiogenesis)	Uncoupling of mitochondria (DNP and CCCP)—OXPHOS inhib (rotenone, oligomycin A, and TTFA)	Haq et al. (2013)
Head and neck squamous cell carcinoma	Xenograft, tumor Samples from patients, Human cell lines	Anti-EGFR therapy such as cetuximab	A rise in xCT expression A rise in intracellular GSH levels	xCT genetic knockdown combined with an inhibitor (sulfasalazine)	Yoshikawa et al. (2013)
Pancreatic cancer	Human cell lines from human	Gemcitabine	A higher expression of xCT—a better absorption of cystines	xCT inhibition (MSG)	Lo et al. (2008)

(continued)

Table 1 (continued)

Cancer	Biological model	Therapy resistance	Metabolic rewiring	Metabolism-interfering therapeutic modalities	References
Melanoma	Xenografts and cell lines from human	BRAF inhib. such as PLX4720 and vemurafenib	Enhanced glutaminolysis and increased mitochondrial bulk and OXPHOS	GLS inhibition (BPTES)	Baenke et al. (2016)
Breast cancer	Xenografts and cell lines from human	Endocrine therapy as tamoxifen and fulvestrant	Upregulation of SLC1A1, SNAAT2/SLC38A2, and SLC7A5 brought on by hypoxia—elevated metabolism of glutamine	SNAT2 genetic deletion plus inhibitor (MelAB)	Morotti et al. (2019)
Breast cancer	Cell lines (T-47D, MCF-7)	Resistance to doxorubicin and tamoxifen	Increased production and storage of fatty acids	Inhibitors of fatty acid synthase and substances that reduce cholesterol	Cioce et al. (2024), Wang et al. (2023b), Yang et al. (2022)
Colorectal cancer	Cell lines (SW480 and HCT116)	Resistance to 5-FU and oxaliplatin	Changed metabolism of phospholipid	LPCAT2 inhibitors and treatments that target SREBP	Hoy et al. (2021), Wang et al. (2023b), Yang et al. (2022)
Ovarian cancer	Cell lines such as OVCAR3 and SKOV3	The EMT-induced chemoresistance	Increased absorption of lipids through CD36	Lipid metabolism modulators and CD36 inhibitors	Vishwa et al. (2024), Yang et al. (2022)
Prostate cancer	Cell lines (LNCaP and PC-3)	Resistance to androgen deficiency treatment	A rise in lipogenesis	Lipid modulators and androgen receptor antagonists together	Cioce et al. (2024), Hoy et al. (2021)
Pancreatic cancer	Cell lines (MiaPaCa-2, Panc-1)	Resistance to gemcitabine	Increased oxidation of fatty acids	Combining gemcitabine with inhibitors of fatty acid oxidation	Vishwa et al. (2024), Yang et al. (2022)
Lung cancer	Cell lines such as H1975 and A549	Resistance to targeted treatments	Modified composition of the lipid raft	Inhibitors of PI3K/AKT pathways	Cioce et al. (2024), Wang et al. (2023b)

Multiple myeloma	Cell line such as RPMI-8226	Resistance to proteasome inhibitors	Enhanced production of cholesterol	Inhibitors of proteasomes combined with medicines that decrease cholesterol	Cioce et al. (2024), Yang et al. (2022)
Renal cell carcinoma	Cell lines (Caki-1,786-O)	Resistance to sunitinib	Improved metabolism of fatty acids	Focusing on mechanisms involved in fatty acid metabolism	Hoy et al. (2021), Vishwa et al. (2024)
Glioblastoma	Cell lines (T98G andU87MG)	Resistance to temozolomide	Lipid metabolism changed for survival	Temozolomide in conjunction with modulators of lipid metabolism	Cioce et al. (2024), Yang et al. (2022)

captures more functional aspects related to metabolic pathways and relationships with cell functions, it describes metabolism in a much more dynamic way than conventional metabolomics. For instance, fluxomics can demonstrate how cancer cells change their metabolism in response to treatment stress by tracking alterations in metabolic pathways. This can be inferred from the measurement of the incorporation of tagged substrates into metabolites, which may indicate a change in metabolic activity, leading to drug resistance (Emwas et al. 2022). Such a technique is increasingly important in medicine and biotechnology in assessing metabolic properties.

6.3 Isotope Labeling

Isotope labeling is a very unique approach applied to metabolomics and fluxomics such that it can be adapted for introducing stable isotopes within metabolites while tracing their further fate in biological systems (Chokkathukalam et al. 2014). The indirect assessment of metabolic fluxes through the tracing of the transformation of isotopically labeled compounds during metabolism makes the process possible. For instance, scientists add ^{13}C -labeled glucose to a cell culture system (Yang et al. 2002). This process allows them to track its conversion and identify the pathways involved, providing insights into how cell metabolism is reprogrammed during cancer treatment (Emwas et al. 2022). Isotopic labeling has helped resolve metabolic pathways and has permitted the better quantification of metabolite concentration and rate of flux more accurately in more complex biological samples. This technique proves to be extremely useful in the identification of biomarkers related to a specific metabolic state or particular therapies.

7 Key Metabolic Biomarkers in Glucose, Amino Acid, and Lipid Metabolism Linked to Resistance

7.1 Glucose Metabolism

Since resistant cancers are associated with elevated lactate, a byproduct of this metabolic switch, anaerobic glycolysis may be harnessed in these cancers to produce energy (Grasso et al. 2017). The glucose transporters, GLUT1, in the case of resistant cancer cells, are usually upregulated to enable enhanced uptake and metabolism of glucose; the same applies for glycolytic enzymes such as hexokinase II (Fadaka et al. 2017; Moldogazieva et al. 2020). These alterations enable the cancer cells to resist therapies that inhibit oxidative phosphorylation and give them the capacity to survive in low-oxygen environments.

7.2 Amino Acid Metabolism

Amino acid metabolism helps maintain the growth and survival of cancer cells during treatment. It has been established that the BCAAs and glutamine are associated with mechanisms of resistance. High levels of BCAAs have been associated with insulin resistance (Wiklund et al. 2016), and it is also associated with metabolic disturbances in cancer patients (Yoon 2016; Zhao et al. 2016). Glutamine is particularly essential as it has been identified as the TCA cycle's main carbon source and supports critical biosynthetic pathways necessary for rapid cell proliferation (Todisco et al. 2019). Drug-resistant cancer cells often have high glutaminolysis, allowing them to sustain energetic fluxes and redox equilibrium under stress conditions (Chen and Russo 2012). Moreover, changes in the metabolism of other amino acids, such as serine and glycine, may also impact lipid metabolism and be a part of a resistance mechanism (Adeva-Andany et al. 2018).

7.3 Lipid Metabolism

Lipid metabolism is gaining recognition in cancer therapy resistance. The effective elevated lipogenesis in cancer cells is further mediated through the increased expression of enzymes responsible for it, that is, FASN and SREBPs (Wu et al. 2014). This causes increased fatty acid and cholesterol synthesis for membrane biogenesis and cell survival signals. For example, in prostate cancer, androgen receptor (AR) signaling controls lipid metabolism directly (Shorning et al. 2020), and under therapeutic stress, it leads to increased lipid accumulation, providing support to tumor growth. The relationship of resistance mechanisms with lipid metabolism indicates the possibility that interventions targeting lipogenic pathways could provide new opportunities to surmount resistance (Bian et al. 2021; Santos and Schulze 2012).

7.4 Therapeutic Targeting of Metabolic Vulnerabilities in Resistant Cancer Cells

Metabolic reprogramming is characteristic of cancer cells, resulting in therapeutic resistance. The focus on targeting the metabolic vulnerability of cancer can help overcome resistance in cancer therapy. There are three prime areas, namely, inhibitors of glycolytic and OXPHOS, metabolism of lipids, and amino acids.

7.5 Targeting Key Glycolytic Enzymes

GLUT1, HK2, and others are some of the very important enzymes in this pathway. PDK1 is the pyruvate dehydrogenase kinase 1; LDHA is the lactate dehydrogenase A. The critical inhibition of such enzymes may affect the energy source supply to

tumors as such and hence treat diseases (Zhang et al. 2022). For instance, on inhibition of GLUT1, uptake of glucose may become inhibited. Further, inhibition of HK2 by their inhibitor would block the first step involved in glycolysis while leaving only a limited source of ATP for the survival of a cancerous cell (Cunha et al. 2023).

7.6 Mitochondria-Targeted Therapies

Mitochondria function is also important for the metabolism of cancer. OXPHOS inhibitors aim mitochondria at the electron transport chain level, thereby cutting off the production of ATP through oxidative phosphorylation. Furthermore, mitochondrial uncouplers can disintegrate proton gradient through the membrane of mitochondria, which further inhibits the synthesis of ATP. Combined glycolysis and mitochondrial respiration inhibition have been presented as a potential strategy over resistance because it forces tumor cells to rely on lower-efficiency energy pathways, rendering them more susceptible to the treatments (Kalyanaraman et al. 2022; Sharma and Borthakur 2023).

8 Amino Acid Metabolism as a Therapeutic Target

8.1 Glutaminase Inhibitors and Amino Acid Mimetics

Cancer cells are particularly dependent on certain amino acids, especially glutamine for survival. Glutaminase inhibitors like CB-839 selectively inhibit the enzymes that convert glutamine to glutamate, thus disrupting cancer cell metabolic flexibility (Stine et al. 2022). Amino acid mimetics can also mimic required amino acids to inhibit their uptake and utilization by cancer cells. This further cripples growth (Nguyen et al. 2022; Sharma and Borthakur 2023).

8.2 Strategies to Prevent Adaptive Resistance

To fight such mechanisms of adaptive resistance that cancer cells develop as a response to treatment, targeting amino acid dependency is a critical strategy. It would be possible through the inhibition of multiple pathways that provide essential nutrients to cancer cells or by blockage of compensatory pathways that the cancer cells would activate, thus enhancing existing therapies and preventing tumor growth (Kalyanaraman et al. 2022).

8.3 Targeting Lipid Metabolism

There exists significant involvement of lipid metabolism in the survival and growth of cancer cells. Fat oxidation inhibitors, such as the CPT1A inhibitors, inhibit the admission of fatty acids into the mitochondria for oxidation processes. This can deprive cancer cells of an auxiliary energy source, thereby easily reacting to other treatments (Mbah et al. 2024; Sharma and Borthakur 2023). Other than inhibition of FAO, a block to lipogenesis—the production of fatty acids—can be effective, by cutting off the lipid supply required for the synthesis of membrane and energy for rapidly proliferating tumor cells (Mbah et al. 2024). The most promising agent in enhancing therapy efficacy remains statins and other cholesterol-modifying agents. They interfere with lipid metabolism and might reduce cholesterol within cancer cells, thus inhibiting the growth and survival mechanisms of such cells (Mbah et al. 2024; Sharma and Borthakur 2023).

Researchers are now working on targeting these metabolic vulnerabilities by carefully combining inhibitors and tailored therapies to develop effective treatment regimens that can overcome resistance in different types of cancers.

9 Role of Diet and Metabolic Modulation in Cancer Therapy

9.1 Ketogenic Diets and Fasting-Mimicking Diets

Altered cancer metabolism can be achieved through dietary strategies, such as a ketogenic diet (KD) or fasting-mimicking diets (FMD), which have appeared to block some pathways of glucose metabolism, potentially critical for many cancer cells (Morita et al. 2021). For example, research has shown that short-term fasting leads to an anti-Warburg effect where the cancer cells switch from glycolysis to oxidative phosphorylation, thereby increasing oxidative stress and apoptosis in the tumor cells (Mercier et al. 2022). Furthermore, caloric restriction and intermittent fasting have been associated with reduced tumor growth and chemotherapy and radiotherapy effectiveness (Locasale 2022).

The modulation of nutrient availability within the tumor microenvironment is thought to be the source of these dietary interventions' effects. Through alterations in metabolic pathways, these diets can affect both tumor biology and immune function as well as the efficacy of conventional therapies (Morita et al. 2021). A good example of this concept is fasting, which has been demonstrated to increase chemotherapy sensitivities in tumors through the modification of metabolic pathways and enhancing immune functions (Locasale 2022).

9.2 Timing and Sequencing with Metabolic Inhibitors

When it comes to cancer treatments, the timing of nutritional interventions will be crucial. Patients' treatment outcomes have been reported to be optimized when

metabolic inhibitors and dietary interventions are combined. For example, a fasting regimen during chemotherapy may increase the effectiveness of the treatment without causing negative side effects (Locasale 2022; Morita et al. 2021). Synergistic effects that could improve patient responses could result from carefully combining the pharmaceutical treatment with the dietary modifications.

9.3 Potential in Therapy-Resistant Cancers

Personalized nutritional interventions are emerging as a new avenue to tackle therapy-resistant cancers. Dietary interventions based on the metabolic signatures of individuals can be tailored to reinforce therapeutic activity and counteract the mechanisms of resistance (Ilerhunmwuwa et al. 2024; Xiao et al. 2024). As the biology of cancer metabolism is better understood, there is increasingly significant interest in taking a precision nutrition strategy that aligns with particular tumor characteristics and patient needs.

10 Conclusion and Future Perspectives

Tumor cells may maintain energy supply and redox balance during treatment by activating the primary pathways of glycolysis, TCA cycle, oxidative phosphorylation, fatty acid oxidation, and amino acid metabolism which mediate metabolic reprogramming for therapy-resistant cancer cells. These modifications give the cancer cell therapeutic resistance by enabling it to take advantage of different metabolic pathways that circumvent the cytotoxic effects of chemotherapy and targeted treatments. New therapeutic strategies that interfere with these altered metabolic pathways are emerging to overcome this resistance. One such strategy is the development of particular inhibitors that target mitochondrial function or glycolytic enzymes. However, the inherent metabolic adaptability of cancer cells presents several difficulties for future research, including the requirement for reprogramming and quick adaptation to shifting conditions and treatments. Finding novel, efficient treatments and eventually making significant strides in the treatment of tumors depend heavily on clinical trials that focus on cancer metabolism. Further understanding of metabolic adaptations in cancers and individualized treatment approaches will be greatly aided by the integration of cutting-edge technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR) screens for functional genomics research, AI-driven predictive models, and the potential for application in single-cell metabolomics to perform a thorough metabolic profiling. When combined, these initiatives could pave the door for more potent therapies that are especially suited to the chemical weaknesses of the resistant cancer cell.

References

- Adeva-Andany M, Souto-Adeva G, Ameneiros-Rodríguez E, Fernández-Fernández C, Donapetry-García C, Domínguez-Montero A (2018) Insulin resistance and glycine metabolism in humans. *Amino Acids* 50:11–27
- Al Tameemi W, Dale TP, Al-Jumaily RMK, Forsyth NR (2019) Hypoxia-modified cancer cell metabolism. *Front Cell Dev Biol* 7:4
- Allen E, Miéville P, Warren CM, Saghafinia S, Li L, Peng M-W, Hanahan D (2016) Metabolic symbiosis enables adaptive resistance to anti-angiogenic therapy that is dependent on mTOR signaling. *Cell Rep* 15(6):1144–1160
- Altman BJ, Stine ZE, Dang CV (2016) From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat Rev Cancer* 16(10):619–634
- Amelio I, Cutruzzolá F, Antonov A, Agostini M, Melino G (2014) Serine and glycine metabolism in cancer. *Trends Biochem Sci* 39(4):191–198
- Apicella M, Giannoni E, Fiore S, Ferrari KJ, Fernández-Pérez D, Isella C et al (2018) Increased lactate secretion by cancer cells sustains non-cell-autonomous adaptive resistance to MET and EGFR targeted therapies. *Cell Metab* 28(6):848–865. e846
- Assaraf YG, Brozovic A, Gonçalves AC, Jurkovicova D, Linē A, Machuqueiro M et al (2019) The multi-factorial nature of clinical multidrug resistance in cancer. *Drug Resist Updat* 46:100645
- Baenke F, Chaneton B, Smith M, Van Den Broek N, Hogan K, Tang H et al (2016) Resistance to BRAF inhibitors induces glutamine dependency in melanoma cells. *Mol Oncol* 10(1):73–84
- Bhardwaj JK, Siwach A, Sachdeva SN (2024) Metabolomics and cellular altered pathways in cancer biology: a review. *J Biochem Mol Toxicol* 38(9):e23807
- Bian X, Liu R, Meng Y, Xing D, Xu D, Lu Z (2021) Lipid metabolism and cancer. *J Exp Med* 218(1)
- Boumahdi S, de Sauvage FJ (2020) The great escape: tumour cell plasticity in resistance to targeted therapy. *Nat Rev Drug Discov* 19(1):39–56
- Bröer A, Fairweather S, Bröer S (2018) Disruption of amino acid homeostasis by novel ASCT2 inhibitors involves multiple targets. *Front Pharmacol* 9:785
- Chen J-Q, Russo J (2012) Dysregulation of glucose transport, glycolysis, TCA cycle and glutaminolysis by oncogenes and tumor suppressors in cancer cells. *Biochim Biophys Acta Rev Cancer* 1826(2):370–384
- Chen W, Zhao H, Li Y (2023) Mitochondrial dynamics in health and disease: mechanisms and potential targets. *Signal Transduct Target Ther* 8(1):333
- Chetta P, Zadra G (2021) Metabolic reprogramming as an emerging mechanism of resistance to endocrine therapies in prostate cancer. *Cancer Drug Resist* 4(1):143
- Chokkathukalam A, Kim D-H, Barrett MP, Breitling R, Creek DJ (2014) Stable isotope-labeling studies in metabolomics: new insights into structure and dynamics of metabolic networks. *Bioanalysis* 6(4):511–524
- Ciccarese F (2022) Cancer metabolism and resistance to cell death: novel therapeutic perspectives. *Biomedicine* 10(8):1828
- Cioce M, Arbitrio M, Polerà N, Altomare E, Rizzuto A, De Marco C et al (2024) Reprogrammed lipid metabolism in advanced resistant cancers: an upcoming therapeutic opportunity. *Cancer Drug Resist* 7:45
- Cunha A, Silva PM, Sarmiento B, Queirós O (2023) Targeting glucose metabolism in cancer cells as an approach to overcoming drug resistance. *Pharmaceutics* 15(11):2610
- Currie E, Schulze A, Zechner R, Walther TC, Farese RV (2013) Cellular fatty acid metabolism and cancer. *Cell Metab* 18(2):153–161
- Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Wong M (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 458(7239):780–783
- Dong C, Wu J, Chen Y, Nie J, Chen C (2021) Activation of PI3K/AKT/mTOR pathway causes drug resistance in breast cancer. *Front Pharmacol* 12:628690

- Eberhardy SR, Farnham PJ (2001) c-Myc mediates activation of the cad promoter via a post-RNA polymerase II recruitment mechanism. *J Biol Chem* 276(51):48562–48571
- Emwas A-H, Szczepski K, Al-Younis I, Lachowicz JJ, Jaremko M (2022) Fluxomics-new metabolomics approaches to monitor metabolic pathways. *Front Pharmacol* 13:805782
- Eniafe J, Jiang S (2021) The functional roles of TCA cycle metabolites in cancer. *Oncogene* 40(19):3351–3363
- Fadaka A, Ajiboye B, Ojo O, Adewale O, Olayide I, Emuowhochere R (2017) Biology of glucose metabolism in cancer cells. *J Oncol Sci* 3(2):45–51
- Fuchs BC, Bode BP (2005) Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? *Semin Cancer Biol* 15:254
- Galluzzi L, Kepp O, Heiden MGV, Kroemer G (2013) Metabolic targets for cancer therapy. *Nat Rev Drug Discov* 12(11):829–846
- Gao P, Tchernyshyov I, Chang T-C, Lee Y-S, Kita K, Ochi T et al (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* 458(7239):762–765
- Geeraerts SL, Heylen E, De Keersmaecker K, Kampen KR (2021) The ins and outs of serine and glycine metabolism in cancer. *Nat Metab* 3(2):131–141
- Gentric G, Kieffer Y, Mieulet V, Goundiam O, Bonneau C, Nemati F et al (2019) PML-regulated mitochondrial metabolism enhances chemosensitivity in human ovarian cancers. *Cell Metab* 29(1):156–173
- Giacomello M, Pyakurel A, Glytsou C, Scorrano L (2020) The cell biology of mitochondrial membrane dynamics. *Nat Rev Mol Cell Biol* 21(4):204–224
- Glaviano A, Foo AS, Lam HY, Yap KC, Jacot W, Jones RH et al (2023) PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol Cancer* 22(1):138
- Grasso C, Jansen G, Giovannetti E (2017) Drug resistance in pancreatic cancer: impact of altered energy metabolism. *Crit Rev Oncol Hematol* 114:139–152
- Grooms AJ, Burris BJ, Badu-Tawiah AK (2024) Mass spectrometry for metabolomics analysis: applications in neonatal and cancer screening. *Mass Spectrom Rev* 43(4):683–712
- Hanahan D (2022) Hallmarks of cancer: new dimensions. *Cancer Discov* 12(1):31–46
- Haq R, Shoag J, Andreu-Perez P, Yokoyama S, Edelman H, Rowe GC et al (2013) Oncogenic BRAF regulates oxidative metabolism via PGC1 α and MITF. *Cancer Cell* 23(3):302–315
- He W (2024) Biophysical characterization of prolyl hydroxylation-dependent HIF-1 α degradation
- Hoy AJ, Nagarajan SR, Butler LM (2021) Tumour fatty acid metabolism in the context of therapy resistance and obesity. *Nat Rev Cancer* 21(12):753–766
- Ilerhunmwuwa NP, Abdul Khader AHS, Smith C, Cliff ERS, Booth CM, Hottel E et al (2024) Dietary interventions in cancer: a systematic review of all randomized controlled trials. *JNCI J Natl Cancer Inst* djae051:1026
- Jacque N, Ronchetti AM, Larrue C, Meunier G, Birsén R, Willems L et al (2015) Targeting glutaminolysis has antileukemic activity in acute myeloid leukemia and synergizes with BCL-2 inhibition. *Blood J Am Soc Hematol* 126(11):1346–1356
- Jing X, Yang F, Shao C, Wei K, Xie M, Shen H, Shu Y (2019) Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Mol Cancer* 18:1–15
- Kalyanaraman B, Cheng G, Hardy M (2022) Therapeutic targeting of tumor cells and tumor immune microenvironment vulnerabilities. *Front Oncol* 12:816504
- Kao T-W, Bai G-H, Wang T-L, Shih I-M, Chuang C-M, Lo C-L et al (2023) Novel cancer treatment paradigm targeting hypoxia-induced factor in conjunction with current therapies to overcome resistance. *J Exp Clin Cancer Res* 42(1):171
- Karakashev SV, Reginato MJ (2015) Progress toward overcoming hypoxia-induced resistance to solid tumor therapy. *Cancer Manag Res*:253–264
- Lin J, Xia L, Liang J, Han Y, Wang H, Oyang L et al (2019) The roles of glucose metabolic reprogramming in chemo-and radio-resistance. *J Exp Clin Cancer Res* 38(1):218
- Lin X, Xiao Z, Chen T, Liang SH, Guo H (2020) Glucose metabolism on tumor plasticity, diagnosis, and treatment. *Front Oncol* 10:317

- Liu R, Chen Y, Liu G, Li C, Song Y, Cao Z et al (2020) PI3K/AKT pathway as a key link modulates the multidrug resistance of cancers. *Cell Death Dis* 11(9):797
- Lo M, Ling V, Wang Y, Gout P (2008) The xc⁻ cystine/glutamate antiporter: a mediator of pancreatic cancer growth with a role in drug resistance. *Br J Cancer* 99(3):464–472
- Locasale JW (2013) Serine, glycine and one-carbon units: cancer metabolism in full circle. *Nat Rev Cancer* 13(8):572–583
- Locasale JW (2022) Diet and exercise in cancer metabolism. *Cancer Discov* 12(10):2249–2257
- Lue H-W, Podolak J, Kolahi K, Cheng L, Rao S, Garg D et al (2017) Metabolic reprogramming ensures cancer cell survival despite oncogenic signaling blockade. *Genes Dev* 31(20):2067–2084
- Luengo A, Gui DY, Vander Heiden MG (2017) Targeting metabolism for cancer therapy. *Cell Chem Biol* 24(9):1161–1180
- Lukey MJ, Cluntun AA, Katt WP, Miao-chong JL, Druso JE, Ramachandran S et al (2019) Liver-type glutaminase GLS2 is a druggable metabolic node in luminal-subtype breast cancer. *Cell Rep* 29(1):76–88. e77
- Maddocks OD, Berkens CR, Mason SM, Zheng L, Blyth K, Gottlieb E, Vousden KH (2013) Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. *Nature* 493(7433):542–546
- Mahmood K, Emadi A (2021) 1-C metabolism – serine, glycine, folates – in acute myeloid leukemia. *Pharmaceuticals* 14(3):190
- Martinez-Outschoorn UE, Pavlides S, Sotgia F, Lisanti MP (2011) Mitochondrial biogenesis drives tumor cell proliferation. *Am J Pathol* 178(5):1949–1952
- Mattaini KR, Sullivan MR, Vander Heiden MG (2016) The importance of serine metabolism in cancer. *J Cell Biol* 214(3):249–257
- Maycotte P, Marín-Hernández A, Goyri-Aguirre M, Anaya-Ruiz M, Reyes-Leyva J, Cortés-Hernández P (2017) Mitochondrial dynamics and cancer. *Tumour Biol* 39(5):1010428317698391
- Mbah NE, Myers AL, Sajjakulnukit P, Chung C, Thompson JK, Hong HS et al (2024) Therapeutic targeting of differentiation-state dependent metabolic vulnerabilities in diffuse midline glioma. *Nat Commun* 15(1):8983
- Menendez JA, Lupu R (2007) Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer* 7(10):763–777
- Mercier BD, Tizpa E, Philip EJ, Feng Q, Huang Z, Thomas RM et al (2022) Dietary interventions in cancer treatment and response: a comprehensive review. *Cancers* 14(20):5149
- Mirveis Z, Howe O, Cahill P, Patil N, Byrne HJ (2023) Monitoring and modelling the glutamine metabolic pathway: a review and future perspectives. *Metabolomics* 19(8):67
- Mishra D, Banerjee D (2019) Lactate dehydrogenases as metabolic links between tumor and stroma in the tumor microenvironment. *Cancers* 11(6):750
- Mishra MK, Kumar A (2024) Fluxomics and metabolic flux analysis. In: *Multi-omics analysis of the human microbiome: from technology to clinical applications*. Springer, pp 171–180
- Moldogazieva NT, Mokhosoev IM, Terentiev AA (2020) Metabolic heterogeneity of cancer cells: an interplay between HIF-1, GLUTs, and AMPK. *Cancers* 12(4):862
- Morita M, Kudo K, Shima H, Tanuma N (2021) Dietary intervention as a therapeutic for cancer. *Cancer Sci* 112(2):498–504
- Morotti M, Bridges E, Valli A, Choudhry H, Sheldon H, Wigfield S et al (2019) Hypoxia-induced switch in SNAT2/SLC38A2 regulation generates endocrine resistance in breast cancer. *Proc Natl Acad Sci* 116(25):12452–12461
- Mouli PK, Twig G, Shrihai OS (2009) Frequency and selectivity of mitochondrial fusion are key to its quality maintenance function. *Biophys J* 96(9):3509–3518
- Mullen AR, Wheaton WW, Jin ES, Chen P-H, Sullivan LB, Cheng T et al (2012) Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature* 481(7381):385–388

- Nguyen T-TT, Katt WP, Cerione RA (2022) Alone and together: current approaches to targeting glutaminase enzymes as part of anti-cancer therapies. *Future Drug Dis* 4(4):FDD79
- Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B et al (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* 136(3):521–534
- Nikiforov MA, Chandriani S, O’Connell B, Petrenko O, Kotenko I, Beavis A et al (2002) A functional screen for Myc-responsive genes reveals serine hydroxymethyltransferase, a major source of the one-carbon unit for cell metabolism. *Mol Cell Biol* 22(16):5793–5800
- Nong S, Han X, Xiang Y, Qian Y, Wei Y, Zhang T et al (2023) Metabolic reprogramming in cancer: mechanisms and therapeutics. *MedComm* 4(2):e218
- Oh SY, Kim J, Lee KY, Lee H-J, Kwon T-G, Kim J-W et al (2024) Chromatin remodeling-driven autophagy activation induces cisplatin resistance in oral squamous cell carcinoma. *Cell Death Dis* 15(8):589
- Ohashi-Fukuda N, Inokuchi R, Sato H, Nakamura K, Iwagami M, Wada T et al (2015) Poorer prognosis with ethylenediaminetetraacetic acid-dependent Pseudothrombocytopenia: a single-center case–control study. *Medicine* 94(15):e674
- Ortmann BM (2024) Hypoxia-inducible factor in cancer: from pathway regulation to therapeutic opportunity. *BMJ Oncol* 3(1)
- Park S, Chang C-Y, Safi R, Liu X, Baldi R, Jasper JS et al (2016) $ERR\alpha$ -regulated lactate metabolism contributes to resistance to targeted therapies in breast cancer. *Cell Rep* 15(2):323–335
- Patgiri A, Skinner OS, Miyazaki Y, Schleifer G, Marutani E, Shah H et al (2020) An engineered enzyme that targets circulating lactate to alleviate intracellular NADH: NAD⁺ imbalance. *Nat Biotechnol* 38(3):309–313
- Pavlova NN, Thompson CB (2016) The emerging hallmarks of cancer metabolism. *Cell Metab* 23(1):27–47
- Pérez-Tomás R, Pérez-Guillén I (2020) Lactate in the tumor microenvironment: an essential molecule in cancer progression and treatment. *Cancers* 12(11):3244
- Pisarsky L, Bill R, Fagiani E, Dimeloe S, Goosen RW, Hagmann J et al (2016) Targeting metabolic symbiosis to overcome resistance to anti-angiogenic therapy. *Cell Rep* 15(6):1161–1174
- Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K et al (2011) Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 476(7360):346–350
- Qiu Z, Deng W, Hong Y, Zhao L, Li M, Guan Y et al (2021) Biological behavior and lipid metabolism of colon cancer cells are regulated by a combination of sterol regulatory element-binding protein 1 and ATP citrate lyase. *Onco Targets Ther* Volume 14:1531–1542
- Qiu H, Shao N, Liu J, Zhao J, Chen C, Li Q et al (2023) Amino acid metabolism in tumor: new shine in the fog? *Clin Nutr* 42(8):1521–1530
- Quenneville J, Feghaly A, Tual M, Thomas K, Major F, Gagnon E (2024) Long-term severe hypoxia adaptation induces non-canonical EMT and a novel Wilms Tumor 1 (WT1) isoform. *Cancer Gene Ther* 31(8):1237–1250
- Raimundo N, Baysal BE, Shadel GS (2011) Revisiting the TCA cycle: signaling to tumor formation. *Trends Mol Med* 17(11):641–649
- Rascio F, Spadaccino F, Rocchetti MT, Castellano G, Stallone G, Netti GS, Ranieri E (2021) The pathogenic role of PI3K/AKT pathway in cancer onset and drug resistance: an updated review. *Cancers* 13(16):3949
- Santos CR, Schulze A (2012) Lipid metabolism in cancer. *FEBS J* 279(15):2610–2623
- Sharma P, Borthakur G (2023) Targeting metabolic vulnerabilities to overcome resistance to therapy in acute myeloid leukemia. *Cancer Drug Resist* 6(3):567
- Shen S, Faouzi S, Souquere S, Roy S, Routier E, Libenciuc C et al (2020) Melanoma persister cells are tolerant to BRAF/MEK inhibitors via ACOX1-mediated fatty acid oxidation. *Cell Rep* 33(8):108421
- Shorning BY, Dass MS, Smalley MJ, Pearson HB (2020) The PI3K-AKT-mTOR pathway and prostate cancer: at the crossroads of AR, MAPK, and WNT signaling. *Int J Mol Sci* 21(12):4507

- Skolariki A, D'Costa J, Little M, Lord S (2022) Role of PI3K/Akt/mTOR pathway in mediating endocrine resistance: concept to clinic. *Explor Target Anti-tumor Ther* 3(2):172
- Soengas MS, Lowe SW (2003) Apoptosis and melanoma chemoresistance. *Oncogene* 22(20):3138–3151
- Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M et al (2013) Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 496(7443):101–105
- Stine ZE, Schug ZT, Salvino JM, Dang CV (2022) Targeting cancer metabolism in the era of precision oncology. *Nat Rev Drug Discov* 21(2):141–162
- Sui X, Chen R, Wang Z, Huang Z, Kong N, Zhang M et al (2013) Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. *Cell Death Dis* 4(10):e838–e838
- Sun L, Suo C, Li S-T, Zhang H, Gao P (2018) Metabolic reprogramming for cancer cells and their microenvironment: beyond the Warburg effect. *Biochim Biophys Acta Rev Cancer* 1870(1):51–66
- Suzuki S, Venkatesh D, Kanda H, Nakayama A, Hosokawa H, Lee E et al (2022) GLS2 is a tumor suppressor and a regulator of ferroptosis in hepatocellular carcinoma. *Cancer Res* 82(18):3209–3222
- Tambay V, Raymond V-A, Bilodeau M (2021) MYC rules: leading glutamine metabolism toward a distinct cancer cell phenotype. *Cancers* 13(17):4484
- Tang K, Zhu L, Chen J, Wang D, Zeng L, Chen C et al (2021) Hypoxia promotes breast cancer cell growth by activating a glycogen metabolic program. *Cancer Res* 81(19):4949–4963
- Tang Q, Wu S, Zhao B, Li Z, Zhou Q, Yu Y et al (2024) Reprogramming of glucose metabolism: the hallmark of malignant transformation and target for advanced diagnostics and treatments. *Biomed Pharmacother* 178:117257
- Todisco S, Convertini P, Iacobazzi V, Infantino V (2019) TCA cycle rewiring as emerging metabolic signature of hepatocellular carcinoma. *Cancers* 12(1):68
- Tran TQ, Lowman XH, Reid MA, Mendez-Dorantes C, Pan M, Yang Y, Kong M (2017) Tumor-associated mutant p53 promotes cancer cell survival upon glutamine deprivation through p21 induction. *Oncogene* 36(14):1991–2001
- Trotta AP, Chipuk JE (2017) Mitochondrial dynamics as regulators of cancer biology. *Cell Mol Life Sci* 74:1999–2017
- Tsun Z-Y, Possemato R (2015) Amino acid management in cancer. In: *Seminars in cell & developmental biology*, vol 43. Academic Press, p 22
- Tu VY, Ayari A, O'Connor RS (2021) Beyond the lactate paradox: how lactate and acidity impact T cell therapies against cancer. *Antibodies* 10(3):25
- Vakifahmetoglu-Norberg H, Ouchida AT, Norberg E (2017) The role of mitochondria in metabolism and cell death. *Biochem Biophys Res Commun* 482(3):426–431
- Varghese E, Samuel SM, Lišková A, Samec M, Kubatka P, Büsselberg D (2020) Targeting glucose metabolism to overcome resistance to anticancer chemotherapy in breast cancer. *Cancers* 12(8):2252
- Vishwa R, BharathwajChetty B, Girisa S, Aswani BS, Alqahtani MS, Abbas M et al (2024) Lipid metabolism and its implications in tumor cell plasticity and drug resistance: what we learned thus far? *Cancer Metastasis Rev* 43(1):293–319
- Wang C, Youle RJ (2009) The role of mitochondria in apoptosis. *Annu Rev Genet* 43(1):95–118
- Wang N, Ma T, Yu B (2023a) Targeting epigenetic regulators to overcome drug resistance in cancers. *Signal Transduct Target Ther* 8(1):69
- Wang Z, Wang Y, Li Z, Xue W, Hu S, Kong X (2023b) Lipid metabolism as a target for cancer drug resistance: progress and prospects. *Front Pharmacol* 14:1274335
- Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y et al (2016) Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. *Sci Rep* 6(1):24540
- Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, Cairns R et al (2011) Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. *J Exp Med* 208(2):313–326

- Wu X, Daniels G, Lee P, Monaco ME (2014) Lipid metabolism in prostate cancer. *Am J Clin Exp Urol* 2(2):111
- Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N et al (2021) The cancer metabolic reprogramming and immune response. *Mol Cancer* 20:1–21
- Xiao Y-L, Gong Y, Qi Y-J, Shao Z-M, Jiang Y-Z (2024) Effects of dietary intervention on human diseases: molecular mechanisms and therapeutic potential. *Signal Transduct Target Ther* 9(1):59
- Xu R, Luo X, Ye X, Li H, Liu H, Du Q, Zhai Q (2021) SIRT1/PGC-1 α /PPAR- γ correlate with hypoxia-induced chemoresistance in non-small cell lung cancer. *Front Oncol* 11:682762
- Yang C, Hua Q, Shimizu K (2002) Metabolic flux analysis in *Synechocystis* using isotope distribution from ¹³C-labeled glucose. *Metab Eng* 4(3):202–216
- Yang Y, Wu J, Cai J, He Z, Yuan J, Zhu X et al (2015) PSAT1 regulates cyclin D1 degradation and sustains proliferation of non-small cell lung cancer cells. *Int J Cancer* 136(4):E39–E50
- Yang W-H, Qiu Y, Stamatatos O, Janowitz T, Lukey MJ (2021) Enhancing the efficacy of glutamine metabolism inhibitors in cancer therapy. *Trends Cancer* 7(8):790–804
- Yang R, Yi M, Xiang B (2022) Novel insights on lipid metabolism alterations in drug resistance in cancer. *Front Cell Dev Biol* 10:875318
- Yi J, Zhu J, Wu J, Thompson CB, Jiang X (2020) Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. *Proc Natl Acad Sci* 117(49):31189–31197
- Yoon M-S (2016) The emerging role of branched-chain amino acids in insulin resistance and metabolism. *Nutrients* 8(7):405
- Yoshikawa M, Tsuchihashi K, Ishimoto T, Yae T, Motohara T, Sugihara E et al (2013) xCT inhibition depletes CD44v-expressing tumor cells that are resistant to EGFR-targeted therapy in head and neck squamous cell carcinoma. *Cancer Res* 73(6):1855–1866
- Yun CW, Lee SH (2018) The roles of autophagy in cancer. *Int J Mol Sci* 19(11):3466
- Zaal EA, Berkers CR (2018) The influence of metabolism on drug response in cancer. *Front Oncol* 8:500
- Zhang J, Pavlova NN, Thompson CB (2017) Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. *EMBO J* 36(10):1302–1315
- Zhang Y, Li Q, Huang Z, Li B, Nice EC, Huang C et al (2022) Targeting glucose metabolism enzymes in cancer treatment: current and emerging strategies. *Cancers* 14(19):4568
- Zhao Y, Butler EB, Tan M (2013) Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis* 4(3):e532–e532
- Zhao X, Han Q, Liu Y, Sun C, Gang X, Wang G (2016) The relationship between branched-chain amino acid related metabolomic signature and insulin resistance: a systematic review. *J Diabetes Res* 2016(1):2794591
- Zhao L, Liu Y, Zhang S, Wei L, Cheng H, Wang J, Wang J (2022) Impacts and mechanisms of metabolic reprogramming of tumor microenvironment for immunotherapy in gastric cancer. *Cell Death Dis* 13(4):378
- Zhao Y, Sepehr E, Vaught C, Yourick J, Sprando RL (2023) Cellular metabolomics: from sample preparation to high-throughput data analysis. *J Agric Food Res* 15:100935
- Zheng Y, Xu R, Chen X, Lu Y, Zheng J, Lin Y et al (2024) Metabolic gatekeepers: harnessing tumor-derived metabolites to optimize T cell-based immunotherapy efficacy in the tumor microenvironment. *Cell Death Dis* 15(10):775
- Zhu L, Zhu X, Wu Y (2022) Effects of glucose metabolism, lipid metabolism, and glutamine metabolism on tumor microenvironment and clinical implications. *Biomol Ther* 12(4):580

A Glance at the Effects of Secondary Metabolites in Fighting Cancer

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Abstract

Cancer is one of the top causes of morbidity and mortality globally. Cancer is a disease of unregulated cell growth that invades and spreads to other cells and tissues. Chemotherapy of cancer has many challenges due to the low selectivity, poor water solubility, instability of molecules, and development of resistance

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against them. Plant-derived natural products exhibit numerous biological activities with unique mechanisms of action, making them attractive for drug development for the management of chronic human diseases, including cancer. Natural products are considered potential cancer therapies either alone or in combination with conventional anticancer medications. Most natural products exhibit own pleiotropic effects when affecting cancer cells and modulating several signal transduction and modulators, in addition to their unique structures. The goal of our study was to elucidate the mechanisms of action of several secondary metabolites' classes on different aspects of the carcinogenesis process. We reviewed the pharmacological actions and molecular targets of the secondary metabolites. Furthermore, we emphasized the influence of secondary metabolites and conventional anticancer drug combinations on several cancer types to understand the mechanisms and advantages underlying these combinations.

Plant-derived natural products fight cancer through different strategies, including apoptosis induction strategy through DNA damage and caspase activation. The antiproliferative strategy inhibits the mitogen-activated protein kinase (MAPK) pathway, immunomodulation, and anti-inflammatory activities. The other strategies target altered metabolism and tumor microenvironment, as well as inhibition of angiogenesis and metastasis. Recently, a novel practice, so-called combinatorial therapy, has been investigated by engaging plant-derived natural substances with conventional chemotherapeutics. It has been reported that plant metabolites may fortify the drug's effective concentration, pile on the collective effect of both administered therapeutics, or increase the selectivity to tumor cells. Moreover, by steering numerous signaling pathways, combinatorial therapy uses several mechanisms to diminish the development of resistance to antitumor drugs. The interaction between plant-derived natural compounds and conventional chemotherapeutics initiates a new aspect in the research and therapy of cancer. It could be a gifted approach to treat cancer and gain fruitful improvements while diminishing adverse effects associated with conventional anticancer medications. The most studied phytochemicals include curcumin, epigallocatechin gallate, and resveratrol. Among the most investigated chemotherapeutics were 5-fluorouracil, doxorubicin, and cisplatin.

A better understanding of the multifaceted mechanisms of action of phytochemical metabolites is anticipated to open new approaches in the coming years for managing all types of cancers. In the present chapter, we report on our efforts to determine the major strategies used to treat cancer by phytochemicals in cancer cells.

Keywords

Chemotherapy · Metabolites · Natural products · Tumor microenvironment

1 Introduction

Cancer is a chief cause of death universally. The number of patients is anticipated to increase to 19.3 million annually by 2025 (Block et al. 2015). Cancer is a multistage disease divided into initiation, proliferation, angiogenesis, and progression (Koh et al. 2020). Investigating molecular mechanisms of cancer development and progression has become a core of cancer research. It can provide a scientific basis for developing cancer prevention strategies and treatment modalities (Ali Syeda et al. 2020).

Normal cells can be gradually developed into neoplastic cells by assimilating multi-processes of tumorigenesis, becoming malignant due to genetic alteration, which, in turn, leads to the initiation of cancer. The major classes of genes that play a key role in initiation are suppressor genes, proto-oncogenes, tumors, and those involved in DNA repair mechanisms. The mutated cells become more vulnerable to promoters that ease cell survival and increase the number of mutated cells (Koh et al. 2020). Furthermore, mutations activate the expression of oncogenes that hasten cell proliferation, deactivate tumor suppressor genes hindering cell death, and alter the genes that engage in DNA repair (Koh et al. 2020). The promotion stage is a reversible phase that relies on nonmutagenic agents known for their capacity to reduce the cells' latency period and enhance the formation of tumors in that tissue (Weiss 2004; Koh et al. 2020).

The progression of the tumor happened after multiple rounds of hyperplasia, clonal selection, and rapid, uncontrollable cell division, which led to an increase in the size of tumor cells. Meanwhile, the tumor gets distant from blood vessels, triggering hypoxia and less access to nutrients. Therefore, in order to promote the creation of blood vessels, angiogenesis factors are required from the tumor itself or surrounding cells to stimulate blood vessel formation (Koh et al. 2020).

The other hallmark of cancer is the reprogramming of cellular metabolic processes and the microenvironment of tumor cells (Ohshima and Morii 2021). Cancer cells work by dysregulated metabolism through increasing glucose uptake. Steering uncontrolled metabolic processes and related signaling pathways is suggested as a critical goal and new strategy in cancer therapy. Further research is required to comprehend and target additional characteristics of cancer cells, such as their capacity to maintain proliferative signaling, resist cell death, evade growth suppressors, enable replicative immortality, induce/access vasculature, activate invasion and metastasis, and avoid immune destruction. These factors are further complicated by the complex pathophysiology of cancer metabolism and the connections between cancer metabolic pathways and signaling pathways (Schiliro and Firestein 2021).

Cancer is treated using different modalities, including conventional anticancer drugs, surgical resection, and radiotherapy (Kikuchi et al. 2019). Natural chemicals are promising multitargeted agents, targeting several receptors, altering signaling pathways in cancer metabolism, and posing minimal side effects. These outcomes have made secondary metabolites promising compounds for treating and preventing cancer. Plant secondary metabolites modulate the metabolism of carbohydrates,

proteins, and lipids, as well as epigenetic changes in cancer. Also, these natural products can target multiple dysregulated signaling pathways and numerous mediators involved in cell overproliferation, and they can hit dysregulated metabolism to hinder carcinogenesis. Cancer cells have developed resistance to traditional anticancer treatments. As a result, discovering effective anticancer agents are instantly needed (Cree and Charlton 2017; Wang et al. 2019; Emran et al. 2022). Several factors participate in the development of resistance against anticancer drugs, including genetic mutations, epigenetic alterations, upregulated drug efflux, and various others (Bukowski et al. 2020; Lei et al. 2023; Sarkar et al. 2023). Other drawbacks of chemotherapeutic agents are the poor water solubility and the instability of molecules, making anticancers unable to permeate through cells (Ioеле et al. 2022). These shortcomings of conventional anticancer medications are the driving force for the development of alternative treatments that offer fewer adverse side effects and improve therapeutic efficacy (Ioеле et al. 2022).

A new, widely accepted, researched approach for treating cancer combines conventional anticancer-phytochemical compounds. The modality aims to improve clinical efficacy, improve selectivity, and overcome cancer cell resistance (Kikuchi et al. 2019).

The goal of our study was to elucidate the mechanisms of action of several secondary metabolites' classes on different aspects of the carcinogenesis process. We reviewed the pharmacological actions and molecular targets of the secondary metabolites. Furthermore, we emphasized the influence of secondary metabolites and conventional anticancer drug combinations on several cancer types to understand the mechanisms and advantages underlying these combinations. This chapter introduces several phytochemicals derived from plants that have been studied for their effect *in vitro* and *in vivo* models.

2 Literature Search Methodology

A search of several electronic databases such as PubMed, Scopus, and EBSCO was executed. A separate Google Scholar search was conducted to locate supplementary articles. This chapter only included articles published in English language. The search keywords were “alkaloids and anticancer activity,” “phenolics and anticancer activity,” and “terpenes and anticancer activity” in the title and abstract. The included articles were checked for the mechanisms of anticancer activity for a specific class of the secondary metabolite.

On the other hand, only articles that followed the IMRAD structure that included an introduction, methods, results, and discussion were included for the part related to combinatorial treatment. The search was narrowed only to include academic articles published from January 2010 to October 2023. Moreover, the studies included in this chapter must have explicitly stated that the plant metabolite and conventional anticancer treatment were combined. The studies chosen were *in vitro* or animal *in vivo* studies investigating the effect of conventional anticancer treatment combined with a plant metabolite. In total, 200 articles were retrieved, among which

51 were left out since they determined the activity of combination therapy on various types of cancers.

3 Results

3.1 Strategies of Cytotoxicity of Plant Secondary Metabolites

Secondary metabolites with potent anticancer properties are widely available in various plant tissues. Eighty percent of the population worldwide use natural compounds contained in medicinal plants and largely depend on them. Naturally occurring compounds target tumor cells by affecting several aspects of the carcinogenesis process (Aung et al. 2017).

3.1.1 Antiproliferative Strategy

Cellular division and DNA replication are major processes that rely on the orchestration of different flows of protein phosphorylation and on various guardians and checkpoints, which lead to these two critical events and eventually proceed to cell cycle completion (Thawabteh et al. 2019; Ponte et al. 2021). Cell cycle dysregulation is one of the ten hallmarks of cancer transformation. Targeting cell cycle key modulators, which include cyclins, cyclin-dependent kinases (CDKs), and tumor suppressor proteins (p53 and Rb), is vital for inducing cell cycle arrest and ultimately causing cancer cell death (Hazafa et al. 2020). Several new alkaloids can impact cell cycle checkpoints, leading to cell cycle arrest (Dey et al. 2019).

Some flavonoids induce G2/M cell cycle arrest by upregulating cyclin-dependent kinases Inhibitor 1 (p21), a protein activated by p53. Lower p21 expression was observed in HeLa cells (cervical cancer), human colon cancer cells (HCT116) treated with genistein, breast cancer cells (MDA-MB-231) treated with luteolin, and colorectal cancer cells (HCT116) treated with taxifolin (Ponte et al. 2021).

Furthermore, terpenes can induce cell cycle arrest in the G1 phase by lessening the expression of cyclin D1 and reducing HIF-1 protein synthesis via mTOR/4E-BP1 signaling pathways and by increasing the expression of p53 and p21 (Ma et al. 2016). Terpenes induce autophagy, which helps control tumor malignancy and metastasis (Martins et al. 2019).

The MAPK pathway performs an integral task in normal cell division and proliferation. It links extracellular signals to cellular growth, differentiation, proliferation, migration, and apoptosis (Sevastre et al. 2022). Several proteins, namely Ras protein, B-Raf protein, and ERK 1 and 2 proteins, and kinases, namely MEK 1 and 2, activate several transcription factors to trigger the expression of genes encrypting growth factors, proteins involved in cell proliferation, in addition to cyclins and cytokines (Sevastre et al. 2022). In cancer cells, the MAPK pathway is dysregulated, leading to uncontrolled cellular division. Numerous secondary metabolite classes can modify and complement these disruptions. For example, flavonoids act as potent inhibitors of the mitogen-activated protein kinase (MAPK) pathway, which includes strategic components such as c-Jun N-terminal kinases (JNKs), p38s, and

extracellular signal-regulated kinases (ERKs). Their ability to modulate this pathway reduces the expression of proinflammatory genes and has promising implications in combating chronic inflammation and potentially inhibiting cancer development (Zhao et al. 2018a, b).

Apoptosis, or programmed cell death, is an integral part of the normal cell cycle and plays an essential role in the advancement and the existence of multicellular organisms. Apoptosis is an ordered morphological and biochemical multisteps of cell collapsing that precedes cell death. It includes DNA breakage, cell contraction, nuclear condensation, membrane protrusions, and modulation of precise signaling circuitry (Koff et al. 2015). Hence, apoptosis represents a natural barrier to cancer development. Any irreversible diversions in the regulators and effector elements of the apoptotic system lead to tumorigenesis. In fact, escaping apoptosis and resisting cell death are among the ten hallmarks of cancer that direct the process of cell immortalization. Nevertheless, any molecule that can induce and activate the apoptotic signaling proteins can be considered a promising remedy for cancer treatment (Habli et al. 2017). However, any compound capable of initiating and triggering the apoptotic signaling proteins may be deemed a promising cancer treatment (Pfeffer and Singh 2018).

Caspases are chief controllers in the programmed cell death pathways (Kopustinskiene et al. 2020). Both intrinsic and extrinsic pathways can activate the caspase pathway (Ponte et al. 2021). They combine and mix apoptotic signals activated by reactive oxygen species production (ROS), DNA damage, abnormal oncogene expression and p53 activation, and cytotoxic agents. Once any of these signals are noticed by cells, the apoptotic machinery is triggered. Several secondary metabolites of polyphenols, alkaloids, and terpenes were reported to stimulate apoptosis in a dose-dependent fashion through increasing expression of p53, cleavage of caspases, and downregulate Bcl2 and Bcl-xL, thus inhibiting their antiapoptotic effect (Letai 2017). Various flavonoids have the capacity to increase the levels of caspase-3, a key protein associated with promoting apoptosis. Also, they elevate the expression of other proapoptotic proteins like caspase-9, BAK, BID, and BAX, further contributing to the activation of pathways leading to programmed cell death (Ponte et al. 2021).

On the other hand, any DNA damage can be detected through cell surveillance machinery and guardian checkpoints. They are capable of halting cell cycle progression until the destruction is fixed. However, if the damage is not repaired, the alternative route would be apoptosis. Tumor cells are capable of deceiving these checkpoints and continuing their proliferation and division without fixing their DNA injury (Koff et al. 2015). In addition, the PI3k/Akt signal transduction cascade is one of the numerous cellular proliferative trails that stimulate normal cell cycle progression by modifying cyclins and proapoptotic proteins. Hence, overexpressed Akt leads to atypical proliferative and antiapoptotic signals that begin the transformation of malignant tumors. Accordingly, many cytotoxic alkaloids, flavonoids, phenolic acids, and terpenes can block cell proliferation and promote apoptosis by inducing DNA damage and targeting the Akt pathway. These options seem to be excellent choices for cancer treatment.

3.1.2 Immune System Modulation and Anti-inflammatory Strategy

Persistent and chronic inflammation eases cancer cells' survival, proliferation, invasion, metastasis, and angiogenesis pathways. Flavonoids have anti-inflammatory effects. They do these effects by regulating immune cells, suppressing chemokines, COX-2, cytokines, and proinflammatory transcription factors, inhibiting PI3K/Akt, and inhibiting kappa kinase/c-Jun amino-terminal kinases (IKK/JNK) (Kopustinskiene et al. 2020). Nuclear factor-kappa NF- κ B inducible signaling pathway is fundamental in regulating inflammation and activating genes involved in cell proliferation, survival, and production of proinflammatory mediators (Leyva-López et al. 2016). However, its downregulation leads directly to apoptosis. The constitutive aberrant activation of this pathway manages the upregulation of proteins engaged in cell adhesion, cell survival, cell invasion, and angiogenesis. Alkaloids can destroy the tumorigenesis process by targeting the NF- κ B pathway precisely by reducing its activation and controlling gene expression. Flavonoids like quercetin and luteolin have been demonstrated to reduce the activity of AP-1 and NF- κ B, which are involved in the expression of proinflammatory cytokines (Khan et al. 2020).

3.1.3 Targeting Reprogrammed Metabolism Strategy

Cancer cells go through extreme metabolic reprogramming to withstand conditions and maintain survival. It depends on metabolizing glucose, lactic acid, fatty acid, and several other molecules for survival. They have the ability to reprogram the metabolic pathways with enhanced glycolytic pathways through the activation of several enzymes like hexokinase, pyruvate kinase, and phosphofructokinase. In addition, they have the ability to recycle and use the catabolites again to overcome a nutrient-depleted environment. Targeting tumor metabolism remains an attractive strategy (Guerra et al. 2018; Luo et al. 2021; Fakhri et al. 2022). Targeting mitochondrial metabolism and glycolytic pathways with a combinatorial approach holds promise as another strategy to disrupt the diverse metabolic compartments within cancer cells (Martinez-Outschoorn et al. 2017). Figure 1 illustrates major reprogrammed metabolic pathways in cancer and targets of plant secondary metabolites.

3.1.4 Targeting Tumor Microenvironment Strategy

Tumor microenvironment implies the cellular environment in which cancer stem cells exist. Cancer stem cells are cells in a tumor with the capability to self-renew and lead the tumorigenesis process (Arneth 2019). Tumor microenvironment involves a variety of cell types, such as tumor cells, immune cells (lymphoid lineage and myeloid lineage), epithelial cells, stromal cells, and fibroblasts, supplemented by hypoxia and various secretory factors and different types of mediators like cytokines, chemokines, lipid mediators, and inflammation-related proteins (Fig. 2) (Cheng et al. 2016; Luo et al. 2021). Key therapeutic targets of the cancer microenvironment include cholesterol synthesis, carbohydrate metabolism, reactive oxygen species, hypoxia, immune cell activation and conversion such as macrophages, dendritic cell (DC) modulation through indoleamine-2,3-dioxygenase regulation (IDO),

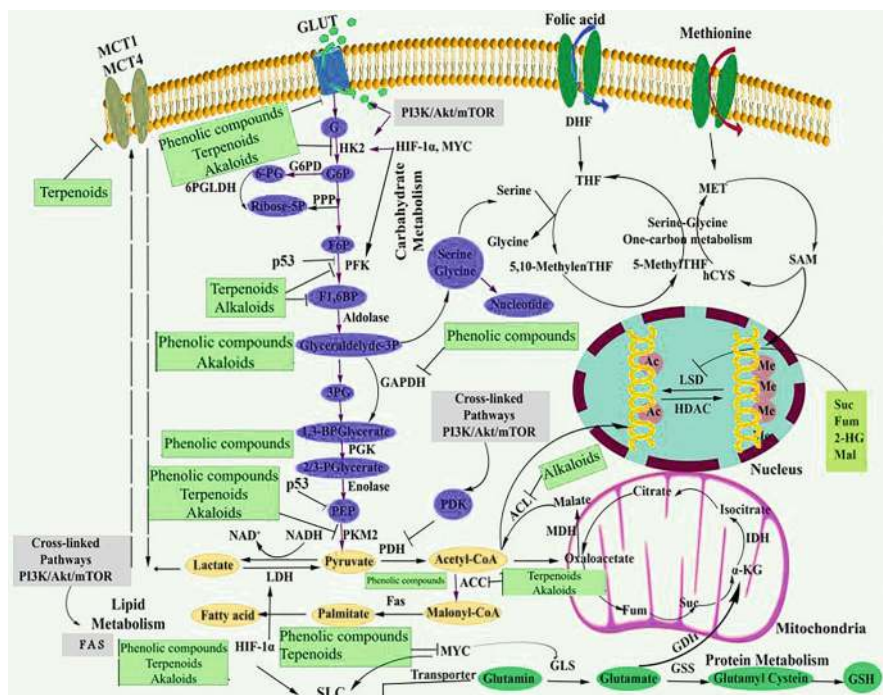


Fig. 1 Major reprogrammed metabolic pathways in cancer and targets of plant secondary metabolites (adopted from (Fakhri et al. 2022) with slight modification). 2-HG 2-hydroxyglutarate, 6PG 6-phosphogluconate, 6PGLDH 6-phosphogluconate dehydrogenase, AC acetyl, ACC acetyl-CoA carboxylase, ACL ATP citrate lyase, α -KG α -ketoglutarate, F fructose, 3PG 3-phosphogluconate, DHF dihydrofolate, Fum fumarate, G glucose, G-6P glucose 6-phosphate, G6PD glucose 6-phosphate dehydrogenase, GAPDH glyceraldehyde-3-phosphate dehydrogenase, GDH glutamate dehydrogenase, GLS glutamate synthase, GLUT glucose transporter, GSH glutathione, GSS glutamylcysteine synthetase, hCYS homocysteine, HDACs histone deacetylases, IDH isocitrate dehydrogenase, LDH lactate dehydrogenase, LSD lysine-specific demethylases, Mal malate, Me methyl, MCT monocarboxylate transporter, MDH malate dehydrogenase, PDH pyruvate dehydrogenase, PDK pyruvate dehydrogenase kinase, PEP phosphoenolpyruvate, PGK phosphoglycerate kinase, SAM S-adenosyl methionine, Suc succinate, SLC solute carrier transporter, TCA tricarboxylic acid cycle, THF tetrahydrofolic acid

angiogenesis regulation through vascular endothelial growth factor (VEGF), fibrosis inhibition, endoglin, and Janus kinase (JAK) signaling.

3.1.5 Antiangiogenic Strategy

Angiogenesis is a multifaceted route of new blood vessels' construction from existing vessels regulated by a balance between stimulators and inhibitor agents (Khalid et al. 2016; Sevastre et al. 2022). It is known that there are many signaling pathways and mediators that induce angiogenesis, including platelet-derived growth

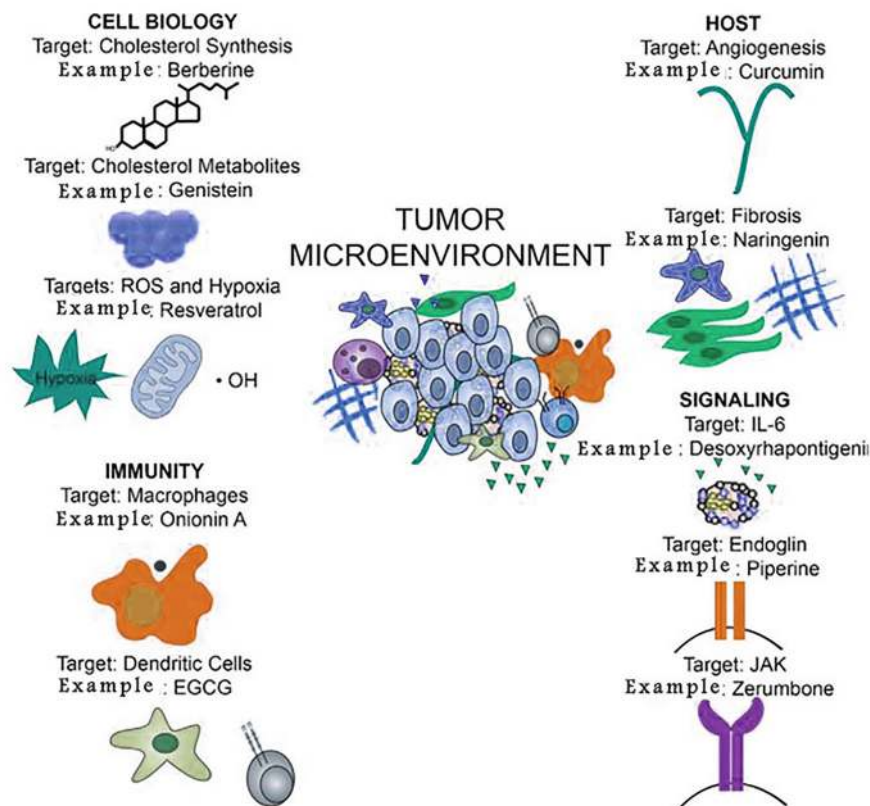


Fig. 2 Tumor microenvironment components that can be targeted by secondary metabolites to prevent or treat cancer (adopted (Guerra et al. 2018) with slight modification)

factor (PDGF), VEGF, fibroblast growth factors (FGF), hepatocyte growth factor (HGF), TKs, PI3K, MAPK, mTOR, Ras, and Ra (Sevastre et al. 2022). Both flavonoids and terpenes decreased the expression of VEGF, a major player in inducing angiogenesis (Chen et al. 2011; Sevastre et al. 2022). Other researchers reported the antiangiogenetic activity of several alkaloids like quinoline and phenanthroindolizidine alkaloids (Jain et al. 2019; Mondal et al. 2019).

3.1.6 Antimetastatic Strategy

Metastasis of cancer cells means the transfer of cells from the primary site to distant parts of the body. It involves a series of multi-step processes regulated by several molecules. Hence, these steps and modulators are understandably considered potential targets for metastasis management (Bag and Bag 2020; Liskova et al. 2020). Figure 1 shows the steps of metastasis and several players that secondary metabolites can target (Liskova et al. 2020).

4 Secondary Metabolite-Conventional Drug Combination

Several conventional anticancer medications are potent and have a broad-spectrum activity. However, patients treated with these medications suffer from serious side effects that limit their benefits. In addition, cancer cells have developed resistance to these drugs, resulting in treatment failure. A new approach of combinatorial anticancer drugs using plant extracts or metabolites may offer a significant advantage over single conventional drug use. Numerous *in vitro* and *in vivo* studies have confirmed that combinations of two or more phytochemical compounds were more effective in inhibiting cancer growth than treatment with a single conventional drug compound. Our review has shown that combinations can increase efficacy, modulate the chemosensitization of conventional compounds, and exhibit pleiotropic effects by concurrently impacting multiple metabolic pathways involved in carcinogenesis. It can decrease the minimum inhibitory concentration of conventional drug therapy and target malignancies resistant to standard conventional drugs resistance (Eid et al. 2012; Cheng et al. 2016; Niedzwiecki et al. 2016).

Our search of the literature yielded 51 studies that included *in vivo* and *in vitro* models. Our review included 49 *in vitro* studies experimenting on grown cancer cell lines, two *in vivo* studies conducted chiefly on rats and mice, and eight studies involved *in vitro* as well as *in vivo* models.

The most studied phytochemicals included curcumin (nine studies), epigallocatechin gallate (six studies), and resveratrol (seven studies). Among the most investigated chemotherapeutics was 5-fluorouracil (5-FU), where combinations improved cancer cells sensitivity and reduced its half maximum inhibitory concentration (IC_{50}). Doxorubicin was vastly studied, and the phytochemical combinations with doxorubicin reversed the resistance and increased its cellular retention, thus improving the cytotoxicity among resistant cancer lines. Also, the phytochemical combinations with cisplatin reversed the resistance, enhanced the cytotoxicity among resistant cancer cell lines, and protected against cisplatin-induced nephrotoxicity. Table 1 summarizes studies executed on a combinatorial approach.

Colorectal cancer is a common type of cancer that causes morbidity and mortality. Several investigations have reported the actions of combinatorial therapy on colon tumorigenesis via regulating several signaling pathways and modulators involved in the management of chronic inflammation, cell cycle, autophagy, apoptosis, metastasis, and angiogenesis (Zhao et al. 2018a, b; Ganesan et al. 2020).

Curcumin, resveratrol, and epigallocatechin gallate are plant-derived compounds that augment the anticancer activity of conventional drugs. Curcumin is a polyphenolic secondary metabolite produced in the *Curcuma longa* species, especially the rhizomes. It is considered a promising compound for the treatment of several types of cancer. It exhibited anticancer activity by targeting different cellular pathways and mediators (Giordano and Tommonaro 2019; Zoi et al. 2021).

Epigallocatechin gallate (EGCG) is a polyphenolic catechin, also known as epigallocatechin-3-gallate, is an ester of epigallocatechin and gallic acid produced in green tea. It has gotten attention for years for its valuable effects on human health,

Table 1 Combinatorial drug-plant secondary metabolite combination effects

Conventional anticancer drug	Secondary metabolite	Type of study	Cell line	Effect of the combination	Reference
Doxorubicin	Sanguinarine	In vitro	Caco-2, CEM/ADR5000	The coadministration of sanguinarine with doxorubicin resulted in a synergistic effect, enhancing the sensitivity of Caco-2 and CEM/ADR5000 cells. This combination also significantly increased the cytotoxicity of doxorubicin by reducing its half-maximal inhibitory concentration (IC ₅₀)	Eid et al. (2012)
Doxorubicin	EGCG	In vitro, in vivo	PC-3ML	EGCG increased the efficacy of doxorubicin and decreased its side effects	Stearns et al. (2010)
Doxorubicin	Quercetin	In vitro	MCF-7, MDA-231	Doxorubicin's maximal inhibitory concentration (IC ₅₀) was decreased by quercetin, resulting in a reduction of its side effects	Staedler et al. (2011)
Doxorubicin	Curcumin	In vitro, in vivo	HeLa, NCI-H460	Curcumin reduced multidrug resistance in cancer cells when combined with doxorubicin, thereby mitigating the adverse effects associated with doxorubicin	Rejinold et al. (2018)
Doxorubicin	Curcumin	In vitro	HT-29	The development of chemoresistance to doxorubicin was significantly reduced by curcumin	Jayarajan et al. (2020)
Doxorubicin	Curcumin	In vitro, in vivo	LL/2, MS1	Doxorubicin's effectiveness was improved by adding curcumin without any adverse effects being observed	Wang et al. (2013)
Doxorubicin	Curcumin	In vitro, in vivo	C26	The combination of doxorubicin and curcumin exhibited a synergistic effect in inhibiting the proliferation of C26 colon carcinoma cells	Sesaman et al. (2019)
Doxorubicin	EGCG, tannic acid, curcumin	In vitro	Caco-2, CEM/ADR 5000	Combining EGCG, curcumin, and tannic acid with doxorubicin can synergistically reduce the activity of P-gp	Li et al. (2018a, b)
Doxorubicin	Piperlongumine	In vitro	DU-145	The combination of doxorubicin and piperlongumine exhibited synergistic antiproliferative, proapoptotic, and antiinvasive properties	Piska et al. (2019)

(continued)

Table 1 (continued)

Conventional anticancer drug	Secondary metabolite	Type of study	Cell line	Effect of the combination	Reference
Doxorubicin	Baicalein	In vitro	LoVo, LoVo/Dx	Combining doxorubicin and glabridin induced apoptosis and displayed synergistic effects on growth inhibition	Palko-Labuz et al. (2019)
Doxorubicin	Glabridin	In vitro	MDA-MB-231, MCF-7	Glabridin inhibited P-gp expression and function, increasing doxorubicin building up in cells and enhancing apoptosis induction and S phase arrest	Qian et al. (2019)
Doxorubicin	Capsaicin, piperine	In vitro	Caco-2, CEM/ADR 5000	As indicated by the results, the modulation of P-gp by capsaicin and piperine exhibited a reversal activity against multidrug resistance	Li et al. (2018a, b)
Cisplatin	Resveratrol	In vitro	A2780, A2780 ^{cisR} , A2780ZD0473 ^R	Resveratrol sensitized the ovarian cancer cells to cisplatin and provided a means of defeating drug resistance	Nessa et al. (2012)
Cisplatin	Escin	In vitro	Panc-1	Escin enhanced the sensitivity of pancreatic cancer cells to cisplatin. The combination decreased pancreatic cancer cell survival, induced apoptosis, and reduced the NF- κ B signal transduction pathway	Rimmon et al. (2013)
Cisplatin	Curcumin	In vitro	HNSCC	The combination of curcumin and cisplatin exhibited enhanced efficacy in inhibiting tumor growth while simultaneously reducing the adverse effects associated with cisplatin administration	Duarte et al. (2010)
Cisplatin	Oxymatrine	In vitro	HeLa/DDP	The combination of cisplatin and oxymatrine could potentially serve as a promising approach to conquer chemoresistance in cervical cancer cases characterized by elevated IMPDH2 expression	Li et al. (2014)
Cisplatin	Genistein	In vitro	MCF-7, HT-29	Co-treatment of MCF-7 cells with cisplatin and genistein resulted in additive effects triggered by increasing apoptosis (sub-G1) and suppression of proliferation (S-phase)	Hu et al. (2014)

Cisplatin	Chrysin	In vivo	HCT116, DLD-1, SW837	The mitigation of oxidative stress and the activation of p38MAPK were found to be the underlying mechanisms through which chrysin protected against colon toxicity induced by cisplatin	Khan et al. (2012)
Cisplatin	NDGA	In vivo	–	The findings demonstrated that the combination of NDGA and cisplatin enhanced the effectiveness of cisplatin in combating breast cancer. The antitumor effects of cisplatin were evident through the reduction in tumor volume and the percentage of tumor inhibition observed in the in vivo model	Mundhe et al. (2015)
Cisplatin	Triptolide	In vitro	SC-M1	The combination of cisplatin and triptolide produced a synergistic anticancer effect	Li et al. (2012)
Cisplatin	TMP	In vivo	–	TMP ameliorated cisplatin-induced nephrotoxicity	Michel and Menze (2019)
Cisplatin	Ginsenoside Rb3	In vitro, in vivo	HEK293	G-Rb3 effectively restored kidney function in vivo and mitigated the cytotoxicity effects induced by cisplatin in renal tubular cells	Xing et al. (2019)
Cisplatin	Dioscin	In vitro, in vivo	NRK-52E, HK-2	Dioscin modulated cisplatin-induced nephrotoxicity through miR-34a/Sirt1 signaling pathway	Zhang et al. (2017)
Centchroman	Resveratrol and curcumin	In vitro	MCF-7, MDA-MB-231	Resveratrol and curcumin augment the apoptotic efficacy of centchroman	Singh et al. (2012)
Dasatinib	Curcumin	In vitro, in vivo	HCT116, HT-29	The combination therapy of dasatinib and curcumin decreased chemoresistance of colon cancer	Nautiyal et al. (2011)
Cisplatin, doxorubicin, docetaxel	Ginsenoside Rg3	In vitro	LNCaP, PC-3, DU145	Ginsenoside Rg3 potentiated the inhibitory effect of docetaxel, cisplatin, and doxorubicin regarding prostate cancer cell growth and NF- κ B activity	Kim et al. (2010)
Gemcitabine	Resveratrol	In vitro, in vivo	AsPC-1, Panc-1, MIA PaCa-2	Resveratrol exhibited a synergistic effect with gemcitabine by effectively inhibiting various indicators of cell proliferation, invasion, angiogenesis, and metastasis. These effects were achieved through the	Harikumar et al. (2010)

(continued)

Table 1 (continued)

Conventional anticancer drug	Secondary metabolite	Type of study	Cell line	Effect of the combination	Reference
Gemcitabine	EGCG	In vitro	AsPC-1, Panc-1	reduction of NF- κ B activity, as well as the downregulation of cyclin D1, Bcl-2, Bcl-xL, COX-2, MMP-9, VEGF, Ki-67, and CD31 expression levels	Tang et al. (2012)
Oxaliplatin	Resveratrol	In vitro	A2780, A2780 ^{cisR} , A2780ZD0473 ^R	EGCG enhanced the therapeutic effects of gemcitabine while reducing side effects	Nessa et al. (2012)
Oxaliplatin	Resveratrol	In vitro	SW480, SW620	Resveratrol enhances the susceptibility of ovarian cancer cells to oxaliplatin-induced apoptosis, offering a potential solution to combat drug resistance	Aires et al. (2013)
Oxaliplatin	Alantolactone	In vitro	HCT116, RKO	Resveratrol counteracted the development of resistance and reduced oxaliplatin side effects	Cao et al. (2019)
Oxaliplatin	Piperlongumine	In vitro	HCT-116 and LoVo	Alantolactone synergized the antitumor effect of oxaliplatin by inducing ROS generation	Chen et al. (2019)
Rapamycin	Resveratrol	In vitro	MCF-7, MDA-MB-231, BT-549	Piperlongumine synergized anticancer effects of oxaliplatin through ROS-mediated ER stress and mitochondrial function impairment	He et al. (2011)
Paclitaxel	EGCG	In vitro	4 T1, MCF-7, MDA-MB-231	Resveratrol potentiated the activity of rapamycin through the activation of mTOR	Luo et al. (2010)
Paclitaxel	Naringin	In vitro	DUI45, PC3, LNCaP	EGCG increased the efficacy of paclitaxel in eradicating extremely aggressive, metastatic tumors or primary tumors originating from tumor-initiating cells	Erdogan et al. (2018)
Paclitaxel	Luteolin	In vitro	MDA-MB-231, MDA-MB-453	Naringin acts as a chemosensitizer, synergistically strengthening paclitaxel's cytotoxic effect	Yang et al. (2014)
5-fluorouracil	Casticin	In vitro	WEHI-3 cells	Luteolin enhanced the effect of paclitaxel by reducing toxicity, activating caspase-8, -3, PARP, upregulating Fas, downregulating Bcl-xL, and inhibiting STAT3	Cheng et al. (2020)

5-fluorouracil	Curcumin	In vitro	HCT-116	A combination of 5-fluorouracil and curcumin counteracted the chemoresistance of 5-fluorouracil mediated via NF-κB/PI-3 K/Src pathways and NF-κB regulated gene products	Shakibaei et al. (2013)
5-fluorouracil	Resveratrol	In vitro	SW620 and HT-29	Resveratrol enhanced the sensitivity of colon cancer cells to 5-fluorouracil by boosting the oxidative stress process, which was linked to the inhibition of AKT and STAT3 proteins, which were recognized as oncogenic factors in colorectal carcinomas	Santandreu et al. (2011)
5-fluorouracil	Polysaccharide	In vitro	SW620 and HT-29	Polysaccharides administered in combination with 5-FU synergistically suppress mutant p53-bearing colorectal cancer cells	Jiang et al. (2017a, b)
5-fluorouracil	EGCG	In vitro	DLD1, SW480and COLO201	EGCG enhanced the effectiveness of 5-FU by stimulating caspase-3/PARP, upregulation of Bad, inhibition of GRP78/ NF-κB/miR-155-5p/MDR1 pathway, and downregulation of Bcl-2	Kumazaki et al. (2013)
5-fluorouracil	Protopanaxadiol	In vitro	HCT-116	Protopanaxadiol synergized 5-fluorouracil through increased the number of apoptotic cells	Wang et al. (2015)
5-fluorouracil	Gossypol	In vitro	HCT116, HT-29 and RKO s	The antitumor effect of 5-fluorouracil was enhanced by the downregulation of TS, cyclin D1, and the mTOR signaling pathways mediated by Gossypol	Yang et al. (2015)
5-fluorouracil	Panaxadiol, irinotecan	In vitro	HCT-116, SW-480	Panaxadiol improved antiproliferative effects of irinotecan on human colorectal cancer cells through caspase-3- and caspase-9-mediated apoptosis	Du et al. (2012)
5-fluorouracil	EGCG	In vitro	HCT-116, DLD1	EGCG improved the responsiveness of colorectal cancer cells to 5-FU by suppressing GRP78/NF-κB/miR-155-5p/MDR1 Pathway (enhanced selectivity)	La et al. (2019)
Irinotecan	Apigenin	In vitro	HT-29 and HRT-18	Apigenin increased in the potency of the irinotecan	Lefort and Blay (2011)

(continued)

Table 1 (continued)

Conventional anticancer drug	Secondary metabolite	Type of study	Cell line	Effect of the combination	Reference
Arsenite	Delphinidin	In vitro	HL-60	Delphinidin-induced sensitization of HL-60 cells to arsenite led to dose reduction and ultimately minimized its side effects	Yoshino et al. (2018)
Arsenite	Delphinidin	In vitro	NB4	Delphinidin selectively sensitized the cells to arsenite, enhancing arsenite cytotoxicity by strengthening intrinsic/extrinsic pathway-mediated apoptosis induction	Yuan et al. (2015)
t- Retinoic Acid	Dihydromyricetin	In vitro	NB4, COS-7 and 293FT	Dihydromyricetin synergized retinoic acid activity	He et al. (2018)
Temozolomide	Quercetin	In vitro	U251 and U87	Quercetin sensitized glioblastoma cells to temozolomide by inhibiting Hsp27	Sang et al. (2014)
Temozolomide	Biochanin A	In vitro	U-87 MG and T98 G	Biochanin A significantly enhanced the anticancer efficacy of temozolomide	Desai et al. (2019)

EGCG epigallocatechin gallate, *NDGA* nordihydroguaiaretic acid, *TMP* tetramethylpyrazine, Caco-2 human colorectal adenocarcinoma cells, CEM/ADR5000 adriamycin-resistant leukemia cells, PC-3ML malignant human prostate cells, MCF-7 and MDA-231 breast cancer cells, HCT116 and HT-29 human colon cancer cell lines, HeLa human cervical cancer cells, NCI-H460 human lung carcinoma cells, LL/2, MS1 lung cancer cells, C26 murine colon carcinoma, DU-145 prostate cancer cells, DU-145 prostate cancer cells, LoVo human colorectal adenocarcinoma cell line, LoVo/Dx doxorubicin-resistant subline, MDA-MB-231/MDR1 and MCF-7/ADR breast cancer cells, A2780, A2780cisR, and A2780ZD0473R ovarian cancer cell lines, HNSCC head and neck squamous cell carcinoma, HeLa/DDP cisplatin-resistant human cervical cells, SC-M1 human gastric adenocarcinoma cells, PC3 prostate cancer cell line, AsPC-1 and PANC-1 human pancreatic cancer cell lines, SW480 and SW620 colon cancer cells, A549 lung, RKO human colon cancer, A2780, A2780cisR, and A2780ZD0473R human ovarian cancer, BT-549 breast cancer cells, DLD1 and COLO201 colorectal cancer cell lines, HL-60 human leukemia cell line, NB4 acute promyelocytic leukemia cells, LNCaP prostate cancer, PC-3 and DU145 prostate cancer, AsPC-1 pancreatic adenocarcinoma, MIA PaCa-2 pancreatic carcinoma, Panc-1 pancreatic duct cell carcinoma, Panc-28 pancreatic carcinoma, MCF-7, MDA-MB-231, and BT-549 breast cancer, 4 T1 breast cancer, DU145, PC3, and LNCaP prostate cancer cell line, MDA-MB231 (ER, HER2/neu), and MDA-MB-453 (ER, HER2/neu+) metastatic breast cancer cell lines, U251 and U87 human glioblastoma cell lines, T98 G glioblastoma multiforme (GBM) cells, MIA PaCa-2, HEK293 cell line (human embryonic kidney epithelial cells), WEHI-3 leukemia cells, *ROS* reactive oxygen species

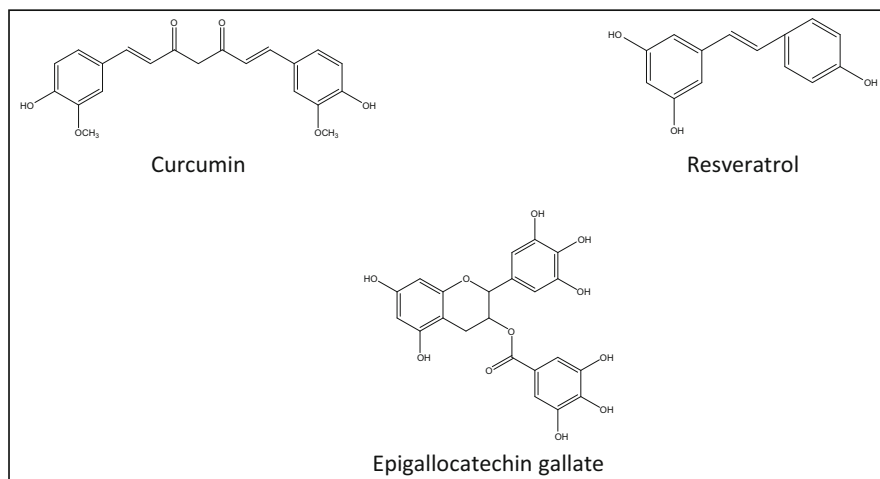


Fig. 3 Most studied phytochemical compounds in a combinatorial approach

including antioxidant and anti-inflammatory properties. The anticancer potential of EGCG has been extensively studied. EGCG impacts apoptosis, cell proliferation, and autophagy pathways. Scientific evidence has shown that EGCG has a promising role in inhibiting tumor cell metastasis and angiogenesis (Aggarwal et al. 2022; Ferrari et al. 2022).

Resveratrol is a miracle natural polyphenolic stilbene with a promising role in ameliorating cancer and proclaiming breakthroughs in cancer therapy. It can target proliferation and cancer progression in several tumor models (Jiang et al. 2017a, b; Ren et al. 2021) (Fig. 3).

5 Conclusion

Phytochemical metabolites reveal plentiful biological activities, making them fascinating as a resource for drug development of human diseases. It is well known that the majority of cancer drugs clinically established originate from nature. Cancer investigators worldwide try to discover novel natural products as lead compounds for cancer therapy. Generally, natural products are considered promising cancer therapeutics, either as single agents or in combinatorial approaches, to enhance the anticancer activity of conventional therapeutic modalities. Most phytochemicals exert pleiotropic effects and modify numerous modulators, intermediates, and signal transduction pathways. But, apoptosis seems a promising target for cancer treatment. Moreover, a better understanding of the multifaceted mechanisms of action of phytochemical metabolites is anticipated to open new approaches for treating all types of cancers in the coming years through a combinatorial approach. Curcumin, resveratrol, and epigallocatechin gallate are plant-derived compounds that augment the anticancer activity of conventional drugs

while minimizing their side effects. In the present chapter, we report on our efforts to determine the major strategies used to treat cancer by phytochemicals in cancer cells.

Compliance with Ethical Standards All authors admitted no conflict of interest.

References

- Aggarwal V, Tuli HS, Tania M, Srivastava S, Ritzer EE, Pandey A, Aggarwal D, Barwal TS, Jain A, Kaur G (2022) Molecular mechanisms of action of epigallocatechin gallate in cancer: recent trends and advancement. *Semin Cancer Biol* 80:256
- Aires V, Limagne E, Cotte AK, Latruffe N, Ghiringhelli F, Delmas D (2013) Resveratrol metabolites inhibit human metastatic colon cancer cells progression and synergize with chemotherapeutic drugs to induce cell death. *Mol Nutr Food Res* 57:1170–1181
- Ali Syeda Z, Langden SSS, Munkhzul C, Lee M, Song SJ (2020) Regulatory mechanism of MicroRNA expression in cancer. *Int J Mol Sci* 21:1723
- Arneth B (2019) Tumor microenvironment. *Medicina* 56:15
- Aung TN, Qu Z, Kortschak RD, Adelson DL (2017) Understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action. *Int J Mol Sci* 18:656
- Bag N, Bag A (2020) Antimetastatic properties of tea polyphenols. *Nutr Cancer* 72:365–376
- Block KI, Gyllenhaal C, Lowe L, Amedei A, Amin AR, Amin A, Aquilano K, Arbisser J, Arreola A, Arzumanyan A (2015) Designing a broad-spectrum integrative approach for cancer prevention and treatment. *Semin Cancer Biol* 35:S276
- Bukowski K, Kciuk M, Kontek R (2020) Mechanisms of multidrug resistance in cancer chemotherapy. *Int J Mol Sci* 21:3233
- Cao P, Xia Y, He W, Zhang T, Hong L, Zheng P, Shen X, Liang G, Cui R, Zou P (2019) Enhancement of oxaliplatin-induced colon cancer cell apoptosis by alantolactone, a natural product inducer of ROS. *Int J Biol Sci* 15:1676
- Chen W, Lu Y, Wu J, Gao M, Wang A, Xu B (2011) Beta-elemene inhibits melanoma growth and metastasis via suppressing vascular endothelial growth factor-mediated angiogenesis. *Cancer Chemother Pharmacol* 67:799–808
- Chen W, Lian W, Yuan Y, Li M (2019) The synergistic effects of oxaliplatin and piperlongumine on colorectal cancer are mediated by oxidative stress. *Cell Death Dis* 10:600
- Cheng Y-T, Yang C-C, Shyur L-F (2016) Phytomedicine—modulating oxidative stress and the tumor microenvironment for cancer therapy. *Pharmacol Res* 114:128–143
- Cheng ZY, Chueh FS, Peng SF, Lin CH, Kuo CL, Huang WW, Chen PY, Way TD, Chung JG (2020) Combinational treatment of 5-fluorouracil and casticin induces apoptosis in mouse leukemia WEHI-3 cells in vitro. *Environ Toxicol* 35:911–921
- Cree IA, Charlton P (2017) Molecular chess? Hallmarks of anticancer drug resistance. *BMC Cancer* 17:1–8
- Desai V, Jain A, Shaghghi H, Summer R, Lai JC, Bhushan A (2019) Combination of biochanin A and temozolomide impairs tumor growth by modulating cell metabolism in glioblastoma multiforme. *Anticancer Res* 39:57–66
- Dey P, Kundu A, Chakraborty HJ, Kar B, Choi WS, Lee BM, Bhakta T, Atanasov AG, Kim HS (2019) Therapeutic value of steroidal alkaloids in cancer: Current trends and future perspectives. *Int J Cancer* 145:1731–1744
- Du G-J, Wang C-Z, Zhang Z-Y, Wen X-D, Somogyi J, Calway T, He T-C, Du W, Yuan C-S (2012) Caspase-mediated proapoptotic interaction of panaxadiol and irinotecan in human colorectal cancer cells. *J Pharm Pharmacol* 64:727–734

- Duarte VM, Han E, Veena MS, Salvado A, Suh JD, Liang L-J, Faull KF, Srivatsan ES, Wang MB (2010) Curcumin enhances the effect of cisplatin in suppression of head and neck squamous cell carcinoma via inhibition of IKK β protein of the NF κ B pathway. *Mol Cancer Ther* 9:2665–2675
- Eid SY, El-Readi MZ, Wink M (2012) Synergism of three-drug combinations of sanguinarine and other plant secondary metabolites with digitonin and doxorubicin in multidrug resistant cancer cells. *Phytomedicine* 19:1288–1297
- Emran TB, Shahriar A, Mahmud AR, Rahman T, Abir MH, Siddiquee M, Ahmed H, Rahman N, Nainu F, Wahyudin E (2022) Multidrug resistance in cancer: understanding molecular mechanisms, immunoprevention and therapeutic approaches. *Front Oncol* 12:891652
- Erdogan S, Doganlar O, Doganlar ZB, Turkecul K (2018) Naringin sensitizes human prostate cancer cells to paclitaxel therapy. *Prostate Int* 6:126–135
- Fakhri S, Moradi SZ, Farzaei MH, Bishayee A (2022) Modulation of dysregulated cancer metabolism by plant secondary metabolites: a mechanistic review. *Semin Cancer Biol* 80:276
- Ferrari E, Bettuzzi S, Naponelli V (2022) The potential of epigallocatechin gallate (EGCG) in targeting autophagy for cancer treatment: a narrative review. *Int J Mol Sci* 23:6075
- Ganesan K, Jayachandran M, Xu B (2020) Diet-derived phytochemicals targeting colon cancer stem cells and microbiota in colorectal cancer. *Int J Mol Sci* 21:3976
- Giordano A, Tommonaro G (2019) Curcumin and cancer. *Nutrients* 11:2376
- Guerra AR, Duarte MF, Duarte IF (2018) Targeting tumor metabolism with plant-derived natural products: Emerging trends in cancer therapy. *J Agric Food Chem* 66:10663–10685
- Habli Z, Toumeh G, Fatfat M, Rahal ON, Gali-Muhtasib H (2017) Emerging cytotoxic alkaloids in the battle against cancer: overview of molecular mechanisms. *Molecules* 22:250
- Harikumar KB, Kunnumakkara AB, Sethi G, Diagaradjane P, Anand P, Pandey MK, Gelovani J, Krishnan S, Guha S, Aggarwal BB (2010) Resveratrol, a multitargeted agent, can enhance antitumor activity of gemcitabine in vitro and in orthotopic mouse model of human pancreatic cancer. *Int J Cancer* 127:257–268
- Hazafa A, Rehman KU, Jahan N, Jabeen Z (2020) The role of polyphenol (flavonoids) compounds in the treatment of cancer cells. *Nutr Cancer* 72:386–397
- He X, Wang Y, Zhu J, Orloff M, Eng C (2011) Resveratrol enhances the antitumor activity of the mTOR inhibitor rapamycin in multiple breast cancer cell lines mainly by suppressing rapamycin-induced AKT signaling. *Cancer Lett* 301:168–176
- He M-h, Zhang Q, Shu G, Lin J-c, Zhao L, Liang X-x, Yin L, Shi F, Fu H-l, Yuan Z-x (2018) Dihydromyricetin sensitizes human acute myeloid leukemia cells to retinoic acid-induced myeloid differentiation by activating STAT1. *Biochem Biophys Res Commun* 495:1702–1707
- Hu X-J, Xie M-Y, Kluxen FM, Diel P (2014) Genistein modulates the antitumor activity of cisplatin in MCF-7 breast and HT-29 colon cancer cells. *Arch Toxicol* 88:625–635
- Ioele G, Chieffallo M, Occhiuzzi MA, De Luca M, Garofalo A, Ragno G, Grande F (2022) Anticancer drugs: recent strategies to improve stability profile, pharmacokinetic and pharmacodynamic properties. *Molecules* 27:5436
- Jain S, Chandra V, Jain PK, Pathak K, Pathak D, Vaidya A (2019) Comprehensive review on current developments of quinoline-based anticancer agents. *Arab J Chem* 12:4920–4946
- Jayarajan J, Angandoor S, Vedula SH, Sritharan S, Ganesan K, War AR, Sivalingam N (2020) Curcumin induces chemosensitization to doxorubicin in Duke's type B coloadenocarcinoma cell line. *Mol Biol Rep* 47:7883–7892
- Jiang Z, Chen K, Cheng L, Yan B, Qian W, Cao J, Li J, Wu E, Ma Q, Yang W (2017a) Resveratrol and cancer treatment: updates. *Ann N Y Acad Sci* 1403:59–69
- Jiang D, Wang L, Zhao T, Zhang Z, Zhang R, Jin J, Cai Y, Wang F (2017b) Restoration of the tumor-suppressor function to mutant p53 by *Ganoderma lucidum* polysaccharides in colorectal cancer cells. *Oncol Rep* 37:594–600
- Khalid EB, Ayman E-ME-K, Rahman H, Abdelkarim G, Najda A (2016) Natural products against cancer angiogenesis. *Tumour Biol* 37:14513–14536

- Khan R, Khan AQ, Qamar W, Lateef A, Tahir M, Rehman MU, Ali F, Sultana S (2012) Chrysin protects against cisplatin-induced colon. toxicity via amelioration of oxidative stress and apoptosis: probable role of p38MAPK and p53. *Toxicol Appl Pharmacol* 258:315–329
- Khan H, Ullah H, Castilho PCMF, Gomila AS, D'Onofrio G, Filosa R, Wang F, Nabavi SM, Daglia M, Silva AS (2020) Targeting NF- κ B signaling pathway in cancer by dietary polyphenols. *Crit Rev Food Sci Nutr* 60:2790–2800
- Kikuchi H, Yuan B, Hu X, Okazaki M (2019) Chemopreventive and anticancer activity of flavonoids and its possibility for clinical use by combining with conventional chemotherapeutic agents. *Am J Cancer Res* 9:1517
- Kim SM, Lee SY, Cho JS, Son SM, Choi SS, Yun YP, Yoo HS, Oh K-W, Han SB, Hong JT (2010) Combination of ginsenoside Rg3 with docetaxel enhances the susceptibility of prostate cancer cells via inhibition of NF- κ B. *Eur J Pharmacol* 631:1–9
- Koff JL, Ramachandiran S, Bernal-Mizrachi L (2015) A time to kill: targeting apoptosis in cancer. *Int J Mol Sci* 16:2942–2955
- Koh Y-C, Ho C-T, Pan M-H (2020) Recent advances in cancer chemoprevention with phytochemicals. *J Food Drug Anal* 28:14–37
- Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J (2020) Flavonoids as anticancer agents. *Nutrients* 12:457
- Kumazaki M, Noguchi S, Yasui Y, Iwasaki J, Shinohara H, Yamada N, Akao Y (2013) Anticancer effects of naturally occurring compounds through modulation of signal transduction and miRNA expression in human colon cancer cells. *J Nutr Biochem* 24:1849–1858
- La X, Zhang L, Li Z, Li H, Yang Y (2019) (–)-Epigallocatechin Gallate (EGCG) enhances the sensitivity of colorectal cancer cells to 5-FU by inhibiting GRP78/NF- κ B/miR-155-5p/MDR1 pathway. *J Agric Food Chem* 67:2510–2518
- Lefort ÉC, Blay J (2011) The dietary flavonoid apigenin enhances the activities of the anti-metastatic protein CD26 on human colon carcinoma cells. *Clin Exp Metastasis* 28:337–349
- Lei ZN, Tian Q, Teng QX, Wurple JN, Zeng L, Pan Y, Chen ZS (2023) Understanding and targeting resistance mechanisms in cancer. *MedComm* 4:e265
- Letai A (2017) Apoptosis and cancer. *Ann Rev Cancer Biol* 1:275–294
- Leyva-López N, Gutierrez-Grijalva EP, Ambriz-Perez DL, Heredia JB (2016) Flavonoids as cytokine modulators: a possible therapy for inflammation-related diseases. *Int J Mol Sci* 17:921
- Li C-J, Chu C-Y, Huang L-H, Wang M-H, Sheu L-F, Yeh J-I, Hsu H-Y (2012) Synergistic anticancer activity of triptolide combined with cisplatin enhances apoptosis in gastric cancer in vitro and in vivo. *Cancer Lett* 319:203–213
- Li M, Su B-S, Chang L-H, Gao Q, Chen K-L, An P, Huang C, Yang J, Li Z-F (2014) Oxymatrine induces apoptosis in human cervical cancer cells through guanine nucleotide depletion. *Anti-Cancer Drugs* 25:161–173
- Li H, Krstin S, Wang S, Wink M (2018a) Capsaicin and piperine can overcome multidrug resistance in cancer cells to doxorubicin. *Molecules* 23:557
- Li H, Krstin S, Wink M (2018b) Modulation of multidrug resistant in cancer cells by EGCG, tannic acid and curcumin. *Phytomedicine* 50:213–222
- Liskova A, Koklesova L, Samec M, Smejkal K, Samuel SM, Varghese E, Abotaleb M, Biringier K, Kudela E, Danko J (2020) Flavonoids in cancer metastasis. *Cancers* 12:1498
- Luo T, Wang J, Yin Y, Hua H, Jing J, Sun X, Li M, Zhang Y, Jiang Y (2010) (–)-Epigallocatechin gallate sensitizes breast cancer cells to paclitaxel in a murine model of breast carcinoma. *Breast Cancer Res* 12:1–10
- Luo Y, Yin S, Lu J, Zhou S, Shao Y, Bao X, Wang T, Qiu Y, Yu H (2021) Tumor microenvironment: a prospective target of natural alkaloids for cancer treatment. *Cancer Cell Int* 21:1–20
- Ma J, Li J, Wang KS, Mi C, Piao LX, Xu GH, Li X, Lee JJ, Jin X (2016) Perillyl alcohol efficiently scavenges activity of cellular ROS and inhibits the translational expression of hypoxia-inducible factor-1 α via mTOR/4E-BP1 signaling pathways. *Int Immunopharmacol* 39:1–9
- Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP (2017) Cancer metabolism: a therapeutic perspective. *Nat Rev Clin Oncol* 14:11–31

- Martins BX, Arruda RF, Costa GA, Jerdy H, de Souza SB, Santos JM, de Freitas WR, Kanashiro MM, de Carvalho ECQ, Sant'Anna NF (2019) Myrtenal-induced V-ATPase inhibition-A toxicity mechanism behind tumor cell death and suppressed migration and invasion in melanoma. *Biochimica et Biophysica Acta (BBA)-Gen Sub* 1863:1–12
- Michel HE, Menze ET (2019) Tetramethylpyrazine guards against cisplatin-induced nephrotoxicity in rats through inhibiting HMGB1/TLR4/NF- κ B and activating Nrf2 and PPAR- γ signaling pathways. *Eur J Pharmacol* 857:172422
- Mondal A, Gandhi A, Fimognari C, Atanasov AG, Bishayee A (2019) Alkaloids for cancer prevention and therapy: current progress and future perspectives. *Eur J Pharmacol* 858:172472
- Mundhe NA, Kumar P, Ahmed S, Jamdade V, Mundhe S, Lahkar M (2015) Nordihydroguaiaretic acid ameliorates cisplatin induced nephrotoxicity and potentiates its antitumor activity in DMBA induced breast cancer in female Sprague–Dawley rats. *Int Immunopharmacol* 28:634–642
- Nautiyal J, Kanwar SS, Yu Y, Majumdar AP (2011) Combination of dasatinib and curcumin eliminates chemoresistant colon cancer cells. *J Mol Signal* 6:1–11
- Nessa MU, Beale P, Chan C, Yu JQ, Huq F (2012) Combinations of resveratrol, cisplatin and oxaliplatin applied to human ovarian cancer cells. *Anticancer Res* 32:53–59
- Niedzwiecki A, Roomi MW, Kalinovsky T, Rath M (2016) Anticancer efficacy of polyphenols and their combinations. *Nutrients* 8:552
- Ohshima K, Morii E (2021) Metabolic reprogramming of cancer cells during tumor progression and metastasis. *Meta* 11:28
- Palko-Labuz A, Środa-Pomianek K, Wesołowska O, Kostrzewa-Susłow E, Uryga A, Michalak K (2019) MDR reversal and proapoptotic effects of statins and statins combined with flavonoids in colon cancer cells. *Biomed Pharmacother* 109:1511–1522
- Pfeffer CM, Singh AT (2018) Apoptosis: a target for anticancer therapy. *Int J Mol Sci* 19:448
- Piska K, Koczurkiewicz P, Wnuk D, Karnas E, Bucki A, Wójcik-Pszczółka K, Jamrozik M, Michalik M, Kołaczowski M, Pękała E (2019) Synergistic anticancer activity of doxorubicin and piperlongumine on DU-145 prostate cancer cells—the involvement of carbonyl reductase 1 inhibition. *Chem Biol Interact* 300:40–48
- Ponte LGS, Pavan ICB, Mancini MCS, da Silva LGS, Morelli AP, Severino MB, Bezerra RMN, Simabuco FM (2021) The hallmarks of flavonoids in cancer. *Molecules* 26:2029
- Qian J, Xia M, Liu W, Li L, Yang J, Mei Y, Meng Q, Xie Y (2019) Glabridin resensitizes p-glycoprotein-overexpressing multidrug-resistant cancer cells to conventional chemotherapeutic agents. *Eur J Pharmacol* 852:231–243
- Rejinold NS, Yoo J, Jon S, Kim Y-C (2018) Curcumin as a novel nanocarrier system for doxorubicin delivery to MDR cancer cells: in vitro and in vivo evaluation. *ACS Appl Mater Interfaces* 10:28458–28470
- Ren B, Kwah MX-Y, Liu C, Ma Z, Shanmugam MK, Ding L, Xiang X, Ho PC-L, Wang L, Ong PS (2021) Resveratrol for cancer therapy: challenges and future perspectives. *Cancer Lett* 515:63–72
- Rimmon A, Vexler A, Berkovich L, Earon G, Ron I (2013) Lev-Ari S (2013) Escin chemosensitizes human pancreatic cancer cells and inhibits the nuclear factor-kappaB signaling pathway. *Biochem Res Int* 2013:1
- Sang D-p, Li R-j, Lan Q (2014) Quercetin sensitizes human glioblastoma cells to temozolomide in vitro via inhibition of Hsp27. *Acta Pharmacol Sin* 35:832–838
- Santandreu FM, Valle A, Oliver J, Roca P (2011) Resveratrol potentiates the cytotoxic oxidative stress induced by chemotherapy in human colon cancer cells. *Cell Physiol Biochem* 28:219–228
- Sarkar S, Deyoung T, Ressler H, Chandler W (2023) Brain tumors: development, drug resistance, and sensitization—an epigenetic approach. *Epigenetics* 18:2237761
- Schiliro C, Firestein BL (2021) Mechanisms of metabolic reprogramming in cancer cells supporting enhanced growth and proliferation. *Cells* 10:1056
- Sesarman A, Tefas L, Sylvester B, Licarete E, Rauca V, Luput L, Patras L, Porav S, Banciu M, Porfire A (2019) Co-delivery of curcumin and doxorubicin in PEGylated liposomes favored the antineoplastic C26 murine colon carcinoma microenvironment. *Drug Deliv Transl Res* 9:260–272

- Sevastre A-S, Manea EV, Popescu OS, Tache DE, Danoiu S, Sfiredel V, Tataranu LG, Dricu A (2022) Intracellular pathways and mechanisms of colored secondary metabolites in cancer therapy. *Int J Mol Sci* 23:9943
- Shakibaei M, Mobasheri A, Lueders C, Busch F, Shayan P, Goel A (2013) Curcumin enhances the effect of chemotherapy against colorectal cancer cells by inhibition of NF- κ B and Src protein kinase signaling pathways. *PLoS One* 8:e57218
- Singh N, Zaidi D, Shyam H, Sharma R, Balapure AK (2012) Polyphenols sensitization potentiates susceptibility of MCF-7 and MDA MB-231 cells to Centchroman. *PLoS One* 7:e37736
- Staedler D, Idrizi E, Kenzaoui BH, Juillerat-Jeanneret L (2011) Drug combinations with quercetin: doxorubicin plus quercetin in human breast cancer cells. *Cancer Chemother Pharmacol* 68: 1161–1172
- Stearns ME, Amatangelo MD, Varma D, Sell C, Goodyear SM (2010) Combination therapy with epigallocatechin-3-gallate and doxorubicin in human prostate tumor modeling studies: inhibition of metastatic tumor growth in severe combined immunodeficiency mice. *Am J Pathol* 177: 3169–3179
- Tang S-N, Fu J, Shankar S, Srivastava RK (2012) EGCG enhances the therapeutic potential of gemcitabine and CP690550 by inhibiting STAT3 signaling pathway in human pancreatic cancer. *PLoS One* 7:e31067
- Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, Karaman R (2019) The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. *Toxins* 11:656
- Wang B-L, Shen Y-M, Zhang Q-W, Li Y-L, Luo M, Liu Z, Li Y, Qian Z-Y, Gao X, Shi H-S (2013) Codelivery of curcumin and doxorubicin by MPEG-PCL results in improved efficacy of systemically administered chemotherapy in mice with lung cancer. *Intl J Nanomed* 3521–3531
- Wang C-Z, Zhang Z, Wan J-Y, Zhang C-F, Anderson S, He X, Yu C, He T-C, Qi L-W, Yuan C-S (2015) Protopanaxadiol, an active ginseng metabolite, significantly enhances the effects of fluorouracil on colon cancer. *Nutrients* 7:799–814
- Wang X, Zhang H, Chen X (2019) Drug resistance and combating drug resistance in cancer. *Cancer Drug Resist* 2:141
- Weiss R (2004) Multistage carcinogenesis. *Br J Cancer* 91:1981–1982
- Xing JJ, Hou JG, Ma ZN, Wang Z, Ren S, Wang YP, Liu WC, Chen C, Li W (2019) Ginsenoside Rb3 provides protective effects against cisplatin-induced nephrotoxicity via regulation of AMPK-mTOR-mediated autophagy and inhibition of apoptosis in vitro and in vivo. *Cell Prolif* 52:e12627
- Yang M-Y, Wang C-J, Chen N-F, Ho W-H, Lu F-J, Tseng T-H (2014) Luteolin enhances paclitaxel-induced apoptosis in human breast cancer MDA-MB-231 cells by blocking STAT3. *Chem Biol Interact* 213:60–68
- Yang D, Qu J, Qu X, Cao Y, Xu L, Hou K, Feng W, Liu Y (2015) Gossypol sensitizes the antitumor activity of 5-FU through down-regulation of thymidylate synthase in human colon carcinoma cells. *Cancer Chemother Pharmacol* 76:575–586
- Yoshino Y, Yuan B, Okusumi S, Aoyama R, Murota R, Kikuchi H, Takagi N, Toyoda H (2018) Enhanced cytotoxic effects of arsenite in combination with anthocyanidin compound, delphinidin, against a human leukemia cell line, HL-60. *Chem Biol Interact* 294:9–17
- Yuan B, Okusumi S, Yoshino Y, Moriyama C, Tanaka S, Hirano T, Takagi N, Toyoda H (2015) Delphinidin induces cytotoxicity and potentiates cytotoxic effect in combination with arsenite in an acute promyelocytic leukemia NB4 cell line. *Oncol Rep* 34:431–438
- Zhang Y, Tao X, Yin L, Xu L, Xu Y, Qi Y, Han X, Song S, Zhao Y, Lin Y (2017) Protective effects of dioscin against cisplatin-induced nephrotoxicity via the microRNA-34a/sirtuin 1 signalling pathway. *Br J Pharmacol* 174:2512–2527
- Zhao Y, Hu X, Zuo X, Wang M (2018a) Chemopreventive effects of some popular phytochemicals on human colon cancer: a review. *Food Funct* 9:4548–4568
- Zhao S, Jiang Y, Zhao J, Li H, Yin X, Wang Y, Xie Y, Chen X, Lu J, Dong Z (2018b) Quercetin-3-methyl ether inhibits esophageal carcinogenesis by targeting the AKT/mTOR/p70S6K and MAPK pathways. *Mol Carcinog* 57:1540–1552
- Zoi V, Galani V, Lianos GD, Voulgaris S, Kyrtisis AP, Alexiou GA (2021) The role of curcumin in cancer treatment. *Biomedicines* 9:1086

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