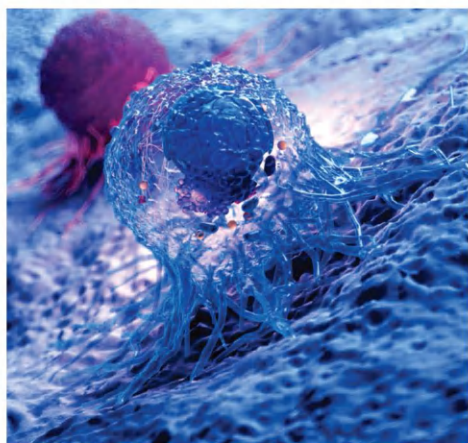


THE **ROLE OF** **PROBIOTICS IN** **CANCER MANAGEMENT**



Md. Faiyazuddin | Sumel Ashique
Editors



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Preface

The role of probiotics in cancer management is an area of ongoing research, and while some studies suggest potential benefits, the evidence is not yet conclusive. Probiotics are live microorganisms, primarily bacteria, that confer health benefits when consumed in adequate amounts. They are commonly known for their positive effects on gut health and the immune system.

Cancer stands as a prominent contributor to global mortality, prompting extensive research. The risk of cancer development hinges on both genetic factors and the immune system's status. The intricate interplay within the intestinal microbiome holds a crucial role in maintaining human body balance.

Probiotics have gained growing medical importance, attributed to their positive effects on the body, particularly in preventing and supporting the treatment of various chronic diseases, including cancer, with minimal side effects. This book seeks to consolidate knowledge surrounding the impact of probiotic microorganisms in cancer prevention. The gut microbiome is recognized for its crucial role in preserving homeostasis within the human body. An imbalance in the gut microbiota can trigger pro-inflammatory immune responses, potentially initiating various disease processes, including cancer. Recent research findings demonstrate that certain strains of probiotics can positively influence the intestinal microbiota and immune responses, suggesting their potential use in cancer prevention and as supportive treatment during anticancer chemotherapy. This book explores the latest advancements in probiotic research, focusing on their effectiveness in preventing and supporting the treatment of cancer. The chapters delve into topics such as the anticancer properties of probiotic microorganisms, the impact of their metabolites, potential mechanisms of probiotic chemoprevention, and the feasibility of incorporating probiotics into cancer care strategies.

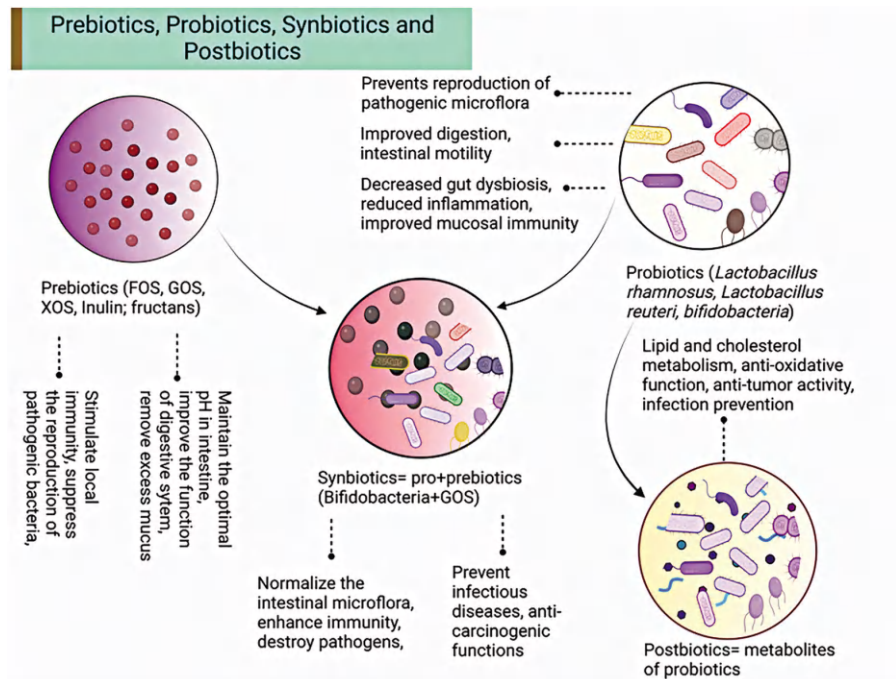


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Introduction



Probiotics are defined as live bacteria that are sufficiently present in the colon to enter an active condition and afterward have a favorable impact on health. Many probiotic microorganisms, such as *Escherichia coli* strain Nissle 1917, specific enterococci (*Enterococcus faecium* SF68), bifidobacteria, *Lactobacillus reuteri*, *Lactobacillus acidophilus* group, and *Lactobacillus rhamnosus* GG, are used in probiotic food, particularly fermented milk products, or have been studied for their potential medicinal benefits. The (temporary) alteration of the host's intestinal microflora and the probiotic bacteria's ability to interact with the immune system, either directly or through the autochthonous microflora, are fundamental processes among the many alleged health advantages of probiotic bacteria. A growing number of *in vitro* and *in vivo* research studies using traditional and molecular biological techniques provide evidence for these benefits. A small number

of randomized, carefully monitored human intervention trials have also been reported in addition to these. Probiotics benefit human organisms in a variety of ways. Their main benefit is the impact on the growth of the microbiota that inhabits the organism by maintaining an adequate balance between bacteria and pathogens, both of which are required for an organism to operate normally. Live microorganisms that meet the requirements are employed to produce functional food and preserve food products. After receiving antibiotic medication, their advantageous effects are used to restore the natural microbiota. Another purpose is to inhibit the activity of intestinal microbiota that are harmful and were introduced into the body by polluted environments and food.

Prebiotics are defined as “selectively fermented ingredients that permit certain changes, both in the composition and/or activity in the gut microbiota that bestow benefits upon host well-being and health,” whereas synbiotics are defined as synergistic mixtures of pro- and prebiotics. Only bifidogenic, non-digestible oligosaccharides currently meet all prebiotic classification requirements, particularly inulin, oligofructose, and (trans)galactooligosaccharides. They are dietary fibers with a proven beneficial effect on the bacteria in the intestines. Prebiotics also have indirect, or mediated by the intestinal microflora, and thus less well-studied, health benefits, such as the prevention of diarrhea or constipation, modulation of the metabolism of the intestinal flora, prevention of cancer, favorable effects on lipid metabolism, stimulation of mineral absorption, and immunomodulatory properties. In recent years, fructo- and (mainly) galactooligosaccharides have been successfully added to infant formula in an effort to mimic breast milk more closely.

The use of prebiotics, probiotics, and postbiotics to modify the gut microbiome has recently drawn interest since an imbalance in the intestinal microbiota can result in the development of numerous diseases (including type 1 diabetes and cancer, among others). Postbiotics are any substances that the microbe releases or produces during its metabolic process and which, either directly or indirectly, have a positive impact on the host. Postbiotics’ potential side effects are reduced because they don’t contain living bacteria. This section only lists the most significant postbiotic-specific mechanisms of action because of the considerable variability of postbiotic chemicals and the study’s constrained scope. It should be stressed that not enough information is now available to fully comprehend the intricate impacts of postbiotics. Postbiotics, however, are probably going to have pleiotropic effects on the human body. Postbiotics take the place of live microbe exposure, which is crucial for kids with developing immune systems and leaky intestinal

barriers. The development of the immune system, the tightness of the intestinal barrier, and the microbiota's indirect structure are all significantly influenced by postbiotics. Postbiotics could therefore be helpful in treating or preventing a variety of disease entities, even some for which an efficient causative therapy has not yet been discovered (such as Alzheimer's disease, inflammatory bowel disease, or multiple sclerosis). Clinical trials are now being conducted, and the preliminary findings are encouraging, with the goal of altering the microbiotas of individuals with the aforementioned disorders. The use of "postbiotics," or metabolites or fragments obtained from bacteria, is an appealing therapeutic and curative approach in contemporary medicine. These postbiotics exhibit pleiotropic effects, including immunomodulatory, anti-inflammatory, antioxidant, and anti-cancer qualities, according to the most recent research. Some of these qualities are even applied in medicine. In several studies, the distinction between probiotics and postbiotics is hazy since their influence on the outcomes is frequently not assessed independently.

Probiotic microorganisms and prebiotic food components are combined to create synbiotic nutritional supplements. Probiotic, prebiotic, and synbiotic therapy allow for the dietary regulation of the makeup of the gut microbiota in both newborns and adults. Prebiotics (mostly oligosaccharides and disaccharides) and probiotic products [dietary supplements containing possibly beneficial bacteria or friendly bacteria] are crucial for maintaining intestinal health. The prebiotics are used by the probiotics as a food supply, allowing them to stay alive inside the human digestive system longer than they otherwise could. Prebiotics are typically concerned with bacteria in the large intestine and colon, whereas probiotics are typically focused on bacteria in the small intestine. In order to increase the survivability of probiotics and provide targeted health advantages, synbiotics [probiotics + prebiotics] are used. The properties of probiotics, prebiotics, and synbiotics include those that are antimicrobial, anticarcinogenic, antidiarrheal, and antiallergenic, as well as those that prevent osteoporosis and ulcerative colitis, lower blood sugar and serum fat levels, regulate the immune system, and treat liver-related brain dysfunction. Severe pediatric surgical cases, liver transplants, biliary malignancy, acute pancreatitis, asthma, and atopic dermatitis, as well as patients receiving emergency treatment, are clinical conditions where probiotic, prebiotic, and synbiotic therapy have been shown to be effective. Probiotics, prebiotics, and synbiotics are becoming more important in the management of lactose intolerance, irritable bowel syndrome, inflammatory bowel disease, and diarrhea caused by a variety of causes, as well as in the reduction of antibiotic-associated diarrhea, rotavirus

diarrhea, and diarrhea due to other causes. Strain-specific effects of probiotic bacteria were discovered. Alternatives to antibiotics in pigs and poultry include probiotics. Prebiotics that are aimed at particular probiotic strains could be developed using synbiotic products to maximize health benefits. It is ideal to have a complete understanding of the probiotic agent's positive benefits in addition to its negative effects in order for it to be a useful tool for medical therapy. Probiotics' safety also requires in-depth scientific research. Given that a probiotic is most active in both the small and large intestines and that a prebiotic's effects are primarily felt in the large intestine, combining the two substances may have an additive impact. Prebiotics are primarily employed as a selective medium for a probiotic strain's growth, fermentation, and intestinal passage. There are hints in the literature that probiotic microbes become more resilient to environmental factors including oxygenation, pH, and temperature in an organism's intestine as a result of the usage of prebiotics.

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CHAPTER 1

Probiotics and Their Potential Effects on Cancer Management

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ABSTRACT

Probiotics are also called “health-friendly bacteria,” which are non-pathogenic microorganisms that have gained growing medical significance as they provide health benefits. They are extensively utilized commercially as functional food. The fame of probiotics nowadays has been ascending due to clinical trial levels showing beneficial effects on health (both locally and for the whole body) and reports that they improve quality of life against numerous diseases without any side effects. It is also reported that the utilization of probiotics can play a significant role in immunomodulation for the prevention of cancer and display anti-cancer properties. They could be responsible for the degradation and detection of possible carcinogens and the formation of short-chain fatty acids that influence proliferation and cell death. Probiotics are able to both decrease and increase the formation of anti-inflammatory cytokines, which play

a major role in carcinogenesis prevention. They may also interfere with phagocyte activation in order to eliminate early-stage cancerous cells. In cancer patients, the probability of probiotic management offers benefits by targeting cancer and minimizing anti-cancer side effects. This review focuses on an overview of probiotics, recent advances in promising health attributes, protective mechanisms of action, and their role in the management of cancer in cancer patients.

1.1 INTRODUCTION

The involvement of live microbial feeds in promoting human welfare has a long history, dating back thousands of years. Probiotics are “*live microorganisms*” that, when consumed in adequate amounts, offer health benefits to the host [1]. These probiotic bacteria, either singly or in combination, are marketed in powder or capsule form or used in the production of various fermented foods. The term “*probiotic*” was first introduced by Parker in 1974, who described it as a substance or organism capable of exerting a beneficial effect on the host by balancing intestinal microbiota. Since then, the definition has evolved multiple times [2]. The first significant study on probiotics was conducted by Metchnikoff and his colleagues, who explored the role of gut flora and the potential health benefits of fermented milk on humans. For his phagocytic cellular theory of immunity, Metchnikoff was awarded the Nobel Prize in Medicine, and his work inspired a generation of scientists and food technologists to focus on transforming toxic intestinal flora into a host-friendly *Bacillus polyfermenticus* KU3 *bulgaricus* colony [3]. Following his research, numerous studies have explored the use of probiotics to treat and prevent gastrointestinal disorders such as irritable bowel syndrome, stomach bloating, antibiotic-related diarrhea, indigestion, and lactose intolerance [4]. Given their proven role in promoting gut health and overall well-being, the demand for probiotic supplements has increased dramatically [5]. Beyond improving gut health, probiotics have been shown to offer additional health benefits, including mitigating chronic conditions such as high cholesterol levels [6], allergies [7], and cancer [8–10]. Figure 1.1 illustrates the fundamental relationship between probiotics and cancer.

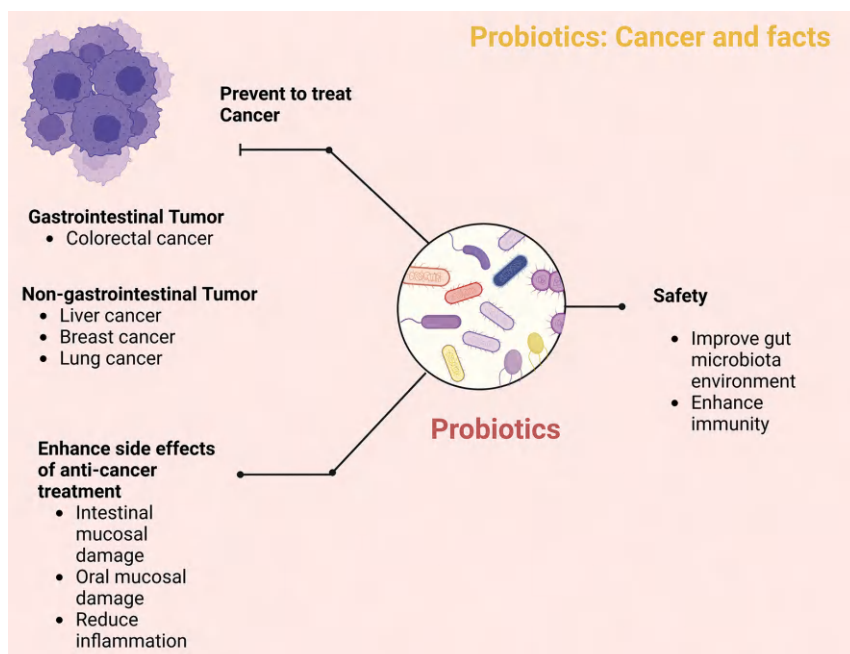


FIGURE 1.1 Interlink between probiotics and cancer.

1.2 PROBIOTICS

1. The term “probiotics” is a combination of the Latin preposition “pro” (meaning ‘in support of’ or ‘for’) and the Greek adjective “biotic” (meaning life). Therefore, probiotics means ‘in support of life’ [11]. Numerous hypotheses have been proposed regarding the evolving definitions of probiotics. Aside from advancements in genomic tools, these contribute to the recognition of novel species of probiotics and their mechanisms of action. The participation of several regulatory organizations and changes contributed by pharmaceutical companies are also significant [12]. The majority of probiotics are commonly referred to as LAB (lactic acid bacteria) because they can generate lactic acid when fermented with sugar-rich substrates. LAB is primarily subdivided into *Streptobacterium*, *Betabacterium*, *Termobacterium*, *Microbacterium*, *Betacoccus*, *Streptococcus*, and *Tetracoccus* based on their phenotypic and morphologic characteristics. At present, only *Streptococcus* species are retained, while several others have been renamed as *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* species [13, 14]. The *Lactobacillus* are

Gram-positive rods, which are lactic acid-secreting bacteria that are facultative and obligate anaerobes, mostly found in the genitourinary and gastrointestinal tracts [15, 16]. Moreover, Bifidobacterium are usually straight anaerobes, pleomorphic rod bacteria, non-spore-forming, and Gram-positive that produce acetic acids and lactic acids, which are the products of fermented carbohydrates [5, 17]. Currently, the interest in the industrialization and research of probiotics is focused on developing associations of various strains and species of probiotics. Several studies have demonstrated that they convey a great impact on human health compared to the utilization of a single strain of probiotics [5]. Probiotics are live microorganisms that provide health benefits when consumed in adequate amounts. They are commonly found in certain foods and dietary supplements. While the specific benefits may vary depending on the strain and dosage of probiotics used, here are some general health benefits associated with their consumption: Improved Digestive Health: Probiotics play a crucial role in maintaining a healthy balance of gut bacteria. They can help alleviate digestive issues such as diarrhea, constipation, and irritable bowel syndrome (IBS). Certain strains of probiotics, like Lactobacillus and Bifidobacterium, have been found to be particularly effective in supporting digestive health.

2. **Enhanced Immune Function:** Probiotics can strengthen the immune system by promoting the production of natural antibodies and stimulating the activity of immune cells. They help maintain a healthy gut barrier, preventing harmful bacteria from entering the bloodstream and triggering immune responses.
3. **Management of Antibiotic-Related Issues:** Antibiotics can disrupt the natural balance of gut bacteria, leading to conditions like antibiotic-associated diarrhea and *Clostridium difficile* (*C. difficile*) infection. Probiotics can help restore the microbial balance in the gut and reduce the risk of these complications.
4. **Potential Reduction in Allergies:** Some research suggests that certain probiotics may help reduce the risk of allergies, particularly in children. They may modulate the immune response and prevent allergic reactions by influencing the development of immune cells and the production of certain antibodies.
5. **Improved Mental Health:** The gut-brain axis is a bidirectional communication pathway between the gut and the brain. Emerging

evidence suggests that probiotics may have a positive impact on mental health by influencing the gut microbiota and the production of neurotransmitters. They have been studied for their potential to reduce symptoms of anxiety, depression, and even certain neurodevelopmental disorders.

- 6. Lowered Blood Cholesterol:** Certain strains of probiotics, such as *Lactobacillus acidophilus* and *Lactobacillus plantarum*, have been associated with a modest reduction in blood cholesterol levels. They can break down bile in the gut, reducing its reabsorption and prompting the liver to use cholesterol to produce more bile.
- 7. Potential Weight Management:** While more research is needed, some studies suggest that certain probiotics may contribute to weight management. They may affect appetite regulation, fat storage, and inflammation, which are factors related to body weight.

It's important to note that the effects of probiotics can vary depending on the individual, the specific strain used, and the dosage. If you're considering using probiotics, it's best to consult with a healthcare professional to determine the most appropriate strain and dosage for your specific health needs. Figure 1.2 depicts the health benefits of probiotics.

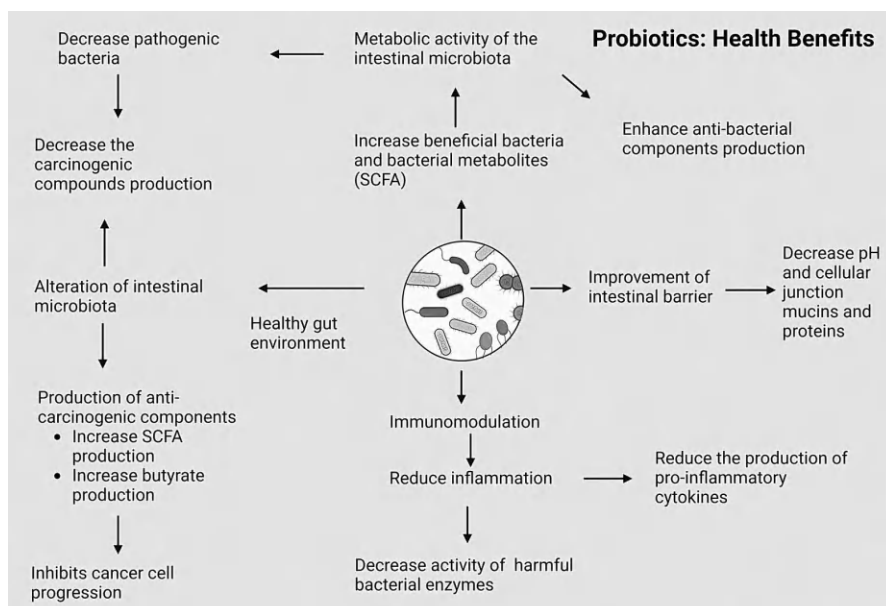


FIGURE 1.2 Various health benefits offered by probiotics.

1.3 PROBIOTICS FOR ALTERNATIVE TREATMENT AND PREVENTION OF CANCER

Cancer is a serious malignancy with elevated clinical consequences and remains one of the chief causes of death and illness [18]. Worldwide, it is the second foremost reason for mortality [19]. The frequency of cases and deaths has been progressively rising all over the globe, mostly in developing countries, with 18.1 million new cancer cases in 2018 and approximately 29.4 million cases expected by 2040 [20]. Various cancer types that can cause significant deaths include prostate, colorectal, stomach, liver, and breast cancers [21]. Essentially, cancer is prompted by deficiencies in DNA repair or mutations during DNA replication, and its key risk factors are lifestyle habits, environmental exposure, and individual genetic backgrounds [22, 23]. Some of the predisposing factors include UV radiation, toxic substances, and infectious agents [24]. Cancer is a condition in which uncontrolled cell division occurs and is usually recognized at a later stage of progression. It initiates from an individual cell and proliferates throughout the body. The transformation of healthy cells into a cancerous condition requires numerous steps for the development of cancerous cells to mature into eventually malignant and tumorigenic forms. Such tumor cells exhibit several physiological changes, including insensitivity to growth-inhibiting signals, resistance to apoptosis, self-sufficiency in growth signals, continuous metastasis and angiogenesis, and unlimited proliferation potential [24]. The present clinical management and treatment of cancer are done with standard drugs. However, the stability and long-term protection of chemotherapeutic medicines and various synthetic medications for cancer treatment are very doubtful. Hence, such hormonal chemotherapeutic substances and multi-drugs not only destroy cancerous cells but also harm healthy cells and create drug resistance [24]. Moreover, cytotoxic drugs are linked with life-threatening side effects that generally worsen the condition of cancer malignancy itself as a result [25]. Since there is no suitable remedy or cure for cancer that exists to date, the number of patients with neoplastic disease is still growing day by day. The recent chemotherapeutic drugs affect the quality of life or develop drug resistance, which is not affordable for the patients, researchers, and scientists who are looking for clinical management and treatment of cancer with negligible side effects and excessive efficiency [26–28]. To avoid and cure different types of cancer efficiently, probiotics play a significant role. This has been reported in a number of studies [5]. Probiotics are microorganisms that are mostly obtained from traditionally fermented foods or from the gut.

Probiotics are known as live microbiological components when utilized as live biotherapeutic substances or in dietary supplements. Probiotics are generally used by several companies, including nutritional supplement and food production companies of probiotics [29]. Dead or lifeless probiotics and their metabolites also express important benefits in the treatment and prevention of cancerous cells [30]. It is essential to find possible probiotic strains, effective doses of administration, and treatment and prevention via molecular mechanisms [31]. Probiotics and their pro-bioactive cellular components provide various beneficial outcomes in the gastrointestinal tract, discharge several enzymes, and offer possible synergistic effects on digestion. In particular, lactic acid bacteria cellular components of probiotics induce adjuvant effects, potentially including modulation of cell-mediated immune responses, stimulation of the reticulo-endothelial system, enhancement of cytokine pathways, and regulation of interleukins and tumor necrosis factors [32]. Most mechanisms of action of probiotics for anti-mutagenic and anti-cancerous activities include: binding/attachment, mutagen degradation and inhibition; prevention of pro-carcinogens and alteration of toxic, dangerous, and highly reactive carcinogens; pH lowering by short-chain fatty acids formed during non-digestible carbohydrate degradation; modulation of the innate immunity of the host; and augmentation through the secretion of anti-inflammatory molecules [33].

1.4 PROBIOTICS RELATIONSHIP, GUT MICROBIOTA, AND FERMENTED FOODS IN THE PREVENTION AND TREATMENT OF CANCER

1.4.1 *FERMENTED FOODS AND PROBIOTICS*

These include products of fermented milk that attract researchers' attention from various fields [34]. Diverse microbes, including molds, yeasts, and bacteria, are responsible for natural fermentation [35]. In particular, lactic acid bacteria include *Streptococcus*, *Bifidobacteria*, and *Lactobacillus* species found in food products (fermented) or naturally [36, 37]. Fermented foods, especially those containing probiotic microbes, are believed to be healthy. Fermented foods have unique functional properties, such as the formation of peptides, anti-microbial and anti-oxidant properties, enzymes, and many more properties of probiotics [38]. Such products show key functions in the treatment and prevention of cancer [34].

Various products of fermentation, like pomegranate juice, have also shown sources of polyphenolic compounds and have antioxidant activity, which is used to prevent the development of cancer cells (K562) [39]. In the process of fermentation, organic compounds are converted into various metabolic products (like acids). Such compounds inhibit the growth of pathogenic microorganisms and degrade several toxins that may give rise to cancer. For example, the fermentation of lactic acid bacteria can completely stop the production of mycotoxins by fungi [40]. Another vital anticancer agent is polyphenols, which are usually linked with fermented beverages and foodstuffs of plant sources like fruits, nuts, vegetables, wine, tea, and soy [41–43]. Blueberries contain anthocyanins, red wine contains resveratrol, soy contains isoflavones, and green tea contains epigallocatechin gallate, which are naturally occurring polyphenols that show anti-cancer activity [44–46]. Such polyphenols possess antioxidant and anti-inflammatory properties, as well as modulation of molecular targets and signaling pathways that are linked with cell proliferation, cell survival, angiogenesis, differentiation, hormonal activities, migration, enzyme detoxification, and immune responses. All these activities are essential for the efficiency of polyphenols' potential in combating cancer [47]. The additional distinctive probiotic features include delaying tumor formation, inhibiting cancer cell proliferation, and avoiding the life-threatening side effects associated with cancer treatment [27]. Administration of probiotics helps in the treatment and prevention of colorectal cancer. They are useful in the protection of therapies for cancer management, such as radiation therapy, surgery, and chemotherapy [48]. The probiotics are generally regarded as more affordable than standard medication, safe, and have an extended usage history [49, 50]. The probiotic yeasts and bacteria are reported to stop the toxicity of carcinogens and induce the death of cancerous cells [51]. Lactic acid bacteria as probiotics are regarded as the most essential microbes in anticancer properties [52]. Their special outcomes depend on metabolic activities, strain specificity, presented molecules, and secretion of components. Anti-proliferative activities of various strains of *Lactobacillus fermentum* and their components that combat cancerous cells *in vivo* and *in vitro* have been documented [53] (Table 1.1).

The probiotic yeasts are utilized as biotherapeutics in the treatment and prevention of colorectal cancer. These yeast probiotics can prevent cancer through mechanisms such as the alteration of the host's immune system, the initiation of apoptotic pathways, and the production of anti-tumorigenic material to scavenge mutagens via the gut. Consuming yeast probiotics in sufficient doses of traditional fermented foodstuffs can diminish the development of colorectal cancer risk [48].

TABLE 1.1 Experimental Model Features in the Live Probiotics Use in Clinical and Preclinical Evidence of Different Cancers

Probiotics	Type of Cancer	Dose	Duration	Evidence Level	Type of Study	Reference
<i>E. faecium</i> RM11 <i>E. faecium</i> RM28	Colorectal	10 ⁸ CFU	24 hrs	Cell line Conditioned media of <i>L. reuteri</i> NCIMB-701359 and NCIMB-702656 caused most significant cytotoxicity in treatment of up to 72 hrs cells	Pre-clinical <i>in-vitro</i>	[54]
<i>L. casei</i> ATCC393	Colorectal	10 ⁸ /10 ⁹ CFU	13 days	Cell lines: CT 26, HT 29	Pre-clinical <i>in-vitro</i> <i>In vivo</i> female BALB/c mice (oral administration)	[55]
<i>L. casei</i> BL23	Colorectal	5 x 10 ⁹ CFU	6–8 days	<i>In vivo</i> animal	Pre-clinical in female mice (oral administration)	[56]
<i>L. cocktail</i>	Colorectal	10 ⁸ CFC	120 hrs	Cell line; Almost complete the cell growth inhibition by the live bacteria at 48 hrs. Adhesion of bacteria to cells observed.	Pre-clinical <i>in vitro</i>	[57]
<i>L. plantarum</i>	Colorectal	10 ⁹ CFC	26 weeks	<i>In vivo</i> animal	Pre-clinical in male rats (oral administration)	[36]
<i>L. acidophilus</i>	Breast cancer	2 x 10 ⁸ CFU	15 days	<i>In vivo</i> animal	Pre-clinical in female mice (intraperitoneal administration)	[26]
<i>L. lactis</i> NK34	Colon, breast, lung, and stomach	10 ⁶ CFU	24 hrs	Cell line; Almost complete the cell growth inhibition by the live bacteria at 48 hrs. Adhesion of bacteria to cells observed.	Pre-clinical <i>in vitro</i>	[58]

TABLE 1.1 (Continued)

Probiotics	Type of Cancer	Dose	Duration	Evidence Level	Type of Study	Reference
<i>S. thermophilus</i> M17PTA496	Colorectal	10 ⁸ CFC	6 days	Cell line; Almost complete the cell growth inhibition by the live bacteria at 48 hrs. Adhesion of bacteria to cells observed.	Pre-clinical <i>in vitro</i>	[59]
<i>S. thermophilus</i> TH982						
Recombinant <i>L. lactis</i> IL-17A	Lung	10 ⁹ CFC	35 days	Cell line; TH17; <i>in vivo</i> animal	Pre-clinical <i>in vitro</i> and mouse (intranasal administration)	[60]
<i>E. faecium</i> CRL 183	Colorectal	10 ⁸ CFC	42 weeks	<i>In vivo</i> animal	Pre-clinical in male rats	[61]
<i>L. casei</i> Shirota	Breast	28.81 43.75 mg/d	Regularly	Human	Clinical trial (oral administration)	[62]
<i>Bifidobacteria</i> strains and <i>Lactobacillus fermentum</i> strains	Colorectal	107 mg, 2 times/day	6 months	Human	Clinical trial (oral administration)	[63]
Yogurt	Colorectal	85–98 g/d	12 years	Human	Clinical trial (oral administration)	[64]

1.4.2 PREBIOTICS, PROBIOTICS, AND GUT MICROBIOTA

Several *in vitro* and animal-based research studies show that probiotics, prebiotics, and synbiotics (a mixture of prebiotics and probiotics) are the ultimate choice for cancer avoidance [33]. Prebiotics are described as food ingredients with fermentable non-digestible compounds that maintain and improve host health. The dietary fibers in unprocessed form, consisting of prebiotics, can combat colorectal cancer [65]. The fibers of dietary supplements are components of edible plants or their extracts that are resistant to absorption and digestion in the small intestine and in the large intestine, with overall or partial fermentation. The majority of them are carbohydrates, which are regarded as prebiotics [66]. In research, the oligosaccharide lactulose (non-digestible) fed to rats causes a decrease in the damage of dimethylhydrazine-induced DNA in the colon of the rats [67]. In a study, feeding 1,2-dimethylhydrazine carcinogen plus the symbiotic to the rats resulted in 100% survival, whereas there was only 70% survival in the rats with only carcinogen administration. The symbiotics are able to combat pre-neoplastic colon lesions in experimental animals [68]. Prebiotics guard against cancer via several mechanisms, such as changing colonic pH, carcinogen adherence to bacteria, fecal bulking, modulation of xenobiotic metabolizing enzymes, modulation of gene expression in the cecum and feces, and modulation of immune response [69]. The relationship between gut microflora and probiotics is also essential in altering gut microbiota composition [56]. The gut microbiota is a heterogeneous population of commensal microbes, mainly comprising bacteria, but also archaea and viruses [70]. Among thousands of species of various bacteria in the gut microbiota, Bacteroidetes and phyla of Firmicutes are found in large quantities [71]. The physiology of humans is regulated by several microorganisms in the gut [72]. The gut microbiota can control numerous features of intestinal health, including cellular features, physiology, metabolism, development, and immune homeostasis [73]. It possesses a dual role in protecting the health of the host by developing a variety of metabolites and bio-products [74]. Residents of intestinal commensals are able to produce hormones such as metabolites like SCFAs, as a result of the fermentation of dietary fibers in the large intestine [75]. The specific composition of microbiota and diet establishes the nature of the synthesized SCFAs. There is a strong relationship among lifestyle, gut microbiota, and diet with the onset of colorectal cancer [75, 76]. Due to bacterial imbalance in the intestine, alterations in diet or the environment can cause colorectal cancer due to virulence factors, inflammatory pathways, and metabolites of microbes.

In the future, probiotics and gut microbiota will be useful in the prevention and treatment of colorectal cancer [48].

1.5 GI CANCER INHIBITION BY PROBIOTICS

Studies demonstrate that probiotics can minimize negative consequences and suffering associated with individuals who are currently undergoing treatment for cancer of the intestinal tract [77–80]. Probiotics strengthen the intestinal barrier and the body's immune system [80, 81]. Similar to study results carried out both *in vitro* and *in vivo*, the medicinal properties of microbes are now linked not only to the alleviation of negative effects from cancer therapy but also to the suppression and prevention of gastrointestinal carcinogenesis. The relationship between colon cancer and gut microbiota is fascinating due to the presence of microbes in the colon. The risk of colon cancer has been correlated with the nature of the gut microbiota. *Streptococcus gallolyticus*, for example [82], *Fusobacterium nucleatum* [83, 84], and more recently, *Providencia* [83], have been observed in tumors of colorectal cancer in humans. Probiotics influence the gut microbiota composition, thus positively affecting the host by improving the integrity of the intestinal barrier, inhibiting pathogen growth, reducing the metabolism of procarcinogenic substances, and much more. Hence, it has been reported and suggested that probiotics are very effective in the inhibition and prevention of the development of colon cancer.

1.5.1 PROBIOTICS EFFECT AGAINST IN-VITRO GASTROINTESTINAL CANCER

Years ago, various probiotic strains were reported to reduce the proliferation and stimulate apoptosis of colon and gastric cancer cells, as represented in Table 1.2. Regarding the mode of action, several studies indicated that the probiotics' ability to generate short-chain fatty acids was vital for the restrictive response [85–87]. In addition to short-chain fatty acids, TNF-related apoptosis-inducing ligand (TRAIL) encourages tumor apoptosis, which was also found to be upregulated by *Lactobacillus fermentum* strains casei ATCC 393, leading to an increase in the expression of TRAIL protein in tumors, which was linked with a reduced prevalence of tumors in mice [55]. Moreover, [88] observed that *Lactobacillus* X12 induced the rise of p27 through its mTOR/4EBP1 signaling pathway, playing a role in the alteration of tumor cells' cell cycle (Table 1.2).

TABLE 1.2 *In-Vitro* Tumor Prophylaxis Provided by Probiotics

Genus	Species and Strains	Outcome	Outcome	Reference
<i>Bacillus polyfermenticus</i> KU3	<i>Bacillus polyfermenticus</i> KU3	>90% proliferation of cancer cell inhibition by the bacterial cell	>90% proliferation of cancer cell inhibition by the bacterial cell	[89]
<i>Enterococcus lactis</i> IW5	<i>Enterococcus lactis</i> IW5	>60% cells died after 24 hrs incubation of cell free supernatant	>60% cells died after 24 hrs incubation of cell free supernatant	[90]
<i>Lactococcus lactis</i> NK34	<i>Lactococcus lactis</i> NK34	HT-29, LoVo, and AGS	Inhibition of >80% proliferation of cell by the 10 ⁶ CFU/well of bacterial cell	[58, 91]
	<i>Lactococcus lactis</i> NK34	Inhibition of >80% proliferation of cell by the 10 ⁶ CFU/well of bacterial cell	[58, 91]	
<i>Lactobacillus fermentum</i> strains	<i>Lactobacillus fermentum</i> strains	Conditioned media of <i>L. fermentum</i> NCIMB 5221 was effective in cell proliferation inhibition and apoptosis induction. The suppression is linked with SCFA production	Conditioned media of <i>L. fermentum</i> NCIMB 5221 was effective in cell proliferation inhibition and apoptosis induction. The suppression is linked with SCFA production	[85, 86]
<i>Lactobacillus fermentum</i> strains	<i>Lactobacillus fermentum</i> strains	Conditioned media of <i>L. reuteri</i> NCIMB-701359 and NCIMB-702656 caused most significant cytotoxicity in treatment of up to 72 hrs	Conditioned media of <i>L. reuteri</i> NCIMB-701359 and NCIMB-702656 caused most significant cytotoxicity in treatment of up to 72 hrs	[87]
	<i>Lactobacillus casei</i> ATCC 393	CT26 and HT29	Almost complete the cell growth inhibition by the live bacteria at 48 hrs. Adhesion of bacteria to cells observed.	[55, 88]
	<i>Lactobacillus fermentum</i> strains	Adhesion of bacteria to cells observed.	Apoptosis induction occurred via upregulation of TRAIL and downregulation of cyclin D1 and BIRC5a.	
	<i>paracasei subsp. Paracasei</i> X12		[55, 88]	

1.5.2 PROBIOTICS EFFECT IN CONTRADICTION OF GI CANCER IN-VIVO

In several studies, tumors were induced either via carcinogen administration, for example, 1,2-dimethylhydrazine dihydrochloride or AOM, or familiarized via tumor cell injection. In previous years, with the exclusion of the studies by Blasingame [92] and [93], the curative effect of probiotics on gut cancer has been demonstrated in all investigations. Many research efforts have centered on the immunomodulation-mediated repression of colon carcinogenesis alongside the decline in tumor rate and volume (Table 1.3).

1.6 BREAST CANCER AND PROBIOTICS

Although breast cancer mortality has decreased, it remains the most common cancer among women globally. As a result, the researcher's interest was immediately piqued in the area of breast tumor prevention and care. Previous research (*in vitro*) has demonstrated that different isolated strains/supernatants of probiotics from probiotic cultures were able to inhibit the Michigan Cancer Foundation-7 breast cancer cell's ability to proliferate [58, 89–91, 104]. Additionally, it was reported that *Enterococcus faecalis* (live) and *Staphylococcus hominis* (live) were retrieved from human milk. Furthermore, it was shown that heat-killed cells and fractions of cytoplasm produced cytotoxicity through the induction of apoptosis and the termination of the G0/G1 cell stages of the cell cycle [104]. Moreover, the probiotics reduced breast cancer cell growth and showed anti-inflammatory properties [58], signifying their anticancer property via immunomodulation. Despite the immune system's involvement, the pathway of hypoxia-inducible factor, which is linked with breast cancer, was also reported to be stifled via the culture of *Lactobacillus supernatant* [105].

1.7 LIVER CANCER AND PROBIOTICS

Liver shortcomings have been correlated with the gut's microbiota, such as non-alcoholic fatty liver disease [106, 107], liver fibrosis [108], and recently, liver cancer [109]. In previous years, it was reported that in mice, probiotics inhibit the progression of hepatocellular carcinoma [110]. Feeding a mixture of probiotics to tumor-injected mice can modify the gut microbiota composition and decrease the size of the tumors in the liver. Additionally, along with tumor size reduction, the angiogenic factors are downregulated

TABLE 1.3 Probiotics Effect Against In-Vivo Gastrointestinal Cancer

Genus	Species and Strains	Treatment	Outcome	Reference
Animal Studies Using Rats				
<i>Lactobacillus</i> <i>Bifidobacteria</i>	<i>Lactobacillus fermentum</i> strains <i>acidophilus</i> , <i>Bifidobacteria infantum</i> , and <i>Bifidobacteria bifidum</i>	Sprague Dawley rats treated via antibiotics DMH were given probiotics for 23 weeks	Tumor incidence reduction and volume by probiotics Suppression was linked with the intestinal integrity improvement and mediated by toll like receptor.	[94, 95]
<i>Lactobacillus fermentum</i> strains	<i>Lactobacillus rhamnus</i> GG CGMCC 1.2134 <i>Lactobacillus plantarum</i> (AdF 10) and <i>Lactobacillus rhamnosus</i> GG <i>Lactobacillus salivarius</i> Ren	SD rats treated with 10 weeks of DMH were given 25 weeks of probiotics SD rats treated with DMH for 4 weeks were given one of the strains for 12 weeks F344 rats inoculated with DMH for 10 weeks were given probiotics 2 weeks before and until the end of experiment	Reduction of tumor incidence, volume and multiplicity Apoptosis induction Decrease in inflammatory cytokines level and angiogenic factor. Reduction of tumor incidence, volume and multiplicity; Decrease in the cyclooxygenase-2 protein expression and total sialic acid level. Reduction of incidence of tumor.	[95–97]
Animal Studies Using Mice				
<i>Pediococcus</i>	<i>Pediococcus pentosaceus</i> GS4	Mice (Swiss albino) treated with AOM were given probiotics for 4 weeks	Cancer progression inhibition. Apoptosis induction in cancerous colonocytes and oxidative stress reduction and NF-KB activity.	[98]

TABLE 1.3 (Continued)

Genus	Species and Strains	Treatment	Outcome	Reference
<i>Lactobacillus fermentum</i> strains	<i>Lactobacillus reuteri</i> isolates 4000 and 4020	A/J mice treated with probiotics before or after receiving AOM	Colon carcinogenesis could not be inhibited by Probiotics. Incidence of reduced cancer by probiotics, accompanied by the proinflammatory cytokines reduction and anti-inflammatory cytokines increment.	[92, 99–102]
	<i>Lactobacillus casei</i> BL23	C57BL/6 mice were treated with DMH and probiotics for 10 weeks	Well balanced Th17-biased immune response could be possible.	
	<i>Lactobacillus plantarum</i> and <i>Lactobacillus rhamnosus</i>	BALB/c mice were injected with AOM and given dextrane sulfatesodium (DSS) to induce cancer, followed by the nonosized (nLp or live bacteria (pLp) for 4 weeks	nLp significant inhibited carcinogenesis and reduced proinflammatory cytokines expression.	
	<i>Lactobacillus fermentum</i> strains acidophilus	BALB/c mice injected with CT26 cells were fed with probiotics	Apoptosis induction, mediated by p53 activation and arrest of cell cycle.	
		Apc (Min/+) mice were gavaged with probiotics	Secretory immunoglobulin level increased.	
<i>Escherichia</i>	Symbioflor-2 (<i>E. coli</i> strains)	BALB/c mice injected with CT26 or RenCa cells were fed with Symbioflor-2	L. plantarum treated group: reduction in size of tumor and induced necrosis and lymphocyte infiltration increase in the cluster of differentiation (CD)4 ⁺ /CD8 ⁺ T-cell ratio, natural killer cells population and interferon (IFN)- γ production.	[103]
VSL#3 (Probiotics mixture)		C57BL/6 mice treated with cycles of DSS were given probiotics before and after the colitis induction	Reduction in small intestinal tumors number and proliferation of cell marker; CD8 cells level increased; No effect on polyp number of large intestine.	
			Colonization of tumors by probiotics; Low therapeutic efficacy on colon tumors.	[103]
			Reduced the dysplasia and tumor incidence in group treated with 15 th -cycle of DSS; cell proliferation marker level reduction including proliferation cell nuclear antigen, and cytokines including tumor necrosis factor- α , interleukin-Increase in IL-10 level	

by the administration of probiotics. In relation to mechanistic pathways, the T helper 17 (Th17) cell levels in the gut and Th17 recruitment to the site of the tumor were decreased in the probiotics-administered mice. The anti-cancer effect of the probiotics is also linked with the production of SCFAs, which can be reflected via the SCFAs enrichment-related pathway shown in probiotic-administered mice. All these findings provide a foundation for research on the potential mechanisms and roles in the treatment or therapy of liver or other types of cancer [111].

1.8 MELANOMA AND PROBIOTICS

Probiotics dietary intake has also been associated with the health of the skin [112–114]. In a study [115] focused on the gut microbiota variation and immunotherapy against melanoma, the effects of *Bifidobacterium* on melanoma were examined when taken alone and in combination with immunotherapy. When used in conjunction, the two methods of treatment were effective at reducing the size of the tumor in mice. In previous years, a study reported how probiotics influence the progression of leukemia. As stated in this investigation, eight unique *Lactobacillus fermentum* strains had an anti-proliferative effect on leukemia cells. Bacteria were predominantly killed by heat, and specific strain genomic DNA and cellular wall separation revealed inhibition of leukemia cells [116].

1.9 OTHER CANCERS

Despite the mentioned types of cancer, other cancers like lung and cervical cancer have been reported to be inhibited by the treatment or therapy of both *in vivo* and *in vitro* [58, 89–91, 117–121]. With much evidence supporting the effectiveness of probiotics in the suppression of cancer, further investigation into various types of cancer and the underlying possible mechanisms is clearly required.

1.10 APPROACHES THAT HELP PROBIOTICS WORK TO AVERT MALIGNANCY

Probiotics may slow the proliferation of abnormalities or carcinomas using a variety of distinctive strategies, such as intestinal microbiota modulation, antioxidant effects, degradation of carcinogenic compounds, as well as gut-associated modulation and systemic immune responses [122]. Among the

studies focused on the probiotic's defensive effects against extra-intestinal cancers, many of them concentrated on the immune-modulatory effects of probiotics concerning their inhibitory effect against extra-intestinal cancers.

1.10.1 CANCER IMMUNITY CYCLE

Anti-tumor responses involves various components of immune system like dendritic cells, natural killer cells, T- cells, and macrophages [123]. Chen and Mellman [124] investigated the tumor resistance cycle as a key way to which immunity can fight cancer. The dendritic cell identifies the antigen on a tumor cell and encourages T-cell activation in the lymph node. The effector T cells start functioning once an antibody is shown to the T cells, and they escape or enter a tumor. After becoming acquainted with the cancer cells, the effector T cells attack it. Dendritic cells are going to eliminate receiving the antigen from the dead cancer cells, restarting the cycle.

1.10.2 IMMUNOTHERAPY AND PROBIOTICS

In recent times, many scientists have centered on chemotherapy in an effort to establish an anti-cancer strategy that makes use of and modifies the patient's immune system. Recent research has primarily concentrated on the possible benefits of drugs that concentrate on proteins that serve as therapeutics, like programmed death-1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CT) that inhibit the activation of T cell and commotion [125]. [126] reported the significance in immunotherapy of gut microbiota. *Bacteroides fragilis* administration restored the effect CTLA-4 blockade, by influencing the interleukin-12 dependent Th1 immune response. [115] When compared to sole medication, *Bifidobacterium*, as well as programmed cell death-ligand-1 (PD-L1) blocking therapy, had a better anti-melanoma impact. It was suggested that certain *Bifidobacterium* species might play a role in defense by boosting the anti-tumor immune response and activating dendritic cells. The growing amount of antigen-specific T cells and tumor-specific T cells indicated that T-cell response activation was a worry.

1.10.3 INFLAMMATORY RESPONSE MODULATION

Probiotics not only help other cancer medicines work better, but they may also have their own anti-tumor effects by altering immune responses. The examined probiotics have demonstrated both anti-inflammatory and a cancer

prevention harm in various *in vitro* trials [58, 89, 91], illustrating that the two distinct influence are interrelated.

Causes of cancer have been hypothesized to embrace persistent aggravation [127–129]. For instance, reactive bowel diseases and colon cancer are substantial causes of risk for these types of cancers. In addition can aggravation play a role in bowel cancer linked to colitis, but it additionally happens in sporadic colon cancer and modify how the disorder develops [130–132]. It was reported that *Lctobacillus rhamnosus* GG prevents carcinogenesis of colon which is accompanied by the NFkB pathway suppression [92], a pro-inflammatory pathway that associated with colon cancer and IBD [133, 134]. Moreover, to the colon cancer, the chronic inflammation also takes part in the other types of the cancer, like HCC. The HCC risk can be augmented by the conditions of inflammation, like infection of hepatitis B, C virus. [110] exhibited a pro-inflammatory cytokine IL-17 reduction by the probiotics in the model of HCC, and skimpy the possible association flanked by anti-cancer consequence and immunomodulatory effect of probiotics.

1.10.4 MODIFICATION OF T-HELPER 17 CELL EXPRESSION AND PROLIFERATION

It is speculated that Th17 cells elevate the chance of concurrent inflamed and soothing responses. In a test, a combination of probiotics that delayed the onset of HCC additionally minimized the levels of IL-17, a proinflammatory cytokine [110]. The assortment of probiotics decreased the frequency of Th17 cells and its development to tumor, causes to a reduced conception of IL-17. From Th17, IL-17A favors angiogenesis [135, 136], IL-17 and Th17 level reduction may contribute to cancer progression inhibition. Despite of HCC evolution inhibition, variation of immune responses and Th17 cell inhabitants was also informed in a study that exhibits defense against carcinogenesis of colon by *L. casei* BL23 [99]. An increase in the population of Th17 cells and in the level of IL-17 was informed, which was opposing to the learning [110]. The anti-inflammatory IL-10 level did rise in both trials, indicating a balanced Th17-mediated response. Furthermore, the quantity of IL-22 climbed, which has a link with Th17 cells and is an anti-angiogenesis cytokine. While perspectives on the significance of IL-17 and Th17 in tumors remain conflicting [135, 136], they have revealed fresh approaches for probiotics' hindering carcinogenesis involving alteration of the general populace and action of Th17 cells.

1.10.5 INTERFERON Γ - LEVEL MODULATION

Despite of the IL-17 level modulation, the relationship between the augment in interferon-g (IFN-g) and decreasing in the prevalence of the cancer in the mice which are treated with the probiotics was also reported [115, 119, 137]. IFN-g is a multifunctional cytokine which is concerned with the fiery retort. It shows so many essential roles in the cycle of cancer-immunity, like up regulation of main histo-compatibility complex class I molecules, infiltration of T-cell and polarization of Th1 that causes various anti-tumor responses [138–140]. It is also accountable for the T cells differentiation into the cytotoxic T lymphocytes that are concerned in several cycle events [124].

1.11 CONCLUSION

Around are numerous in-vivo and in-vitro statistics through the dated revised regarding the anti-tumorigenic/anti-carcinogenic effects of strains of probiotics against the extra-intestinal and gastrointestinal cancers. Various mechanisms can be concerned in such beneficial effects, mostly via the gut microbiota modulation, which thereby affects the immunity and metabolism of host. Studies focusing on the utilization of probiotics as bio-therapeutics to treat malignancy have remained rare, nevertheless. In order to determine probable strains, administration, and quantity regimens for various stages and types of cancer, significant scientific studies are required. Similar to this, biomechanical research in rat or clinical models may reveal upgrades in dietary strategies and the development of brand-new molecular targets of therapy for avoidance and cure of cancer.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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KEYWORDS

- **anti-carcinogenic**
- **anti-tumorigenic**
- **bio-therapeutics**
- **cancer managements**
- **cancer therapy**
- **gut microbiota**
- **immunomodulation**
- **probiotics**

REFERENCES

1. Joint, F. (2002). *WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food*. London, Ontario, Canada.
2. Fuller, R. (1999). Probiotics for farm animals. In *Probiotics: A Critical Review* (pp. 15–22).
3. Metchnikoff, E. (1907). *The Prolongation of Life: Optimistic Studies*. G.P. Putman's Sons. Mitsuoka, T. (1992). The human gastrointestinal tract. In B.J.B. Wood (Ed.), *The Lactic Acid Bacteria in Health and Disease* (Vol. 1, pp. 69–114). Elsevier Applied Science.
4. Ritchie, M. L., & Romanuk, T. N. (2012). A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLOS ONE*, 7(4), e34938.
5. Nazir, Y., Hussain, S. A., Abdul Hamid, A., & Song, Y. (2018). Probiotics and their potential preventive and therapeutic role for cancer, high serum cholesterol, and allergic and HIV diseases. *BioMed Research International*, 2018, 1–17.
6. Ettinger, G., MacDonald, S. M., Reid, G., & Burton, J. P. (2014). The influence of the human microbiome and probiotics on cardiovascular health. *Gut Microbes*, 5(6), 719–728.
7. Di Felice, G., Barletta, B., Butteroni, C., & Corinti, S. (2008). Use of probiotic bacteria for prevention and therapy of allergic diseases: Studies in mouse model of allergic sensitization. *Journal of Clinical Gastroenterology*, 42, S130–S132.
8. Rossi, M., Keshavarzian, A., & Bishehsari, F. (2018). Nutraceuticals in colorectal cancer: A mechanistic approach. *European Journal of Pharmacology*, 833, 396–402.
9. Ma, E. L., Choi, Y. J., Choe, Y. H., & Lee, D. S. (2010). The anticancer effect of probiotic *Bacillus polyfermenticus* on human colon cancer cells is mediated through ErbB2 and ErbB3 inhibition. *International Journal of Cancer*, 127(4), 780–790.
10. Verhoeven, V., Renard, N., Makar, A., Lardon, F., Apers, L., Van Royen, P., & Bogers, J. P. (2013). Probiotics enhance the clearance of human papillomavirus-related cervical lesions. *European Journal of Cancer Prevention*, 22(1), 46–51.

11. Ezema, C. (2013). Probiotics in animal production: A review. *Journal of Veterinary Medicine and Animal Health*, 5(11), 308–316.
12. Reid, G. (2016). Probiotics: Definition, scope and mechanisms of action. *Best Practice & Research Clinical Gastroenterology*, 30(1), 17–25.
13. Holzapfel, W. H., Haberer, P., Snel, J., Schillinger, U., & Huis in't Veld, J. H. J. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. *The American Journal of Clinical Nutrition*, 73(2), 365S–373S.
14. Harzallah, D., & Belhadj, H. (2013). Lactic acid bacteria as probiotics: Characteristics, selection criteria and role in immunomodulation of human GI mucosal barrier. In M. Kongo (Ed.), *Lactic Acid Bacteria—R & D for Food, Health and Livestock Purposes* (pp. 197–216).
15. Fujisawa, T., Benno, Y., Yaeshima, T., & Mitsuoka, T. (1992). Taxonomic study of the *Lactobacillus acidophilus* group, with recognition of *Lactobacillus gallinarum* sp. nov. and *Lactobacillus johnsonii* sp. nov. and synonymy of *Lactobacillus acidophilus* group A3 (Johnson et al., 1980) with the type strain of *Lactobacillus amylovorus* (Nakamura, 1981). *International Journal of Systematic and Evolutionary Microbiology*, 42(3), 487–491.
16. Gupta, K., Stapleton, A. E., Hooton, T. M., Roberts, P. L., Fennell, C. L., & Stamm, W. E. (1998). Inverse association of H₂O₂-producing lactobacilli and vaginal *Escherichia coli* colonization in women with recurrent urinary tract infections. *Journal of Infectious Diseases*, 178(2), 446–450.
17. Klein, G., Pack, A., Bonaparte, C., & Reuter, G. (1998). Taxonomy and physiology of probiotic lactic acid bacteria. *International Journal of Food Microbiology*, 41(2), 103–125.
18. Ferlay, J., Steliarova-Foucher, E., Lortet-Tieulent, J., Rosso, S., Coebergh, J. W. W., Comber, H., Forman, D., & Bray, F. (2013). Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. *European Journal of Cancer*, 49(6), 1374–1403.
19. Fitzmaurice, C., Allen, C., Barber, R. M., Barregard, L., Bhutta, Z. A., Brenner, H., & Naghavi, M. (2017). Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the global burden of disease study. *JAMA Oncology*, 3(4), 524–548.
20. World Health Organization. (2020). *WHO Report on Cancer: Setting Priorities, Investing Wisely and Providing Care for All*.
21. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., & Bray, F. (2015). Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*, 136(5), E359–E386.
22. Shaheen, M., Allen, C., Nickoloff, J. A., & Hromas, R. (2011). Synthetic lethality: Exploiting the addiction of cancer to DNA repair. *Blood, The Journal of the American Society of Hematology*, 117(23), 6074–6082.
23. Anand, P., Kunnumakkara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., & Aggarwal, B. B. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research*, 25, 2097–2116.
24. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5), 646–674.

25. Vivarelli, S., Salemi, R., Candido, S., Falzone, L., Santagati, M., Stefani, S., & Libra, M. (2019). Gut microbiota and cancer: From pathogenesis to therapy. *Cancers*, 11(1), 38.
26. Maroof, H., Hassan, Z. M., Mobarez, A. M., Mohamadabadi, M. A., Ostad, S. N., & Bayati, K. (2012). *Lactobacillus acidophilus* could modulate the immune response against breast cancer in murine model. *Journal of Clinical Immunology*, 32, 1353–1359.
27. Hassan, Z. (2019). Anti-cancer and biotherapeutic potentials of probiotic bacteria. *Journal of Cancer Science and Therapy*, 11(1), 9–13.
28. Górská, A., Przystupski, D., Niemczura, M. J., & Kulbacka, J. (2019). Probiotic bacteria: A promising tool in cancer prevention and therapy. *Current Microbiology*, 76, 939–949.
29. O'Toole, P. W., Marchesi, J. R., & Hill, C. (2017). Next-generation probiotics: The spectrum from probiotics to live biotherapeutics. *Nature Microbiology*, 2(5), 1–6.
30. Rasouli, B. S., Khosravi, A., Mashkani, B., Taheripak, G., Hosseini, M., & Talebi, A. (2017). *In vitro* activity of probiotic *Lactobacillus reuteri* against gastric cancer progression by downregulation of urokinase plasminogen activator/urokinase plasminogen activator receptor gene expression. *Journal of Cancer Research and Therapeutics*, 13(2), 246–251.
31. Georgiev, K., Iliev, I., Stefanova, S., Koycheva, I., & Dimitrova, E. (2015). Antiproliferative effect of Bulgarian spring water probiotics (Lakter a Nature Probiotics®), against human colon carcinoma cell line. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(6), 130–136.
32. Kumar, M., Nagpal, R., & Hemalatha, R. (2010). Cancer-preventing attributes of probiotics: An update. *International Journal of Food Sciences and Nutrition*, 61(5), 473–496.
33. Raman, M., Ambalam, P., Doble, M., & Vyas, M. (2013). Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. *Gut Microbes*, 4(3), 181–192.
34. Sharifi, M., Moridnia, A., Mortazavi, D., Keikha, M., & Shoja, S. (2017). Kefir: A powerful probiotic with anticancer properties. *Medical Oncology*, 34(1), 1–7.
35. Antony, U., & Chandra, T. S. (1997). Microbial population and biochemical changes in fermenting finger millet (*Eleusine coracana*). *World Journal of Microbiology and Biotechnology*, 13, 533–537.
36. Kumar, R. S., Kanmani, P., Yuvaraj, N., Paari, K. A., Pattukumar, V., Arul, V., & Thirunavukkarasu, K. S. (2012). *Lactobacillus plantarum* AS1 isolated from South Indian fermented food Kallappam suppress 1,2-dimethyl hydrazine (DMH)-induced colorectal cancer in male Wistar rats. *Applied Biochemistry and Biotechnology*, 166, 620–631.
37. El Sheikha, A. F., & Hu, D. M. (2020). Molecular techniques reveal more secrets of fermented foods. *Critical Reviews in Food Science and Nutrition*, 60(1), 11–32.
38. Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2016). Functional properties of microorganisms in fermented foods. *Frontiers in Microbiology*, 7, 578.
39. Filannino, P., Di Cagno, R., & Gobbetti, M. (2013). Exploitation of the health-promoting and sensory properties of organic pomegranate (*Punica granatum* L.) juice through lactic acid fermentation. *International Journal of Food Microbiology*, 163(2, 3), 184–192.
40. Pereira, A. L. F., Feitosa, W. A. O., Abreu, V. K. G., Rodrigues, S., & Narain, N. (2017). Impact of fermentation conditions on the quality and sensory properties of a probiotic cupuassu (*Theobroma grandiflorum*) beverage. *Food Research International*, 100, 603–611.

41. Zhou, Y., Zheng, J., Li, Y., Xu, D. P., Li, S., Chen, Y. M., & Li, H. B. (2016). Dietary natural products for prevention and treatment of liver cancer. *Nutrients*, 8(3), 156.
42. Fu, L., Xu, B. T., Xu, X. R., Qin, X. S., Gan, R. Y., & Li, H. B. (2010). Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. *Molecules*, 15(12), 8602–8617.
43. Deng, G. F., Lin, X., Xu, X. R., Gao, L. L., Xie, J. F., Li, H. B., & Li, H. B. (2013). Antioxidant capacities and total phenolic contents of 56 vegetables. *Journal of Functional Foods*, 5(1), 260–266.
44. Kausar, H., Jeyabalan, J., Aqil, F., Gupta, R. C., & Agarwal, M. L. (2012). Berry anthocyanidins synergistically suppress growth and invasive potential of human non-small-cell lung cancer cells. *Cancer Letters*, 325(1), 54–62.
45. Li, A. N., Li, S., Li, H. B., Xu, D. P., Xu, X. R., & Chen, F. (2014). Resources and biological activities of natural polyphenols. *Nutrients*, 6(12), 6020–6047.
46. Shi, J., Liu, F., Zhang, W., Zhang, C., Wang, S., Zhang, X., & Zhou, G. (2015). Epigallocatechin-3-gallate inhibits nicotine-induced migration and invasion by the suppression of angiogenesis and epithelial-mesenchymal transition in non-small cell lung cancer cells. *Oncology Reports*, 33(6), 2972–2980.
47. Li, F., Wu, X., Tsang, W. P., Zhang, Z., & Wong, M. S. (2013). Antiproliferative activities of tea and herbal infusions. *Food & Function*, 4(4), 530–538.
48. Bedada, T. L., Lyu, J. K., Kim, G. D., & Choi, J. S. (2020). Probiotics for cancer alternative prevention and treatment. *Biomedicine & Pharmacotherapy*, 129, 110409.
49. Choi, S., Kim, Y., Han, K. S., You, S., Oh, S., & Kim, S. H. (2006). Effects of *Lactobacillus* strains on cancer cell proliferation and oxidative stress *In vitro*. *Letters in Applied Microbiology*, 42(5), 452–458.
50. Kim, J. E., Woo, J. Y., & Kim, Y. S. (2007). Cancer chemo preventive effects of lactic acid bacteria. *Journal of Microbiology and Biotechnology*, 17(8), 1227–1235.
51. Albookkarami, H. (2017). Investigating the role of probiotics in coping with cancer and health in society. *Cancer Press Journal*, 3(4), 188–196.
52. Zhong, L., Zhang, X., & Covasa, M. (2014). Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World Journal of Gastroenterology*, 20(24), 7878–7886.
53. Liu, C. T., Pan, T. M., & Tsai, Y. C. (2011). Antiproliferative and anticytotoxic effects of cell fractions and exopolysaccharides from *Lactobacillus casei* 01. *Mutation Research/ Genetic Toxicology and Environmental Mutagenesis*, 721(2), 157–162.
54. Thirabunyanon, M., Boonprasom, P., & Niamsup, P. (2009). Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. *Biotechnology Letters*, 31, 571–576.
55. Tiptiri-Kourpeti, A., Spyridopoulou, K., Santarmaki, V., Aindelis, G., Lamprianidou, E., Saxami, G., & Lamprianidou, E. (2016). *Lactobacillus casei* exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. *PLOS ONE*, 11(2), e0147960.
56. Jacouton, E., Chain, F., Sokol, H., Langella, P., & Bermúdez-Humarán, L. G. (2017). Probiotic strain *Lactobacillus casei* BL23 prevents colitis-associated colorectal cancer. *Frontiers in Immunology*, 8, 1553.
57. Ghanavati, R., Nobakht, M., Sabzi, M., & Ebrahimi, M. (2020). Inhibitory effects of *Lactobacilli* cocktail on HT-29 colon carcinoma cells growth and modulation of the Notch and Wnt/ β -catenin signaling pathways. *Microbial Pathogenesis*, 139, 103829.

58. Han, K. J., Lee, J. Y., Park, M. H., Jeong, Y. J., Park, S. H., & Kim, J. E. (2015). Anticancer and anti-inflammatory activity of probiotic *Lactococcus lactis* NK34. *Journal of Microbiology and Biotechnology*, 25(10), 1697–1701.
59. Tarrah, A., da Silva Duarte, V., de Castilhos, J., dos Reis, A. R., & Peralta, R. M. (2018). *In vitro* probiotic potential and anti-cancer activity of newly isolated folate-producing *Streptococcus thermophilus* strains. *Frontiers in Microbiology*, 9, 2214.
60. Jacouton, E., Michel, M. L., Chain, F., & Bermúdez-Humarán, L. G. (2019). Anti-tumoral effects of recombinant *Lactococcus lactis* strain secreting IL-17A cytokine. *Frontiers in Microbiology*, 9, 3355.
61. Sivieri, K., Bacchi, V. D., Machado, A. M., Berto, D. A., Correia, L. L., & Gilliland, S. E. (2008). Probiotic *Enterococcus faecium* CRL 183 inhibit chemically induced colon cancer in male Wistar rats. *European Food Research and Technology*, 228, 231–237.
62. Toi, M., Hirota, S., Tomotaki, A., Hozumi, Y., Anan, K., Takada, M., & Ohno, S. (2013). Probiotic beverage with soy isoflavone consumption for breast cancer prevention: A case-control study. *Current Nutrition & Food Science*, 9(3), 194–200.
63. Zaharuddin, L., Mokhtar, N. M., Hashim, N. M., & Nasir, N. M. (2019). A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterology*, 19(1), 1–8.
64. Pala, V., Krogh, V., Berrino, F., Sieri, S., Grioni, S., Tjønneland, A., & Vineis, P. (2011). Yogurt consumption and risk of colorectal cancer in the Italian European prospective investigation into cancer and nutrition cohort. *International Journal of Cancer*, 129(11), 2712–2719.
65. Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *The Journal of Nutrition*, 125(6), 1401–1412.
66. Lim, C. C., Ferguson, L. R., & Tannock, G. W. (2005). Dietary fibres as "prebiotics": Implications for colorectal cancer. *Molecular Nutrition & Food Research*, 49(6), 609–619.
67. Rowland, I. R., Bearne, C. A., Fischer, R., & Pool-Zobel, B. L. (1996). The effect of lactulose on DNA damage induced by DMH in the colon of human flora-associated rats. *Nutrition and Cancer*, 26(1), 37–47.
68. Gavresea, F., Bulea, D., Păunescu, V., Mureșan, R., & Nagy, A. (2018). Beneficial effect of symbiotic on experimental colon cancer in rats. *The Turkish Journal of Gastroenterology*, 29(4), 494.
69. Harris, P. J., & Ferguson, L. R. (1993). Dietary fiber: Its composition and role in protection against colorectal cancer. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 290(1), 97–110.
70. Vaahtovuori, J., Munukka, E., Korkeamäki, M., Luukkainen, R., & Toivanen, P. (2008). Fecal microbiota in early rheumatoid arthritis. *The Journal of Rheumatology*, 35(8), 1500–1505.
71. Russell, S. L., Gold, M. J., Hartmann, M., Willing, B. P., Thorson, L., Wlodarska, M., & Finlay, B. B. (2012). Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Reports*, 13(5), 440–447.
72. Lin, C., Wan, L., Geng, Y., & Jin, L. (2019). Role of gut microbiota in the development and treatment of colorectal cancer. *Digestion*, 100(1), 72–78.

73. Dos Reis, S. A., da Conceição, L. L., Siqueira, N. P., Rosa, D. D., da Silva, L. L., & Peluzio, M. d. C. G. (2017). Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutrition Research*, 37, 1–19.
74. Manichanh, C., Borruel, N., Casellas, F., & Guarner, F. (2012). The gut microbiota in IBD. *Nature Reviews Gastroenterology & Hepatology*, 9(10), 599–608.
75. Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana, L., & Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, 332(6032), 970–974.
76. Shamekhi, S., Zare-Zardini, H., Yaghoubi, A., Bahramian, H., & Ashraf, M. J. (2020). An overview of yeast probiotics as cancer biotherapeutics: Possible clinical application in colorectal cancer. *Clinical and Translational Oncology*, 22, 1227–1239.
77. Kotzampassi, K., Giamarellos-Bourboulis, E. J., Voudouris, A., Kazamias, P., & Eleftheriadis, E. (2015). A four-probiotics regimen reduces postoperative complications after colorectal surgery: A randomized, double-blind, placebo-controlled study. *World Journal of Surgery*, 39, 2776–2783.
78. Liang, S., Xu, L., Zhang, D., Wu, Z., Zhang, G., & Liu, H. (2016). Effect of probiotics on small intestinal bacterial overgrowth in patients with gastric and colorectal cancer. *Turkish Journal of Gastroenterology*, 27(3), 227–232.
79. Yang, Y., Xia, Y., Chen, H., Hong, L., Feng, J., Yang, J., & Cao, H. (2016). The effect of perioperative probiotics treatment for colorectal cancer: Short-term outcomes of a randomized controlled trial. *Oncotarget*, 7(7), 8432–8440.
80. Liu, D., Jiang, P., & Lei, D. (2016). Effects of probiotics on intestinal mucosa barrier in patients with colorectal cancer after operation: Meta-analysis of randomized controlled trials. *Medicine*, 95(15), e3342.
81. Aisu, N., et al. (2015). Impact of perioperative probiotic treatment for surgical site infections in patients with colorectal cancer. *Experimental and Therapeutic Medicine*, 10(3), 966–972.
82. Abdulmir, A. S., Hafidh, R. R., & Bakar, F. A. (2010). Molecular detection, quantification, and isolation of *Streptococcus gallolyticus* bacteria colonizing colorectal tumors: Inflammation-driven potential of carcinogenesis via IL-1, COX-2, and IL-8. *Molecular Cancer*, 9(1), 1–18.
83. Burns, M. B., et al. (2015). Virulence genes are a signature of the microbiome in the colorectal tumor microenvironment. *Genome Medicine*, 7(1), 1–12.
84. Ahn, J., et al. (2013). Human gut microbiome and risk for colorectal cancer. *Journal of the National Cancer Institute*, 105(24), 1907–1911.
85. Kahouli, I., et al. (2015). *In-vitro* characterization of the anti-cancer activity of the probiotic bacterium *Lactobacillus fermentum* NCIMB 5221 and potential against colorectal cancer. *Journal of Cancer Science and Therapy*, 7(7), 224–235.
86. Kahouli, I., et al. (2015). Identification of *Lactobacillus fermentum* strains with potential against colorectal cancer by characterizing short chain fatty acids production, anti-proliferative activity and survival in an intestinal fluid: *In vitro* analysis. *Journal of Bioanalysis & Biomedicine*, 7, 1–6.
87. Kahouli, I., et al. (2015). Screening and *in-vitro* analysis of *Lactobacillus reuteri* strains for short chain fatty acids production, stability and therapeutic potentials in colorectal cancer. *Journal of Bioequivalence & Bioavailability*, 7(1), 39.

88. Huang, L., et al. (2016). Effects of *L. paracasei* subsp. *paracasei* X12 on cell cycle of colon cancer HT-29 cells and regulation of mTOR signaling pathway. *Journal of Functional Foods*, 21, 431–439.
89. Lee, N. K., et al. (2015). The prophylactic effect of probiotic *Bacillus polyfermenticus* KU3 against cancer cells. *Journal of Functional Foods*, 14, 513–518.
90. Nami, Y., et al. (2015). The prophylactic effect of probiotic *Enterococcus lactis* IW5 against different human cancer cells. *Frontiers in Microbiology*, 6, 1317.
91. Lee, N. K., et al. (2015). Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi. *LWT-Food Science and Technology*, 64(2), 1036–1041.
92. Blasingame, C. A., et al. (2016). Modulation of colorectal cancer by the probiotic organism *Lactobacillus reuteri*. *Professional Agricultural Workers Journal (PAWJ)*, 3(174-2016-2152).
93. Kocijancic, D., et al. (2016). Therapy of solid tumors using probiotic *Symbioflor-2*—restraints and potential. *Oncotarget*, 7(16), 22605–22621.
94. Kuugbee, E. D., et al. (2016). Structural change in microbiota by a probiotic cocktail enhances the gut barrier and reduces cancer via TLR2 signaling in a rat model of colon cancer. *Digestive Diseases and Sciences*, 61, 2908–2920.
95. Gamallat, Y., et al. (2016). *Lactobacillus rhamnosus* induced epithelial cell apoptosis, ameliorates inflammation and prevents colon cancer development in an animal model. *Biomedicine & Pharmacotherapy*, 83, 536–541.
96. Walia, S., et al. (2015). Cyclooxygenase as a target in chemoprevention by probiotics during 1, 2-dimethylhydrazine induced colon carcinogenesis in rats. *Nutrition and Cancer*, 67(4), 603–611.
97. Zhang, M., et al. (2015). Effects of *Lactobacillus salivarius* Ren on cancer prevention and intestinal microbiota in 1, 2-dimethylhydrazine-induced rat model. *Journal of Microbiology*, 53, 398–405.
98. Dubey, V., et al. (2016). Appraisal of the anti-cancer potential of probiotic *Pediococcus pentosaceus* GS4 against colon cancer: *In vitro* and *in vivo* approaches. *Journal of Functional Foods*, 23, 66–79.
99. Lenoir, M., et al. (2016). Retracted Article: *Lactobacillus casei* BL23 regulates T reg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. *Journal of Gastroenterology*, 51, 862–873.
100. Lee, H. A., et al. (2015). Dead nano-sized *Lactobacillus plantarum* inhibits azoxymethane/dextran sulfate sodium-induced colon cancer in Balb/c mice. *Journal of Medicinal Food*, 18(12), 1400–1405.
101. Hu, J., et al. (2015). Anti-tumor immune effect of oral administration of *Lactobacillus plantarum* to CT26 tumor-bearing mice. *Journal of Biosciences*, 40, 269–279.
102. Urbanska, A. M., et al. (2016). Orally delivered microencapsulated probiotic formulation favorably impacts polyp formation in APC (Min/+) model of intestinal carcinogenesis. *Artificial Cells, Nanomedicine, and Biotechnology*, 44(1), 1–11.
103. Talero, E., et al. (2015). Inhibition of chronic ulcerative colitis-associated adenocarcinoma development in mice by VSL# 3. *Inflammatory Bowel Diseases*, 21(5), 1027–1037.
104. Hassan, Z., et al. (2016). Anti-breast cancer effects of live, heat-killed and cytoplasmic fractions of *Enterococcus faecalis* and *Staphylococcus hominis* isolated from human breast milk. *In vitro Cellular & Developmental Biology-Animal*, 52, 337–348.

105. Esfandiary, A., et al. (2016). Lactobacilli modulate hypoxia-inducible factor (HIF)-1 regulatory pathway in triple negative breast cancer cell line. *Cell Journal (Yakhteh)*, 18(2), 237.
106. Raman, M., et al. (2013). Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clinical Gastroenterology and Hepatology*, 11(7), 868–875.e3.
107. Wong, V. W. S., et al. (2013). Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis—A longitudinal study. *PLOS One*, 8(4), e62885.
108. De Minicis, S., et al. (2014). Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. *Hepatology*, 59(5), 1738–1749.
109. Yoshimoto, S., et al. (2013). Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature*, 499(7456), 97–101.
110. Li, J., et al. (2016). Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proceedings of the National Academy of Sciences*, 113(9), E1306–E1315.
111. So, S. S., Wan, M. L., & El-Nezami, H. (2017). Probiotics-mediated suppression of cancer. *Current Opinion in Oncology*, 29(1), 62–72.
112. Bouilly-Gauthier, D., et al. (2010). Clinical evidence of benefits of a dietary supplement containing probiotic and carotenoids on ultraviolet-induced skin damage. *British Journal of Dermatology*, 163(3), 536–543.
113. Viljanen, M., et al. (2005). Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: A double-blind placebo-controlled trial. *Allergy*, 60(4), 494–500.
114. Hoang, B. X., et al. (2010). *Lactobacillus rhamnosus* cell lysate in the management of resistant childhood atopic eczema. *Inflammation & Allergy-Drug Targets*, 9(3), 192–196.
115. Sivan, A., et al. (2015). Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*, 350(6264), 1084–1089.
116. Tuo, Y., et al. (2015). Antiproliferative effect of 8 different *Lactobacillus* strains on K562 cells. *Journal of Dairy Science*, 98(1), 106–110.
117. Nouri, Z., et al. (2016). Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. *Cell Journal (Yakhteh)*, 18(2), 127.
118. Motevaseli, E., et al. (2016). The effect of *Lactobacillus crispatus* and *Lactobacillus rhamnosus* culture supernatants on expression of autophagy genes and HPV E6 and E7 oncogenes in the HeLa cell line. *Cell Journal (Yakhteh)*, 17(4), 601.
119. Gui, Q., et al. (2015). Well-balanced commensal microbiota contributes to anti-cancer response in a lung cancer mouse model. *Genetics and Molecular Research*, 14(2), 5642–5651.
120. Abd El Ghany, K., et al. (2015). A potential role of *Lactobacillus acidophilus* LA1 and its exopolysaccharides on cancer cells in male albino mice. *Biotechnology & Biotechnological Equipment*, 29(5), 977–983.
121. Shin, R., et al. (2016). Anti-tumor activity of heat-killed *Lactobacillus plantarum* BF-LP284 on Meth-A tumor cells in BALB/c mice. *International Journal of Food Sciences and Nutrition*, 67(6), 641–649.
122. de Moreno, M. A. (2014). The administration of probiotics and fermented products containing lactic acid bacteria exert beneficial effects against intestinal and non-intestinal cancers.

123. Liu, Y., & Zeng, G. (2012). Cancer and innate immune system interactions: Translational potentials for cancer immunotherapy. *Journal of Immunotherapy (Hagerstown, Md.: 1997)*, 35(4), 299.
124. Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: The cancer-immunity cycle. *Immunity*, 39(1), 1–10.
125. Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer*, 12(4), 252–264.
126. Vétizou, M., et al. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*, 350(6264), 1079–1084.
127. Mantovani, A., et al. (2008). Cancer-related inflammation. *Nature*, 454(7203), 436–444.
128. Kundu, J. K., & Surh, Y. J. (2008). Inflammation: Gearing the journey to cancer. *Mutation Research/Reviews in Mutation Research*, 659(1, 2), 15–30.
129. Perwez Hussain, S., & Harris, C. C. (2007). Inflammation and cancer: An ancient link with novel potentials. *International Journal of Cancer*, 121(11), 2373–2380.
130. Grivennikov, S. I., et al. (2012). Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumor growth. *Nature*, 491(7423), 254–258.
131. Tosolini, M., et al. (2011). Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, Th2, Treg, Th17) in patients with colorectal cancer. *Cancer Research*, 71(4), 1263–1271.
132. West, N. R., et al. (2015). Emerging cytokine networks in colorectal cancer. *Nature Reviews Immunology*, 15(10), 615–629.
133. Grivennikov, S., et al. (2009). IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell*, 15(2), 103–113.
134. Greten, F. R., et al. (2004). IKK β links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell*, 118(3), 285–296.
135. Murugaian, G., & Saha, B. (2009). Protumor vs antitumor functions of IL-17. *The Journal of Immunology*, 183(7), 4169–4175.
136. Bailey, S. R., et al. (2014). Th17 cells in cancer: The ultimate identity crisis. *Frontiers in Immunology*, 5, 276.
137. Fooladi, A. A. I., et al. (2015). Th1 cytokine production induced by *Lactobacillus acidophilus* in BALB/c mice bearing transplanted breast tumor. *Jundishapur Journal of Microbiology*, 8(4).
138. Zaidi, M. R., & Merlino, G. (2011). The Two Faces of Interferon- γ in Cancer. *Clinical Cancer Research*, 17(19), 6118–6124.
139. Mandai, M., et al. (2016). Dual faces of IFN γ in cancer progression: A role of PD-L1 induction in the determination of pro-and antitumor immunity. *Clinical Cancer Research*, 22(10), 2329–2334.
140. Beatty, G. L., & Paterson, Y. (2001). Regulation of tumor growth by IFN- γ in cancer immunotherapy. *Immunologic Research*, 24, 201–210.



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CHAPTER 2

Probiotics as a Promising Candidate for the Management of Colorectal Cancer

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ABSTRACT

Bacteria included in probiotics, particularly *Bifidobacterium* and *Lactobacillus*, have been shown to cause an anti-tumor effect by enhancing apoptosis of cancer cells and protecting against oxidative stress. *Fusobacterium*, a cancer-producing bacteria, is also reduced by probiotic supplementation. The *Bifidobacterium* present in probiotic supplements also diminishes the mucositis and diarrhea induced by chemotherapy and radiation, respectively. The next generation of probiotics, including *Bacteroides fragilis*, *A. muciniphila*, and *B. pullicaecorum*, exhibit significant anticancer effects. Some of the probiotic strains and their metabolites have the capability to improve the intestinal immune system by attenuating the development of tumor cells. The probiotics encourage the differentiation of macrophages and dendritic cells, escalating the activation of NK and cytotoxic T cells. A potential method to enhance the tumor's receptivity to chemotherapy drugs

is to alter the gut flora composition. Probiotics may contribute to the prevention and treatment of colon cancer through one of three ways: colonization resistance, immunity modulation, and improved function of the gut barrier. Probiotic supplements strengthen the immune system and intestinal health in colorectal patients, boost antimicrobial defense, and neutralize carcinogenic chemicals. The positive effects of probiotic supplementation depend on the host's physiology, the severity of the condition, the strain, the dosage, the length of the intervention, and other dietary supplements.

2.1 INTRODUCTION

Cancer of the colon or rectum, part of the intestine, is considered the most common cancer of the gastrointestinal tract. Decades ago, the incidence of colorectal cancer was low. Nonetheless, it has emerged as a common malignancy and currently represents approximately 10% of deaths in Western nations due to cancer. The most prevalent form of gastrointestinal cancer is colorectal cancer, which is one of the globe's biggest health issues. Colorectal cancer ranks among the top three most prevalent malignancies and is within the top four most frequent root causes of cancer-related mortality. Around 4%–5% of people may acquire colorectal cancer, and personal traits or behaviors are linked to a higher chance of acquiring the disease [1–4].

Colorectal cancer is a prominent example of cancer associated with a sedentary lifestyle and other external factors like unhealthy food habits, obesity, consumption of red meat and alcohol, metabolic disorders, and heredity. In contrast, fruits and vegetables alleviate the situation in colorectal cancer [5]. This type of cancer is more prevalent in industrialized developed countries, including Australia, Europe, New Zealand, and North America. According to the American Cancer Society, the estimated number of colorectal cancer cases is 106,970 new cases in the United States for 2023, and it is more common in men than in women. In India, the incidence of colorectal cancer is rising, although the overall incidence from a global perspective is low. In India, colorectal cancer caused 35,385 deaths in 2020, accounting for 4.15% of overall cancer-related deaths. It is assumed that by 2030, new cases of colorectal cancer will exceed 2.2 million. The current treatments include local surgical resection, followed by chemotherapy, which may involve single-drug therapy with fluoropyrimidine and multiple-drug therapy consisting of oxaliplatin, irinotecan, and capecitabine. Targeted therapy, where monoclonal antibodies play a role, includes cetuximab and

panitumumab for the epidermal growth factor receptor, while bevacizumab targets the vascular endothelial growth factor-A receptor [6].

Some bacteria can promote colorectal cancer, including species such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Escherichia coli*, and *Salmonella* sp., which contribute to the progression of colorectal cancer. These bacteria release toxic substances that damage the host's DNA. Examples include colibactin, released from *Escherichia coli*, and typhoid toxin, released from *Salmonella*. Other mechanisms include influencing the E-cadherin and SMO/p38 MAPK/RAS signaling pathways. These bacteria and toxins act as biomarkers of colorectal cancer. Specific antibiotics for these bacteria, like metronidazole and the macrolide class of drugs, can kill the microorganisms [7].

The human body consists of diversified microflora. Gastrointestinal microflora is established from birth and balanced throughout a lifetime to maintain a healthy human body. Gut microflora is comprised predominantly of four kinds of microorganisms: *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*. The mode of baby delivery from the mother's womb is also a contributing factor to microbial colonization in the baby's gut. If the baby is delivered vaginally, they are colonized with lactobacilli, and their fecal microbiota will resemble their mother's fecal microbiota by 72%. However, if the baby is delivered via C-section, they are depleted of the *Bacteroides* genus, and their fecal microbiota resembles their mother's by only 41% [8].

The microbial ecosystem consists of more than 10^{12} colony forming units (CFU/g) with 1,000 microorganism species. Microorganisms help in the metabolism and fermentation of various non-digestible fibers, produce vitamins, create metabolites beneficial to the host's body, degrade bile salts and cholesterol, and resist pathogens. The intestinal barrier encompasses the epithelial and mucus layers, limiting the exposure of gut microflora to the host immune system, which contributes to protection and maintains homeostasis. If the small intestine and large intestine are constantly exposed to pathogenic bacteria, it creates a higher chance of gastric, cervical, and colorectal cancer. Several factors modify the microbial ecosystem in the gut, including antibiotics and poor diet. Altered and compromised gut microflora, termed dysbiosis, can cause DNA damage, changes in molecular activities, and alterations in homeostasis, and it has a strong relationship with the induction of irritable bowel syndrome (IBS) as well as colorectal cancer [9].

Figure 2.1 depicts majorly used genera of microbial organisms in probiotic products.

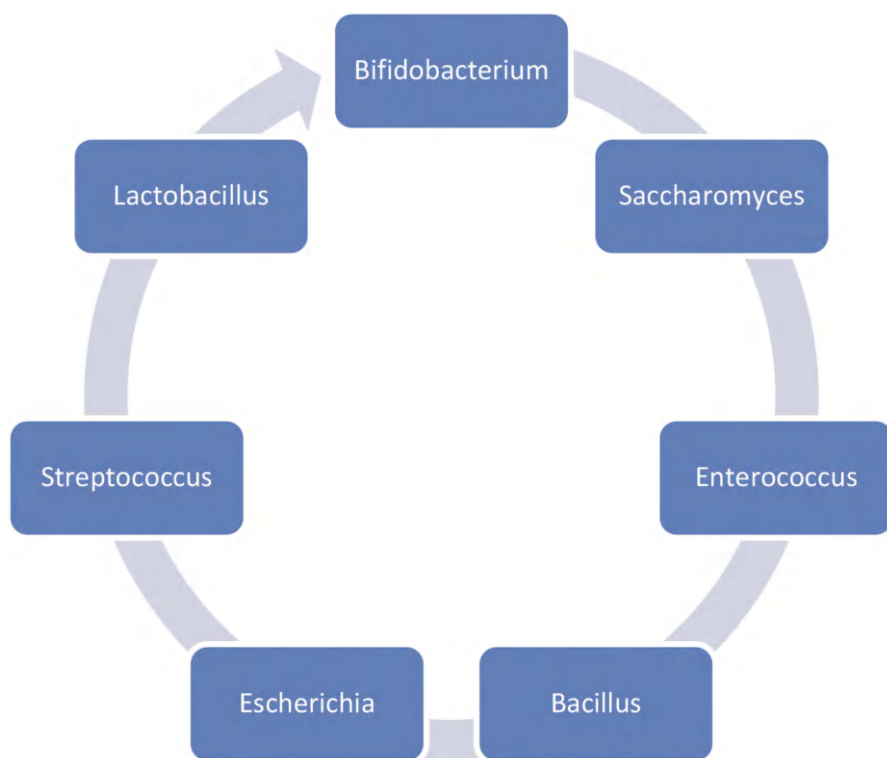


FIGURE 2.1 Majorly used genera of microbial organisms in probiotic products.

Colorectal cancer starts within the lining of the colon or rectum in the form of lumps of cells called polyps. These polyps are usually not carcinogenic but may sometimes develop into cancer; the frequency is increasing in the modernized world. The growth starts in the innermost layer of the gastrointestinal tract and spreads to the outer layer, and then to the blood and lymph, through which it can spread to various parts of the body [3, 4].

2.1.1 TYPES OF COLORECTAL CANCER

Adenocarcinomas make up most colon cancers. These tumors begin in the cells that produce the mucus that coats the rectum and colon. This is the kind of cancer that doctors are usually referring to when they discuss colorectal cancer. The prognosis for some subtypes of adenocarcinoma, such as signet ring and mucinous, may be worse than for other subtypes of adenocarcinoma

[10]. The colon and the rectum can also be the site of other, considerably less typical tumor forms. Among them are:

- **Carcinoid tumors:** They originate in the gut from distinct hormone-producing cells.
- **Gastrointestinal stromal tumors (GISTs):** Interstitial cells of Cajal, a unique type of cell found in the wall of the colon, are the origin of gastrointestinal stromal tumors. Some are benign (not cancerous). These tumors can develop anywhere throughout the digestive tract, although the colon is rarely where they occur.
- **Lymphomas:** Immune system cell malignancies include lymphomas. However, they can also begin in the colon, rectum, or other organs. They often start in lymph nodes. Non-Hodgkin lymphoma contains details on lymphomas of the gastrointestinal tract.
- **Sarcomas:** Sarcomas can begin in the wall of the colon and rectum, in blood vessels, muscle layers, or other connective tissues. Colon or rectal sarcomas are uncommon.

Figure 2.2 illustrates the types of colorectal cancers.

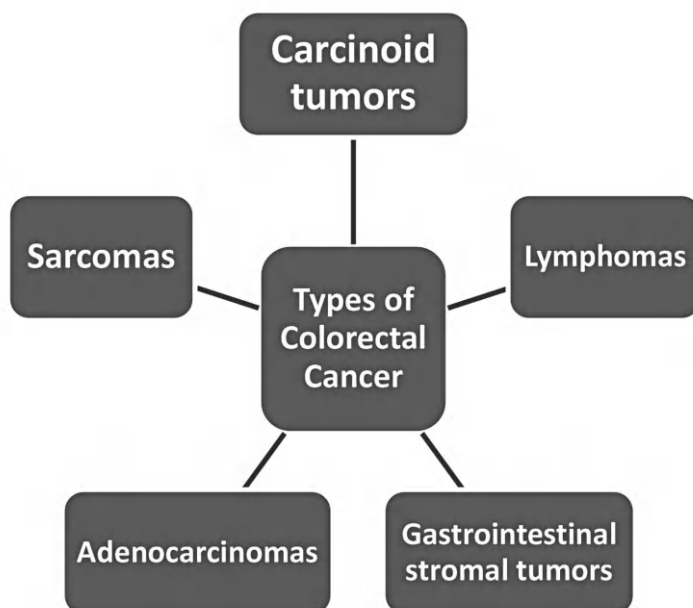


FIGURE 2.2 Types of colorectal cancers.

2.1.1.1 SCREENING OF COLORECTAL CANCER

The procedure of checking for cancer in people who have no symptoms is known as screening. Several tests are available to screen for colorectal cancer. These examinations are classified as stool-based visual assessments, chemical or instrumental testing, and straight radiological or endoscopic techniques. A colonoscopy should be performed if any abnormal test results are found. When colon cancer is detected early, the patient typically has a better prognosis. The US Preventive Services Task Force (USPSTF) advised screening for colorectal cancer beginning at the age of 50 and lasting until the age of 75 in 2016. The task group advised that screening for colorectal cancer in individuals aged 76 to 85 years be individualized, taking into consideration the patient's general health and past screening history [11–14].

Stool examination is the first step toward the examination of colorectal cancer. As colorectal cancer increases in size, it starts bleeding, and the presence of blood in stool can be used as an identification test. Fecal immunohistochemical testing involves the examination of the presence of blood in stool using monoclonal or polyclonal antibody reactions. Antibodies form a complex with hemoglobin. Other stool-based tests include stool DNA testing, which is still an area for researchers to work on, in addition to the use of RNA or protein biomarkers.

Probiotics are living microorganisms, consumed in adequate amounts to restore the gut microflora, and they claim to improve some medical conditions such as diarrhea and inflammation. To improve colorectal cancer conditions, the bacteria present in the probiotics must have that capacity, as lactic acid bacteria (LAB) have reportedly modulated the natural defense mechanism and improved the mucosal barrier of the host [15]. Probiotics can increase the number of strains of bacteria. They increase mucus secretion to prohibit colonization by pathogenic bacteria. Fecal samples can provide some idea of intestinal flora [16].

2.1.1.2 MECHANISM INVOLVED OF PROBIOTICS AND ITS DERIVATIVES AGAINST COLORECTAL CANCER

Tumor growth consists of several stages. First, in stage 0, the lesion of cancer remains located where it started. In stage 1, cancer is small and hasn't spread to any parts of the body. In stage 2, cancer cells grow up to 5 cm. In stage 3, cancer cells invade the surrounding cells or lymph nodes. Lastly, cancer cells travel or metastasize to another organ [17]. Probiotics attach to the epithelial

layer of the host intestine and exhibit antibacterial activity towards pathogenic microorganisms, as well as compete with pathogenic bacteria for their required space and nutrition. In return, the desired microbes from probiotics release bacteriocins, deconjugated bile acids, and lactic acid. These chemicals inhibit pathogenic microbes from causing cancer [18]. Microbes can associate with cancer cells in the first three stages, initiating an inflammatory response that alters the tumor microenvironment. Cytokines and protease growth factors are released from inflammatory responses, responsible for gene mutation and enhanced cancer growth. As a result, epithelial cells become hyperplastic, which may lead to adenocarcinoma [19]. If adenocarcinoma invades the submucosa and spreads in the colon, it transforms into prominent colon cancer [20]. Colorectal cancer can be treated by modifying the microbial composition with probiotics, which can build up the integrity of the epithelial barrier, improve mucus cell production, and antagonize pathogenic microorganisms [18]. Examples of pathogenic microorganisms that can induce colorectal cancer include *Bacteroides*, *Salmonella*, *Prevotella*, *Eubacterium*, and *Proteobacteria*. Probiotics increase the number of *Bifidobacterium* and *Lactobacillus*, which improve the immune system of the host, modify metabolism, and stop the formation of carcinogenic metabolites such as N-methyl-N-nitro-N-nitrosoguanidine, aromatic amines, sodium azides, benzopyrene, and reactive oxygen species (ROS), as well as mutagens such as deoxycholic acid, produced from bile acid. Probiotics can alleviate the removal of carcinogenic and mutagenic metabolites, and in place of those toxic metabolites, they generate anti-carcinogenic antioxidant metabolites like glutathione reductase and superoxide dismutase [21]. Employing probiotics on a daily basis can regulate gastric pH and improve dietary fiber fermentation.

2.1.1.3 PREVENTIVE ACTION PROVIDED BY LACTOBACILLUS BACTERIA IN DYSBIOSIS

Lactobacilli species, including *Lactobacillus casei*, induce the production of interleukin-12 (IL-12) from macrophages and influence the production of interferon-gamma (IFN- γ). The IL-12 receptor is present on T-cells and NK cells, and it shows antiangiogenic properties, exerting antiangiogenic chemicals like inducible protein-10 (IP-10) and monokine induced by gamma interferon (MIG), which inhibit tumor growth. *Lactobacillus casei* and *Bifidobacterium longum* can suppress human colorectal cancer [22].

This bacterial species shows an inhibitory effect in bladder cancer and protects against intestinal disorders. *Lactobacillus pentosus* and *Lactobacillus plantarum* show decreased cell proliferation and arrest the cell cycle in the G1 phase in Caco-2 and HT-29 cells. *Lactobacillus lactis* at 1×10^{10} CFU decreases cell proliferation. *Lactobacillus rhamnosus* GG lowers the level of IL-8, a cytokine that causes angiogenesis and tumor progression. *L. acidophilus* and *L. casei*, in the presence of 5-fluorouracil, induce apoptosis. Overall, lactobacilli are capable of preventing carcinogen and mutagen formation, removing toxic metabolites, and improving the overall metabolism of the host. *Lactobacillus plantarum* 2592 and *Lactobacillus paracasei* F19 generate antioxidants similar to 100 mg of vitamin C. These antioxidants can prevent free radicals from promoting tumor formation. *Lactobacillus fermentum* has high antiproliferative activity against colorectal cancer [23, 24].

2.1.1.4 OTHER MICROORGANISMS IN COLORECTAL CANCER

Some microorganisms are reportedly found in patients with colorectal cancer and are associated with its progression. Species such as *Bacteroides*, *Prevotella*, *Clostridium*, and *Salmonella* are found in higher numbers in colorectal cancer patients. Some bacteria cause DNA damage by forming reactive oxygen species (ROS); for example, *Enterococcus faecalis* and *Bacteroides fragilis*. DNA is also destroyed by colibactin and fragilysin, toxins synthesized from *Escherichia coli* and *Bacteroides fragilis*, respectively. A toxic chemical, fragilysin, produced by *Bacteroides fragilis* forms a biofilm over the intestinal tract, produces ROS, and induces inflammatory pathways, particularly the cyclooxygenase-2 (COX-2) pathway, releasing prostaglandin E2 (PGE-2). This leads to the activation of the transcriptase 3 pathway, resulting in prolonged inflammation. This chronic inflammation can cause cellular changes and promote angiogenesis [25].

Meanwhile, certain bacteria, such as *Bacillus polyfermenticus*, *Bacillus subtilis*, and *Bifidobacterium lactis*, have been shown to reduce cell proliferation and induce apoptosis in cancer cells, including colorectal cancer cells like Caco-2, SW1116, and DLD-1 [26]. *Bifidobacterium longum*, in doses of 10^8 CFU/g, can suppress the proliferation of human colorectal cancer cells as well as gastric carcinoma cells [27]. Additionally, *Fusobacterium nucleatum*, a bacterium found in larger numbers in colorectal cancer patients, is considered one of the biomarkers of colon cancer. This bacterium is associated

with adenocarcinoma in the colon by producing inflammatory responses and activating the Wnt signaling/beta-catenin pathway, which promotes tumor formation. This pathway can cause mutations in the adenomatous polyposis coli (APC) gene, leading to overactive T-cell factor (TCF)4/ β -catenin signaling, directly connected to colorectal cancer [28].

Furthermore, *Fusobacterium nucleatum* prevents anti-tumorigenic activity and promotes tumor development by inhibiting the recruitment of tumor-infiltrating lymphocytes (TILs) and destroying natural killer cells. This bacterium has also been linked to chemotherapy resistance [29]. Some strains of *Escherichia coli*, particularly the B2 and D groups, are involved in inflammatory bowel disease, which can lead to colorectal cancer. These strains produce toxins such as cytotoxic necrotizing factors and cytolethal distending toxins that affect cell cycle regulation [30].

2.1.1.5 METABOLITES HAS DIRECT INFLUENCE IN COLORECTAL CANCER

Microorganisms play a vital role in protecting the gut environment by synthesizing vitamins like folate, niacin, and biotin. Probiotic bacteria ferment undigested food particles, including dietary fiber, protein, and short-chain fatty acids. They also deconjugate bile acids and metabolize plant cell walls, glycans, and fatty acids. Disturbances in protein breakdown and mutations in certain genes can lead to colorectal cancer. Physiologically, protein synthesis regulates cell growth, but alterations in signaling pathways, such as over-expression of the Wnt, RAS/MAPK, and PI3K/Akt pathways, followed by downregulation in translation processes, can contribute to cancer [31].

Bile acids are crucial for lipid metabolism and fat-soluble vitamin absorption. In the liver, bile acids conjugate with glycine or taurine and are stored in the gallbladder. Once secreted into the large intestine, bile acids undergo deconjugation by bacterial enzymes, producing secondary bile acids. These secondary bile acids can induce ROS, inhibit apoptosis, and promote angiogenesis by binding to the epidermal growth factor receptor (EGFR) and activating the PI3K/Akt pathway, leading to epithelial cell proliferation and DNA damage in the gut [32].

In contrast, the fermentation of fatty acids, especially butyrate, acetate, and propionate, provides energy to colonocytes. Butyrate, in particular, regulates the balance between proliferation, multiplication, and apoptosis by promoting proteins like Bcl-2 and Bak and activating caspases 3 and 7. This

regulation decreases the likelihood of disease by promoting apoptosis and reducing inflammatory cytokines. It also strengthens the intestinal barrier. Butyrate and other metabolites promote acidosis, resulting in lower fecal pH, which is often observed in healthy individuals compared to colorectal cancer patients. A decrease of 1 $\mu\text{g/L}$ in fecal butyrate concentration is estimated to increase the risk of colon cancer by 84.2% and adenoma by 71.3% [33].

Some bacteria, such as *Clostridium leptum*, *Roseburia* spp., and *Coprococcus* spp., produce butyrate, while *Eubacterium hallii* and *Anaeripes caccae* can convert acetate or lactate into butyrate. In colorectal cancer, mutations in the APC gene contribute to adenomatous polyp formation by regulating the Wnt pathway. Phytochemicals like polyphenols, produced from plant foods, inhibit tumorigenic signaling pathways. Polyphenols and antioxidants suppress histone acetylase and tumor proliferation while enhancing natural killer cell activity and inhibiting interleukin 6 (IL-6) and the tyrosine kinase pathway, thereby regulating apoptosis [34].

2.1.1.6 MODIFICATION IN PHYSICAL AND CHEMICAL PROPERTIES IN COLORECTAL CANCER

In colorectal cancer patients, disturbances in physicochemical properties like pH and viscosity are observed. Probiotic bacteria lower the pH and modify the gut microenvironment, reducing hypersensitivity in the intestine. Bacterial enzymes such as azoreductase, beta-glucosidase, and nitroreductase are released from intestinal dysbiosis, inducing carcinogen production. These enzymes lead to the creation of aromatic amines, hydrogen sulfide, acetaldehyde, and reactive oxygen species (ROS). Probiotics neutralize these compounds and generate anticarcinogenic substances like saromycin and neocarcinomycin [35].

In the liver, toxic compounds conjugate with glucuronic acid, produced internally from glucose, helping neutralize and eliminate the toxic compounds from the body. Heterocyclic aromatic amines (HCA), carcinogenic compounds formed during the cooking of meat, affect the mucous membrane of the large intestine. Probiotics, such as lactic acid bacteria (LAB), detoxify these compounds and decrease the risk of colorectal cancer [36]. LAB also neutralizes 1,2-dimethylhydrazine (DMH) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), absorbing reactive intermediate products like superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), and catalase (CAT) [23].

A diet rich in fiber from vegetables and fruits reduces the transit time of food from the stomach to the intestine, helping dilute colonic contents and enhancing fermentation. In Africa and Asia, the incidence of colorectal cancer is lower due to the high consumption of fiber-rich foods. Bacterial fermentation of fiber results in the production of beneficial metabolites such as acetate, propionate, and butyrate. Fiber has anti-inflammatory properties, decreasing the production of IL-6, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS), as well as tumor necrosis factor-alpha (TNF- α) [37].

The intestinal barrier, composed of an epithelial layer, junction proteins, goblet cells, a mucus layer, and immune cells, is crucial to preventing colorectal cancer. An imbalance in this composition can lead to disease. Some probiotics improve the intestinal barrier by lowering pH, which inhibits pathogenic growth. Fecal pH can serve as an indicator of pathogenic bacteria and colorectal cancer [5].

2.1.1.7 RELATION WITH PROBIOTICS WITH INTESTINAL BARRIER

A healthy intestinal mucosal barrier protects the body from pathogens, toxins, and physical damage. A weakened or leaky barrier can allow allergens to enter, causing an inflammatory response at the site. Pathogenic bacteria disrupt the permeability of the epithelial membrane either by direct injury or by releasing toxins, leading to the recruitment of leukocytes and protease enzymes that compromise the intestinal epithelium and increase colorectal cancer risk [38].

Probiotics can strengthen the epithelial membrane's integrity and inhibit damage caused by TNF- α . For instance, bacteria from the *Bifidobacterium* genus boost the epithelial membrane's integrity, while *Lactobacillus* promotes the integrity of tight junction proteins in the intestinal barrier [39]. Proteins such as claudin-3 and occludin, essential for intestinal permeability, are improved by *Lactobacillus rhamnosus* GG and *Lactobacillus reuteri*, which reduce oxidative stress and inflammation. Probiotics also reorganize tight junction proteins, reduce epithelial gaps, and promote mucus production by goblet cells, thus enhancing the intestinal barrier [40].

2.1.1.8 PROBIOTICS THAT REDUCE INFLAMMATORY RESPONSE

Probiotic bacteria can control the immune response and help in the proper functioning of the intestinal immune response. They distinguish between

different immune cells, including dendritic cells, T lymphocytes, and B lymphocytes. Probiotics help in the modulation of immunological tolerance, which produces beneficial effects for the host's body. Probiotic microorganisms or their proteins, including flagellin, lipopolysaccharide, and other metabolites, interact with the Toll-like and NOD-like receptors of the epithelial cells in the gastrointestinal tract. After attachment to the particular receptor, some anti-inflammatory cytokines are released, for example, IL-10 and transforming growth factor beta (TGF- β). These cytokines neutralize the inflammatory cytokines IL-1 β , IL-6, IL-17, IL-8, and tumor necrosis factor- α (TNF- α) [41, 42].

Additionally, probiotics stimulate the production of IgA, prevent the breakdown of intestinal barrier proteins, and limit the entry of carcinogenic compounds and pathogens. Probiotics maintain an anti-inflammatory environment in the colon by downregulating the COX-2 enzyme and decreasing the production of prostaglandins. Prostaglandin E2 (PGE-2) is the major inflammatory agent responsible for the development of arthritis, inflammatory bowel disease, and the growth of solid tumors, including colon cancer. Whereas the COX-1 enzyme maintains the gastric mucosal layer and stimulates platelet aggregation, Prostacyclin or PGI-2, produced by the COX-1 enzyme in gastric epithelial cells, provides protection from chronic inflammation [43].

If a patient is infected with *Helicobacter pylori*, chronic hepatitis B, or any other immune-related disorder like ulcerative colitis, which causes prolonged inflammatory reactions in the gastrointestinal tract that lead to colorectal cancer, treatment with NSAIDs and probiotics can improve these conditions [44].

There are some probiotics that can act by activating phagocytosis through the activation of macrophages that remain in the Peyer patches of the peritoneum against pathogenic microorganisms. Macrophages release different pro-inflammatory cytokines such as IL-1, IL-6, IL-18, and tumor necrosis factor (TNF). These chemicals can kill the pathogenic microbes and damage the surrounding tissue of the infected site, ultimately causing apoptosis of neutrophils, which are then removed by macrophages. The pathogen *Aggregatibacter actinomycetemcomitans* alters this normal pathway, and the pathogen acquires survival techniques such as escaping from the phagosome, lysis of neutrophils, and delay in apoptosis to promote the proliferation of intracellular pathogens. This *Aggregatibacter actinomycetemcomitans* bacteria is inhibited by probiotic bacteria containing lactobacilli [45].

2.1.1.9 LIFESTYLE CHANGES AND IMPLEMENTATION OF PROBIOTICS ON THE INCIDENCE OF COLORECTAL CANCER

A diet containing fiber-rich food and phytochemicals, as well as green vegetables containing folic acid and vitamin B, reduces the incidence of adenoma and colorectal cancer. Vitamin B has DNA repair, synthesis, and methylation properties. Folate deprivation below physiological levels can activate the Sonic Hedgehog signaling pathway by hampering the one-carbon (1C) metabolism pathway, which causes hypomethylation in DNA and chromosome instability, leading to carcinogenesis in the colon. However, an overdose of folic acid supplements is harmful. Regular intake of folic acid at a dosage of 1 mg/day can increase the risk of adenomas by 2.3 times; high dosages (1,000 µg/day) of folic acid supplements in the elderly can increase the chances of neoplasms [46].

Consumption of tobacco can increase the risk of colonic adenoma as well as colorectal cancer. The carcinogenic compounds aromatic amines, aromatic hydrocarbons, nitrosamines, and heterocyclic amines found in tobacco can lead to gene mutations after metabolism. Nitrosamines can cause an increase in ROS concentration, which can initiate inflammatory pathways.

Probiotic bacteria numbers must be high so that they can compete with pathogenic bacteria in the intestinal epithelium. The probiotic bacteria concentration must be more than 10^6 CFU/ml in the ileum part of the small intestine and in the large intestine 10^{12} CFU/g. Consumption of meals with 10^9 – 10^{12} CFU probiotic bacteria for a few weeks can increase the number and activity of macrophages, lymphocytes, and immunoglobulins in the blood. Intake of 10^{10} – 10^{11} CFU *Lactobacillus* and *Bifidobacterium* per day for 4–6 weeks can decrease the risk of colorectal cancer [47].

The connection between the host and resistant microflora at the mucus membrane strengthens the immune system and influences gut-associated lymphoid tissue (GALT), which helps in the development of systemic immunity. LAB can reportedly synergize with the chemotherapeutic agent 5-fluorouracil and induce apoptosis. A study was performed with 150 colon cancer patients for 24 weeks. They were randomly divided into two groups. The test group was given *Lactobacillus rhamnosus* GG (1 – 2×10^{10} CFU) per day along with 11 g of guar gum, continuing with chemotherapy, while the standard group received all the treatment except the probiotics. The test group patients experienced no discomfort or only mild discomfort, and they did not have acute diarrhea. Additionally, they required fewer chemotherapy doses compared to the standard treatment group. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* improve the condition in radiotherapy-induced

diarrhea. *Lactobacillus rhamnosus* reduces radiation-related gastrointestinal toxicity [48].

2.2 IN VITRO STUDIES INVOLVING PROBIOTICS FOR COLORECTAL CANCER

With the extraordinary frequency of mortality, colorectal cancer is a life-threatening melanoma linked with genetic and environmental factors, in addition to modern lifestyle [49]. Studies suggest that the incidence, growth, and management of different types of melanomas are closely linked to the gut flora. In a study, Qi et al. clinically showed that the use of recombinant phycoerythrin can have improved anti-tumor and immunomodulatory effects. They examined the effect of recombinant phycoerythrin on gut flora in mice bearing H22 tumor cells. They suggested that the use of recombinant phycoerythrin in dietary supplements improves the good microbiota of the gut, modulating disease resistance in the animals [50].

Baldwin et al. demonstrated that the anti-tumor activity of 5-fluorouracil in colorectal cancer can be regulated with different concentrations of *Lactobacillus acidophilus* and *L. casei*. This co-administration of live or inactive strains of *Lactobacillus* increased the efficacy of 5-fluorouracil treatment by about 40%. In the case of inactive strains, a dose-related decrease in anti-tumor activity was observed when the bacterial strain was inactivated by microwave treatment. In contrast, for γ -radiation-inactivated strains, the activity was dose-independent compared to live strains of the probiotics [51].

Escamilla et al. highlighted the tumor suppressor effect of probiotic cell-free supernatants from *Lactobacillus* species in human colorectal cancer cells. The matrix metalloproteinase-9 level decreases, while the zona occludens-1 level increases in human colorectal cancer cells upon exposure to cell-free supernatants from *Lactobacillus* species. The metabolites of *Lactobacillus* strains were found to exhibit tumor suppressor activity in human colorectal cancer cells [52].

Orlando et al. studied the tumor suppressor effect of both active and inactive *Lactobacillus paracasei* IMPC2.1 compared to *Lactobacillus rhamnosus* GG in colorectal cancer cells (DLD-1). They showed the sensitivity of cancerous cells toward both strains of *Lactobacillus*, which can be used in inactive form in protein drinks or other probiotic beverages for their anti-tumor activity [53].

JaeJin An and Eun-Mi Ha confirmed the selective inhibitory activity of *Lactobacillus plantarum* and *Lactobacillus* supernatant against 5-fluorouracil-resistant colorectal cancerous cells (HT29 and HCT116). Markers

CD44, 133, 166, and ALDH1 expression are suppressed in cancerous stem cells. The blend treatment of *Lactobacillus plantarum* and *Lactobacillus* supernatant with 5-fluorouracil prompts caspase-3 action in carcinogenic stem cells and leads to their demise by hindering Wnt/ β -catenin signaling in chemo-resistant cancerous stem cells, demonstrating the use of probiotics as an alternative tool for cancer therapy [54].

Dallal et al. showed that probiotics based on *Lactobacilli* strains not only enhance the immune system but also suppress the malignant cells of colorectal cancer. Compared to supernatants, extracts have similar effects but cause necrosis of cancerous cells, which is not seen in the case of *Lactobacilli* supernatants. On the other hand, they have similar detrimental effects on migration, invasion, proliferation, and apoptosis of cancerous cells [55].

Chen et al. examined the cancer cell line of human colon HT29 for the inhibitory effect of seven *Lactobacillus* strains and supernatants. The co-culture of the BCRC17010 strain and HT29 cells presented a significant supportive apoptotic impact on the HT-29 cells, while the co-culture with the BCRC14625 strain resulted in a critical increase in lactate dehydrogenase activity, leading to the destruction of the HT29 cell membrane [56].

Cousin et al. reported a synergistic pro-apoptotic effect, where metabolites or supernatants of propionibacteria were responsible for the production and release of propionate and acetate, short-chain fatty acid substituents, resulting in intrinsic apoptosis of colon cancer cells. These propionibacteria, when co-studied with TNF-Related Apoptosis-Inducing Ligand (responsible for extrinsic apoptosis of colon cancer cells), induced a synergistic effect on the apoptosis of colon cancer cells [57].

Kahouli et al. discussed the constructive effect of probiotics from *Lactobacillus acidophilus* ATCC 314 and *Lactobacillus fermentum* NCIMB 5221 on colorectal tumor cells, inhibiting the growth and proliferation of tumor cells in a mouse model. When both strains of *Lactobacillus* were used in combination, a significant increase in apoptosis with a decrease in proliferation was observed, while having no toxic effect on normal colon cells [58].

Saber et al. evaluated the anti-cancer activity of secretion metabolites from *Pichia kudriavzevii* AS-12 by inhibiting the growth and proliferation of cancerous cells of the human colon and rectum. FACS-flow cytometry tests, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide assay, and 4',6-diamidino-2-phenylindole staining techniques were used to evaluate the anti-cancer activity [59].

Sambrani et al. considered the antiproliferative activity due to the suppression of Bcl-xL and RelA, and a real-time PCR test was conducted

for enhanced expression of PTEN and Caspase-3 by *Saccharomyces cerevisiae* supernatant in human colorectal cancerous cell line HT29. The co-culture of *Saccharomyces cerevisiae* and HT29 cells was studied for their apoptosis by 4',6-diamidino-2-phenylindole (DAPI) staining, growth by 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and metastasis via scratch wound-healing assay [60].

Gong et al. studied four strains of microorganisms, including *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Enterococcus faecalis*, for their inhibitory effect on human colorectal cancer proliferation and growth [61].

2.3 IN VIVO STUDIES INVOLVING PROBIOTICS FOR COLORECTAL CANCER

Kahouli and colleagues investigated the effect of a probiotic mixture [*L. fermentum* (NCIMB 5221) and *L. acidophilus* (ATCC 314)] against a colorectal mouse model (APCMin/+). The dose of probiotic supplementation NCIMB 5221 (0.5×10^{10} CFU/g) and ATCC 314 (0.5×10^{10} CFU/g) was given per day for 4 months to the mouse model. The researchers observed that probiotic supplementation reduced the severity of colorectal cancer. Compared to the control group, the probiotic-treated group showed a significant decrease in the ratio and multiplicity of tumors, as well as in the expression of markers for cellular proliferation. The findings suggest that this probiotic preparation could be employed as a biotherapeutic agent to prevent colorectal cancer [62].

Another researcher, Leu et al., investigated the effect of *B. lactis* probiotic (1×10^{11} CFU/g) against azoxymethane-mediated colon neoplasm in Sprague-Dawley rats. The probiotic preparation significantly reduced colonic neoplasm proliferation in rats after 4 months of intervention. Probiotic treatment also caused a change in pH and an alteration in short-chain fatty acid content. The study demonstrated that probiotic regimens could serve as a protective agent for treating colorectal cancer [63].

The ability of several probiotics to protect Sprague-Dawley rats from colon cancer initiated by 1,2-dimethylhydrazine dihydrochloride (DMH) was evaluated. One week of daily oral treatment with 1×10^9 lactobacilli was administered to animals from various probiotic groups, followed by six weeks of weekly intraperitoneal injections of DMH along with daily probiotic administration. The aberrant crypt foci (ACF) levels greatly diminished in animals treated with *Lactobacillus GG* and *L. acidophilus*. A considerable reduction was seen

in fecal nitroreductase in rats treated with DMH + *L. plantarum* and DMH + *L. casei*. DMH + *L. acidophilus* and DMH + *Lactobacillus GG* diminished the activity of β -glucuronidase in rats. Glucosidase activity was also significantly reduced with *B. bifidum* in treated rats. However, a negligible difference was seen in the rats' colon morphology when treated with DMH + probiotic. The researchers concluded that *L. acidophilus* and *L. GG* have the potential to be used as prophylactic agents against colon cancer [64].

The researchers investigated how the probiotic bacterium strain *Lactobacillus plantarum* (AS-1) and its anticancer and antioxidant characteristics affect colon cancer caused by DMH. AS-1 was administered to Wistar rats during the selection or promotion phases of colon cancer. AS-1 supplementation was found to be beneficial in changing lipid peroxidation, antioxidant enzyme activity, and marker enzymes to a statistically significant level, evaluated both in the colon and plasma. The rats treated with AS-1 before and after treatment showed significantly fewer tumors overall and a smaller mean tumor volume. Additionally, histological analysis revealed a marked difference between the control and treated groups. AS-1 exhibits promising antioxidant properties, according to an *in vitro* antioxidant experiment. These findings suggest that the AS-1 strain has the ability to control the progression of colon cancer induced by DMH through an antioxidant-dependent mechanism [65].

The anticancer effect of a probiotic mixture was examined against colon cancer cell lines (CT26). The researchers employed different assays such as colony formation, migration and invasion assays, and cell counting (Kit-8) to characterize the anti-cancer and anti-metastasis effects of probiotics. CT26 cells were implanted into mice (BALB/c) to provide an animal model of a transplanted tumor. The mice were randomly split into two groups: the probiotic mixture intragastric injection group and the control group. Immunohistochemistry (IHC) and Hematoxylin and Eosin (HE) staining were used to assess tumor size and immune cell infiltration in the spleen tissues after 3 weeks of injection. Compared to control cells, the probiotic mixture dramatically reduced the capacity of CT26 cells to multiply, invade, and migrate. In animal studies, the tumor volume of probiotic mixture-fed mice was significantly lower than that of the control group. More immune cell infiltration and apoptotic cells were seen in the tumor tissues of mice treated with the probiotic mixture compared to control mice. Additionally, an increase in CD8⁺ cells was observed in the spleen tissues and tumor, but negligible changes were seen in other tissues. These findings suggest that the probiotic mixture may hinder the proliferation of CT26 tumors and trigger an immune reaction *in vivo* [66].

Table 2.1 summarizes the impact of probiotic supplementation against colorectal cancer.

TABLE 2.1 Impact of Probiotic Supplementation Against Colorectal Cancer

Probiotics	Cancer Cell Line/ Animal Model	Outcome	References
<i>L. casei</i> and <i>L. acidophilus</i>	LS 513 cell line	Enhancement of apoptotic efficiency, Decline of expression of p21, Escalation of caspase-3 activation	[51]
Cell free supernatants from <i>L. casei</i> and <i>L. rhamnosus</i>	HCT-16 cell line	Decline in cell invasion and MMP-9, Increase in ZO-1	[52]
Cell free supernatants from <i>S. cerevisiae</i>	HT-29 cell line	Decline in growth of cancer cells, Increase caspase 3 expression, Decline Bclxl genes expression	[60]
Methanolic extract of <i>Pichia Kudriavzevii</i>	Caco ₂ & HT-29 cell line	Decline in growth of cancer cells, Surge apoptosis	[59]
<i>L. casei</i> (BL23)	1,2- dimethylhydrazine induced colorectal cancer mice	Decline in tumor growth, Decrease in plaque lesions, Changes in IL-6, IL-10 and IL-17 expression	[64]
Probiotic mixture s	CT-26 cell line	Surged the CD8+ cells, Inhibition in proliferation of CT26 cell lines	[66]
<i>L. plantarum</i> & <i>5-Flurouracil</i>	HCT-116& HT-26 cell lines	Decline CD44, CD166 and CD133 expression, Surge caspase-3 activity, Diminish the colonospheres formation	[54]
<i>L. casei</i> ATCC & <i>L. acidophilus</i> ATCC	Caco ₂ cell line	Surge apoptosis, Decline proliferation of cancer cell line	[55]
<i>L. rhamnosus</i> & <i>L. paracasei</i>	DLD-1 & HGC-27 cell line	Inhibition of cell growth and apoptosis induction	[53]
<i>L. fermentum</i> & <i>L. acidophilus</i>	Caco ₂ cell line	Increase apoptosis and decline in proliferation of cancer cells	[62]

The antiproliferative activity of 40 probiotic extracts was screened against two colorectal cancer cell lines, including HRT-18 and Caco-2. The screening was carried out by employing Trypan Blue (TBE) and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assays. The outcome revealed that *Lactobacillus acidophilus* (LA-102) exhibited

significant anticancer activities with 38% and 69% against Caco-2 and HRT-18 cell lines, respectively. Similarly, *Lactobacillus casei* (LC-232) also inhibited proliferation with 49% and 46% for Caco-2 and HRT-18 cell lines, respectively. The values of IC₅₀ for anticancer activity were 1.7 and 2.7 µg/ml for LA-102 and 15.5 and 6.5 µg/ml for LC-232 against HRT-18 and Caco-2 cell lines, respectively. In addition, results revealed that the normal Vero cells were not cytotoxically affected by the probiotic isolates LA-102 and LC-232 [67].

In numerous experimental trials, it has been discovered that colorectal cancer (CRC) can be prevented by either taking probiotics or the non-steroidal anti-inflammatory drug celecoxib (CLB), which suppresses early pro-carcinogenic indicators. Hence, the objective of the current investigation was to characterize the prophylactic potential of combining CLB with probiotics, including *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus*, and treatment against experimental colon carcinogenesis. One week before 1,2-dimethylhydrazine (DMH)-induced tumor induction, probiotics *L. acidophilus* and *L. rhamnosus* GG were given to Sprague Dawley rats (six groups), and the treatment was sustained for 18 weeks. By using a variety of techniques, including tumor burden, tumor incidence, multiplicity of tumors, apoptosis, caspase activity, proto-oncogene *K-ras* expression, and the tumor suppressor gene *p53* in colonic tumors, it was possible to determine the prophylactic capability of probiotics and CLB. Intriguingly, it was observed that probiotics and CLB supplementation given one week prior to surgery decreased tumor burden and tumor multiplicity. The researchers also saw a decline in proto-oncogene *K-ras* expression and downregulation of the anti-apoptotic *Bcl-2*. There was enhancement in the expression of the tumor suppressor *p53* and pro-apoptotic *Bax* in *L. rhamnosus* GG + CLB + DMH animals compared to control and DMH-treated animals. It can be recapitulated that this combinatorial approach may be helpful in lowering the burden and severity in highly sensitive patients of CRC [68].

Those who have inflammatory bowel disease are at a greater risk of developing cancer related to colitis (CRC). Inflammatory bowel disease can lead to CRC; hence, strategies to stop its progression should be taken into account. The objective of the experiment is to examine the anticancer potential of the probiotic cocktail of Bifico and its probable mechanism in mice with CRC caused by azoxymethane and dextran sodium sulfate. Bifico was given as an oral pretreatment in order to assess its protective effects. To examine the extent of tumorigenesis and inflammation, the researchers used the colorectums of 35 C57BL/6 mice. The 16S rRNA sequencing was

conducted to look for Bifico-target changes in microbiota structure and gene expression. The researchers revealed that pretreatment with Bifico reduced tumor growth and diminished the inflammation present in the intestine. A genus *Lactobacillus* bloom was found by the investigators, and the 16S rRNA sequencing revealed that Bifico reduced the abundance of the genera *Desulfovibrio*, *Odoribacter*, and *Mucispirillum*. Notably, the researchers observed a strong correlation between certain Bifico-target taxa and the expression of *CXCR2* ligand genes. According to the research, Bifico can treat CRC in mice when administered orally by potentially interfering with the relationship between *CXCR2* signaling and *Desulfovibrio*, *Mucispirillum*, *Odoribacter*, and *Lactobacillus* [69].

Chemotherapy-treated cancer patients frequently experience the adverse effect of intestinal mucositis (IMCS). This investigation looked at how *Bifidobacterium infantis* (*B. infantis*) affected the rats' T cell subsets with CRC to reduce the intestinal mucositis severity brought on by chemotherapy. To create a CRC model, 30 male Sprague-Dawley rats were subcutaneously injected with dimethyl hydrazine (DMH) for 10 weeks. The rats were then arbitrarily divided into three groups: the Control group (saline and saline), the Chemotherapy group (saline and 5-FU + Oxaliplatin), and the *B. infantis* group (*B. infantis* + 5-FU + Oxaliplatin). The researchers used crypt depth, intestinal villus height, pro-inflammatory cytokines (IL-6, IL-1, TNF- α), T cell subsets (CD4⁺ IL17A⁺ cells and CD4⁺ CD25⁺ Foxp3⁺ Tregs), and associated cytokine profiles to assess IMCS. The findings revealed that the *B. infantis* group outperformed the Chemotherapy group in terms of body weight (BW), intestinal villus height, and crypt depth. Chemotherapy-induced increases in IL-6, IL-1, and TNF- α were decreased by administering *B. infantis*. The *B. infantis* decreased the relative cytokine expression related to Th17 and Th1 cells while increasing the relative expression of cytokines associated with CD4⁺ CD25⁺ Foxp3⁺ Tregs, as revealed by real-time reverse transcription-polymerase chain reaction (RT-PCR). The *B. infantis* reduces chemotherapy-induced IMCS by lowering Th1 and Th17 responses while enhancing responses of CD4⁺ CD25⁺ Foxp3⁺ Tregs [70].

Pre-neoplastic lesions called aberrant crypt foci (ACF) and mucin-depleted foci (MDF) have been seen in the colons of rodents treated with carcinogens and in people who are at high risk of developing colon cancer. The goal of the current investigation was to determine whether the probiotic Dahi, which contains *Lactobacillus acidophilus* LaVK2 and *Bifidobacterium bifidum* BbVK3, alone or in combination with piroxicam (PRX), could prevent the emergence of early biomarkers of colorectal carcinogenesis

in male rats given 1,2-dimethylhydrazine (DMHD). The DMHD was administered subcutaneously twice weekly for two weeks at a dose of 40 mg/kg body weight for each animal. A total of 120 male rats were divided into five groups at random, with 24 animals per group. For 32 weeks, the rats were given buffalo milk or a probiotic supplement (20 g), either alone or in conjunction with PRX. Group I, which acted as the control group, received buffalo milk (BFM). Group II acted as the DMHD-control group and received DMHD along with BFM; group III received BFM-DMHD-PRX, which included PRX in addition to BFM-DMHD administration. Probiotic LaBb Dahi and DMHD were given to group IV, while probiotic LaBb Dahi and PRX were also given to group V together with DMHD. At the 8th, 16th, and 32nd weeks of the experiment, the rats were euthanized. The researchers examined the growth of ACF, aberrant crypts per ACF (AC/ACF), mucin-depleted foci (MDF), large MDF, and the proliferating cell nuclear antigen (PCNA) labeling index in the rats. Pre-neoplastic lesions (ACF and MDF) were generated by DMHD administration in rats, and the PCNA index was elevated in the colorectal tissue. Comparing the probiotic LaBb Dahi group to the DMHD control group, it was shown that there was a substantial decrease in the number of ACF, AC/ACF, MDF, big MDF, and PCNA labeling index. The onset and progression of DMHD-induced pre-neoplastic lesions, as well as the PCNA index, were reduced in rats fed LaBb Dahi or treated with PRX, but the combination of LaBb Dahi and PRX was noticeably more effective. In the rat-DMHD model, the dietary intervention of probiotics and PRX considerably delays the onset of CRC. These findings imply that probiotic LaBb Dahi, either by itself or in conjunction with PRX, may have anti-proliferative and anti-neoplastic properties. Additionally, probiotic LaBb Dahi has therapeutic characteristics that can stop the development of CRC [71].

In the case of colon cancer, probiotics have been recommended as a preventative measure. This study sought to determine how colonic microbiota structure and colon cancer incidence in a rat model following injection with 1,2-dimethylhydrazine (DMHD) were affected by *Lactobacillus salivarius* Ren (Ren). The outcomes showed that oral Ren treatment could successfully inhibit DMHD-induced colonic carcinogenesis. Rats administered a dosage of 5×10^{10} CFU/kg body weight per day showed a considerable reduction in cancer incidence (from 88.4% to 23%). The researchers demonstrated that DMHD injection significantly altered the rat gut microbiota using denaturing gradient gel electrophoresis, real-time PCR, and multivariate statistical methods, whereas Ren counteracted these DMHD-induced negative effects

and encouraged reversion of the gut microbiota close to a healthy state. DMHD and Ren had a significant impact on 21 bacterial strains, according to t-value biplots and band sequencing. The quantity of *Ruminococcus* species (sp.) and *Clostridiales* bacteria greatly increased after DMHD injection, while *Prevotella* species significantly decreased. Ren administration enhanced the amount of *Prevotella* while decreasing the amounts of *Ruminococcus* sp., *Clostridiales* bacteria, and *Bacteroides dorei*. The real-time PCR outcomes agreed with those obtained from t-value biplots. These results indicated that Ren may be a viable colon cancer preventive agent. The findings of the present investigation, in summary, point to a prospective treatment strategy based on probiotics' manipulation of intestinal microbiota as being helpful to prevent CRC [72].

One of the most common types of cancer, colorectal cancer (CRC), has a high mortality rate and a growing prevalence. While there are many effective CRC treatment options, such as surgery, radiotherapy, chemotherapy, and immunotherapy, their drawbacks and limitations are significant. Through the promotion of host innate and adaptive immunity, probiotics may be a useful method for preventing and suppressing tumor growth. Hu and his colleagues examined and assessed possible anti-cancer immune responses of *Lactobacillus plantarum* A and *Lactobacillus rhamnosus* by pre-inoculating mice with these two *Lactobacillus* strains for a period of 14 days. The researchers utilized CT26 murine cells to produce subcutaneous and orthotopic intestinal tumors in the pre-inoculated mice, and tumor response was evaluated. The investigators showed that oral administration of *L. plantarum* to BALB/c mice reduced the proliferation of CT26 cells and increased the survival duration of tumor-bearing mice as compared to mice given *L. rhamnosus*. *L. plantarum* showed protective immunity against CT26 cells by enhancing the CD8⁺ effector functions and natural killer (NK) cell infiltration into tumor tissue, improving the differentiation of Th1-type CD4⁺ T cells, and upregulating the production of IFN- γ . As a result, the researchers concluded that *L. plantarum* can boost the anti-tumor immune response and delay the development of tumors [73].

The goal of the current work is to assess the effects of probiotics *Bifidobacterium bifidum* and *Lactobacillus acidophilus* on the expression of miRNAs 135b, 26b, 18a, and 155 and their target genes, including PTEN, APC, KRAS, and PU1 in mice colon cancer (CC) caused by azoxymethane (AZOM). To examine the effects of the probiotics on the miRNAs and their target genes, 38 male BALB/c mice were randomly allocated into four groups: control, AZOM, *Lactobacillus acidophilus*, and *Bifidobacterium*

bifidum. AZOM (20 mg/kg) was administered weekly to the other groups (except the control group) for three consecutive weeks to develop mouse colon cancer. For five months, the animals in the linked groups received 1.5 g powders containing *L. acidophilus* (1×10^9 CFU/g) and *B. bifidum* (1×10^9 CFU/g) in drinking water (30 cc). The animals were sacrificed at the conclusion of the investigation, and samples of their blood and colon were taken for the molecular examinations. The findings demonstrated that ingestion of probiotics reduced the expression of miR-135b, miR-155, and *KRAS* in both colon tissue and plasma samples taken from the AZOM group compared to the control group. Additionally, the AZOM group's expressions of miR-26b, miR-18a, *APC*, *PUI*, and *PTEN* were lower than those of the control group, and the ingestion of probiotics increased those expressions. It appears that *L. acidophilus* and *B. bifidum* can enhance colon cancer treatment by upregulating tumor suppressor miRNAs and their target genes while downregulating oncogenes [74].

The consistent intestinal inflammation modifies the physiology of the host and may cause CRC. The probiotic strain of *Lactobacillus casei* (BL23) has been shown to have positive benefits in the past in various mouse models of intestinal inflammation. Additionally, there is growing interest in the possible advantages of probiotics in the treatment of CRC. Thus, we investigated whether BL23 has anti-CRC properties. Ten-week subcutaneous injections of 1,2-dimethylhydrazine (DMHD) and oral administration of *L. casei* BL23 in drinking water were given to mice. Large intestinal plaque lesions were counted and observed macroscopically, and intestinal samples also underwent histological examination. To test the immunomodulatory effects of this bacterium, populations of T-cells and production of cytokines were assessed after co-incubation of spleen cells with BL23 from untreated mice. The researchers demonstrated that oral administration of this probiotic bacterium modifies host immune responses and greatly protects mice from CRC caused by DMHD. This protection may be related to the modulation of regulatory T-cells toward a Th17-biased immune response associated with the regulatory cytokines such as IL-6, IL-10, IL-17, and TGF- β . This protection is also associated with IL-22 colonic expression in BL23-treated mice, found *in vivo*, pointing to the production of a precisely tailored Th17-biased response. These findings collectively point to BL23's strong therapeutic potential for CRC and open up new research avenues for the investigation of probiotics' immunomodulatory properties [75].

The anticancer potential of probiotic *L. paracasei* was evaluated against an *in vivo* model of 60 male Wistar rats for a period of 8 weeks. Male Wistar

rats treated with DMHD (30 mg/kg) for 12 weeks were used to study the effects of *L. paracasei* B-14 on CRC-induced animals. After treatment, all groups (treated and control groups) had their tumor incidence, number, size, histological examinations, apoptosis frequency, and cell proliferation evaluated. The *L. paracasei* treatment for malignant rats reduced CRC symptoms such as diarrhea, rectal bleeding, hair loss, and atrophy. Compared to the untreated control (2.5%) and DMHD (6.2%) groups, *L. paracasei* with 5-FU resulted in a significant reduction in tumor size and quantity, as well as a rise in total apoptotic cells (52.2%). In comparison to the DMHD-only treated group, the *L. paracasei* treatment group displayed fewer dysplastic changes in the crypts, lower grades of dysplasia, and reduced proliferative and metastatic activity into deeper layers. The group treated with both *L. paracasei* and 5-FU had the lowest AgNOR counts. In rats treated with *L. paracasei*, apoptosis-inducing and anti-apoptotic genes were differentially altered. According to the findings of the current investigation, *L. paracasei* has the therapeutic potential to slow the spread of colorectal cancer and improve the anti-tumor effects of 5-FU [76].

This investigation looked at the preventive benefits of a ricotta-derived isolate of *Lactococcus lactis* subsp. *lactis* (R7) on CRC, as well as the treatment's impact on biochemical and hematological markers. Wistar rats were given oral suspensions of R7, a hypercaloric diet, and subcutaneous doses of 1,2-dimethylhydrazine (DMHD), a chemical carcinogen known to cause carcinogenesis in experimental models. The outcomes showed that R7 has the potential to be anti-carcinogenic against CRC by stabilizing the cancer cells that had been artificially infected into the colorectal epithelium. The biochemical data demonstrated that R7 alleviated the stress brought on by DMHD and a hypercaloric diet, and an immunological response was also observed. Regarding R7's behavior in connection to the treatment of CRC, the results are encouraging [77].

Kim et al. demonstrated the antitumor mechanism of p8 in a mouse xenograft model of CRC. The p8 protein induces apoptotic cells and cleaved PARP1 expression. Furthermore, by utilizing RNA sequencing, they discovered *Ring Finger Protein 152* (RNF152) as a potential target of p8. Additionally, after being treated with p8 both *in vivo* and *in vitro*, the expression levels of RNF152 were elevated. Moreover, they found that p8 causes CRC cells to accumulate cleaved PARP1. These findings imply that p8 inhibits the growth of CRC by inducing apoptosis via modulation of RNF152 [78].

Guo and his colleagues examined the anticancer properties of *Lactobacillus acidophilus* CICC 6074, and its underlying mechanism of action

was evaluated. In a dose- and time-dependent manner, *L. acidophilus* CICC 6074 reduced the activity of HT-29 cells and triggered their apoptosis. The mitochondrial membrane potential of HT-29 cells exposed to *L. acidophilus* CICC 6074 was reduced, and Cyt c was released from the mitochondria into the cytoplasm, upregulating Bax, Caspase-9, and Caspase-3 while down-regulating Bcl-2. A mouse model of colon cancer caused by HT-29 cells was employed to further investigate the mechanism through which *L. acidophilus* CICC 6074 exerts its anti-cancer effects. The high-dose *L. acidophilus* CICC 6074 group had evident colon cancer cell death, as assessed by histological morphology, electron microscopy, and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay in comparison to a colon cancer model control group. Activating Caspase-3 and Caspase-9, releasing Cyt c from the mitochondria into the cytoplasm, and upregulating Bax were the mechanisms used by *L. acidophilus* CICC 6074 to cause colon cancer apoptosis in *in vivo* research. Bcl-2 was also downregulated. These findings show that *L. acidophilus* CICC 6074 exerts its anticancer properties by activating mitochondrial pathways that subsequently trigger apoptosis. The study may offer fresh perspectives and a theoretical foundation for the growth of the probiotics functional food sector [79].

The purpose of this study was to examine the potential anti-cancer benefits of probiotic cell-free supernatant (CFS) treatment for CRC in 3D culture systems utilizing *Lactobacillus fermentum*. MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assays were used to measure the viability of the cells, while flow cytometry analysis of the *Lactobacillus* cell-free supernatant (LCFS) treatment and RT-qPCR analysis of the expression of Bax and Bak were conducted. The researchers demonstrated that the 3D culture of HCT-116 cells significantly boosted the anti-cancer effect of LCFS on cell viability, which was associated with the elevated level of cleaved caspase-3. Moreover, 3D cultured cells significantly outperformed 2D systems in the activation of apoptotic marker gene mRNA transcription. This study concludes by arguing that LCFS increases the intrinsic apoptotic activation in HCT-116 cells and the potential anti-cancer benefits of *Lactobacilli* combinations in 3D culture systems. Overall, investigators emphasize the advantages of 3D culture models over 2D culture modeling for researching probiotics' anti-cancer properties [80].

Dong et al. investigated the anticancer potential and the mechanism of the probiotic *Lactobacillus salivarius* (LS). The researchers observed that both the initial and post-initial stages of DMHD-induced CRC were successfully

prevented by the oral administration of LS. Excellent anticancer and pro-apoptotic effects were seen, along with the prevention of tumor development when LS was consumed through food. In addition, LS metabolites prevented HT-29 cells from growing, stopped their cell cycle, and caused apoptosis. Also, in both *in vitro* and *in vivo* studies, the treatment with LS dramatically reduced protein kinase B (AKT) phosphorylation as well as the downstream proteins of cyclooxygenase-2 (COX-2) and cyclin D1. The researchers demonstrated that LS suppressed colorectal carcinogenesis by blocking the AKT signaling pathway, which in turn reduced cell proliferation and triggered cell apoptosis. The research suggests that this probiotic may function as a preventative measure against CRC [81].

Yue et al. investigated the anticancer mechanism of the probiotic *Lactobacillus plantarum* strain YYC-3 in a colon cancer mouse model (APCMin/+). In APCMin/+ mice fed a high-fat diet, both strain YYC-3 and the YYCS treatment reduced the development of colon tumors and mucosal damage; however, YYC-3 showed a higher anti-cancer impact. The immune system was modulated as part of the strategy, which also included reduced infiltration of inflammatory cells and downregulation of the inflammatory cytokines interleukin (IL)-6, IL-17F, and IL-22 expression. Moreover, YYC-3 inhibited the activation of the NF- κ B and Wnt signaling pathways and returned the altered gut microbiota to closely resemble that of mice with wild-type genotypes. These findings provide a theoretical framework for the use of YYC-3 in colon cancer prevention [82].

The antiproliferative and antitumor activity of *Faecalibacterium prausnitzii* was evaluated by the researchers in the rats' gut microbiota. The results demonstrated that the probiotic *F. prausnitzii* significantly decreased the incidence and development of colonic aberrant crypt foci in rats with CRC caused by azoxymethane (AOM). The *F. prausnitzii* injection also reduced the lipid peroxidation levels in the tissues of the colon. The *F. prausnitzii* cell-free supernatant inhibited the growth of colorectal cancer cells (HCT116), as confirmed by the MTT assay in a dose-dependent manner. The samples of rat feces employed for gene sequencing of 16S rRNA demonstrated that *F. prausnitzii* changed and enhanced the diversity of the rats' gut microbiota. Therefore, these outcomes suggested that *F. prausnitzii* could be employed as a supplement of probiotics to prevent and treat CRC. Though extensive investigation is needed for a complete understanding of the molecular and cellular mechanisms of action [83].

KEYWORDS

- **bifidobacterium**
- **colon cancer**
- **gut microbiota**
- **oxidative stress**
- **probiotics**

REFERENCES

1. Kuipers, E. J., Grady, W. M., Lieberman, D., Seufferlein, T., Sung, J. J., Boelens, P. G., & Watanabe, T. (2015). Colorectal cancer. *Nature Reviews Disease Primers*, 1, 15065. <https://doi.org/10.1038/nrdp.2015.65>.
2. Mármol, I., Sánchez-de-Diego, C., Pradilla Dieste, A., Cerrada, E., & Rodríguez Yoldi, M. J. (2017). Colorectal carcinoma: A general overview and future perspectives in colorectal cancer. *International Journal of Molecular Sciences*, 18(1), 197. <https://doi.org/10.3390/ijms18010197>.
3. Sawicki, T., Ruszkowska, M., Danielewicz, A., Niedźwiedzka, E., Arłukowicz, T., & Przybyłowicz, K. E. (2021). A review of colorectal cancer in terms of epidemiology, risk factors, development, symptoms, and diagnosis. *Cancers*, 13(9), 2025. <https://doi.org/10.3390/cancers13092025>.
4. Hossain, M. S., Karuniawati, H., Jairoun, A. A., Urbi, Z., Ooi, D. J., John, A., & Ming, L. C. (2022). Colorectal cancer: A review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. *Cancers*, 14(7), 1732. <https://doi.org/10.3390/cancers14071732>.
5. Uccello, M., Malaguarnera, G., Basile, F., D'Agata, V., Malaguarnera, M., Bertino, G., & Biondi, A. (2012). Potential role of probiotics on colorectal cancer prevention. *Bioscience Reports*, 12, 1–8. <https://doi.org/10.1042/BSR20110756>.
6. Rodríguez, J., Zarate, R., Bandres, E., Viudez, A., Chopitea, A., Garcia-Foncillas, J., & Gil-Bazo, I. (2007). Combining chemotherapy and targeted therapies in metastatic colorectal cancer. *World Journal of Gastroenterology*, 13(44), 5867–5878. <https://doi.org/10.3748/wjg.v13.i44.5867>.
7. Li, S., Liu, J., Zheng, X., Ren, L., Yang, Y., & Li, W. (2022). Tumorigenic bacteria in colorectal cancer: Mechanisms and treatments. *Journal of Medicine and Life*, 19(2), 147. <https://doi.org/10.25122/jml-2021-0194>.
8. Thursby, E., & Juge, N. (2017). Introduction to the human gut flora. *Biotechnology Journal*, 474(11), 1823–1836. <https://doi.org/10.1042/BSR20170477>.
9. Sivamaruthi, B. S., Kesika, P., & Chaiyasut, C. (2020). The role of probiotics in colorectal cancer management. *Evidence-Based Complementary and Alternative Medicine*, 2020, 1–14. <https://doi.org/10.1155/2020/3535982>.

10. Alves Martins, B. A., de Bulhões, G. F., Cavalcanti, I. N., Martins, M. M., de Oliveira, P. G., & Martins, A. M. A. (2019). Biomarkers in colorectal cancer: The role of translational proteomics research. *Frontiers in Oncology*, 9, 1284. <https://doi.org/10.3389/fonc.2019.01284>.
11. Bretthauer, M., Løberg, M., Wieszczy, P., Kalager, M., Emilsson, L., Garborg, K., & Bugajski, M. (2022). Effect of colonoscopy screening on risks of colorectal cancer and related death. *New England Journal of Medicine*, 387(17), 1547–1556.
12. Helsing, L. M., & Kalager, M. (2022). Colorectal cancer screening—Approach, evidence, and future directions. *Nature Reviews Disease Primers*, 1(1), EVIDra2100035.
13. Bevan, R., & Rutter, M. D. (2018). Colorectal cancer screening—Who, how, and when. *Clinical Excellence*, 51(1), 37–49.
14. Bretthauer, M., Løberg, M., Wieszczy, P., Kalager, M., Emilsson, L., Garborg, K., & Bugajski, M. (2022). Effect of colonoscopy screening on risks of colorectal cancer and related death. *New England Journal of Medicine*, 387(17), 1547–1556.
15. Kahouli, I., Tomaro-Duchesneau, C., & Prakash, S. (2013). Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. *Journal of Microbiology and Microbial Technology*, 62(8), 1107–1123.
16. Torres-Maravilla, E., Boucard, A. S., Mohseni, A. H., Taghinezhad-S, S., Cortes-Perez, N. G., & Bermúdez-Humarán, L. G. (2021). Role of gut microbiota and probiotics in colorectal cancer: Onset and progression. *Journal of Microbiology*, 9(5), 1021.
17. Compton, C. C., & Greene, F. L. (2004). The staging of colorectal cancer: 2004 and beyond. *Cancer*, 54(6), 295–308.
18. Hendler, R., & Zhang, Y. (2018). Probiotics in the treatment of colorectal cancer. *Microorganisms*, 5(3), 101.
19. Drewes, J. L., Housseau, F., & Sears, C. L. (2016). Sporadic colorectal cancer: Microbial contributors to disease prevention, development, and therapy. *British Journal of Cancer*, 115(3), 273–280.
20. Aarons, C. B., Shanmugan, S., & Bleier, J. I. (2014). Management of malignant colon polyps: Current status and controversies. *Journal of Gastroenterology and Hepatology*, 20(43), 16178.
21. Khaneghah, A. M., Abhari, K., Eş, I., Soares, M. B., Oliveira, R. B., Hosseini, H., & Technology, B. (2020). Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review. *Food Science and Technology*, 95, 1–9.
22. Zhong, L., Zhang, X., & Covasa, M. (2014). Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World Journal of Gastroenterology*, 20(22), 7878–7886.
23. Di Cerbo, A., Palmieri, B., Aponte, M., Morales-Medina, J. C., & Iannitti, T. (2016). Mechanisms and therapeutic effectiveness of lactobacilli. *Frontiers in Microbiology*, 7, 1209.
24. Sánchez-Alcoholado, L., Ramos-Molina, B., Otero, A., Laborda-Illanes, A., Ordóñez, R., Medina, J. A., & Queipo-Ortuño, M. I. (2020). The role of gut microbiota on colorectal cancer development and therapy response. *Cancers*, 12(6), 1406.
25. Cheng, W. T., Kantilal, H. K., & Davamani, F. (2020). The mechanism of *Bacteroides fragilis* toxin contributes to colon cancer formation. *Microorganisms*, 8(1), 68.
26. Sánchez-Alcoholado, L., Ramos-Molina, B., Otero, A., Laborda-Illanes, A., Ordóñez, R., Medina, J. A., & Queipo-Ortuño, M. I. (2020). The role of gut microbiota on colorectal cancer development and therapy response. *Cancers*, 12(6), 1406.

27. Buc, E., Dubois, D., Sauvanet, P., Raisch, J., Delmas, J., Darfeuille-Michaud, A., & Bonnet, R. (2013). High prevalence of mucosa-associated *Escherichia coli* producing cyclomodulin and *Fusobacterium nucleatum* in colon cancer. *PLOS ONE*, 8(12), e83701.
28. Cheng, W. T., Kantilal, H. K., & Davamani, F. (2020). The mechanism of *Bacteroides fragilis* toxin contributes to colon cancer formation. *Microorganisms*, 8(1), 68.
29. Lee, D.-W., Han, S. W., Bae, J. M., Jang, H., Han, H., Kim, H. S., & Oh, D. Y. (2018). *Fusobacterium nucleatum* and clinical outcomes in colorectal cancer with microsatellite instability. *Annals of Oncology*, 29(1), 139–144.
30. Nouri, M., Bredberg, A., West, C. E., & Larsson, E. (2021). Pathogenic *Escherichia coli* and colorectal cancer—A role for cyclomodulins. *Pathogens*, 10(1), 32.
31. Wu, W. K. K., Chen, C. C., Panyod, S., Chen, R. A., Wu, M. S., & Sheen, L. Y. (2018). Interaction between the gut microbiota and tumour-associated immune cells in colorectal cancer. *Journal of Gastroenterology*, 53(1), 10–20.
32. Nguyen, H. D., Lapp, S., Wagner, C., Pastuschek, J., Carvalho, R. A., & Meinhardt, A. (2022). Bile acid-induced ROS production promotes colon cancer and biliary disease progression. *Gut*, 71(1), 65–72.
33. Gonçalves, P., & Martel, F. (2013). Butyrate and colorectal cancer: The role of microRNAs. *Gut Microbes*, 4(1), 1–6.
34. Zhu, W., Zhang, J., Huang, Y., & Lin, Y. (2021). Dietary polyphenols supplementation and cancer risk reduction: Mini review of the effects on the gut microbiota. *Nutrients*, 13(10), 3476.
35. Drago, L. (2019a). Probiotics and colon cancer. *Microorganisms*, 7(3), 66.
36. Santarelli, R. L., Pierre, F., & Corpet, D. E. (2008). Processed meat and colorectal cancer: A review of epidemiologic and experimental evidence. *Nutrition and Cancer*, 60(2), 131–144.
37. Uccello, M., Malaguarnera, G., Basile, F., D'Agata, V., Malaguarnera, M., Bertino, G., & Biondi, A. (2012). Potential role of probiotics on colorectal cancer prevention. *Bioscience Reports*, 32(1), 45–52.
38. Camilleri, M. (2021). Leaky gut: Mechanisms, measurement and clinical implications in humans. *Gut*, 70(8), 1517–1526.
39. Cheng, Y., Ling, Z., & Li, L. (2020). The intestinal microbiota and colorectal cancer. *Frontiers in Immunology*, 11, 3100.
40. Drago, L. (2019b). Probiotics and colon cancer. *Microorganisms*, 7(3), 66.
41. Fasano, A., Ziegler, T. R., & Williamson, J. R. (2021). Gut health and the immune system: A balanced relationship. *Nature Reviews Immunology*, 21(1), 13–26.
42. Plaza-Díaz, J., Ruiz-Ojeda, F. J., Vilchez-Padial, L. M., & Gil, A. (2017). Evidence of the anti-inflammatory effects of probiotics and synbiotics in intestinal chronic diseases. *Nutrients*, 9(6), 555.
43. Fasano, A., Ziegler, T. R., & Williamson, J. R. (2021). Gut health and the immune system: A balanced relationship. *Nature Reviews Immunology*, 21(1), 13–26.
44. Butt, J., & Epplein, M. (2019). *Helicobacter pylori* and colorectal cancer: A bacterial link to colon carcinogenesis. *Gastroenterology*, 156(2), 626–628.
45. Braster, M., Bögels, M., Beelen, R. H. J., & Van Egmond, M. (2017). The delicate balance of macrophages in colorectal cancer; their role in tumour development and therapeutic potential. *Immunobiology*, 222(1), 21–24.

46. Wang, J., Hsu, S. M., Feng, H., & Huang, C. J. (2012). Folic acid supplementation and colorectal cancer risk: An interaction between DNA methylation and folic acid. *Journal of Cancer Research and Clinical Oncology*, 138(2), 227–233.
47. Han, S., Cheng, S., Liu, Y., Sun, H., Zhang, Z., Fan, R., & Liu, S. (2021). Probiotics improve immunity and inhibit tumor growth in colorectal cancer patients. *Journal of Clinical Oncology*, 40(7), 134–141.
48. Banna, G. L., Torino, F., Marletta, F., Santagati, M., Salemi, R., Cannarozzo, E., & Ferrau, F. (2017). *Lactobacillus rhamnosus* GG: An effective strategy to prevent diarrhea during radiotherapy in colorectal cancer patients. *Radiation Oncology*, 12(1), 155.
49. Talukdar, F. R., Rashid, M. I., Kabir, A., Islam, S. M. A., Ali, Y., Hafiz, M., & Tanvir, R. (2023). Genetics and epigenetics of colorectal cancer: A review. *BioMed Research International*, 2023, 1–15.
50. Qi, J., Tang, J., Wang, Z., & Zhao, Y. (2019). Effects of recombinant phycoerythrin on gut microbiota and immune function of mice bearing H22 tumors. *Frontiers in Microbiology*, 10, 1772.
51. Baldwin, C., Millette, M., Oth, D., Ruiz, M. T., & Luquet, F. M. (2010). Probiotic strains in cancer prevention and treatment: Potential influence of live versus inactivated probiotics. *International Journal of Oncology*, 37(6), 1697–1708.
52. Escamilla, J., Lane, M. A., & Maitin, V. (2012). Cell-free supernatants from probiotic *Lactobacillus* inhibit colon cancer invasion and metastasis. *World Journal of Gastroenterology*, 18(34), 4651–4658.
53. Orlando, A., Linsalata, M., Russo, F., et al. (2012). Probiotic *Lactobacillus paracasei* IMPC2.1 displays anti-tumor activity in colorectal cancer cells. *Journal of Cancer Therapy*, 3(10), 1507–1517.
54. An, J., & Ha, E. (2016). *Lactobacillus plantarum* selectively suppresses tumor stem cells and enhances the anti-cancer effect of chemotherapy in colorectal cancer. *Journal of Microbiology and Biotechnology*, 26(2), 365–373.
55. Dallal, M. M. S., Yazdi, M. H., Hassan, Z. M., et al. (2015). Comparison of the effects of *Lactobacillus* supernatant and extract on colorectal cancer cells in vitro. *Iranian Journal of Immunology*, 12(1), 1–8.
56. Chen, Y., Hsieh, H. H., Huang, Y., & Tsai, C. C. (2017). Cytotoxic effects of *Lactobacillus* strains and their supernatants on human colon cancer cells. *Experimental Biology and Medicine*, 242(15), 1638–1645.
57. Cousin, F. J., Jouan-Lanhouet, S., Thepaut, M., et al. (2016). Probiotic *Propionibacteria* inhibit colon cancer cell proliferation via short-chain fatty acid production. *Nutrition and Cancer*, 68(7), 1161–1168.
58. Kahouli, I., Malhotra, M., & Prakash, S. (2017). Biotherapeutic effect of *Lactobacillus acidophilus* and *Lactobacillus fermentum* on colorectal cancer in a mouse model. *Frontiers in Microbiology*, 8, 1917.
59. Saber, A. M., Alipour, B., Faghfoori, Z., & Khosroushahi, A. Y. (2017). Probiotic *Pichia kudriavzevii* inhibits colon cancer cell growth and promotes apoptosis via modulating gut microbiota. *Iranian Journal of Basic Medical Sciences*, 20(12), 1390–1401.
60. Sambrani, R., Abdolalizadeh, J., Kohan, S., & Jafari, A. (2019). *Saccharomyces cerevisiae* supernatant induces apoptosis and inhibits the metastasis of human colorectal cancer HT29 cells. *Journal of Clinical Medicine*, 8(6), 844.

61. Gong, J., Cheng, L., Jiang, Y., & Zhang, Y. (2017). Effects of probiotics on the proliferation and metastasis of human colorectal cancer cells. *Journal of Clinical Oncology*, 35(3), 92–98.
62. Kahouli, I., Malhotra, M., & Prakash, S. (2017). Biotherapeutic effect of *Lactobacillus acidophilus* and *Lactobacillus fermentum* on colorectal cancer in a mouse model. *Frontiers in Microbiology*, 8, 1917.
63. Leu, R. K., Hu, J. L., Brown, R. S., Woodman, R. J., & Young, G. P. (2010). Probiotics and prevention of colorectal cancer: The role of butyrate. *Cancer Prevention Research*, 3(9), 1271–1279.
64. Verma, A., Shukla, G., & Gupta, P. K. (2013). Probiotic *Lactobacillus* GG and *L. acidophilus* as prophylactic agents against 1,2-dimethylhydrazine-induced colon cancer in rats. *Journal of Cancer Research and Therapeutics*, 9(3), 527–533.
65. Kumar, M., Nagpal, R., Verma, V., & Kumar, A. (2012). Probiotic *Lactobacillus plantarum* reduces DMH-induced oxidative stress and suppresses colon carcinogenesis. *Nutrition and Cancer*, 64(4), 520–526.
66. Shang, H., Wei, Y., Zhang, D., Chen, X., & Li, H. (2020). Anti-cancer activity of a probiotic mixture against colon cancer in an animal model. *Frontiers in Oncology*, 10, 238.
67. Awaisheh, S. S., Khalaf, R. A., & Al-Dababseh, M. M. (2016). Anticancer properties of probiotic isolates from dairy products and their effects on colorectal cancer cell lines. *Journal of Dairy Science*, 99(3), 2152–2162.
68. Sharaf, R. K., Sharma, N., Chandel, D., & Shukla, G. (2018). Prophylactic potential of *Lactobacillus* GG and celecoxib in DMH-induced colon carcinogenesis: A multi-step approach. *Journal of Cancer Research and Clinical Oncology*, 144(6), 1109–1120.
69. Song, X., Xin, T., Zhou, S., Feng, J., & Li, W. (2018). The protective effect of Bifico on colorectal cancer in azoxymethane-treated mice: Involvement of gut microbiota. *Oncology Reports*, 40(5), 2793–2803.
70. Mi, H., Wang, L., Huang, X., & Huang, J. (2017). The protective effect of Bifidobacterium infantis on chemotherapy-induced mucositis and its regulatory effect on T cell subsets in rats. *Oncology Letters*, 13(6), 4569–4575.
71. Mohania, D., Kansal, V. K., Kruzliak, P., & Kumari, A. (2014). Modulatory role of probiotic Dahi and piroxicam on the development of pre-neoplastic lesions induced by 1,2-dimethylhydrazine in rat colon. *Journal of Dairy Research*, 81(3), 287–295.
72. Zhang, H., Liu, J., Wang, L., Chen, H., & Zheng, Y. (2015). Effects of *Lactobacillus salivarius* Ren on gut microbiota and colonic carcinogenesis in a rat model. *Oncology Reports*, 34(6), 3151–3159.
73. Hu, Y., Hu, T., Liu, Y., & Ren, J. (2015). *Lactobacillus plantarum* A enhances CD8+ T-cell and NK cell immune responses and reduces tumor growth in a CT26 mouse colon cancer model. *Journal of Immunology Research*, 2015, 489825.
74. Heydari, Z., Jeddi, F., & Salekdeh, G. H. (2019). Modulation of miRNA expression and signaling pathways by probiotics in a colon cancer model: A mechanistic insight. *Journal of Functional Foods*, 62, 103556.
75. Lenoir, M., Del Carmen, S., Cortes-Perez, N., Lozano-Ojalvo, D., & Chain, F. (2016). *Lactobacillus casei* BL23 regulates Th17/Treg immune balance and promotes colonic mucosal healing in *in vivo* models of colorectal cancer. *Journal of Clinical Medicine*, 5(12), 118.

76. Jam, A., Talebi, M., Alipour, B., & Khosroushahi, A. Y. (2021). Probiotic *L. paracasei* and 5-FU combination reduces colorectal cancer progression in an *in vivo* rat model. *Oncology Reports*, 46(6), 2552–2562.
77. Kim, H., Yoon, H., Ryu, H., & Chung, M. J. (2021). Evaluation of the anticancer potential of *Lactococcus lactis* R7 in an *in vivo* colorectal cancer model. *Journal of Functional Foods*, 75, 104265.
78. Jaskulski, S., Huang, T., McGregor, J., & Li, Z. (2020). Mechanism of p8-induced apoptosis in colorectal cancer: RNF152 regulation. *Oncology Letters*, 19(2), 1029–1036.
79. Guo, X., Chen, Y., Wang, X., Li, Z., & Qiu, Y. (2020). *Lactobacillus acidophilus* CICC 6074 induces apoptosis through mitochondrial pathways in HT-29 cells and in a murine model of colon cancer. *Microbial Cell Factories*, 19(1), 183.
80. Lee, J.-E., Yun, H. S., & Kim, D. Y. (2019). Probiotic *Lactobacillus fermentum* cell-free supernatant increases apoptosis in 3D cultured colon cancer cells. *Journal of Applied Microbiology*, 127(6), 1850–1862.
81. Dong, X., Xu, W., Zhang, G., & Zhao, J. (2020). *Lactobacillus salivarius* prevents colorectal cancer progression by inhibiting the AKT signaling pathway. *Journal of Translational Medicine*, 18(1), 53.
82. Yue, S., Huang, W., Liang, J., Wang, Z., & Zhang, Y. (2020). Probiotic *Lactobacillus plantarum* YYC-3 alleviates colon cancer in *APCMin/+* mice by modulating gut microbiota and immune responses. *Frontiers in Microbiology*, 11, 826.
83. Dikeocha, I. J., Al-Kabsi, A. M., Chiu, H. T., & Alshawsh, M. A. (2022). Anticancer properties of *Faecalibacterium prausnitzii* in azoxymethane-induced colorectal cancer rats and its effect on gut microbiota. *International Journal of Molecular Sciences*, 23(11), 5984.

CHAPTER 3

Correlation Between Gut Microbiota and Cancer

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ABSTRACT

Modifications in the relationships between the intestinal epithelium, gut microbiota, and host immune system have an impact on a number of disorders, including cancer. We discuss the relationship between oncogenesis and tumor development and how altering the intestinal microbiota may expand the therapeutic effectiveness of chemotherapy medications. It is widely acknowledged that germs may cause a number of illnesses, including cancer, the second biggest cause of death worldwide. It is well acknowledged that the host's health and physiology are significantly influenced by the intestinal bacteria. The intestinal microbiota and their byproducts may activate cellular and immunological pathways to remove invading infections and start an immune response to fight cancer. The gut microbiota can affect even how well chemotherapy drugs work. The intestinal microbiome has been associated with around 20% of all malignancies, while commensal microbiota activate inflammasomes to defend the host's biological and intestinal stability. Describe the impact of the gut microbiota on cancer initiation and prevention. This book chapter discusses a few microorganisms that might have a role in the growth of malignancy. In order to repopulate the microbial population and stimulate the immune system to combat cancer, future research should

examine the potential use of numerous bacteria with probiotic properties as bio-therapeutic agents.

3.1 INTRODUCTION

One of the potentially most mixed populations of the billions of commensal microorganisms is found in the human gut [1]. These microorganisms include viruses, protists, fungi, bacteria, and archaea, with bacteria making up the majority of the residents of the intestine [2]. The microbiota has a significant role in preventing many illnesses and may have an effect on human health by producing important metabolites, metabolizing nutrients, and many toxicants that inhibit microbial attackers, limit their development, and produce important microbial products [3]. Numerous essential regulatory roles are played by the contact of the intestinal microbiota with epithelial cells and stromal cells [4]. These include preventing pathogen colonization, preventing pathogen incursion and infection, preserving metabolism, and serving as a barrier [5].

The severe acute respiratory coronavirus-related pandemic has highlighted the significance of gut microbiome makeup and diversity [6]. Surprisingly, a current research study found that the prevalence of two bacteria, *Porphyromonas endodontics* (oral) and *Enterococcus faecalis* (fecal), in the oral and stool microbiomes is a sign of severity in COVID-19 patients [7]. COVID-19 intensity is inversely linked with the prevalence of *Ruminococcus gnavus*, an anaerobic gram-positive gut resident that acts as an inflammatory indicator [8]. Microbes and malignancy have been linked in a well-known manner. The gut microbiota, which is a crucial actor, affects not only the whole gut but also a number of lateral organs, including the brain, pancreas, and liver [9]. In reality, alterations in the intestinal microbiota's composition and activity may have an effect on the development of different illness conditions, such as diabetes, obesity, malignancy, and other neurological ailments [10]. The risk of cancer may be increased, in particular, if specific microorganisms are present [11]. For instance, numerous viruses (such as human papillomavirus and Epstein-Barr virus) and microbial pathogens (such as *Fusobacterium nucleatum* and *Helicobacter pylori*) are responsible for around 20% of the world's cancer burden [12]. A number of other microbes, such as *Bacteroides fragilis*, *Parvimonas micra*, *Alistipes finegoldii*, *Thermanaerovibrio acidaminovorans*, *Prevotella intermedia*, and *Porphyromonas asaccharolytica*, might be utilized as microbial markers for diagnosis [13].

Here, we talk about the connection between malignancy and the gut flora [14]. In reality, alterations in the composition and operation of the intestinal microbiota may hasten the development of a variety of illnesses, including malignancy, diabetes, obesity, and other neurological issues. Additionally, we discuss possible mechanisms that the gut microbiome may use to bilaterally regulate the immune response, which may aid in the growth of cancer and be utilized as effective treatment options for cancer [15].

3.2 GUT MICROBIOTA

It is well recognized that the human natural homeostasis is significantly influenced by gut bacteria [16]. For instance, the number of microbial cells in an individual body ranges from 40 to 100 trillion, which is a 10-fold increase over individual somatic cells (i.e., thirty trillion) [17]. A healthy person's gut contains between 300 and 500 distinct bacterial species [18]. In fact, it's thought that the bacteria in the stomach work as a kind of digital human bodily organ [19]. Numerous pathways are used by the linked intestinal bacteria to communicate with the host [20]. For example, it promotes immune system development and enhances epithelial protection against harmful bacteria, as well as dietary nutrient absorption [21]. The gut bacteria support immune function by identifying preserved regions of Gram-negative infective bacteria, which is likely to trigger the production of immunoglobulin G (IgG) antibodies to eliminate these bacteria, despite mucus (the biological filters of the immune reaction to bacteria) containing a number of antibacterial peptides and other large molecules [22]. Actinobacteria, Proteobacteria, Bacteroidetes, and Firmicutes make up most of the phyla in a healthy digestive system [23]. However, this microcommunity exhibits diversity at the species and genus levels [24].

3.2.1 FUNCTION OF THE NORMAL GUT FLORA

According to theories, the microbiota in the digestive system significantly contributes to sustaining gut health and is crucial for preserving general physiological health [25]. Studies on humans and germ-free rats and mice provide plenty of data to support this notion [26]. Information from the US Human Microbiome Research and numerous other studies have shown the genetic advantages of a healthy intestinal microbiota [27]. For instance,

genes from the intestinal microbiota have been related to the digestion of human milk oligosaccharide compounds [28, 29].

Additionally, the large number of intestinal flora coexist peacefully with the host immune system and are non-infective and environmentally friendly (especially with intestinal cells) [30]. The probiotic bacteria of the gut participate in this relationship by producing all the nutritional requirements for defense, preventing pathogen growth, and managing medication and nutrient breakdown [31]. The host immune system does its duty simultaneously in fighting off invading harmful microorganisms. Immune cells, including macrophages, phagocytes, and dendritic cells, have a close connection to the intestinal bacteria and its metabolites and are crucial for preserving gut homeostasis and spotting dangerous pathogens [32].

On the other hand, bacterial migration in healthy people might result in moderate to extreme clinical symptoms [33]. For instance, when toxins from living or dead microbes very briefly traverse the gut's epithelial mucosa (EM), they might stimulate the body's reticulo-endothelial system (RES), especially in the liver's Kupffer cells. Gram-negative bacteria, for example, may begin to migrate into the epithelial mucosa in a dysbiosis scenario and subsequently move to extra-intestinal locations and tissues. As a result, a variety of clinical signs, such as sepsis, septic shock, organ failure, and even host death, may appear [34].

3.2.2 THE MICROBIOTA AND CANCER

The leading cause of death globally is cancer [35]. The population of cancer patients in the United States of America (USA) alone has risen dramatically from 13.8 to 18.1 million in the last ten years, placing a \$158 billion financial strain on the country's economy [36]. Even with early detection screening and innovative therapy approaches, cancer-related death rates in the USA fell by 26% between 1991 and 2015 [37]. It is predicted that by 2030, there may be 24 million active cases annually [38]. The development of cancer may be significantly influenced by exposure to carcinogens and other melanoma chemicals. Recent research found that high fat intake by normal people seems to be connected with undesired alterations in intestinal microbiota, which might influence the host's general health. An increased, highly processed Western diet is also thought to have an impact on gut bacteria diversity [39].

A few bacteria are known to encourage cancer under unfavorable conditions in this respect. According to several studies, changing the intestinal flora

may raise the risk of acquiring different kinds of malignancies via a number of distinct routes. Additionally, research has shown that microbes and their metabolites may interfere with the pharmacodynamics of anti-cancer medications in addition to contributing to the growth of cancer. Inflammation may be caused by bacteria penetrating the stomach lining and surrounding tissues when the intestinal microbiota's homeostasis is disturbed. In this context, evidence suggests that persistent inflammation may play a role in the growth of malignancies. As the inflammatory process promotes tumor growth, tumor cell invasion is accelerated and ultimately results in metastasis. Numerous inflammatory mediators have the potential to directly damage epithelial cells' DNA, which can result in cancers linked to inflammation [40].

Additionally, it is known that the gut microbiome influences various cancer types [41]. Samples from the GI tract and feces were subjected to 16S rRNA sequencing analysis, which demonstrated that dysregulation of the intestinal microbiota may promote the growth of malignancy [42]. For instance, *Helicobacter pylori* may trigger immunological reactions and persistent inflammation, which can ultimately result in stomach malignancy [43]. A number of *H. pylori* genes dysregulate normal cell/tissue balance in *H. pylori*-infected individuals, leading to elevated levels of cytokine accumulation and various melanoma signaling factors, ultimately causing gastric cancer [44].

Esophageal carcinoma may potentially be caused by *H. pylori* [45]. Streptococcus and various Gram-positive Firmicutes are heavily prevalent in the distal oesophagus of healthy people [46]. When the microbiome is dysbiotic, many Gram-negative microorganisms (anaerobic and micro-aerophiles) compete with the Gram-positive occupants. Later stages of Gram-negative species might result in esophagitis [47]. The main phyla that produce esophageal malignancies among other intestinal microbiota include Firmicutes, Proteobacteria, and Bacteroidetes [48].

Additionally, the large intestine's microbiota, which produces carcinogens, co-carcinogens, and pro-carcinogens, may have a significant impact on the occurrence of colorectal malignancy [49]. In terms of health issues, colorectal malignancy ranks third in terms of frequency of detection among malignancies that affect both sexes. Numerous metabolites produced by the residing microbiota (Firmicutes, Bacteroidetes, and Proteobacteria) may have both malignant and anti-cancer characteristics. Recent research claims that a variety of bacteria are related to the growth of colon malignancy [50]. These include *Clostridium septicum*, *B. fragilis*, *E. coli*, *Fusobacterium* spp., *E. faecalis*, *Helicobacter pylori*, and *Streptococcus bovis*, etc. [51]. The

decrease in butyrate-producing bacteria in the intestinal microbiota is one reason that could be applicable. The colon, also known as the large intestine, contains ten times more bacteria than the intestinal tract, and as a result, studies have shown that there is an approximately 12-fold increased risk of developing colorectal malignancy. This emphasizes the importance of the colon bacteria in the development of malignancy [52]. According to studies, animals with dysbiotic gut microbiomes may acquire cancer. This is based on the observation that alteration of the intestinal microbiota in a mouse model was associated with swelling of the internal lining of the colon and the development of tumors [54]. However, it has also been reported that using germ-free mice, colitis and melanoma advancement could be prevented [55]. Additionally, interleukin IL-10 and IL-2 knockout mice that developed colitis and cancer on their own under normal circumstances were less common in germ-free mice [56]. Overall, these results point to the negative effects of gut microbiome dysbiosis in promoting colitis and carcinogenesis. Even as intestinal bacteria have demonstrated their significance in preventing many cancers and other diseases, on the other hand [57], with a mortality risk of more than 40,000 per year, breast malignancy is the second most common cause of mortality among women. Over the past 20 years, about 5.6 billion dollars have been spent on studies into breast cancer, which is the second most prevalent cause of death in women, with a death risk of over 40,000 each year, but the cause is still unknown [58]. It has been suggested that this syndrome may really be influenced by bacteria. The bacteria itself or its byproducts might be the catalyst for breast carcinoma. For instance, the tissues of breast tumors contain large amounts of *Methylobacterium radiotolerans* [59]. According to another study, there may be a link between obesity, the gut microbiota, and hepatic malignancies. Obesity damages epithelial connections, making it possible for commensal bacteria to enter the bloodstream and cause systemic infections. As a result, the gut flora may alter, which might result in the emergence of hepatocellular carcinoma associated with obesity [60]. A change in gut bacteria may trigger the onset of a number of liver disorders. The following are among them: liver malignancy, non-alcoholic steatohepatitis, cirrhosis, and fatty liver disease, both alcoholic and nonalcoholic [61]. Hepatic carcinoma is the most common significant hepatocellular malignancy and the leading cause of mortality in cirrhotic patients [62]. Bile acids (BA), lipoteichoic acid (LTA), lipopolysaccharides (LPS), and other tiny molecules that are produced and secreted by intestinal microbiota help the liver's immune response remain under control [63]. A key connection between the intestine, liver, and gut bacterial flora

may be provided by BA specifically [64]. Before being absorbed in the GI epithelium, lipids and other various water-insoluble vitamins [65], such as *C. hylemonae*, *C. scindens*, *C. sordellii*, and *Clostridium hiranonis*, change the primary bile acids (7 α -hydroxyl BAs, chenodeoxycholic acid, and cholic acid) into the secondary bile acids: deoxycholic acid via 7 α -dehydroxylation [66]. It has been shown that excessive concentrations of secondary bile acids produce reactive nitrogen species and reactive oxygen species, which damage DNA and alter the mitochondria and plasma membrane, resulting in colon cancer [67]. Liver cancer may arise as a result of LPS activating the toll-like receptor (TLR) 4 on human cells [68]. Of all gynecological malignancies, ovarian malignancy has the highest death rate in the world. With 0.28 million new cases and 0.19 million malignancy deaths worldwide in 2018, ovarian malignancy was the most common reason for death from gynecologic malignancies [69]. Due to the absence of nearly effective identification and screening methods, the majority of ovarian malignancies are discovered at an advanced stage [70]. The histopathology, molecular biology, and other characteristics of ovarian cancer are quite diverse. Type II cancers are usually aggressive, high grade, and in advanced stages. There are two types of epithelial ovarian malignancy, with type II tumors being the most aggressive, rising, and progressed of all ovarian malignancies, accounting for 90% of all malignancies [71]. Currently, ovarian malignancy is treated with cytoreductive surgery and platinum-based chemotherapy, but these aggressive surgical protocols and subsequent cancer treatments may cause gastrointestinal diseases, particularly mucosal inflammation [72]. Treatments involving chemotherapy, such as cisplatin regimens (because of their antibiotic-like action), and other chemotherapy agents are cytotoxic, producing GI mucositis and upsetting the commensal bacteria community that lives in the gut [73]. Losing weight and heart failure have been associated with cisplatin-related side effects, which are thought to be caused by alterations in the intestinal microbiota, notably a decline in Firmicutes. By lowering inflammation, Lactobacillus supplementation may also assist in lessening the cardiotoxicity caused by cisplatin [74]. It was shown that *Ruminococcus gnavus* abundance was significantly decreased in mice given cisplatin and was restored with orally administered fecal pellets [75].

A number of things, such as bad eating habits, cigarette use, poor oral cleanliness, and excessive alcohol use, may lead to oral cancer. Microbial infections have been shown to have a significant role in the growth of oral malignancy [76]. More than 90% of oral malignancies are believed to be caused by oral squamous cell carcinoma [77]. Acetaldehyde is a crucial

byproduct of the metabolism of ethanol [78]. Its usage has historically been connected to the growth of oral cancers since it is prevalent in alcoholic drinks [79]. By manufacturing a variety of hydrolases that convert alcohol into the carcinogenic acetaldehyde, a species of *Candida* present in the oral microbiota is considered to encourage the growth of tumors. In a recent investigation, the saliva of individuals with oral squamous cell malignancy was isolated from the *Candida* class, with *C. albicans* being the most common species [80]. Additionally, the effects of the mortality rate were investigated. Inflammation brought on by pathogens is thought to be essential for the growth of cancer. Elevated pro-inflammatory cytokine release as a consequence of microbial infection of the oral mucosal layer contributed to the swelling. For example, *C. albicans* causes malignancy genesis by triggering inflammation and the Th-17 reaction, among other ways [81]. A portion of Th-17 cells and the Th-17-dependent signaling pathway activate the NF- κ B and Wnt pathways, which provide an inflammatory milieu that promotes the growth of malignancies [82]. Additionally, increased pro-inflammatory cytokine levels, primarily IL-6 and IL-8, contribute to the inflammatory response and, consequently, oncogenesis in cancer cells. For example, IL-6 inhibits the apoptosis of cancerous cells [83]. The fourth most common cause of malignancy death in women worldwide and the leading cause of gynecological cancers in emerging countries is cervical malignancy [84]. According to recent research, more than 270,000 people die from cervical malignancy each year, with over 500,000 new cases identified [85]. Previous studies have shown that cervical cancers are caused by a host-dependent immune reaction and immunological avoidance caused by the human papillomavirus (HPV) [86]. Through the use of 16S rRNA sequencing analysis, a current study examined the differences in intestinal bacteria between cervical malignancy patients and healthy controls. The fecal DNA microbiol profiles of five healthy persons and eight cervical malignancy patients were examined. The abundance of seven different genera, including *E. Shigella*, *Roseburia*, *Pseudomonas*, *Lachnoclostridium*, *Dorea*, and *Succinivibrio*, was also shown to be substantially different in cervical malignancy. Proteobacteria, the genera *Parabacteroides*, *E. Shigella*, and *Roseburia*, according to the author, may serve as helpful biomarkers for cervical malignancy [87].

3.2.3 TUMOR-PROMOTING EFFECTS OF THE GUT MICROBIOTA

A subset of morbidic microbes is either explicitly or implicitly related to the growth and activation of malignancies, despite the fact that it is well

known that the commensal microbiota is important for human health and that it strengthens the immune system of the host to prevent the onset of melanoma. Table 3.1 illustrates the part that various microorganisms play in the development of tumors and the management of malignancy.

Pathogenic microorganisms in the dysbiotic gut negatively affect the host body in a number of ways to promote cancer. Dysbiosis in the gastrointestinal system, in particular, has been linked to the establishment of benign and malignant cancers [89]. Previous research has shown that the intestinal flora has the ability to extensively penetrate bodily tissues and induce carcinogenesis in hygienic animal models by decreasing interleukin-10, a crucial cytokine in the host's fight against cancer [101]. The most powerful cytokine, interleukin-10, is well recognized. Additionally, it is important for the control of malignancy and tumor immunologic monitoring [102]. CagA, a protein of microbial ancestry, was the first to be connected to human carcinoma [88]. This protein is generated by *H. pylori*, a class 1 carcinogen. Bacteria create and produce a wide range of toxins when there is a microbial imbalance in the intestinal flora [103]. These toxins may cause damage to the host's DNA, the onset of tumors, and their growth in cells and tissues that are predisposed to them [104]. Similar to how *E. coli* produces colibactin, cdt-positive strains of bacteria produce cytolethal distending toxin (CDT), which causes the target cells to ultimately undergo apoptosis due to nuclear and cellular enlargement caused by DNase activity. Toxins released by bacteria that are close to the intestinal epithelium cause double-stranded breaks in the host deoxyribonucleic acid, which leads to gene variation and the growth of tumors. *Shigella flexneri* produces its essential enzymes, cysteine protease and IpgD, which cause the breakdown of the host cell's p53 gene and also affect the deoxyribonucleic acid repair process [105]. Similar to this, CagA is used by *H. pylori* to break down p53 in gastric cells. By disrupting the host's serine/threonine kinase pathway, the cytotoxin promotes stomach cancer [106]. Additionally, it is recognized that intestinal bacteria can induce carcinogens by altering a variety of host cellular pathways [92]. Numerous pathogenic bacterial proteins, enzymes, and toxins bind to cells, change their standard functions, and promote a variety of tumors and malignancies [93]. As an example, the effector adhesin A (FadA) protein of *Fusobacterium*, CagA of *Helicobacter pylori*, and metalloproteinase toxin of *Bacteroides fragilis* adhere to the host tissue epithelial protein, rupturing cell connections and activating β -catenin signaling. The stimulation intensifies the transformation of the affected cells' and tissues' tumorigenic properties [91].

TABLE 3.1 The Function of the Gut Microbiota in Cancer Therapy and Carcinogenesis

Bacteria	Toxin/Metabolite/ Enzyme	Effects	Type(s) of Cancer/ Tumor	Reference
Microbiota with Tumor Promotion Effects				
<i>Helicobacter pylori</i>	Cytotoxin linked gene A	Promotes gastric epithelial cells to undergo neoplastic mutation. Alternatively, disrupt the host's serine/threonine kinase (AKT) pathway to cause gastric malignancy	Human melanoma Or Gastric cancer	88
<i>Escherichia coli</i>	Cytotoxic distending toxin and Colibactin	Genomic instabilities cause breakdowns in the DNA of the host	Tumor	89
<i>Shigella flexneri</i>	Cysteine protease like Inositol phosphate phosphatase D and virulence gene A	DNA destruction and host cell p53 gene degradation	Necrosis causing cell death and the formation of cancer	90
<i>Fusobacterium nucleatum</i>	Effector adhesin A (FadA)	Disrupts cell connections by binding to E-cadherin and stimulation β -catenin.	Colo-rectal cancer	91
<i>Helicobacter pylori</i>	Cag A protein	E-cadherin interaction and discharge The catenin β -cat	Stomach epithelial cell carcinogenesis	92
<i>Bacteroides fragilis</i>	Metalloprotease toxin	Triggers cell division by adhering to intestinal E-cadherin	Colorectal carcinogenesis	93
<i>Helicobacter pylori</i>	Cag A protein	Spermine oxidase overexpression, which causes an increase in H_2O_2 , ROS, and DNA damage	Gastric cancer	94
<i>Bacteroides fragilis</i>	Bacteroides fragilis toxin	Spermine oxidase activation, which causes an rise in Reactive Oxygen Species, Hydrogen peroxide, and DNA damage	Colon tumorigenesis	95
<i>Fusobacterium nucleatum</i>	<i>Fusobacterium nucleatum</i> virulence factor	Inhibits the natural killer cells by binding to TGIT (natural killer inhibitory receptor) and the ITIM domain.	Colon adenocarcinoma	89

TABLE 3.1 (Continued)

Bacteria	Toxin/Metabolite/ Enzyme	Effects	Type(s) of Cancer/ Tumor	Reference
Microbiota with Anti-Tumor Properties				
<i>Bifidobacterium longum</i> and <i>Bifidobacterium breve</i>	-	Enhancing the role of dendritic cells, causes cytotoxic T-cell infiltration in cancerous locations	Anti-tumor	11
<i>B. rodentium</i>	-	Cut down on the UPR signaling pathway	Melanoma immunity to chemotherapeutic drugs	11
Heat killed <i>Streptococcus</i> and <i>Serratia</i>	Endotoxins	Engenders enduring immunity	Antineoplastic effects on sarcoma	96
<i>Bacteroides</i>	-	Restore the ability of inhibitors of CTLA-4, the cytotoxic T cells associated antigen.	Prevent solid tumorigenesis	97
<i>Propionibacteria</i> , <i>Acidipropionici</i> , and <i>Propionibacteria. freudenreichi</i>	SCFAs (propionate and butyrate)	Impede the tumor cells' histone deacetylases. In order to eradicate the tumor, butyrate activates the GPR 109A, which is then followed by IL-18 and CD8+ cells.	Anticarcinogenic for lymphoma and colorectal malignancy	98
Gram-negative bacteria	LPS	Activate TLR4, which increases the immune system's T-cell defense against malignancy cells.	Melanoma-specific anti-tumor action	99
<i>Lactocaseibacillus casei</i>	Ferrirome	Jun N-terminal kinase signaling pathway activation and apoptosis induction are the last steps.	Colorectal cancer	100

Studies utilizing a mouse model in both *in vivo* and *in vitro* have demonstrated that Fad, a protein from *F. nucleatum*, binds to the E-cadherin receptor, triggering catenin, which promotes the development of cancer. The growth of cancer cells may be reduced by inhibiting *F. nucleatum* from attaching to E-cadherin using synthetic peptide molecules. Pathogenic microorganisms may indirectly influence the carcinogenesis of host cells via a variety of methods. For instance, pathogenic bacteria create a number of chemicals that may suppress the host's immunological response and increase inflammation, enabling the malignancy to escape the immune system [107]. Similar to how they do, bacteria also cause oxidative stress, which leads to self-directed genetic changes in the host cell [108]. Both *B. fragilis* and *H. pylori* have the ability to activate the spermine oxidase flavoenzyme in the host. Reactive oxygen species and hydrogen peroxide are formed during this activation, leading to the accumulation of DNA damage [95]. *Enterococcus faecalis* enters the host cell by creating external superoxide and oxygen species derivatives to hasten the modification of the host's DNA [94].

The intestinal bacteria have a critical role in both fostering and preventing cancer. A healthy gut's bacterial population prevents pathogen penetration, implantation, and infections while also suppressing the immune system. By inducing the innate (myeloid cells) and adaptive (CD4⁺ cells and CD8⁺ cells) immune responses, as well as programmed cell death, short-chain fatty acids (SCFAs), which are potent remnants of bacteria and include propionate and butyrate, play a significant role. Conversely, using antimicrobials in combination with a variety of pathobionts, their metabolic products, and toxicants leads to a dysbiotic intestine that is incapable of preventing the colonization of pathogens, increases inflammation through various pathways, and ultimately results in the development of tumor cells in various body tissues. Prebiotics and probiotic microbes may enhance the capacity of dendritic cells to further infiltrate CD8⁺ cells and the tumor microenvironment, inhibiting the development of tumors.

By destroying tumor cells, natural killer (NK) cells are essential for preventing the development of tumors. The virulence factor of *F. nucleatum* (Fap2) inhibits NK cells from attacking malignant cells by interacting with TGIT (Natural Killer inhibitory receptor) and the ITIM domain [109].

3.2.3.1 GUT MICROBIOTA INHIBITING TUMOR GROWTH

The intestinal microbiota has been shown to be significant in balancing the effects and results of anticancer medicines in addition to their function in

carcinogenesis (Figure 3.1). There are many trillions of bacteria in the human body, and they are constantly interacting with the tissues of the host. So, it comes as no surprise that the microbiota has a significant impact on how the host's body functions [110]. For instance, the host immune system and intestinal microbiota work together to regulate food digestion and the release of metabolites that are antigen-specific in the GI tract. This interaction also enables immune cells to recognize and eradicate opportunistic superbugs before they infiltrate the host's body[111].

Both the innate and adaptive immune systems are affected by the microbiome on a systemic level. Studies conducted *in vivo* on animals without the gut microbiota and devoid of germs support this notion. Modified immunoglobulin A, a deficiency in mucus, and mesenteric lymphadenopathy have all been shown in mouse models. Investigations have also shown that the lack of germs has a detrimental influence on the host's ability to heal itself [112]. The efficiency of immunotherapy and chemotherapy medications may be altered by commensal microorganisms.

When combined with an integrated microbiome, biological treatment stops the progression of cancer by inducing immune cells, such as myeloid cells and cytotoxic T-lymphocytes, to create tumor necrosis factor. On the other hand, the antibiotic therapy that slows tumor deterioration in a mouse model inhibits the generation of cytokines and TNF by immune cells. For instance, research on mice fed an antibiotic cocktail changed the gut flora, which reduced the effectiveness of chemotherapy and CpG-oligonucleotide immunotherapy. Preclinical mouse models have helped us understand how the gut microbiota may cause inflammation while receiving treatment. However, the scenario may vary based on the kind of malignancy, the animal model, and should be cautiously understood. Moving forward, individual cohorts should serve as models for future research [113].

An authorized medication called cyclophosphamide (CTX) is used to cure certain cancers by enhancing the host's T-cell immune reaction. The mutualistic flora of the gut relocates to the mesenteric lymph nodes and spleen as a consequence of this chemotherapy drug's abridgment of the intestine and breaching of the epithelial membrane, stimulating helper T cells and further triggering antineoplastic effects against carcinogenesis [114]. Research using the mouse model has shown that a wide variety of bacterial species boost CTX's anticancer efficacy. These include *Enterococcus hirae*, *Lactobacillus johnsonii*, and *Brucella intestinihominis*. The absence of Gram-positive bacteria in the intestines of mice treated with antibiotics or mice raised without germs results in a diminished immune reaction [97].

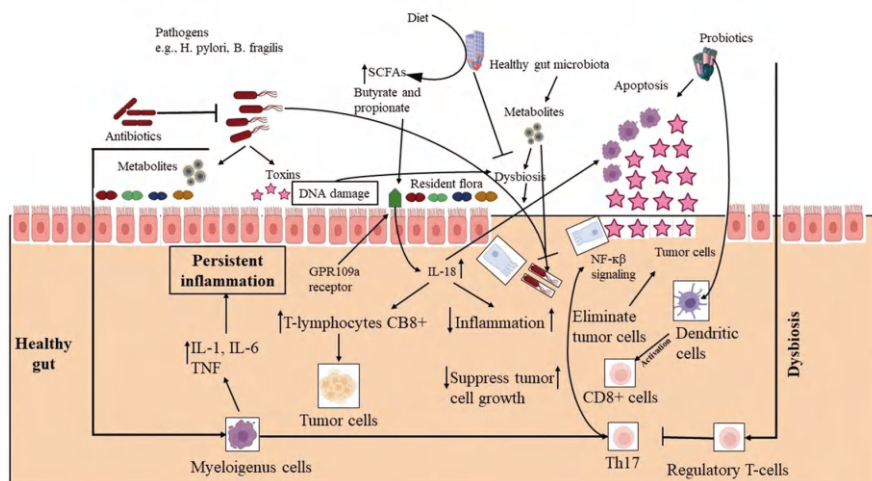


FIGURE 3.1 Gut microbiota inhibiting tumor growth.

It has been shown that *Bifidobacterium longum* and *B. breve* improve dendritic cell functionality. Cytotoxic T lymphocytes are then drawn to the tumor microenvironment as a result of dendritic cell activation. A biological process known as the unfolded protein response (UPR) has been related to a number of human illnesses and disorders, including several tumor forms. By lowering the UPR signaling pathway, certain bacterial species may help the immune system fight melanoma by facilitating the anticancer immune response. When it comes to chemotherapy, certain gut flora show extreme resistance in cases of colorectal cancer. For instance, *F. nucleatum* uses certain forms of Toll-like receptor (TLR) binding sites and micro-RNA to activate the autophagy process to impart drug resistance [97].

According to several studies, *Streptococcus* and *Serratia pyogenes* may increase sarcoma patients' survival rates by 70% for up to 5 years [115]. Additionally, it is thought that heat-killed microbes trigger the enduring immune reaction and may have anti-cancer effects on sarcomas [96]. In the process of solid carcinogenesis, CD8⁺ T cells are expected to efficiently infiltrate infected cells and tissues as a result of the intestinal microbiota. For instance, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockers depend on *Bacteroides*, a kind of intestinal microbiota, to have anti-cancer effects. The anti-cancer efficacy of CTLA-4 inhibitors is correlated with the T-cell response to *Bacteroides*. It's interesting to note that CTLA-4 had no anti-cancer effects in germ-free animals, but these effects were recovered when *Bacteroides* were given to sterile mice. [116]

Molecules and chemicals produced from microorganisms have powerful anticancer effects [98]. Short-chain fatty acids (SCFAs) formed by the microbiota may have a key role in the prevention of tumors and malignancies [117]. Common SCFAs with potent anti-cancer properties include butyrate and propionate, which are developed by commensal bacteria in the gut [118]. These chemicals cause apoptosis (programmed cell death) and inhibit the histone deacetylases of cancerous cells. Individuals with colonic cancer had lower quantities of butyrate-producing microorganisms [119]. The stomach lining's epithelial cells create interleukin-18 as a result of butyrate's stimulation of the GPR109A receptor, which may initiate the healing mechanisms for mucosal tissue [120].

By altering the host's immune response, metabolites from probiotics may cause an indirect immune reaction against cancer genesis. For instance, TLR4 may be activated by lipopolysaccharides (LPS), which then activate T-cell-mediated immune reactions against tumor cells [121]. Similar to this, *Salmonella enterica*'s monophosphoryl lipid A has been employed as an adjuvant in vaccine formulation and is very effective against cervical cancer. Some gut microbes could create molecules derived from probiotics that have anti-tumor properties [122]. For instance, *L. casei* generates ferricrome, which causes programmed cell death in malignant cells by activating the JNK signaling pathway [123]. Additionally, it is thought that lactobacilli stimulate the host immune system (NK, TH1, or DC), which may help to eradicate tumor-infected cells [124]. According to a new study, some individuals with malignancies that do not respond to immunotherapy or medication may benefit from changing the bacteria in their gut microbiota via the use of fecal transplantations [125]. Chemotherapy, immunology, the immune response, and the immunological response are all impacted by bacteria and viruses in the stomach [126]. For instance, studies have shown that mice with tumors do not often react to vaccinations but may start to respond when fed certain gut microbes from mice that did react to the medications [127]. Immune checkpoint suppression using mAbs directing apoptosis provided long-term therapeutic benefits in around 40% of individuals with metastatic melanoma [128]. In addition to the malignancy internal processes that promote sensitivity to anti-PD-1 mAbs, the gut flora plays a crucial role in the tumor's external regulation of anti-programmed cell death protein 1 reactions [129]. In mice with cancer, the treatment of particular gut bacteria or fecal microbiota transplantation (FMT) boosts anti-PD-1 efficacy. Animals' responses to anti-programmed death-ligand 1 (PD-L1) and anti-PD-1 therapies are influenced by the composition of their gut flora [130]. Actinobacteria

(*Bifidobacteriaceae* and *Coriobacteriaceae*) and Firmicutes (*Ruminococcaceae* and *Lachnospiraceae* species) are significant microorganisms from many phyla that are linked to a beneficial reaction to PD-1-targeting mAbs in cancer [131]. Moreover, in the Interleukin-10 knockout mouse model administered with azoxymethane, the overabundance of *E. coli* NC101 in the gut may purposefully promote invasive malignancy [132]. Furthermore, this bacterium's genotoxic islands were removed, which markedly decreased the prevalence of malignancy and its development [133].

3.2.4 RELATIONSHIP BETWEEN THE GUT MICROBIOME AND OTHER MICROORGANISMS

Different types and quantities of bacteria are present in the GI microbiome and oral microbiome, but their interconnectivity implies microbial transmission channels [134]. There are two ways that the oral microbiota can move from the mouth to the gut: (1) oral bacteria go from the gastric to the intestine through the intestinal route, and (2) the hematogenous pathway, via which oral bacteria colonize the GI system after entering lesions and traveling throughout the body [135]. In individuals with inflammatory bowel illness, for instance, an antibiotic-sensitive *Klebsiella* species from the mouth may move from the oral cavity to the gut. This results from the antibiotic use's destruction of the GI tract's bacteria [136]. In individuals with liver cirrhosis, colorectal carcinoma, autoimmune diseases, and other intestinal disorders, various bacterial species, including *Fusobacterium*, *Porphyromonas*, *Actinomyces*, *Parvimonas*, and *Veillonella*, move from the mouth cavity to the gut [137].

Similar to how the gut flora is connected to gastrointestinal malignancies, the vaginal microbiome is linked with malignancies of the female reproductive system. Increased vaginal pH and *Lactobacillus* spp. abundance are, respectively, adversely and favorably related to high concentrations of malignancy indicators in the cervicovaginal micro-ecosystem in females with invasive cervical cancer [138]. Aggressive cervical malignancy development, persistent human papillomavirus infection, and cervical epithelial dysplasia are all correlated with a dysbiotic vaginal microbiome that is low in lactobacilli and high in pH [139]. Additionally, the absence of lactobacilli and the existence of *Mobiluncus*, *Prevotella*, *Streptococcus*, *Atopobium*, and *Ureaplasma* in the vaginal flora are linked with a higher risk of malignancy of the ovaries or factors that affect that risk, such as age and mutations in the

BRCA1 gene [140]. Inflammation brought on by cancers of the reproductive and digestive systems triggers dysbiosis, which in turn causes dysbiosis, generating a disease-promoting positive feedback loop [141].

Understanding how dysbiosis results in illness can help with the creation of novel bacterial treatments that control the microbiota to reestablish bodily balance [142]. For instance, a *Clostridium difficile* infection results in fewer resident, beneficial bacteria in the gut, which promotes *C. difficile* growth and results in gastrointestinal microbial dysbiosis [143]. Through fecal microbiota transplantation (FMT), the diseased patient's intestines receive the advantageous microbes from a healthy person, aiding in recolonization [144].

3.2.5 INFLAMMASOME ACTIVATION AND GUT MICROBIOTA

In addition to interacting with immune cells, intestinal epithelial cells may also be influenced by gut microbiota via inflammasome stimulation. Through the use of receptors with an oligomerization domain-like structure, inflammasomes are able to distinguish between non-toxic and toxic molecules formed by non-pathogenic and pathogenic bacteria in the host's intestine and immune cells [145]. Inflammasomes play an important role in maintaining the body's balance and the health of its tissues and cells. When the body's balance is disturbed for whatever reason, the inflammasomes activate and mediate a potent immunological response. Inflammasomes, in short, trigger caspase-1, which then promotes the liberation of cytokines (Interleukins-1), IL-18, and ultimately leads to apoptosis [146]. There is proof that inflammasome dysregulation causes a variety of diseases, such as autoimmune, neurological, metabolic, and cancer [147].

In this context, inflammasome stimulation is crucial for the development of malignancy [148]. This is because inflammasomes have the ability to both induce and repress malignancy [149]. Regarding their potential involvement in carcinogenesis, there are several considerations [150]. This covers both the stage and kind of cancers, as well as the impact of chemicals, expression patterns, and tumor stage. Inflammasome stimulation is considered to be largely influenced by the gut flora [151]. Similar to how certain essential components are thought to have an essential role in how inflammasomes operate [152]. The growth of colon cancer is suppressed by IL-18, whereas lung, breast, pancreatic, and skin cancers are all proven to be adversely affected by IL-1 [153]. Several different types of binding site proteins with

inflammasomal receptors are present in host epithelial and immune cells. These receptors have been designed specifically to detect the microbiota and its secretory byproducts. When microbiota or their metabolites trigger inflammasomes, interleukin-18 accumulates in the intestine and may eventually govern gut dysbiosis [156]. Contrarily, when gut dysbiosis results from decreased IL-18 synthesis (as a result of insufficient inflammasome elements), the intestinal microbes may pass the epithelial protection and induce significant inflammation, which may promote the growth of malignancy [157]. Lactobacilli, as was mentioned in the part above, may strengthen the host immunological reaction [158]. This genus of macrophages and gut epithelial cells has the ability to activate inflammasomes, which may defend the gut from harm and avoid viral infections [159].

3.3 PROBIOTICS AND PREBIOTICS' INVOLVEMENT IN PREVENTING CANCER

Live probiotics are usually considered to be harmless bacteria with enormous health advantages. Probiotics may be essential in avoiding a variety of illnesses, especially several tumor forms [160]. Probiotics may be employed as microbiological components or biotherapeutic agents in the food and pharmaceutical sectors, respectively. Dead probiotics, such as bacterial cell walls, offer significant advantages in preventing and treating various illnesses, including cancer [161]. Finding a powerful probiotic strain, however, might be difficult. The release of the digestive tract's vital enzymes for food digestion may be significantly increased by probiotic variations or their bioactive elements [162]. Although probiotics are usually considered to be safe, caution should be used when administering them to chemotherapy patients who have compromised immune systems since there may be a chance of increasing antibiotic resistance and creating new infections [163].

However, probiotics can be given to people with malignancy because they have a variety of advantages and can also inhibit diarrhea and other gut-related issues, enabling the regeneration of a healthy microbiota and subsequent antineoplastic therapy [164]. The effects shown in clinical studies are considerable, and taking probiotics increases the number of good bacteria in the stomach. For instance, *L. johnsonii* and *B. longum* were administered to a patient in a double-blind study. The study found that the earlier probiotic strain adhered to the gut mucosa and cleared harmful bacteria by inducing a localized immune reaction [165]. In related research,

an investigational approach was employed to evaluate the probiotic effectiveness of *Lactobacillus acidophilus* and *Bifidobacterium longum*. Extreme dysentery following pelvic irradiation was significantly reduced by using these species [166]. Similarly, a combination of 10 probiotic bacteria decreased chemotherapy-mediated cytotoxicity while also preventing diarrhea in patients with metastatic colon malignancy [167]. By decreasing the gut enzyme actions that transform a number of amines and complex aromatic hydrocarbons into active carcinogens, probiotic microbes may indirectly eradicate colorectal malignancy [168]. Another research study showed that *Bifidobacterium* species, specifically subspecies *Lactis*, and other probiotic bacteria control the immune reaction of the host by stimulating phagocytic cells and then eliminating cancer cells in their early phases of growth [169]. Individuals with colorectal malignancy who consume probiotics have a clear correlation between the enhancement of pro-apoptotic action. For instance, *L. delbrueckii* greatly increases the production of caspase-3 in human colorectal carcinoma cells, which is accompanied by the activation of programmed cell death [170]. Despite their substantial advantages, it's crucial to understand any probiotic strain's clinical effectiveness before, during, and after antitumor treatments. As a result, probiotic bacteria have the potential to be powerful biotherapeutic agents in the treatment of several illnesses, particularly cancers, by preventing intestinal dysbiosis, enhancing the host immune reaction, and more.

3.4 THE LINK BETWEEN MALIGNANCY AND THE GUT MICROBIOTA

The emergence of cancer is influenced by a variety of variables. It is progressively becoming clear that the makeup and alterations in the gut microbiota also matter, in addition to lifestyle choices, epigenetic and genetic modifications, and ecological variables [171]. The importance of the mammalian intestinal microbiota on human health is undeniable. It is made up of more than a thousand different species and 100 trillion archaeal and microbial cells. When a person is healthy, their gut maintains homeostasis, avoiding the invasion of bacteria as a result of connections between the host and its flora [171]. However, changes to the gut microbiota may throw off this equilibrium and harm the host, including the growth and proliferation of tumors. Depending on a substantial body of research, we discovered that the gut microbiota not only contributes locally to the formation of intestinal

tumors but also indirectly influences the growth of extra-gastrointestinal tumors. Hepatocellular carcinoma may be affected by intestinal microbiota-related chemicals, antigens produced by the intestinal microbiota, and microbe biochemical patterns, for instance, which may be transported to the liver through the portal vein. The gut microbiota may go to other closely related organs and affect the development of tumors. Mice were gavaged with fluorescently tagged commensal bacteria, which indicated the transfer of microorganisms from the gastrointestinal compartments to the pancreas. In a nutshell, the growth of many human systems, including the gastrointestinal system, genitourinary system, cardiovascular system, and respiratory system, is linked to the gut microbiota. In this paper, we review current findings on the processes behind the association between malignancy and the gut microbiota. Studies showing a connection between intestinal imbalance and cancer have been conducted thus far. Therefore, even though our understanding of the processes is still limited, more and more people have started to investigate them further. According to studies, gut microbiota biofilms may significantly contribute to the development and maintenance of colorectal malignancy [171]. The study offered a convincing bacterial driver-passenger concept to explain how biofilms work. According to this concept, bacterial drivers use genotoxicity to initiate CRC development, while bacterial passengers use metabolites to sustain the CRC process. Recently, it has been claimed that biofilms may develop in tissues with urothelial malignancy, and it has been shown that biofilm development is connected to bladder malignancy. Numerous studies have shown that infectious disease, which may be related to the *Helicobacter pylori* biofilm, may prevent the excision of stomach cancer (see Table 3.2) [171].

3.5 RADIOTHERAPY AND GUT MICROBIOTA INTERACTIONS

A bigger function for the intestinal microbiota in host homeostasis has been steadily revealed via the advent of novel molecular tools, including 16S ribosomal RNA sequencing, deoxyribonucleic acid sequencing, and metagenomics, as well as the use of mice. In the human body, certain microorganisms have useful or negative effects (Table 3.3). As the link between the intestinal microbiota and the development of cancer becomes increasingly clear, so has the importance of the intestinal microbiota in modern anti-cancer therapies, as well as its potential to affect the efficacy and toxicity of tumor treatment. The gut flora is influenced by a variety of variables. One of these numerous

TABLE 3.2 Dysbiosis of Gut Microbiota in the Development and Progression of Cancer

Cancer	Object of Research	Sample Type	Method	Gut Microbiota Dysbacteriosis in Malignancy Patients	Reference
colorectal malignancy	Brazilian colorectal malignancy patients	stool samples	Sequencing of the 16S rRNA gene Amplicon	Prevotella grew whereas Megamonas and Ruminococcus declined, considerably increasing the α -diversity.	172
gastric malignancy	45 gastric malignancy cases from Shenyang, China	malignant lesions and adjacent non-malignant tissues	Analysis of ITS rDNA gene	<i>Arcopilus aureus</i> , and <i>Fusicolla aquaeductuum</i> were more abundant, whereas <i>Aspergillus montevicensis</i> , <i>Candida glabrata</i> , and <i>Penicillium arenicola</i> were clearly less abundant in GC.	173
hepatocellular malignancy	143 hepatocellular malignancy patients and 40 healthy volunteers	stool samples	16S rRNA sequencing	Reduced -diversity; a greater average frequency of Actinobacteria and a considerably lower average number of Bacteroidetes in the HCC group	174
cervical malignancy	42 cervical malignancy patients and 46 healthy female controls	stool samples	16S rRNA gene sequencing	Prevotella, and Dialister were considerably supplemented in older women with cervical malignancy; alpha diversity was maximum in these patients.	175

factors is radiation therapy, which results in dysregulation of the intestinal microbiota, frequently exhibited by a reduction in the variety and abundance of the intestinal flora, a boost in the proteobacteria and fusobacteria that are harmful microorganisms, and a decline in the beneficial microbiota. Due to this characteristic, the intestinal microbiota may be used as a new biomarker of radiation exposure, which might significantly enhance biological dose estimation and complement the current method of chromosomal aberration evaluation [171]. Currently, numerous studies have looked at how radiotherapy affects the microbiota in various cancers [171].

TABLE 3.3 Harmful and Beneficial Bacteria Commonly Found in Humans

Beneficial Bacteria	Harmful Bacteria
<i>Akkermansia muciniphila</i>	Acetothermia
<i>Clostridium casei</i>	Cryptomycota
<i>Bacteroides fragilis</i>	Desulfovibrio
<i>Christensenella minuta</i>	Enterococcus
<i>Bifidobacterium</i>	Deferribacteres
<i>Faecalibacterium prausnitzii</i>	Lachnospiraceae
<i>Turicibacter etc.</i>	Rikenellaceae_RC9_gut_group etc.
<i>Bacteroides thetaiotaomicron</i>	Fusobacterium
<i>Prevotella copri</i>	Pseudomonas
<i>Lactobacillus acidophilus</i>	Porhyromonas
<i>Parabacteroides goldsteinii</i>	Peptococcaceae
<i>Saccharomycetes</i>	Proteobacteria

3.6 DYSBIOSIS AND CARCINOGENESIS

In addition to pathogenic microorganisms and passing commensals, environmental variables including age, antibiotics, smoking, xenobiotics, hormones, and nutritional cues may also promote dysbiosis. These variables are also known to increase the chance of developing extraintestinal or intestinal neoplasms [176]. Additionally, since they promote inflammatory disorders like Crohn’s disease, which increase the risk of the host developing neoplastic transformation, genetic defects that impact lymphoid, myeloid, or epithelial cells of the gastrointestinal immune response favor dysregulation. As a result, a number of variables that encourage tumorigenesis also encourage dysbiosis [177]. The relevance of the connection between dysregulation and intestinal malignancy in terms of medicine is shown by epidemiological studies that relate intra-abdominal illnesses, antibiotic usage, or both, to a

rise in the risk of colorectal malignancy. In fact, there are several methods in which the gut microbiota affects the development of colorectal malignancy. Elimination or selective alteration of the gut microbiota alters the prevalence and progression of colorectal cancer in both hereditary and carcinogen-induced models of carcinogenesis. Several intestinal epithelial cell (IEC)-targeting products of the intestinal microbiota have also been discovered to either induce carcinogenic effects (as documented for hydrogen sulfide and the *Bacteroides fragilis* toxin) or prevent tumor formation (as established for short-chain fatty acids) [178]. Intestinal bugs have a role in the development of various malignancies in addition to colorectal carcinogenesis. Through inflammatory and metabolic pathways, experimental changes to the intestinal microbiota may also affect the emergence and development of extraintestinal malignancies, such as breast and hepatic carcinoma. These findings are consistent with epidemiological investigation conclusions that extracolonic neoplasms, especially breast cancer, are more common when dysbiosis is present, as are their effects or causes (particularly the misuse of antibiotics). These results could be a result of bacterial and bacterial byproducts spreading throughout the body as a result of inflammatory reactions that weaken the intestinal barrier's integrity. Therefore, both locally and systemically, the gut microbiota affects oncogenesis and tumor progression. The capacity of dysbiosis to induce carcinogenesis may be attributed to other, as of yet unidentified pathways, even if inflammatory and metabolic signals encourage this phenomenon (see Figure 3.2) [179].

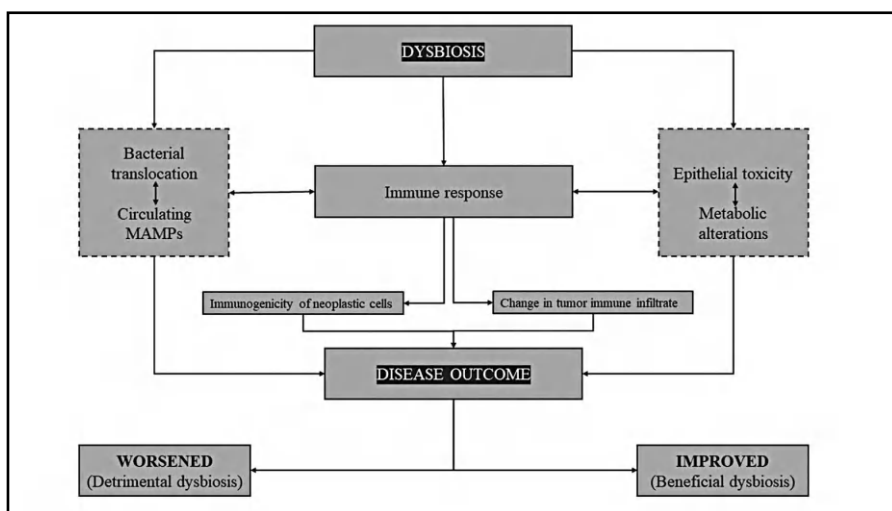


FIGURE 3.2 Links between dysbiosis and cancer.

3.7 RELATIONSHIP STATUS: CHALLENGING

The gut microbes and anticancer drugs interact in a two-way manner during cancer treatment [180]. On the one side, numerous treatments for neoplastic disorders now in use have cytotoxic effects on gut microorganisms, *de facto* encouraging dysbiosis [180]. Radiotherapy, allogeneic stem cell transplantation, and a number of chemotherapy drugs, including 5-fluorouracil and irinotecan, can be harmful to the intestinal microbiota and thereby change its proportion, either directly or by inducing an immune reaction. Furthermore, the intestinal barrier is adversely impacted by these (and other) therapeutic interventions [180]. On the other side, mounting research suggests that the gut bacteria, through pharmacological and immunological pathways, affect both the therapeutic effectiveness and the adverse effects of chemotherapeutic drugs (see Figure 3.3).

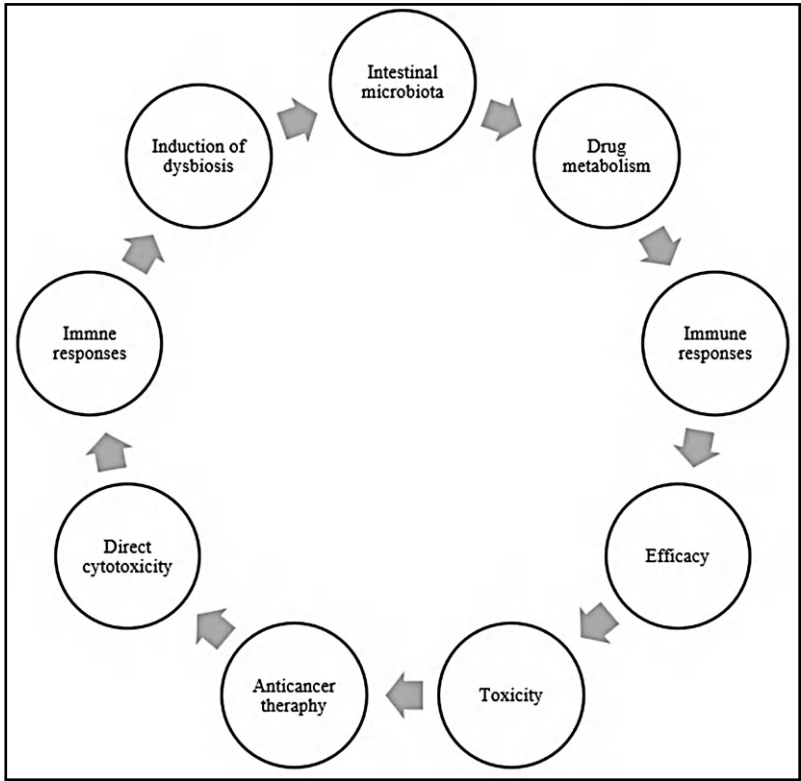


FIGURE 3.3 Relationship between the gut bacteria and antineoplastic treatment Gut bacteria interact in both directions with chemotherapeutic, radiotherapeutic, and immunotherapeutic anticancer treatments.

3.7.1 PHARMACODYNAMIC EFFECTS

Intestinal bacteria are numerous and exhibit considerable metabolic activity, which allows them to assess the bioavailability and bioactivities of ingested xenobiotics to evaluate whether they are justified (efficacy) or not (toxicity) [181]. This has been shown for a number of medications, including irinotecan. Irinotecan's dose-limiting diarrhea has been linked to the intestinal microbiota's capacity to regionally reactivate the medication. Additionally, the digestive toxicity of irinotecan is decreased by administering a Chinese herbal medication (PHY906) that only stimulates the regrowth of gastrointestinal progenitor cells following transformation by bacteria-glucuronidase (which is highly expressed by the gut microbiota). In addition to oral molecules, systemic therapies may also be implicated in how the gut microbiota affects the pharmacodynamics of chemotherapeutic medications (which come into direct contact with intestinal bacteria). In fact, a large panel of hepatic genes implicated in xenobiotic metabolism are expressed differently in germ-free (GF) mice than in their typical, pathogen-free counterparts. The gut flora may potentially contribute to acute graft-versus-host disease (GVHD), a key obstacle to the successful treatment of allogeneic cell therapy. According to several studies, Paneth cell death and changes in the TLR9/MYD88 signaling axis play an important role in the link between intestinal GVHD and dysbiosis, which is most often characterized by an enrichment in Enterobacteriaceae species. Dysbiosis may thus reduce the therapeutic effectiveness of several anticancer therapies in addition to affecting their gastrointestinal adverse effects. However, eubiotic gut flora may lessen the unnecessary side effects of a number of anticancer drugs [181].

3.7.2 IMMUNOLOGICAL EFFECTS

There is growing evidence that, particularly in mice, the gut microbiota affects how different tumor types respond to cancer treatment by altering immune circuitry. Lymphodepleting whole-body radiation, for example, is thought to increase intestinal epithelium-crossing of the intestinal microbiota, or at least several of its products. This helps irradiation boost the effectiveness of adoptively transplanted CD8⁺ T cells, and it also corresponds with increased dendritic upregulation and raised amounts of blood-borne proinflammatory cytokines. As a result, mice treated with antibiotics, those injected with an antibody that neutralizes lipopolysaccharide

(LPS), Tlr4 and Cd14 animals (which do not react to lipopolysaccharide normally), as well as their control counterparts, are less vulnerable to lymphodepleting irradiation. When given intravenously to animals kept in pathogen-free environments, the immunostimulatory alkylating drug cyclophosphamide causes mucosal damage and the spread of certain Gram-positive bacteria through the intestinal epithelium [182]. This phenomenon was connected to TH1 and TH17 immunological reactions in the spleen, which are significant for therapeutic purposes. Mice with tumors given GF and antibiotics were less able to mount such bactericidal T cell-mediated responses, making them more vulnerable to the curative effects of cyclophosphamide than control animals. Furthermore, adoptive transfer of TH17 cells generated and expanded *in vitro* allowed cyclophosphamide's full-blown anticancer activity to be recovered in mice receiving antibiotic treatment. However, not all Gram-positive microorganisms were effective in triggering helpful TH17 immune reactions in this scenario. The therapeutic effects of anti-cancer treatment were instead lessened by certain prokaryotes, such as *Parabacteroides distasonis* [which exhibits regulatory T (Treg) cell-activation actions] and branched filamentous bacteria [which generate typical TH17 reactions]. The therapeutic effects of platinum substances and a potential therapeutic regimen based on CpG oligodeoxynucleotides have been shown to be enhanced by a healthy gut flora, which is consistent with these results. When combined with a specific antibody that neutralizes the interleukin-10 receptor, CpG oligodeoxynucleotides' capacity to trigger a therapeutically significant innate immune response against cancer cells was altered by the intestinal microbiota (IL10RA). In addition, the immunostimulatory platinum salt oxaliplatin, which is authorized for treatment in people with colorectal malignancy, needed a eubiotic intestinal flora to promote cancer infiltration by myeloid cells, which mediated anti-cancer benefits via creating reactive oxygen species (ROS). The Cybb/genotype (corresponding to the absence of an enzyme that produces reactive oxygen species) and systematic antioxidant supply may mimic the chemotherapy-impairing effects of antibiotics, claims this idea. Mice missing Tlr4 or Myd88 (encoding crucial sections of the apparatus recognizing molecular patterns correlated with microbes) were likewise more unaffected by oxaliplatin-based therapy than their wild-type counterparts. Thus, oxaliplatin's full-fledged therapeutic action entails the immune system's identification of gut microbiota constituents, enabling the development of tumor-infiltrating myeloid cells with anticancer properties [114].

Overall, these findings suggest that antineoplastic therapy can encourage two types of dysbiosis that are functionally opposed to each other: detrimental dysbiosis, which reduces therapeutic efficacy or raises treatment toxicity, and helpful dysbiosis, which is necessary for, or at least noticeably enhances, medical activity. This shows that improving the therapeutic index of anticancer treatment by the pharmacological modulation of the gut microbiota has significant potential [59].

3.8 MICROBIOTA MANIPULATION FOR CANCER THERAPY

There are four possible approaches to alter how the gut microbiota impacts anticancer therapy: Probiotics are live bacteria or other germs that, when administered in large enough doses, provide positive health effects. Prebiotics are non-digestible compounds that improve certain properties of the gut microbiota, such as development. Chemicals known as antibiotics have a cytotoxic action that is preferred for one or more species of bacteria. Postbiotics are untenable byproducts of the intestinal microbiota that influence the host's bioactivities. It may not be possible, due to specificity concerns, to employ conventional antibiotics to induce a condition of dysbiosis that enhances the effectiveness of chemotherapeutic medicines instead of working against it [183].

However, if a harmful dysbiosis has already developed, it might be possible to reverse it with antibiotics. According to recent research, proteinaceous bacteriocin antibiotics generated by certain bacterial strains may be used to target and therapeutically reduce one or more gut bacteria components [142]. Additionally, some chemotherapeutics' pharmacodynamics may be improved by using certain compounds to lessen the negative impact of the gut flora. As proof of concept, it has been shown that mice may be protected from the gastrointestinal adverse effects of irinotecan, prolonging its therapeutic window, by a potent inhibitor of microbial (but not human) glucuronidase [184]. Promising findings have been obtained from comprehensive testing of probiotics' capacity to inhibit (mostly intestinal) carcinogenesis in animal tumor models. Additionally, at least in animal studies, genetically altered probiotics have been used to deliver immunomodulatory molecules, tumor-linked antigens, or enzymes that lessen the toxicity of chemotherapeutic medications [185]. The efficacy and safety of these therapies, as well as their capacity to stimulate biologically relevant immune responses in cancer patients, are presently being studied [186]. Epidemiological research has not

been able to conclusively prove whether probiotics may lower the incidence of colorectal cancer in certain patient groups [187]. The use of probiotics to lessen the induced gastric toxicity of radiation therapy and certain chemotherapies is beneficial, but there isn't enough clinical data to make a clear decision about it [188]. Even though prebiotics (like oligofructose or inulin) and postbiotics (like butyrate) have drawn interest as likely colorectal cancer preventative measures, their capacity to extend the therapeutic window of chemotherapeutics is still little understood [189].

It is encouraging to speculate that the analytical profile of at least some chemotherapy agents can be enhanced by combinational treatments relying on one or more antimicrobials, prebiotics, probiotics, or postbiotics in light of recent studies demonstrating that modifications in the intestinal microbiota are beneficial rather than harmful to the effectiveness of antineoplastic chemotherapy. Intestinal microorganisms affect oncogenesis, tumor growth, and therapeutic response, according to growing research. Therefore, selectively altering the intestinal microbiota may be a practical way to reduce the prevalence of particular tumors in the population and boost the effectiveness of different antineoplastic drugs. Although the primary hypothesis has been tested in a number of oncogenesis models with encouraging outcomes, it is still unknown whether anti-, pre-, pro-, and postbiotics actually have any onco-preventive effects in humans. On the other hand, the idea of intentionally altering the gut microbiota's composition as a route to the best possible responses to chemotherapy, radiotherapy, or immunotherapy in the clinic is a relatively recent one, and more research is needed to fully grasp its therapeutic use [190]. The low selectivity of the majority of conventional antibiotics and the high inter-individual variation of the gut flora may pose significant challenges in this situation. These problems could be mitigated, at least in part, by the development of new technologies and the use of highly particular antibiotics such as bacteriocins and extremely specialized antimicrobials. The clinical effectiveness of anticancer chemotherapy, radiotherapy, and immunotherapy may be enhanced by altering the gut flora [191].

3.8.1 EFFECTIVENESS OF RADIOTHERAPY AND GUT MICROBIOTA

There is strong proof that the body's sensitivity to anti-cancer therapies like chemotherapy, vaccination, radiation, and surgeries may be influenced by the gut flora. The intestinal bacteria and these therapies participate in both directions; hence, various treatments will result in different alterations in the intestinal microbiota, which will, in turn, result in varied treatment reactions.

Radiotherapy is similarly comparable to them as an efficient way to treat malignancies. The efficiency of tumor radiation is influenced by the gut microbiota either negatively or favorably, according to recent study findings. Although several studies have shown that the gut microbiota may hold the key to reducing radiation-related problems, the immediate influence of the intestinal flora on the efficacy of radiotherapy is still poorly understood, making this a significant and intriguing field of radiology study [192]. A study has shown that changes in the intestinal microbiota's composition increased the vulnerability of mice to gamma radiation. Another research discovered that, in an animal model of cancer, whole-body radiation boosted the migration of gut microbes to mesenteric lymph nodes (LNs), resulting in a higher antineoplastic response. There is preliminary evidence that individuals who receive broad-spectrum antibiotics have less effective anticancer radiation. Antibiotic-induced changes to the intestinal microbiota may be the cause of this condition. However, the limited information suggests a negative link between the two [193]. FOXQ1 was shown to be highly expressed in colorectal cells and tissues, along with higher levels of catenin and nuclear displacement, which were both advantageous to the intestinal pathogenic bacteria associated with CRC. This increased radio-resistance in colon cancer cells was positively connected with poor prognostications in colon cancer patients, pointing to a possible causal relationship involving radio-resistance and the intestinal microbiota. This implies a negative correlation between gut flora imbalance and therapeutic efficacy and partly describes the pharmacodynamic process of Shengmai curcumin powder in radiosensitivity. Shengmai curcumin powder may shield the gut flora against radiotherapy's negative effects, according to current pharmacological studies. More investigation is still required to ascertain how the intestinal bacteria influence the body's response to radiation, despite emerging indications that the mammalian gut microbiota contributes to radio-resistance. Fast-induced adipokine (Fiaf), which is typically produced from the villous epithelium of the small intestine, is often suppressed by the intestinal microbiota, which causes a rise in the deposition of fatty acids in the liver and adipose tissues. Villous endothelium and lymphocyte groups have been demonstrated to lose their susceptibility to radiation-induced apoptosis as a result of Fiaf deficiency, according to studies. It was shown that grape polyphenols (GP) increased the expression of genes related to Fiaf and improved intestinal barrier performance. Additionally, GP markedly accelerated *Akkermansia muciniphila*'s development rate and decreased the ratio of Firmicutes to Bacteroidetes [194]. In conclusion, it is possible that changes to the intestinal

microbiota may have an impact on Fiaf's metabolism and, therefore, radiation sensitivities. Enhancing autophagy might have a big impact on how radiotherapy-resistant cancers are treated. X-ray exposure has been shown in certain studies to greatly increase autophagy in parental cells, but radiation-tolerant cells seldom experience autophagic cell death, indicating a connection between autophagic cell death and radiation sensitivity. It's also unclear whether the gut bacteria are involved. The prevalence of *F. nucleatum* in high concentrations in repeated CRC tissues following treatment was also shown in other investigations to be a factor in CRC's chemoresistance. Regarding the mechanism, *F. nucleatum* activates the autophagy system by targeting the innate immune signals TLR4 and MYD88, as well as certain microRNAs, which changes the therapeutic responses for colorectal cancer. It is now well known how vitamin D, the gut flora, and radiation responses interact. The altered gut flora brought on by radiotherapy eventually affected blood vitamin D levels, as well as the body's distribution and metabolism. Therefore, variations in vitamin D levels have an effect on a patient's capacity to react to radiation, which may be regulated by the intestinal milieu, immunological molecules in the gut, intestinal microbial metabolites, and vitamin D receptor signaling pathways. Radiation is a well-known palliative treatment for cancer, and it is also believed to cause an antitumor immune response; thus, immunomodulation may be one way that the gut bacteria affect the radiotherapy response. We still don't know enough about the abscopal impact at this time. Ionizing radiation has the potential to provide both the maturation stimuli required by dendritic cells to stimulate tumor-specific T lymphocytes as well as tumor-specific antigens from dying cells. This phenomenon, known as the abscopal effect, is caused by ionizing radiation's ability to restrict tumor development beyond the radiation-treated area. It is unclear, nevertheless, whether this radiotherapy-related impact involves the intestinal flora and its metabolites. Vancomycin is a gut-specific antibiotic that mostly affects Gram-positive bacteria. Vancomycin has been shown to improve the RT-induced tumor growth inhibition and antitumor immune function. IFN- and the cross-presentation of TTA to cytolytic CD8⁺ T cells are required for this interaction. As a result, it could be possible to increase RT's anticancer effect by getting rid of vancomycin-sensitive microorganisms. Additionally, research has revealed that SCFAs generated by the intestinal microbiota can be absorbed into the blood and exert their effects throughout the body. These effects include inhibiting histone deacetylase action, regulating the expression of genes in distal organs, and controlling the effector function of CD8⁺ T cells. The tumor microenvironment (TME),

which is made up of the tumor vascular system and cells of the immune system, is also impacted by radiotherapy in addition to cancer cells. Endothelial cells may be destroyed, and an inflammatory reaction may result. Damaged arteries block CD8⁺ T cell entry into tumors and activate immunosuppressive pathways [194]. It's interesting to note that prior research has revealed that radiation may also encourage a pro-immunogenic environment inside the tumor, which prompts the host's immune system to respond specifically to cancer. The delicate balance between immune reactions and immunosuppression brought on by radiotherapy may have a substantial impact on effectiveness. Unexpectedly, it was discovered that intestinal bacteria have a substantial role in controlling the TME externally. It has been shown to play a significant part in the immunological reprogramming of innate and/or adaptive immune cells to the TME, which may impact the efficacy of traditional chemotherapy and immunotherapy for malignancy. It is uncertain, however, if this impact results from radiation. The immunosuppressive milieu typical of advanced malignancies often outweighs the initial immunological responses produced by radiation *in vivo* and clinically; therefore, the initial immune effects are suppressed. However, when preexisting immunosuppression is diminished or eliminated, like in the case of combining immune checkpoint inhibitors with local irradiation, the pro-immunogenic actions of ionizing radiation play a role and boost immune-mediated cancer resistance. In "hot" tumors, which are tumors that have been invaded by lymphocytes, immune checkpoint inhibitors (ICI), effective cancer medicines, restore anticancer T cell activity. In contrast, "cool" tumors lack lymphocyte infiltration and are resistant to immunotherapy. Radiation may increase the quantity of antitumor T cells and render resistant tumors more susceptible to immune checkpoint blockers, according to preclinical studies. Recent studies on ICI treatment have shown that changing gut microbiota may reduce tumor patients' antitumor immunity, which in turn impacts their resistance to ICI, while increasing the number of bacteria in the stomach may recover their susceptibility to anticancer drugs. The efficiency of ICI and local and systemic anticancer immune reactions may be influenced by the intestinal microbiota via the transfer of small-molecule metabolites from the gut to other parts of the body [195].

3.9 CONCLUSION

Trillions of bacteria that protect the host's body from harmful effects reside in the human GI tract. Among them, commensal bacteria and the elements

of their secretions play a crucial role in the destruction of harmful superbugs and the prevention of many illnesses, including cancer. While not all microorganisms are involved in tumor suppression, it is now widely recognized that many of them promote malignancy. Microbiota in a dysbiotic gut can penetrate deeply into host tissues and cross the epithelial barrier, leading to cancer. Some of these microbiotas may release compounds upon penetration that could prompt the host immune system to target infected tissues and halt cancer progression. Probiotic species are essential in this process, as they can effectively aid in both the removal of harmful bacteria and the repopulation of beneficial bacteria in the gut. In animal models, probiotics have also demonstrated a preventive effect against the spread of cancer. Additionally, clinical studies suggest that certain probiotic species may reduce the risk of inflammation in cancer patients. However, the precise optimal gut microbiota composition needed to sustain immune responses and anti-tumor effects remains unclear. Whether altering the gut microbiota can be used as a therapeutic strategy requires further investigation. More research is needed to identify the metabolites produced by the gut microbiota and assess their potential effects on harmful pathogens or the host's immune system.

KEYWORDS

- **cancer**
- **dysbiosis**
- **gut microbiota**
- **immune reaction**
- **microbiome**
- **probiotics**

REFERENCES

1. Meng, C., Bai, C., Brown, T. D., Hood, L. E., & Tian, Q. (2018). Human gut microbiota and gastrointestinal cancer. *Genomics, Proteomics & Bioinformatics*, 16, 33–49.
2. Zitvogel, L., Daillère, R., Roberti, M. P., Routy, B., & Kroemer, G. (2017). Anticancer effects of the microbiome and its products. *Nature Reviews Microbiology*, 15(8), 465–478.

3. Vaishnava, S., Behrendt, C. L., Ismail, A. S., Eckmann, L., & Hooper, L. V. (2008). Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proceedings of the National Academy of Sciences*, 105(52), 20858–20863.
4. Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature Reviews Immunology*, 14(3), 141–153.
5. De Santis, S., Cavalcanti, E., Mastronardi, M., Jirillo, E., & Chieppa, M. (2015). Nutritional keys for intestinal barrier modulation. *Frontiers in Immunology*, 6, 612.
6. Sommer, F., & Bäckhed, F. (2015). The gut microbiota engages different signaling pathways to induce Duox2 expression in the ileum and colon epithelium. *Mucosal Immunology*, 8(2), 372–379.
7. Chung, W. S. F., Walker, A. W., Louis, P., Parkhill, J., Vermeiren, J., Bosscher, D., Duncan, S. H., & Flint, H. J. (2016). Modulation of the human gut microbiota by dietary fibers occurs at the species level. *BMC Biology*, 14(1), 1–13.
8. Ward, D. V., Bhattarai, S., Rojas-Correa, M., Purkayastha, A., Holler, D., Da Qu, M., Mitchell, W. G., Yang, J., Fountain, S., & Zeamer, A. (2021). The intestinal and oral microbiomes are robust predictors of COVID-19 severity: The main predictor of COVID-19-related fatality. *medRxiv*, 2001–2021.
9. Gasmi, A., Tippairote, T., Mujawdiya, P. K., Peana, M., Menzel, A., Dadar, M., Benahmed, A. G., & Björklund, G. (2021). The microbiota-mediated dietary and nutritional interventions for COVID-19. *Clinical Immunology*, 226, 108725.
10. Siddiqui, R., Qaisar, R., Goswami, N., Khan, N. A., & Elmoselhi, A. (2021). Effect of microgravity environment on gut microbiome and angiogenesis. *Life*, 11(10), 1008.
11. Cheng, W. Y., Wu, C. Y., & Yu, J. (2020). The role of gut microbiota in cancer treatment: Friend or foe? *Gut*, 69(10), 1867–1876.
12. Parlato, M., & Yeretssian, G. (2014). NOD-like receptors in intestinal homeostasis and epithelial tissue repair. *International Journal of Molecular Sciences*, 15(6), 9594–9627.
13. Siddiqui, R., Akbar, N., & Khan, N. A. (2021). Gut microbiome and human health under the space environment. *Journal of Applied Microbiology*, 130(1), 14–24.
14. Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. *Current Opinion in Gastroenterology*, 31(1), 69.
15. Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology*, 14(8), e1002533.
16. Gao, R., Gao, Z., Huang, L., & Qin, H. (2017). Gut microbiota and colorectal cancer. *European Journal of Clinical Microbiology & Infectious Diseases*, 36, 757–769.
17. Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ*, 361.
18. Leeming, E. R., Louca, P., Gibson, R., Menni, C., Spector, T. D., & Le Roy, C. I. (2021). The complexities of the diet-microbiome relationship: Advances and perspectives. *Genome Medicine*, 13(1), 1–14.
19. Gomma, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: A review. *Antonie Van Leeuwenhoek*, 113(12), 2019–2040.
20. Chung, H., Pamp, S. J., Hill, J. A., Surana, N. K., Edelman, S. M., Troy, E. B., Reading, N. C., Villablanca, E. J., Wang, S., & Mora, J. R. (2012). Gut immune maturation depends on colonization with a host-specific microbiota. *Cell*, 149(7), 1578–1593.
21. Kamada, N., Chen, G. Y., Inohara, N., & Núñez, G. (2013). Control of pathogens and pathobionts by the gut microbiota. *Nature Immunology*, 14(7), 685–690.

22. Sellon, R. K., Tonkonogy, S., Schultz, M., Dieleman, L. A., Grenther, W., Balish, E. D., Rennick, D. M., & Sartor, R. B. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infection and Immunity*, 66(11), 5224–5231.
23. Zeng, M. Y., Cisalpino, D., Varadarajan, S., Hellman, J., Warren, H. S., Cascalho, M., Inohara, N., & Núñez, G. (2016). Gut microbiota-induced immunoglobulin G controls systemic infection by symbiotic bacteria and pathogens. *Immunity*, 44(3), 647–658.
24. Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., & Mele, M. C. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*, 7(1), 14.
25. Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Reddy, D. N. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology*, 21(29), 8787.
26. Chen, D., Jin, D., Huang, S., Wu, J., Xu, M., Liu, T., Dong, W., Liu, X., Wang, S., & Zhong, W. (2020). *Clostridium butyricum*, a butyrate-producing probiotic, inhibits intestinal tumor development through modulating Wnt signaling and gut microbiota. *Cancer Letters*, 469, 456–467.
27. Vannucci, L., Stepankova, R., Kozakova, H., Fiserova, A., Rossmann, P., & Tlaskalova-Hogenova, H. (2008). Colorectal carcinogenesis in germ-free and conventionally reared rats: Different intestinal environments affect the systemic immunity. *International Journal of Oncology*, 32(3), 609–617.
28. Li, Y., Kundu, P., Seow, S. W., de Matos, C. T., Aronsson, L., Chin, K. C., Kärre, K., Pettersson, S., & Greicius, G. (2012). Gut microbiota accelerate tumor growth via C-Jun and STAT3 phosphorylation in APC Min/+ mice. *Carcinogenesis*, 33(6), 1231–1238.
29. Gagnière, J., Raisch, J., Veziant, J., Barnich, N., Bonnet, R., Buc, E., Bringer, M. A., Pezet, D., & Bonnet, M. (2016). Gut microbiota imbalance and colorectal cancer. *World Journal of Gastroenterology*, 22(2), 501–518.
30. Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., et al. (2010). A human gut microbiome gene catalog established by metagenomic sequencing. *Nature*, 464, 59–65.
31. Structure, function and diversity of the healthy human microbiome. (2012). *Nature*, 486(7402), 207–214.
32. Jiao, Y., Wu, L., Huntington, N. D., & Zhang, X. (2020). Crosstalk between gut microbiota and innate immunity and its implication in autoimmune diseases. *Frontiers in Immunology*, 11, 282.
33. Guarner, F., Casellas, F., Borruel, N., Antolín, M., Videla, S., & Vilaseca, J. (2003). Gut flora in health and disease. *The Lancet*, 361, 512–519.
34. Jeyamogan, S., Khan, N. A., Anwar, A., Shah, M. R., & Siddiqui, R. (2018). Cytotoxic effects of benzodioxane, naphthalene diimide, porphyrin and acetamol derivatives on HeLa cells. *SAGE Open Medicine*, 6, 2050312118781962.
35. Jeyamogan, S., Khan, N. A., & Siddiqui, R. (2021). Application and importance of theranostics in the diagnosis and treatment of cancer. *Archives of Medical Research*, 52(2), 131–142.
36. Bultman, S. J. (2014). Emerging roles of the microbiome in cancer. *Carcinogenesis*, 35(2), 249–255.
37. Siegel, R. L., Miller, K. D., & Jemal, A. (2019). CA: A cancer journal for clinicians. *Cancer Statistics*, 69(1), 7–34.

38. Agrawal, B. (2019). New therapeutic targets for cancer: The interplay between immune and metabolic checkpoints and gut microbiota. *Clinical and Translational Medicine*, 8(1), 23.
39. Wan, Y., Wang, F., Yuan, J., Li, J., Jiang, D., Zhang, J., Li, H., Wang, R., Tang, J., & Huang, T. (2019). Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: A 6-month randomized controlled-feeding trial. *Gut*, 68(8), 1417–1429.
40. Hattori, N., & Ushijima, T. (2016). Epigenetic impact of infection on carcinogenesis: Mechanisms and applications. *Genome Medicine*, 8, 1–13.
41. Doorakkers, E., Lagergren, J., Engstrand, L., & Brusselaers, N. (2016). Eradication of *Helicobacter pylori* and gastric cancer: A systematic review and meta-analysis of cohort studies. *JNCI: Journal of the National Cancer Institute*, 108(9).
42. Chen, W., Liu, F., Ling, Z., Tong, X., & Xiang, C. (2012). Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLOS One*, 7(6), e39743.
43. Wu, N., Yang, X., Zhang, R., Li, J., Xiao, X., Hu, Y., Chen, Y., Yang, F., Lu, N., & Wang, Z. (2013). Dysbiosis signature of fecal microbiota in colorectal cancer patients. *Microbial Ecology*, 66, 462–470.
44. Odenbreit, S., Püls, J., Sedlmaier, B., Gerland, E., Fischer, W., & Haas, R. (2000). Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science*, 287(5457), 1497–1500.
45. Moyat, M., & Velin, D. (2014). Immune responses to *Helicobacter pylori* infection. *World Journal of Gastroenterology*, 20(19), 5583.
46. Khatoon, J., Rai, R. P., & Prasad, K. N. (2016). Role of *Helicobacter pylori* in gastric cancer: Updates. *World Journal of Gastrointestinal Oncology*, 8(2), 147.
47. Sadowski, D. C., Ackah, F., Jiang, B., & Svenson, L. W. (2010). Achalasia: Incidence, prevalence and survival. A population-based study. *Neurogastroenterology & Motility*, 22(9), e256–e261.
48. Nasrollahzadeh, D., Malekzadeh, R., Ploner, A., Shakeri, R., Sotoudeh, M., Fahimi, S., Nasser-Moghaddam, S., Kamangar, F., Abnet, C. C., & Winckler, B. (2015). Variations of gastric corpus microbiota are associated with early esophageal squamous cell carcinoma and squamous dysplasia. *Scientific Reports*, 5(1), 1–6.
49. Abdulmir, A. S., Hafidh, R. R., & Bakar, F. A. (2011). The association of *Streptococcus bovis*/Gallolyticus with colorectal tumors: The nature and the underlying mechanisms of its etiological role. *Journal of Experimental & Clinical Cancer Research*, 30(1), 1–13.
50. Arthur, J. C., Perez-Chanona, E., Mühlbauer, M., Tomkovich, S., Uronis, J. M., Fan, T. J., Campbell, B. J., Abujamel, T., Dogan, B., & Rogers, A. B. (2012). Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science*, 338(6103), 120–123.
51. Wang, T., Cai, G., Qiu, Y., Fei, N., Zhang, M., Pang, X., Jia, W., Cai, S., & Zhao, L. (2012). Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME Journal*, 6(2), 320–329.
52. Proctor, L. M. (2011). The Human Microbiome Project in 2011 and beyond. *Cell Host & Microbe*, 10(4), 287–291.
53. Couturier-Maillard, A., Secher, T., Rehman, A., Normand, S., De Arcangelis, A., Haesler, R., Huot, L., Grandjean, T., Bressenot, A., & Delanoye-Crespin, A. (2013).

- NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *The Journal of Clinical Investigation*, 123(2), 1–6.
54. Hu, B., Elinav, E., Huber, S., Strowig, T., Hao, L., Hafemann, A., Jin, C., Wunderlich, C., Wunderlich, T., & Eisenbarth, S. C. (2013). Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer. *Proceedings of the National Academy of Sciences*, 110(24), 9862–9867.
 55. Kang, X., Zhan, L., Lu, X., Song, J., Zhong, Y., Wang, Y., Yang, Y., Fan, Z., Jiang, X., & Sun, R. (2020). Characteristics of gastric microbiota in GK rats with spontaneous diabetes: A comparative study. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 13, 1435.
 56. Uronis, J. M., Mühlbauer, M., Herfarth, H. H., Rubinas, T. C., Jones, G. S., & Jobin, C. (2009). Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLOS One*, 4(6), e6026.
 57. Zhan, Y., Chen, P. J., Sadler, W. D., Wang, F., Poe, S., Núñez, G., Eaton, K. A., & Chen, G. Y. (2013). Gut microbiota protects against gastrointestinal tumorigenesis caused by epithelial injury. *Cancer Research*, 73(24), 7199–7210.
 58. Madigan, M. P., Ziegler, R. G., Benichou, J., Byrne, C., & Hoover, R. N. (1995). Proportion of breast cancer cases in the United States explained by well-established risk factors. *JNCI: Journal of the National Cancer Institute*, 87(22), 1681–1685.
 59. Xuan, C., Shamonki, J. M., Chung, A., DiNome, M. L., Chung, M., Sieling, P. A., & Lee, D. J. (2014). Microbial dysbiosis is associated with human breast cancer. *PLOS One*, 9(1), e83744.
 60. Pollard, R. T., Salter, I., Sanders, R. J., Lucas, M. I., Moore, C. M., Mills, R. A., Statham, P. J., Allen, J. T., Baker, A. R., & Bakker, D. C. E. (2009). Southern Ocean deep-water carbon export enhanced by natural iron fertilization. *Nature*, 457(7229), 577–580.
 61. Tilg, H., Cani, P. D., & Mayer, E. A. (2016). Gut microbiome and liver diseases. *Gut*, 65(12), 2035–2044.
 62. Balogh, J., Victor III, D., Asham, E. H., Burroughs, S. G., Bektour, M., Saharia, A., Li, X., Ghobrial, R. M., & Monsour Jr, H. P. (2016). Hepatocellular carcinoma: A review. *Journal of Hepatocellular Carcinoma*, 41–53.
 63. Loo, T. M., Kamachi, F., Watanabe, Y., Yoshimoto, S., Kanda, H., Arai, Y., Nakajima-Takagi, Y., Iwama, A., Koga, T., & Sugimoto, Y. (2017). Gut microbiota promotes obesity-associated liver cancer through PGE2-mediated suppression of antitumor immunity. *Cancer Discovery*, 7(5), 522–538.
 64. Ma, C., Han, M., Heinrich, B., Fu, Q., Zhang, Q., Sandhu, M., Agdashian, D., Terabe, M., Berzofsky, J. A., & Fako, V. (2018). Gut microbiome–Mediated bile acid metabolism regulates liver cancer via NKT cells. *Science*, 360(6391), eaan5931.
 65. Singh, V., San Yeoh, B., Chassaing, B., Xiao, X., Saha, P., Olvera, R. A., Lapek, J. D., Zhang, L., Wang, W. B., & Hao, S. (2018). Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. *Cell*, 175(3), 679–694.
 66. Fiorucci, S., Cipriani, S., Mencarelli, A., Renga, B., Distrutti, E., & Baldelli, F. (2010). Counter-regulatory role of bile acid activated receptors in immunity and inflammation. *Current Molecular Medicine*, 10(6), 579–595.
 67. Ridlon, J. M., Kang, D. J., & Hylemon, P. B. (2006). Bile salt biotransformation's by human intestinal bacteria. *Journal of Lipid Research*, 47(2), 241–259.
 68. Ajouz, H., Mukherji, D., & Shamseddine, A. (2014). Secondary bile acids: An underrecognized cause of colon cancer. *World Journal of Surgical Oncology*, 12(1), 1–5.

69. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68(6), 394–424.
70. Radu, M. R., Prădatu, A., Duică, F., Micu, R., Crețoiu, S. M., Suciu, N., Crețoiu, D., Varlas, V. N., & Rădoi, V. E. (2021). Ovarian cancer: Biomarkers and targeted therapy. *Biomedicines*, 9(6), 693.
71. Kurman, R. J., & Shih, I. M. (2016). The dualistic model of ovarian carcinogenesis: Revisited, revised, and expanded. *American Journal of Pathology*, 186(4), 733–747.
72. Perales-Puchalt, A., Perez-Sanz, J., Payne, K. K., Svoronos, N., Allegrezza, M. J., Chaurio, R. A., Anadon, C., Calmette, J., Biswas, S., & Mine, J. A. (2018). Frontline science: Microbiota reconstitution restores intestinal integrity after cisplatin therapy. *Journal of Leukocyte Biology*, 103(5), 799–805.
73. Joyce, K., Saxena, S., Williams, A., Damurjian, C., Auricchio, N., Aluotto, S., Tynan, H., & Demain, A. L. (2010). Antimicrobial spectrum of the antitumor agent, cisplatin. *Journal of Antibiotics (Tokyo)*, 63(8), 530–532.
74. Zhang, S., & Chen, D. C. (2019). Facing a new challenge: The adverse effects of antibiotics on gut microbiota and host immunity. *Chinese Medical Journal (English)*, 132(10), 1135–1138.
75. Zhao, L., Xing, C., Sun, W., Hou, G., Yang, G., & Yuan, L. (2018). Lactobacillus supplementation prevents cisplatin-induced cardiotoxicity possibly by inflammation inhibition. *Cancer Chemotherapy and Pharmacology*, 82, 999–1008.
76. Arzmi, M. H., Dashper, S., & McCullough, M. (2019). Polymicrobial interactions of *Candida albicans* and its role in oral carcinogenesis. *Journal of Oral Pathology & Medicine*, 48(7), 546–551.
77. Pushalkar, S., Mane, S. P., Ji, X., Li, Y., Evans, C., Crasta, O. R., Morse, D., Meagher, R., Singh, A., & Saxena, D. (2011). Microbial diversity in saliva of oral squamous cell carcinoma. *FEMS Immunology and Medical Microbiology*, 61(3), 269–277.
78. Alnuaimi, A. D., Ramdzan, A. N., Wiesenfeld, D., O'Brien-Simpson, N. M., Kolev, S. D., Reynolds, E. C., & McCullough, M. J. (2016). *Candida* virulence and ethanol-derived acetaldehyde production in oral cancer and non-cancer subjects. *Oral Diseases*, 22(8), 805–814.
79. Stornetta, A., Guidolin, V., & Balbo, S. (2018). Alcohol-derived acetaldehyde exposure in the oral cavity. *Cancers (Basel)*, 10(1), 20.
80. Mäkinen, A., Nawaz, A., Mäkitie, A., & Meurman, J. H. (2018). Role of non-*albicans* *Candida* and *Candida albicans* in oral squamous cell cancer patients. *Journal of Oral and Maxillofacial Surgery*, 76(12), 2564–2571.
81. Ramirez-Garcia, A., Rementeria, A., Aguirre-Urizar, J. M., Moragues, M. D., Antoran, A., Pellon, A., Abad-Diaz-de-Cerio, A., & Hernando, F. L. (2016). *Candida albicans* and cancer: Can this yeast induce cancer development or progression? *Critical Reviews in Microbiology*, 42(2), 181–193.
82. Dai, Z., Zhang, J., Wu, Q., Chen, J., Liu, J., Wang, L., Chen, C., Xu, J., Zhang, H., & Shi, C. (2019). The role of microbiota in the development of colorectal cancer. *International Journal of Cancer*, 145(8), 2032–2041.
83. Dongari-Bagtzoglou, A., & Kashleva, H. (2003). *Candida albicans* triggers interleukin-8 secretion by oral epithelial cells. *Microbial Pathogenesis*, 34(4), 169–177.

84. Arbyn, M., Weiderpass, E., Bruni, L., de Sanjosé, S., Saraiya, M., Ferlay, J., & Bray, F. (2020). Estimates of incidence and mortality of cervical cancer in 2018: A worldwide analysis. *Lancet Global Health*, 8(2), E191–E203.
85. Basu, P., Mittal, S., Vale, D. B., & Kharaji, Y. C. (2018). Secondary prevention of cervical cancer. *Best Practice & Research Clinical Obstetrics & Gynecology*, 47, 73–85.
86. Conesa-Zamora, P. (2013). Immune responses against virus and tumor in cervical carcinogenesis: Treatment strategies for avoiding the HPV-induced immune escape. *Gynecologic Oncology*, 131(2), 480–488.
87. Wang, Z., Wang, Q., Zhao, J., Gong, L., Zhang, Y., Wang, X., & Yuan, Z. (2019). Altered diversity and composition of the gut microbiome in patients with cervical cancer. *AMB Express*, 9, 1–9.
88. Hatakeyama, M. (2017). Structure and function of *Helicobacter pylori* CagA, the first-identified bacterial protein involved in human cancer. *Proceedings of the Japan Academy, Series B*, 93(4), 196–219.
89. Vivarelli, S., Salemi, R., Candido, S., Falzone, L., Santagati, M., Stefani, S., Torino, F., Banna, G. L., Tonini, G., & Libra, M. (2019). Gut microbiota and cancer: From pathogenesis to therapy. *Cancers (Basel)*, 11(1), 38.
90. Bergounioux, J., Elisee, R., Prunier, A. L., Donnadieu, F., Sperandio, B., Sansonetti, P., & Arbibe, L. (2012). Calpain activation by the *Shigella flexneri* effector VirA regulates key steps in the formation and life of the bacterium's epithelial niche. *Cell Host & Microbe*, 11(3), 240–252.
91. Rubinstein, M. R., Wang, X., Liu, W., Hao, Y., Cai, G., & Han, Y. W. (2013). *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/ β -catenin signaling via its FadA adhesin. *Cell Host & Microbe*, 14(2), 195–206.
92. Murata-Kamiya, N., Kurashima, Y., Teishikata, Y., Yamahashi, Y., Saito, Y., Higashi, H., Aburatani, H., Akiyama, T., Peek, R. M., & Azuma, T. (2007). *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the β -catenin signal that promotes intestinal trans differentiation in gastric epithelial cells. *Oncogene*, 26(32), 4617–4626.
93. Wu, S., Rhee, K. J., Zhang, M., Franco, A., & Sears, C. L. (2007). *Bacteroides fragilis* toxin stimulates intestinal epithelial cell shedding and γ -secretase-dependent E-cadherin cleavage. *Journal of Cell Science*, 120(11), 1944–1952.
94. Chaturvedi, R., Asim, M., Romero-Gallo, J., Barry, D. P., Hoge, S., De Sablet, T., Delgado, A. G., Wroblewski, L. E., Piazuelo, M. B., & Yan, F. (2011). Spermine oxidase mediates the gastric cancer risk associated with *Helicobacter pylori* CagA. *Gastroenterology*, 141(5), 1696–1708.
95. Goodwin, A. C., Destefano, Shields, C. E., Wu, S., Huso, D. L., Wu, X., Murray-Steward, T. R., Hacker-Prietz, A., Rabizadeh, S., Woster, P. M., Sears, C. L., & Casero, R. A. (2011). *Proceedings of the National Academy of Sciences USA*, 108, 15–354.
96. Lin, C., Cai, X., Zhang, J., Wang, W., Sheng, Q., Hua, H., & Zhou, X. (2019). Role of gut microbiota in the development and treatment of colorectal cancer. *Digestion*, 100(1), 72–78.
97. Daillère, R., Vétizou, M., Waldschmitt, N., Yamazaki, T., Isnard, C., Poirier-Colame, V., Duong, C. P. M., Flament, C., Lepage, P., & Roberti, M. P. (2016). *Enterococcus hirae* and *Barnesiella intestinihominis* facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. *Immunity*, 45(4), 931–943.

98. Jan, G., Belzacq, A. S., Haouzi, D., Rouault, A., Metivier, D., Kroemer, G., & Brenner, C. (2002). Propionibacteria induce apoptosis in colon carcinoma cells via short-chain fatty acids action on mitochondria. *Cell Death & Differentiation*, 9, 179–188.
99. Paulos, C. M., Wrzesinski, C., Kaiser, A., Hinrichs, C. S., Chieppa, M., Cassard, L., Palmer, D. C., Boni, A., Muranski, P., & Yu, Z. (2007). Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8⁺ T cells via TLR4 signaling. *Journal of Clinical Investigation*, 117(8), 2197–2204.
100. Konishi, H., Fujiya, M., Tanaka, H., Ueno, N., Moriichi, K., Sasajima, J., Ikuta, K., Akutsu, H., Tanabe, H., & Kohgo, Y. (2016). Probiotic-derived ferriochrome inhibits colon cancer progression via JNK-mediated apoptosis. *Nature Communications*, 7(1), 12365.
101. Nougayrède, J. P., Homburg, S., Taieb, F., Boury, M., Brzuszkiewicz, E., Gottschalk, G., Buchrieser, C., Hacker, J., Dobrindt, U., & Oswald, E. (2006). *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. *Science*, 313(5788), 848–851.
102. Dennis, K. L., Blatner, N. R., Gounari, F., & Khazaie, K. (2013). Current status of IL-10 and regulatory T-cells in cancer. *Current Opinion in Oncology*, 25, 637–645.
103. Yao, Y., & Dai, W. (2014). Genomic instability and cancer. *Journal of Carcinogenesis & Mutagenesis*, 5.
104. Frisan, T. (2016). Bacterial genotoxins: The long journey to the nucleus of mammalian cells. *Biochimica et Biophysica Acta (BBA)–Biomembranes*, 1858(3), 567–575.
105. Kemp, K., Griffiths, J., Campbell, S., & Lovell, K. (2013). An exploration of the follow-up needs of patients with inflammatory bowel disease. *Journal of Crohn's and Colitis*, 7(9), e386–e395.
106. Buti, L., Spooner, E., Van der Veen, A. G., Rappuoli, R., Covacci, A., & Ploegh, H. L. (2011). *Helicobacter pylori* cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of P53 (ASPP2) tumor suppressor pathway of the host. *Proceedings of the National Academy of Sciences*, 108(22), 9238–9243.
107. Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157(1), 121–141.
108. Nagarajan, U. M., Prantner, D., Sikes, J. D., Andrews Jr, C. W., Goodwin, A. M., Nagarajan, S., & Darville, T. (2008). Type I interferon signaling exacerbates *Chlamydia muridarum* genital infection in a murine model. *Infection and Immunity*, 76(10), 4642–4648.
109. Huycke, M. M., Moore, D., Joyce, W., Wise, P., Shepard, L., Kotake, Y., & Gilmore, M. S. (2001). Extracellular superoxide production by *Enterococcus faecalis* requires demethylmenaquinone and is attenuated by functional terminal quinol oxidases. *Molecular Microbiology*, 42(3), 729–740.
110. Huttenhower, C. (2012). Chapter 12: Human microbiome analysis. *PLoS Computational Biology*, 8, e1002808.
111. Johansson, M. E. V., Jakobsson, H. E., Holmén-Larsson, J., Schütte, A., Ermund, A., Rodríguez-Piñeiro, A. M., & Hansson, G. C. (2015). Normalization of host intestinal mucus layers requires long-term microbial colonization. *Cell Host & Microbe*, 18, 582–592.
112. Spiljar, M., Merkler, D., & Trajkovski, M. (2017). The immune system bridges the gut microbiota with systemic energy homeostasis: Focus on TLRs, mucosal barrier, and SCFAs. *Frontiers in Immunology*, 8, 1353.

113. Ghaly, S., Kaakoush, N. O., & Hart, P. H. (2020). Effects of UVR exposure on the gut microbiota of mice and humans. *Photochemical & Photobiological Sciences*, 19, 20–28.
114. Viaud, S., Saccheri, F., Mignot, G., Yamazaki, T., Daillère, R., Hannani, D., & Pittet, M. J. (2013). The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science*, 342(6161), 971–976.
115. Akbar, N., Khan, N. A., Muhammad, J. S., & Siddiqui, R. (2022). The role of gut microbiome in cancer genesis and cancer prevention. *Health Science Review*, 100010.
116. Vétizou, M., Pitt, J. M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., & Roberti, M. P. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*, 350, 1079–1084.
117. Wei, W., Sun, W., Yu, S., Yang, Y., & Ai, L. (2016). Butyrate production from high-fiber diet protects against lymphoma tumor. *Leukemia & Lymphoma*, 57(10), 2401–2408.
118. Zhang, J., Xia, Y., & Sun, J. (2021). Breast and gut microbiome in health and cancer. *Genes & Diseases*, 8(5), 581–589.
119. Sánchez-Alcoholado, L., Ramos-Molina, B., Otero, A., Laborda-Illanes, A., Ordóñez, R., Medina, J. A., & Queipo-Ortuño, M. I. (2020). The role of the gut microbiome in colorectal cancer development and therapy response. *Cancers*, 12(6), 1406.
120. Schwerd, T., Twigg, S. R. F., Aschenbrenner, D., Manrique, S., Miller, K. A., Taylor, I. B., & McGowan, S. J. (2017). A biallelic mutation in IL6ST encoding the GP130 co-receptor causes immunodeficiency and craniosynostosis. *The Journal of Experimental Medicine*, 214(9), 2547–2562.
121. Paavonen, J., Naud, P., Salmerón, J., Wheeler, C. M., Chow, S. N., Apter, D., Kitchener, H., Castellsague, X., Teixeira, J. C., & Skinner, S. R. (2009). Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): Final analysis of a double-blind, randomised study in young women. *Lancet*, 374(9686), 301–314.
122. Davar, D., Dzutsev, A. K., McCulloch, J. A., Rodrigues, R. R., Chauvin, J. M., Morrison, R. M., Deblasio, R. N., Menna, C., Ding, Q., & Pagliano, O. (2021). Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*, 371(6529), 595–602.
123. Brahmer, J., Reckamp, K. L., Baas, P., Crinò, L., Eberhardt, W. E. E., Poddubskaya, E., Antonia, S., Pluzanski, A., Vokes, E. E., & Holgado, E. (2015). Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. *New England Journal of Medicine*, 373(2), 123–135.
124. Larkin, J., Lao, C. D., Urban, W. J., McDermott, D. F., Horak, C., Jiang, J., & Wolchok, J. D. (2015). Efficacy and safety of nivolumab in patients with BRAF V600 mutant and BRAF wild-type advanced melanoma: A pooled analysis of 4 clinical trials. *JAMA Oncology*, 1(4), 433–440.
125. Robert, C., Schachter, J., Long, G. V., Arance, A., Grob, J. J., Mortier, L., Daud, A., Carlino, M. S., McNeil, C., & Lotem, M. (2015). Pembrolizumab versus ipilimumab in advanced melanoma. *New England Journal of Medicine*, 372(26), 2521–2532.
126. Ribas, A., Hamid, O., Daud, A., Hodi, F. S., Wolchok, J. D., Kefford, R., Joshua, A. M., Patnaik, A., Hwu, W. J., & Weber, J. S. (2016). Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA*, 315(15), 1600–1609.
127. Robert, C., Ribas, A., Schachter, J., Arance, A., Grob, J. J., Mortier, L., Daud, A., Carlino, M. S., McNeil, C. M., & Lotem, M. (2019). Pembrolizumab versus ipilimumab

- in advanced melanoma (KEYNOTE-006): Post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. *Lancet Oncology*, 20(9), 1239–1251.
128. Perez-Chanona, E., & Trinchieri, G. (2016). The role of microbiota in cancer therapy. *Current Opinion in Immunology*, 39, 75–81.
 129. Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillère, R., Fluckiger, A., Messaoudene, M., Rauber, C., & Roberti, M. (2018). Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science*, 359(6371), 91–97.
 130. Matson, V., Fessler, J., Bao, R., Chongsuwat, T., Zha, Y., Alegre, M. L., Luke, J. J., & Gajewski, T. F. (2018). The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*, 359(6371), 104–108.
 131. Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews*, 90(3), 859–904.
 132. Frankel, A. E., Coughlin, L. A., Kim, J., Froehlich, T. W., Xie, Y., Frenkel, E. P., & Koh, A. Y. (2017). Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. *Neoplasia*, 19(10), 848–855.
 133. Peters, B. A., Wilson, M., Moran, U., Pavlick, A., Izsak, A., Wechter, T., Weber, J. S., Osman, I., & Ahn, J. (2019). Relating the gut metagenome and metatranscriptome to immunotherapy responses in melanoma patients. *Genome Medicine*, 11, 1–14.
 134. Rojas-Tapias, D., Vlamakis, H., & Xavier, R. (2017). El enriquecimiento de un organismo oral en el intestino es favorecido por inflamación. *Scientia Spiritus*, 358(359), 22.
 135. Qin, N., Yang, F., Li, A., Prifti, E., Chatelier, E. L., Yao, J., Wu, L., Zhou, J., Ni, S., Liu, L., Pons, N., Batto, J. M., Kennedy, S. P., Leonard, P., Yuan, C., Ding, W., Chen, Y., Hu, X., Zheng, B., Qian, G., Xu, W., Ehrlich, S. D., Zheng, S., & Li, L. (2014). Alterations of the human gut microbiome in liver cirrhosis. *Nature*, 513, 59–64.
 136. Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., Wu, X., Li, J., Tang, L., & Li, Y. (2015). The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nature Medicine*, 21(8), 895–905.
 137. Komiya, Y., Shimomura, Y., Higurashi, T., Sugi, Y., Arimoto, J., Umezawa, S., Uchiyama, S., Matsumoto, M., & Nakajima, A. (2019). Patients with colorectal cancer have identical strains of *Fusobacterium nucleatum* in their colorectal cancer and oral cavity. *Gut*, 68(7), 1335–1337.
 138. Gut Microbiota, Obesity and Metabolic Disorders. (2017). *Minerva Gastroenterology & Dietology*, 63(4), 337–344.
 139. Kitamoto, S., Nagao-Kitamoto, H., Hein, R., Schmidt, T. M., & Kamada, N. (2020). The bacterial connection between the oral cavity and the gut diseases. *Journal of Dental Research*, 99(9), 1021–1029.
 140. Keute, M., Demirezen, M., Graf, A., Mueller, N. G., & Zaehle, T. (2019). No modulation of pupil size and event-related pupil response by transcutaneous auricular vagus nerve stimulation (TaVNS). *Scientific Reports*, 9(1), 11452.
 141. Nené, N. R., Reisel, D., Leimbach, A., Franchi, D., Jones, A., Evans, I., Knapp, S., Ryan, A., Ghazali, S., & Timms, J. F. (2019). Association between the cervicovaginal microbiome, BRCA1 mutation status, and risk of ovarian cancer: A case-control study. *Lancet Oncology*, 20(8), 1171–1182.
 142. Schwabe, R. F., & Jobin, C. (2013). The microbiome and cancer. *Nature Reviews Cancer*, 13(11), 800–812.

143. Chase, D. M., Kauderer, J., Wenzel, L., Ramondetta, L., Cella, D., Long, H. J., & Monk, B. J. (2015). Factors associated with grade 3 or 4 treatment-related toxicity in women with advanced or recurrent cervical cancer: An exploratory analysis of NRG Oncology/Gynecologic Oncology Group trials 179 and 204. *International Journal of Gynecologic Cancer*, 25(2).
144. Agito, M. D., Atreja, A., & Rizk, M. K. (2013). Fecal microbiota transplantation for recurrent *C. difficile* infection: Ready for prime time. *Cleveland Clinic Journal of Medicine*, 80(2), 101–108.
145. Levy, M., Thaïss, C. A., Katz, M. N., Suez, J., & Elinav, E. (2015). Inflammasomes and the microbiota—Partners in the preservation of mucosal homeostasis. In *Seminars in Immunopathology* (Vol. 37, pp. 39–46). Springer.
146. Franchi, L., Eigenbrod, T., Muñoz-Planillo, R., & Núñez, G. (2009). The inflammasome: A caspase-1 activation platform regulating immune responses and disease pathogenesis. *Nature Immunology*, 10, 241–247.
147. Zitvogel, L., Kepp, O., Galluzzi, L., & Kroemer, G. (2012). Inflammasomes in carcinogenesis and anticancer immune responses. *Nature Immunology*, 13(4), 343–351.
148. Kanton, M., & Guo, B. (2017). Inflammasomes and cancer: The dynamic role of the inflammasome in tumor development. *Frontiers in Immunology*, 8, 1132.
149. Nishimura, K., Sano, M., Ohtaka, M., Furuta, B., Umemura, Y., Nakajima, Y., Ikehara, Y., Kobayashi, T., Segawa, H., & Takayasu, S. (2011). Development of defective and persistent Sendai virus vector: A unique gene delivery/expression system ideal for cell reprogramming. *Journal of Biological Chemistry*, 286(6), 4760–4771.
150. McLoed, A. G., Sherrill, T. P., Cheng, D. S., Han, W., Saxon, J. A., Gleaves, L. A., Wu, P., Polosukhin, V. V., Karin, M., & Yull, F. E. (2016). Neutrophil-derived IL-1 β impairs the efficacy of NF- κ B inhibitors against lung cancer. *Cell Reports*, 16(1), 120–132.
151. Zhong, F. L., Mamaï, O., Sborgi, L., Boussofara, L., Hopkins, R., Robinson, K., Szeverényi, I., Takeichi, T., Balaji, R., & Lau, A. (2016). Germline NLRP1 mutations cause skin inflammatory and cancer susceptibility syndromes via inflammasome activation. *Cell*, 167(1), 187–202.
152. Mai, S., Pollum, M., Martínez-Fernández, L., Dunn, N., Marquetand, P., Corral, I., Crespo-Hernández, C. E., & González, L. (2016). The origin of efficient triplet state population in sulfur-substituted nucleobases. *Nature Communications*, 7(1), 13077.
153. Daley, D., Mani, V. R., Mohan, N., Akkad, N., Pandian, G. S. D. B., Savadkar, S., Lee, K. B., Torres-Hernandez, A., Aykut, B., & Diskin, B. (2017). NLRP3 signaling drives macrophage-induced adaptive immune suppression in pancreatic carcinoma. *Journal of Experimental Medicine*, 214(6), 1711–1724.
154. Zaki, M. H., Boyd, K. L., Vogel, P., Kastan, M. B., Lamkanfi, M., & Kanneganti, T. D. (2010). The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity*, 32(3), 379–391.
155. Allen, I. C., TeKippe, E. M., Woodford, R. M. T., Uronis, J. M., Holl, E. K., Rogers, A. B., Herfarth, H. H., Jobin, C., & Ting, J. P. Y. (2010). The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *Journal of Experimental Medicine*, 207(5), 1045–1056.
156. Zaki, M. H., Vogel, P., Body-Malapel, M., Lamkanfi, M., & Kanneganti, T. D. (2010). IL-18 production downstream of the NLRP3 inflammasome confers protection against colorectal tumor formation. *Journal of Immunology*, 185(8), 4912–4920.

157. Williams, T. M., Leeth, R. A., Rothschild, D. E., Coutermarsh-Ott, S. L., McDaniel, D. K., Simmons, A. E., Heid, B., Cecere, T. E., & Allen, I. C. (2015). The NLRP1 inflammasome attenuates colitis and colitis-associated tumorigenesis. *Journal of Immunology*, 194(7), 3369–3380.
158. Man, S. M. (2018). Inflammasomes in the gastrointestinal tract: Infection, cancer, and gut microbiota homeostasis. *Nature Reviews Gastroenterology & Hepatology*, 15(12), 721–737.
159. Miettinen, M., Pietilä, T. E., Kekkonen, R. A., Kankainen, M., Latvala, S., Pirhonen, J., Österlund, P., Korpela, R., & Julkunen, I. (2012). Nonpathogenic *Lactobacillus rhamnosus* activates the inflammasome and antiviral responses in human macrophages. *Gut Microbes*, 3(6), 510–522.
160. Bedada, T. L., Feto, T. K., Awoke, K. S., Garedew, A. D., Yifat, F. T., & Birri, D. J. (2020). Probiotics for cancer alternative prevention and treatment. *Biomedicine & Pharmacotherapy*, 129, 110409.
161. Rasouli, B. S., Ghadimi-Darsajini, A., Nekouian, R., & Iragian, G. R. (2017). *In vitro* activity of probiotic *Lactobacillus reuteri* against gastric cancer progression by downregulation of urokinase plasminogen activator/urokinase plasminogen activator receptor gene expression. *Journal of Cancer Research and Therapeutics*, 13(2), 246–251.
162. Georgiev, K., Georgieva, M., Iliev, I., Peneva, M., & Alexandrov, G. (2015). Antiproliferative effect of Bulgarian spring water probiotics (Lakter a Nature Probiotics®) against human colon carcinoma cell line. *World Journal of Pharmaceutical and Pharmaceutical Sciences*, 4(6), 130–136.
163. Redman, M. G., Ward, E. J., & Phillips, R. S. (2014). The efficacy and safety of probiotics in people with cancer: A systematic review. *Annals of Oncology*, 25(10), 1919–1929.
164. Mego, M., Holec, V., Drgona, L., Hainova, K., Ciernikova, S., & Zajac, V. (2013). Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy. *Complementary Therapies in Medicine*, 21(6), 712–723.
165. Gianotti, L., Morelli, L., Galbiati, F., Rocchetti, S., Coppola, S., Beneduce, A., & Braga, M. (2010). A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. *World Journal of Gastroenterology*, 16(2), 167.
166. Demers, M., Dagnault, A., & Desjardins, J. (2014). A randomized double-blind controlled trial: Impact of probiotics on diarrhea in patients treated with pelvic radiation. *Clinical Nutrition*, 33(5), 761–767.
167. Mego, M., Chovanec, J., Vochyanova-Andrezalova, I., Konkolovsky, P., Mikulova, M., & Medvecova, L. (2015). Prevention of irinotecan-induced diarrhea by probiotics: A randomized double-blind, placebo-controlled pilot study. *Complementary Therapies in Medicine*, 23(3), 356–362.
168. Hatakka, K., Mutanen, M., Holma, R., Saxelin, M., & Korpela, R. (2008). *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp. *shermanii* JS administered in capsules is ineffective in lowering serum lipids. *Journal of the American College of Nutrition*, 27(4), 441–447.
169. Chen, Q., Wang, C., Chen, G., Hu, Q., & Gu, Z. (2018). Delivery strategies for immune checkpoint blockade. *Advanced Healthcare Materials*, 7(20), 1800424.
170. Chaput, N., Lepage, P., Coutzac, C., Soularue, E., Le Roux, K., & Collins, M. (2017). Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Annals of Oncology*, 28(6), 1368–1379.

171. Wang, Y., & Li, H. (2022). Gut microbiota modulation: A tool for the management of colorectal cancer. *Journal of Translational Medicine*, 20(1), 178.
172. Berbert, L., Santos, A., Magro, D. O., Guadagnini, D., Assalin, H. B., & Coy, C. S. R. (2022). Metagenomics analysis reveals universal signatures of the intestinal microbiota in colorectal cancer, regardless of regional differences. *Brazilian Journal of Medical and Biological Research*, 55.
173. Zhong, M., Xiong, Y., Zhao, J., Gao, Z., Ma, J., & Hong, X. (2021). *Candida albicans* disorder is associated with gastric carcinogenesis. *Theranostics*, 11(10), 4945.
174. Deng, T., Li, J., He, B., Chen, B., Liu, F., & Zhang, T. (2022). Gut microbiome alteration as a diagnostic tool and associated with inflammatory response marker in primary liver cancer. *Hepatology International*, 16(1), 99–111.
175. Sims, T. T., Colbert, L. E., Zheng, J., Medrano, A. Y. D., Hoffman, K. L., & Daniel, C. R. (2019). Gut microbial diversity and genus-level differences identified in cervical cancer patients versus healthy controls. *Gynecologic Oncology*, 155(2), 237–244.
176. Kamada, N., Seo, S. U., Chen, G. Y., & Núñez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology*, 13(5), 321–335.
177. Wang, J., Chang, C., Lin, J., Wu, L., Chuang, L., & Lai, M. (2014). Infection, antibiotic therapy, and risk of colorectal cancer: A nationwide nested case–control study in patients with type 2 diabetes mellitus. *International Journal of Cancer*, 135(4), 956–967.
178. Bonnet, M., Buc, E., Sauvanet, P., Darcha, C., Dubois, D., & Darfeuille-Michaud, A. (2014). Colonization of the human gut by *E. coli* and colorectal cancer risk. *Clinical Cancer Research*, 20(4), 859–867.
179. Louis, P., Hold, G. L., & Flint, H. J. (2014). The gut microbiota, bacterial metabolites, and colorectal cancer. *Nature Reviews Microbiology*, 12(10), 661–672.
180. Touchefeu, Y., Montassier, E., Nieman, K., Gastinne, T., Potel, G., & de La Cochetière, M. (2014). Systematic review: The role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis—current evidence and potential clinical applications. *Alimentary Pharmacology & Therapeutics*, 40(5), 409–421.
181. Wallace, B. D., Wang, H., Lane, K. T., Scott, J. E., Orans, J., Koo, J. S., & Mani, S. (2010). Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science*, 330(6005), 831–835.
182. Iida, N., Dzutsev, A., Stewart, C. A., Smith, L., Bouladoux, N., Weingarten, R. A., & Cramer, S. (2013). Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science*, 342(6161), 967–970.
183. Zackular, J. P., Baxter, N. T., Iverson, K. D., Sadler, W. D., Petrosino, J. F., Chen, G. Y., & Schloss, P. D. (2013). The gut microbiome modulates colon tumorigenesis. *mBio*, 4(6), e00692-13.
184. Liong, M. T. (2008). Roles of probiotics and prebiotics in colon cancer prevention: Postulated mechanisms and *in-vivo* evidence. *International Journal of Molecular Sciences*, 9(5), 854–863.
185. Capurso, G., Marignani, M., & Delle Fave, G. (2006). Probiotics and the incidence of colorectal cancer: When evidence is not evident. *Digestive and Liver Disease*, 38(Suppl), S277–S282.
186. Bermúdez-Humarán, L. G., Aubry, C., Motta, J. P., Deraison, C., Steidler, L., & Langella, P. (2013). Engineering lactococci and lactobacilli for human health. *Current Opinion in Microbiology*, 16(3), 278–283.

187. Senovilla, L., Vacchelli, E., Garcia, P., Eggermont, A., Fridman, W. H., & Galluzzi, L. (2013). Trial watch: DNA vaccines for cancer therapy. *Oncoimmunology*, 2(4), e23803.
188. Sandoval, F., Terme, M., Nizard, M., Badoual, C., Bureau, M. F., & Fraisse, G. (2013). Mucosal imprinting of vaccine-induced CD8⁺ T cells is crucial to inhibit the growth of mucosal tumors. *Science Translational Medicine*, 5(172), 172ra20.
189. Hamad, A., Fragkos, K. C., & Forbes, A. A systematic review and meta-analysis of probiotics for the management of radiation-induced bowel disease. *Clinical Nutrition*, 32(3), 353–360.
190. Taper, H. S., & Roberfroid, M. B. (2005). Possible adjuvant cancer therapy by two prebiotics—Inulin or oligofructose. *In vivo*, 19(1), 201–204.
191. Holmes, E., Kinross, J., Gibson, G. R., Burcelin, R., Jia, W., & Nicholson, J. K. (2012). Therapeutic modulation of microbiota-host metabolic interactions. *Science Translational Medicine*, 4(137), 137rv6.
192. Crawford, P. A., & Gordon, J. I. (2005). Microbial regulation of intestinal radiosensitivity. *Proceedings of the National Academy of Sciences*, 102(37), 13254–13259.
193. Yang, M., Liu, Q., Dai, M., Peng, R., Li, X., & Liu, H. (2022). FOXQ1-mediated SIRT1 upregulation enhances stemness and radio-resistance of colorectal cancer cells and restores intestinal microbiota function by promoting β -catenin nuclear translocation. *Journal of Experimental & Clinical Cancer Research*, 41(1), 1–20.
194. Roopchand, D. E., Carmody, R. N., Kuhn, P., Moskal, K., Rojas-Silva, P., & Raskin, I. (2015). Dietary polyphenols promote growth of the gut bacterium *Akkermansia muciniphila* and attenuate high-fat diet-induced metabolic syndrome. *Diabetes*, 64(8), 2847–2858.
195. Makaranka, S., Scutt, F., Frixou, M., Wensley, K. E., Sharma, R., & Greenhowe, J. (2022). The gut microbiome and melanoma: A review. *Experimental Dermatology*, 31(9), 1292–1301.



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CHAPTER 4

Importance of Probiotics in Gut Microbiota for the Management of Cancer

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ABSTRACT

Various live microbes that are beneficial to your health when consumed in proper amounts are denoted as probiotics. The gut microbiome is significantly influenced by probiotics, which are essential for sustaining the body's general health, according to recent studies. A complex ecology of bacteria called the gut microbiota interacts with the immune system, controls metabolism, and affects a number of physiological functions. A current focus of research is how the function of the gut microbiome relates to the development and spread of cancer. Various pieces of evidence suggest that some types of cancer may arise because of an imbalance in the gut flora. Probiotics have been demonstrated to assist in re-establishing this equilibrium and may thus play a part in the treatment of cancer. The chapter elaborates on the importance of probiotics, which the gut microbiota contains, for the management of cancer. We explore the potential mechanisms through which probiotics may exert anti-cancer effects, including the alteration of the immune system, the reduction of inflammation, and the inhibition of cancer cell growth. It also goes over the most recent research

on probiotics as an adjuvant treatment for cancer and discusses their safety and possible negative effects.

4.1 INTRODUCTION

Cancer is a disease with a low chance of survival. Poor early diagnosis and treatment resistance continue to be the main contributors to illness and the second greatest cause of death globally [1, 2]. The incidence of cancer and related deaths is gradually increasing, and the predicted number of new cases by 2040 will be 29.4 million [3]. The deadliest types of cancer are lung, colorectal, prostate, liver, pancreatic, and breast cancer [4]. Cancer cells exhibit common physiological conditions such as uncontrolled cell growth, proliferation, resistance to apoptosis, invasiveness, metastasis, constant angiogenesis, and altered signaling pathways [5]. The major reasons for cancer progression include DNA repair deficiency, mutations during replication, genetic background, environmental factors (including toxic substances, infectious agents, and UV radiation) [5], and lifestyle changes [6, 7].

Currently available therapeutic modalities include surgery, chemotherapy, radiotherapy, and combinations of these. Despite optimal therapy, long-term survival remains poor due to chemoresistance, the stability and safety of chemotherapeutic medications, and synthetic treatment agents [8]. Drug combinations or hormonal chemotherapeutic treatments have severe side effects that not only impact normal cells but also target cancer cells, resulting in symptoms that can be worse than the malignancy itself [9]. Therefore, it is critical to acquire more knowledge regarding the incidence of cancer, early detection, and management [10].

The GIT of human beings is a complex reservoir that consists of the dynamic microbial population known as gut microbiota. Microbial cells, totaling more than 100 trillion in number, are present in the human gut microbiome, the majority of which are bacterial populations. Viral, fungal, and archaeal colonization are also widely distributed in this environment [11, 12]. The chemical composition of these in-body microorganisms is connected to homeostasis and various disease presentations. Due to the massive amount of gut bacteria, the human body consumes prokaryotic cells ten times more frequently than eukaryotic cells. The gut microbiome develops very early in the first three years of human life [13]. Plant-based complex carbohydrates are challenging for the host to digest; the intestinal microbiome has a role in their breakdown [14]. Additionally, they regulate the equilibrium

of the microbiome in the colon by producing or breaking down SCFAs, or short-chain fatty acids, including organic acids like propionate, acetate, and butyrate [15]. The proportion of microbiota in the intestine also maintains the host body's energy balance. The foundation of the human body's molecular and metabolic processes is a healthy gut microbiota. Many factors are responsible for the imbalance of the microbiome, and this intestinal dysbiosis leads to embolic disease progression (obesity and diabetes), intestinal disorders (AAD, IBD, Crohn's disease, ulcerative colitis), proinflammatory immune response, gastrointestinal tumors, colorectal cancer, and tumorigenesis in other parts of the body [16, 17].

The human gut microbiota benefits from the utilization of probiotics as adjuvant treatment. The main function of probiotics is the maintenance of homeostasis and the restoration of the necessary microbiome in cases of imbalance in the human gut. Probiotics are becoming a valuable factor in cancer treatment and prevention. Oral delivery of probiotics also plays an essential role in lowering the side effects associated with anticancer therapy, commonly diarrhea and mucositis [9]. Figure 4.1 shows the function of probiotics present in the gut microbiota and their role in cancer prevention/inhibition.

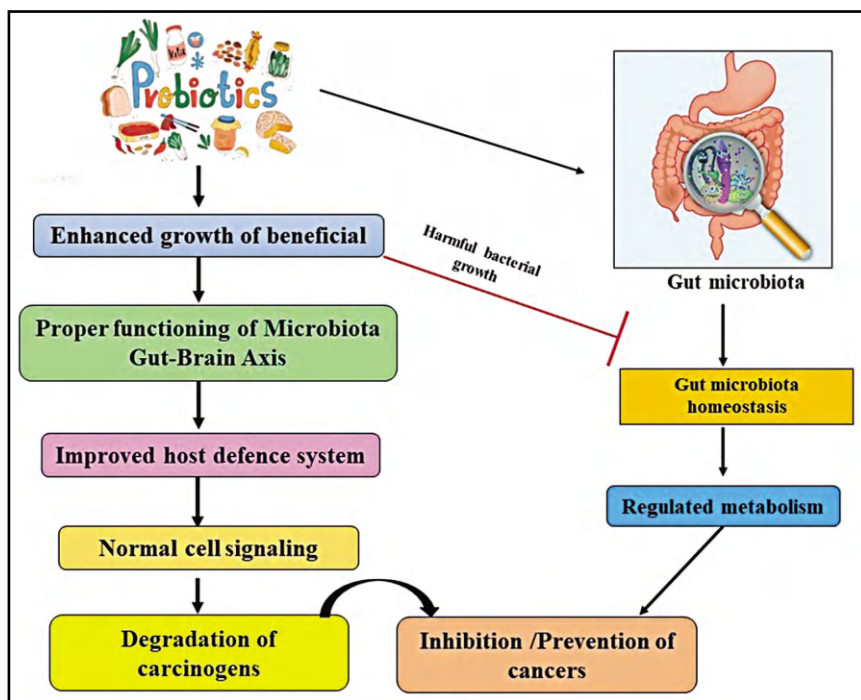


FIGURE 4.1 Role of probiotic in gut microbiota for inhibition of cancer.

4.2 PROBIOTICS

Live, non-pathogenic bacteria are called probiotics. When given in the right quantity, they can have constructive gut-related effects on the microbiota of the host. A large population of these probiotics consists of non-pathogenic, acid-producing microorganisms such as *Lactobacillus*, *Streptococcus*, *Propionibacterium*, *Bifidobacterium*, and *Enterococcus*, as well as non-pathogenic yeasts (*Saccharomyces boulardii*) and some strains of *Bacillus* and *Saccharomyces* [18]. Probiotics are effective for different gastrointestinal conditions, such as inflammatory bowel disease, diarrhea, and symptoms related to *Salmonella* and *Helicobacter pylori*, as well as for bacteriocins and immunomodulation [19]. However, dead probiotics and their metabolites are notably responsible for biological activities [3].

Probiotic microbes are considered to be native strains or sometimes genetically modified forms. The major sources of probiotics are fermented foods (yogurt, kefir), silage (cabbage and cucumbers), and the gut microbiome [3]. Probiotic microbes in fermented foods are considered healthy foods that lead to the production of antimicrobial peptides, enzymes, and antioxidants [20]. These products play a substantial role in chemoprevention and treatments. Recent reports have mentioned that dietary probiotics, particularly lactic acid bacteria (LAB), have therapeutic effects in several disease conditions, including cancer. LAB has different anticancer properties, such as antiproliferative effects, apoptosis induction, anti-inflammatory effects, antioxidative effects, and antimutagenic effects [21–24].

Probiotic microbes have different mechanisms of action at the cellular level. They can kill or inhibit the activity of pathogenic bacteria, such as lactobacilli species, which play a vital role in this mode of action [25, 26]. Secondly, probiotic bacteria can compete with pathogenic bacteria and prevent them from attaching to the wall of the gastrointestinal tract [27]. Major anticancer and antimutagenic mechanisms include binding and degradation by probiotics. They can stop the production of toxic, reactive, and dangerous carcinogens, as well as short-chain fatty acids, which can lower the pH of the stomach. Probiotics also modulate host innate immunity and increase the secretion of anti-inflammatory molecules [28].

4.3 PROBIOTICS' ROLE IN CANCER CHEMOTHERAPY PREVENTION

Cancer is a highly invasive and proliferative disease triggered by an unhealthy and unbalanced diet. Therefore, research on dietary factors and dietary supplements is extremely important. Probiotic bacteria are an important

dietary factor that could modulate the defense mechanism and protect tumor cells from neoplastic progression [29]. Recent reports depict that a dietary probiotic, kefir grains, have vast potential for chemoprevention and treatment. It is a type of fermented milk product prepared by combining raw milk with kefir grains, and it has a combination of yeast and bacteria that coexist harmoniously. The bioactive substances, including peptides and polysaccharides, make kefir a potential anti-proliferative agent and apoptosis inducer for tumor cells. Kefir shows anticancer effects on several cancers, including lung carcinoma, breast cancer, malignant T cells, and colorectal cancer [21].

4.3.1 PROBIOTICS IN COLORECTAL CANCER

The fourth most common malignant tumor in the United States is colorectal cancer, which is also a leading cause of cancer-related death. The rapid increase in colorectal cancer (CRC) is attributed to certain genetic abnormalities and environmental factors such as lifestyle changes, dietary routines, and obesity. CRC development is a multistep process that progresses from normal epithelium to adenoma and then transforms into carcinoma. These histological alterations are connected to tumor suppressor gene loss and oncogenic mutations caused by mutagens and carcinogenic substances [30, 31]. A lack of balance in DNA breakdown and repair is the leading cause of CRC [28].

Other than the conventional therapy options, probiotics are a potential approach for CRC management and prevention. There are several commercially available bacterial species used as probiotics for CRC treatment [32]. Lactic acid bacteria are commonly applied to utilize their colonization resistance and immunomodulatory ability [33, 34]. Oral delivery of *Lactobacillus casei* BL23, as a probiotic strain, significantly suppresses tumor progression in a mouse model by utilizing its anti-inflammatory and anticancer properties [35]. In a separate study, this probiotic displayed an immunomodulatory effect by suppressing colonic IL-22, antiproliferative ability via increased caspase-7, caspase-9, and Bik, and loss of Ki67 expression [36]. Another research study has demonstrated that adjuvant 5-FU-based chemotherapy caused intestinal mucositis and diarrhea, and *L. rhamnosus* (Lcr35) has effectively reduced these side effects in the CRC mouse model. This treatment strategy also enhanced the apoptotic BCL2-associated X protein population and NF- κ B activated cells associated with overexpression of TNF- α and IL-6 [37]. Colorectal cancer cells underwent apoptosis when exposed to the peptides m2163 and m2386 produced by *Lactobacillus casei*

(*L. casei*) [38]. Two research studies exhibited that ferrichrome, a product of *L. casei* ATCC334, displayed antitumor activity by inhibiting the JKN pathway, and apoptosis in colorectal and gastric cancer is mediated by DNA damage-inducible transcript 3 (DDIT3) [39, 40]. These studies demonstrated the tumor suppressor effect of ferrichrome, which showed a greater effect compared to the conventional antitumor drugs such as cisplatin and 5-FU in colorectal cancer [41].

4.3.2 PROBIOTICS IN BLADDER CANCER

First, research on the antitumor activity of the probiotic *L. casei* (LC9018) displayed inhibition of MBT-2 by orally administering *L. casei* [42]. *Lactobacillus rhamnosus* strain GG (LGG) also demonstrated a reduction in tumor size and deceleration of tumor progression in a bladder cancer mouse model. LGG modulates the immune response by reducing CD3 T-cells and subsequently enhancing the growth of CD8 and CD4 T-cells [43]. Another study showed how *L. casei* Shirota (LcS) works on increasing splenic NK cell activity in mice and inhibiting tumor development. This study also confirmed that the anticancer effect of LcS is specifically on splenic NK cell activity induced by 3-methylcholanthrene [44, 45].

By adding 50 patients who had transurethral resection of bladder tumors (TURBT), a human clinical trial was set up. Equal numbers of patients were included in the treatment and control groups. An oral *L. casei* bacterium preparation (1×10^{10} viable cells) was given three times each day for 427 days, and data revealed 50% recurrence-free patients, and none of them received any chemotherapy. The treated patient group has been shown to vary significantly from the control group [46]. The treated patient group has been shown to vary significantly from the control group. By disease stage, patients were divided into groups, and each group was given a placebo or an oral *Lactobacillus casei* preparation (BLP) at random. The study result demonstrated that BLP showed significant effective compared to placebo in primary and recurrent tumor groups but not in recurrent multiple tumor subgroup [47].

4.3.3 PROBIOTICS IN BREAST CANCER

One of the prevalent types of cancer in women is breast cancer [48]. According to statistics, one in eight women who live to be 85 years old may acquire breast cancer [49]. Many *in vivo* and *in vitro* studies have been

conducted to analyze the effect of probiotics in breast cancer (BC) therapy and prevention. Several *in vitro* studies were carried out to investigate the effect of probiotics on breast cancer cell lines. *Lactobacillus crispatus* and *Lactobacillus acidophilus* showed an antiproliferative effect in BC cells, MDA-MB-231 [50]. Treatment with *Lactobacillus plantarum* downregulated the expression of NF- κ B and induced apoptosis in MDA-MB-231 [51]. There are numerous probiotic bacterial species, including *Enterococcus lactis* IW5, *Lactococcus lactis* NK34, *Lactococcus lactis* KC24, *Enterococcus faecalis*, *Staphylococcus hominis*, *Lactobacillus crispatus*, and *Lactobacillus rhamnosus*, which showed effects on MCF-7, a BC cell line, in different research studies and displayed inhibition of cell proliferation, potent cytotoxic effects on cancer cells, cell cycle arrest, and enhanced apoptotic populations [52–56]. Well-known kefir grains (a combination of *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus lactis*) have shown anti-tumor activity in human cancer cells, 4T1 [57].

By enhancing the immunological response, oral dosing of *Lactobacillus acidophilus* prolonged survival in an animal model. The treated group has shown stimulation of pro-inflammatory cytokines (IFN- γ) and inhibition of anti-inflammatory cytokines (IL-4 and IL-10) in spleen cells [58]. Another study analyzed that daily intake of *Lactobacillus acidophilus* can modulate the IL-12 cytokine level in BALB/c mice bearing transplanted breast tumors [59]. Oral suspension of *Lactobacillus casei* displayed a significant reduction of tumor growth and enhanced survival by increasing cytokine production (Th1) in mouse spleen cells [60].

4.3.4 PROBIOTICS IN HEPATOCELLULAR CARCINOMA

In the world, hepatocellular carcinoma (HCC) is one of the prevalent causes of death from cancer. The utilization of probiotics to alter the gut microbiota is a newer strategy for liver cancer therapy. A research team has examined a brand-new probiotic combination known as Prohep, which contains *L. rhamnosus* GG, live *E. coli* Nissle 1917, and heat-inactivated VSL#3. The results demonstrated that the probiotic-fed mice showed a significant reduction in tumor growth and tumor size by down-regulation of cytokine IL-17 and its producer cell Th17. The probiotic-treated group had significantly decreased migrating Th17 lymphocytes in the peripheral blood and intestines. Additionally, probiotics promote the gut microbial population, such as *Prevotella* and *Oscillibacter*, also

including propionate synthesis and IL-10-mediated regulatory T cell homeostasis, resulting in the creation of anti-inflammatory metabolites [61]. This study further revealed the anti-angiogenesis and anticancer effect of Prohep by downregulation of VEGFA, ANGPT2, Fms-related receptor tyrosine kinase 1, and kinase insert domain receptor. Heydari et al., looked into how probiotic bacteria like *Lactobacillus acidophilus* and *B. bifidum* affected hepatocellular carcinoma's emergence in Balb/c mice. The study demonstrated a significant downregulation of miR-221 and miR-155 and repression of oncogenes like Bcl-w and KRAS when treated with probiotics in the liver cancer mice model induced with azoxymethane, a well-known carcinogen. The probiotics also enhanced the tumor suppressor's expression of microRNAs, miR-26b, miR-18a, and tumor suppressor PU [62].

4.3.5 PROBIOTICS IN ORAL CANCER

Few studies have been conducted on the topic, and probiotics in the treatment of oral cancer are still in their infancy. A recent study evaluated the enhanced antitumor effects of Geniposide (derived from *Gardenia jasminoides*), which showed anticancer ability against oral squamous cell carcinoma (HSC-3 OSCC), using *L. rhamnosus* GG. The study revealed increased apoptosis due to the combined effect of Geniposide and LGG on oral cancer cells [63]. Another research study displayed the inhibitory role of *L. plantarum* on oral cancer KB cells. *L. plantarum* modulates the activation of PTEN pathways and inhibits MAPKs for cancer therapeutics [64]. Another study investigated the cytotoxicity of *Acetobacter syzygii* strain supernatant against human oral cancer cell KB and found that it dramatically increased apoptotic potential, similar to cisplatin (75.7%) [65]. Several other microbes, such as *Neisseria sicca*, *Parvimonas micra*, and *S. anginosus*, are associated with oral microbiota, which helps to prevent OSCC progression. *L. salivarius* Ren down-regulated COX-2 expression, preventing proliferative growth and inducing apoptosis in TCA-8113 (oral cancer cells) [69].

4.3.6 PROBIOTICS IN CERVICAL CANCER

Probiotics are equally efficient in several gynecological diseases [70]; therefore, research has been performed to investigate the effect of probiotics in diagnosis, cervical cancer therapy, and prevention [71].

By promoting S phase cells and decreasing the G2/M cell population, *L. supernatants* (LS) (*L. crispatus*, *L. jensenii*, and *L. gasseri*) effectively alter the cell cycle phases, which results in reduced Caski cell proliferation and an anticancer impact. Additionally, the combination of microorganisms helps to reduce the expression of cyclin A, CDK2, E6, and E7 (HPV oncogene) [72]. *L. plantarum* bacteria and human milk-isolated *Lactobacillus* strains (*L. casei* SR1, *L. casei* SR2, and *L. paracasei* SR4) displayed potent anticancer activity against the HeLa cervical cancer cell line. These probiotics also showed antioxidant properties, low pH resistance, antibiotic susceptibility, and high-level bile salt resistance [73, 74]. In a study, Sungur et al., demonstrated that human vaginal *L. gasseri* strains reduced the development of HeLa cells, elevated the expression of Bax and Caspase 3, and generated an apoptotic population. As an anticancer probiotic, *L. gasseri* also showed anti-inflammatory effects by reducing TNF- α expression [75]. It has been observed that *L. rhamnosus* and *L. crispatus* downregulate the expression of MMP2 and MMP9, consequently inhibiting metastasis [76].

4.3.7 PANCREATIC CANCER AND PROBIOTICS

Cancer of the pancreas is a deadly malignancy, and management is extremely difficult due to late diagnosis, poor response to available therapeutics, and drug resistance. Several studies have explained the role of microbiota in pancreatic cancer (PanCa) progression. By stimulating p53 through a cell cycle change, Kita et al., examined the impact of ferrichrome produced from probiotics, significantly reducing tumor development in pancreatic cancer cells. Furthermore, it could overcome the 5-FU mediated resistance in pancreatic *in vitro* and xenograft mouse models. Recent studies revealed that *L. casei*-derived ferrichrome significantly altered tumor-associated macrophages, which led to the infiltration of CD8⁺ T cells into tumors in the PanCa mouse model, resulting in the suppression of tumor development. Additionally, ferrichrome treatment improves the combination therapeutic modality against PanCa by enhancing the anti-PD-L1 response [77]. A combination of next-generation probiotics, including *F. prausnitzii*, *P. goldsteinii*, *P. copri*, and *A. muciniphila*, has shown a promising therapeutic response against pancreatic cancer [78]. A gram-negative bacterium, *A. muciniphila*, displayed inhibition of rat pancreatic islet tumor cell proliferation, subsequently improving gut immunity and the release of cytokines [79].

4.4 PROBIOTICS' MECHANISMS OF ACTION IN TREATING AND PREVENTING CANCER [80–84]

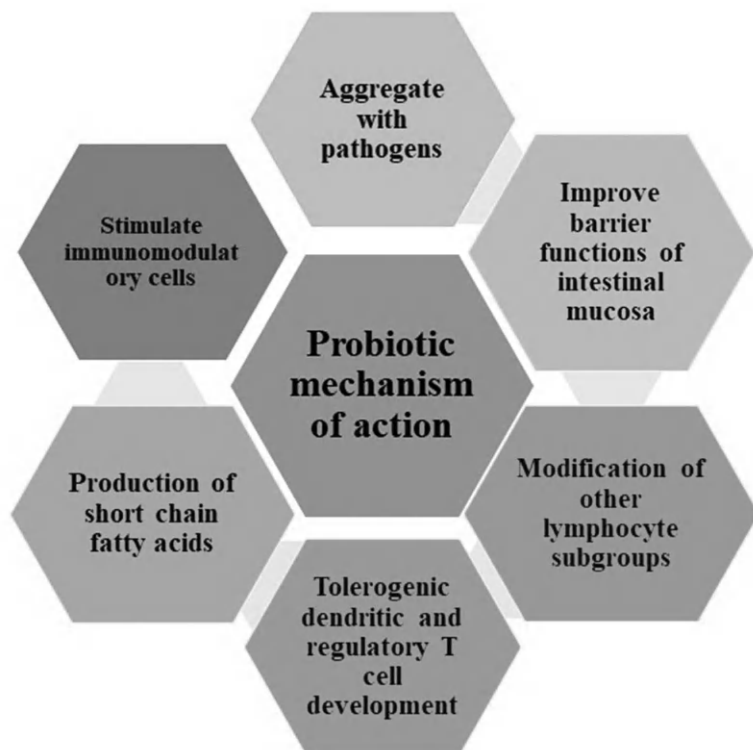


FIGURE 4.2 Probiotics' cancer-fighting mechanisms.

4.4.1 MODIFICATION OF INTESTINAL MICROBIOTA COMPOSITION

Additionally, probiotics can alter the gut immune system, leading to reduced inflammation and improved gut barrier function. Probiotics can improve the conditions for the expansion of beneficial microorganisms in the gut by lowering inflammation, which can in turn further improve gut health [85].

Overall, probiotics are a promising strategy for modifying digestive tract microbiota composition in order to advance gut health and prevent or treat a range of health conditions. Probiotics affect the intestinal microbiome in several ways, but more study is required to fully understand these mechanisms and to choose the most effective strains and doses for various medical diseases.

4.4.2 METABOLIC ACTIVITY OF THE INTESTINAL MICROBIOTA

The combined biochemical processes comprise the gut microbiota's metabolic activity and the processes carried out by the microorganisms that reside in the gut. These metabolic activities are crucial for sustaining general health and the healthy operation of the stomach. Numerous metabolic processes are carried out by the intestinal microbiota, such as the digestion of complex carbohydrates, the creation of vitamins, the process of short-chain fatty acid synthesis (SCFAs), and the control of bile acid metabolism.

These processes are necessary for nutrient digestion and absorption, immune system regulation, and infection prevention. The gut microbiota ferments food fibers to create SCFAs such as acetate, propionate, and butyrate. By supplying colonocytes with energy, SCFAs support gut health by regulating immune function and gastrointestinal pH. Furthermore, research has shown that SCFAs have anti-inflammatory and anti-cancer properties [86]. The liver produces bile acids, which aid in the breakdown and absorption of dietary lipids. The metabolism of these bile acids also involves the gut bacteria. Bile acid content can be altered by the gut flora, which may have an impact on how well they work and are absorbed in the gut.

4.4.3 PRODUCTION OF MOLECULES WITH ANTI-CARCINOGENIC PROPERTIES, SUCH AS CONJUGATED LINOLEIC ACIDS AND FATTY ACIDS, AS WELL AS SHORT CHAIN

A significant method for lowering the risk of cancer formation is the generation of short-chain fatty acids (SCFAs) along with conjugated linoleic acids (CLAs) produced by the intestinal microbiome, as they possess anticarcinogenic activities.

Dietary fibers are fermented by gut bacteria, producing SCFAs such as butyrate, acetate, and propionate. It has been established that these SCFAs have strong anticarcinogenic properties, such as the ability to control the immunological response and induce apoptosis (programmed cell death), which results in a reduction in the growth of cancer cells [88]. A class of fatty acids known as CLAs has been found to possess strong anticarcinogenic effects. These substances are created in the stomach by bacteria like *Bifidobacterium* and *Lactobacillus* species, which biohydrogenate linoleic acid [89].

According to research, CLAs can stop the growth of cancer cells, trigger apoptosis, and lessen inflammation. The production of compounds with anti-carcinogenic activity by the intestinal microbiota, such as SCFAs and CLAs, is an important mechanism for reducing the risk of cancer development. A

diet rich in dietary fibers and beneficial bacteria, along with regular consumption of fermented foods and probiotics, can help promote the production of these compounds in the gut [90].

4.4.4 INTESTINAL LUMEN'S CARCINOGENIC COMPOUNDS: BINDING AND DEGRADATION

The binding followed by the degradation of carcinogenic compounds present in the intestinal lumen by probiotics is another important mechanism for reducing the risk of cancer development. Some probiotic strains have the capacity to bind and metabolize carcinogenic substances, such as those created when meat and fish are cooked, including heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs)[91].

HCAs and PAHs have been shown to have mutagenic and carcinogenic effects and are considered to be important contributors to the emergence of colon cancer. The synthesis of enzymes like nitro reductases and azoreductases by some probiotic strains, including those from the *Lactobacillus* and *Bifidobacterium* species, has been demonstrated to break down and inactivate HCAs and PAHs [92].

Probiotics can bind to and detoxify other carcinogenic substances, such as aflatoxins, which are formed by specific molds and are present in contaminated food, in addition to binding to and metabolizing HCAs and PAHs. Probiotics can help prevent these substances from being absorbed into the body and lower the risk of cancer development by binding to and detoxifying them. Regular consumption of probiotic-rich foods, such as fermented foods and yogurt, may help encourage the development and activity of these helpful intestinal microorganisms.

4.5 SELECTION CRITERIA OF PROBIOTICS

Probiotics have been demonstrated to modulate gut microbiota composition, potentially helping manage cancer more successfully. Probiotics used in cancer management come with numerous selection criteria; each criterion holds special significance. These selection criteria are based on the probiotic strains' ability to survive in the gut, their potential to modulate immune systems, produce bioactive compounds, and alter host metabolism. Before selecting a probiotic strain for use, one should carefully evaluate its safety, viability, and efficacy, as well as its resistance to gastrointestinal stresses and stability during processing and storage. Selecting appropriate probiotic

strains is paramount to creating safe and effective probiotic products designed to combat cancer. Studies are essential for identifying probiotic strains most suitable for treating various forms of cancer and creating personalized therapies utilizing probiotics. Furthermore, standardization must occur regarding identification and characterization processes to guarantee reproducibility as well as quality control of probiotic products. Selecting appropriate strain(s) of probiotics for cancer management requires great consideration. Therefore, this chapter discusses selection criteria with examples and citations as part of this discussion.

4.5.1 STRAIN SPECIFICITY

Probiotic strains have different impacts on gut microbiota. Therefore, it's essential to select probiotic strains that benefit cancer management. When selecting probiotic strains that will have beneficial results on gut microbiota modulation, *B. bifidum* has been demonstrated to effectively suppress the growth of colon cancer cells in culture and in living organisms [94], while *L. acidophilus* has been proven to lower incidence rates for colon tumors [95]. Certain strains may also be more effective at modulating gut microbiota in cancer patients than in healthy individuals; one study discovered that a mixture of *L. plantarum*, *L. acidophilus*, and *B. bifidum* improved gut microbiota composition among colon cancer patients but not healthy controls [96].

4.5.2 SAFETY

Safety should always be at the forefront when selecting probiotics for cancer management, with all strain(s) selected having a documented safety record, particularly among immunocompromised patients. *Lactobacillus rhamnosus* GG has been extensively researched, with positive safety records in critically ill patients as well. Conversely, other strains could pose risks, including *Lactobacillus casei* Shirota, which has been associated with sepsis cases [97]. Thus, it is important that probiotic strains that have undergone rigorous safety studies be selected when choosing probiotic strain(s) suitable for target patient populations [98].

4.5.3 MECHANISM OF ACTION

Understanding how probiotic strains exert their effects is of critical importance in cancer management. Select probiotic strains with well-documented

mechanisms relevant to cancer care management – for instance, some strains could modulate your gut microbiota by producing short-chain fatty acids, which have anticancer properties [99], while others may produce antimicrobial peptides called bacteriocins, which inhibit cancer cell growth [100]. Ultimately, it's vital that you select probiotic strains with clear mechanisms relevant for cancer management when making selection decisions related to cancer management.

4.5.4 EVIDENCE-BASED

When selecting probiotics for cancer management, it is crucial that strains have been thoroughly studied and have proven effects. In a critical review, the randomized controlled trial meta-analysis demonstrated how some strains, specifically *Lactobacillus* and *Bifidobacterium* species, reduced colorectal adenomas, which are precursors to colorectal cancer [101], while another research study concluded that *Lactobacillus acidophilus* decreased colon tumor incidence [102]. Therefore, it is important that cancer practitioners select probiotic strains that have proven effects against cancer with high-quality documented research evidence.

4.5.5 DELIVERY SYSTEM

A good delivery system for probiotics is also key; the selection of probiotic strains must have enough survival rates in the human gut environment in order to reach their site of action and exert a therapeutic effect in adequate numbers. Some strains are more resistant to gastric acid and bile salts than others, thus increasing their survival in the digestive tract. Furthermore, any delivery system used should be practical and convenient for patient consumption. Some patients may prefer capsules or tablets over dairy products and fermented foods as an effective probiotic source, making probiotics even more convenient and practical to take daily. Therefore, selecting strains suitable to survive the harsh conditions in their gut is key if patients want the benefits.

4.5.6 SYNERGISM WITH OTHER THERAPIES

Probiotics have the potential to have a synergistic effect when used alongside chemotherapy and radiotherapy; as such, it's wise to select probiotic strains that have demonstrated this capability in clinical studies.

To maximize synergy with cancer therapies and ensure maximum benefit, studies have discovered that the probiotic *Lactobacillus rhamnosus* GG significantly enhanced chemotherapy's antitumor activity against colon cancer [103]. Studies have also demonstrated how pairing *Bifidobacterium lactis* with the anticancer medication 5-fluorouracil may significantly decrease colon cancer cell proliferation [104]. Therefore, when selecting probiotic strains as cancer therapies, it is crucial to choose ones with synergistic effects, such as those suggested above. Probiotics have demonstrated effective results in cancer management by altering the composition and functioning of gut microbiota, playing an integral part in maintaining immune homeostasis and shaping cancer development and progression. Probiotics may affect this balance by producing bioactive compounds, modulating immune responses, or directly engaging gut epithelial cells to regulate gut microbial communities.

Probiotics in cancer management must satisfy several selection criteria, such as being able to survive the digestive environment, offering immunomodulatory properties, producing bioactive compounds, having metabolic effects on their host body, being safe and viably effective, being resistant to gastrointestinal stressors during processing/storage, and providing resistance against digestive upset during processing/storage. One such probiotic that meets these requirements includes *Lactobacillus* and *Bifidobacterium* strains, which have been extensively researched for their potential in cancer therapy management. Future research should aim at identifying and characterizing probiotic strains with proven effectiveness for cancer management, along with their mechanisms of action and personalized probiotic-based therapy solutions tailored specifically for individuals based on gut microbiota composition and cancer type. Furthermore, standardization should take place during probiotic strain identification and characterization to guarantee reproducibility and quality control for probiotic products.

Probiotics offer an innovative strategy for cancer management by altering gut microbiota composition and function. Selecting effective probiotic strains based on the selection criteria outlined above is crucial in developing safe products targeted specifically toward managing cancer; further research must also take place to identify highly effective strains as well as to tailor personalized probiotic-based therapies.

Table 4.1 summarizes studies of probiotic strain in different cell lines to study the effect of cancer cells.

TABLE 4.1 Studies of Probiotic Strain in Different Cell Lines to Study the Effect of Cancer Cells

Probiotic Strain	Cell Line	Effect on Cancer Cells	Reference
<i>L. acidophilus</i>	HT-29 (human colon cancer cells)	Apoptosis and cell cycle arrest are induced, which slows the development of colon cancer cells.	[105]
<i>B. lactis</i>	MCF-7 (human breast cancer cells)	Induces apoptosis and inhibits cell migration to slow the development of breast cancer cells.	[106]
<i>L. rhamnosus GG</i>	HT-29 (human colon cancer cells)	Inhibits the expression of pro-inflammatory cytokines and slows the development of colon cancer cells.	[107]
<i>S. thermophilus</i>	HT-29 (human colon cancer cells)	Arrests the cell cycle and inhibits the development of colon cancer cells.	[108]
<i>L. casei</i>	Caco-2 (human colon cancer cells)	Reduces the growth of colon cancer cells and induces apoptosis	[109]
<i>L. plantarum</i>	SW620 (human colon cancer cells)	Stops the growth of colon cancer cells and causes apoptosis	[110]
<i>B. bifidum</i>	HT-29 (human colon cancer cells)	Inhibits the development of colon cancer cells and causes apoptosis	[111]
<i>L. reuteri</i>	HT-29 (human colon cancer cells)	Inhibits the growth of colon cancer cells and blocks the production of pro-inflammatory cytokines.	[112]
<i>B. longum</i>	HT-29 (human colon cancer cells)	Reduces colon cancer cell proliferation and promotes apoptosis	[113]
<i>E.coli Nissle 1917</i>	HT-29 (human colon cancer cells)	Prevents colon cancer cells from growing and triggers apoptosis	[114]

4.6 CONCLUSION

In conclusion, probiotics are crucial for cancer treatment in the intestinal microbiota. Gut microbiota is a complex ecosystem of bacteria that has a significant impact on many facets of human health, including the management and prevention of cancer. The benefits of probiotics have been demonstrated; they can modify the gut microbiota's composition and activity in ways that can lower the chance of contracting cancer and increase the effectiveness of cancer treatment. This includes the generation of substances with anticarcinogenic action, such as SCFAs and CLAs, as well as the binding and destruction of carcinogenic chemicals. Modifications in the gut flora are one of the ways through which probiotics exercise their anticancer benefits.

Probiotics have the potential to prevent and cure a variety of cancers, including colon, breast, and liver cancer, according to animal and cell culture research. While the evidence supporting the role of probiotics in cancer management is promising, more research is needed to fully understand the underlying mechanisms and to choose the best strains, doses, and duration of probiotic supplementation for cancer prevention and therapy. Nonetheless, regular consumption of probiotic-rich foods, such as fermented foods and yogurt, can be a simple and effective way to promote the development and function of helpful bacteria in the gut and support overall health, including cancer prevention and therapy.

KEYWORDS

- **Cancer**
- **cell lines**
- **ecosystem**
- **immune system**
- **microbiota**
- **probiotics**

REFERENCES

1. Ferlay, J., Steliarova-Foucher, E., Lortet-Tieulent, J., Rosso, S., Coebergh, J. W. W., Comber, H., & Bray, F. (2013). Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. *European Journal of Cancer*, 49(6), 1374–1403. <https://doi.org/10.1016/j.ejca.2012.12.027>.
2. Collaboration GBoDC. (2019). Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: A systematic analysis for the Global Burden of Disease Study. *JAMA Oncology*, 5(12), 1749–1768. <https://doi.org/10.1001/jamaoncol.2019.2996>.
3. Legesse Bedada, T., Feto, T. K., Awoke, K. S., Garedew, A. D., Yifat, F. T., & Birri, D. J. (2020). Probiotics for cancer alternative prevention and treatment. *Biomedicine & Pharmacotherapy*, 129, 110409. <https://doi.org/10.1016/j.biopha.2020.110409>.
4. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., & Bray, F. (2015). Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*, 136(5), E359–E386. <https://doi.org/10.1002/ijc.29210>.

5. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5), 646–674. <https://doi.org/10.1016/j.cell.2011.02.013>.
6. Shaheen, M., Allen, C., Nickoloff, J. A., & Hromas, R. (2011). Synthetic lethality: Exploiting the addiction of cancer to DNA repair. *Blood*, 117(23), 6074–6082. <https://doi.org/10.1182/blood-2011-01-313734>.
7. Anand, P., Kunnumakkara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., & Aggarwal, B. B. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research*, 25(9), 2097–2116.
8. Raguz, S., & Yagüe, E. (2008). Resistance to chemotherapy: New treatments and novel insights into an old problem. *British Journal of Cancer*, 99(3), 387–391. <https://doi.org/10.1038/sj.bjc.6604510>.
9. Vivarelli, S., Salemi, R., Candido, S., Falzone, L., Santagati, M., Stefani, S., & Libra, M. (2019). Gut microbiota and cancer: From pathogenesis to therapy. *Cancers (Basel)*, 11(1). <https://doi.org/10.3390/cancers11010038>.
10. Jiang, Z., Li, L., Chen, J., Wei, G., Ji, Y., Chen, X., & Huo, J. (2021). Human gut-microbiome interplay: Analysis of clinical studies for the emerging roles of diagnostic microbiology in inflammation, oncogenesis, and cancer management. *Infection, Genetics and Evolution*, 93, 104946.
11. Śliżewska, K., Markowiak-Kopeć, P., & Śliżewska, W. (2020). The role of probiotics in cancer prevention. *Cancers (Basel)*, 13(1). <https://doi.org/10.3390/cancers13010020>.
12. Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, 124(4), 837–848. <https://doi.org/10.1016/j.cell.2006.02.017>.
13. Gonzalez, A., Stombaugh, J., Lozupone, C., Turnbaugh, P. J., Gordon, J. I., & Knight, R. (2011). The mind-body-microbial continuum. *Dialogues in Clinical Neuroscience*, 13(1), 55–62. <https://doi.org/10.31887/DCNS.2011.13.1/agonzalez>.
14. Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, 3(4), 289–306. <https://doi.org/10.4161/gmic.19897>.
15. Apajalahti, J. (2005). Comparative gut microflora, metabolic challenges, and potential opportunities. *Journal of Applied Poultry Research*, 14(2), 444–453. <https://doi.org/10.1093/japr/14.2.444>.
16. Goodman, B., & Gardner, H. (2018). The microbiome and cancer. *Journal of Pathology*, 244(5), 667–676. <https://doi.org/10.1002/path.5047>.
17. Wen, H., Yin, X., Yuan, Z., Wang, X., & Su, S. (2018). Comparative analysis of gut microbial communities in children under 5 years old with diarrhea. *Journal of Microbiology and Biotechnology*, 28(4), 652–662. <https://doi.org/10.4014/jmb.1711.11065>.
18. Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and symbiotic on human health. *Nutrients*, 9(9). <https://doi.org/10.3390/nu9091021>.
19. Adams, C. A. (2010). The probiotic paradox: Live and dead cells are biological response modifiers. *Nutrition Research Reviews*, 23(1), 37–46. <https://doi.org/10.1017/S0954422410000090>.
20. Tamang, J. P., Shin, D. H., Jung, S. J., & Chae, S. W. (2016). Functional properties of microorganisms in fermented foods. *Frontiers in Microbiology*, 7, 578. <https://doi.org/10.3389/fmicb.2016.00578>.

21. Sharifi, M., Moridnia, A., Mortazavi, D., Salehi, M., Bagheri, M., & Sheikhi, A. (2017). Kefir: A powerful probiotic with anticancer properties. *Medical Oncology*, 34(11), 183. <https://doi.org/10.1007/s12032-017-1044-9>.
22. Van t Veer, P., Van Leer, E. M., Rietdijk, A., Kok, F. J., Schouten, E. G., Hermus, R. J., & Sturmans, F. (1991). Combination of dietary factors in relation to breast-cancer occurrence. *International Journal of Cancer*, 47(5), 649–653.
23. Lê, M. G., Moulton, L. H., Hill, C., & Kramar, A. (1986). Consumption of dairy produce and alcohol in a case-control study of breast cancer. *J Natl Cancer Inst*, 77(3), 633–636. <https://doi.org/10.1093/jnci/77.3.633>.
24. Cramer, D., Willett, W., Bell, D., Ng, W., Harlow, B., Welch, W., Scully, R., & Knapp, R. (1989). Galactose consumption and metabolism in relation to the risk of ovarian cancer. *The Lancet*, 334(8654), 66–71.
25. Svetoch, E. A., Eruslanov, B. V., Pereygin, V. V., Mitsevidh, E. V., Mitsevidh, I. P., Borzenkov, V. N., Levchuk, V. P., Svetoch, O. E., Kovalev, Y. N., Stepanshin, Y. G., Siragusa, G. R., Seal, B. S., & Stern, N. J. (2008). Diverse antimicrobial killing by *Enterococcus faecium* E 50–52 bacteriocin. *Journal of Agricultural and Food Chemistry*, 56(6), 1942–1948. <https://doi.org/10.1021/jf073284g>.
26. Siggers, R. H., Siggers, J., Boye, M., Thymann, T., Mølbak, L., Leser, T., Jensen, B. B., & Sangild, P. T. (2008). Early administration of probiotics alters bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing enterocolitis in preterm pigs. *J Nutr*, 138(8), 1437–1444. <https://doi.org/10.1093/jn/138.8.1437>.
27. Candela, M., Perna, F., Carnevali, P., Vitali, B., Ciatì, R., Gionchetti, P., Rizzello, F., Campieri, M., & Brigidi, P. (2008). Interaction of probiotic *Lactobacillus* and *Bifidobacterium* strains with human intestinal epithelial cells: Adhesion properties, competition against enteropathogens and modulation of IL-8 production. *International Journal of Food Microbiology*, 125(3), 286–292. <https://doi.org/10.1016/j.ijfoodmicro.2008.04.012>.
28. Raman, M., Ambalam, P., Kondepudi, K. K., Pithva, S., Kothari, C., Patel, A. T., Purama, R. K., Dave, J. M., & Vyas, B. R. (2013). Potential of probiotics, prebiotics and symbiotic for management of colorectal cancer. *Gut Microbes*, 4(3), 181–192. <https://doi.org/10.4161/gmic.23919>.
29. Sheikhi, A., Jafarzadeh, A., Kokhaei, P., & Hojjat-Farsangi, M. (2016). Whole tumor cell vaccine adjuvants: Comparing IL-12 to IL-2 and IL-15. *Iranian Journal of Immunology*, 13(3), 148–166.
30. Ferguson, P. J., Kurowska, E., Freeman, D. J., Chambers, A. F., & Koropatnick, D. J. (2004). A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. *J Nutr*, 134(6), 1529–1535. <https://doi.org/10.1093/jn/134.6.1529>.
31. Sankpal, U. T., Pius, H., Khan, M., Shukoor, M. I., Maliakal, P., Lee, C. M., Abdelrahim, M., Connelly, S. F., & Basha, R. (2012). Environmental factors in causing human cancers: Emphasis on tumorigenesis. *Tumor Biology*, 33(5), 1265–1274. <https://doi.org/10.1007/s13277-012-0413-4>.
32. Perillo, F., Amoroso, C., Strati, F., Giuffrè, M. R., Díaz-Basabe, A., Lattanzi, G., & Facciotti, F. (2020). Gut microbiota manipulation as a tool for colorectal cancer management: Recent advances in its use for therapeutic purposes. *International Journal of Molecular Sciences*, 21(15). <https://doi.org/10.3390/ijms21155389>.
33. Salminen, S., Nybom, S., Meriluoto, J., Collado, M. C., Vesterlund, S., & El-Nezami, H. (2010). Interaction of probiotics and pathogens—Benefits to human health?

- Current Opinion in Biotechnology*, 21(2), 157–167. <https://doi.org/10.1016/j.copbio.2010.03.016>.
34. Paolillo, R., Romano Carratelli, C., Sorrentino, S., Mazzola, N., & Rizzo, A. (2009). Immunomodulatory effects of *Lactobacillus plantarum* on human colon cancer cells. *International Immunopharmacology*, 9(11), 1265–1271. <https://doi.org/10.1016/j.intimp.2009.07.008>.
 35. Rochat, T., Bermúdez-Humarán, L., Gratadoux, J. J., Fourage, C., Hoebler, C., Corthier, G., & Langella, P. (2007). Anti-inflammatory effects of *Lactobacillus casei* BL23 producing or not a manganese-dependent catalase on DSS-induced colitis in mice. *Microbial Cell Factories*, 6, 22. <https://doi.org/10.1186/1475-2859-6-22>.
 36. Jacouton, E., Chain, F., Sokol, H., Langella, P., & Bermúdez-Humarán, L. G. (2017). Probiotic strain *Lactobacillus casei* BL23 prevents colitis-associated colorectal cancer. *Frontiers in Immunology*, 8, 1553. <https://doi.org/10.3389/fimmu.2017.01553>.
 37. Chang, C. W., Liu, C. Y., Lee, H. C., Huang, Y. H., Li, L. H., Chiau, J. C., Wang, T. E., Chu, C. H., Shih, S. C., Tsai, T. H., & Chen, Y. J. (2018). *Lactobacillus casei* variety rhamnosus probiotic preventively attenuates 5-fluorouracil/oxaliplatin-induced intestinal injury in a syngeneic colorectal cancer model. *Frontiers in Microbiology*, 9, 983. <https://doi.org/10.3389/fmicb.2018.00983>.
 38. Tsai, T. L., Li, A. C., Chen, Y. C., Liao, Y. S., & Lin, T. H. (2015). Antimicrobial peptide m2163 or m2386 identified from *Lactobacillus casei* ATCC 334 can trigger apoptosis in the human colorectal cancer cell line SW480. *Tumor Biology*, 36(5), 3775–3789. <https://doi.org/10.1007/s13277-014-3018-2>.
 39. Konishi, H., Fujiya, M., Tanaka, H., Ueno, N., Moriichi, K., Sasajima, J., Ikuta, K., Akutsu, H., Tanabe, H., & Kohgo, Y. (2016). Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. *Nature Communications*, 7, 12365. <https://doi.org/10.1038/ncomms12365>.
 40. Ijiri, M., Fujiya, M., Konishi, H., Tanaka, H., Ueno, N., Kashima, S., Moriichi, K., Sasajima, J., Ikuta, K., & Okumura, T. (2017). Ferrichrome identified from *Lactobacillus casei* ATCC334 induces apoptosis through its iron-binding site in gastric cancer cells. *Tumour Biology*, 39(6), 1010428317711311. <https://doi.org/10.1177/1010428317711311>.
 41. Kita, A., Fujiya, M., Konishi, H., Tanaka, H., Kashima, S., Iwama, T., Ijiri, M., Murakami, Y., Takauji, S., Goto, T., Sakatani, A., Ando, K., Ueno, N., Ogawa, N., & Okumura, T. (2020). Probiotic derived ferrichrome inhibits the growth of refractory pancreatic cancer cells. *International Journal of Oncology*, 57(3), 721–732. <https://doi.org/10.3892/ijo.2020.5096>.
 42. Asano, M., Karasawa, E., & Takayama, T. (1986). Antitumor activity of *Lactobacillus casei* (LC 9018) against experimental mouse bladder tumor (MBT-2). *Journal of Urology*, 136(3), 719–721. [https://doi.org/10.1016/s0022-5347\(17\)45035-x](https://doi.org/10.1016/s0022-5347(17)45035-x).
 43. Lim, B. K., Mahendran, R., Lee, Y. K., & Bay, B. H. (2002). Chemopreventive effect of *Lactobacillus rhamnosus* on growth of a subcutaneously implanted bladder cancer cell line in the mouse. *Japanese Journal of Cancer Research*, 93(1), 36–41. <https://doi.org/10.1111/j.1349-7006.2002.tb01198.x>.
 44. Takagi, A., Matsuzaki, T., Sato, M., Nomoto, K., Morotomi, M., & Yokokura, T. (2001). Enhancement of natural killer cytotoxicity delayed murine carcinogenesis by a probiotic microorganism. *Carcinogenesis*, 22(4), 599–605. <https://doi.org/10.1093/carcin/22.4.599>.

45. Ng, S. C., Hart, A. L., Kamm, M. A., Stagg, A. J., & Knight, S. C. (2009). Mechanisms of action of probiotics: Recent advances. *Inflammatory Bowel Diseases*, 15(2), 300–310. <https://doi.org/10.1002/ibd.20602>.
46. Aso, Y., & Akazan, H. (1992). Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. BLP Study Group. *Urologia Internationalis*, 49(3), 125–129. <https://doi.org/10.1159/000282409>.
47. Aso, Y., Akaza, H., Kotake, T., Tsukamoto, T., Imai, K., & Naito, S. (1995). Preventive effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. The BLP Study Group. *European Urology*, 27(2), 104–109. <https://doi.org/10.1159/000475138>.
48. Sung, H., Ferlay, J., Siegel, R. L., 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: A Cancer Journal for Clinicians*, 71(3), 209–249. <https://doi.org/10.3322/caac.21660>.
49. Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians*, 68(1), 7–30. <https://doi.org/10.3322/caac.21442>.
50. Azam, R., Ghafouri-Fard, S., Tabrizi, M., Modarressi, M. H., Ebrahimzadeh-Vesal, R., Daneshvar, M., Mobasheri, M. B., & Motevaseli, E. (2014). *Lactobacillus acidophilus* and *Lactobacillus crispatus* culture supernatants downregulate expression of cancer-testis genes in the MDA-MB-231 cell line. *Asian Pacific Journal of Cancer Prevention*, 15(10), 4255–4259. <https://doi.org/10.7314/apjcp.2014.15.10.4255>.
51. Kadirareddy, R. H., Vemuri, S. G., & Palempalli, U. M. (2016). Probiotic conjugated linoleic acid mediated apoptosis in breast cancer cells by downregulation of NFκB. *Asian Pacific Journal of Cancer Prevention*, 17(7), 3395–3403.
52. Nami, Y., Haghshenas, B., Haghshenas, M., Abdullah, N., & Yari Khosroushahi, A. (2015). The prophylactic effect of probiotic *Enterococcus lactis* IW5 against different human cancer cells. *Frontiers in Microbiology*, 6, 1317.
53. Han, K. J., Lee, N. K., Park, H., & Paik, H. D. (2015). Anticancer and anti-inflammatory activity of probiotic *Lactococcus lactis* NK34. *Journal of Microbiology and Biotechnology*, 25(10), 1697–1701.
54. Lee, N. K., Han, K. J., Son, S. H., Eom, S. J., Lee, S. K., & Paik, H. D. (2015). Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi. *LWT—Food Science and Technology*, 64(2), 1036–1041.
55. Hassan, Z., Mustafa, S., Rahim, R. A., & Isa, N. M. (2016). Anti-breast cancer effects of live, heat-killed, and cytoplasmic fractions of *Enterococcus faecalis* and *Staphylococcus hominis* isolated from human breast milk. *In vitro Cellular & Developmental Biology—Animal*, 52(3), 337–348. <https://doi.org/10.1007/s11626-015-9978-8>.
56. Bharti, V., Mehta, A., Singh, S., Jain, N., & Ahirwal, L. (2015). Cytotoxicity of live whole cell, heat-killed cell, and cell-free extract of *Lactobacillus* strain in U-87 human glioblastoma cell line and MCF-7 breast cancer cell line. *International Journal of Probiotics and Prebiotics*, 4.
57. Zambari, N. R., Abu, N., Mohamed, N. E., Nordin, N., Keong, Y. S., Beh, B. K., Zakaria, Z. A., Nik Abdul Rahman, N. M., & Alitheen, N. B. (2016). The antimetastatic and antiangiogenesis effects of kefir water on murine breast cancer cells. *Integrative Cancer Therapies*, 15(4), Np53-np66. <https://doi.org/10.1177/1534735416642862>.
58. Imani Fooladi, A. A., Yazdi, M. H., Pourmand, M. R., Mirshafiey, A., Hassan, Z. M., Azizi, T., Mahdavi, M., & Soltan Dallal, M. M. (2015). Th1 cytokine production

- induced by *Lactobacillus acidophilus* in BALB/c mice bearing transplanted breast tumor. *Jundishapur Journal of Microbiology*, 8(4), e17354. [https://doi.org/10.5812/jjm.8\(4\)2015.17354](https://doi.org/10.5812/jjm.8(4)2015.17354).
59. Yazdi, M. H., Soltan Dallal, M. M., Hassan, Z. M., Holakuyee, M., Agha Amiri, S., Abolhassani, M., & Mahdavi, M. (2010). Oral administration of *Lactobacillus acidophilus* induces IL-12 production in spleen cell culture of BALB/c mice bearing transplanted breast tumor. *British Journal of Nutrition*, 104(2), 227–232. <https://doi.org/10.1017/s0007114510000516>.
 60. Soltan Dallal, M. M., Yazdi, M. H., Holakuyee, M., Hassan, Z. M., Abolhassani, M., & Mahdavi, M. (2012). *Lactobacillus casei* ssp. *casei* induced Th1 cytokine profile and natural killer cells activity in invasive ductal carcinoma bearing mice. *Iranian Journal of Allergy, Asthma and Immunology*, 11(2), 183–189.
 61. Li, J., Sung, C. Y. J., Lee, N., Ni, Y., Pihlajamäki, J., Panagiotou, G., & El-Nezami, H. (2016). Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proceedings of the National Academy of Sciences*, 113(9), E1306–E1315. <https://doi.org/10.1073/pnas.1518189113>.
 62. Heydari, Z., Rahaie, M., & Alizadeh, A. M. (2019). Different anti-inflammatory effects of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in hepatocellular carcinoma cancer mouse through impact on microRNAs and their target genes. *Journal of Nutrition & Intermediary Metabolism*, 16, 100096. <https://doi.org/10.1016/j.jnim.2019.100096>.
 63. Cheng, Z., Xu, H., Wang, X., & Liu, Z. (2017). *Lactobacillus* raises *In vitro* anticancer effect of geniposide in HSC-3 human oral squamous cell carcinoma cells. *Experimental and Therapeutic Medicine*, 14(5), 4586–4594. <https://doi.org/10.3892/etm.2017.5105>.
 64. Asoudeh-Fard, A., Barzegari, A., Dehnad, A., Bastani, S., Golchin, A., & Omid, Y. (2017). *Lactobacillus plantarum* induces apoptosis in oral cancer KB cells through upregulation of PTEN and downregulation of MAPK signaling pathways. *Bio Impacts*, 7(3), 193–198. <https://doi.org/10.15171/bi.2017.22>.
 65. Aghazadeh, Z., Pouralibab, F., & Yari Khosroushahi, A. (2017). The prophylactic effect of *Acetobacter syzygii* probiotic species against squamous cell carcinoma. *Journal of Dentistry Research, Dental Clinics, Dental Prospects*, 11(4), 208–214. <https://doi.org/10.15171/joddd.2017.037>.
 66. Perera, M., Al-Hebshi, N. N., Speicher, D. J., Perera, I., & Johnson, N. W. (2016). Emerging role of bacteria in oral carcinogenesis: A review with special reference to perio-pathogenic bacteria. *Journal of Oral Microbiology*, 8, 32762. <https://doi.org/10.3402/jom.v8.32762>.
 67. Hayes, R. B., Ahn, J., Fan, X., Peters, B. A., Ma, Y., Yang, L., Agalliu, I., Burk, R. D., Ganly, I., Purdue, M. P., Freedman, N. D., Gapstur, S. M., & Pei, Z. (2018). Association of oral microbiome with risk for incident head and neck squamous cell cancer. *JAMA Oncology*, 4(3), 358–365. <https://doi.org/10.1001/jamaoncol.2017.4777>.
 68. Morita, E., Narikiyo, M., Yano, A., Nishimura, E., Igaki, H., Sasaki, H., Terada, M., Hanada, N., & Kawabe, R. (2003). Different frequencies of *Streptococcus anginosus* infection in oral cancer and esophageal cancer. *Cancer Science*, 94(6), 492–496. <https://doi.org/10.1111/j.1349-7006.2003.tb01471.x>.
 69. Wang, Y., Zhang, M., Fang, B., & Chen, L. (2016). Effects of *Lactobacillus salivarius* on oral cancer cell proliferation and apoptosis *In vitro*. *Carpathian Journal of Food Science & Technology*, 8(1).

70. Badehnoosh, B., Karamali, M., Zarrati, M., Jamilian, M., Bahmani, F., Tajabadi-Ebrahimi, M., Jafari, P., Rahmani, E., & Asemi, Z. (2018). The effects of probiotic supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes. *Journal of Maternal-Fetal & Neonatal Medicine*, 31(9), 1128–1136. <https://doi.org/10.1080/14767058.2017.1310193>.
71. Jahanshahi, M., Maleki Dana, P., Badehnoosh, B., Asemi, Z., Hallajzadeh, J., Mansournia, M. A., Yousefi, B., Moazzami, B., & Chaichian, S. (2020). Anti-tumor activities of probiotics in cervical cancer. *Journal of Ovarian Research*, 13(1), 68. <https://doi.org/10.1186/s13048-020-00668-x>.
72. Wang, K. D., Xu, D. J., Wang, B. Y., Yan, D. H., Lv, Z., & Su, J. R. (2018). Inhibitory effect of vaginal *Lactobacillus* supernatants on cervical cancer cells. *Probiotics and Antimicrobial Proteins*, 10(2), 236–242. <https://doi.org/10.1007/s12602-017-9339-x>.
73. Nami, Y., Abdullah, N., Haghsheenas, B., Radiah, D., Rosli, R., & Khosroushahi, A. Y. (2014). Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium *Lactobacillus plantarum* 5BL. *Microbiology and Immunology*, 58(9), 492–502. <https://doi.org/10.1111/1348-0421.12175>.
74. Riaz Rajoka, M. S., Zhao, H., Lu, Y., Lian, Z., Li, N., Hussain, N., Shao, D., Jin, M., Li, Q., & Shi, J. (2018). Anticancer potential against cervix cancer (HeLa) cell line of probiotic *Lactobacillus casei* and *Lactobacillus paracasei* strains isolated from human breast milk. *Food Function*, 9(5), 2705–2715. <https://doi.org/10.1039/c8fo00547h>.
75. Sungur, T., Aslim, B., Karaaslan, C., & Aktas, B. (2017). Impact of exopolysaccharides (EPSs) of *Lactobacillus gasseri* strains isolated from human vagina on cervical tumor cells (HeLa). *Anaerobe*, 47, 137–144. <https://doi.org/10.1016/j.anaerobe.2017.05.013>.
76. Nouri, Z., Karami, F., Neyazi, N., Modarressi, M. H., Karimi, R., Khorramzadeh, M. R., Taheri, B., & Motevaseli, E. (2016). Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. *Cell Journal*, 18(2), 127–134. <https://doi.org/10.22074/cellj.2016.4307>.
77. Chaib, M., Hafeez, B. B., Mandil, H., Daria, D., Pingili, A. K., Kumari, S., Sikander, M., Kashyap, V. K., Chen, G. Y., Anning, E., Tripathi, M. K., Khan, S., Behrman, S., Yallapu, M. M., Jaggi, M., Makowski, L., & Chauhan, S. C. (2022). Reprogramming of pancreatic adenocarcinoma immunosurveillance by a microbial probiotic siderophore. *Communications Biology*, 5(1), 1181. <https://doi.org/10.1038/s42003-022-04102-4>.
78. Chang, C. J., Lin, T. L., Tsai, Y. L., Wu, T. R., Lai, W. F., Lu, C. C., & Lai, H. C. (2019). Next generation probiotics in disease amelioration. *Journal of Food and Drug Analysis*, 27(3), 615–622. <https://doi.org/10.1016/j.jfda.2018.12.011>.
79. Liu, M. N., Zhang, L., Dong, X. Y., Liu, M., Cheng, G., Zhang, X. L., He, F., & Wang, G. Q. (2020). Effects of *Akkermansia muciniphila* on the proliferation, apoptosis and insulin secretion of rat islet cell tumor cells. *Sichuan Da Xue Xue Bao Yi Xue Ban*, 51(1), 13–17.
80. Liu, Y., Wang, J., & Wu, C. (2022). Modulation of gut microbiota and immune system by probiotics, prebiotics, and postbiotics. *Frontiers in Nutrition*, 8, 1155.
81. Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M., & Margolles, A. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research*, 61(1), 1600240.
82. Eslami, M., Yousefi, B., Kokhaei, P., Hemati, M., Nejad, Z. R., Arabkari, V., & Namdar, A. (2019). Importance of probiotics in the prevention and treatment of colorectal cancer. *Journal of Cellular Physiology*, 234(10), 17127–17143.

83. Aghebati-Maleki, L., Hasannezhad, P., Abbasi, A., & Khani, N. (2021). Antibacterial, antiviral, antioxidant, and anticancer activities of postbiotics: A review of mechanisms and therapeutic perspectives. *Biointerface Research in Applied Chemistry*, 12, 2629–2645.
84. Osadchiy, V., Martin, C. R., & Mayer, E. A. (2019). The gut–brain axis and the microbiome: Mechanisms and clinical implications. *Clinical Gastroenterology and Hepatology*, 17(2), 322–332.
85. Masanta, W. O., Heimesaat, M. M., Bereswill, S., Tareen, A. M., Lugert, R., Groß, U., & Zautner, A. E. (2013). Modification of intestinal microbiota and its consequences for innate immune response in the pathogenesis of campylobacteriosis. *Clinical and Developmental Immunology*, 2013, Article ID 109170.
86. Zeng, H., Lazarova, D. L., & Bordonaro, M. (2014). Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. *World Journal of Gastrointestinal Oncology*, 6(2), 41–51.
87. Palla, M., Conte, G., Grassi, A., Esin, S., Serra, A., Mele, M., Giovannetti, M., & Agnolucci, M. (2021). Novel yeasts producing high levels of conjugated linoleic acid and organic acids in fermented doughs. *Foods*, 10(9), 2087.
88. Park, Y., Albright, K. J., Cai, Z. Y., & Pariza, M. W. (2001). Comparison of methylation procedures for conjugated linoleic acid and artifact formation by commercial (trimethylsilyl) diazomethane. *Journal of Agricultural and Food Chemistry*, 49(3), 1158–1164.
89. Field, C. J., & Schley, P. D. (2004). Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: Lessons from n–3 fatty acids. *The American Journal of Clinical Nutrition*, 79(6), 1190S–1198S.
90. O'Shea, E. F., Cotter, P. D., Stanton, C., Ross, R. P., & Hill, C. (2012). Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: Bacteriocins and conjugated linoleic acid. *International Journal of Food Microbiology*, 152(3), 189–205.
91. Yang, R., Shan, S., Zhang, C., Shi, J., Li, H., & Li, Z. (2020). Inhibitory effects of bound polyphenol from foxtail millet bran on colitis-associated carcinogenesis by the restoration of gut microbiota in a mice model. *Journal of Agricultural and Food Chemistry*, 68(11), 3506–3517.
92. Tripathy, A., Dash, J., Kancharla, S., Kolli, P., Mahajan, D., Senapati, S., & Jena, M. K. (2021). Probiotics: A promising candidate for management of colorectal cancer. *Cancers*, 13(13), 3178.
93. Yousefi, M., Shariatifar, N., Tajabadi Ebrahimi, M., Mortazavian, A. M., Mohammadi, A., Khorshidian, N., Arab, M., & Hosseini, H. (2019). *In vitro* removal of polycyclic aromatic hydrocarbons by lactic acid bacteria. *Journal of Applied Microbiology*, 126(3), 954–964.
94. Motevaseli, E., Shirzad, M., Akrami, S. M., et al. (2019). The probiotic mixture VSL#3 has anti-cancer effects on genetically predisposed colitis-associated colorectal cancer in mice. *Iranian Journal of Basic Medical Sciences*, 22(10), 1188–1195.
95. Kim, Y., Kim, H., Kim, S. W., et al. (2007). Protective effect of *Lactobacillus acidophilus* LA1 on N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis in rats. *Letters in Applied Microbiology*, 44(4), 351–356.

96. Cai, H., Rodríguez, B. T., Zhang, W., et al. (2019). Differential effects of probiotics on the composition of the gut microbiota in colon cancer patients. *World Journal of Gastroenterology*, 25(38), 5846–5858.
97. Petrof, E. O., Dhaliwal, R., Manzanares, W., Johnstone, J., Cook, D., & Heyland, D. K. (2012). Probiotics in the critically ill: A systematic review of the randomized trial evidence. *Critical Care Medicine*, 40(12), 3290–3302.
98. Salminen, M. K., Rautelin, H., Tynkkynen, S., et al. (2002). Lactobacillus bacteremia during a rapid increase in probiotic use of *Lactobacillus rhamnosus* GG in Finland. *Clinical Infectious Diseases*, 35(10), 1155–1160.
99. Singh, R., Chandrashekarappa, S., Bodduluri, S. R., et al. (2019). Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. *Nature Communications*, 10(1), 89.
100. Nami, Y., Abdullah, N., Haghshenas, B., Radiah, D., Rosli, R., & Khosroushahi, A. Y. (2014). Probiotic assessment of *Enterococcus durans* 6HL and *Lactococcus lactis* 2HL isolated from vaginal microflora. *Journal of Medical Microbiology*, 63(Pt 8), 1044–1051.
101. Hevia, A., Delgado, S., Sánchez, B., et al. (2014). Probiotic administration attenuates hepatocellular damage in a rat model of nonalcoholic fatty liver disease. *The Journal of Nutrition*, 144(4), 574–580.
102. Khaledi, S., Sun, J., Buys, N., & Jayasinghe, R. (2014). Effect of probiotics on blood pressure: A systematic review and meta-analysis of randomized, controlled trials. *Hypertension*, 64(4), 897–903.
103. Raza, S. Q., Khan, J. A., Faisal, S. M., et al. (2017). Anti-cancer and anti-angiogenic properties of various lactic acid bacteria isolated from dairy products. *Microbial Pathogenesis*, 107, 411–420.
104. Shi, L., Sheng, J., Wang, M., Luo, H., Zhu, J., Zhang, B., Liu, Z., & Yang, X. (2019). Combination therapy of TGF- β blockade and commensal-derived probiotics provides enhanced antitumor immune response and tumor suppression. *Theranostics*, 9(14), 4115–4128.
105. Guo, Y., Zhang, T., Gao, J., Jiang, X., Tao, M., Zeng, X., Wu, Z., & Pan, D. (2020). *Lactobacillus acidophilus* CICC 6074 inhibits growth and induces apoptosis in colorectal cancer cells *In vitro* and in HT-29 cells induced-mouse model. *Journal of Functional Foods*, 75, 104290.
106. Razak, N. A., Abu, N., Ho, W. Y., Zambari, N. R., Tan, S. W., Alitheen, N. B., Long, K., & Yeap, S. K. (2019). Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells via cell cycle arrest, anti-angiogenesis and induction of apoptosis. *Scientific Reports*, 9(1), 1, 2.
107. Karimi Ardestani, S., Tafvizi, F., & Tajabadi Ebrahimi, M. (2019). Heat-killed probiotic bacteria induce apoptosis of HT-29 human colon adenocarcinoma cell line via the regulation of Bax/Bcl2 and caspases pathway. *Human & Experimental Toxicology*, 38(9), 1069–1081.
108. Li, F., Jiao, X., Zhao, J., Liao, X., Wei, Y., & Li, Q. (2022). Antitumor mechanisms of an exopolysaccharide from *Lactobacillus fermentum* on HT-29 cells and HT-29 tumor-bearing mice. *International Journal of Biological Macromolecules*, 209, 552–562.
109. Dallal, M. M., Mojarad, M., Baghbani, F., Raoofian, R., Mardaneh, J., & Salehipour, Z. (2015). Effects of probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* on colorectal tumor cells activity (CaCo-2). *Archives of Iranian Medicine*, 18(3), 0–0.

110. Wan, Y., Xin, Y., Zhang, C., Wu, D., Ding, D., Tang, L., Owusu, L., Bai, J., & Li, W. (2014). Fermentation supernatants of *Lactobacillus delbrueckii* inhibit growth of human colon cancer cells and induce apoptosis through a caspase 3 dependent pathway. *Oncology Letters*, 7(5), 1738–1742.
111. Coakley, M., Johnson, M. C., McGrath, E., Rahman, S., Ross, R. P., Fitzgerald, G. F., Devery, R., & Stanton, C. (2006). Intestinal bifidobacteria that produce trans-9, trans-11 conjugated linoleic acid: A fatty acid with antiproliferative activity against human colon SW480 and HT-29 cancer cells. *Nutrition and Cancer*, 56(1), 95–102.
112. Zaharuddin, L., Mokhtar, N. M., Muhammad Nawawi, K. N., & Raja Ali, R. A. (2019). A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterology*, 19(1), 1–8.
113. Saber, A., Alipour, B., Faghfoori, Z., & Khosroushahi, A. Y. (2017). Secretion metabolites of probiotic yeast, *Pichia kudriavzevii* AS-12, induces apoptosis pathways in human colorectal cancer cell lines. *Nutrition Research*, 41, 36–46.
114. Mack, D. R., Michail, S., Wei, S., McDougall, L., & Hollingsworth, M. A. (1999). Probiotics inhibit enteropathogenic *E. coli* adherence *In vitro* by inducing intestinal mucin gene expression. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 276(4), G941–G950.

CHAPTER 5

Role of Prebiotics and Probiotics in the Protection Against Cervical Cancers

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ABSTRACT

Worldwide, cervical cancer is regarded as a significant cancer that affects women. According to reports, patients who receive current cervical cancer treatments experience cytotoxicity. Additionally, these treatments have manifested unfavorable side effects that may shorten women's lives with this illness. Therefore, fewer hazardous anti-tumor medicines are required than conventional therapeutic treatments. Furthermore, cervical cancer patients' quality of life may be improved by using drugs that prevent or lessen the negative effects of their treatments. Numerous health advantages of probiotic supplements, frequently including live bacteria, have been well-researched in studies. Major research areas have included the impact of probiotic supplements on chemotherapy toxicity and effectiveness, as well as cancer prevention. The

usefulness of probiotics used to treat cervical cancer has recently attracted attention among various studies. Including prebiotics, indigestible compounds broken down by gut microbiota, can increase the effectiveness of probiotics on human health. Prebiotics are fermentable and nondigestible carbohydrates that preferentially affect the development of probiotic organisms, which inhibit malignant cell development through immunomodulation. By attracting natural killer cells and cytotoxic T cells, and triggering apoptosis through oxidative stress in the tumor microenvironment, bacteria are able to suppress tumor growth. This book chapter reviews the microorganisms utilized as probiotics, either alone or in conjunction with prebiotics or medications, to lessen the unintended consequences of cervical cancer therapy.

5.1 INTRODUCTION

Cervical cancer is a crucial concern for women's health globally, with an elevated rate of incidence and mortality. The disease often goes undetected until it has reached an advanced stage, making prevention an important strategy in reducing cervical cancer's burden [1]. The key instigator of cervical cancer is a persistent infection with HPV, which can lead to alterations in cervical cells that may advance to cancer over time [1]. The availability of vaccines targeting these viruses has significantly contributed to reducing the incidence of this disease, but their effectiveness can be influenced by the individual's immune response [1].

In the current era, prebiotics and probiotics have emerged as potential tools for preventing cervical cancer [2]. Probiotics refer to living microorganisms that, when ingested in adequate amounts, provide advantages for the host's health. These microorganisms have the ability to regulate the gut microbiome, leading to favorable effects on the immune system. On the other hand, prebiotics are indigestible components present in food that promote the growth of beneficial bacteria in the intestinal tract. These two elements together are known as synbiotics and have been shown to offer superior health benefits compared to probiotics or prebiotics alone [1, 2].

According to the studies, the usage of synbiotics can help in the prevention of cervical cancer. They can modulate the gut microbiome, which subsequently influences the immune system's response to HPV infection [3]. The microbiome shows a significant role in preventing the progress of pathogens (including viruses) and sustaining a healthy immune system. Research has shown that alterations to the microbiome can impact the threat of the development of cervical cancer [4–6]. One study examined the potential usage of

synbiotics in the prevention of the development of cervical cancer along with HPV infection in women.

The research found that the use of synbiotics resulted in a reduction in HPV viral load and an improvement in immune system function [3]. An additional study revealed that synbiotic use improved the effectiveness of HPV vaccines in preventing cervical cancer by modulating the gut microbiome [7]. The potential of synbiotics in preventing cervical cancer highlights the essentiality of maintaining a healthy gut microbiome. The gut microbiome plays a key role in the immune system of the human body, and microbiome alterations might have various side effects on health outcomes. The use of synbiotics is a promising strategy for enhancing immune system function and decreasing the possibility of cervical cancer development [4, 5, 7].

However, improved research will be helpful in determining the proper dosage and duration of synbiotic intake for cervical cancer prevention. Additionally, further analysis is required to scrutinize the potential usage of synbiotics in combination with other preventive approaches, such as ordered screening for cervical cancer and vaccination for HPV. Nevertheless, the potential benefits of synbiotics in preventing cervical cancer make it a promising area of research for the future [8].

5.2 OVERVIEW OF CERVICAL CANCER AND ITS RISK FACTORS

Since cervical cancer ranks as the fourth most commonly occurring cancer globally, it is considered a global health issue. Worldwide, cervical cancer accounts for 4% of all cancer diagnoses. Because there are no programs to vaccinate against HPV and screen for cancer, the incidence is higher in non-developing nations [9]. Three main subtypes of cervical cancer have been identified based on their characteristics: adenocarcinoma, squamous cell carcinoma, and adenosquamous carcinoma. Nearly 25% and 70% of cervical cancers are caused by adenocarcinoma and squamous cell carcinoma, respectively. Compared to healthy cells, cancer cells typically exhibit several abnormal characteristics [10]. These characteristics indicate malignancies at the cellular level: immortalization, loss of anchorage dependence, decreased density-dependent inhibition of proliferation, loss of contact inhibition, invasiveness, and metastasis. Over time, cervical cancer develops slowly. Dysplasia is a process by which the cells change in appearance in the cervix. Long-term HPV infection of the cervix can cause cancer or lead to the formation of large tumors. A malignant tumor has the potential to spread throughout the body. Some findings show that a cell's initial changes

are abnormal and not cancerous; they are sometimes called “atypical cells.” While some atypical cells are harmless and go away without treatment, others can cause cancer. Certain findings exhibit these unusual changes at the beginning of gradual alterations that may lead to malignant growth. This phase of precancerous illness is called ‘cervical dysplasia.’ There are two parts to the cervix: the endocervix and the ectocervix. The primary development site of cervical cancer is the transformation zone, the junction point between these two areas. It is vital to be aware of the risk factors for cervical cancer because doing so helps those who have started exhibiting these symptoms to undergo routine screening procedures to identify the condition as early as possible. Several risk factors could increase the likelihood of cervical cancer [11]. One of the chief reasons for increasing the risk of cervical cancer is HPV. These viruses can affect skin surface cells and the lining of the genitals but not blood or internal organs. There are 150 HPV-related viruses linked to cervical cancer. Other factors, such as chlamydia infection, a compromised immune system, smoking, and young-age pregnancy, raise the risk of developing cervical cancer [12]. Refer to Figure 5.1.

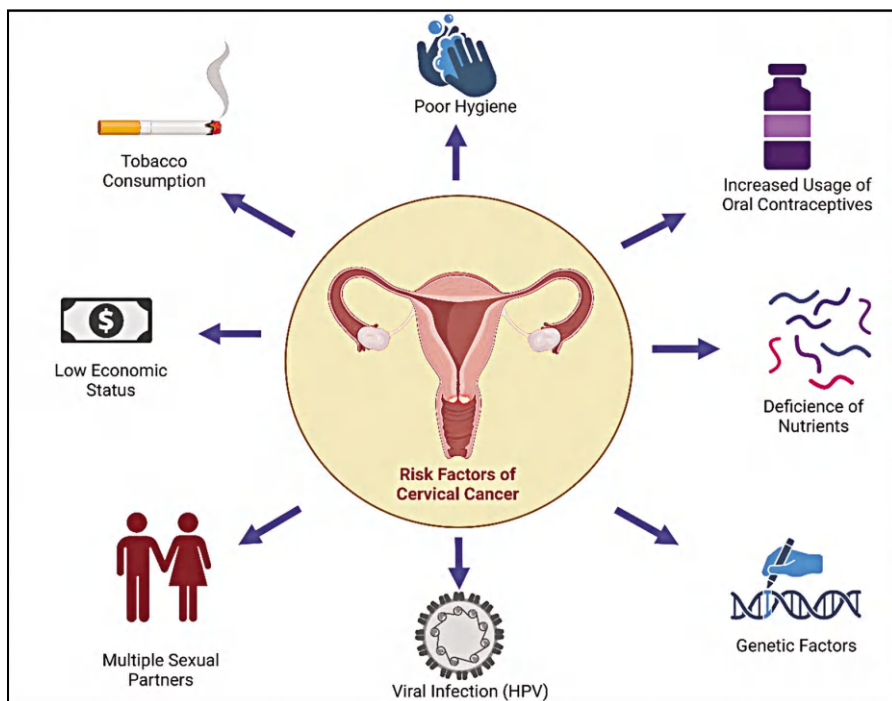


FIGURE 5.1 Risk factors of cervical cancer. (The diagram clearly showing the different causes that are responsible for the onset of cervical cancer) (Created in BioRender.com).

5.3 THE ASSOCIATION BETWEEN DYSBIOSIS AND CERVICAL CANCER

The relationship between human microbiota and human health has been extensively studied for the past two decades [13]. The human microbiota comprises the complete collection of microorganisms, such as bacteria, viruses, fungi, and archaea, that inhabit or exist within the human body [14]. These microbes underlie many human physiological processes, like nutrient absorption, regulation of the immune and endocrine systems, and defense against pathogen attacks [15]. These organisms exist in a careful balance inside the human body; however, when this balance is disturbed, it causes dysbiosis. For instance, a portion of the human microbiota, such as the gut flora, vaginal flora, or even the skin flora, can fall out of balance, resulting in the decrease of normally dominant species and the increase of normally outcompeted or contained species in their place [16]. This imbalance in the microbiota can influence the outcome of a number of inflammatory pathways and may cause systemic chronic inflammation [17]. Recent findings also suggest that microbial dysbiosis plays a significant role in cancer initiation and progression.

Cervical cancer is one such malignancy that has been found to be linked with dysbiosis. The gut microbiome includes viruses and other microbes, which can influence cancer risks [13]. For example, infection with HPVs has been found to be a major cause of cervical cancer in women worldwide. HPVs have developed various methods to escape the host's adaptive response, secreting fewer proteins and modifying the antigen-processing machinery [18, 19]. The vaginal microbiota possesses the metabolic and enzymatic machinery needed to clear off these infections and preserve the integrity of the cervical epithelium. However, dysbiosis of the vaginal microbiota makes the invasion of sexually transmitted pathogens like HPVs easier, as the vaginal dysbiosis-associated bacteria frequently produce mucin-degrading enzymes and elicit a pro-inflammatory response [20], with weakening of the mucosal barrier (21), thus increasing the chances of cervical cancer. Studies have shown that viral antigens E6 and E7 are the major contributors to malignancy in HPV-induced cervical cancer [22]. However, estrogen and estrogen receptor α (ER α) are required for this development [23], as estrogen increases the transcription of viral antigens E6 and E7, promoting cervical carcinogenesis. Estrogen levels in women are affected by intestinal microbes [24]; thus, dysbiosis of the intestinal microbiota essentially contributes to cervical cancer development from HPV infection. These findings suggest that a link between dysbiosis and cervical cancer exists. However, the effects

of dysbiosis may vary from person to person, depending on the nature of the dysbiotic community and the host's underlying genetic predisposition to the disease. Hence, further studies to understand the dysbiotic signature of carcinogenesis are necessary.

5.4 THE POTENTIAL OF PREBIOTICS AND PROBIOTICS IN PREVENTING CERVICAL CANCER

Probiotics are nourishments or supplements that contain live microorganisms to maintain the balance of “good” microscopic organisms within the body. In contrast, prebiotics are high-fiber foods that feed the human microbiome. It has been discovered that some probiotic strains, such as *Bifidobacterium* and *Lactobacillus*, commonly found in fermented food products, have various therapeutic effects [25]. Maintaining the reproductive health of women and reducing gynecological diseases, as well as enhancing immunity, play a fundamental role in the use of probiotics and prebiotics. Together, probiotics and prebiotics have been demonstrated to have various beneficial properties in the immunological process, such as enhancing antibody response and reducing the proliferation of mononuclear cells. These characteristics are potentially used as innovative therapy and an alternative to more invasive chemotherapy treatments [26].

If a person is infected with HPV, the microecological balance is destroyed, and fewer *Lactobacillus* strains exist. This enhances the upregulation of HPV protein expression [27]. Two HPV-encoded genes, E6 and E7 [28], promote P53 expression, which is necessary for cancer development. HPV oncogenes (E6 & E7) can be efficiently suppressed by *Lactobacillus supernatants*. One of the main approaches for treating cervical cancer is radiotherapy; however, this treatment has a number of side effects that add to the burden on patients. Probiotics may alleviate certain issues that cervical cancer patients experience due to the disease itself or while undergoing treatment, as well as mitigate the negative effects of radiotherapy. Bacteria can be utilized as promising diagnostic tools by sensing a specific chemical in the human body and producing a signal. It is an invasive method where probiotics can be programmed to detect metastases and transfer gene circuits to unhealthy tissue microenvironments. Probiotics inhibited the growth of tumors when combined with anti-infective medications. These combination therapies improve cervical cancer diagnosis [29]. The high risk of HPV infection can be efficiently prevented with the HPV vaccine; however, treatment outcomes

for cervical cancer cannot be improved. It has been demonstrated in several preclinical studies that using probiotics is generally safe and has the capacity to deliver recombinant antigens. It can elicit cellular and humoral immunity in the host and destroy the HPV virus.

5.4.1 PROBIOTICS IN CERVICAL CANCER PREVENTION AND THEIR CLINICAL EVIDENCE

Regarding cancer-related deaths among females, cervical cancer ranks third among the most common causes, with about 80% of cases arising from developing or less developed countries [30, 31]. Adenocarcinoma and squamous cell carcinoma are the two main types of cervical cancer, with the former developing from the ectocervix and the latter from the glandular cells of the endocervix. Probiotic sources containing useful bacteria, such as fermented dairy products or vegetables, can be traced back to our traditional food. In this chapter, we will look into the potential roles of probiotics in preventing cervical cancer, the current research evidence, and future prospects [32]. Probiotics are living microorganisms that impart health benefits to their hosts when given in adequate quantities [33]. This definition was adopted by the WHO and the United Nations Food and Agriculture Organization in 2002 and has been widely accepted by the scientific community. Probiotics are known to improve the host body by balancing the microflora of the intestine [34]. They help strengthen the epithelial layer of the intestine, thus preventing the entry of pathogens into the host [35]. They also have anti-proliferative and anti-mutagenic effects against tumor cells [36].

5.4.1.1 CLASSES OF PROBIOTICS

Bacillus species, *Bifidobacterium*, *Clostridiales*, *Bacteroides*, *Escherichia coli*, *Enterococcus*, *Lactobacillus*, and *Lactococcus* are prominent lactic acid bacteria that make up probiotics [37, 38]. Table 5.1 summarizes effective probiotics. These probiotics utilize non-digestible substrates like barley, carbohydrates, oats, soybeans, and wheat that selectively stimulate their growth and activity in the gut. These are known as prebiotics, and they enhance the host's health by helping gut microbes flourish [39]. Often, a combination of probiotics and prebiotics, known as synbiotics, works synergistically to provide health benefits to the host. Synbiotics enhance the

survival and growth of probiotic bacteria in the gut, leading to improved gut health and overall well-being [40].

TABLE 5.1 Lists of Microorganisms Used as Probiotics (Adapted with permission from Ref. [41])

Group of Microorganisms	Examples
<i>Lactobacillus</i> sp.	<i>L. acidophilus</i> , <i>L. amylovorus</i> , <i>L. brevis</i> , <i>L. bulgaricus</i> , <i>L. casei</i> , <i>L. crispatus</i> , <i>L. cromoris</i> , <i>L. delbrueckii</i> , <i>L. fermentum</i> , <i>L. gasseri</i> , <i>L. helveticus</i> , <i>L. iners</i> , <i>L. jensenii</i> , <i>L. johnsonii</i> , <i>L. kefiranoferiens</i> , <i>L. kefir</i> , <i>L. lactis</i> , <i>L. mucosae</i> , <i>L. paracasei</i> , <i>L. pentosus</i> , <i>L. plantarum</i> , <i>L. racemosus</i> , <i>L. reuteri</i> , <i>L. rhamnosus</i> , <i>L. salivarius</i>
<i>Bifidobacterium</i> sp.	<i>B. adolescentis</i> , <i>B. animalis</i> , <i>B. bifidum</i> , <i>B. breve</i> , <i>B. infantis</i> , <i>B. lactis</i> , <i>B. longum</i> , <i>B. thermophilum</i>
Other bacteria	<i>Bacillus clausii</i> , <i>B. coagulans</i> , <i>B. cereus</i> , <i>B. licheniformis</i> , <i>Clostridium butyricum</i> , <i>Enterococcus faecium</i> SF68, <i>faecalis</i> , <i>Escherichia coli</i> Nissle 1917, <i>Lactococcus lactis</i> , <i>Leuconostoc cremoris</i> , <i>Pediococcus acidilactici</i> , <i>P. pentosaceus</i> , <i>Propionibacterium freudenreichii</i> , <i>Streptococcus lactis</i> , <i>S. thermophilus</i> <i>Candida</i>
Yeasts	<i>Candida kefir</i> , <i>C. sphaerica</i> , <i>Kluyveromyces marxianus</i> , <i>Pichia kudriavzevii</i> , <i>Saccharomyces cerevisiae</i> , <i>S. fragilis</i>

5.4.1.2 PROBIOTIC SOURCES AND INTAKE RECOMMENDATIONS

There are many natural sources of probiotics, including fermented foods and specific supplements. Fermented dairy products such as buttermilk, cheese, kefir, and yogurt are excellent sources of probiotics. They contain *Lactobacillus* and *Bifidobacterium*, which help to improve gut health [42]. Fermented vegetables such as kimchi, sauerkraut, and pickles are also good sources of probiotics that improve digestion and boost the immune system [43–45]. In addition, fermented soy products like miso, tempeh, tofu, and natto, fermented foods like sourdough bread, fermented meat products like salami, and probiotic supplements are rich sources of beneficial bacteria that are believed to have probiotic effects [46–48]. The optimal dose of probiotics can vary depending on the individual, the specific condition being treated, and the strain of probiotics used. Factors such as an incorrect diet, alcohol consumption, and aging are also essential to consider [49, 50]. Therefore, probiotics should be taken as part of a regular diet.

5.4.1.3 MECHANISMS BY WHICH PROBIOTICS MAY PREVENT CERVICAL CANCER

Various bioactive compounds, metabolites, and nutrients produced by probiotics contribute to cancer treatment. One of the ways lactic acid bacteria tackle cervical cancer is by activating apoptosis signaling pathways through both intrinsic and extrinsic mechanisms [49]. Lactic acid bacteria promote apoptosis by activating Bax and Bak proteins of the B-cell lymphoma 2 (Bcl-2) family, leading to the permeabilization of the mitochondrial outer membrane (MOMP). As a result, cytochrome c is released, which activates caspases, resulting in cell death. In addition, they induce Beclin-1 and GRP78 proteins to promote apoptosis [6]. They also activate death ligands on the cell surface, such as FasL (Fas ligand) or TNF- α (tumor necrosis factor), to their respective receptors, TNFR1 and Fas. This binding triggers apoptosis through caspase activity by forming a death-inducing signaling complex (DISC) [51]. Several cell proliferation genes are expressed when nuclear factor-kappaB (NF- κ B) is activated. These include Bcl-xL, Bcl-2, cyclin D1, and Cox-2, whose overexpression is associated with cervical cancer progression. Lactic acid bacteria have been shown to downregulate NF- κ B-dependent gene products [52]. The occurrence of diarrhea is a significant side effect for people undergoing chemotherapy and radiotherapy [53]. It is suggested that in patients with cervical cancer, probiotics may reduce radiotherapy-induced diarrhea (RID) [54].

Cervical smears with mildly abnormal and unsatisfactory results have been shown to be reduced by the strains of probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 [55]. In a study, it was found that probiotics can help clear cervical lesions related to HPV for up to 6 months [56]. CaSki cells express HPV genes and produce high levels of the oncoproteins E7 and E6, which are known to play a role in the progression of cervical cancer. Overexpression of cyclin-dependent kinase 2 (CDK2) and cyclin A has been linked to various types of cancer, including cervical cancer. *Lactobacillus crispatus*, *Lactobacillus gasseri*, and *Lactobacillus jensenii* supernatants have been shown to reduce the expression of HPV E6 and E7 oncogenes, cyclin A, and CDK2 [57, 6]. *Lactobacillus gasseri* and *Lactobacillus plantarum* isolates from vaginal secretions of young adults and adolescents have been found to have probiotic properties against HeLa cell lines by reducing MMP9, MMP2, and CASP3 expression [58–60]. It was found that *Bifidobacterium adolescentis* strain SPM1005-A inhibited the expression of E7 and E6 oncogenes in SiHa cervical cancer cell line expressing HPV type 16 [61]. Radiation-induced diarrhea was reduced in cervical cancer patients

when *Lactobacillus acidophilus* was combined with *Bifidobacterium bifidum* [62, 63]. A diagrammatic representation is given in Figure 5.2.

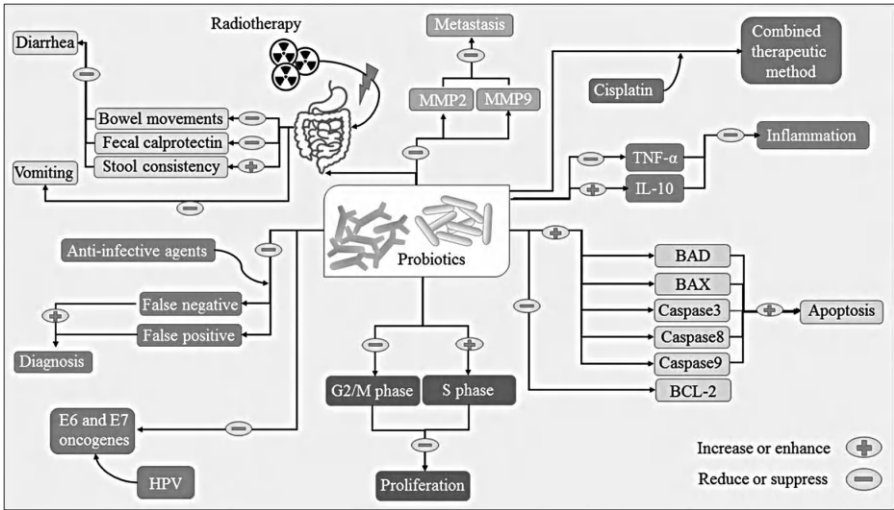


FIGURE 5.2 Representation of how probiotics are involved in the prevention, diagnosis, and treatment of cervical cancer.

Source: Adapted with permission from Ref. [1]

5.4.2 PREBIOTICS IN CERVICAL CANCER PREVENTION AND THEIR CLINICAL EVIDENCE

Prebiotics are food components that are not easily digestible by the human body but serve as a source of nutrients for the beneficial bacteria residing in the gut. They promote the growth and activity of these bacteria, improving overall health. Prebiotics can be found in various plant-based foods such as vegetables, fruits, and whole grains. They are known to enhance the functioning of the digestive system, strengthen the immune system, and stimulate the development of useful bacteria, including *Bifidobacterium* and *Lactobacillus* [64].

Cervical cancer is a significant global health issue, with over 500,000 new cases and 300,000 deaths reported annually. Most cases of cervical cancer are caused by HPV, a sexually transmitted infection that can lead to abnormal changes in cervical cells. While there are effective prevention strategies for cervical cancer, such as HPV vaccination and regular screening, there is increasing evidence to suggest that prebiotics may also play a role in cervical cancer prevention [65].

5.4.2.1 PREBIOTICS AND THE MICROBIOME

The human body is home to trillions of microorganisms, including viruses, bacteria, and fungi, collectively known as the microbiome. The microbiome plays a crucial role in maintaining human health, including immune system function and metabolism. The gut microbiome, in particular, has been found to influence various aspects of human health, including mental health, obesity, and cancer risk.

Prebiotics support the activity and growth of beneficial gut bacteria, such as *Bifidobacterium* and *Lactobacillus*. These bacteria produce short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate, which have been shown to have anti-inflammatory and anti-cancer properties. Research has demonstrated that SCFAs promote cancer cell death and inhibit cancer cell growth *in vitro* [66].

5.4.2.2 PREBIOTICS AND CERVICAL CANCER RISK

The consumption of prebiotics has been found to reduce the risk of cervical cancer through several mechanisms. One important way prebiotics may prevent cervical cancer is by promoting the growth of beneficial gut bacteria. These bacteria produce short-chain fatty acids (SCFAs) with anti-cancer and anti-inflammatory properties. SCFAs have been shown to promote cancer cell death and inhibit cancer cell growth *in vitro* [67].

Prebiotics may also help reduce cervical cancer risk by improving the barrier function of the gut. This barrier serves as a biochemical and physical separation between the gut's contents and the bloodstream. A healthy gut barrier helps prevent harmful bacteria and toxins from entering the bloodstream, which can trigger inflammation and promote cancer development. Prebiotics have been shown to enhance the barrier function of the gut by stimulating the growth of beneficial bacteria and by increasing mucus production, which helps protect the gut lining [68].

In addition to improving gut barrier function and stimulating the growth of beneficial bacteria, prebiotics may also help reduce inflammation to decrease the risk of cervical cancer. Persistent inflammation is a key factor in developing many types of cancer, including cervical cancer. Prebiotics have been shown to reduce gut inflammation and systemic inflammation by promoting the production of anti-inflammatory cytokines and modulating the immune system [65]. Multiple research studies have explored the connection between cervical cancer risk and prebiotics. A study published in the *Journal of Nutrition* revealed that women who consumed high levels of dietary fiber,

a type of prebiotic, had a significantly lower risk of developing cervical cancer compared to those with lower dietary fiber intake. Similarly, another research paper in the *International Journal of Cancer* found that women with high dietary fiber intake had a significantly reduced risk of developing cervical intraepithelial neoplasia, a precursor to cervical cancer [33].

Other prebiotics have also demonstrated anti-cancer properties. For example, a study published in the *Journal of Functional Foods* found that inulin, a type of prebiotic found in chicory root, reduced the growth of cervical cancer cells in laboratory experiments. Another study published in the *Journal of Nutritional Biochemistry* showed that oligofructose, a prebiotic found in many fruits and vegetables, inhibited the growth of cervical cancer cells in both *in vitro* and animal studies.

5.4.2.3 PREBIOTICS AND HPV INFECTION

Infection by HPV is the primary cause of cervical cancer, and several research groups have investigated the role of prebiotics in preventing HPV infection. According to a study published in the *Journal of Clinical Gastroenterology*, researchers found that women who consumed a prebiotic supplement containing inulin and oligofructose significantly reduced HPV infection compared to women who did not receive the supplement [69]. Similarly, another study published in the *Journal of Applied Microbiology* found that *Lactobacillus crispatus*, a beneficial bacteria promoted by prebiotics, reduced the adhesion of HPV to cervical cells *in vitro* [70].

While the evidence linking prebiotics to cervical cancer prevention is promising, further investigation is required to fully understand the mechanisms by which prebiotics exert their anti-cancer effects and to determine the ideal dosage and duration of prebiotic supplementation for cervical cancer prevention [71]. It is important to note that prebiotics should not be used as a substitute for other established cervical cancer prevention strategies, such as regular screening and HPV vaccination. However, incorporating prebiotic-rich foods into the diet, such as fruits, vegetables, and whole grains, may offer additional benefits for cervical cancer prevention and overall health [72].

5.4.3 SYNBIOTICS IN CERVICAL CANCER PREVENTION AND CLINICAL EVIDENCE

Cervical cancer is a significant public health concern among women worldwide. It is the second most common cancer in developing countries and the

fourth most common globally, following breast cancer. One approach to reducing cervical cancer cases is through the use of synbiotics [1].

Synbiotics are a combination of probiotics and prebiotics that work together to enhance the growth and activity of beneficial bacteria in the gut. Probiotics are living microorganisms that provide health benefits when consumed in appropriate quantities, while prebiotics are non-digestible carbohydrates that promote the growth of gut probiotics [5, 6].

Synbiotics are available in various forms such as capsules, tablets, powders, and functional foods. Common sources of synbiotics include dairy products like kefir, yogurt, and cheese, as well as fermented vegetables like kimchi and sauerkraut. The recommended intake of synbiotics varies based on an individual's gender, age, and health status. However, a general recommendation is to consume at least 1 billion CFU (colony-forming units) of probiotics and 5–10 grams of prebiotics daily [4, 5, 7].

Several mechanisms have been proposed to explain how synbiotics may help prevent cervical cancer. First, synbiotics can boost the immune system by increasing the production of antibodies and cytokines, which are essential for fighting off infections. This is important because cervical cancer is mainly caused by persistent infection with HPV [4, 5, 8].

Second, synbiotics may reduce inflammation in the cervix by altering the gut microbiota. Inflammation has been identified as a common risk factor in cervical cancer cases, and studies have shown that women with HPV-related cervical lesions have higher levels of inflammatory cytokines in their cervical mucus. By modulating the gut microbiota, synbiotics can reduce inflammation and potentially prevent the development of cervical cancer [8].

Third, synbiotics can enhance the efficacy of HPV vaccines by improving the immune response to the vaccine. While HPV vaccines have been proven to be highly effective in preventing cervical cancer, their effectiveness is greatly influenced by the individual's immune response. Studies have shown that synbiotics can increase the production of specific antibodies in response to the HPV vaccine, thereby improving its effectiveness [8].

In conclusion, synbiotics offer a promising approach to preventing cervical cancer by enhancing the immune system and improving the efficacy of HPV vaccines. However, additional research is needed to determine the ideal quantity and duration of synbiotic consumption. It's important to note that synbiotics should not replace regular cervical cancer screening and HPV vaccination [4, 5, 7].

5.4.4 POTENTIAL CHALLENGES AND LIMITATIONS OF USING PREBIOTICS AND PROBIOTICS FOR CERVICAL CANCER PREVENTION AND FUTURE DIRECTIONS FOR RESEARCH AND PRACTICE

The usage of probiotics and prebiotics for the inhibition and treatment of several health conditions, including cervical cancer, has been a hot topic of discussion over the last decade. However, their use in this context is still early, and several challenges and limitations must be addressed. The first major problem is the lack of clinical evidence and the challenges associated with their production as therapeutics [73]. While prebiotics and probiotics have shown major benefits in gut health and preventing other diseases, their effectiveness in cervical cancer management is still inconclusive [74]. The safety of prebiotics and probiotics in cervical cancer patients has not yet been thoroughly demonstrated; hence, they should be used cautiously, as some probiotic strains may have negative interactions with chemotherapy and radiation therapy [75, 76]. Furthermore, the studies investigating the impacts of prebiotics and probiotics on cervical cancer have been small, of short duration, and focused on certain demographics, making the generalization of results to a broader population difficult [77]. Thus, additional research is required to determine their safety and efficacy.

Another major issue with using prebiotics and probiotics is that their sole application cannot cure any type of cancer; instead, they need to be taken in conjunction with other existing drugs [41]. Thus, to avert any adverse reactions, it is critical to consult with a medical professional before taking these supplements. In some people, prebiotics have been known to cause bloating, gas, distension, and other gastrointestinal symptoms. This may limit their use in individuals with sensitive digestive systems [50]. In rare cases, probiotics have been linked to infections, especially in people with compromised immune systems; thus, they should be used with caution since their safety is not fully demonstrated [78].

Variability in bacterial strains is also an issue that needs to be addressed. Diverse probiotic strains may have different effects on health, and the same strain may have distinct effects on different individuals. This makes it difficult to predict the effectiveness of the supplements. It is very important to have an extensive human database and analyze the correlation between various strains and clinical responses to detect specific combinations or strains that will be able to lessen the adverse side effects of cancer treatment and enhance treatment activity [75].

The prebiotics and probiotics have a limited shelf life and may lose potency during storage. Probiotics are live microorganisms that are sensitive to environmental conditions; thus, they must be delivered in sufficient quantities and in a way that ensures they reach their target site on the cervix. This may be difficult because, before they reach the cervix, stomach acids and bile may destroy the probiotics [79].

Finally, using probiotics and prebiotics in preventing and treating cervical cancer has drawn some ethical criticism from researchers. They have expressed worries about the possibility of false hope and the exploitation of vulnerable patients.

Overall, while probiotics and prebiotics show promise as potential preventive or therapeutic agents for cervical cancer, their use in this context is still at the initial stages, and further research is required to establish their safety and effectiveness. It is important to consult a doctor before using these supplements, especially if one has an underlying medical condition or is taking medications.

5.5 CONCLUSION

In conclusion, probiotics and prebiotics have successfully presented hopeful outcomes in protecting against cervical cancer. The microbiota of the gut also shows a prominent role in modifying the immune system, and disparities in the microbiota have been associated with the emergence and progression of various cancers, including cervical cancer. Both probiotics and prebiotics, along with their combination as synbiotics, can help restore a healthy balance of gut microbiota, leading to improved immune function and a decreased risk of cervical cancer. The studies discussed above have shown that the production of interferon-gamma is enhanced by probiotics. It is a cytokine that plays a pivotal role in the immune response to fight against HPV, the virus causing cervical cancer.

Furthermore, probiotics have been scientifically proven to moderate inflammation, a significant factor in the development of several cancers, including cervical cancer. Additionally, prebiotics can fuel the production of beneficial gut bacteria, improving the overall health of the gut microbiota. This can lead to a reduced risk of cervical cancer development as well as other diseases.

While more research is needed for a complete understanding of the role of prebiotics and probiotics in protecting against cervical cancer, the

evidence is promising. Incorporating prebiotics and probiotics into one's diet can have numerous health benefits, including a lowered risk of cervical cancer development.

KEYWORDS

- **Prebiotics**
- **Probiotics**
- **Cervical Cancers**
- **Chemotherapy**
- **Cancer Therapy**

REFERENCES

1. Jahanshahi, M., Maleki Dana, P., Badehnoosh, B., Asemi, Z., Hallajzadeh, J., Mansournia, M. A., Yousefi, B., Yousefi, B., Moazzami, B., & Chaichian, S. (2020). Anti-tumor activities of probiotics in cervical cancer. *Journal of Ovarian Research*, 13(1), 1–11. <https://doi.org/10.1186/S13048-020-00668-X>.
2. Górka, A., Przystupski, D., Niemczura, M. J., & Kulbacka, J. (2019). Probiotic bacteria: A promising tool in cancer prevention and therapy. *Current Microbiology*, 76(8), 939–949. <https://doi.org/10.1007/s00284-019-01679-8>.
3. Li, Y., Yu, T., Yan, H., Li, D., Yu, T., Yuan, T., Rahaman, A., Ali, S., Abbas, F., Dian, Z., Wu, X., & Baloch, Z. (2020). Vaginal microbiota and HPV infection: Novel mechanistic insights and therapeutic strategies. *Infection and Drug Resistance*, 13, 1213–1220. <https://doi.org/10.2147/IDR.S210615>.
4. González-Herrera, S. M., Bermúdez-Quiñones, G., Ochoa-Martínez, L. A., Rutiaga-Quiñones, O. M., & Gallegos-Infante, J. A. (2021). Symbiotic: A technological approach in food applications. *Journal of Food Science and Technology*, 58(3), 811–824. <https://doi.org/10.1007/s13197-020-04532-0>.
5. Nazir, Y., Hussain, S. A., Abdul Hamid, A., & Song, Y. (2018). Probiotics and their potential preventive and therapeutic role for cancer, high serum cholesterol, and allergic and HIV diseases. *BioMed Research International*, 2018. <https://doi.org/10.1155/2018/3428437>.
6. Śliżewska, K., Markowiak-Kopeć, P., & Śliżewska, W. (2020). The role of probiotics in cancer prevention. *Cancers*, 13(1), 20. <https://doi.org/10.3390/CANCERS13010020>.
7. Dahiya, D., & Nigam, P. S. (2022). Probiotics, prebiotics, symbiotic, and fermented foods as potential biotics in nutrition improving health via microbiome-gut-brain axis. *Fermentation*, 8(7), Article 303. <https://doi.org/10.3390/fermentation8070303>.
8. Kamolratanakul, S., & Pitisuttithum, P. (2021). Human papillomavirus vaccine efficacy and effectiveness against cancer. *Vaccines*, 9(12), 1413. <https://doi.org/10.3390/VACCINES9121413>.

9. Marlow, L. A. V., Waller, J., & Wardle, J. (2007). Public awareness that HPV is a risk factor for cervical cancer. *British Journal of Cancer*, 97(5), 691. <https://doi.org/10.1038/SJ.BJC.6603927>.
10. Kashyap, N., Krishnan, N., Kaur, S., & Ghai, S. (2019). Risk factors of cervical cancer: A case-control study. *Asia-Pacific Journal of Oncology Nursing*, 6(3), 308. https://doi.org/10.4103/APJON.APJON_73_18.
11. De Freitas, A. C., Gurgel, A. P. A. D., Chagas, B. S., Coimbra, E. C., & Do Amaral, C. M. M. (2012). Susceptibility to cervical cancer: An overview. *Gynecologic Oncology*, 126(2), 304–311. <https://doi.org/10.1016/j.ygyno.2012.03.047>.
12. Johnson, C. A., James, D., Marzan, A., & Armaos, M. (2019). Cervical cancer: An overview of pathophysiology and management. *Seminars in Oncology Nursing*, 35(2), 166–174. <https://doi.org/10.1016/J.SONCN.2019.02.003>.
13. Sheflin, A. M., Whitney, A. K., & Weir, T. L. (2014). Cancer-promoting effects of microbial dysbiosis. *Current Oncology Reports*, 16(10), 1–9. <https://doi.org/10.1007/S11912-014-0406-0/FIGURES/1>.
14. Dekaboruah, E., Suryavanshi, M. V., Chettri, D., & Verma, A. K. (2020). Human microbiome: An academic update on human body site specific surveillance and its possible role. *Archives of Microbiology*, 202(8), 2147–2167. <https://doi.org/10.1007/s00203-020-01931-x>.
15. Borella, F., Carosso, A. R., Cosma, S., Preti, M., Collemi, G., Cassoni, P., Bertero, L., & Benedetto, C. (2021). Gut microbiota and gynecological cancers: A summary of pathogenetic mechanisms and future directions. *ACS Infectious Diseases*, 7(5), 987–1009. <https://doi.org/10.1021/acsinfecdis.0c00839>.
16. Moos, W. H., Faller, D. V., Harpp, D. N., Kanara, I., Pernokas, J., Powers, W. R., & Steliou, K. (2016). Microbiota and neurological disorders: A gut feeling. *BioResearch Open Access*, 5(1), 137. <https://doi.org/10.1089/BIORES.2016.0010>.
17. Tyszkowski, R., & Mehrzad, R. (2023). Inflammation: A multifaceted and omnipresent phenomenon. In *Inflammation and Obesity: A New and Novel Approach to Manage Obesity and Its Consequences* (pp. 19–30). <https://doi.org/10.1016/B978-0-323-90960-0.00009-6>.
18. Jee, B., Yadav, R., Pankaj, S., & Shahi, S. K. (2020). Immunology of HPV-mediated cervical cancer: Current understanding. *Critical Reviews in Oncology/Hematology*, 40(5), 359–378. <https://doi.org/10.1080/08830185.2020.1811859>.
19. Nicolò, S., Tanturli, M., Mattiuz, G., Antonelli, A., Baccani, I., Bonaiuto, C., Baldi, S., Nannini, G., Menicatti, M., Bartolucci, G., Rossolini, G. M., Amedei, A., & Torcia, M. G. (2021). Vaginal lactobacilli and vaginal dysbiosis-associated bacteria differently affect cervical epithelial and immune homeostasis and anti-viral defenses. *International Journal of Molecular Sciences*, 22(12), 6487. <https://doi.org/10.3390/IJMS22126487/S1>.
20. Amabebe, E., & Anumba, D. O. C. (2018). The vaginal microenvironment: The physiologic role of Lactobacilli. *Frontiers in Medicine*, 5, Article 181. <https://doi.org/10.3389/fmed.2018.00181>.
21. Torcia, M. G. (2019). Interplay among vaginal microbiome, immune response, and sexually transmitted viral infections. *International Journal of Molecular Sciences*, 20(2), 266. <https://doi.org/10.3390/IJMS20020266>.
22. Mitrani-Rosenbaum, S., Tsvieli, R., & Tur-Kaspa, R. (1989). Oestrogen stimulates differential transcription of human papillomavirus type 16 in SiHa cervical

- carcinoma cells. *Journal of General Virology*, 70(8), 2227–2232. <https://doi.org/10.1099/0022-1317-70-8-2227/CITE/REFWORKS>.
23. Chung, S. H., Wiedmeyer, K., Shai, A., Korach, K. S., & Lambert, P. F. (2008). Requirement for estrogen receptor α in a mouse model for human papillomavirus-associated cervical cancer. *Cancer Research*, 68(23), 9928–9934. <https://doi.org/10.1158/0008-5472.CAN-08-2051>.
 24. Lombardi, P., Goldin, B., Boutin, E., & Gorbach, S. L. (1978). Metabolism of androgens and estrogens by human fecal microorganisms. *Journal of Steroid Biochemistry*, 9(8), 795–801. [https://doi.org/10.1016/0022-4731\(78\)90203-0](https://doi.org/10.1016/0022-4731(78)90203-0).
 25. George Kerry, R., Patra, J. K., Gouda, S., Park, Y., Shin, H. S., & Das, G. (2018). Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis*, 26(3), 927–939. <https://doi.org/10.1016/j.jfda.2018.01.002>.
 26. Hanson, L., Vandevusse, L., Jermé, M., Abad, C. L., & Safdar, N. (2016). Probiotics for treatment and prevention of urogenital infections in women: A systematic review. *Journal of Midwifery & Women's Health*, 61(3), 339–355. <https://doi.org/10.1111/jmwh.12472>.
 27. Harper, A., Vijayakumar, V., Ouwehand, A. C., ter Haar, J., Obis, D., Espadaler, J., Binda, S., Desiraju, S., & Day, R. (2021). Viral infections, the microbiome, and probiotics. *Frontiers in Cellular and Infection Microbiology*, 10, Article 925. <https://doi.org/10.3389/fcimb.2020.596166>.
 28. Wang, K. D., Xu, D. J., Wang, B. Y., Yan, D. H., Lv, Z., & Su, J. R. (2018). Inhibitory effect of vaginal *Lactobacillus* supernatants on cervical cancer cells. *Probiotics and Antimicrobial Proteins*, 10(2), 236–242. <https://doi.org/10.1007/S12602-017-9339-X>.
 29. Tsuda, N., Watari, H., & Ushijima, K. (2016). Chemotherapy and molecular targeting therapy for recurrent cervical cancer. *Chinese Journal of Cancer Research*, 28(2), 241. <https://doi.org/10.21147/J.ISSN.1000-9604.2016.02.14>.
 30. Afifirad, R., et al. (2022). Effects of pro/prebiotics alone over pro/prebiotics combined with conventional antibiotic therapy to treat bacterial vaginosis: A systematic review. *International Journal of Clinical Practice*, 2022, 4774783. <https://doi.org/10.1155/2022/4774783>.
 31. Akamine, I. T., et al. (2023). Probiotics in the sourdough bread fermentation: Current status. *Fermentation*, 9(2), 90. <https://doi.org/10.3390/fermentation9020090>.
 32. Cha, M. K., et al. (2012). Antiviral activity of *Bifidobacterium adolescentis* SPM1005-A on human papillomavirus type 16. *BMC Medicine*, 10, 72. <https://doi.org/10.1186/1741-7015-10-72>.
 33. Chandel, D., et al. (2019). Isolation, characterization, and identification of antigenotoxic and anticancerous indigenous probiotics and their prophylactic potential in experimental colon carcinogenesis. *Scientific Reports*, 9(1), 51361. <https://doi.org/10.1038/s41598-019-51361-z>.
 34. Chang, C. J., et al. (2019). Next generation probiotics in disease amelioration. *Journal of Food and Drug Analysis*, 27(3), 615–622. <https://doi.org/10.1016/j.jfda.2018.12.011>.
 35. Chitapanarux, I., et al. (2010). Randomized controlled trial of live *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. *Radiation Oncology*, 5(1), 31. <https://doi.org/10.1186/1748-717X-5-31>.

36. Einhorn, N., et al. (2003). A systematic overview of radiation therapy effects in cervical cancer (cervix uteri). *Acta Oncologica*, 42(5–6), 546–556. <https://doi.org/10.1080/02841860310014660>.
37. Gibson, G. R., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14(8), 491–502. <https://doi.org/10.1038/nrgastro.2017.75>.
38. Heydari, A., et al. (2022). Functional foods and antioxidant effects: Emphasizing the role of probiotics. *Current Topics in Functional Food*. <https://doi.org/10.5772/intechopen.104322>.
39. Hill, C., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, 11(8), 506–514. <https://doi.org/10.1038/nrgastro.2014.66>.
40. Hu, S., et al. (2021). *Lacticaseibacillus casei* LH23 suppressed HPV gene expression and inhibited cervical cancer cells. *Probiotics and Antimicrobial Proteins*, 1–8. <https://doi.org/10.1007/S12602-021-09848-7>.
41. Jampilek, J., et al. (2022). Probiotics and prebiotics in the prevention and management of human cancers (colon cancer, stomach cancer, breast cancer, and cervix cancer). In *Probiotics in the Prevention and Management of Human Diseases* (pp. 187–212). <https://doi.org/10.1016/B978-0-12-823733-5.00009-X>.
42. Ranadheera, C. S., et al. (2010). Probiotic viability and physicochemical and sensory properties of plain yogurt made from bovine and goat milk. *Food Science and Technology International*, 16(3), 227–239. <https://doi.org/10.1177/1082013210367954>.
43. Park, K. Y., et al. (2014). Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. *Journal of Medicinal Food*, 17(1), 6–20. <https://doi.org/10.1089/jmf.2013.3083>.
44. Ragul, S., et al. (2017). Potential probiotics from traditional Indian fermented foods. *Journal of Microbiology, Biotechnology and Food Sciences*, 7(1), 46–50. <https://doi.org/10.15414/jmbfs.2017.7.1.46-50>.
45. Touret, T., et al. (2018). Immunological and microbiota modulation properties of sauerkraut: Impact of fermentation conditions. *Food Microbiology*, 76, 169–177. <https://doi.org/10.1016/j.fm.2018.05.014>.
46. Akamine, I. T., et al. (2023). Probiotics in the sourdough bread fermentation: Current status. *Fermentation*, 9(2), 90. <https://doi.org/10.3390/fermentation9020090>.
47. Pérez-Burillo, S., et al. (2020). Bioaccessibility of phenolic compounds and potential antioxidant activity of 12 species of edible seaweeds. *Food Chemistry*, 302, 125553. <https://doi.org/10.1016/j.foodchem.2019.125553>.
48. Rezac, S., et al. (2018). Fermented foods as a dietary source of live organisms. *Frontiers in Microbiology*, 9, 1785. <https://doi.org/10.3389/fmicb.2018.01785>.
49. Kandati, S., et al. (2022). Role of probiotics in the management of gut microbiota and colorectal cancer. *Journal of Food and Drug Analysis*, 30(2), 125–137. <https://doi.org/10.38212/2224-6614.3371>.
50. Rao, K., et al. (2018). Probiotic therapy: Current status and future perspectives. *Frontiers in Bioscience (Landmark Edition)*, 23, 1407–1445. <https://doi.org/10.2741/4662>.
51. Sungur, T., et al. (2017). Probiotics: A novel strategy for management of cervical cancer. *BioMed Research International*, 2017, 1790256. <https://doi.org/10.1155/2017/1790256>.

52. Riaz Rajoka, M. S., et al. (2018). Antioxidant potential of lactic acid bacteria isolated from indigenous dahi. *Journal of Dairy Science*, 101(12), 10836–10844. <https://doi.org/10.3168/jds.2018-14927>.
53. Stein, A., et al. (2009). Managing chemotherapy-induced diarrhea: Optimal strategies for successful treatment. *Cancer Treatment Reviews*, 35(6), 452–460. <https://doi.org/10.1016/j.ctrv.2009.03.007>.
54. Qiu, J., et al. (2019a). Probiotics for radiation-induced diarrhea: A meta-analysis of randomized controlled trials. *European Journal of Clinical Nutrition*, 73(1), 27–37. <https://doi.org/10.1038/s41430-018-0311-0>.
55. Ou, C. C., et al. (2019). Efficacy of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* probiotics for cervical lesions associated with human papillomavirus: A randomized controlled trial. *Probiotics and Antimicrobial Proteins*, 11(3), 692–700. <https://doi.org/10.1007/s12602-018-9466-3>.
56. Verhoeven, V., et al. (2013). Can probiotics reduce the risk of recurrent vaginal infections? *American Journal of Clinical Nutrition*, 97(5), 1247–1254. <https://doi.org/10.3945/ajcn.112.037770>.
57. Hu, S., et al. (2021). *Lacticaseibacillus casei* LH23 suppressed HPV gene expression and inhibited cervical cancer cells. *Probiotics and Antimicrobial Proteins*, 13(5), 1335–1345. <https://doi.org/10.1007/s12602-021-09848-7>.
58. Nami, Y., et al. (2014). Probiotic properties of *Lactobacillus* strains isolated from various biotopes in Iran: Biodiversity, fitness and antagonistic activities. *Anaerobe*, 28, 12–17. <https://doi.org/10.1016/j.anaerobe.2014.04.007>.
59. Nouri, Z., et al. (2016). Assessment of probiotic *Lactobacillus* strains for the control of cervical cancer cells and the production of bacteriocins. *Iranian Journal of Basic Medical Sciences*, 19(11), 1199–1206.
60. Sungur, T., et al. (2017). Antimicrobial activity of *Lactobacillus* strains isolated from vaginitis cases in Turkey. *European Journal of Microbiology and Immunology*, 7(4), 258–264. <https://doi.org/10.1556/1886.2017.00015>.
61. Cha, M. K., et al. (2012). Antiviral activity of *Bifidobacterium adolescentis* SPM1005-A on human papillomavirus type 16. *BMC Medicine*, 10, 72. <https://doi.org/10.1186/1741-7015-10-72>.
62. Chitapanarux, I., et al. (2010). Randomized controlled trial of live *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. *Radiation Oncology*, 5(1), 31. <https://doi.org/10.1186/1748-717X-5-31>.
63. Einhorn, N., et al. (2003). A systematic overview of radiation therapy effects in cervical cancer (cervix uteri). *Acta Oncologica*, 42(5–6), 546–556. <https://doi.org/10.1080/02841860310014660>.
64. Pandey, K. R., et al. (2015). Probiotics, prebiotics and synbiotics—a review. *Journal of Food Science and Technology*, 52(12), 7577–7587. <https://doi.org/10.1007/s13197-015-1921-1>.
65. Jampilek, J., et al. (2022). Probiotics and prebiotics in the prevention and management of human cancers. *Probiotics in the Prevention and Management of Human Diseases*, 187–212. <https://doi.org/10.1016/B978-0-12-823733-5.00009-X>.
66. Gibson, G. R., et al. (2017). Expert consensus document: The ISAPP consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14(8), 491–502. <https://doi.org/10.1038/nrgastro.2017.75>.

67. Marcel B. Roberfroid. (2008). Prebiotics: Concept, definition, criteria, methodologies, and products. In *The Prebiotic Approach to Health* (pp. 9–31). Springer. https://doi.org/10.1007/978-1-4020-8837-1_2.
68. Kyrgiou, M., & Moscicki, A. B. (2022). The microbiome and HPV-induced cervical neoplasia: Old friends in a new perspective. *Vaccines*, 10(2), 1–8. <https://doi.org/10.3390/vaccines10020307>.
69. Kudela, P., et al. (2021). Prebiotics: Novel protective therapy for HPV infections? *Journal of Clinical Gastroenterology*, 55(8), 673–679. <https://doi.org/10.1097/MCG.0000000000001504>.
70. Kudela, P., et al. (2023). Probiotics, prebiotics, and symbiotics: Immunological properties and protection against cervical infections. *Journal of Applied Microbiology*, 134(3), 1–10. <https://doi.org/10.1111/jam.15605>.
71. Wei, W., et al. (2022). Mechanisms of action of prebiotics in cancer prevention and therapy: A review. *Cancer Therapy and Research*, 18, 57–69.
72. Afifrad, R., et al. (2022). Prebiotic-rich foods in cervical cancer prevention and treatment. *International Journal of Clinical Practice*, 2022, 1–13. <https://doi.org/10.1155/2022/1234567>.
73. Mejía-Caballero, A., et al. (2021). Gut microbiota and HPV in the development of cervical cancer: A complex interaction. *Cancer Letters*, 519, 65–73. <https://doi.org/10.1016/j.canlet.2021.07.009>.
74. Thantsha, M. S., et al. (2012). Evaluating the anti-cancer potential of probiotics: The role of host immune responses. *South African Journal of Science*, 108(11–12), 23–29.
75. Lu, X., et al. (2021). Probiotic supplements in cancer therapy: Interactions with radiation and chemotherapy. *Cancer Biology & Therapy*, 22(7), 488–498. <https://doi.org/10.1080/15384047.2021.1933150>.
76. Qiu, J., et al. (2019b). Probiotics in radiotherapy: Clinical trials and evidence. *Radiation Oncology Journal*, 35(3), 203–208. <https://doi.org/10.3857/roj.2019.00283>.
77. Legesse Bedada, W., et al. (2020). A systematic review of clinical trials on prebiotics and probiotics. *Systematic Reviews*, 9(1), 45. <https://doi.org/10.1186/s13643-020-01306-4>.
78. Liong, M. T. (2008). Safety of probiotics in cancer patients. *Critical Reviews in Food Science and Nutrition*, 48(3), 247–259. <https://doi.org/10.1080/10408390701280564>.
79. Sarao, L. K., & Arora, M. (2016). Probiotics, prebiotics, and synbiotics: An overview. *Journal of Food Science and Technology*, 53(8), 3561–3575. <https://doi.org/10.1007/s13197-016-2321-1>.



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CHAPTER 6

Probiotics for the Mitigation of Inflammation-Based Lung Cancer

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ABSTRACT

On a global scale, the number of lung cancer-related deaths is leading. Earlier studies have reported that probiotics are responsible for the treatment and detection of various tumors. Probiotics may be a game-changer in contrast to conventional and chemotherapeutic tactics, and the particular microbiome of the lung may be vital in the onset and progression of lung cancer. This microbiome may develop into a brand-new target in cancer treatments, and probiotic medication may improve lung metastasis immunity. Moreover, *Lactobacillus* decreased tumor development, and the effectiveness of cisplatin-based treatment was reduced by antibiotics; through genotoxicity, systemic inflammation, and impaired immunosurveillance, gut dysbiosis has an impact on the onset and progression of lung carcinoma. A unique biomarker that may be utilized to foresee an individual's reaction to immunotherapy and its side effects may also be present in the gut flora. Additionally, the lung microbiota may be used as a biomarker for lung cancer treatment. Probiotics, along with fecal microbiota transplantation (FMT), may alter the gut microbiota, enhancing the effectiveness and reducing the toxicity of immune checkpoint inhibitors. Because of this, gut microflora also has a major impact on the development and treatment of lung cancer. This clinical trial (operable) is focused on the gut and lung pathway and

may have implications for the efficacy of PD-1-based immunotherapy for lung cancer. The immune system, specifically the immune system's ability to identify cancer, may be improved by having a range of bacterial species inside the gut. Taking probiotics may modify the kind and diversity of intestinal bacteria as well as the immune system's reaction to lung cancer.

6.1 INTRODUCTION

One out of every five cancer-related deaths is caused by lung cancer. Moreover, this amount is approximately 20% throughout the world [1]. Non-small cell lung cancer (NSCLC) grows more slowly than small cell lung cancer (SCLC) [2]. It has also been reported in earlier studies that the responsible genomic variance, cigarette smoking, and air pollution are risk factors for lung cancer, as well as exposure to certain substances like heavy metal ingestion, radon gas, asbestos, and silica dust [3]. Smoking is identified as the main reason for the development of lung cancer [4–6]. Additionally, it was shown that if lung cancer is identified in the early stage, it may be curable [7].

Trillions of bacteria have been found to reside in the gastrointestinal system, and these microbes are crucial for preserving immunological balance in the gut milieu [6, 8]. Probiotics are live microorganisms that have potential nutritional and health benefits. The benefits of gut microbiota include nutrient utilization, infection resistance, control of intestinal microbial communities, development of the immune system, and control of host metabolism [9, 10]. Several different microbial species are found in the gut and are susceptible to outside factors like age, nutrition, stress, sickness, drugs, and lifestyle [11]. Since the mortality and incidence rates of cancer are increasing significantly throughout the world, earlier studies reported that the use of probiotics can be helpful for the therapy of many forms of cancer [12]. In light of this, the present study was conducted to determine whether there is, in fact, a connection between probiotics and lung cancer.

6.2 PROBIOTICS

Probiotics are thought to have a number of positive benefits, including the regulation of metabolism, immunity, and inflammatory disorders [13]. They may also provide protection against cancer, infections, illnesses, etc. According to Kandyliis, probiotics are present in dairy products (such as yogurt, cultured buttermilk, and cheese) as well as other foodstuffs like wheat, soybeans, sorghum, grains, corn, and others [14, 15]. Natural biopolymers, glycans/

polysaccharides (carbohydrates) generated by bacteria that produce lactic acid, are available in a wide range of shapes and structural variations [16].

Prospective probiotic strains should be able to tolerate acid and endure the bile and gastric juice produced by humans. They must have the capacity to persist in large enough numbers and stick to the gut mucosal surface. They should also activate the immune system, which will benefit the host. The ability to create strains on a wide scale and their rapid multiplication are required. Probiotics are a class of bacteria that include the first and most numerous members of the genera *Lactobacillus* and *Bifidobacterium*. These microbes naturally thrive in the human gastrointestinal tract (GIT) and, unlike other gut bacteria, are known to have no adverse effects. Probiotic strains must be highly sensory and phage-resistant [17].

6.2.1 PROBIOTICS CHALLENGES

Healthy probiotic strains that are readily accessible on the market can be added to cultured food products, taken as dietary supplements, used as medications, or provided as freeze-dried supplements. Probiotics must retain a precise proportion of living cells and have particular strains in order to be beneficial to the host's health. Some countries have established the absolute minimum amount of live cells a probiotic product must contain to be effective. There are major financial and technological barriers to using probiotic cultures in functional foods because of their viability. Excessive amounts of 50–200% more cells have been introduced to products to make up for cells that degrade during preservation. Before reaching their final destination, the colon, probiotics are exposed to circumstances prevalent in the stomach and small intestine after being ingested. When the tablets are created, for instance, in the case of dry cell pills, the producer must include an excess of cells in order to be safe. Producers of probiotics must develop probiotic microorganisms that can last for a long time and can withstand the acidity of the stomach [18].

6.2.2 TECHNIQUES TO INCREASE PROBIOTICS VIABILITY

With their positive effects on health, probiotics are attracting more and more attention. The recovery of live bacteria in products that make this promise is low. Micronutrients like peptides and amino acids can also be added, and probiotic cultures can also be pre-exposed to stressors with low lethal doses. Other possible methods for improving probiotic viability include genetic

modification, immobilization, and two-step fermentations. To extend the shelf life of probiotics and ensure their survival throughout processing and storage, microencapsulation is the procedure by which they are enclosed in tiny particles. Probiotic cells don't die because of microparticles [19]. According to studies, probiotic microorganisms preserved in solid fat microcapsules still function and are viable. The walls of the microcapsules might grow, break, or disintegrate as a result of these changes. Although there are some negative aspects to this procedure, microencapsulation mainly offers positive effects. Some of the generated microparticles might contract and lose mechanical strength because of their susceptibility to acids. Some foods in other countries cannot contain some of the polysaccharides that are utilized. Supercritical fluids contain a variety of solvent characteristics, allowing them to be used as solvents in a number of processes [20].

6.3 CONCERNS OF LUNG CANCER

6.3.1 FACTORS RESPONSIBLE FOR THE PROGRESS OF LUNG CARCINOMA

Smoking poses a risk that is changeable. Certain factors, including a particular age or family history, cannot be changed. It is possible that some individuals who get the disease have few or no identified potential risks. Lung cancer risk is generally influenced by a variety of factors [21–24] that could be relevant to SCLC, while some of them might not be.

6.3.1.1 CIGARETTE SMOKE

Smoking is a contributing factor in about 80% of lung cancer fatalities. Using pipes and cigars increases your likelihood of developing lung carcinoma almost as much as smoking. Lung cancer is more likely to occur if you smoke both regular cigarettes and low-tar or “light” cigarettes. As menthol cigarettes may support heavier breaths, smoking them may significantly raise the danger.

Exposure to passive smoking may also be responsible for the emergence of cancer in the lungs. According to the American Lung Association (ALSA), passive smoking appears to be the third most frequent cause of lung cancer in the US. Radon is a radioactive gas that is created mostly by uranium within minerals and earth spontaneously decaying. Small quantities of radiation

may enter your lungs when people inhale it, raising one's likelihood of getting lung cancer. High indoor radon levels are prevalent throughout the country, especially within basements, homes, and other structures [25].

6.3.1.2 ASBESTOS EXPOSURE

Lung carcinoma is much more likely to arise in asbestos workers than in non-asbestos workers [26–29].

6.3.1.3 RADIATION THERAPY FOR THE LUNGS IN THE PAST

Individuals who have undergone thoracic radiotherapy for other malignancies are much more prone to develop tumors. There is no proof that women who have breast cancer treatment after a lumpectomy are more likely to acquire lung cancer [28].

6.3.1.4 AIR POLLUTION

The incidence of lung carcinoma is somewhat increased in urban areas because of air pollution; in the US, air pollution is said to be a factor in between one and two percent of lung cancer fatalities [30].

6.3.1.5 PERSONAL AND FAMILY HISTORY

The chance of acquiring lung carcinoma may somewhat rise if an individual or a family member has previously experienced it. Although it is unclear how much of this is attributable to common genes and how much may be due to exposures in the home, inheritance definitely seems to play a part in some families where carcinoma occurs with prevalence.

6.3.1.6 OTHER CANCER-CAUSING CHEMICAL EXPOSURE

Other carcinogens (substances that cause cancer) have indeed been found in a number of jobs, some of which might raise the chance of developing emphysema. These substances include chromium and nickel complexes, mustard gas, charcoal products, radon, beryllium, Cd, cadmium, silica, vinyl chloride, and chloromethyl ethers.

6.3.1.7 *DIETARY SUPPLEMENTS AND DRINKING WATER*

According to research, persons who smoke and use beta-carotene supplements may be more likely to develop emphysema. Water consumption could also raise this threat.

6.3.2 *UNCERTAIN OR UNTESTED RISK FACTORS*

6.3.2.1 *SMOKING MARIJUANA*

There seem to be valid concerns that marijuana use may raise the chance of developing lung cancer. Those chemotherapeutics have more time to accumulate in your lungs when people breathe in marijuana vapor deeply and retain it there for a prolonged period. Cannabis users frequently consume fewer cigarettes per day or per week than cigarette smokers. Tar and a few of the cancer-influencing agents present in cigarette smoke are also present in marijuana fumes. It has proven challenging to carry out research to ascertain if there is any connection. Since marijuana has been illegal for so long, there is no known link between cannabis and lung cancer. Understanding the potential cancer risks associated with marijuana use requires more investigation [29].

6.3.2.2 *E-CIGARETTES*

E-cigarettes are called “tobacco products” as most of them contain nicotine. As e-cigarettes remain in their infancy, further research is necessary to ascertain possible significant long-term effects, such as an increased chance of developing lung cancer.

6.3.2.3 *TALCUM POWDER*

Talc that is found in its natural form might contain asbestos. There isn't any proof that the use of talcum powder for aesthetic purposes makes individuals more likely to develop lung cancer. According to several studies, inhaling commercial-grade talc may increase the risk of lung cancer and other respiratory diseases. Some studies, however, have not found an increase in lung cancer rates.

6.4 CAUSES OF LUNG CANCER

Regular cigarette smoking is the main cause of lung carcinoma. Individuals exposed to smoking, radon, and asbestos are significantly more susceptible to risk. Although lung carcinoma in non-smokers is uncommon, people without a history of smoking can also develop small cell lung cancer (SCLC), which can be diagnosed [31]. Exposure to radon, secondhand smoke, smog, or other factors can result in lung cancer in non-smokers. Only a small minority of lung cancer cases are caused by patients who have no recognized risk factors. Others may be the result of unknown factors, while some may merely be random occurrences with no external explanation. Without smoking, lung cancer tends to appear in younger patients and has distinct gene mutations from tumors seen in people who smoke [32].

6.4.1 CHANGES IN GENE

Our DNA, the genetic code that makes us what we are, might influence the likelihood that we will experience certain illnesses, along with some forms of cancer. Different risk factors responsible for the emergence of cancer in the lungs have been shown to alter the DNA of lung cells, according to scientists. These alterations may result in cancer and uncontrolled cell proliferation. Certain genes have an impact on how quickly cells replicate to generate new cells and how long they live. Those genes are referred to as oncogenes. Whenever necessary, tumor suppressor genes can cause cell death or regulate the division of cells. DNA alterations that activate oncogenes or silence tumor suppressor genes can cause cancer. Gene alterations are typically the cause of lung cancer.

6.4.1.1 INHERITED GENE CHANGES

Considering that not all occurrences of lung cancer are thought to be inherited, genes may have an impact on the disease. Changes in DNA or DNA mutations in certain people inherited from their family can lead to the rapid development of lung cancer. Some types of cancer may occur upon exposure to chemicals, such as those found in cigarette smoke, and appear to be inherited by some individuals as a diminished ability of the body to break them down or eliminate them. Others acquire weak DNA repair mechanisms, which raises the possibility that they might have genetic changes [33–36].

6.4.1.2 ACQUIRED GENE ALTERATION

Gene changes linked to lung carcinoma are developed rather than inherited. Exposure to environmental stimuli, such as cigarette smoke, commonly leads to lung cell acquired mutations. A cell's internal processes can occasionally result in random gene changes that have no outside cause. Changes in chromosome 3 and the tumor inhibitor gene TP53 are present in both NSCLC and SCLC. The oncogene (K-RAS) and the tumor-suppressor protein (p16) are two examples of acquired changes that are anticipated to have the greatest influence on both types of cancer, considering that not all lung tumors display the same gene alterations.

6.5 ROLE OF INFLAMMATIONS IN LUNG CANCER

Inflammation is also responsible for the development of cancer cells [37]. The identifying characteristics of cancer include retaining proliferative signals, eluding growth inhibitors, permitting replicative immortality, resisting cell death, encouraging angiogenesis, and suppressing cell death. These traits are unique and complementary, promoting tumor development and spread [38]. Systemic inflammation is responsible for the development of tumor growth, anorexia, inflammation, disability, and poor survival, in addition to promoting the growth of tumors [39]. Systemic inflammation may be identified through different indices, namely the Glasgow Prognostic Score (GPS), hypoalbuminemia, plasma C-reactive protein (CRP), absolute white cell count, and its subsets (neutrophils, neutrophil/lymphocyte ratio (NLR), and platelet/lymphocyte ratio (PLR)), which are all indicators of prognosis [40, 41]. NLR has been demonstrated to function as a single predictor of survival and to be associated with a higher stage in individuals with totally resected NSCLC [42].

Leukocyte infiltration, angiogenesis, tissue remodeling, and the presence of cytokines and chemokines are all indicators of pro-tumor inflammation, which is associated with cancer. Extrinsic or intrinsic inflammations are two different types of inflammation [43]. Certain stimuli have the ability to cause carcinogenesis and persistent inflammation. In fact, carcinoma and COPD are both correlated with smoking-related lung damage, inflammation, and DNA fragmentation. It is known that the inflammatory process caused by alcohol use or hepatitis B or C leads to the development of cirrhosis and fibrosis, which are connected to hepatocellular carcinoma. These are examples of extrinsic inflammation-causing mechanisms [43]. Oncogene activation, which results in cytokine production and tissue remodeling, and tumor suppressor gene

inactivation, which also controls the generation of cytokines, are the two primary factors that cause inflammation induced by tumors [43]. These stimuli have been linked in several preclinical models to the induction of genetic and epigenetic processes necessary for the onset and development of cancer [44]. Cells interact with one another and the extracellular matrix in addition to releasing cytokines, chemokines, enzymes, and extracellular vesicles like exosomes [45]. Angiogenesis, extracellular matrix remodeling, cancer cell migration, proliferation, and survival are all induced by CAFs, which are produced by a number of cell types. Additionally, CAFs contribute to the development of an immunosuppressive environment [45–47]. During angiogenesis, pericytes modify the basement membrane and control lymphocyte activation. Blood vessels are lined by endothelial cells, which also control angiogenesis, immune cell infiltration, and tumor growth and metastasis [45–50].

6.5.1 NON-SMALL CELL LUNG CANCER PATHOGENESIS: INVOLVEMENT OF INFLAMMATORY PROCESS

6.5.1.1 CHRONIC IMMUNE ACTIVATION AND CARCINOGENESIS

Immunity that is both humoral and cell-mediated are two crucial and complementary parts of the adaptive immune response. Both systems depend on the CD4⁺ T-helper lymphocyte, which also orchestrates two distinct but overlapping cytokine patterns. Regarding the molecular mechanisms driving the division of developed naive (TH0) CD4⁺ cells into genetically distinct cells that display either a CMI or HI phenotype, questions remain. Mature dendritic cells that are already producing IL-12 cause CMI to be upregulated in effector and memory cells. Immune detournement in favor of a predominant TH1 response promotes cancer cell death and tumor rejection. In order to get rid of the damaging stimuli, both systems are engaged in response to an inflammatory stimulus (such as coming into contact with a viral infection). Following the eradication of the offending infection, HI is upregulated and CMI is comparatively decreased. Malignancies frequently develop under conditions of prolonged immune activation, which includes an elevation in immune activation and a shift in immune reaction to one characterized by a wound-healing, pro-angiogenic, pro-apoptotic cytokine program. Cancer cells adapt as the malignant process progresses to thwart the CMI response. When considered as a whole, these data show how important a role the immune system plays in dictating carcinogenesis outcomes [51].

6.5.1.2 THE CONTRIBUTION OF CHRONIC INFLAMMATION MAKES TO LUNG TUMOR PROGRESSION

There is growing proof that breathing in tobacco smoke causes extensive inflammation and mutagenesis consequences in the lungs, which encourage an immunological response that favors cancer. Lung cancer is also associated with a systemic and localized inflammatory response that is abnormally out of control, characterizing Chronic Obstructive Pulmonary Disease (COPD). Smoking tobacco considerably increases the risk of developing COPD and lung cancer in susceptible individuals. Ten percent of lung cancer cases are found in nonsmokers who have never smoked, indicating that there may be additional factors at play in the occurrence of lung cancer [52–57]. Those who were previously confirmed to have tuberculosis had an almost 50% greater chance of developing lung cancer, with the impact being most pronounced in people who had recently been infected. Nickel, chromium, beryllium, arsenic, and silica are other known or suspected risk factors. It has been hypothesized that the *p53* gene's overexpression and subsequent mutation may help individuals with idiopathic pulmonary fibrosis develop a pro-tumor environment [58–62].

6.5.1.3 CYCLOOXYGENASE, EGFR, AND EML4-ALK MUTATION

This is acknowledged as COX regulates a number of the crucial elements in tumorigenesis as well as the progression of cancer. Protracted consumers of anti-inflammatory drugs had a roughly 50% decreased risk of colon cancer. Measuring COX-2 production in patients with resected NSCLC might produce useful, independent predictive information. Though this hasn't been thoroughly researched to date, host immunological dysregulation may have a part in the cause of lung cancer in non-smokers. Further clinical research is needed to help show a possible link among exposure to cigarette smoke and developmental diseases [63–67].

6.5.2 THE MECHANISMS LINKING INFLAMMATION AND CANCER: TUMOR INITIATION

Chronic autoimmune diseases increase the risk of getting cancer, promote its beginning, penetration, and metastasis of carcinoma, and are responsible for 15–20% of all melanoma mortality [68]. The cytokines damage DNA in growing cells and the epithelium that surrounds them, increasing the likelihood that they may become cancerous.

6.5.2.1 EXTRINSIC PATHWAYS

Macrophages can activate in a variety of ways, with M1 and M2 becoming the two circumstances. Anti-inflammatory cytokines, immunosuppressive agents, and matrix-degrading protease are produced by the body. They aid in revascularization, extracellular matrix remodeling, and repair (Figure 6.1).

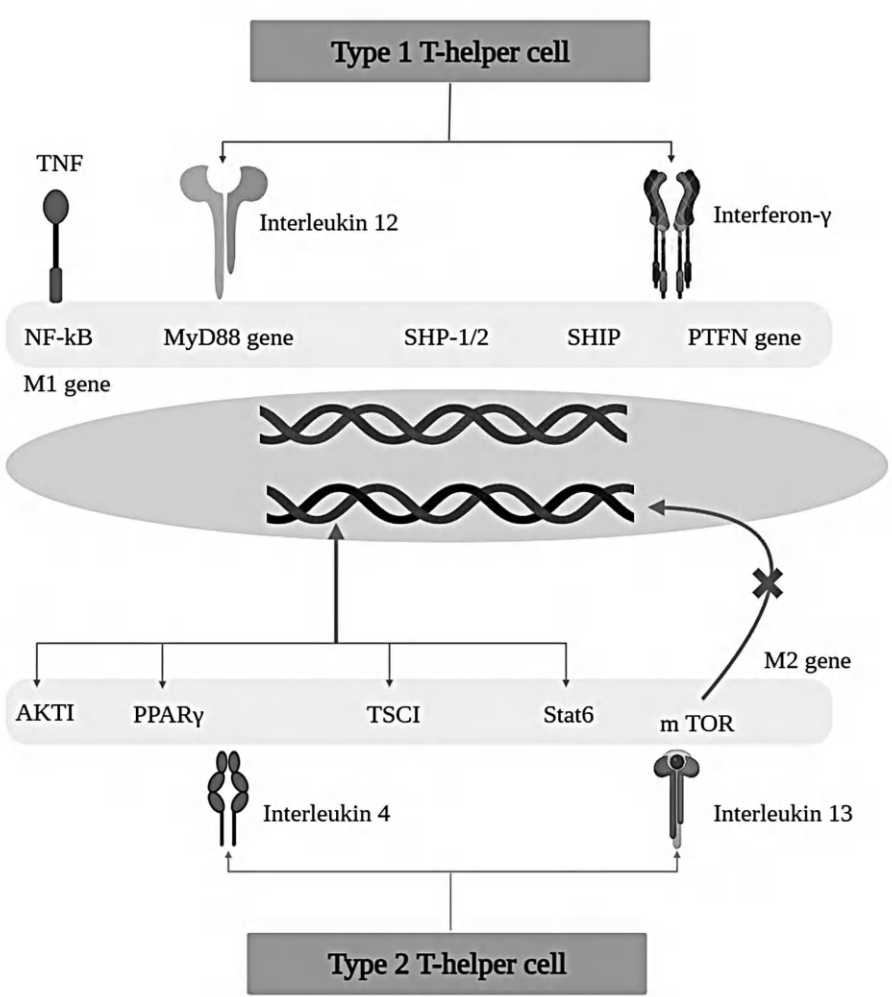


FIGURE 6.1 The polarization states of the macrophages in the tumor microenvironment.
Source: Modified from Ref. [69]

6.5.2.2 INTRINSIC PATHWAY (GENETIC)

Metabolic pathways necessary for cell multiplication, longevity, division, and DNA damage repair become dysregulated when tumor suppressor genes are repressed while gene mutations are activated. Such changes commonly disrupt innate immunological and inflammatory signaling pathways, which decrease anti-tumor immunity and encourage carcinogenesis. TAMs typically support tumor development whenever malignancies arise, whether there is persistent inflammation present or not. (Figure 6.2). M1 macrophages that are found in regions of the inflammatory process promote the growth of tumors by releasing inflammatory cytokines, causing cellular damage, and creating mediators that trigger a response. Hypoxia-inducible factor 1 α (HIF1 α) levels increase as a result of the loss of tumor suppressor genes von Hippel-Lindau (VHL) and phosphatase and tensin homolog (PTEN), which also favor metastasis.

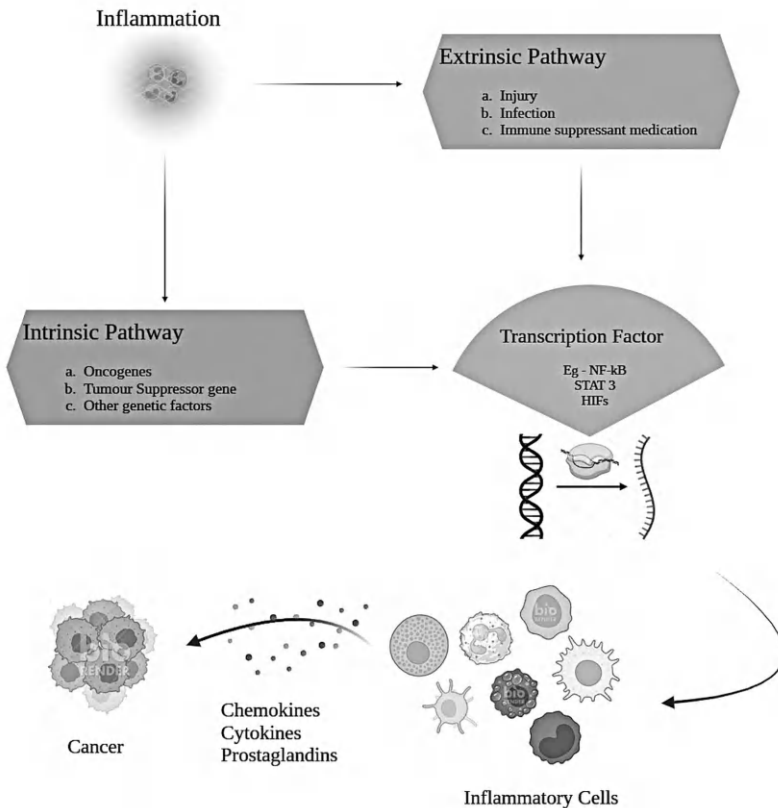


FIGURE 6.2 Pathways between cancer and inflammation.

Source: Modified from Ref. [70]

6.5.2.3 INTERACTION OF THE INTERNAL AND EXTERNAL PATHWAYS

Critical signaling pathways notably the oxygen deprivation factor-1A, STAT3, and Nacrotic Factor-kB can converge if they are active in tumor cells, despite the fact that both intrinsic and external pathways are derived from separate biochemical functions. Such translational regulators are already in charge of controlling the proinflammatory messengers' synthesis, which draws and activates inflammatory cells and intensifies the already existing inflammatory milieu. The majority of solid tumors, if not all, exhibit inflammation, which is explained by the confluence of an inflammatory pathways signaling pathways.

6.6 EXISTING TUMORS: ROLE OF MACROPHAGES IN MALIGNANCY PROMOTION

6.6.1 CELL MOTILITY, SURVIVAL, AND PROLIFERATION

According to theory, M2-like macrophages predominate in the immune infiltration of malignancies that have already grown, whereas M1-like macrophages prevail at the time a tumor first appears. Growth factors and cytokines are secreted by M2 TAMs to aid in immune escape, tumor cell multiplication, survivability, and endothelial transformation (Figure 6.1). Due to the slower growth of inflammatory cytokines and the generation of reactive oxygen species, TAMs are much less capable of causing irritation due to intermediates (IL-12 and IL-23), in addition to troublesome proteins (CD80 and CD86) [71]. They also have diminished allergen capacity.

Tumor-associated macrophages' (TAMs') crucial function in the development of malignancy involves TAMs aggressively attracting certain T-cell subgroups, such as T-helper type 2 [Th2] cells and T cells with regulatory functions that lack cytotoxic capabilities, which supports the development of malignancies. TAMs also prevent an adaptive immune response from being recruited and activated, which has anticancer activity. Whenever the immunotherapeutic molecule programmed death ligand-1 (PD-L1) is triggered, immunologic repression results, leading active T cells to degrade and die [72].

6.6.2 LOCATION AND CONCENTRATION OF MACROPHAGES IN LUNG CANCER

In the early 2000s, many studies investigated the immunohistochemistry analysis of macrophage infiltration in primary cancers. According to

one study, individuals who passed away had considerably more tumor-infiltrating macrophages (TAMs) than those who avoided recurrence (LC). TAM density and prognosis were not found to be significantly correlated in this study or any other study. Those with stromal and tumor cell islets of adenocarcinomas, including CD68+ macrophages, had twice as long survival times as patients with full sections. Stage, grade, or lymph node histology were not linked with macrophage counts. Patients' cumulative survival was considerably improved when their macrophage islet/stromal ratio was above the median. Stage IV NSCLC participants who received initial treatment had the same first-line survival rate as those who received less chemotherapy. Furthermore, patients who received irradiation and chemotherapy treatments had the same first-line survival rate as those who received less chemotherapy. Macrophages were discovered in carcinoma colonies in 195 of 199 stromal tumors and 194 of 199 tumors, respectively. HLA-DR, CD-68, and CD-163 staining were employed to gauge the number of macrophages present in tumor islets and surgically removed NSCLC. This is well within limitations because HLA-DR can distinguish between M1 and M2 macrophages, while CD68 can be most clearly categorized as non-M2 macrophages. There is currently no known particular cell-surface sensor for M1 macrophages. The four M1 markers and CD163 (M2) were initially stained to see if they could distinguish between M1 and M2 macrophage subsets. With a median of 6.45% in 4003 fields, CD163+ HLA-DR+ macrophages were quite common, although CD163 did not co-occur with any of the other M1 marker fields. These findings again imply that M1 macrophages are not the only cells that express HLA-DR. The number of M1 TAMs in research including 65 individuals with adenocarcinomas failed to demonstrate clinical significance. In a study of patients with adenocarcinoma, M2 (CD68+ CD206+) TNM staging, lymph node metastases, and overall survival were all correlated with TAM invasion. A larger percentage of circulating M2 cells (CD14+ CD204+) obtained from the pulmonary circulation of lungs that had undergone excision correlated with both the abundance of CD204+ TAM in the tumor and was indicative of an early recurrence, according to a separate study. Despite the fact that just one labeling procedure was used, it was shown that there was a substantial correlation between above-median CD204 expression and characteristics such as stage, nodal involvement, vascular invasion, and pleural incursion that imply malignant aggression [73].

6.7 LUNG AND PROBIOTICS

6.7.1 LUNG FLORA DETECTION IN DIAGNOSIS

A population of bacteria that resides inside the lungs has been connected with lung cancer, according to reviewed studies by specialists. They arrive at the conclusion that people who have cancer may have a greater chance of surviving if the disease is caught early. Yet, there are still numerous questions about how specific germs induce lung cancer. Asthma, COPD, and cystic fibrosis are among them. The interplay of the oral, pulmonary, and gut microbiomes with each other may cause lung disease. An earlier study also reported the corpus of evidence on the connections between microbiomes and lung cancer. Many respiratory conditions are linked to the pulmonary microbiome, a type of bacteria, fungus, and virus that live in the lungs. These include chronic obstructive pulmonary disease (COPD), cystic fibrosis, and asthma. It is extremely likely that pulmonary illness is brought on by the interaction of the microbiomes in the mouth, lungs, and stomach. The majority of the time, the absence of proper early onset results in around 75% of instances simply progressing when discovered by medical specialists [74].

6.7.2 ROLE OF MICROBIOME IN LUNG CARCINOMA

Currently, the concept of a typical lung microbiota is largely accepted. 16S rRNA gene decoding was the earliest biochemical technique used to examine different microbial communities prevalent inside the human body. The microbiota found in the lungs is mimicked by the oropharynx and intrathoracic airways. Surprisingly, little bacteria are seen in healthy lungs, although this number can rise with respiratory illness. The microbiome of the lung changes as a result of the pro-inflammatory cascade that might cause fibrosis and lung cancer. The lung is continually exposed to the environment. *Mycobacterium tuberculosis*, whose high occurrence is linked to treatment resistance offered by the makeup of mycosides, is one of the greatest instances. *Streptococcus* colonization was a reliable indicator of asthmatic illness [75].

6.7.2.1 LUNG MICROBIOME

It is yet unknown whether microorganisms may play a part in the emergence of lung carcinoma, notwithstanding numerous research studies relating the microbiota and malignancy. Pathogenic bacteria can, in fact, lead to inflammation in a lung cancer assay. The communication pathways phosphoinositide

3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) have been suggested in this because *Streptococcus* and *Veillonella* were common in lung cancer patients. There was a significant difference between the oral microbial populations of those with lung cancer and those with non-small cell lung carcinoma, indicating a strong association between the two diseases. Bacteria can migrate from nearby, distant mucosal cavities into a necrotic, malignant environment, which can change the lung niche and lessen immune surveillance. A dysbiotic airway microbiota is linked to chronic lung illnesses, which frequently co-occur with gastrointestinal disorders [76].

6.7.2.2 MICROBIOME-BASED LUNG CANCER RISK FACTORS

The pulmonary microbiome's tendency to cause tumors is regulated by a number of important pathways. COPD enables colonization and bacterial dysbiosis, a bacterial imbalance that fosters an ongoing inflammatory environment and frequently results in cancer. Smoke is among the largest risk factors for lung cancer since it is connected to respiratory problems like nicotine use. Additionally, *Helicobacter pylori* has been linked to a spike in the generation of cytokines that promote inflammation, such as tumor necrosis factor, IL-1, and IL-6. The chronic bronchitis that typically precedes lung cancer could develop from this edema. The lung microbiome maintains homeostasis when immune sensing and microbial tolerance are in balance. Three main pathways allow the immune system to protect itself from pathogen invasion. For instance, the chronic inflammation-associated carcinogenesis was the mechanistically responsible link between *Mycobacterium tuberculosis* and lung cancer [72–76]. Dysbiosis or the emergence of cancer is probably the result of a dynamic interplay between a number of immunological, microbial, and environmental variables. Several bacterial substances, sometimes known as “bacteriotoxins,” have been connected to genotoxicity and the development of tumors. Nuclear factors and calcineurin are produced by activated T cells as a result of TLR communication, which promotes cancer stem cell survival and expansion [77].

6.7.2.3 LUNG CANCER MICROBIOME CHALLENGES

Lung diagnosis and microbiota assessment remain clinical difficulties. Nutrition, culture, and genetic makeup, among others, have an impact on the microbiome. The use of cigarettes is a known risk factor for lung cancer and is commonly found in the microbial flora of the airways. In reality,

conditions including oxygen saturation, pH, and immunological function encourage bacterial growth in the lung. In patients with lung cancer, tumor variability has a considerable impact on tumor categorization, prognosis, and available treatments (Figure 6.3). It is conceivable that the microbiome of lung cancer patients may have an effect on carcinogenesis [78].

6.7.3 PROBIOTICS' EFFECTS ON THE IMMUNE SYSTEM IN PATIENTS WITH LUNG CANCER

The randomized controlled trial examines if altering the gut microbiota with probiotics might affect how effectively the body's immune system responds to phases of breast or lung cancer that are treatable by surgery (operable). The ability of the immune system to identify cancer may potentially be enhanced by the presence of a range of microbial species in the gut. Utilizing probiotics could alter the type and variety of gut bacteria, in addition to the way the immune system reacts to mammary or lung cancer [79].

6.8 THERAPY AND MANAGEMENT

Environmental, smoking, and genetic factors are well-known risk factors for lung cancer and are a normal component of the lung microbiota. Indeed, factors such as oxidative stress, pH, and immunological state support the colonization of bacteria in the lung. Tumor heterogeneity strongly influences tumor classification, prognosis, and treatment options in lung cancer patients. The microbial community could have an effect on the heterogeneity of tumors in lung cancer. In a laboratory model of inflammatory mediators' colorectal cancer, probiotic treatment decreased the growth of tumor cells and adversely affected nuclear factor-kappa light-chain activator of activated B cells activation [80]. Changes in the microbiota can also affect how cancer develops. Another study reported that probiotic medications may enhance protection against lung metastasis, highlighting the importance of the microbiome in cancer recurrence [81]. The microbiome can be utilized as an additional cancer therapy option [82]. *Lactobacillus* decreased tumor development and enhanced longevity, while the efficacy of cisplatin-based therapy was diminished by antibiotics [83]. These findings suggest that the microbiome may be beneficial for the treatment of lung cancer, both in terms of therapeutic results and in terms of cancer prevention and effect amplification. There is still research suggesting that the microbiota can affect treatment approaches. Antibiotic usage lowered the therapeutic efficacy in patients who had advanced NSCLC, indicating that such people

had a higher likelihood of disease progression and a shorter PFS and OS [84]. Additionally, regardless of smoking habits or the frequency of illnesses, it has been demonstrated that taking antibiotics such as penicillin and cephalosporins increases the likelihood of developing pulmonary, gastric, mammary, and prostate cancers [83]. This demonstrates that changes to the microbiome may play a significant role in the development of cancer. It has been questioned whether the decline in the usage of immune checkpoint inhibition (ICI) in people with lung cancer is due to the microbiome in the intestinal axial direction or a more straightforward anti-neoplastic impact [83]. Examples of monoclonal antibodies used in the therapy of cancer include programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) [85–88]. A T fibroblast immune response occurs when ICIs are administered, primarily as a consequence of the blockage of connections between T cell-suppressing receptors. Similar receptors were found on tumor cells [89]. Conversely, it is known that melanoma cells that express PD-L1 cause active CD8⁺ lymphocytes that are cytotoxic to activate and kill the cancer cells [89] and that an anti-human PD-L1 monoclonal antibody can block the above engagement. According to recent studies, the microbiome may significantly affect the effectiveness of cancer immunotherapy [89–93]. In relation to immune checkpoint inhibitors, animal studies have highlighted the significance of the intestinal microbiota, demonstrating that it affects the effectiveness of cancer immunotherapy targeting PD-1 and CTLA-4 [94]. Surprisingly, oral administration of *Burkholderia cepacia* or *Bacteroides thetaiotaomicron*, along with biological properties, improved anti-CTLA-4 immunotherapy [94]. Additionally, oral *Bacillus* bacterial contamination seems to increase tumor sensitivity to anti-PD-L1 treatment and ICI therapy in mice by encouraging dendritic cell maturation and anti-tumor CD8⁺ cytotoxic T cell activity [89].

The effectiveness of PD-1-based immunotherapeutic appears to be significantly influenced by this gut-lung axis [95]. Poorer outcomes with ICIs have been linked to earlier antibiotic usage, which may be due to alterations in the gut wall, microbiota of the feces, and the immune system's anti-tumor response [95]. By decreasing the variety of the microbiota, the dysbiosis brought on by antibiotics may lead to ICI resistance [96]. A gut microbiota with high diversity promotes the development of Th1 lymphocytes and M1 macrophages, as well as the proliferation of supportive and cytotoxic T cells, and also an increase in PD-1 transcription on lymphocytes, according to a prior study. To improve clinical outcomes with ICIs, controlling antibiotic-related dysbiosis with the composition of the gut microbiota may be a key strategy [96]. Regardless of traditional prognostic indicators in NSCLC, antibiotic usage predicted PD-1

or PD-L1 blockade resistance. In contrast to NSCLC patients who were sensitive to nivolumab, *Rikenellaceae*, *Prevotella*, *Streptococcus*, *Lactobacillus*, *Bacteroides plebeius*, *Oscillospira*, and *Enterobacteriaceae* have been found at low concentrations in control subjects, while *Akkermansia muciniphila*, *Bifidobacterium longum*, *Faecalibacterium prausnitzii*, and *Propionibacterium* were more prevalent. This work emphasized the crucial relationship between both the microbiota and ICIs by demonstrating how much the microbiota influences the immunological therapeutic efficacy [96]. Increased microbiome diversity enhances the effectiveness of immunotherapy by re-establishing the microbiome composition in cancer patients, which is promoted by antimicrobial treatment.

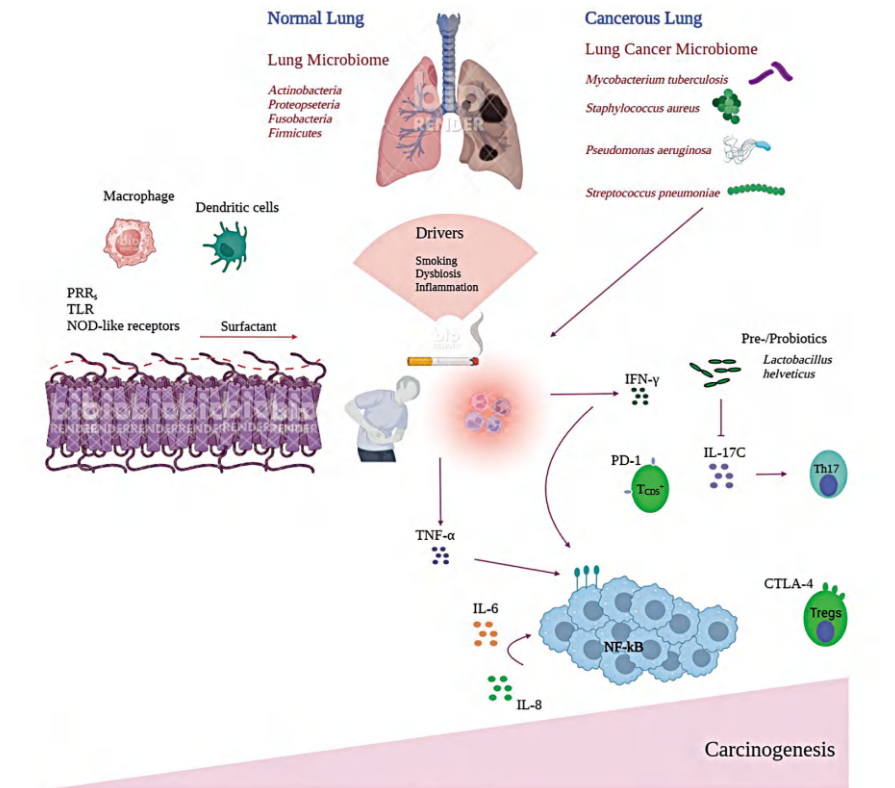


FIGURE 6.3 Demonstrates the relationship connecting carcinoma and the lung microbiome, including lung carcinoma carcinogenesis, etiology, and treatment. Cytotoxic CTLA-4 stands for T lymphocyte-associated protein 4. Chemical substances include interleukin, interferon gamma, nuclear factor of kappa-light chain enhancer of activated B cells, regulatory T cells, and NF-B. T helper 17 (Th17), PD-L1 (planned death ligand 1), PRRs (pattern recognition receptors), TLR (toll-like receptor), and TNF (tumor necrosis factor) are all related terms.

Source: Modified from Ref. [97]

6.9 PROBIOTICS' EFFECTS ON CHEMOTHERAPY IN LUNG CANCER PATIENTS: AN INVESTIGATIONAL STUDY

Chemotherapy is an unpleasant digestive side effect because it damages the intestinal mucosa. The prevalence of diarrhea is reduced in digestive illnesses like irritable bowel disease caused by *Clostridium butyricum*. The current study aimed to determine the precise mechanism of *C. butyricum* action in chemotherapy-treated cancer patients. The *C. butyricum* (CB) or placebo groups were randomly assigned to lung cancer patients, yielding 20 CB and 21 placebo participants. Blood and stool samples were taken on the first and last days of the three-week treatment and examined. The stool flora was analyzed using 16S ribosomal RNA sequencing. Overall, the diarrhea caused by chemotherapy was less severe in the CB group than in the control group. The fibrin ratio and white blood cell count (PLR), antibody levels, and changes in lymphocyte subsets were not significantly different. Overall variations in albumin (ALB) level and mass between the two groups were not particularly significant. After three weeks, there was a decrease in neither group's overall vegetation variety. The proportion of Firmicutes significantly decreased in the CB group compared to the placebo group, although phyla scarcely altered. Although there was no statistically significant difference between the two groups, in the CB group, the harmful genera began to decline, and the genera that produce short-chain fatty acids tended to increase, which was almost the exact reverse of what happened in the control group. The CB group had much more beneficial bacteria, including *Clostridium* and *Lactobacillus* species, compared to the placebo group. This was proven during a course study on operational taxonomy.

6.10 GASTROINTESTINAL MICROBES AND LUNG CANCER: FROM CARCINOGENESIS TO IMMUNOTHERAPY

The effect of intestinal flora on host physiology and illness, as well as the chemicals it produces, on adult immunological balance, has garnered enough study. Several studies have demonstrated that gut dysbiosis, or a change in the composition and operation of the gut microbiota, compromises the balance of the respiratory immune system and increases illness susceptibility. The intestines and lungs are the key sites of passage in this connection. Through genotoxicity, chronic inflammation, and other means, gut dysbiosis influences the growth and dissemination of lung cancer. The gut flora may provide a special diagnostic tool for evaluating the effectiveness and adverse outcomes of chemotherapy in lung cancer patients. In order

to more effectively prevent and treat lung cancer, research was carried out to examine the fundamental processes that control how gut microbiota affects immunology and carcinogenesis (Figure 6.4). It also offers novel methods for managing the bacteria in the intestines [98].

6.11 IMMUNOTHERAPY

Durability and diversity of the intestinal flora are thought to be biological indicators of a patient's likelihood to respond to anti-PD-1 therapy. Increased ICI effectiveness was linked to the presence of *Bifidobacterium longum*, *Alistipes putredinis*, and *Prevotella copri*. Metagenomic analysis of patient fecal samples revealed a correlation between the therapeutic benefits of ICIs and the presence of *Akkermansia muciniphila*. Anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, among other ICIs, are now the standard of care for a number of malignant tumors. Immune-related negative reactions (irAEs) have been associated with a number of medications, especially when used in combination. IrAEs, which include mucositis, pneumonia, colitis, fatigue, rash/itching, and diarrhea, can be present in 15 to 90% of patients. Apparently, clinical applications of ICIs in NSCLC patients might well be impacted mostly by the stability of gut bacteria brought about through the use of antibiotics. The gut microbiome may have some bearing on the general effectiveness of immunotherapy for lung cancer, as well as immunorelated toxicity and side effects. PD-1/PD-L1 blockade-treated patients with NSCLC who had

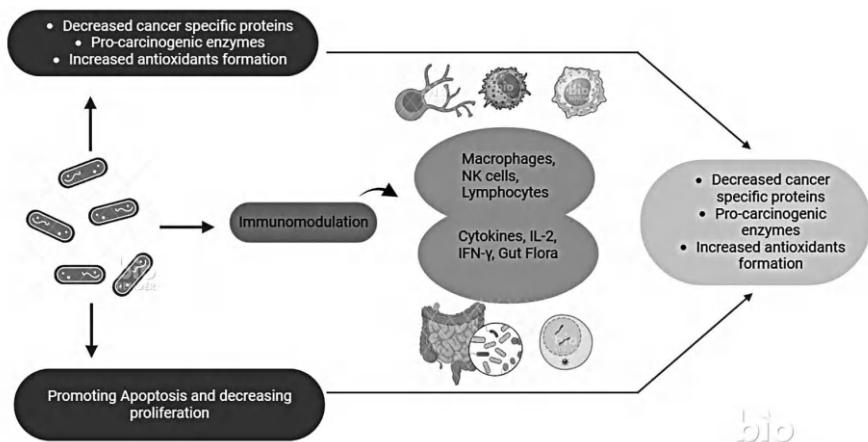


FIGURE 6.4 Probiotics responsible for minimizing lung cancer progression.

Source: Modified from Ref. [100]

previously used antibiotics had obviously worse survival rates, according to a meta-analysis that examined the effect of antibiotic use on survival. Giulia Galli et al. defined the antibiotic-immunotherapy exposures ratio (AIER) as “days of antibiotic agents of IO” throughout the immunotherapy treatment phase (WIOP) for patients with breast cancer [99].

6.12 TWO-HEADED COIN: PROBIOTICS

The intensity of lung problems can be lessened, and lung health can be preserved using probiotics. Probiotic-based remote control of respiratory homeostasis supports the idea that advantageous gut bacteria and lungs can regulate immune function. In mouse models infected with *Klebsiella pneumoniae*, *Lactobacillus plantarum* CIRM653 reduced the response of lung inflammation. However, because they may produce a variety of toxins and carry migratory antibiotic-resistant alleles, probiotics may pose a health concern. Several studies have uncovered numerous “pneumotypes” of humans. For instance, a prior study found a connection between a bacteriome rich in oral taxa (*Prevotella* and *Veillonella*) and the local inflammation that is dependent on Th17 and necessary for altering lung immune status in both healthy and pathological conditions [101].

6.13 THE IMPACT OF MICROBIOTA ON LUNG CANCER TREATMENT AND PREVENTION

Due to interaction with the surrounding environment, the lungs’ extensive mucosal surface is exposed to microbes, but the number of bacteria found there (5–8.25 log copies/mL) seems to be rather modest. The next genome microbiome could contain more than 90% nonviable microbes, according to research [102]. This finding corresponds with both the microbial biomass concentration found inside the lung region, which would probably be the consequence of a robust immune response that is primarily controlled by macrophages, and the capacity of the respiratory epithelium to vigorously remove microscopic organisms, as well as undesirable environmental conditions inside the respiratory tract for bacterial growth. The equilibrium of particular growth conditions, such as oxygen level, temperature, pH, nutrition, cell activity, inflammation, and localized bacterial competitiveness, contributes to the maintenance of this overall population of lung bacteria [103].

Although it is well accepted that the lung microbiome contributes to the development of many respiratory disorders, it is still unclear if there is a direct connection between the composition of the lung microbial population and homeostasis, and further study is probably required. Those with very different gut flora than those who don't may experience significant differences. The gut microbiota may have a significant influence on immunotherapeutics and modify immunostimulatory pathways by altering the generation of regulatory T cells. *Akkermansia muciniphila* supplementation boosts receptivity to anti-PD-1 treatment, and therapeutic resistance is linked to a disorganized microbiome.

The responsiveness to anti-PD-1 treatment is enhanced by the variety of gut flora in fecal matter microorganisms (*Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*). Nivolumab-responding patients with NSCLC had much more consistent and varied gut bacteria compositions. Additionally, research demonstrated that individuals who had elevated microbiome variety outlived those with lower microbial variety in terms of movement survival periods. Moreover, the gut microbiota affects how successfully chemotherapy treats lung cancer. In mouse models of lung cancer, for instance, orally ingested *Lactobacillus acidophilus* in combination with cisplatin therapy improved anticancer activity and success rates. Lung cancer patients' gut microbiotas were compared to those of healthy individuals. Fewer individuals from the probiotic genera *Blautia*, *Coprococcus*, *Bifidobacterium*, and *Lachnospiraceae* were present in each lung cancer category, and microbial biodiversity had reduced in each group. Increased yogurt intake has been associated with a 30% lower lung cancer risk, according to many investigations.

6.14 POSSIBILITY OF USING PROBIOTICS TO TREAT LUNG CANCER

Based on a scientific experiment and a plethora of studies, the probiotics found within the stomach are essential for both the prevention and treatment of different forms of tumors. These helpful microbes create compounds that affect distant organs like the lungs and control intestinal immunity. Hence, digestive problems that are present in lung diseases best illustrate the bi-directional relationship between the intestine and lungs (known as the Gut-Lung axis). According to recent research, bacterial load helps patients in a wide range of ways, notably reducing how people experience cancer

symptoms and minimizing the duration of the illness. Many probiotics have supposedly shown therapeutic promise in clinical trials, albeit it is entirely unclear how. The discussion of laboratory or clinical trials in the current chapter includes a remark on the use of probiotics and recent findings [102].

6.15 INFLUENCE OF PROBIOTIC USAGE ON IMMUNE CHECKPOINT: CLINICAL OUTCOMES

Clinical studies show that probiotics are strongly linked to better overall survival (OS) and progression-free survival (PFS) in non-small cell lung cancer (NSCLC) patients using immune checkpoint inhibitors (ICIs), indicating potential advantages of probiotic use during ICI treatment. Nevertheless, research data did not reveal a statistically significant link between probiotics and objective response rate (ORR). This may be partly attributable to the fact that only two studies—and their combined sample sizes—published the results of ORR; it's likely that the results may have been different had additional studies recruited participants for the prospective analysis. Probiotics were substantially linked to better OS and PFS in patients with NSCLC using ICIs, pointing to potential advantages of taking probiotics while undergoing ICI treatment. Nevertheless, our data did not reveal a statistically significant link between probiotics and ORR. Clinical trials have shown a strong relationship between probiotics and better OS and PFS in NSCLC patients using ICIs, pointing to potential advantages of probiotic use during ICI treatment. However, the investigation did not demonstrate a statistically significant connection between probiotics and ORR. This may be partly due to the fact that only two studies—and their pooled sample sizes—published the findings of ORR; it's likely that outcomes may have been different if more studies had recruited participants for the program. Probiotics were substantially linked to better OS and PFS in NSCLC patients using ICIs, pointing to potential advantages of taking probiotics while undergoing ICI treatment. Nevertheless, our data failed to find a statistically significant connection between probiotics and ORR.

Since it has been shown to have a direct role in the biology of tumors, including the process of transformation, tumor growth, and response to anti-cancer drugs, the microbiota of the gut is widely accepted to be crucial in the management of tumors. To fully comprehend how bacteria may impact the many characteristics of cancer, characterizing the intra-tumoral microbiota may be a crucial first step. Numerous studies have been conducted on the involvement of the commensally present microbiota in carcinogenesis related

to mucous membranes that come into contact with the outside environment, such as the lung. It is critical to focus more on how the microbiome may influence how the illness progresses in order to improve patient survival and treatment outcomes. However, it is widely acknowledged that lung cancer patients' intestinal microbiota differs significantly from that of healthy people and that lung cancer patients who respond to immunotherapy have a different intestinal microbiota than those who do not.

These findings suggest that lung cancer therapy and prognosis may be influenced by the gut microbiome. The gut microbiota (GLA) is a vital dialogue between our body's mucosal tissues that influences both health and illness, changes the gut environment, and potentially has a big impact on the etiology and treatment of diseases in other organ systems. The potential for treating diseases of the airways with specific intestinal probiotic strains is also attractive. In an attempt to find phyla or genera with probiotic qualities and single species connected to improved response to therapy and disease-free survival, studies have looked at the correlations between the relative abundance of specific genera of bacteria and inflammation-related blood indicators. Data confirming the observations of positive gut microbiota are highly associated with more bacterial family variety and better tumor prognosis, better treatment response, and disease-free survival. Data supporting all observations of favorable connections show that higher bacterial family diversity is highly associated with improved tumor prognosis, better treatment responsiveness, and disease-free survival of the gut microbiota.

The main objective is to return the entire bacterial microbiota to homeostasis and eubiosis. Even though many of the above-mentioned probiotics are still inaccessible for human consumption, encouraging a healthy lifestyle and diet is a daily practice that can affect microbiota composition. Through the careful selection of particular prebiotics, probiotics, and integrated post-biotics, the health of the host can be best promoted.

6.17 FUTURE PROSPECT

Probiotics have the best possibility of reviving organ tissues in the future, especially lung tissue that has already suffered partial damage or is anatomically inert. In addition, the following measures must be taken:

- Under some circumstances, the development of a probiotic adsorbent insertion process inside pleural fluid may be able to neutralize pleural fluid infection.

- Auto RTI (Respiratory Tract Infection) fight mechanisms development in organ transfer using probiotic slurry.
- The use of probiotic treatment in conjunction with bacterial quorum-sensing strategies.
- Key performance indicators (KPIs) for the industrial production of nutrient-formulated preparations and the switching off or altering of antibiotics will be probiotics.

KEYWORDS

- **Anti-inflammatory**
- **gut dysbiosis**
- **gut microbiota**
- **inflammation**
- **lung cancer**
- **lung carcinogen**
- **metabolites**
- **microbiome**
- **probiotics.**

REFERENCES

1. Chen, Z., et al. (2015). Global and regional burden of cancer in 2013, with projections to 2030. *Cancer Research*, 75(15), 2883–2897.
2. Toyoda, Y., et al. (2008). Prognostic factors in non-small cell lung cancer. *Journal of Thoracic Oncology*, 3(3), 251–256.
3. Islami, F., Torre, L. A., & Jemal, A. (2015). Global trends of lung cancer mortality and smoking prevalence. *Translational Lung Cancer Research*, 4(4), 327–338.
4. Alberg, A. J., et al. (2013). Epidemiology of lung cancer: Diagnosis and management. *The Lancet*, 382(9893), 187–197.
5. Kim, H., et al. (2014). Cigarette smoking and risk factors for lung cancer in Korea. *International Journal of Cancer*, 134(2), 480–486.
6. Kim, S. J., et al. (2014). Smoking and lung cancer in East Asian women. *Journal of Thoracic Disease*, 6(7), 1054–1060.

7. Viktorsson, K., et al. (2014). Molecular mechanisms of apoptosis resistance in cancer: The role of oncogenes and tumor suppressors. *Journal of Experimental & Clinical Cancer Research*, 33(1), 1–10.
8. Serban, D. E. (2014). Gastrointestinal microbiota and its role in the immune system: Probiotics as an adjuvant in cancer therapy. *Journal of Gastroenterology and Hepatology Research*, 3(6), 1042–1052.
9. Brestoff, J. R., & Artis, D. (2013). Immune regulation by commensal microbiota in the gastrointestinal tract. *Nature Reviews Immunology*, 13(5), 321–335.
10. Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science*, 336(6086), 1268–1273.
11. Yu, G., & Li, X. (2016). The human gut microbiome: A new frontier in personalized medicine. *Nature Medicine*, 22(1), 1–9.
12. Kumar, M., et al. (2010). Probiotics and cancer therapy: An overview. *Probiotics and Antimicrobial Proteins*, 2(3), 147–158.
13. Mitropoulou, G., et al. (2013). Probiotics and cancer: A critical appraisal of in vitro and in vivo studies. *International Journal of Molecular Sciences*, 14(4), 6336–6372.
14. Kandyliis, P., et al. (2016). The probiotic potential of dairy products and other foods. *International Journal of Food Microbiology*, 216, 82–88.
15. Sanders, M. E., et al. (2016). Probiotics and their role in the prevention of infections. *Journal of Clinical Gastroenterology*, 50(S2), S85–S89.
16. Bernal, V., & Llamas, A. (2012). The role of polysaccharides in probiotics. *Journal of Functional Foods*, 4(1), 78–86.
17. Bhat, M. I., et al. (2013). Bacterial strains in probiotics: A review of their beneficial effects. *Journal of Microbial Biotechnology*, 20(4), 299–310.
18. Panesar, P. S., Anal, A. K., & Kaur, G. (2022). Probiotic cultures and their challenges in the food industry. *Trends in Food Science & Technology*, 124, 68–82.
19. Krasakoopt, W., Bhandari, B., & Deeth, H. (2004). The encapsulation of probiotics: A review. *Food Biotechnology*, 18(2), 121–136.
20. Corona-Hernandez, R., et al. (2013). Encapsulation of probiotics for food applications: Challenges and opportunities. *Current Opinion in Food Science*, 2, 84–89.
21. Amos, C. I., et al. (2010). Genome-wide association study identifies novel lung cancer susceptibility loci in never-smoking women. *Journal of the National Cancer Institute*, 102(23), 1822–1831.
22. Berthiller, J., et al. (2008). A pooled analysis of case-control studies on the effect of tobacco smoking on lung cancer. *International Journal of Cancer*, 122(5), 1188–1194.
23. Cohen, A. J., et al. (2005). Outdoor air pollution and lung cancer: A systematic review and meta-analysis. *Environmental Health Perspectives*, 113(12), 1628–1634.
24. The effect of vitamin E and beta carotene on male smokers' risk of developing lung cancer and other malignancies (1994). *New England Journal of Medicine*, 330(15), 1029–1035.
25. Hashibe, M., et al. (2006). Passive smoking and risk of lung cancer: A meta-analysis of epidemiologic studies. *British Medical Journal*, 336(7635), 173–177.
26. Dracham, C. B., Shankar, A., & Madan, R. (2018). Radiotherapy for lung cancer: Present and future. *Lung Cancer*, 7, 141–149.
27. Ghias, N., et al. (2021). Occupational exposure to asbestos and the risk of lung cancer. *European Respiratory Journal*, 58(3), 2100169.

28. Kaufman, D. W., et al. (2008). The risk of lung cancer in individuals with asbestos exposure. *Journal of Occupational and Environmental Medicine*, 50(7), 776–781.
29. Kaufman, D. W., et al. (2008). The relationship between marijuana use and lung cancer risk. *Journal of the National Cancer Institute*, 100(8), 559–566.
30. Malhotra, J., et al. (2016). Air pollution and the risk of lung cancer: A meta-analysis of studies. *American Journal of Epidemiology*, 183(9), 1–9.
31. DeVita, V. T., Lawrence, T. S., & Rosenberg, S. A. (2008). *Cancer: Principles and Practice of Oncology*. Lippincott Williams & Wilkins.
32. Araujo, L. H., et al. (2020). Smoking, mutations, and lung cancer: An exploration of mechanisms. *Journal of Thoracic Oncology*, 15(3), 304–312.
33. Gazdar, A. F. (2009). DNA repair genes and the development of lung cancer. *Journal of the National Cancer Institute*, 101(7), 441–443.
34. Board, P. G. (2022). Genetic susceptibility to lung cancer. *Oncology Reviews*, 7(2), 33–44.
35. Richardson, L. A., et al. (2003). p53 gene mutations and lung cancer. *Journal of Thoracic Oncology*, 8(7), 517–528.
36. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers (1994). *New England Journal of Medicine*, 330(15), 1029–1035.
37. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5), 646–674.
38. MacDonald, T. T. (2007). The immunology of inflammation and cancer. *Journal of Experimental Medicine*, 204(11), 2569–2574.
39. Zahorec, R. (2001). Ratio of neutrophil to lymphocyte counts: A simple parameter for monitoring systemic inflammation and predicting the outcome. *British Journal of Haematology*, 123(3), 476–479.
40. Sarraf, K. M., et al. (2009). Inflammation and cancer: Glasgow prognostic score. *Clinical Cancer Research*, 15(4), 1562–1569.
41. Del Prete, M., et al. (2011). Systemic inflammation and prognosis in lung cancer: The role of neutrophil-lymphocyte ratio. *Cancer*, 117(18), 4135–4141.
42. Lu, H., Ouyang, W., & Huang, C. (2006). Inflammation, a key event in cancer development. *Molecular Cancer Research*, 4(4), 221–233.
43. Baghban, R., et al. (2020). Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling*, 18(1), 59.
44. Hinshaw, D. C., & Shevde, L. A. (2019). The tumor microenvironment innately modulates cancer progression. *Cancer Research*, 79(18), 4557–4566.
45. Zhang, Y., et al. (2019). The tumor microenvironment and immunotherapy. *Frontiers in Cell and Developmental Biology*, 7, 152.
46. Greten, F. R., & Grivennikov, S. I. (2019). Inflammation and cancer: Triggers, mechanisms, and consequences. *Immunity*, 51(1), 27–41.
47. Christofi, T., et al. (2019). Cancer-associated fibroblasts in the immunosuppressive tumor microenvironment. *Frontiers in Immunology*, 10, 1838.
48. Gelfo, V., et al. (2020). Roles of IL-6 in cancer progression: Challenges and opportunities. *Journal of Cancer Metastasis and Treatment*, 6, 23.
49. Mantovani, A., et al. (2019). Cancer-related inflammation. *Nature*, 457(7225), 798–804.
50. Zhang, M., Borcherdig, N., & Kolb, R. (2020). Cancer-associated fibroblasts: The architects of tumor immune evasion. *Frontiers in Immunology*, 11, 593221.

51. Cardenas, M., et al. (1997). The role of the immune system in chronic inflammation and cancer progression. *Cancer Immunology and Immunotherapy*, 45(2), 95–105.
52. Skillrud, D. M., Offord, K. P., & Miller, R. D. (1986). Higher risk of lung cancer in non-smokers with chronic bronchitis. *American Review of Respiratory Disease*, 133(4), 505–509.
53. Nomura, A., et al. (1991). Tuberculosis and the risk of lung cancer in humans: A review of the evidence. *European Respiratory Journal*, 4(5), 493–498.
54. Saetta, M., et al. (2001). Chronic obstructive pulmonary disease and risk of lung cancer. *American Journal of Respiratory and Critical Care Medicine*, 163(5), 1290–1295.
55. Vestbo, J. (2007). The relationship between COPD and lung cancer. *Respiratory Medicine*, 101(5), 945–949.
56. Mannino, D. M., et al. (2003). Chronic obstructive pulmonary disease and mortality in a cohort of United States adults. *American Journal of Respiratory and Critical Care Medicine*, 168(7), 695–700.
57. Loganathan, R. S., et al. (2006). A reappraisal of the risk factors for lung cancer in nonsmokers. *Journal of Clinical Oncology*, 24(8), 1229–1233.
58. Alberg, A. J., Ford, J. G., & Samet, J. M. (2007). Epidemiology of lung cancer: ACCP evidence-based clinical practice guidelines. *Chest*, 132(3_suppl), 29S–55S.
59. Brenner, D. R., et al. (2001). The role of p53 gene mutations in the pathogenesis of lung cancer. *Journal of Thoracic Oncology*, 2(4), 397–402.
60. Masferrer, J. L., et al. (2000). Cyclooxygenase-2 expression in human lung cancer and preinvasive lesions. *Cancer Research*, 60(6), 1516–1520.
61. Richardson, A., et al. (2003). Mechanisms of p53 mutation in cancer. *Frontiers in Oncology*, 3, 46.
62. Martins, D., Mendes, F., & Schmitt, F. (2021). Microbiome: A supportive or a leading actor in lung cancer? *Pathobiology*, 88(2), 198–207.
63. Williams, C. S., Mann, M., & DuBois, R. N. (1999). The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene*, 18(55), 7908–7916.
64. Lu, H., et al. (2004). EGFR mutations in non-small cell lung cancer: Prevalence and implications. *Molecular Cancer Research*, 2(7), 422–433.
65. Allavena, P., et al. (2008). The inflammatory micro-environment in tumor progression: The role of tumor-associated macrophages. *Critical Reviews in Oncology/Hematology*, 66(1), 1–9.
66. Cendrowicz, E., et al. (2021). Inflammatory mechanisms in cancer pathogenesis. *Immunology Letters*, 232, 59–67.
67. Wang, D., et al. (2021). Programmed death ligand-1 and immunotherapy for lung cancer. *Cancer Research*, 81(9), 2268–2280.
68. Gerthoffer, W. T., et al. (2012). Chronic inflammatory diseases increase cancer risk and promote tumor progression. *Journal of Experimental & Clinical Cancer Research*, 31(1), 4.
69. Boutilier, A. J., & ElSawa, S. F. (2021). Macrophage polarization states in the tumor microenvironment. *International Journal of Molecular Sciences*, 22(13), 6995. DOI: 10.3390/ijms22136995.
70. Porta, C., et al. (2009). Cellular and molecular pathways linking inflammation and cancer. *Immunobiology*, 214(9–10), 761–777. DOI: 10.1016/j.imbio.2009.06.014.
71. Conway, E. M., et al. (2016). The role of tumor-associated macrophages in cancer progression. *Nature Reviews Cancer*, 16(12), 751–764.

72. Le Noci, V., et al. (2018). Gut microbiota modulation to improve immunotherapy responses in breast and lung cancer. *Journal of Clinical Oncology*, 36(15_suppl), e15159.
73. Wang, L., et al. (2021). Programmed death ligand 1 and programmed death 1 expression in tumor-associated macrophages: Correlation with prognosis in non-small cell lung cancer. *Cancer Immunology, Immunotherapy*, 70(4), 1239–1251.
74. Huang, Y. J., & Huang, Y. (2022). The lung microbiome in health and disease. *European Respiratory Journal*, 60(1), 2200200.
75. Zhou, L., & Chen, H. (2022). The role of lung microbiota in lung cancer development. *Translational Lung Cancer Research*, 11(6), 1084–1096.
76. Ocariz-Diez, R., et al. (2020). The lung microbiome and its implications in lung cancer. *Cancer Research*, 80(18), 4046–4056.
77. Mendoza, J. L. (2019). The role of the microbiome in cancer therapy. *Cancer Discovery*, 9(5), 546–548.
78. Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, 9(9), 1021.
79. Picardo, S. L., Coburn, B., & Hansen, A. R. (2019). The microbiome and lung cancer treatment. *Journal of Thoracic Disease*, 11(S1), S24–S28.
80. Gui, Q., et al. (2015). The role of probiotics in cancer prevention and treatment: A review. *Critical Reviews in Food Science and Nutrition*, 55(7), 1016–1022.
81. Derosa, L., et al. (2018). Gut bacteria composition and response to immune checkpoint inhibitors in cancer patients. *Science*, 359(6371), 1366–1370.
82. Wojas-Krawczyk, K., et al. (2019). Probiotics and their potential use in lung cancer treatment. *Frontiers in Oncology*, 9, 53.
83. Stancu, A. M. (2018). Antibiotic use and cancer: A review. *Journal of Thoracic Disease*, 10(Suppl 23), S2762–S2765.
84. Galli, G., et al. (2019). The impact of antibiotic-immunotherapy exposures ratio on the outcome of breast cancer patients. *Cancer Immunology, Immunotherapy*, 68(12), 2225–2232.
85. Liu, J., et al. (2021). Anti-PD-1/PD-L1 therapies in lung cancer. *Lung Cancer*, 153, 55–65.
86. Galli, G., et al. (2019). Microbiome modulation as a novel therapeutic approach in cancer. *Current Opinion in Oncology*, 31(4), 242–248.
87. Mortaz, E., et al. (2013). The lung microbiome and the effectiveness of immunotherapy. *Current Respiratory Medicine Reviews*, 9(3), 164–173.
88. Jiang, X., et al. (2019). Anti-PD-1/PD-L1 therapy and its role in lung cancer. *Lung Cancer*, 129, 45–53.
89. Redman, J. M., Ward, R. J., & Phillips, G. K. (2014). The role of the PD-1/PD-L1 pathway in the immune response. *Journal of Thoracic Oncology*, 9(12), 1624–1635.
90. Costantini, L., et al. (2019). The role of the gut microbiome in lung cancer: Current knowledge and potential therapeutic implications. *Cancers*, 11(9), 1313.
91. Yu, Y., et al. (2016). Microbiota and immunotherapy: Potential therapeutic implications. *Frontiers in Immunology*, 7, 505.
92. Ramírez-Labrada, A. G., et al. (2020). The role of the microbiome in cancer immunotherapy. *Nature Communications*, 11, 4161.
93. Botticelli, A., et al. (2018). The gut microbiota and cancer: From prevention to immunotherapy. *Immunotherapy*, 10(8), 735–745.

94. Pitt, J. M., et al. (2016). Targeting the microbiome to improve cancer immunotherapy. *Cancer Research*, 76(21), 6035–6046.
95. Botticelli, A., et al. (2018). The gut microbiome and cancer immunotherapy: Prognostic implications. *International Journal of Molecular Sciences*, 19(12), 4066.
96. Pitt, J. M., et al. (2016). Targeting microbiota dysbiosis to enhance cancer immunotherapy efficacy. *Nature Reviews Immunology*, 16(8), 456–466.
97. Martins, D., Mendes, F., & Schmitt, F. (2021). Microbiome: A supportive or a leading actor in lung cancer? *Pathobiology*, 88(2), 198–207.
98. Mortaz, E., et al. (2013). Microbiome dysbiosis and lung cancer: Implications for immunotherapy. *Respiratory Medicine*, 8(5), 431–437.
99. Ramírez-Labrada, A. G., et al. (2020). Targeting the microbiome to improve cancer immunotherapy. *Nature Communications*, 11(1), 4161.
100. Sharma, A., Viswanath, B., & Park, Y.-S. (2018). Role of probiotics in the management of lung cancer and related diseases: An update. *Journal of Functional Foods*, 40, 625–633.
101. Dickson, R. P., & Huffnagle, G. B. (2015). The lung microbiome: New principles for respiratory bacteriology in health and disease. *PLoS Pathogens*, 11(7), e1004923.
102. Gebrayel, P., et al. (2022). The human microbiome in lung cancer: A potential biomarker and therapeutic target. *Cancer*, 128(15), 2814–2823.
103. Wan, Y., et al. (2023). Lung microbiome and cancer: Progress, challenges, and potential therapies. *Journal of Thoracic Oncology*, 18(1), 115–128.



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CHAPTER 7

Importance of Synbiotic for the Management of Colorectal Cancers

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ABSTRACT

Colorectal cancer (CRC) is the third leading cause of death globally. The enormous rise in the number of CRC cases observed in the last decade is evident in cancer statistics. The lack of nutrients from food, intake of heavy metals and chemicals through vegetables, fruits, and other food items, a sedentary lifestyle, mutations in genes, and DNA damage accelerate the chances of CRC. CRC is also known as bowel cancer, which initiates in the colon and extends to the rectum. CRC is characterized by frequent bowel activities, including diarrhea, constipation, blood in the stool, abdominal cramps and pain, a feeling of incomplete bowel movements, and sometimes weakness or fatigue. Currently, certain well-known biomarkers have been recognized that predict occurrences of CRC, including Micro-RNA, BRAF, KRAS, PI3K, TP53, and stool biomarkers. Moreover, FOBT, FIT, and colonoscopy are the screening techniques for the marked reduction of severe progression of CRC. Synbiotics are comprised of a combination of prebiotics and probiotics that work synergistically to minimize the chances of cancer

and other diseases. Synbiotics are responsible for modulating the colonic microbiome, which results in improved metabolic functions. Moreover, the prompt minimization of exposure to tumor-inducing agents and reduced cell proliferation are contributed by synbiotics. Synbiotics show antioxidant and immunomodulatory characteristics, thereby reducing the chances of CRC. The well-balanced composition of human gut microbiota contributes significantly to the minimization of chances of CRC. Moreover, the generation of SCFA, the neutralizing capability of several enzymes responsible for inducing tumors, and the suppression of inflammation as well as pathogen activity also help prevent CRC. Certain synergistic combinations reported for the prevention of CRC include *Bifidobacterium* and *Lactobacillus*, as well as *Lactobacillus gasseri* 505 and *Cudrania tricuspidata* leaf extract.

7.1 INTRODUCTION

Cancer is regarded as the second largest disease affecting the health and mortality of a huge number of people in the world [1]. The uncontrolled development and proliferation of cells inside the body result in the initiation of cancer. The high mortality rates in cancers are due to a lack of diagnosis at the initial level for unobserved symptoms [2]. In the previous two years of the pandemic (January 2020 to 2022), due to the widespread Covid-19 virus, fewer cancer cases were recorded, either due to fear or lockdown conditions, which later resulted in a significant increase in the stage of cancer. These conditions were responsible for high mortality rates due to the widespread of tumor cells throughout the body [3]. According to the cancer statistics data published by the American Chemical Society (ACS), the new cases of colon cancer registered in 2021–22 were 106,180 and 44,850 for rectal cancers. An approximately equal number of colon cancer cases were observed in males (54,040) and females (52,140), while mortality rates were quite high in males (28,400) compared to females (24,180) [4]. The third emerging type of tumor with a high death rate in the United States is colorectal cancer (CRC). It was observed in both sexes, with a five-year survival rate of sixty-three percent for patients without metastatic illness and ten percent for those with metastatic disease. Over 80% of these neoplasms are sporadic, emerging from adenomatous polyps through the accumulation of mutations in genes like APC, K-ras, and TP53 over time [5]. Diet plays a significant role in CRC risk, suggesting that the disease's risks may be reduced [6].

The medical appearance of CRC patients was assessed by the site, size, and the existence or nonappearance of tumor progression. The notable symptoms

include abdominal pain, alterations in prolonged bowel habits, fluctuations in bowel movements, unintended weight loss, nausea, vomiting, malaise, and abdominal tightness [7] [8]. In contrast to proximal malignancies, which can generate mixed blood in the stool, distal cancers cause obvious rectal bleeding. As a result, anemia may present as a subsequent symptom.

The inhibitors or traditional methods, known as mutators, are included in the carcinogenic models of CRC. The chromosomal instability pathway, which follows a pattern of adenoma progression in 80% of sporadic colorectal cancer, and the microsatellite instability pathway, which includes a wide variety of gene mutations found in 20% of sporadic cancer and 80% of hereditary colorectal cancer, primarily in the APC (60%), p53 (70%), DCC (70%), and KRAS (40%) [9].

Sometimes, CRC is referred to as colon, rectal, or bowel cancer, depending on the region where it originates. The digestive system of the body constitutes the large intestine, which is comprised of two sections: the colon and the rectum. The colon is recognized as a long muscular tube (1.5 m), and the total area is divided into three compartments: ascending, transverse, descending, and sigmoid colon, respectively [10]. The main function of the colon is the absorption of fluids and electrolytes from food, while the remaining waste materials pass into the rectum and are finally released through the anus.

The initiation of growth during CRC inside the cells of the colon and rectum resulted in the development of unwanted growth cells known as polyps. These polyps, greater than three in number and longer than 1 cm, later on mostly convert into CRC. Three types of polyps have been identified in the colorectal region, namely adenomatous, hyperplastic, and inflammatory sessile serrated polyps. Among them, adenomatous polyps are mostly responsible for the induction of CRC [3].

7.2 EPIDEMIOLOGY

CRC can be triggered by sporadic or non-hereditary factors such as DNA mutations, transcriptional silencing of tumor suppressor genes, and dysregulation of genes involved in the cell cycle, repair, and apoptosis. Genetic factors, including mutations in tumor suppressor genes like APC, DCC, BRAF, PIK3CA, AKT, and TP53, or the activation of oncogenes such as K-RAS and CTNNB1 [11], also contribute to CRC development. Sporadic CRC is often caused by activation of the K-RAS oncogene on chromosome 12, with mutations occurring predominantly in codons 12 and 13 (95% of cases), while only 5% are found in codons 61, 146, and 154, mainly seen in

metastatic cases. Mutations in codon 12 are associated with mucinous cancer, whereas codon 13 mutations are linked to non-mucinous cancer, which is highly aggressive and more prone to metastasis [12]. Metastasis-protective genes, such as *KISS1*, which binds to the KISS1R receptor, play a crucial role in metastasis control. Elevated levels of KISS1 and KISS1R have been shown to significantly increase survival rates by inhibiting metastasis [13]. Table 7.1 summarizes the gene mutation and function.

TABLE 7.1 Gene Mutation and Function [9]

Gene	Role	Mutation
APC	Organizes a protein that inhibits the cancerous cells and worked as an opponent of Wnt signal pathway. immigration and cellular bonding.	CRC
DCC	Codifies netrin-1 protein, Tumor suppressor	CRC, Esophageal cancer
TP53	Tumor suppressor	CRC
BRAF	Map/Kinase pathway, related to cellular division and differentiation and secretion.	CRC, Non Hodgkin lymphoma, Malignant melanoma, Thyroid cancer, Lung carcinoma of non-small cells
KISS1	Metastasis suppressor by joining KISS1R	CRC
AKT1	Signal pathway, oncogenesis, cellular proliferation, cellular survival apoptosis and angiogenesis. Codifies serine and threonine kinase PKB	CRC
SMAD4	Pathway of growing beta transforming factor (TGF- β), controls the adhesion of DNA for particular genes and suppressor genes.	Polyposis syndromes, Colon cancer, Pancreas cancer
K-RAS	Oncogene Codifies KRAS, a GTPASE include in the cellular division diversity and apoptosis.	CRC
CTNNB1	Cellular adhesion essential for formation and preservation of epithelial layers for directive of cellular growth.	CRC, Medulloblastoma Ovary tumor
P53	Tumor suppressor, stopping the cellular cycle, apoptosis and DNA repair.	Hereditary tumor
PIK3CA	Codifies p110 alpha working as a kinase in cellular pathway, cellular proliferation division, migration and cellular survival.	CRC, Ovary cancer, Breast cancer, Stomach cancer, Lung cancer, Brain cancer

7.2.1 SIGN AND SYMPTOMS FOR CRC

- Alterations in bowel habits and sometimes includes constipation, and diarrhea.

- Occurrence of blood in stool.
- Abdominal pain, bloating and cramps.
- Unsatisfactory feeling of incomplete bowel passage.
- Weakness, fatigue followed by weight loss.
- Iron deficiency [14, 15].

7.2.2 STAGES OF CRC

Depending on the progress of the proliferation of polyps, there are the following stages of CRC.

Stage 0: This is the beginning stage, where polyps are present in the innermost region of the colon or rectum.

- **Stage I:** Polyps or tumor cells are formed and growing rapidly inside the colon or rectum but have not spread to the outer boundary wall of the colon and rectum.
- **Stage II:** The tumor cells cover most of the area of the colon and rectum but have not spread to the lymph nodes.
- **Stage III:** After completely covering the overall area of the colon and rectum, the tumor cells show evidence in the lymph nodes.
- **Stage IV:** These tumor cells have started to proliferate and spread to most of the other body parts or organs [16].

7.2.3 RISK FACTORS

The rapid rise in the worldwide burden of CRC is multifaceted, with specific hereditary disorders and environmental variables, such as dietary behaviors and lifestyle modifications, including excessive meat and fatty food consumption, increased alcohol intake, cigarette use, and obesity, being the main contributors. Carcinogenic chemicals and mutagens cause a succession of precise histological alterations (the adenoma-carcinoma sequence), which are accompanied by mutational instigation of malignancy and loss of heterozygosity of cancer suppressor genes.

This occurs when the balance between DNA damage and DNA repair is disrupted, resulting in cell progeny with mutagenic and/or unrepaired DNA containing variations that evade the DNA repair process. It starts in the inner lining of the small intestine and extends to the inner lining, muscular tissue, and other organs, causing metastasis. Colorectal cancer risk is determined by

the potency of carcinogens, the rate of exposure, and the individual's genetic makeup [17].

Diet has long been connected to CRC occurrence, as evidenced by global differences in CRC incidence and the comparatively quick changes in risk among immigrant communities in the United States. Folate consumption, whether from food or supplements, appears to have a complicated association with CRC risk, potentially promoting tumor growth while preventing tumor formation in healthy tissue.

1. **Age factor:** Currently, any type of cancer can be detected at any age group. In case of colon cancer, it was mostly observed in person above the age of 50 years.
2. **Racism:** The chances of occurrence of CRC was higher in American and African persons comparatively with other countries.
3. **Family history:** The person suffered with CRC have higher chances for occurrence of cancer in their next generations.
4. **Inflammatory conditions:** Persons suffering from swelling conditions in the gastrointestinal tract have a higher chance of developing CRC. Moreover, the presence of long-term diseases such as IBS, ulcerative colitis, Crohn's disease, etc., increases the likelihood of CRC.
5. **Diet:** A fatty diet and high carbohydrate intake increase the risk associated with CRC. In contrast, a diet containing high fiber minimizes the occurrences of CRC. Additionally, it has also been noted that individuals consuming high amounts of red meat have a higher incidence of colon cancer.
6. **Lifestyle:** A lack of physical exercise and activity can lead to the development of CRC. A healthy lifestyle can minimize the chances of CRC.
7. **Diseases:** The existence of certain diseases such as diabetes mellitus and obesity can predispose individuals to the initiation of CRC.
8. **Faulty habits:** Smoking and drinking may accelerate the chances of CRC.
9. **Medication:** Chronic use of certain medications, as well as exposure to radiation, also increases the risk factor for CRC [18].

7.3 HUMAN GUT MICROBIOTA (HGM).

The human gut is recognized as one of the most complex and dynamic communities of microbes designated as microbiota [19]. The microbiota comprises around 100 trillion microbes, including bacteria, viruses, fungi, archaea, phages, yeast, and protozoa [20]. The genome sequencing of microbes contains approximately 3×10^6 genes, which is considered to be 150 times the length of the mammalian genome [21]. The encoding genes by microbiota are referred to as the microbiome [22]. The most abundantly observed bacteria in the gut microbiota belong to phyla that include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*, among others [23].

The composition of gut microbiota slightly differs from individual to individual and becomes stagnant after a particular age. The changes in the progress of microbiota result from their interactions with the physiological processes of the body and the availability of microbes [24]. The gut microbiota contributes significantly to the regulation of health aspects of individuals. Its prime roles include the modulation of biotransformation, immunity, and homeostasis. The absorption of nutrients from ingested foods is also carried out by the gut microbiota [23]. Moreover, microbiota extensively contributes to reducing the chances of several diseases by creating vital metabolites and thereby promoting a healthy lifestyle. Additionally, the monitoring of the growth of several pathogenic microbes and their irradiation is also performed by the microbiota [25]. Routinely, the conversion of complex polysaccharides into simple monomers is also performed by the microbiota [19].

Conferring to the recommendations of “International Agency for Research on Cancer” (IARC), certain typical species of microbes are seems to be carcinogenic namely “*Helicobacter pylori*, *Hepatitis B and C virus*, *HIV type I virus*, *Human papillomavirus*, *Epstein-Barr virus*, *Human herpes virus type 8*, *Human T-cell lymphotropic virus*, *Opisthorchis viverrini*, *Clonorchis sinensis*, and *Schistosoma haematobium*,” etc. [26]. Any alteration in the microbial compositions are termed as dysbiosis.

7.4 GUT MICROBIOME AND CRC

The number of cancer patients is rising very sharply and is predicted to reach up to 24 million by 2030. The large intestine, comprised of the gut microbiome, is regarded as the most complex community in human beings. The well-known bacterial species responsible for the induction of colorectal

cancer (CRC) are Firmicutes (Gram-positive), Bacteroidetes, and Proteobacteria (Gram-negative) [22]. It is assumed that CRC is initiated from the polyps, precursors of adenoma and serrated type [27]. The contact between body tissues and carcinogens mostly results in the development of cancer. Various environmental factors contribute greatly to the occurrence of CRC. Moreover, several reported case studies have also revealed that dysbiosis is the prime cause of the initiation of CRC. Bacterial secretions or metabolic products are also well-known causes of CRC.

Additionally, during dysbiosis of the gut, several bacteria affect the neighboring cells or tissues, thereby inducing inflammation. These types of chronic inflammation further result in tumor formation. Additionally, due to the release of inflammatory cytokines, impairment of DNA occurs, and upon continuation, this results in metastasis. The collection of samples from the gut and fecal matter indicated the initiation of tumor formation, confirmed by 16S rRNA sequential estimation [25].

Mutation is another reason for occurrences of CRC. The mutation of the adenomatous polyposis coli (APC) gene provokes tumor generation in the colon, which later results in metastasis. Another classical example of mutation is observed in “Knudson’s two-hit hypothesis,” which resulted in the development of CRC due to gene mutation [28][29]. Sometimes, rapid interactions occur between colon epithelial cells (CEC) and microbiota, resulting in the destruction of DNA and increasing the chances of CRC. Furthermore, certain species of bacteria are recognized as genotoxic due to their production of toxins, namely colibactin, which is responsible for the destruction of the DNA helical structure and cell arrest, e.g., pks and *E. coli* [28].

Diet plays a pivotal role in both the prevention and occurrence of CRC. Most of the diet and essential nutrients get absorbed in the intestine and are further circulated for vital functioning in the body. A diet consisting of a high amount of fibers and proteinaceous materials is constituted in the colon. Depending on the quantity of fatty materials, the liver secretes bile acid. Upon digestion, the fibers are converted into fatty acids containing small chains. These fatty acids are capable of producing colonocytes, which possess antitumor, anti-inflammatory, immunomodulatory, antiproliferative properties, and maintain homeostasis as well as genetic regulation. In contrast to a balanced diet, excessive consumption of fatty foods (Western culture) leads to the fermentation of foodstuffs and thereby the deconjugation of bile acids. These reactions accelerate the harmful effects on the colon by damaging it and producing inflammation with tumor formation, which later results in the development of CRC [30].

The GM contributed a dual character in human beings, depending on the composition responsible for the prevention of tumor formation as well as the induction of tumors. The presence of pathogenic microbes is responsible for inducing tumors, such as benign and malignant types. Studies illustrate that IL-10 is highly effective in the minimization and eradication of tumors due to its anti-inflammatory characteristics. The host lacking IL-10 in gut microbiota favors the chances for the initiation of cancer. The metabolic products of certain microorganisms are also the prime cause of inducing cancer. For example, *Helicobacter pylori* generates cytotoxin-associated gene A (CagA), which is carcinogenic in nature. Similarly, *E. coli* also produces colibactin and cytolethal distending toxin (CDT). The metabolites produced by these organisms cause distortion or damage to DNA strands and sometimes lead to apoptosis. Another classical piece of evidence was reported by *Shigella flexneri*, which resulted in damage to p53 genes and the double helical structure of DNA by releasing the component virulence gene A. The defense mechanism of the body significantly contributes to the eradication of tumor cells. Sometimes, certain organisms like *F. nucleatum* block the action of NK cells and thereby prevent the destruction of tumor cells.

At the same time, gut microbiota contributed significantly to the eradication of tumor cells from the body by activating innate and adaptive immunity. Currently, immunotherapy has gained tremendous significance for minimizing the occurrences of cancer and thus boosting the lifespan of an individual. An alkylating agent, cyclophosphamide, is recommended for the therapy of several types of tumors in the body and has shown its antitumor characteristics owing to the suppression of immunity. The marked antitumor characteristic is due to the triggering of Helper T cells. The reported microbiota species possessing antitumor activity are “*Branesiella intestinihominis*, *Enterococcus hirae*, and *Lactobacillus johnsonii*,” et al., Similarly, *Bifidobacterium longum* has been reported to stimulate dendritic cells, which work against tumor cells [25].

7.5 DIAGNOSIS OF CRC

Among the several cancers, CRC ranks in the top 3 and is hence associated with a large number of mortalities worldwide. Prompt diagnosis of CRC is of utmost importance in this era, as it will minimize not only the development of higher stages but also save the lives of individuals. The symptoms associated with CRC often resemble normal conditions, and hence many patients cannot utilize critical instrumental procedures for their recognition [31].

Currently, several novel biomarkers have been identified that can predict the initiation of CRC occurrence and are also found to be beneficial from the patient's perspective due to their non-invasive nature.

The dysbiosis of gut microbiota contributes significantly to the diagnosis of CRC. Preliminary screening of gut microbiota enhances the survival rate. The well-known screening methods employed are the "fecal occult blood test (FOBT)" and the "fecal immunochemical test (FIT)," which reduce the chances of CRC by 14% and 59%, respectively [32][33].

The most significant measures for preventing colorectal cancer are life-style changes, recognition of persons at risk, and elimination of pre-neoplastic lesions. Several medications, including aspirin and hormone replacement therapy, have shown a protective effect in randomized trials [15].

7.5.1 SCREENING FOR OCCULT BLOOD

CRC usually progresses over a long period of time. If adenomas are identified and eradicated before they grow into cancer, the disease can be avoided. Furthermore, colon cancer is almost always treatable if caught early. Compared to other malignancies, colorectal cancer screening and prompt recognition are effective secondary prevention methods for colorectal cancer and mortality. Screening programs can help reduce CRC morbidity and mortality. This has been established for occult blood detection in stool tests and flexible sigmoidoscopy. gFOBTs (guaiac fecal occult blood tests) and FITs (fecal immunochemical tests for hemoglobin) are non-invasive stool diagnostics. These assessments focus on either heme (gFOBTs) or human globin to identify tiny amounts of serum (FITs) [34, 35].

7.5.2 ENDOSCOPY

A single round of flexible sigmoidoscopy screening resulted in a reduction in colorectal cancer incidence (18–23%) and mortality (22–31%) in four randomized controlled trials. Colonoscopy is the primary diagnostic instrument, and it is the technique of choice for following up on stool tests and sigmoidoscopies. Colonoscopy combined with polypectomy has been shown in cohort studies to reduce the incidence and mortality of CRC by 68% in a recent study [36].

The appearance of interval malignancies following a previously negative screening colonoscopy is a serious concern. Colorectal cancers found within five years of the last colonoscopy, with either a deleterious result or excision

of all adenomas, are known as interval cancers. The incidence of colon cancer is caused by three key factors: i) advanced adenomas or cancer were overlooked during colonoscopy; ii) polypectomy was inadequate; and iii) rapid de novo carcinogenesis occurred [37]. The list of several biomarkers is depicted in Table 7.2.

TABLE 7.2 List of Biomarkers for the Diagnosis of CRC

Sr. No	Name of Biomarker	Characteristics	References
1	Micro-RNA	An abnormal concentration of micro-RNA with their up and down regulation indicated as biomarker for the diagnosis of CRC. Moreover, micro-RNA significantly contributed in the tumor formation, proliferation and metastasis.	[38–40]
2	sHLA-G	Human leukocyte antigen gene (HLA-G) is available in the form of soluble (sHLA-G), membrane (mHLA-G) and conjugated form with extracellular vesicles (HLA-Gev). The overexpression of all these forms in plasma served as prognostic biomarker for the CRC.	[41]
3	CDX2 expression	CDX2 is a homeobox protein liable for the preserving of intestinal phenotype. The down regulation of CDX2 was observed in 20% patients suffering with CRC. Hence, served as useful biomarker for CRC.	[42]
4	Long non-coding RNA (Lnc-RNA)	Studies revealed that upregulation of lnc-RNA in plasma observed during CRC and hence, served as biomarker.	[43]
5	Carcinoembryonic antigen (CEA)	These are membrane-bound glycoprotein usually overexpressed in gut epithelium. Further, several patients with CRC found elevated level of CEA.	[44]
6	BRAF	BRAF gene mutation served as prognostic biomarker for several type of cancers and identified 10% in CRC. These mutants are highly expressed in tumors and can be prognostic biomarker for CRC.	[45]
7	KRAS	These genes contributed in the cell growth and differentiation. Mutation observed in KRAS resulted in metastasis behavior.	[45]
8	Phosphatidylinositide-3-kinases (PI3K)	These enzymes are involved in cell modulation, proliferation and survival. Whereas, mutant type of the PI3K resulted in tumor formation and metastasis of CRC.	[45]

TABLE 7.2 (Continued)

Sr. No	Name of Biomarker	Characteristics	References
9	TP53 gene	These type of gene are responsible for regulation of cell cycle, apoptosis, and DNA repair. Any mutational changes associated with these genes resulted in development tumor. These mutants' genes observed around 60% in CRC patients.	[46]
10	Circulating cell-free DNA	These cells are received in plasma after apoptosis or necrosis. Normally, the length of these cells are 180 bp but tumor containing cells are slightly longer than normal cells. The quantitative estimation of these cells can be beneficial in the diagnosis of CRC.	[47]
11	Stool biomarkers	Estimation of stool DNA samples, miRNA, and methylated DNA served as good indicator for diagnosis of CRC.	[47]

7.6 SYNBIOTICS

These are characterized as a grouping of probiotics and prebiotics, serving as a material for the progress and development of beneficial bacteria that are either native to the colon or introduced. Synbiotics have lately gained popularity as a nutritional complement for avoiding both contagious and non-contagious diseases. This is a new topic in functional foods and nutraceuticals gaining popularity due to its numerous health benefits [48].

Synbiotics comprise prebiotics and probiotics intended to promote the “friendly flora” of the human intestine and are of economic value (“Syn” means “together” and “bios” means “life”). The primary reason for using synbiotics is that the gastrointestinal tract is occupied with a huge quantity and diversity of bacteria with specific nutrient needs (bifidobacteria), and it is possible to increase the amounts of these target bacteria by selecting specific foods or food ingredients [17].

Synbiotics promote probiotic organism growth by supplying the probiotic organism with a specific substrate for fermentation. As a result, the synbiotic concept was developed as a blend of probiotics and prebiotics that benefit the person by refining the persistence and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively encouraging the growth and/or activating the metabolism of one or a small number of health-promoting bacteria, and thus improving host welfare. A suitable single or multi-strain probiotic and a variety of prebiotics would make up an

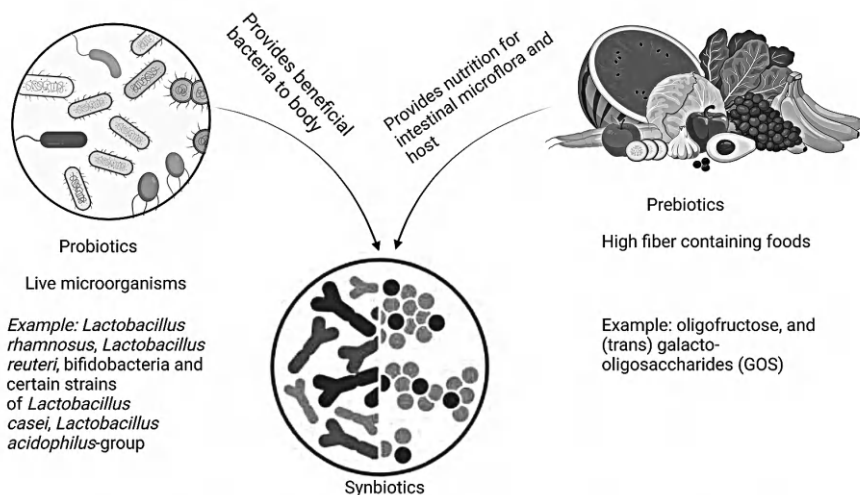
optimal synergistic synbiotic solution. Prebiotics encourage ingested probiotics, resulting in an additional or synergistic impact. They also promote the formation of endogenous good bacteria while lowering the amount of cancer-causing or pathogenic bacteria.

Bifidobacteria and fructo-oligosaccharides (FOS), Lactobacillus GG and inulin, and bifidobacteria and lactobacilli with FOS or inulin are some of the presently available and well-known synbiotic products on the market. FOS is a potential prebiotic since it is fermented by healthy gut bacteria.

Listing of common synbiotics

- Lactobacilli + lactitol.
- Lactobacilli + inulin.
- Lactobacilli + fructo-oligosaccharide or inulin.
- Lactobacillus rhamnosus GG + inulin.
- Bifidobacteria + fructo-oligosaccharide.
- Bifidobacteria + galacto-oligosaccharide.
- Bifidobacteria and lactobacilli + fructo-oligosaccharide or inulin [49].

Figure 7.1 illustrates fate of synbiotics.



Synbiotics= Probiotics + Prebiotics: Mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and activity of beneficial microorganisms in the gut

FIGURE 7.1 Fate of synbiotics.

7.6.1 GUT HEALTH AND SYNBIOTICS

The stomach is a common target for a variety of stressors such as sepsis, trauma, burns, shock, bleeding, and infection. Foreign substances, such as food particles and microbial antigens, attack the gut and cause infectious and multiple organ dysfunction syndrome; thus, GM plays a vital part in health and disease. Infectious problems such as colon cancer and others are triggered and aggravated by unbalanced gut bacteria, a damaged intestinal epithelium, a compromised immune system, and commensal bacteria. The disruption of normal gut flora may result in death. Synbiotics have been shown in research to improve gut flora. *In vitro* and *in vivo*, it has been discovered that combining prebiotics with probiotics like FOS and *Bifidobacterium* or lactitol and *Lactobacillus* stimulates the microorganisms' survival and activity. The survival, implantation, and growth of newly introduced probiotic strains are improved by certain synbiotic combinations. Synbiotics containing the *Bifidobacterium* brave strain Yakult and *Lactobacillus* as probiotics and galacto-oligosaccharides as prebiotics produced significantly higher levels of beneficial *Bifidobacterium* and *Lactobacillus*, as well as short-chain fatty acids (SCFA), and reduced the risk of communicable complications [50].

7.6.2 SYNBIOTICS IN COLORECTAL CANCER

Several studies suggest that altering the gut bacteria through these dietary changes may interfere with the carcinogenesis process, lowering the risk of colorectal cancer (CRC). Probiotics and prebiotics have been shown in epidemiological and experimental research in humans, as well as *in vivo* investigations, to reduce the risk of carcinogenesis by modifying bacterial enzyme activity, antigenotoxic effects, and precancerous lesions.

- When compared to individual dosing, a synbiotic combination of *Bifidobacteria* and oligofructose (FOS) has been shown to reduce colon tumorigenesis in rats.
- When inulin and *B. longum* were given together to azoxymethane (AOM)-treated Sprague Dawley rats, the total aberrant crypt foci (ACF) were reduced by 26% compared to when they were given separately. Consumption of *B. lactis* and resistant starch increased the apoptotic response to AOM in rats, which was likely due to the resistant starch acting as the substrate for the probiotic species' optimal action.

- A mixture of oligofructose-enriched inulin, *L. rhamnosus*, and *B. lactis* protects Peyer's patches from AOM-induced reduction of NK-cell activity, whereas individual components were ineffective.
- These findings suggest that synbiotics are important in the therapy of CRC. However, when given alone, prebiotics have shown conflicting results in carcinogen-induced ACF, which could be related to variances in the type of carcinogen/mutagen and treatment regimens [51].
- Recently, a synergistic combination of prebiotics consisting of leaf extract of *Cudrania tricuspidata* and probiotic *Lactobacillus gasseri* 505 showed protection against CRC and also demonstrated significant immunomodulatory and antioxidant action [52].
- The Greek company Elpen Pharma prepared synbiotics for CRC, which comprised 6 species of probiotics and one prebiotic, namely FOS [53].

7.6.3 GUT BARRIER FUNCTION ENHANCEMENT

Increased tight junction permeability, or gut barrier dysfunction, is a prevalent symptom of CRC. However, a leaky gut facilitates microbial translocation and promotes endotoxemia, resulting in cachexia. Tight junction protein deficiency has also been linked to EMT and metastasis in CRC. Several probiotic strains have been demonstrated to enhance gut barrier function by upregulating or normalizing tight junction protein expression, boosting mucin synthesis, decreasing inflammation, and promoting epithelial repair. Probiotics may have a favorable effect on CRC patients by restoring epithelial integrity [54].

7.7 USE OF PROBIOTICS IN DISEASE CONDITIONS

Although probiotics are usually seen as harmless and well-tolerated in fit people, their safety has been called into question in patients with underlying health issues. One of the most serious concerns is probiotic translocation, which is the introduction of live bacteria into extraintestinal locations and the subsequent systemic or localized illnesses. Although bacterial translocation can occur in healthy individuals, bacteria are generally sequestered and eliminated in the mesenteric lymph nodes by a healthy immune system, thus

there is little risk. Such physiological protection, on the other hand, may be disrupted in patients with a compromised intestinal barrier or a weakened immune system—both of which are clinical symptoms seen in cancer patients, making them vulnerable. Despite the fact that consumed probiotics have low-virulence and non-pathogenic qualities, several case reports of probiotic-associated bacteremia, fungemia, endocarditis, liver abscess, and pneumonia have been described [55].

7.7.1 MECHANISM OF PROBIOTIC BACTERIA MAY INHIBIT COLORECTAL CANCER

Probiotic strains' ability to prevent CRC is becoming well-documented. Intake of significant amounts of fermented milk products containing *Lactobacillus* or *Bifidobacteria* has been linked to a lower risk of colon cancer in some epidemiological research, but ingestion of fermented dairy foodstuffs was linked to little or no protection in other studies. The methods by which probiotics may protect against colon cancer are still being studied. Nevertheless, there is indication for altering the metabolic actions of intestinal microflora, altering physicochemical circumstances in the colon, binding of potential carcinogens, creation of SCFA, construction of anti-tumorigenic or anti-mutagenic compounds, uplifting the hosts' immune response, and shifting the hosts' physiology.

7.7.1.1 INHIBITION OF CONVERSION OF PROCARCINOGEN INTO CARCINOGENS

In comparison to *Bacteroides*, *Clostridia*, and *Enterobacteriaceae*, which mediate carcinogenesis, *bifidobacteria* and *lactobacilli* reduced the countenance of xenobiotic-metabolizing enzymes—glucuronidase, azoreductase, and other enzymes involved. The activity of these enzymes was reduced by some strains of *Lactobacillus acidophilus* and *Bifidobacterium* spp., lowering the risk of tumor growth. By reducing enzyme levels, short-chain fatty acids (SCFAs) generated from colonic non-digestible carbohydrate fermentation promote *lactobacilli* and *bifidobacteria* growth and limit the production of carcinogenic products from procarcinogens. Beneficial bacteria modulating the adaptation of procarcinogens to carcinogens is an additional fascinating field that requires more extensive research at the cellular and molecular levels [17, 56].

7.7.1.2 REDUCTION OF INTESTINAL PH

Lactic acid and other SCFAs are produced by probiotic bacteria as metabolic products of non-digestible carbohydrate fermentation in the gut. These SCFAs reduce pathobiont load, assist in sustaining homeostasis, and minor gut pH. It also helps to reduce bile acid solubility and ammonia absorption while increasing mineral absorption.

7.7.1.3 ACTIVATION OF IMMUNE SYSTEM OF HOST

At the systemic level, the defense system is complicated and acts and reacts locally. The directive of the immune system, as well as the connections inside and among the constituents of inflammatory cascades, has recently been the subject of investigation. Dissimilar indicators are utilized to investigate these cascades, and they are used to deal with environmental microbial threats as well as common and severe illnesses. In the early stages of cancer defense, dendritic cells (DCs) and natural killer (NK) cells are crucial. The effect of probiotics on carcinogenesis is discussed elsewhere; NK cells have been found to be operative against tumor cells, and reduced NK cell movement has been linked to a lesser cancer hazard. Oral administration of *L. casei* strain Shirota (LcS) to mice with methylcholanthracene-induced tumors improved host innate immunity by exciting splenic NK cell activity, resulting in a delay in tumor progression [57].

7.8 PREBIOTICS AND CANCER

“A selectively fermentable non-digestible oligosaccharide or substance that causes specific changes in the composition and/or activity of the gastrointestinal microflora, giving health benefits” is how the term “prebiotic” is defined. Fructooligosaccharides (FOS) and galactooligosaccharides (GOS) are the two most commonly reported prebiotics that are “Generally Regarded as Safe” (GRAS). Other oligosaccharides include xylooligosaccharides (XOS), isomaltoligosaccharides (IMOS), glucooligosaccharides, pectin oligosaccharides (POS), mannanooligosaccharides (MOS), gentiooligosaccharides (GTO), and chitooligosaccharides (CHOS), as well as soybean oligosaccharides [58].

7.8.1 PREBIOTICS: ANTICARCINOGENS AND MECHANISM OF ACTION

The following properties of prebiotics contribute to their ACA:

1. Motivation of valuable indigenous gut bacteria.
2. Generation of SCFAs and lactic acid as fermentation products.
3. Alteration of gene expression in the cecum, colon, and faces.
4. Increased micronutrient absorption in the colon.
5. Inflection of xenobiotic metabolizing enzymes.
6. Inflection of immune response [59, 60].

7.8.2 MECHANISM OF ACTION OF SYNBIOTICS TO INHIBIT COLORECTAL CANCER

Synbiotics, unlike probiotics, are not known to have anticarcinogenic properties. Butyrate and other SCFAs are produced when prebiotic oligosaccharides are fermented by probiotic bacteria and other bacteria in the colon, resulting in butyrate-mediated anticarcinogenic effects. The mechanism of action of synbiotics to inhibit colorectal cancer can be normally explained as follows:

- Synergistic action: Prebiotics provide a substrate for the survival and growth of specific beneficial allochthonous and autochthonous bacteria.
- Alteration in intestinal metabolic activity due to changes in the colonic microbiota.
- The prebiotics in synbiotics may raise mineral levels in the colon, such as calcium and magnesium, and promote bifidobacteria and lactobacilli growth in the large intestine.
- Furthermore, *in vitro* and animal studies have revealed that these bacteria bind and inactivate carcinogens, implying that they can directly suppress tumor growth and prevent pre-carcinogen conversion to carcinogens.
- The creation of cancer-preventive agents and, thus, the reduction of the risk of colorectal cancer may be linked to bacterial fermentation of dietary components in the gut system.
- The major byproducts of bacterial fermentation are SCFAs, namely acetate, propionate, and butyrate, as well as gases. Gases are removed, and SCFAs, along with nutrients and growth signals, play an important role in CRC prevention.

- Secondary bile acid levels are lowered, and a synbiotic blend of fructan and *B. lactis* or *L. rhamnosus* GG was found to attenuate AOM-induced colorectal adenomas and carcinogenesis by boosting SCFA levels. The proliferative activity and expression levels of GST placental enzyme pi-type, inducible NO-synthase, and cyclooxygenase-2 enzymes, which are involved in colorectal carcinogenesis, are also reduced [61] [62].

7.8.2.1 IMMUNOMODULATION AND ANTI-INFLAMMATORY

Synbiotics help to produce more SCFA than probiotics, which protects against the development of colorectal cancer. One possible reason is that the immunomodulation capabilities of probiotic bacteria interact with butyrate, which is created during prebiotic fermentation, causing apoptosis to be upregulated. Probiotics are also involved in the creation of conjugated linoleic acids (CLA), which have been proven to have a variety of health advantages, including anti-inflammatory and anticarcinogenic properties. Synbiotics, unlike probiotics, do not have an overt anticarcinogenic effect. Butyrate and other SCFA are generated when prebiotic oligosaccharides are fermented by probiotic bacteria in the colon, resulting in butyrate-mediated anticarcinogenic effects [63].

7.8.2.2 EFFECT OF SYNBIOTIC ON TUMOR CELL INVASION AND EPITHELIAL BARRIER FUNCTION

Increased tight junction permeability and decreased epithelial barrier function are two common side effects of tumor promoters. As a result, the effect of the synbiotic intervention on fecal water's ability to change these variables in colonic cells was examined. The Caco-2 monolayer's barrier performance was dramatically improved by fecal water from polyp patients during the synbiotic intervention. In the cancer patients, there were no notable effects of synbiotic treatment. The Madrigal invasion assay was used to investigate the effect of the synbiotic intervention on fecal water's ability to affect the invasive capability of colonic carcinoma cells. In the cancer and polyp patients, there was no effect of the intervention on fecal water's ability to affect HT115 cell invasion [64]. The role of synbiotics is shown in Figure 7.2.

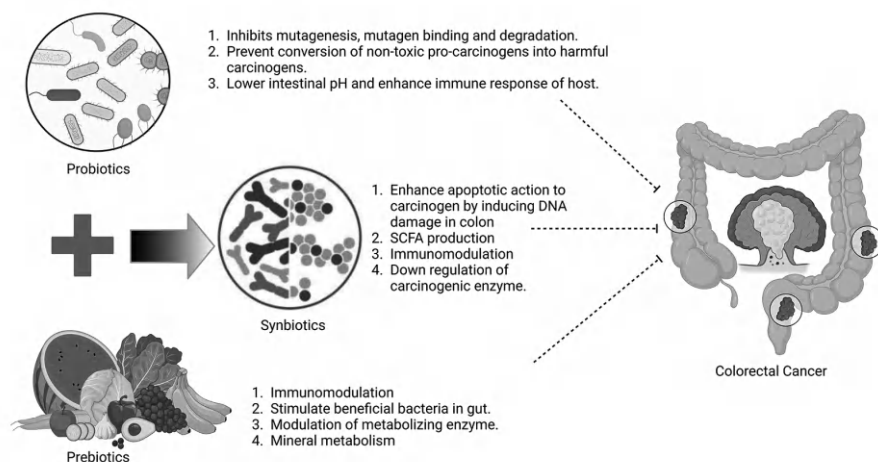


FIGURE 7.2 Role of synbiotics in the prevention of CRC.

7.8.2.3 SYNBIOTIC AND COMPLICATIONS

The goal of the current research is to provide a larger evidence base for the effects of probiotic/synbiotic therapy on functional outcomes and postoperative complications. The quick translation of scientific research into clinical practice enables doctors to make more informed decisions concerning the treatment of patients with adverse resection consequences or the future avoidance of postoperative problems. Treatment of postsurgical symptoms with antidiarrheal medications and bulk-forming agents, for instance, is only partially effective. According to the results of this meta-analysis, synbiotics can benefit CRC patients with gastrointestinal discomfort and postoperative problems since they are easy to use, have fewer side effects, and are less expensive than alternative treatments.

A lower quality of life and post-CRC resection issues are related to long-term survival (QoL). Preoperative administration of probiotics and/or synbiotics to CRC patients may lessen adverse effects and improve QoL and survival. A total of 16 probiotic/synbiotic clinical trials involving patients after CRC surgery were randomized, placebo-controlled, and examined postoperative complications and QoL side effects. Meta-analyses using a random-effects model were performed on data from 11 studies to estimate the effect of probiotics and synbiotics on typical CRC resection postoperative side effects and complications. The findings of this systematic review and meta-analysis lend support to the use of probiotics and synbiotics in

CRC patients to reduce and prevent complications, adverse side effects, and postoperative symptoms. Additional large RCTs could provide clinical practitioners with much-needed more comprehensive data, illuminating options for improving the burden of cancer, its symptoms, QoL, and perhaps even long-term prognosis [65, 66].

7.9 CLINICAL TRIALS

Currently, several molecules are in CT showing significant minimization in chances of CRC. The Synbiotics in CT are depicted in Table 7.3.

TABLE 7.3 The List of Products in Clinical Trials [67]

Sr. No	Compound	Condition	CT Number	Phase	Status
1	Probiotic (La1, BB536)	CRC	NCT00936572	Phase 2	Completed
2	Probiotic capsule	CRC	NCT03742596	Phase 2,3	Recruiting
3	Omni-biotic 10	Metastatic CRC	NCT03705442	Phase 2	Unknown
4	Probiotic-Hexbio	CRC	NCT03782428	-	Completed
5	Bifico, chemotherapy and targeted therapy	Unresectable metastatic CRC	NCT04131803	-	Yet not recruiting
6	<i>Saccharomyces boulardi</i>	CRC	NCT01895530	-	Completed
7	<i>Saccharomyces boulardi</i>	CRC	NCT01609660	Phase 4	Completed
8	Probiotic-Colon Dophilus	CRC	NCT01410955	Phase 3	Completed
9	<i>Lactobacillus Rhamnosus</i> suppliments	CRC	NCT00197873	-	Completed
10	Synbiotics-multistrain and multibiotic	Colorectal neoplasm	NCT01479907	-	Completed
11	Synbiotics-Oat bran and blueberry husk	Rectal cancer	NCT03420443	-	Completed
12	ProBion Clinica	Colon cancer	NCT03072641	-	Completed
13	Mechnicov Probiotics	Sigmoid colon cancer	NCT03531606	-	Completed
14	Icosapent ethyl oral capsule	Colon cancer liver metastasis	NCT04682665	-	Recruiting
15	Dietary supplements with meal and snacks	CRC prevention	NCT01929122	-	Completed

7.10 MARKETED BRANDS

Significant policies used by companies in the Synbiotic Marketplace include product launches, unions and purchases, and R&D actions. The top 9 competitors in the field are fragmenting the Synbiotic market share in 2020. The top 9 brands in the Synbiotic market are as follows. The market scenario of Synbiotics is shown in Figure 7.3.

- 1. Seed health, Inc.
- 2. Sabinsa corporation.
- 3. Biomin America Inc.
- 4. United naturals.
- 5. Probiotical SpA.
- 6. Yakult Honsha co., ltd.
- 7. Pfizer Inc.
- 8. Daflorn ltd.
- 9. Chobani global holdings, llc [68, 69].

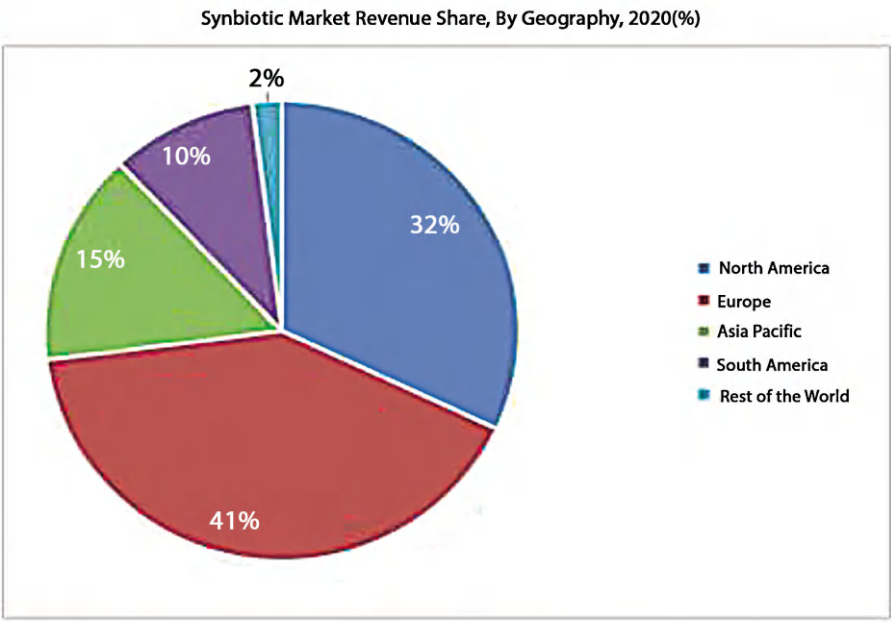


FIGURE 7.3 Representation of synbiotics market revenue.

Source: Adapted from Ref. [31]

7.11 CONCLUSION

CRC seems to be the emerging type of cancer affecting both males and females equally. During this global era, lifestyle changes, busy lives, and stressful conditions at the workplace create challenges that lead to ignorance towards a healthy life and the maintenance of nutritional balance. Family history, gene mutations, and associated risk factors provoke the occurrences of CRC. The human gut microbiota, comprised of trillions of microbes, significantly contributes to minimizing the chances of CRC by demolishing harmful pathogens, enzymes, metabolic products, and cancer-causing agents. Sometimes, dysbiosis of the microbiota favors the initiation of CRC. Currently, several liquid biomarkers have been identified, which are invasive, simple, and convenient methods for the early prognosis of CRC. Moreover, if raised concentrations occur, FOBT, FIT, and colonoscopy enable prompt diagnosis and increase survival rates in several patients. The synergistic combination of prebiotics and probiotics, designated as “synbiotics,” combines the benefits of both and significantly works to minimize the chances of CRC. Currently, many synbiotic products are helpful in maintaining healthy gut microbiota, reducing inflammatory responses, and generating antioxidant and immunomodulatory actions that eliminate tumor-inducing agents from the body. Hence, it is concluded that an adequate nutritional diet or the consumption of synbiotics during the initial observation of any risk factors can help avoid the chances of CRC.

KEYWORDS

- **Biomarkers**
- **colorectal cancer**
- **gut microbiota**
- **prebiotics**
- **probiotics**
- **synbiotics**

REFERENCES

1. Kanugo, A., Gautam, R. K., & Kamal, M. A. (2021). Recent advances of nanotechnology in the diagnosis and therapy of triple-negative breast cancer (TNBC). *Current Pharmaceutical Biotechnology*, 23. <https://doi.org/10.2174/1389201023666211230113658>.

2. Kanugo, A. (2022). Recent advances of nanotechnology in the treatment of skin cancer. *Current Pharmaceutical Biotechnology*, 23. <https://doi.org/10.2174/1389201023666220404113242>.
3. American Cancer Society. (2022). What is colorectal cancer? How does colorectal cancer start? *Cancer.org*. <https://www.cancer.org/cancer/colon-rectal-cancer/about/what-is-colorectal-cancer.html> (accessed on 27 September 2024).
4. Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2022). Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians*, 72(1), 7–33. <https://doi.org/10.3322/caac.21708>.
5. Cefali, M., Epistolio, S., Palmarocchi, M. C., Frattini, M., & De Dosso, S. (2021). Research progress on KRAS mutations in colorectal cancer. *Journal of Cancer Metastasis and Treatment*, 7, 26. <https://doi.org/10.20517/2394-4722.2021.61>.
6. Fotiadis, C. I., Stoidis, C. N., Spyropoulos, B. G., & Zografos, E. D. (2008). Role of probiotics, prebiotics and synbiotics in chemoprevention for colorectal cancer. *World Journal of Gastroenterology*, 14(39), 6453–6457. <https://doi.org/10.3748/wjg.14.6453>.
7. Basini, J., Rayadurgam, S., & Dakshinamurthy, S. (2019). An overview of colorectal cancer: Implication of two medicinal plants in their treatment. *Asian Journal of Pharmaceutical and Clinical Research*, 12(7), 47–52. <https://doi.org/10.22159/ajpcr.2019.v12i7.33287>.
8. McCulloch, S. M., Aziz, I., Polster, A. V., Pischel, A. B., Stålsmeden, H., Shafazand, M., Block, M., Byröd, G., Lindkvist, B., Törnblom, H., et al. (2020). The diagnostic value of a change in bowel habit for colorectal cancer within different age groups. *United European Gastroenterology Journal*, 8(2), 211–220. <https://doi.org/10.1177/2050640619888040>.
9. Granados-Romero, J. J., Valderrama-Treviño, A. I., Contreras-Flores, E. H., Barrera-Mera, B., Enríquez, M. H., Uriarte-Ruíz, K., Ceballos-Villalba, J. C., Estrada-Mata, A. G., Rodríguez, C. A., & Arauz-Peña, G. (2017). Colorectal cancer: A review. *International Journal of Research in Medical Sciences*, 5(11), 4667–4676. <https://doi.org/10.18203/2320-6012.IJRMS20174914>.
10. Mariani, G., Pauwels, E. K. J., AlSharif, A., Marchi, S., Boni, G., Barreca, M., Bellini, M., Grosso, M., De Bortoli, N., Mumolo, G., et al. (2008). Radionuclide evaluation of the lower gastrointestinal tract. *Journal of Nuclear Medicine*, 49(5), 776–787. <https://doi.org/10.2967/jnumed.107.040113>.
11. Burnett-Hartman, A. N., Lee, J. K., Demb, J., & Gupta, S. (2021). An update on the epidemiology, molecular characterization, diagnosis, and screening strategies for early-onset colorectal cancer. *Gastroenterology*, 160(4), 1041–1049. <https://doi.org/10.1053/j.gastro.2020.12.068>.
12. Nguyen, H. T., & Duong, H. Q. (2018). The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. *Oncology Letters*, 16(1), 9–18. <https://doi.org/10.3892/ol.2018.8679/html>.
13. Zhu, N., Zhao, M., Song, Y., Ding, L., & Ni, Y. (2022). The KiSS-1/GPR54 system: Essential roles in physiological homeostasis and cancer biology. *Genes & Diseases*, 9(1), 28–40. <https://doi.org/10.1016/j.gendis.2020.07.008>.
14. Elshami, M., Ayyad, M. M., Alser, M., Al-Slaibi, I., Ahmed Naji, S., Mohamad, B. M., Isleem, W. S., Shurrah, A., Yaghi, B., Qabaja, Y. A., et al. (2022). Awareness of colorectal cancer signs and symptoms: A national cross-sectional study from Palestine. *BMC Public Health*, 22(866), 1–9. <https://doi.org/10.1186/s12889-022-13285-8>.

15. Kuipers, E. J., Grady, W. M., Lieberman, D., Seufferlein, T., Sung, J. J., Boelens, P. G., Van De Velde, C. J. H., & Watanabe, T. (2015). Colorectal cancer. *Nature Reviews Disease Primers*, 1, 1–25. <https://doi.org/10.1038/nrdp.2015.65>.
16. Cancer.Net. (2022). Colorectal cancer: Stages. *Cancer.Net*. <https://www.cancer.net/cancer-types/colorectal-cancer/stages> (accessed on 27 September 2024).
17. Raman, M., Ambalam, P., Kondepudi, K. K., Pithva, S., Kothari, C., Patel, A. T., Purama, R. K., Dave, J. M., & Vyas, B. R. M. (2013). Potential of probiotics, prebiotics and symbiotics for management of colorectal cancer. *Gut Microbes*, 4(3), 181–192. <https://doi.org/10.4161/gmic.23919>.
18. Mayo Clinic. (2022). Colon cancer: Symptoms and causes. *Mayo Clinic*. <https://www.mayoclinic.org/diseases-conditions/colon-cancer/symptoms-causes/syc-20353669> (accessed on 27 September 2024).
19. Wang, F., Song, M., Lu, X., Zhu, X., & Deng, J. (2021). Gut microbes in gastrointestinal cancers. *Seminars in Cancer Biology*. <https://doi.org/10.1016/j.semcancer.2021.03.037>.
20. Pothuraju, R., Chaudhary, S., Rachagani, S., Kaur, S., Roy, H. K., Bouvet, M., & Batra, S. K. (2021). Mucins, gut microbiota, and postbiotics role in colorectal cancer. *Gut Microbes*, 13(1), 1–17. <https://doi.org/10.1080/19490976.2021.1974795>.
21. Shi, N., Li, N., Duan, X., & Niu, H. (2017). Interaction between the gut microbiome and mucosal immune system. *Military Medical Research*, 4, 1–7. <https://doi.org/10.1186/s40779-017-0122-9>.
22. Zhang, J., Xia, Y., & Sun, J. (2021). Breast and gut microbiome in health and cancer. *Genes & Diseases*, 8, 581–589.
23. Kalasabail, S., Engelman, J., Zhang, L. Y., El-Omar, E., & Yim, H. C. H. (2021). A perspective on the role of microbiome for colorectal cancer treatment. *Cancers*, 13. <https://doi.org/10.3390/cancers13184623>.
24. 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 Journal of Gastroenterology*, 22, 501–518. <https://doi.org/10.3748/wjg.v22.i2.501>.
25. Akbar, N., Khan, N. A., Muhammad, J. S., & Siddiqui, R. (2022). The role of gut microbiome in cancer genesis and cancer prevention. *Health Science Reviews*, 2, 100010. <https://doi.org/10.1016/j.hsr.2021.100010>.
26. Meng, C., Bai, C., Brown, T. D., Hood, L. E., & Tian, Q. (2018). Human gut microbiota and gastrointestinal cancer. *Genomics, Proteomics & Bioinformatics*, 16, 33–49. <https://doi.org/10.1016/j.gpb.2017.06.002>.
27. Weng, M. T., Chiu, Y. T., Wei, P. Y., Chiang, C. W., Fang, H. L., & Wei, S. C. (2019). Microbiota and gastrointestinal cancer. *Journal of the Formosan Medical Association*, 118(S32–S41). <https://doi.org/10.1016/j.jfma.2019.01.002>.
28. Allen, J., & Sears, C. L. (2019). Impact of the gut microbiome on the genome and epigenome of colon epithelial cells: Contributions to colorectal cancer development. *Genome Medicine*, 11, 1–18. <https://doi.org/10.1186/s13073-019-0621-2>.
29. Knudson, A. G. (1971). Mutation and cancer: Statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America*, 68, 820–823. <https://doi.org/10.1073/PNAS.68.4.820>.
30. Noshio, K., Sukawa, Y., Adachi, Y., Ito, M., Mitsuhashi, K., Kurihara, H., Kanno, S., Yamamoto, I., Ishigami, K., Igarashi, H., et al. (2016). Association of *Fusobacterium nucleatum* with immunity and molecular alterations in colorectal cancer. *World Journal of Gastroenterology*, 22, 557–566. <https://doi.org/10.3748/wjg.v22.i2.557>.

31. Stott, K. J., Phillips, B., Parry, L., & May, S. (2021). Recent advancements in the exploitation of the gut microbiome in the diagnosis and treatment of colorectal cancer. *Bioscience Reports*, 41, 1–20. <https://doi.org/10.1042/BSR20204113>.
32. Navarro, M., Nicolas, A., Ferrandez, A., & Lanás, A. (2017). Colorectal cancer population screening programs worldwide in 2016: An update. *World Journal of Gastroenterology*, 23, 3632–3642. <https://doi.org/10.3748/wjg.v23.i20.3632>.
33. Saus, E., Iraola-Guzmán, S., Willis, J. R., Brunet-Vega, A., & Gabaldón, T. (2019). Microbiome and colorectal cancer: Roles in carcinogenesis and clinical potential. *Molecular Aspects of Medicine*, 69, 93–106.
34. Shen, L., Lu, W., Huang, Y., He, J., Wang, Q., Zheng, X., & Wang, Z. (2022). SNORD15B and SNORA5C: Novel diagnostic and prognostic biomarkers for colorectal cancer. *Biomedical Research International*, 2022, 1–10. <https://doi.org/10.1155/2022/8260800>.
35. Meester, R. G. S., van de Schootbrugge-Vandermeer, H. J., Breekveldt, E. C. H., de Jonge, L., Toes-Zoutendijk, E., Kooyker, A., Nieboer, D., Ramakers, C. R., Spaander, M. C. W., van Vuuren, A. J., et al. (2022). Faecal occult blood loss accurately predicts future detection of colorectal cancer: A prognostic model. *Gut*. <https://doi.org/10.1136/gutjnl-2022-327188>.
36. Issa, I. A., & NouredDine, M. (2017). Colorectal cancer screening: An updated review of the available options. *World Journal of Gastroenterology*, 23, 5086. <https://doi.org/10.3748/wjg.v23.i28.5086>.
37. Kolligs, F. T. (2016). Diagnostics and epidemiology of colorectal cancer. *Visceral Medicine*, 32, 158. <https://doi.org/10.1159/000446488>.
38. Zhu, J., Xu, Y., Liu, S., Qiao, L., Sun, J., & Zhao, Q. (2020). MicroRNAs associated with colon cancer: New potential prognostic markers and targets for therapy. *Frontiers in Bioengineering and Biotechnology*, 8, 176. <https://doi.org/10.3389/fbioe.2020.00176>.
39. Yang, G., Zhang, Y., & Yang, J. (2019). A five-microRNA signature as prognostic biomarker in colorectal cancer by bioinformatics analysis. *Frontiers in Oncology*, 9. <https://doi.org/10.3389/fonc.2019.01207>.
40. Hossain, M. S., Quadery Tonmoy, M. I., Islam, M. N., Islam, M. S., Afif, I. K., Singha Roy, A., Fariha, A., Al Reza, H., Bahadur, N. M., & Rahaman, M. M. (2021). MicroRNAs expression analysis shows key affirmation of synaptopodin-2 as a novel prognostic and therapeutic biomarker for colorectal and cervical cancers. *Heliyon*, 7. <https://doi.org/10.1016/j.heliyon.2021.e07347>.
41. Dhouioui, S., Boujelbene, N., Chelbi, H., Zemni, I., Ben Safta, I., Ouzari, H. I., Mezlini, A., Harrath, A. H., Rebmann, V., & Zidi, I. (2022). SHLA-G as a biomarker for colorectal cancer pathogenesis. *Journal of King Saud University–Science*, 34, 101708. <https://doi.org/10.1016/j.jksus.2021.101708>.
42. Graule, J., Uth, K., Fischer, E., Centeno, I., Galván, J. A., Eichmann, M., Rau, T. T., Langer, R., Dawson, H., Nitsche, U., et al. (2018). CDX2 in colorectal cancer is an independent prognostic factor and regulated by promoter methylation and histone deacetylation in tumors of the serrated pathway. *Clinical Epigenetics*, 10, 120. <https://doi.org/10.1186/s13148-018-0548-2>.
43. Ye, B., Li, F., Chen, M., Weng, Y., Qi, C., Xie, Y., Zhang, Q., Ding, H., Zhang, J., & Gao, X. (2022). A panel of platelet-associated circulating long non-coding RNAs as potential biomarkers for colorectal cancer. *Genomics*, 114, 31–37. <https://doi.org/10.1016/j.ygeno.2021.11.026>.

44. Parent, P., Cohen, R., Rassy, E., Svrcek, M., Taieb, J., André, T., & Turpin, A. (2020). A comprehensive overview of promising biomarkers in stage II colorectal cancer. *Cancer Treatment Reviews*, 88, 102059. <https://doi.org/10.1016/j.ctrv.2020.102059>.
45. Vacante, M., Borzi, A. M., Basile, F., & Biondi, A. (2018). Biomarkers in colorectal cancer: Current clinical utility and future perspectives. *World Journal of Clinical Cases*, 6, 869. <https://doi.org/10.12998/wjcc.v6.i15.869>.
46. Wang, P., Liang, J., Wang, Z., Hou, H., Shi, L., & Zhou, Z. (2017). The prognostic value of p53 positive in colorectal cancer: A retrospective cohort study. *Tumor Biology*, 39. <https://doi.org/10.1177/1010428317703651>.
47. Oh, H. H., & Joo, Y. E. (2020). Novel biomarkers for the diagnosis and prognosis of colorectal cancer. *Intestinal Research*, 18, 168–183. <https://doi.org/10.5217/ir.2019.00080>.
48. Markowiak, P., & Ślizewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, 10, 1–20. <https://doi.org/10.1186/s13099-018-0250-0>.
49. Larsen, M. B., Njor, S., Ingeholm, P., & Andersen, B. (2018). Effectiveness of colorectal cancer screening in detecting earlier-stage disease: A nationwide cohort study in Denmark. *Gastroenterology*, 155, 99–106. <https://doi.org/10.1053/j.gastro.2018.03.062>.
50. Ale, E. C., & Binetti, A. G. (2021). Role of probiotics, prebiotics, and symbiotics in the elderly: Insights into their applications. *Frontiers in Microbiology*, 11, 19. <https://doi.org/10.3389/fmicb.2021.631254>.
51. Fotiadis, C. I., Stoidis, C. N., Spyropoulos, B. S., & Zografos, E. D. (2008). Role of probiotics, prebiotics, and symbiotics in chemoprevention for colorectal cancer. *World Journal of Gastroenterology*, 14(42), 6453–6457. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2773329/> (accessed on 27 September 2024).
52. Oh, N. S., Lee, J. Y., Kim, Y. T., Kim, S. H., & Lee, J. H. (2020). Cancer-protective effect of a symbiotic combination between *Lactobacillus gasseri* 505 and a *Cudrania tricuspidata* leaf extract on colitis-associated colorectal cancer. *Gut Microbes*, 11, 1–20. <https://doi.org/10.1080/19490976.2020.1785803>.
53. Gavresea, F., Vagianos, C., Korontzi, M., Sotiropoulou, G., Dadioti, P., Triantafillidis, J. K., & Papalois, A. E. (2018). Beneficial effect of synbiotics on experimental colon cancer in rats. *Turkish Journal of Gastroenterology*, 29, 494–501. <https://doi.org/10.5152/tjg.2018.17469>.
54. Aponte, M., Murru, N., & Shoukat, M. (2020). Therapeutic, prophylactic, and functional use of probiotics: A current perspective. *Frontiers in Microbiology*, 11, 2120. <https://doi.org/10.3389/fmicb.2020.562048>.
55. Liu, Y., Tran, D. Q., & Rhoads, J. M. (2018). Probiotics in disease prevention and treatment. *Journal of Clinical Pharmacology*, 58(S164), <https://doi.org/10.1002/jcph.1121>.
56. Molska, M., & Reguła, J. (2019). Potential mechanisms of probiotics action in the prevention and treatment of colorectal cancer. *Nutrients*, 11. <https://doi.org/10.3390/nu11102453>.
57. Yan, F., & Polk, D. B. (2011). Probiotics and immune health. *Current Opinion in Gastroenterology*, 27, 496–501. <https://doi.org/10.1097/mog.0b013e32834baa4d>.
58. Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Berenjian, A., & Ghasemi, Y. (2019). Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods*, 8, 92. <https://doi.org/10.3390/foods8030092>.

59. Halloran, K., & Underwood, M. A. (2019). Probiotic mechanisms of action. *Early Human Development*, 135, 58–65. <https://doi.org/10.1016/j.earlhumdev.2019.05.010>.
60. Batista, V. L., da Silva, T. F., de Jesus, L. C. L., Coelho-Rocha, N. D., Barroso, F. A. L., Tavares, L. M., Azevedo, V., Mancha-Agresti, P., & Drumond, M. M. (2020). Probiotics, prebiotics, symbiotic, and Para probiotics as a therapeutic alternative for intestinal mucositis. *Frontiers in Microbiology*, 11, 2246. <https://doi.org/10.3389/fmicb.2020.544490>.
61. Yadav, M. K., Kumari, I., Singh, B., Sharma, K. K., & Tiwari, S. K. (2022). Probiotics, prebiotics, and symbiotic: Safe options for next-generation therapeutics. *Applied Microbiology and Biotechnology*, 106, 505–521. <https://doi.org/10.1007/s00253-021-11646-8>.
62. Markowiak, P., & Ślizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, 9(1021). <https://doi.org/10.3390/nu9091021>.
63. Mirzaei, R., Afaghi, A., Babakhani, S., Sohrabi, M. R., Hosseini-Fard, S. R., Babolhavaeji, K., Khani Ali Akbari, S., Yousefimashouf, R., & Karampoor, S. (2021). Role of microbiota-derived short-chain fatty acids in cancer development and prevention. *Biomedicine & Pharmacotherapy*, 139, 111619. <https://doi.org/10.1016/j.biopha.2021.111619>.
64. Fong, W., Li, Q., & Yu, J. (2020). Gut microbiota modulation: A novel strategy for prevention and treatment of colorectal cancer. *Oncogene*, 39, 4925–4943. <https://doi.org/10.1038/s41388-020-1341-1>.
65. Rafter, J., Bennett, M., Caderni, G., Clune, Y., Hughes, R., Karlsson, P. C., Klinder, A., O'Riordan, M., O'Sullivan, G. C., Pool-Zobel, B., et al. (2007). Dietary symbiotic reduce cancer risk factors in polypectomized and colon cancer patients. *American Journal of Clinical Nutrition*, 85, 488–496. <https://doi.org/10.1093/ajcn/85.2.488>.
66. Amitay, E. L., Carr, P. R., Gies, A., Laetsch, D. C., & Brenner, H. (2020). Probiotic/synbiotic treatment and postoperative complications in colorectal cancer patients: Systematic review and meta-analysis of randomized controlled trials. *Clinical and Translational Gastroenterology*, 11, e00268. <https://doi.org/10.14309/ctg.0000000000000268>.
67. Search of: Colorectal cancer | Probiotics - List results - ClinicalTrials.gov. (n.d.). *ClinicalTrials.gov*. <https://clinicaltrials.gov/ct2/results?cond=probiotics&term=colorectal+cancer&cntry=&state=&city=&dist=> (accessed on 27 September 2024).
68. Maftai, N. M. (2019). Probiotic, prebiotic, and symbiotic products in human health. In *Frontiers in New Trends Science Fermented Food Beverages*. <https://doi.org/10.5772/intechopen.81553>.
69. Synbiotic market share, size, and industry growth analysis 2021-2026. (n.d.). *IndustryARC*. <https://www.industryarc.com/Research/Synbiotic-Market-Research-507360> (accessed on 27 September 2024).

CHAPTER 8

Correlation Between Reactive Oxygen Species (ROS), Inflammation, and Probiotics

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ABSTRACT

Key signaling molecules called Reactive Oxygen Species (ROS) are crucial in the development of inflammatory diseases. Endothelial dysfunction and tissue damage are brought on by polymorphonuclear neutrophils (PMNs), which increase the production of ROS at the site of inflammation. The body's immunological response is triggered by infections and damage. Free radicals are created by immune cells called macrophages as they combat foreign pathogens. Inflammation can result from the damage these free radicals can do to healthy cells. Probiotic bacteria have the ability to both increase and decrease the production of anti-inflammatory cytokines, which play an important role in the prevention of carcinogenesis. They are also capable of activating phagocytes to eliminate early-stage cancer cells. The use of

probiotics could improve the gut microbial population, increase mucus secretion, and prevent the destruction of tight junction proteins by decreasing the number of lipopolysaccharides. Additionally, probiotic treatment may lessen the formation of inflammatory biomarkers and reduce unnecessary immune system activation by reducing gut dysbiosis and intestinal permeability. Application of heat-killed probiotic bacteria coupled with radiation had a positive influence on enhancing immunological recognition of inflamed cells. The notion of employing probiotic bacteria as medication delivery vehicles has recently gained traction due to multiple articles revealing promising outcomes. Probiotic bacteria and gut microbiota are anticipated to play a significant role in immunomodulation and the management of inflammation during the next several years. The paper describes how, with the help of thorough literature investigation, the beneficial supplementation of probiotics on oxidative stress enhances antioxidant resistance and increases the quantity of antioxidant enzymes in the human body.

8.1 INTRODUCTION

Probiotic literally means “for life” in Greek. With the growth of interest in taking supplements containing live bacteria, as well as research into the mechanisms of action of these bacteria, we have gained a greater understanding of what a probiotic is. The term was first applied to substances produced by one microorganism that induced the growth of other microorganisms, and it was later applied to tissue extracts that induced microbial growth, as well as to feed additives that had a positive impact on animals by balancing their intestinal flora [1]. It has been shown that probiotics and the fermented metabolites of them have antioxidative properties that control oxidative stress and shield cells from damage caused by oxidants. Inflammation is broadly categorized into acute and chronic, which might be due to various diseases like arthritis, bowel disease, diabetes, cardiovascular diseases, etc. Probiotics show promise as anti-inflammatory agents that may help to treat chronic inflammation and may be used as a preventative measure. One of the most well-researched ways that probiotics may reduce inflammation is through the repair of the mucosal barrier against pathogens. Enhanced intracellular reactive oxygen species (ROS) levels that harm lipids, proteins, and DNA are known as oxidative stress. Oxygen free radicals, prostaglandins, leukotrienes, and nitric oxide are all important mediators of inflammation, including proteins, peptides, glycoproteins, and cytokines. Various cell types, including epithelia, endothelium, and inflammatory cells,

produce these chemicals [2]. Reactive oxygen species (ROS) are central in the development of inflammatory diseases. Inflammatory sites can be damaged by polymorphonuclear neutrophils that produce ROS and cause endothelial dysfunction. High ROS levels, together with decreased levels of cellular antioxidant enzyme systems, cause malignant transformation via many molecular targets, including NF- κ B and nuclear factor (erythroid-derived 2)-like 2 factor (Nrf2). These critical components govern signaling cascades that create an inflammatory milieu that suppresses apoptotic cell death, tumor growth, angiogenesis, and metastasis, all of which contribute to the initiation, development, and progression of malignant neoplasms [2]. Recent research has proven the pivotal role of probiotics in regulating inflammation and the generation of ROS. The current review focuses on the role of probiotics in controlling various inflammatory mediators, including ROS, with the aid of research evidence [2, 1].

8.2 ROS IN INFLAMMATORY DISEASE

A living mammalian tissue's protective response to injury caused by any substance is inflammation, which helps to restore (or replace) damaged tissues as well as localize, get rid of, and remove harmful stimuli. Reactive oxygen species (ROS) are now being demonstrated to play a part in the beginning, progression, and resolution of an allergic response. Moreover, ROS function in intracellular signaling as second messengers and microbicidal agents. Nonetheless, there is convincing evidence that excessive ROS production can cause tissue and cell damage, as well as the chronic inflammation that underlies many neurodegenerative, cardiovascular, and metabolic illnesses. Angiogenesis, respiratory burst, phagocytosis, and increased vascular permeability are all crucial aspects of inflammation, and some of these events also contribute to the resolution of inflammation [3].

In order to initiate signaling cascades that could trigger the emergence and evolution of inflammatory diseases, ROS can either oxidize biomolecules or structurally alter proteins and genes. Inflammation starts when ROS activate transcription factors and genes that promote inflammation. To draw additional immunological cells to the area of contamination or oxidative stress, immunological cells release a variety of chemokines and cytokines during inflammation. Instinctively, immune cells near the site of inflammation produce more ROS, which leads to tissue damage and oxidative stress [4]. Reactive oxygen species, also known as ROS, are free radicals formed via

molecules or molecular fragments of molecular oxygen, such as hydrogen peroxide (H_2O_2), hydroperoxyl radicals, hypochlorous acid (HOCl), nitric oxide (NO), singlet oxygen ($^1\text{O}_2$), and superoxide anion hydroxyl ($\text{HO}\bullet$) [3]. The main source of ROS in various cell types is the cellular mitochondria. The electron transport chain, dehydrogenases in the matrix of mitochondrial cells, the p66^{shc} protein within the space between membranes, and monoamine oxidase in the outer membrane (from complexes of the mitochondrial respiratory chain: complex I—NADH dehydrogenase complex and complex III—cytochrome bc_1 complex) are used to produce reactive oxygen species in this situation. Xanthine oxidase, cyclooxygenases, myeloperoxidase, NADPH oxidase, cytochrome P450 enzymes, lipoxygenases, and oxidoreductase 1 are additional cell enzymes that can produce ROS [5].

DNA, lipids, and proteins are harmed by high quantities of ROS. Yet, ROS function as significant mediators in the control of cell proliferation, adhesion, development, and death. By posttranslational alteration of redox-sensitive proteins, such as different receptors and channels, kinases and phosphatases, caspases, and transcription factors, ROS are implicated in intracellular signaling. Redox-sensitive proteins are those that have functionally important cysteine residues that are susceptible to oxidation. Therefore, sulfhydryl groups of cysteine (Cys-SH) can be oxidized by H_2O_2 , leading to the generation of sulfonic ($\text{Cys-SO}_3\text{H}$), sulfinic ($\text{Cys-SO}_2\text{H}$), and sulfenic (Cys-SOH) acids. Any of these alterations change how proteins behave and impact how they work in the signal transduction pathway [6]. The onset, progression, and resolution of inflammation are all influenced by ROS.

8.2.1 ROS'S ROLE IN CRUCIAL MOMENTS

- 1. Leukocyte Extravasation and Vascular Permeability:** When injurious substances activate cells of innate immunity (mostly macrophages and mast cells), they release a variety of inflammatory mediators that enhance vascular permeability and cause leukocyte migration to the site of injury [7].
- 2. Phagocytosis and Respiratory Burst:** The most well-known function of ROS in inflammation is their involvement in the respiratory burst phenomenon. At the site of the inflammation, neutrophils and macrophages phagocytize pathogens and cellular debris. This results in the assembly and activation of the NADPH oxidase at

the phagosome membrane. This enzyme turns O_2 into ROS, which releases additional ROS. ROS produced during respiratory bursts are discharged into the surrounding tissues as well as phagosomes. Most of their actions are bactericidal [8].

3. **Angiogenesis:** Angiogenesis, the act of creating fresh blood vessels, is a critical phase in the proliferative stage of inflammation. There is proof that ROS produced by the oxidase enzyme NADPH are essential for this stage of an inflammatory response. Human umbilical vein endothelial cells (HUVECs) contain NADPH oxidase and have been connected to actin and the protein IQGAP1 in the upper region of migratory cells (Ras GTPase-activating-like protein). When this connection is broken, NADPH oxidase is translocated from the leading edge, which impairs endothelial cells' ability to migrate [9].
4. **Resolution of Inflammation:** ROS are involved in a number of procedures that aid in the healing of inflammation. Massive neutrophil death is one of the major processes that occur before inflammation resolves. Granulocytes and other cells undergo apoptosis through signaling pathways that depend on ROS. A mouse model of antigen-induced arthritis has therefore demonstrated that H_2O_2 causes leukocyte mortality and remission of the response to inflammation [10].
5. **Oxidative Stress:** ROS are crucial regulators in almost all stages of the inflammatory process, but excessive ROS produced by leukocyte mitochondria and NADPH oxidases in mice, which is not countered by antioxidant systems, may cause serious cellular and tissue damage and promote the chronic inflammation that underlies many neurodegenerative, cardiovascular, and metabolic diseases [11].

8.3 ROS IN TUMORIGENESIS

8.3.1 SIGNIFICANCE OF ROS IN TUMORIGENESIS

Cancer intricacy is due to its multifaceted and multifactorial appearance. In recent decades, it has been demonstrated that the disturbance of redox balance is one of the most significant causes underpinning cancer formation, development, and metastasis in human cells. This disproportionality in redox balance has been linked to a rise in free radicals, specifically ROS [12].

Intracellular oxidative metabolism produces a group of molecules called ROS [13]. Singlet oxygen (primary excited state), superoxide anion (single electron state), hydroxyl radical (three-electron state), and hydrogen peroxide (H_2O_2) are among these compounds. It is essential to note that ROS are crucial signaling molecules and can, at low levels, regulate a variety of biological and physiological processes. It is generally thought that intracellular ROS levels remain stable due to the fact that ROS are powerful oxidants responsible for increasing intracellular damage and oxidation to lipids, DNA, and proteins during oxidative stress events [14].

ROS plays a significant part in signaling, cell proliferation, and revascularization in the tumor microenvironment. The progressive increase in ROS levels can similarly stimulate cancer cell growth, diffusion, and metastasis. On the other hand, the damaged DNA of cancer cells partially causes apoptosis and tumor necrosis when the amount of ROS is excessive [15].

The amount of ROS has varying impacts on genetic roles. While cytostatic ROS levels support biological activities, cytotoxic ROS levels trigger cell death and immunological deregulation. Tumors are promoted by ROS when they reach super-physiological or cytostatic levels while avoiding cell death levels. As previously stated, tumor cells can cause reactive stress [16].

8.3.2 REACTIVE OXYGEN SPECIES -MEDIATED INDUCTION OF OXIDATIVE STRESS

ROS levels in a typical cell are maintained through a variety of detoxification processes controlled by antioxidant enzymes [17]. In regular circumstances, the intracellular antioxidant system, which comprises several enzymatic and non-enzymatic components, balances ROS in cells, hence ensuring cellular homeostasis [18]. However, under illness or pathological conditions, the quantity of ROS rises, depleting antioxidants and eventually leading to a disruption in homeostasis or oxidative stress. As a result, during ROS homeostasis, the cells are well regulated, which helps to preserve redox balance in healthy cells. Ketoglutarate dehydrogenase, monoamine oxidase, mitochondrial $p66^{Shc}$ (pro-apoptotic protein), sirtuins, Nrf2, and fork-head box O3 (FOXO3), in addition to the primary redox cycling reactions, are hypothesized to be the source of ROS. This increased ROS generation or defective protective mechanisms may also result in increased oxidative stress, which can contribute to a variety of health issues. Inflammatory cells like macrophages and neutrophils produce ROS as a result of mitochondrial oxidative phosphorylation, P450 metabolism, and peroxisomes [19].

8.3.3 ACTIVATION OF ROS BY INFLAMMATION MARKERS

Inflammatory cytokines and chemokines are inflammation-related factors. The levels of these inflammation-related factors play a significant role in regulating cancer growth [20]. These factors either produce an antitumor response or induce malignant transformation during cancer development. ROS has been shown to play a significant role in the link between cancer and chronic inflammation, as it can influence the existence, amounts, and types of inflammation-modulating factors such as AP-1, β -catenin/Wnt (wingless-related integration site), HIF-1 (hypoxia-induced factor-1 alpha), NF- κ B, PPAR-gamma, p53, inflammatory cytokines, chemokines, and growth regulators [21]. During the process of inflammation within the tumor microenvironment, ROS is produced by enzymes that generate oxidants, namely, inducible nitric oxide synthase (iNOS), myeloperoxidase (MPO), and xanthine oxidase (XO) [22]. In order to remove and disrupt biological, chemical, and biological potential threats, cyclooxygenase 2 (COX-2) and lipoxygenase (LOX) are used. The oxidative damage to DNA, RNA, proteins, lipids, and mitochondria is due to the generous amount of ROS accumulation. This results in an increased mutation stress signal-transduction error, apoptosis inactivation, and an overwhelming production of extra ROS that triggers inflammation-modulating factors, inflammatory cytokines, and chemokines [14]. Aside from inducing persistent inflammation through ROS-mediated NF- κ B activation, active NF- κ B is thought to be a crucial factor in the progression of treatment-resistant cancers toward intermittent gamma-irradiation therapy and chemotherapeutic drugs. Tumor-associated chronic inflammation and cancer-development hallmarks may be treated by targeting ROS as a treatment. The proliferative signaling, evasion of development restrainers, metastasis, replication immortality, angiogenesis, and intransigence to cell death are all facilitated by ROS [23].

8.3.4 ROS IN CANCER METASTATIC PROCESSES

In cancer, metastasis is the main cause of morbidity and mortality. It involves the spreading of cancer cells to nearby tissues and distant organs. According to research, tumor metastasis is a complicated and multidimensional event caused by the inherent mutational burden of cancerous cells and bidirectional contact distinguishing cancerous and noncancerous cells [24]. The presence of ROS has been shown to contribute to epithelial-to-mesenchymal transition

(EMT) in numerous studies. In addition, TGF- β 1 regulates uPA (urokinase-type plasminogen activator). The ROS-dependent migration and invasion of cells are facilitated by PFA (plasminogen activator) and MMP-9. Hypoxia-mediated MMPs and cathepsin expression are induced by ROS, increasing tumor migration. ROS triggers Nrf2, which promotes Klf9 (Kruppel-like factor 9), activating ERK1/2 and increasing ROS generation in cancer cells [25]. Inhibiting premalignant development can be accomplished by targeting KLF9 with antioxidants.

8.3.5 ANGIOGENIC AND ROS-PRODUCING CELLS

A tumor's blood vessels form from preexisting ones through the preliminary stages of tumorigenesis. The formation of new blood vessels that aid tumor growth and survival occurs in the process of angiogenesis. Angiogenesis dependent on ROS is initiated by cancer proliferation. Cancer growth triggers ROS-dependent angiogenesis, which raises the metabolic rate and results in high levels of ROS [26]. Angiogenic modulators are secreted in response to the elevated ROS levels within the tumor microenvironment. Growth factors are promoted by both endogenous and exogenous ROS. VEGF and hypoxia-inducible factors are two cytokines and transcription factors that promote tumor growth and progression through ROS-dependent cellular signaling (HIF-1) [18]. Many investigations using cancer cell lines (MCF-7, HepG2, H-1299, and PC-3) have demonstrated that ROS works via the PI3K/Akt signaling cascade, boosting HIF-1 expression and angiogenesis. As a result of downregulating neovascularization in accordance with the body of information demonstrating ROS's angiogenic potential, therapeutic targets with antioxidant properties and targeting consequent communication pathways will be useful in managing and treating patients. Another way to improve the course of the disease is through various redox imbalance restoration techniques. Further investigation into disease-specific ROS may have therapeutic implications for future medication development, given the failure of interventional trials using small antioxidants [27].

8.3.6 ROS IN TUMOR CELL PROLIFERATION

Hydrogen peroxide and superoxide at low levels have a significant impact in assisting cell growth in a broad range of cancer cell types. For instance, intracellular oxidative stress in breast cancer cells increases as a result of the

transformation of estrogen to the mitochondria. ROS formed by mitochondria govern both cell development and inactivity [28]. This is accomplished by MnSOD activity, which acts as a mitochondrial ROS regulator. Higher superoxide and low hydrogen peroxide levels promote proliferation due to reduced manganese superoxide dismutase (MnSOD) activity; however, improved MnSOD activity causes proliferating cells to enter inactivity due to increased hydrogen peroxide production. Inhibiting the enzyme known as the mitochondrial uniporter prevents ROS formation and declines estrogen-induced cell proliferation in breast cancer cells, indicating a role for mitochondrial ROS in tumor progression. ROS-mediated activation of the Erk1/2 MAPK signaling pathway, along with the transcription factor, promotes estrogen-induced cell growth. CREB is a protein that binds to the cyclic AMP response element (CRE). There is an increase in the mRNA levels of cyclins that are involved in the cell cycle due to reactive oxygen species, such as cyclin B2, cyclin D3, cyclin E1, and cyclin E2 [29]. There has been evidence that MCF-10A cells undergo abnormal multiplication due to a lack of redox regulation of the cell series. There was a major variance between the basal stages of endogenous oxidative stress in pancreatic cancer cell lines and normal cells. Higher ROS levels have been associated with higher proliferating cells. A sustained ectopic production of the extremely potent antioxidant enzyme MnSOD slows pancreatic tumor cell development [15].

ATM (ataxia telangiectasia mutated) is one of the proteins that are triggered by ROS in cell cycle regulation. It is possible to reverse equivalent effects in ATM knockout mice with antioxidant supplementation, whereas patients missing ATM have greater levels of oxidative damage. As a result of altering critical proteins involved in the development of cell cycles, ROS might modulate tumor cell proliferation [30].

Table 8.1 summarizes the progression of cancer caused by ROS and Table 8.2 summarizes the contribution of ROS in cancer cell death.

8.4 INTERLINK BETWEEN ROS AND INFLAMMATION IN CANCER

All stages of carcinogenesis are aided by inflammation, which also increases the risk of developing cancer. A complex network of interacting cells, including stromal fibroblasts, many immune cell types, endothelial cells, and cancer cells that infiltrate the host, makes up the tumor microenvironment (TME) [32]. Because of their high plasticity, within the TME, cells' phenotypic and

TABLE 8.1 Progression of Cancer Caused by ROS [31]

Influence	Mechanism	Cell line
Induces DNA damage	ROS can damage DNA directly, leading to mutations that can promote cancer growth.	HeLa, MCF-7, A549
Activates oncogenes	ROS can activate oncogenes, which are genes that promote cancer growth.	KRAS, BRAF, MYC
Inhibits tumor suppressor genes	ROS can inhibit tumor suppressor genes, which are genes that help to prevent cancer growth.	TP53, PTEN, BRCA1
Increases cell proliferation	ROS can increase cell proliferation by stimulating the cell cycle.	MCF-7, A549
Induces cell survival	ROS can induce cell survival by inhibiting apoptosis.	HeLa, A549
Promotes angiogenesis	ROS can promote angiogenesis, which is the growth of new blood vessels that supply tumors with nutrients.	HeLa, MCF-7
Increases metastasis	ROS can increase metastasis, which is the spread of cancer cells from the primary tumor to other parts of the body.	HeLa, MCF-7

TABLE 8.2 Contribution of ROS in Cancer Cell Death [15]

Influence	Mechanism	Cell line
Induces apoptosis	ROS can induce apoptosis, which is a type of programmed cell death.	HeLa, MCF-7, A549
Stimulates autophagy	ROS can stimulate autophagy, which is a process of cellular self-digestion.	HeLa, MCF-7, A549
Increases ROS sensitivity	Cancer cells can become more sensitive to ROS, which makes them more likely to die.	HeLa, MCF-7, A549
Inhibits cell survival pathways	ROS can inhibit cell survival pathways, such as the PI3K/AKT pathway.	HeLa, MCF-7, A549
Promotes cell death signaling pathways	ROS can promote cell death signaling pathways, such as the caspase cascade.	HeLa, MCF-7, A549

functional features are continually modifying. Chronic inflammation is a key factor in the growth of tumors by producing a TME that is supportive. Many physiological processes depend on ROS, which can operate as messengers inside cells. When ROS reach supraphysiological levels, they are thought to be powerful DNA-damaging agents. Many malignancies have been discovered to have elevated ROS levels, and these elevated levels have been linked to practically every facet of cancer growth. Chronic inflammation and cancer have long been linked to ROS, according to the theory [33].

The extracellular matrix and blood vessels are acellular components of the tumor microenvironment (TME), while epithelial cells, fibroblasts, and immune cells are examples of cellular components surrounding the tumor mass. In addition to other roles that contribute to the hallmarks of cancer, cancer cells secrete chemicals that modify the TME and promote the progression of the disease through immune evasion, metastatic niche formation, and neo-angiogenesis, among other mechanisms [34].

8.5 AN OVERVIEW OF CANCER ASSOCIATED CHANGES

One of the most concerning diseases in the world is still cancer. By 2030, there will likely be 13 million cancer-related deaths worldwide, demonstrating the critical need for scientific cancer therapies [33]. A group of cells that grow out of control and ignore the usual laws of cell division is what is referred to as cancer, and these cells are referred to as cancer cells [35]. According to recent studies, the loss of redox equilibrium serves a crucial role in the initiation, growth, and eventual spread of cancer in human cells. Free radicals, which are mostly identified as reactive species of oxygen, are overexpressed, causing this disruption in redox equilibrium (ROS). The control of cellular differentiation, communication, and their existence, as well as tissue homeostasis, depend greatly on ROS. Furthermore, ROS controls the homeostasis of cells and plays a crucial role in the development of disease by regulating the mechanisms by which cells malfunction. Abnormally high levels of ROS promote the formation and spread of cancer, as well as other unfavorable processes (Figure 8.1). Because ROS has both pro- and anti-tumorigenic actions, it plays both a negative and a positive role in the biology of cancer. Increased ROS and RNS levels, which are commonly associated with persistent inflammation, cause a range of epigenetic changes, DNA mutations, and genomic instability. All these changes support the genesis, growth, progression, metastatic spread, and treatment resistance of tumors.

It has long been recognized that inflammation and the emergence of cancer are related [36]. The body's defenses against germs are coordinated by the inflammatory response, which also regulates tissue regeneration and repair that can occur after either infectious or non-infectious tissue injury. According to epidemiological data, chronic inflammation causes dysplasia, which in turn increases the risk that cancer may spread [37].

A small tumor can collect adequate oxygen and nutrients from its environment when it first develops from a few stray cells. Yet, as it expands,

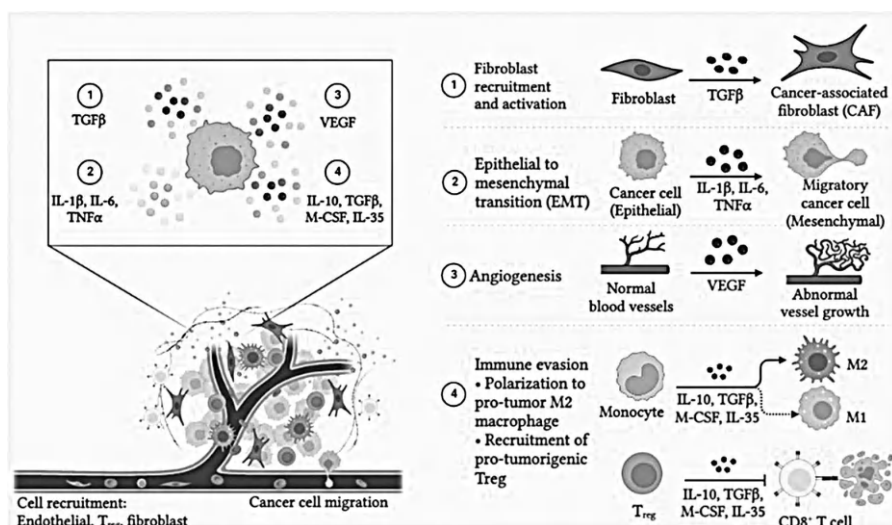


FIGURE 8.1 Overview of cancer associated changes.

supply begins to fall short of demand, leading to an abnormal situation. The cancer cells generate chemical signals that encourage immune cells such as macrophages and granulocytes to colonize the tumor as they struggle to survive and as they acquire more and more genetic flaws [38]. When these cells reach the tumor's interior, they release substances known as cytokines, which trigger the angiogenesis process, resulting in the inflow of vital nutrients and oxygen. Other cytokines promote the formation of the stroma, which acts as a cellular "cushion" for the tumor. As a result of chemicals (free radicals) released by other inflammatory cells, the tumor's DNA is further damaged. With the release of molecules that aid tumor cells in separating from their environment, inflammation may also serve as the catalyst for metastasis. Combined, these results show how cancer development is enhanced by inflammation that is being controlled by growing tumors.

Table 8.3 summarizes the role of ROS and inflammation in cancer.

8.5 ROLE OF PROBIOTICS IN OXIDATIVE STRESS AND INFLAMMATION

The body's reactive oxygen species are intimately related to biological aging. It is possible for free radicals to damage DNA, proteins, lipids, and other molecules of cells, causing gradual physiological decline and diseases

TABLE 8.3 Role of ROS and Inflammation in Cancer

Effect	Mechanism	References
Inflammation and Tumor	Myeloid cell infiltration may enhance the mutational load of existing neoplasms because ROS have been demonstrated to cause DNA damage in epithelial cells <i>in vitro</i> .	[39]
	The higher cancer risk in those with conditions like Crohn's and inflammatory bowel disease may be explained by the possibility that myeloid cell-derived ROS cause driver mutations.	[40]
	Tumor growth is enhanced in wounded limbs by the induction of paracrine factors such as TGF- β and bFGF in wound fluid.	[41]
	Administration of the phorbol ester TPA and the mutagen DMBA causes skin tumors.	[42]
	Hepatocellular carcinoma caused by a mutagen after diethyl nitrosamine (DEN) administration.	[43]
	Chronic inflammatory circumstances can cause myeloid cells to create ROS, which can promote the development of cancer.	[44]

such as cancer and cardiovascular disease [45]. A rise in oxidative stress is widely believed to be associated with cancer, diabetes, Parkinson's disease, Alzheimer's disease, cardiovascular disease, neurological disorders, and psychiatric disorders. Oxidative stress occurs due to an imbalance in the body's prooxidant/antioxidant status, which is normally regulated by the liver. The past few years have seen a rise in interest in probiotics due to their potential to treat diseases caused by inflammation. Probiotics may successfully lower both peripheral and central inflammation through a variety of routes, according to several studies. The root cause is connected to gut flora restoration, increased gut permeability, immune system regulation, and reduced pro-inflammatory cytokine production [46]. Additionally, probiotics have beneficial effects on a number of diseases, including inflammatory bowel disease, obesity, and neurodegenerative conditions. They can control the maturation and activity of microglia and possibly stop neuroinflammatory processes. In addition, it has been found that probiotics and/or bacterial metabolites can influence the amount of endogenous and exogenous ROS produced by the host, leading to an improvement in oxidative status. In the gastrointestinal tract, there are ten times as many bacteria as human cells, and their genomic content exceeds 3 million, compared to 23,000 genes in the human genome [47]. Research over time has shown that probiotic *Bifidobacteria* and *Lactobacilli* strains have antioxidant capabilities.

8.5.1 PROBIOTICS AND OXIDATIVE STRESS

The current study looked at a number of publications from clinical trials that examined how probiotics affected oxidative stress and redox potential in studies involving humans. According to studies, probiotics strengthen the body's antioxidant function and promote oxidative stability. In clinical studies, malondialdehyde (MDA), 7,8-dihydroxy-8-oxoguanosine (8-OXO-G), glutathione (GSH), superoxide dismutase (SOD), nitric oxide (NO), catalase (CAT), and glutathione peroxidase (GPx) were primarily evaluated [48]. Probiotic supplementation's impact on TAC was examined in randomized studies. According to the preliminary and main meta-analysis findings, probiotic medicines significantly raised TAC levels in blood by 52.46 mmol/L [49]. The quantity of TAC in the body is influenced by a variety of enzymatic and non-enzymatic variables. These elements can make up the body's antioxidant structure by fending off oxidizing agents. The presence of vitamin E, SOD, and glutathione are the major components of the TAC structure [50]. It has been demonstrated that different probiotic species can control inflammatory and immunological responses in the host by changing the makeup of the gut microflora [51]. Probiotics work to increase TAC by boosting GSH levels, scavenging superoxide and hydroxyl radicals, and decreasing inflammatory IL-6 expression [52]. Considering the findings of this research, patients with diabetes or gestational diabetes who supplement with probiotics or synbiotics experience a reduction in blood sugar or insulin levels, as well as improvements in insulin resistance and HbA1c. Research on TAC has shown the benefits of probiotic supplementation on blood glucose in these individuals [53]. As high levels of insulin and insulin resistance are associated with elevated levels of oxidative and inflammatory factors, probiotic supplementation appears to reduce the level of insulin resistance by lowering blood glucose, insulin, and oxidants in the body, as well as increasing TAC.

8.5.2 PROBIOTICS AND INFLAMMATION

It has been demonstrated that different probiotic strains have a variety of impacts on the human body and its defenses. In numerous *in vitro* and *ex vivo* models, as well as in mouse models, their crucial function in the control of inflammation has been thoroughly described. These models demonstrate the catastrophe of the immune regulatory networks in blood circulation, which sets off a chain of actions that result in an inflammatory reaction. Certain types of bacteria can influence the intestinal mucosal barrier, the

gastrointestinal luminal environment, and the mucosal immune system. Probiotics may have an impact on a variety of innate and acquired immune system cells, including monocytes, killer cells, macrophages, lymphocytes, and epithelial cells (Figure 8.2). The pattern recognition receptors (PRRs) expressed on immune cells and non-immune cells, like M cells in Peyer's patches and intestinal epithelial cells, may be specifically activated by them. TLRs, which may trigger signaling cascades that result in cell proliferation and the release of cytokines to control the immune system, are the PRRs that have been the subject of the most research.

Additionally, some particular strains secrete substances that could cause the immune system to become activated. M cells specifically phagocytose

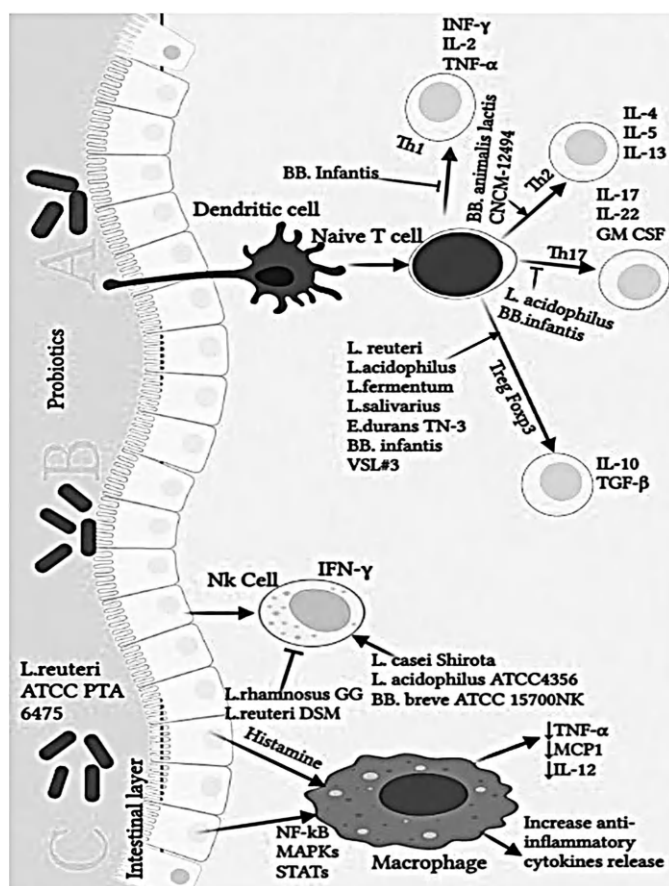


FIGURE 8.2 Representation of GI-based probiotic immune-modulating pathways A. Naïve T-Cells, B. NK cells C. Immune cells like MAP kinase cascade, STATs and NF-kB.

or internalize the probiotic and the antigenic components derived from its metabolites to generate endosomes. Certain probiotic strains can cause dendritic cells to become activated, which then transport antigens to nearby lymph nodes and release IL-10 and IL-12. Here, dendritic cells use their arsenal of cytokines to encourage naïve T and B cells to differentiate into their respective subpopulations. In more detail, naïve Th cells can develop into Treg, Th1, and Th2 immune cells, while B cells can change into plasma cells that are actively involved in humoral responses or regulatory B (Breg) cells that produce IL-10 or TGF. In addition, by producing cytokines like IL-12 and IL-15, dendritic cells could promote the activation of NK cells. Many lactic acid bacteria, in particular, encourage the IFN- γ synthesis by NK cells through dendritic cells in the presence of other probiotic microorganisms.

TABLE 8.4 Probiotics in Control of Inflammation

Probiotic Strain	Model Used for the Study	Cytokines and Inflammatory Mediators Involved	Reference
<i>L. acidophilus</i>	DSS-induced colitis in mice	IL-17, IL-23, TGF- β 1, TNF- α	[54]
<i>L. reuteri</i> ATCC PTA6495	TNBS-induced colitis in mice	IL-1 β , IL-6	[55]
<i>E. durans</i> TN-3	DSS-induced colitis in mice	Treg, IL-10, IL-1 β , IL-6, IL-17A, IFN- γ	[56]
<i>L. rhamnosus</i> RC007	TNBS-induced colitis in mice	IL-10/TNF- α ratio	[57]
<i>L. casei</i> Lbs2	TNBS-colitis-induced in mice	Treg	[58]
<i>L. fermentum</i> CECT5716, <i>L. salivarius</i> CECT5713	DSS-induced colitis in mice	IL-1 β , IL-12, TGF- β , NOS	[59]
<i>BB. infantis</i>	Colorectal cancer induced by dimethylhydrazine in mice	IL-2, IL-12, IFN- γ	[60]
<i>B. breve</i> DM8310, <i>L. acidophilus</i> DM8302, <i>L. casei</i> DM8121, <i>S. thermophilus</i> DM8309	5-FU-induced mucositis in mice	IL-6, TNF- α	[61]
<i>L. acidophilus</i>	DSS-induced colitis in mice	Treg, IL-10, IL-17	[62]

TABLE 8.4 (Continued)

Probiotic Strain	Model Used for the Study	Cytokines and Inflammatory Mediators Involved	Reference
<i>L. fermentum</i> CQPC04	DSS-induced colitis in mice	IL-10, TNF- α , IFN- γ , IL-1 β , IL-6, IL-12, NF- κ Bp65, COX-2	[63]
<i>L. plantarum</i> CAU1055	DSS-induced colitis in mice	TNF- α , IL-6, NOS, COX-2	[64]
<i>L. plantarum</i> C88	Aflatoxin B1-induced liver injury in mice	IL-1 β , IL-6, IL-8, TNF- α , IFN- γ , NF- κ B	[65]
<i>L. lactis</i> ML2018	DSS-induced colitis in mice	NF- κ B, MAPK	[66]
<i>L. reuteri</i> LMG P-2748	<i>C. difficile</i> -induced colitis in mice	IL-10	[67]

According to studies, the immunological modulation brought on by probiotic bacteria may be caused by the anti-inflammatory cytokines secreted in the stomach. However, the precise molecular interactions between the host and the probiotics are not well understood. The three genera *Bifidobacterium* (*BB*), *Bacillus* (*B.*), and *Lactobacillus* (*L.*) are the most often utilized probiotics in humans, but the genus *Saccharomyces* (*S.*) is also frequently employed in commercial goods [68]. *Bifidobacterium* causes the acquisition of tolerance, whereas some strains of *Lactobacillus* might modify the cytokine generation by immune cells.

Each probiotic strain exhibits such distinctive regulatory behaviors, which are related to their structure, the range of mediators they release, and the numerous pathways they concurrently activate [69]. Probiotics' ability to reduce inflammation has been examined *in vitro*, *ex vivo*, and in animal studies to assess cytokine generation and immune cell propagation. We discuss current research topics that show certain probiotic strains have an anti-inflammatory impact in the sections that follow (Table 8.4).

8.6 PROBIOTICS IN THE PREVENTION OF CARCINOGENESIS

The World Health Organization and the Food and Agriculture Organization of the United Nations define probiotics as live bacteria that, when supplied to a host in a suitable proportion, confer an advantage for health [70]. Figure 8.3 illustrates the mechanism of action of probiotics.

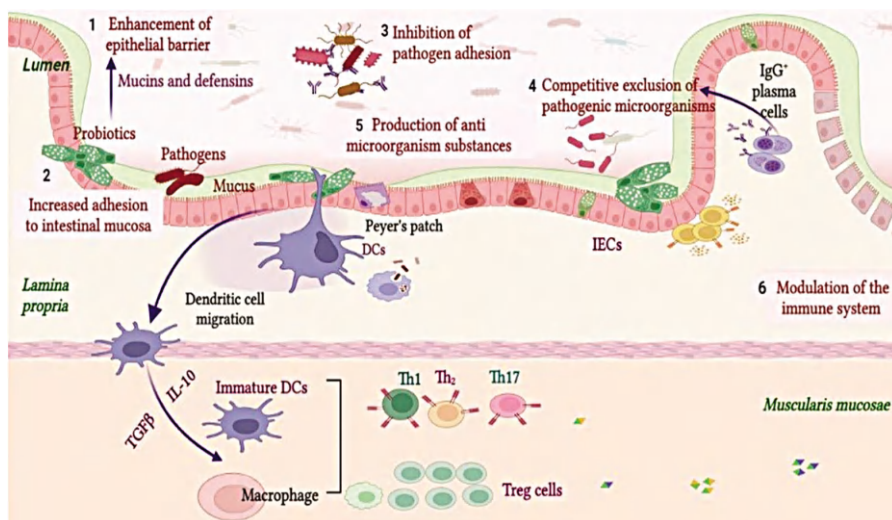


FIGURE 8.3 Mechanism of action of probiotics.

For the body to remain in a state of equilibrium, the gut microbiota can be crucial. Interruption of disease processes, such as cancer, and pro-inflammatory immune reactions might result from an imbalance in the gut microbiota. The probiotic's efficiency in preventing, treating, and supporting cancer is examined using the most recent findings in scientific research [71]. One of the first to point out a link between a diet rich in *Lactobacillus* and a 37% reduction in the risk of colorectal cancer compared to controls was Goldin and Gorbach [72]. Probiotics may benefit cancer cells by controlling their development and mortality, according to the results of numerous *in vitro* investigations on myeloid leukemia, gastric, colon, and other cancer cells. The majority of *in vitro* studies using human colon cancer cells were performed. One of the key mechanisms underlying the antagonistic effects of probiotics on various microorganisms is the modification of the gut microbiota. Other significant mechanisms include competitive adhesion to the mucosa and epithelium, strengthening of the gut epithelial barrier, and immune system modulation to the host's advantage [73].

8.6.1 POTENTIAL MECHANISMS BY WHICH PROBIOTICS PREVENT CARCINOGENESIS (FIGURE 8.4)

- 1. Modification of the Composition of the Gut Microbiota:** To maintain eubiosis, the gut microbiota needs to be appropriately

diverse and balanced. An imbalance among harmful and beneficial microbes in the intestinal microbiota is known as dysbiosis. The risk of developing colorectal cancer is further increased by dysbiosis, resulting in chronic inflammation and increased synthesis of carcinogenic substances [74]. Probiotics are advantageous for the host by increasing intestinal barrier integrity, lowering pathogen development, and slowing the metabolism of chemicals that promote cancer.

2. **Intestinal Microbiota Metabolic Activity:** Numerous bacteria found in the human gut are capable of producing endogenous bile salts as well as tumor-causing compounds from dietary sources. These substances can have genotoxic and cytotoxic effects, leading to unusual colonocyte growth and the stimulation of anti-apoptotic pathways, which aid in the progression of colon cancer. By modulating the functioning of such digestive enzymes, probiotics can change bacterial metabolism and reduce the risk of colon cancer. Probiotics can cause cell lysis, interfere with or inhibit the synthesis of DNA, proteins, and RNA, and cause the transmembrane potential to dissipate as well as the leakage of K^+ ions, ATP, and amino acids from the cytoplasm of the attacked cells in sensitive microorganisms. Bacteriocins produced by probiotics can be lethal or bacteriostatic and impact bacterial cell membranes with receptors that may adhere to them, causing cell death [75].
3. **The Generation of Substances Having Anticarcinogenic Properties, Including CLAs and SCFAs:** Although short-chain fatty acids (SCFAs) have immunomodulatory properties that influence inflammation, conjugated linoleic acids (CLAs) regulate the proliferation of genes that control apoptosis (Bcl-2, caspase 3 and 9) and the response of cells to proliferation factors. Additionally, colonocytes' ability to produce eicosanoids can be suppressed by CLAs [76].
4. **Suppression of Cancer Cell Growth and Induction of Apoptosis:** The rate at which cancer develops is influenced by the apoptosis and proliferation of cancer cells. Cancer cells experience greater proliferation than apoptosis as the malignancy develops. Since apoptosis doesn't affect nearby cells or result in inflammation, cancer cells can be eliminated less violently. Bcl-2 and Bcl-xl, two NF- κ B-dependent genes that regulate cell viability and proliferation, may be inhibited by probiotic bacteria such as lactic acid bacteria (LAB), working to prevent cancer [77].

- 5. Impact on Additional Mutagenic and Carcinogenic Factors:** Free radicals and cancer-causing substances are less active when probiotics are present because they can change the functioning of many enzymes involved in the detoxification process. Probiotics can impact other mutagenic and carcinogenic factors, helping prevent cancer and contributing to overall health. Probiotics modify microbial enzyme activity in the intestines through various mechanisms, including their capacity to create acids that might decrease the pH of the colon, synthesize antibiotic substances that hinder the spread of other microbes, and exclude infectious microbiota [78].
- 6. Degradation and Binding of Cancer-Causing Substances in the Gut Lumen:** Carcinogenic compounds may adhere to the cell walls of some probiotic bacteria. A cationic exchange between the cancer-causing agents and the peptidoglycan molecules found within their cell walls might take place. Through the feces, microorganisms and potentially cancer-causing chemicals would be eliminated similarly [79].
- 7. Immunomodulation:** For the lymphoid tissues of the body's immune response to develop, the body's immune response must be activated by the microbes in the gut microbiota. These microbes induce and maintain immune responses against bacterial and viral infections, environmental antigens (food and inhalation), and prevent autoimmune and allergic reactions. The intestinal microbiota, which contains probiotic bacteria, has immunomodulatory action through these three seemingly incompatible processes [80].
- 8. Strengthening the Intestinal Barrier:** The intestinal barrier and permeation can be altered by the microbes in the gut microbiota. Probiotics can decrease intestinal permeability due to their potential to alter the intestinal barrier's constituents, including intracolonic pH, mucin formation, and cellular junction proteins. A more acidic (lower intracolonic pH) environment prevents the growth of putrefactive and pathogenic microbes along with microbial enzymes that produce cancer-causing substances. When the incubation medium's pH was acidic, inhibition occurred, but there was no growth inhibition when it was neutral.

Figure 8.4 depicts the mechanisms of probiotics in prevention of carcinogenesis.

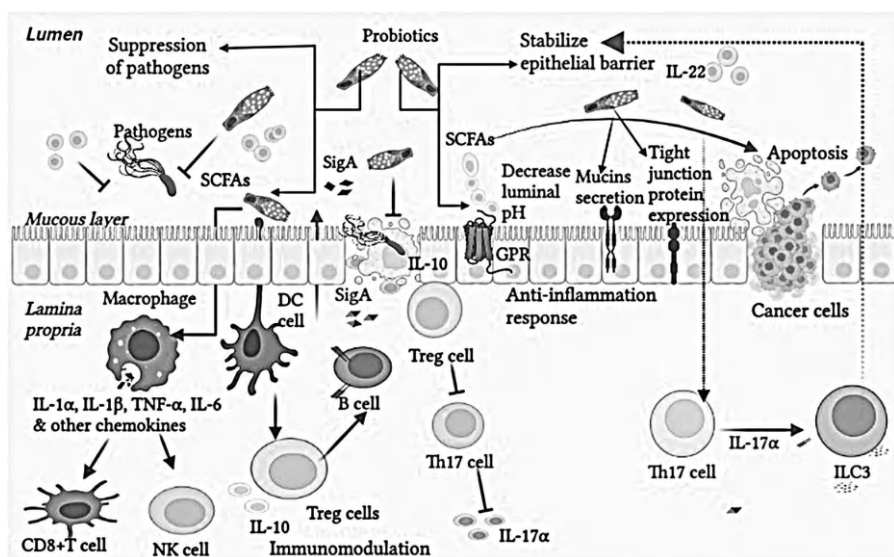


FIGURE 8.4 Potential mechanisms of probiotics in prevention of carcinogenesis.

8.7 CLINICAL TRIALS IN PROBIOTICS FOR DECREASING ROS

When reactive oxygen species (ROS) are formed in surplus or are not cleaved enough, it results in oxidative damage, which can lead to DNA damage, lipid peroxidation, enzyme inactivation, cell necrosis, and apoptosis [81]. Interventional research [82–85] has looked at how probiotic supplementation affects oxidative stress reduction and antioxidant biomarker improvement. The effects of probiotic and synbiotic supplementation on oxidative stress indices in healthy participants were also studied in a meta-analysis, and the scientists concluded that these products enhance antioxidant resistance and boost the body's supply of antioxidant enzymes [86]. One more meta-analysis of individuals with Chronic Kidney Disease (CKD) revealed that bacteriological therapies significantly decreased blood levels of C-Reactive Protein (CRP), Malondialdehyde (MDA), Total Antioxidant Capacity (TAC), and Total Glutathione (GSH) [87]. A recent comprehensive analysis found that probiotic administration may reduce oxidative stress and systemic inflammation in people with diabetic nephropathy (DN) without having any appreciable negative effects [88].

8.7.1 CLINICAL STUDY

In one investigation, the impact of several *Lactobacillus* strains on athletes' oxidative stress was assessed [89]. In this study, one group drank

Lactobacillus paracasei IMC 502 and *Lactobacillus rhamnosus* IMC 501 daily over the course of four weeks. Both bacterial strains were tested at the beginning of the study using the thiobarbituric acid (TBA) technique to measure oxidative stress resistance and identify *in vitro* antioxidative activity. Thus, the antioxidative impact of both *Lactobacillus* strains, which mostly manifests in intracellular extracts, was proven by the same linoleic acid peroxidation suppression in comprehensive cells. Furthermore, both strains have adequate tolerance to oxidative stress. The biological antioxidant capacity of plasma, as well as the quantity of blood reactive oxygen metabolic compounds, was next assessed in the probiotic and control groups. Members were asked to engage in vigorous physical exercise, which increases oxidative stress and ROS generation.

It was observed that the two strains under investigation have significant antioxidant potential. Probiotic supplementation helps raise plasma antioxidant levels, which neutralize ROS and present a fantastic potential for probiotic application on a larger scale. Analysis of the feces following probiotic treatment further indicated that these bacteria's capacity for colonization is probably host-specific. Other studies' findings support the positive effects of probiotics [90]. In this intricate and thorough investigation, healthy volunteers' responses to probiotic pills and fermented goat milk comprising *Lactobacillus fermentum* ME-3 were evaluated for their antioxidant potency. Total antioxidative status (TAS), glutathione redox fraction, and total antioxidative activity (TAA) of participants' blood serum were examined to assess the oxidative status of the human body. Probiotic pills and fermented goat milk both showed improvements in oxidative stress indicators.

However, the TAA and TAS indicators were found in considerably greater concentrations in the *Lactobacillus fermentum* ME-3 fermented goat milk cluster compared to the capsule group. Moreover, a decline in the glutathione redox fraction was seen, nonetheless only in those who had fermented goat milk. The present suggests that there may be a connection between the formulation type and the effects of probiotic antioxidants within the host. Further research, however, does not support probiotics' beneficial effects on oxidative stress. The fast production of physiological amounts of ROS against an unidentified source by some classes of commensal gut bacteria in human epithelial cells has regulatory consequences [91-94]. NADPH oxidases' catalytic activity is frequently used to create cellular ROS. Furthermore, it was shown that lactobacilli are particularly strong inducers of ROS formation both *in vivo* and in cultured cells. This impact was established, in accordance with Neish [95], by an upsurge in the oxidoreductase response of transcription

factor stimulations (NF- κ B), Nrf2, and the antioxidant response element, demonstrating a controlled cell response to enhanced ROS generation.

The third trimester of pregnancy was the subject of another investigation. Contrary to normal yogurt, probiotic yogurt enhanced through *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12 had not substantially altered serum 8-oxo-G levels, erythrocyte GPx, plasma GSH, or TAC levels after nine weeks of consumption [96]. Only the level of erythrocyte glutathione reductase (GR) increased considerably from the control group.

In accordance with probiotic supplementation, Chamari et al., [97] evaluated the amount of CAT in the plasma of healthy women. In comparison to the untreated or control groups, the probiotic clusters exhibited a statistically significant rise in catalase levels. In contrast to placebo, *Lactobacillus casei* 01 supplements had not significantly enhanced oxidative status in rheumatoid arthritis patients [98]. Finally, a sample of smokers was studied to see if probiotics had any positive benefits. Plasma F2-isoprostane concentrations were significantly lower (31%) after *Lactobacillus plantarum* supplementation compared to the control group. These biochemical indicators can accurately and efficiently detect oxidative stress and lipid peroxidation [99] (Table 8.5).

TABLE 8.5 Clinical Studies

Strains	Study Groups	Main Conclusion	References
<i>Lactobacillus paracasei</i> IMC 502 and <i>L. rhamnosus</i> IMC 501	Athletes	Exert strong antioxidant activity	[89]
<i>Lactobacillus fermentum</i> ME-3	Healthy volunteer	Increases counts of intestinal lactobacilli and reduces oxidative stress	[90]
<i>Lactobacillus acidophilus</i> LA5 and <i>Bifidobacterium lactis</i> BB12	Pregnant women	Do not affect oxidative stress markers, only significantly increase erythrocyte GR level	[96]
<i>Lactobacillus acidophilus</i> LA5 and <i>Bifidobacterium lactis</i> BB12	Healthy women	Consumption of selected probiotic strain increases antioxidant enzyme levels and decreases oxidative stress	[97]
<i>Lactobacillus casei</i> 01	Rheumatoid arthritis patients	No significant improvement of oxidative status	[98]
<i>Lactobacillus plantarum</i> 299v	Healthy smokers	Reduction of plasma F2-isoprostane concentrations	[99]

Forty patients with a brucellosis diagnosis, between the ages of 8 and 15, were registered in a randomized, double-blind, and placebo-controlled experiment. Probiotic supplements or placebos were given to study participants in one of two groups ($n = 20$ per group) over an eight-week period. To measure biochemical markers, plasma specimens were taken beforehand and subsequently after the 8-week intervention. A pediatric infectious disease expert assessed the clinical state. Probiotic supplementation after the 8-week intervention markedly increased malondialdehyde ($P < 0.001$) and overall antioxidant capacity ($P < 0.001$). Although probiotic intake had no statistically significant impacts on highly sensitive CRP, total glutathione, and other experimental conclusions related to placebo, there was a variance between the probiotic group and the placebo group for the duration of fever ($P < 0.02$) and musculoskeletal pain ($P < 0.001$), respectively. Overall, probiotic supplementation had a positive effect on clinical status and the body's natural antioxidant defenses in pediatric brucellosis patients [98].

Another double-blind, randomized controlled study with forty critical care unit admissions was conducted. For seven days, they were arbitrarily allocated to either a probiotic or a placebo. Acute Physiology & Chronic Health Evaluation records, malondialdehyde (MDA), total antioxidant capacity, and CRP levels in serum were assessed prior to the study's start and on day seven. By the conclusion of the trials, a notable disparity existed ($P = 0.003$ and 0.001 , respectively) between the two groups' CRP levels and APACHE II scores. TAC and MDA levels had not varied significantly between the two groups. Probiotics were given to very unwell individuals, which decreased inflammation and improved clinical outcomes. The indicators of oxidative stress did not, however, vary significantly [99].

8.8 FUTURE ASPECTS AND CONCLUSION

In addition to being naturally occurring byproducts of distinct cellular compartments' metabolic processes, ROS also operate as signaling molecules that control particular biological processes essential to healthy cell function and survival. The pathogenesis of a disease can, however, be affected by deregulation of ROS signaling or excessive generic ROS generation. The importance of ROS in cell metabolism and inflammatory signaling has been underlined in this study. Therefore, it is not unexpected that disorders connected to metabolic dysregulation and inflammation also involve ROS significantly.

There are several areas that need more research. It's significant that whereas cytoROS and mitoROS signaling have received a lot of attention, less is known about the role of ROS produced by peroxisomes and the endoplasmic reticulum in maintaining cellular homeostasis. To better understand the role of organelle/compartment-specific ROS and its impact on important cell processes like fatty acid biosynthesis, protein translation systems, and post-translational modifications, novel organelle-targeted probes and antioxidant substances will be used. Due to the connection between inflammatory and metabolic signaling, such techniques will correspondingly shed light on how reactive oxygen species involved in inflammatory signaling might influence and be influenced through this pathway. Additionally, investigation must continue to define the tissue-specific function of ROS signaling in metabolism and cardiac illnesses in light of the emergence of tissue-specific and inducible transgenic mouse models and Cre-lox technologies. Understanding tissue-specific redox signaling would be vital in our endeavor to discover novel and innovative medicines to treat disease. The etiology of cardiovascular and metabolic diseases is complicated. Understanding the role and impact of ROS on metabolism is an intriguing topic for scientific investigation because metabolic dysregulation is a major contributor to cellular dysfunction and the development of disease [100].

Within biological systems, ROS and free radical behavior have become more complicated. Free radicals and ROS were formerly thought to be harmful; however, modern mechanisms describe both beneficial and harmful effects. With the onset of reperfusion and in relation to damage, a burst of ROS has been well documented. Yet, ROS could also act as a signaling preconditioning defense and trigger stress reactions that aid in survival. It is known that ROS synthesis occurs through ischemia, even with the short oxygen tension from a source that is most likely the mitochondria, and reactive oxygen species-induced ROS release might intensify its signal. Unidentified cellular sources may be responsible for the burst of ROS observed after reperfusion, which differs from the source detected during ischemia.

Energy metabolic processes, survival/stress responses, apoptosis, inflammatory responses, oxygen sensing, and many other important processes are all controlled by ROS and cellular redox conditions. Even though cellular systems may exhibit reperfusion damage, whole organ and animal models continue to show conflicting findings on reperfusion injury and the therapeutic value of antioxidants. Together, these findings may shed light on why therapeutic trials of antioxidants have shown such inconsistent and generally unfavorable outcomes. Imminent antioxidant therapies are likely to be successful; however,

they should: be more specific for the site of action; not have negative effects on other signaling pathways; be targeted to a specific ROS or cellular compartment; and be “time-sensitive” so they provide the right treatment at exactly the right moment in ischemia and other conditions [101].

The efficacy of probiotics’ antioxidative intrusion for a certain clinical state depends on careful assessment of the most suitable species and strains. Probiotics can be “potentially” useful in a number of conditions, yet the European Food Safety Authority hasn’t approved any health claims on them [102]. By lowering lipid peroxidation and ROS production while enhancing SOD activity, probiotics may be able to treat ischemia-reperfusion damage. Irritable bowel syndrome may be another condition that probiotics might help. The most popular strains are Lactobacilli and Bifidobacteria, which are said to release SOD enzymes as well as metal-complexing and antioxidant compounds. These strains may also shield the bowel from IBD and even colorectal carcinoma. Probiotic bacteria with the ability to provide SOD locally may offer a novel treatment choice for bowel illness with more ROS formation. The antioxidant properties of probiotics can also help individuals with liver illness that is marked by severe oxidative stress. The hunt for particular probiotic species that provide the best anticipation and justification of oxidative stress must go on if new probiotic products are to be created with the capacity to prevent it. To fully understand the probiotics’ antioxidative potential, further research is also required.

KEYWORDS

- **Probiotics**
- **Reactive Oxygen Species (ROS)**
- **Inflammation**
- **Polymorphonuclear neutrophils (PMNs)**
- **Gut dysbiosis**

REFERENCES

1. Kechagia, M., Basoulis, D., Konstantopoulou, S., et al. (2013). Health benefits of probiotics: A review. *ISRN Nutrition*, 2013, 481651. <https://doi.org/10.5402/2013/481651>.

2. Bhattacharyya, A., Chattopadhyay, R., Mitra, S., & Crowe, S. E. (2014). Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiological Reviews*, 94(2), 329–354. <https://doi.org/10.1152/physrev.00019.2013>.
3. Feniouk, B. A., & Skulachev, V. P. (2017). Cellular and molecular mechanisms of action of mitochondria-targeted antioxidants. *Current Aging Science*, 10(1), 41–48. <https://doi.org/10.2174/1874609810666170329112952>.
4. Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. (2016). Oxidative stress and inflammation: What polyphenols can do for us? *Oxidative Medicine and Cellular Longevity*, 2016, 7432797. <https://doi.org/10.1155/2016/7432797>.
5. Holmström, K. M., & Finkel, T. (2014). Cellular mechanisms and physiological consequences of redox-dependent signaling. *Nature Reviews Molecular Cell Biology*, 15(6), 411–421. <https://doi.org/10.1038/nrm3807>.
6. Kalinina, E. V., Chernov, N. N., & Novichkova, M. D. (2014). Role of glutathione, glutathione transferase, and glutaredoxin in regulation of redox-dependent processes. *Biochemistry (Moscow)*, 79(13), 1562–1583. <https://doi.org/10.1134/S0006297914130086>.
7. Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature*, 454(7203), 428–435.
8. Segal, A. W. (2008). The function of the NADPH oxidase of phagocytes and its relationship to other NOXs in plants, invertebrates, and mammals. *International Journal of Biochemistry & Cell Biology*, 40(4), 604–618.
9. Ikeda, S., Yamaoka-Tojo, M., Hilenski, L., et al. (2005). IQGAP1 regulates reactive oxygen species-dependent endothelial cell migration through interacting with Nox2. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25(11), 2295–2300. <https://doi.org/10.1161/01.ATV.0000183221.61199.71>.
10. Lopes, F., Coelho, F. M., Costa, V. V., et al. (2011). Resolution of neutrophilic inflammation by H₂O₂ in antigen-induced arthritis. *Arthritis & Rheumatism*, 63(9), 2651–2660.
11. Wagener, F. A., Carels, C. E., & Lundvig, D. M. (2013). Targeting the redox balance in inflammatory skin conditions. *International Journal of Molecular Sciences*, 14(5), 9126–9167.
12. Jorgenson, T. C., Zhong, W., & Oberley, T. D. (2013). Redox imbalance and biochemical changes in cancer. *Cancer Research*, 73(20), 6118–6123. <https://doi.org/10.1158/0008-5472.CAN-13-1122>.
13. Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology*, 24(10), R453–R462.
14. Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., & 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. *Antioxidants*, 10(9), 4642. <https://doi.org/10.3390/antiox100904642>.
15. Liou, G. Y., & Storz, P. (2010). Reactive oxygen species in cancer. *Free Radical Research*, 44(5), 479–496.
16. Weinberg, F., Ramnath, N., & Nagrath, D. (2019). Reactive oxygen species in the tumor microenvironment: An overview. *Cancers (Basel)*, 11(8), 1191.
17. Snezhkina, A. V., Kudryavtseva, A. V., Kardymon, O. L., et al. (2019). ROS generation and antioxidant defense systems in normal and malignant cells. *Oxidative Medicine and Cellular Longevity*, 2019, 6175804.

18. Khan, A. Q., Rashid, K., AlAmodi, A. A., et al. (2021). Reactive oxygen species (ROS) in cancer pathogenesis and therapy: An update on the role of ROS in anticancer action of benzophenanthridine alkaloids. *Biomedicine & Pharmacotherapy*, 143, 112142. <https://doi.org/10.1016/j.biopha.2021.112142>.
19. Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity*, 2016, 1245049. <https://doi.org/10.1155/2016/1245049>.
20. Wang, J., Li, D., Cang, H., & Guo, B. (2019). Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. *Cancers*, 8(10), 4709–4721.
21. Vallée, A., & Lecarpentier, Y. (2018). Crosstalk between peroxisome proliferator-activated receptor gamma and the canonical WNT/ β -catenin pathway in chronic inflammation and oxidative stress during carcinogenesis. *Frontiers in Immunology*, 9, 745.
22. Kennel, K. B., & Greten, F. R. (2021). Immune cell-produced ROS and their impact on tumor growth and metastasis. *Redox Biology*, 42, 101891. <https://doi.org/10.1016/j.redox.2021.101891>.
23. Gabryelska, M. M., & Conn, S. J. The RNA interactome in the hallmarks of cancer. *n/a* (n/a), e1786. <https://doi.org/10.1016/j.ebiom.2019.101786>.
24. Baghban, R., Roshangar, L., Jahanban-Esfahlan, R., et al. (2020). Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling*, 18(1), 59. <https://doi.org/10.1186/s12964-020-00602-2>.
25. Zhu, M. M., Ma, Y., Tang, M., Pan, L., & Liu, W. L. (2022). Hypoxia-induced upregulation of matrix metalloproteinase 9 increases basement membrane degradation by downregulating collagen type IV alpha 1 chain. *Physiological Research*, 71(6), 825–834.
26. Perillo, B., Di Donato, M., Pezone, A., et al. (2020). ROS in cancer therapy: The bright side of the moon. *Experimental & Molecular Medicine*, 52(2), 192–203.
27. Liu, Z., Ren, Z., Zhang, J., et al. (2018). Role of ROS and nutritional antioxidants in human diseases. *Nutrients*, 9(9), 1092.
28. Arfin, S., Jha, N. K., Jha, S. K., et al. (2021). Oxidative stress in cancer cell metabolism. *Nutrients*, 10(5), 642. <https://doi.org/10.3390/nu10050642>.
29. Zhang, J., Wang, X., Vikash, V., et al. (2016). ROS and ROS-mediated cellular signaling. *Oxidative Medicine and Cellular Longevity*, 2016, 4350965.
30. Blignaut, M., Harries, S., Lochner, A., & Huisamen, B. (2021). Ataxia telangiectasia mutated protein kinase: A potential master puppeteer of oxidative stress-induced metabolic recycling. *Oxidative Medicine and Cellular Longevity*, 2021, 8850708. <https://doi.org/10.1155/2021/8850708>.
31. Aggarwal, V., Tuli, H. S., Varol, A., et al. (2019). Role of reactive oxygen species in cancer progression: Molecular mechanisms and recent advancements. *Biomolecules*, 9(11). <https://doi.org/10.3390/biom9110680>.
32. Aboeella, N. S., Brandle, C., Kim, T., Ding, Z. C., & Zhou, G. (2021). Oxidative stress in the tumor microenvironment and its relevance to cancer immunotherapy. *Cancers*, 13(5). <https://doi.org/10.3390/cancers13050953>.
33. Wu, H., Zhong, M., & Wang, Y. (2022). The interdependence of inflammation and ROS in cancer. In S. Chakraborti, B. K. Ray, & S. Roychoudhury (Eds.), *Handbook*

- of Oxidative Stress in Cancer: Mechanistic Aspects* (pp. 29–58). Singapore: Springer Nature Singapore.
34. Greten, F. R., & Grivennikov, S. I. (2019). Inflammation and cancer: Triggers, mechanisms, and consequences. *Immunity*, 51(1), 27–41. <https://doi.org/10.1016/j.immuni.2019.06.018>.
 35. Russo, M. M., & Sundaramurthi, T. (2019). An overview of cancer pain: Epidemiology and pathophysiology. *Seminars in Oncology Nursing*, 35(3), 223–228.
 36. Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: Back to Virchow? *Lancet*, 357(9255), 539–545. [https://doi.org/10.1016/S0140-6736\(00\)04046-0](https://doi.org/10.1016/S0140-6736(00)04046-0).
 37. Coussens, L. M., & Werb, Z. (2002). Inflammation and cancer. *Nature*, 420(6917), 860–867. <https://doi.org/10.1038/nature01322>.
 38. Rakoff-Nahoum, S. (2006). Why cancer and inflammation? *Yale Journal of Biology and Medicine*, 79(3, 4), 123–130.
 39. Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell*, 140(6), 883–899. <https://doi.org/10.1016/j.cell.2010.01.025>.
 40. El-Kenawi, A., & Ruffell, B. (2017). Inflammation, ROS, and mutagenesis. *Cancer Cell*, 32(6), 727–729. <https://doi.org/10.1016/j.ccell.2017.10.008>.
 41. Hofer, S. O., Shrayder, D., Reichner, J. S., Hoekstra, H. J., & Wanebo, H. J. (1998). Wound-induced tumor progression: A probable role in recurrence after tumor resection. *Archives of Surgery*, 133(4), 383–389. <https://doi.org/10.1001/archsurg.133.4.383>.
 42. Moore, R. J., Owens, D. M., Stamp, G., et al. (1999). Mice deficient in tumor necrosis factor- α are resistant to skin carcinogenesis. *Nature Medicine*, 5(7), 828–831.
 43. Luo, J. L., Kamata, H., & Karin, M. (2005). IKK/NF- κ B signaling: Balancing life and death—A new approach to cancer therapy. *The Journal of Clinical Investigation*, 115(10), 2625–2632.
 44. Canli, Ö., Nicolas, A. M., Gupta, J., et al. (2017). Myeloid cell-derived reactive oxygen species induce epithelial mutagenesis. *Cancer Cell*, 32(6), 869–883.e5. <https://doi.org/10.1016/j.ccell.2017.10.009>.
 45. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8), 118–126.
 46. Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., & Rotondo, J. C. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*, 12(1), 134.
 47. Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology*, 14(8), e1002533.
 48. Kim, D. S., Park, Y., Choi, J. W., Park, S. H., Cho, M. L., & Kwok, S. K. (2021). *Lactobacillus acidophilus* supplementation exerts a synergistic effect on tacrolimus efficacy by modulating Th17/Treg balance in lupus-prone mice via the SIGNR3 pathway. *Frontiers in Immunology*, 12, 696074. <https://doi.org/10.3389/fimmu.2021.696074>.
 49. Wang, Z. H., Gao, Q. Y., & Fang, J. Y. (2013). Meta-analysis of the efficacy and safety of *Lactobacillus*-containing and *Bifidobacterium*-containing probiotic compound preparation in *Helicobacter pylori* eradication therapy. *Journal of Clinical Gastroenterology*, 47(1), 25–32.
 50. Burton, G. J., & Jauniaux, E. (2011). Oxidative stress. *Best Practice & Research Clinical Obstetrics & Gynecology*, 25(3), 287–299. <https://doi.org/10.1016/j.bpobgyn.2011.01.007>.

51. Mazidi, M., Rezaie, P., Ferns, G. A., & Vatanparast, H. (2017). Impact of probiotic administration on serum C-reactive protein concentrations: Systematic review and meta-analysis of randomized control trials. *Nutrients*, 9(1), 14.
52. Hegazy, S. K., & El-Bedewy, M. M. (2010). Effect of probiotics on pro-inflammatory cytokines and NF-kappaB activation in ulcerative colitis. *World Journal of Gastroenterology*, 16(33), 4145–4151. <https://doi.org/10.3748/wjg.v16.i33.4145>.
53. Musazadeh, V., Zarezadeh, M., Ghalichi, F., et al. (2022). Anti-obesity properties of probiotics; a considerable medical nutrition intervention: Findings from an umbrella meta-analysis. *European Journal of Pharmacology*, 928, 175069.
54. Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Environmental Research and Public Health*, 11(5), 4745–4767. <https://doi.org/10.3390/ijerph110504745>.
55. Das, T. K., Pradhan, S., Chakrabarti, S., Mondal, K. C., & Ghosh, K. (2022). Current status of probiotic and related health benefits. *Applied Food Research*, 2(2), 100185. <https://doi.org/10.1016/j.afres.2022.100185>.
56. Chen, L., Zou, Y., Peng, J., et al. (2015). *Lactobacillus acidophilus* suppresses colitis-associated activation of the IL-23/Th17 axis. *Journal of Immunology Research*, 2015, 909514. <https://doi.org/10.1155/2015/909514>.
57. Gao, C., Major, A., Rendon, D., et al. (2015). Histamine H2 receptor-mediated suppression of intestinal inflammation by probiotic *Lactobacillus reuteri*. *mBio*, 6(6), e01358-15. <https://doi.org/10.1128/mBio.01358-15>.
58. Kanda, T., Nishida, A., Ohno, M., et al. (2016). Enterococcus durans TN-3 induces regulatory T cells and suppresses the development of dextran sulfate sodium (DSS)-induced experimental colitis. *PLoS ONE*, 11(7), e0159705. <https://doi.org/10.1371/journal.pone.0159705>.
59. Dogi, C., García, G., De Moreno de LeBlanc, A., Greco, C., & Cavaglieri, L. (2016). *Lactobacillus rhamnosus* RC007 intended for feed additive: Immune-stimulatory properties and ameliorating effects on TNBS-induced colitis. *Beneficial Microbes*, 7(4), 539–547. <https://doi.org/10.3920/BM2015.0075>.
60. Thakur, B. K., Saha, P., Banik, G., et al. (2016). Live and heat-killed probiotic *Lactobacillus casei* Lbs2 protects from experimental colitis through Toll-like receptor 2-dependent induction of T-regulatory response. *International Immunopharmacology*, 36, 39–50.
61. Rodríguez-Nogales, A., Algieri, F., Garrido-Mesa, J., et al. (2017). Differential intestinal anti-inflammatory effects of *Lactobacillus fermentum* and *Lactobacillus salivarius* in DSS mouse colitis: Impact on microRNAs expression and microbiota composition. *Molecular Nutrition & Food Research*, 61(11), 1700047.
62. Mi, H., Dong, Y., Zhang, B., et al. (2017). *Bifidobacterium infantis* ameliorates chemotherapy-induced intestinal mucositis via regulating T cell immunity in colorectal cancer rats. *Cell Physiology and Biochemistry*, 42(6), 2330–2341.
63. Tang, Y., Wu, Y., Huang, Z., et al. (2017). Administration of probiotic mixture DM#1 ameliorated 5-fluorouracil-induced intestinal mucositis and dysbiosis in rats. *Nutrition*, 33, 96–104.
64. Park, J. S., Choi, J. W., Jhun, J., et al. (2018). *Lactobacillus acidophilus* improves intestinal inflammation in an acute colitis mouse model by regulation of Th17 and Treg cell balance and fibrosis development. *Journal of Medicinal Food*, 21(3), 215–224.

65. Zhou, X., Liu, H., Zhang, J., et al. (2019). Protective effect of *Lactobacillus fermentum* CQPC04 on dextran sulfate sodium-induced colitis in mice is associated with modulation of the nuclear factor- κ B signaling pathway. *Journal of Dairy Science*, 102(11), 9570–9585.
66. Choi, S. H., Lee, S. H., Kim, M. G., Lee, H. J., & Kim, G. B. (2019). *Lactobacillus plantarum* CAU1055 ameliorates inflammation in lipopolysaccharide-induced RAW264.7 cells and a dextran sulfate sodium-induced colitis animal model. *Journal of Dairy Science*, 102(8), 6718–6725. <https://doi.org/10.3168/jds.2018-15873>.
67. Huang, L., Zhao, Z., Duan, C., et al. (2019). *Lactobacillus plantarum* C88 protects against aflatoxin B1-induced liver injury in mice via inhibition of NF- κ B-mediated inflammatory responses and excessive apoptosis. *BMC Microbiology*, 19(1), 170. <https://doi.org/10.1186/s12866-019-1581-0>.
68. Sgheddu, V., Uggeri, F., Belogi, L., et al. (2020). The biotherapeutic potential of *Lactobacillus reuteri* characterized using a target-specific selection process. *Frontiers in Microbiology*, 11, 532.
69. Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llrente, C., & Gil, A. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, 61(2), 160–174. <https://doi.org/10.1159/000342079>.
70. Śliżewska, K., Markowiak-Kopeć, P., & Śliżewska, W. (2020). The role of probiotics in cancer prevention. *Cancers*, 13(1), 30.
71. Goldin, B. R., & Gorbach, S. L. (1980). Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. *Journal of the National Cancer Institute*, 64(2), 263–265. <https://doi.org/10.1093/jnci/64.2.263>.
72. Dos Reis, S. A., da Conceição, L. L., Siqueira, N. P., da Silva, L. L., & Peluzio, M. D. (2017). Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutritional Research*, 37, 1–19. <https://doi.org/10.1016/j.nutres.2016.11.005>.
73. Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews*, 12(1–3), 39–85. <https://doi.org/10.1111/j.1574-6976.1993.tb00018.x>.
74. Duc, N. M., Kim, H. R., & Chung, K. Y. (2015). Structural mechanism of G protein activation by G protein-coupled receptor. *European Journal of Pharmacology*, 763(Pt B), 214–222. <https://doi.org/10.1016/j.ejphar.2015.05.023>.
75. Fearon, E. R. (2011). Molecular genetics of colorectal cancer. *Annual Review of Pathology*, 6, 479–507. <https://doi.org/10.1146/annurev-pathol-011811-132435>.
76. Commane, D., Hughes, R., Shortt, C., & Rowland, I. (2005). The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mutation Research*, 591(1, 2), 276–289. <https://doi.org/10.1016/j.mrfmmm.2005.03.001>.
77. Burns, A. J., & Rowland, I. R. (2004). Antigenotoxicity of probiotics and prebiotics on faecal water-induced DNA damage in human colon adenocarcinoma cells. *Mutation Research*, 551(1, 2), 233–243. <https://doi.org/10.1016/j.mrfmmm.2004.01.008>.
78. Santillo, A., Annicchiarico, G., Caroprese, M., Marino, R., Sevi, A., & Albenzio, M. (2012). Probiotics in milk replacer influence lamb immune function and meat quality. *Animal*, 6(2), 339–345.
79. Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, 5(1), 9–19. <https://doi.org/10.1097/WOA.0b013e31824f2c58>.

80. Mafi, A., Namazi, G., Soleimani, A., Bahmani, F., Aghadavod, E., & Asemi, Z. (2018). Metabolic and genetic response to probiotics supplementation in patients with diabetic nephropathy: A randomized, double-blind, placebo-controlled trial. *Food & Function*, 9(9), 4763–4770.
81. Mazruei Arani, N., Emam-Djomeh, Z., Tavakolipour, H., Sharafati-Chaleshtori, R., Soleimani, A., & Asemi, Z. (2019). The effects of probiotic honey consumption on metabolic status in patients with diabetic nephropathy: A randomized, double-blind, controlled trial. *Probiotics and Antimicrobial Proteins*, 11, 1195–1201.
82. Miraghajani, M., Zaghian, N., Mirlohi, M., Feizi, A., & Ghiasvand, R. (2017). The impact of probiotic soy milk consumption on oxidative stress among type 2 diabetic kidney disease patients: A randomized controlled clinical trial. *Journal of Renal Nutrition*, 27(5), 317–324.
83. Soleimani, A., Zarrati Mojarad, M., Bahmani, F., et al. (2017). Probiotic supplementation in diabetic hemodialysis patients has beneficial metabolic effects. *Kidney International*, 91(2), 435–442.
84. Heshmati, J., Farsi, F., Shokri, F., et al. (2018). A systematic review and meta-analysis of the probiotics and symbiotics effects on oxidative stress. *Journal of Functional Foods*, 46, 66–84. <https://doi.org/10.1016/j.jff.2018.04.015>.
85. Zheng, H. J., Guo, J., Wang, Q. H., et al. (2021). Probiotics, prebiotics, and symbiotics for the improvement of metabolic profiles in patients with chronic kidney disease: A systematic review and meta-analysis of randomized controlled trials. *Critical Reviews in Food Science and Nutrition*, 61(4), 577–598.
86. Vlachou, E., Ntikoudi, A., Govina, O., et al. (2020). Effects of probiotics on diabetic nephropathy: A systematic review. *Current Clinical Pharmacology*, 15(3), 234–242.
87. Martarelli, D., Verdenelli, M. C., Scuri, S., et al. (2011). Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. *Current Microbiology*, 62, 1689–1696.
88. Songisepp, E., Kals, J., Kullisaar, T., et al. (2005). Evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. *Nutrition Journal*, 4(1), 1–10.
89. Kumar, A., Wu, H., Collier-Hyams, L. S., et al. (2007). Commensal bacteria modulate cullin-dependent signaling via generation of reactive oxygen species. *The EMBO Journal*, 26(21), 4457–4466.
90. Kumar, A., Wu, H., Collier-Hyams, L. S., Kwon, Y. M., Hanson, J. M., & Neish, A. S. (2009). The bacterial fermentation product butyrate influences epithelial signaling via reactive oxygen species-mediated changes in cullin-1 neddylation. *The Journal of Immunology*, 182(1), 538–546. <https://doi.org/10.4049/jimmunol.182.1.538>.
91. Swanson, P. A., Kumar, A., Samarin, S., et al. (2011). Enteric commensal bacteria potentiate epithelial restitution via reactive oxygen species-mediated inactivation of focal adhesion kinase phosphatases. *Proceedings of the National Academy of Sciences*, 108(21), 8803–8808.
92. Wentworth, C. C., Alam, A., Jones, R. M., Nusrat, A., & Neish, A. S. (2011). Enteric commensal bacteria induce extracellular signal-regulated kinase pathway signaling via formyl peptide receptor-dependent redox modulation of dual specific phosphatase 3. *Journal of Biological Chemistry*, 286(44), 38448–38455.
93. Jones, R. M., & Neish, A. S. (2013). Redox signaling mediated by the gut microbiota. *Free Radical Research*, 47(11), 950–961. <https://doi.org/10.3109/10715762.2013.835990>.

94. Asemi, Z., Jazayeri, S., Najafi, M., et al. (2012). Effect of daily consumption of probiotic yogurt on oxidative stress in pregnant women: A randomized controlled clinical trial. *Annals of Nutrition and Metabolism*, 60(1), 62–68. <https://doi.org/10.1159/000335389>.
95. Chamari, M., Djazayeri, A., Jalali, M., Sadrzadeh Yeganeh, H., Hosseini, S., & Heshmat, R. (2008). The effect of daily consumption of probiotic and conventional yoghurt on some oxidative stress factors in plasma of young healthy women. *ARYA Atherosclerosis Journal*, 4(4), 175–179.
96. Vaghef-Mehrabany, E., Alipour, B., Homayouni-Rad, A., Sharif, S. K., Asghari-Jafarabadi, M., & Zavvari, S. (2014). Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis. *Nutrition*, 30(4), 430–435.
97. Naruszewicz, M., Johansson, M. L., Zapolska-Downar, D., & Bukowska, H. (2002). Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers. *The American Journal of Clinical Nutrition*, 76(6), 1249–1255.
98. Zamani, N., Ganjy, Z., Sharif, M. R., et al. (2022). The effects of probiotics supplementation on clinical status and biomarkers of oxidative damage and inflammation in children with brucellosis: A randomized, double-blind, and placebo-controlled trial. *Evidence-Based Complementary and Alternative Medicine*, 2022, 6943631.
99. Ebrahimi-Mameghani, M., Sanaie, S., Mahmoodpoor, A., & Hamishehkar, H. (2013). Effect of a probiotic preparation (VSL# 3) in critically ill patients: A randomized, double-blind, placebo-controlled trial (Pilot Study). *Pakistan Journal of Medical Sciences*, 29(2), 490. <https://doi.org/10.12669/pjms.292.3725>.
100. Forrester, S. J., Kikuchi, D. S., Hernandez, M. S., Xu, Q., & Griendling, K. K. (2018). Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Research*, 122(6), 877–902. <https://doi.org/10.1161/CIRCRESAHA.117.311401>.
101. Becker, L. B. (2004). New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovascular Research*, 61(3), 461–470. <https://doi.org/10.1016/j.cardiores.2003.11.004>.
102. Hoffmann, A., Kleniewska, P., & Pawliczak, R. (2021). Antioxidative activity of probiotics. *Archives of Medical Science: AMS*, 17(3), 792–800. <https://doi.org/10.5114/aoms.2021.102872>.



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CHAPTER 9

Probiotics: The Nonpathogenic Microorganisms with a Functional Role in Bladder Cancer Management

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ABSTRACT

Treatment of bladder cancer (BlCa) typically involves removing tumors from the bladder through a procedure called transurethral resection of the bladder tumor. In addition, affected individuals at intermediate-to-high risk of recurrence and progression may receive adjunct intravesical therapy, including chemotherapy or immunotherapy with BCG (Bacillus Calmette-Guerin). There has been a growing interest in the potential of probiotics in treating BlCa. Probiotics are live microorganisms that provide health advantages when administered in appropriate quantities. Evidence has shown that probiotics can regulate the immune system, reduce inflammation, and promote gut microbiota diversity, collectively hindering the development and progression of BlCa. The underlying mechanism by which probiotics exert their beneficial effects in BlCa needs to be clarified. Probiotics might enhance the immune response against cancer cells to impede their growth and proliferation. Additionally, probiotics may increase the efficacy of conventional therapies and reduce the risk of adverse events. Overall, probiotics as a complementary therapeutic option for BlCa show promise. Further research is required to identify the most suitable strains and doses of probiotics, along with the most effective delivery methods. With further investigation, probiotics may become an important adjunct therapy in the treatment of BlCa.

9.1 INTRODUCTION

In terms of incidence, bladder carcinoma ranks 17th among women and 7th among men in the realm of oncopathology and constitutes the most

common malignant tumor of the urinary system. The incidence of bladder cancer (BlCa) varies by around ten times across nations, depending on the geographic region [1]. As a result, the incidence is higher in Western Europe than in the United States, and it is lower in Eastern Europe than in Asia. In the European Union, the age-standardized prevalence rates for males are 19.1, while for females, it is 4.0. On a global scale, the age-standardized death rates are 3.2 for males and 0.9 for females per 100,000 people [2]. BlCa ranks as the ninth most prevalent disease among men and the sixteenth most common cancer among women in India [3]. Men had an incidence rate of 13.2, and women had an incidence rate of 2.3 per 100,000 individuals. BlCa affects men more frequently than women (3:1), which is correlated with men's higher prevalence of tobacco use and occupations linked to carcinogens that raise the risk of acquiring the disease [4]. The prevalence of carcinoma of the bladder varies depending on ethnicity. For instance, it is 2 and 8 times lower among Black men and American Indians, respectively, in the United States, and it is 60% lower in Asian communities than it is among White Americans [1].

Reducing the toxicities of BlCa treatment is becoming more critical. Significant treatment-related morbidity and mortality are possible with surgery, chemotherapy, immunological therapy, and radiation therapy. Adverse events affecting the gastrointestinal tract, like nausea, diarrhea, and flu-like symptoms (e.g., fever, aches, chills, and fatigue), can impact a patient's quality of life, make it impossible for them to receive further treatment, and occasionally even put their lives in danger [5]. Several research studies conducted over the past few decades have shown that particular microorganisms, also referred to as probiotics, when introduced to the human body in sufficient amounts, can have a positive impact on health [6]. According to the definition given above, a probiotic must satisfy three requirements to be considered effective: first, the organisms must be alive when consumed; second, they must survive in the body in sufficient quantities to have a beneficial effect; and third, they must benefit the host. *Bifidobacterium* and *Lactobacillus* make up the great majority of probiotics, as they are the most typical types of normal human microflora [7]. It should be noted that there are 56 species of *Lactobacillus* in the genus, while there are 32 species of *Bifidobacterium*. The main benefits of probiotics include improvements in metabolic and synthetic processes; an improvement in the barrier properties of the intestinal lining; and detoxification (offsetting of exogenous and endogenous toxins) [8]. Pathogenic germs and the formation of a protective crust on the outer surface of the intestinal lining increase the body's anti-infectious defense. It is crucial to mention that different probiotics, even ones from the same genus, affect people's bodies in various ways. The

advantages of probiotics are strain-specific; thus, it is difficult to ascribe the positive traits of any strain to others of the same type of bacteria [9].

The key intention of this analysis was to thoroughly examine the effects of probiotics on the host's immune system, the extent to which probiotics can fortify the body against infections while simultaneously mitigating the risk of exaggerated immune responses, and whether probiotics have a beneficial effect on BlCa and provide protection against bladder tumor recurrence.

9.2 DESCRIPTION OF PROBIOTICS AND THEIR MECHANISM OF ACTION

Probiotics are live microorganisms, which are usually bacteria or yeast. Consuming sufficient quantities of these microorganisms can offer health benefits to individuals. Foods like yogurt, kefir, kimchi, sauerkraut, and kombucha, which are fermented, usually contain probiotics. They can also be found in dietary supplements [10]. Probiotics confer health benefits through a variety of mechanisms, including modulating gut microbiota, enhancing immune components, and producing bioactive compounds. The gut microbiota refers to the microorganisms living in the gastrointestinal tract, which are essential for maintaining gut health, regulating immune function, and providing protection against harmful pathogens [11]. Probiotics can boost the immune system by promoting the production of antibodies and immune cells. There are many different strains of probiotics, each with unique properties and potential health benefits. Some of the most commonly studied probiotics include *Bifidobacterium* and *Lactobacillus* species, which are found in many fermented foods and dietary supplements. Probiotics are generally considered safe for healthy individuals, although they may cause mild gastrointestinal symptoms such as bloating, gas, and diarrhea in some people. It is imperative that individuals with a compromised immune system or specific medical issues consult a healthcare professional before taking probiotics [12].

Evidence supports that probiotics have a beneficial effect on reducing inflammation in bladder cancer (BlCa). It has been established that inflammation can enhance the development and progression of malignancies, including BlCa [13]. Friedrich et al. demonstrated the effects of a probiotic supplement on inflammation markers in patients with BlCa. According to the study, the use of probiotic supplements led to a substantial reduction in the levels of inflammatory markers in the patients' urine samples, suggesting that it may have a beneficial effect on inflammation in BlCa [14]. Another study published in 2022 investigated the impacts of a certain probiotic strain on inflammation

and oxidative stress in patients with BlCa who are undergoing surgery. The study found that the probiotic supplement reduced oxidative-induced inflammation, suggesting that it may have a beneficial effect on BlCa [15].

Evidence supports that probiotics can modulate the cytokine storm in cancer via the cellular pathway, through the activation of T regulatory cells (Tregs), which are a subset of immune cells that can suppress immune responses and prevent excessive inflammation. Studies have shown that certain probiotic strains can stimulate the production of Tregs and promote their activity [16]. Tuohy et al. demonstrated that a specific probiotic strain increased the number of Tregs in the serum of healthy volunteers and can modulate the gut microbiota [17]. Research has substantiated that the gut microbiota is indispensable in modulating the immune response and preserving immune homeostasis. When there is an imbalance in the gut microbiota, called dysbiosis, it has been associated with numerous inflammatory illnesses, such as cancer [18]. Probiotics can help restore gut microbiota balance and reduce inflammation by promoting beneficial bacteria growth and inhibiting harmful bacteria growth. This can lead to a reduction in pro-inflammatory cytokines and a more balanced immune response [19].

Additional research is required to comprehensively understand the explicit impact of probiotics on the cellular pathways related to cytokine storms and cancer, despite the potential role these mechanisms suggest probiotics may have in modulating the cytokine storm in cancer. It is also imperative to note that probiotics should not be used as an adjunct to standard health treatment for cancer. Probiotics have been shown to generate several anti-inflammatory metabolites (e.g., short-chain fatty acids [SCFAs]), which can inhibit the production of pro-inflammatory cytokines, including interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α [20]. According to a study published in 2023, probiotics may protect the intestine and offer an advanced therapeutic approach to supplement the chemotherapy treatment of tumors by activating the Toll-like receptors (TLRs) signaling pathway [21]. TLRs are a family of receptors that play a critical role in the recognition of pathogens and the initiation of the immune response. The activation of TLRs by probiotics can result in the production of anti-inflammatory cytokines while also thwarting the production of pro-inflammatory cytokines [22]. In 2019, a study discovered that the probiotic LGG can activate TLR signaling in a mouse model of colorectal cancer. This activation led to the creation of anti-inflammatory cytokines and inhibited tumor growth [23].

Moreover, probiotics have been shown to modulate several molecular pathways involved in cancer, including the PI3K/AKT/mTOR and

Wnt/ β -catenin pathways, as well as nuclear factor-kappa B (NF- κ B) [24]. These pathways can regulate the immune response, cell proliferation, and caspase-mediated cell death. The dysregulation of these pathways can promote the progression and advancement of cancer [25]. Although probiotics have the potential to modulate the cytokine storm in cancer through molecular pathways, further research is necessary to fully comprehend their specific effects on these pathways and their potential role in cancer. It is also imperative to note that probiotics should not be used as an adjunct to standard health treatment for cancer.

9.3 PATHOGENESIS OF BLADDER CANCER

It has been demonstrated that BICa is a multipart and multifactorial ailment that arises from the cells facing the bladder wall. Globally, it ranks as the sixth most common malignancy and the fourth most common cancer in men. The morbidity of BICa is the interplay between environmental and inherited influences that promote the advancement of the disease [26]. In the following, the pathogenesis of BICa is discussed in detail. Figure 9.1 depicts the molecular mechanism of BICa pathogenesis.

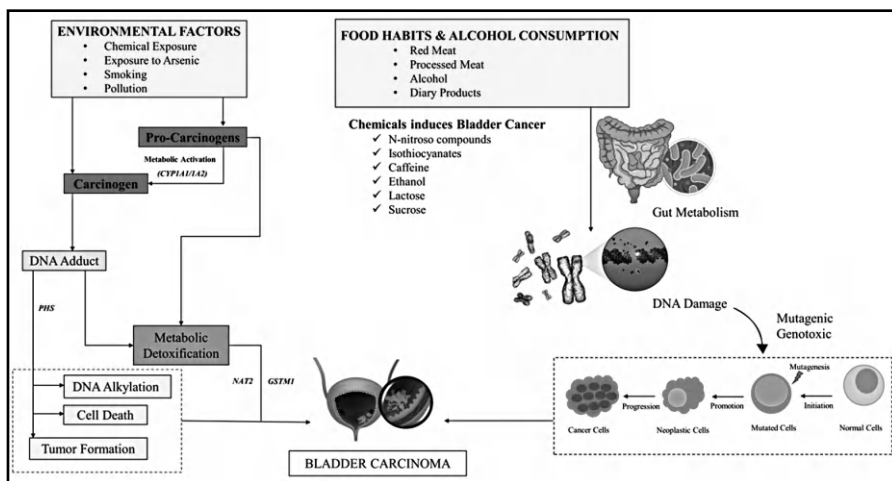


FIGURE 9.1 Various molecular pathogenesis and prognostics of bladder cancer. Ellipses: CYP1A1/CYP1A2, NAT2, N-acetyltransferase 2; cytochrome P-450 1A1/1A2; UGT, PHS, prostaglandin H-synthase; UDP-glucuronosyltransferase; GSTM1, glutathione-S-transferase μ -1.

9.3.1 ENVIRONMENTAL FACTORS

Exposure to certain environmental factors (e.g., chemicals such as aromatic amines and polycyclic aromatic hydrocarbons [PAHs]) is a significant risk factor for the development of bladder cancer (BlCa) [26]. These chemicals are found in tobacco smoke, industrial chemicals, and certain dyes. Chronic exposure to these chemicals can lead to DNA damage and mutations, leading to the development of BlCa. Other risk factors of BlCa include radiation and certain medications [27].

9.3.2 CHEMICAL EXPOSURE

Occupational exposure to several chemicals, such as polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, and aromatic amines, is the second most preventable cause of BlCa [28]. These substances are frequently present in the industrial production of rubber, dyes, paint, metals, and petroleum-based products. There was a 253% greater mortality risk among those working in the rubber industry's "storage and shipment" division, and a 159% higher mortality risk among individuals performing "general work" in the division [29]. BlCa is more prevalent among hairdressers, firefighters, and farmers who use fungicides in their profession. Studies show that occupational exposures cause 18% of BlCa cases. Even two years of exposure can increase the risk, but the disease may not appear until many years later, similar to the effects of tobacco smoke [30].

9.3.3 EXPOSURE TO ARSENIC

BlCa can be caused by exposure to arsenic, which is a naturally occurring element found in soil and rocks. According to a study conducted by Hayashi et al., arsenic can be released into the environment through industrial processes like mining and smelting [31]. Consuming drinking water with high levels of arsenic increases the risk of BlCa due to DNA damage and mutations caused by the element. However, the exact mechanism is not fully understood [32]. A substantial prospective observational study in Chile indicated that exposure to arsenic amplified the risk of BlCa [33]. Another study from Finland discovered a synergistic relationship between cigarette smoke and low concentrations of arsenic (0.5 µg/L), increasing BlCa development [34]. Various factors, including exposure to carcinogens in water and the

consumption of metals like selenium and zinc, can increase the risk of BICa. Nitrates and disinfection byproducts in water may also contribute to this risk.

9.3.4 CIGARETTE SMOKING

Research shows that smoking tobacco is the leading cause of new cases of BICa, accounting for 50 to 65% of annual occurrences. Furthermore, smoking is known to increase the risk of developing BICa by three to four times [35]. Tobacco smoke contains harmful substances like polycyclic aromatic hydrocarbons and beta-naphthylamine, which are known to cause cancer. These particles create inflammation and can lead to DNA adduct production and irreversible genetic mutations in the bladder and other parts of the body [36]. Such mutations can increase the risk of cancer by either triggering oncogenes or silencing tumor suppressor genes. Studies have shown that individuals who smoke have a higher chance of having specific genetic variations that are associated with impaired detoxification enzymes. BICa usually appears at an older age, indicating that there is a delay of around 30 years between the start of smoking and the diagnosis of cancer. While smoking has been associated with a connection between the number of years smoking and the incidence of BICa, it has been proven that quitting smoking can reduce the risk of BICa by 40% or more in as little as 1 to 4 years. The risk of BICa can return to the same level as non-smokers after 20 years of quitting smoking [27]. It has been demonstrated that exclusive tobacco smokers are at a higher risk than exclusive tobacco pipe or cigar smokers. Due to their lower temperatures than cigarettes, lower chemical content, and reduced inhalation of carcinogenic particles, pipes and cigars are associated with a lower risk of head, neck, and lung cancers [37].

9.3.5 CHRONIC INFLAMMATION

Chronic inflammation is associated with BICa formation. Chronic inflammation can be due to chronic infections, bladder stones, or exposure to irritants such as catheters or other medical devices [31]. Inflammation can promote the development of cancer by creating an environment that facilitates the growth and spread of malignant cells. Inflammatory cells release growth factors and cytokines that promote the cancer cells' growth and angiogenesis [38].

9.3.6 HEREDITARY & GENETIC FACTORS

BlCa risk is associated with numerous genetic loci, as revealed through genome-wide association studies. Among these loci are GSTM1, an enzyme that helps detoxify environmental carcinogens, NAT2, a slow acetylator that detoxifies aromatic amines, and MYC, a common oncogene and cell-signaling molecule. The latter two (NAT2 and GSTM1) also appear to interact with tobacco use in a carcinogenic manner [39]. Although BlCa is typically not thought to be hereditary, some genetic factors can increase the risk of BlCa. For example, a genetic deficiency in the tumor-suppressor gene PTEN causes Cowden's syndrome, which significantly elevates the risk of developing neoplasms such as transitional and squamous cell urothelial carcinoma [40]. Another example is Lynch syndrome, which increases the likelihood of developing BlCa. It is often associated with non-polypoid colorectal cancer caused by a malfunction in DNA mismatch repair. Patients with Lynch syndrome are suitable candidates for treatment using checkpoint inhibitors [41]. Up to 20% of patients with recurrent BlCa have reported having somatic mutations of the fibroblast growth factor receptor (FGFR), which led to the approval of erdafitinib, a tyrosine kinase inhibitor, as a later-line treatment [42]. It has been shown that P53 mutations are associated with BlCa formation, as they are in many neoplasms, and they may even be useful prognostic indicators [43]. Mutations in tumor suppressor genes (e.g., RB1, TP53, and PTEN) and oncogenes (e.g., HRAS and FGFR3) are associated with BlCa [44]. TP53 mutations are the main genetic alteration found in BlCa, occurring in up to 70% of cases [39]. FGFR3 mutations are also prevalent in BlCa and are associated with low-grade non-muscle invasive BlCa (NMIBC).

Epigenetic alterations, such as DNA methylation and histone modification, have been observed in BlCa. These alterations can activate oncogenes and silence tumor suppressor genes, resulting in the development of the disease. Promoter hypermethylation of tumor suppressor genes, such as RASSF1A and CDKN2A, is a common epigenetic alteration observed in BlCa [45].

9.3.7 FOOD HABITS

A hot meta-analysis has indicated that consuming high amounts of processed and red meat increases the chances of developing BlCa by 10% and 17%, respectively [46]. In another investigation, processed meat consumption increased by 22%, but red meat consumption did not increase statistically

significantly. The difference can be explained by the limit of meat consumption used to define high consumption. It has been demonstrated that rodent bladder tumors are directly caused by the nitrosamines from nitrates in processed meat. The International Agency for Research on Cancer (IARC) classified red meat as a likely cancer-causing agent in 2015 and processed meat as a Group 1 cancer-causing agent due to their significant links to colorectal cancer prevalence. Nevertheless, pork and poultry have not been linked to an increased risk of BICa [47].

Being pre-obese increases the risk of BICa by 7%, while having a high-fat diet and being obese raises the risk by 10%. It has been discovered that body mass index (BMI) and BICa risk are directly linked, with a 4.2% increase in BICa risk for every 5000 g/m² increase in weight [48]. It was discovered that the threat was unrelated to food, alcohol, physical activity, or smoking. Despite the fact that obesity is linked to a variety of cancer types, the underlying process is not fully known. Insulin and IGF-1 (insulin-like growth factor-I) are produced in greater quantities as a result of being overweight and obese, and these molecules alter cell division, angiogenesis, and apoptosis. By modifying cytokine levels, obesity also encourages chronic inflammation, which sets off an immunological cascade that eventually promotes carcinogenesis. Independent of obesity, physical activity can reduce the risk of BICa.

9.4 ANTI-CARCINOGENIC MECHANISM OF PROBIOTICS

Live bacteria described as probiotics have a number of positive health impacts, including an anti-carcinogenic effect when taken in sufficient levels. These helpful bacteria use a variety of intricate mechanisms to exert their anti-cancer capabilities. Immune regulation, antioxidant activity, detoxification, reduction of inflammation, activation of apoptosis, reduction of angiogenesis, cell cycle control, metabolic control, epigenetic control, and microbial-host interaction are a few of the main processes. Probiotics also aid in competitive exclusion, improved gut barrier performance, host hormone level regulation, autophagy modulation, and cellular senescence induction. Probiotics have anti-cancer properties that involve complex interactions with the body's cells, immune system, and gut bacteria. These processes work in tandem to prevent and inhibit the growth and spread of cancer, showing that probiotics can be a useful adjunct treatment and preventive measure for cancer [49].

Staldoni de Oliveira et al. [50] demonstrated that a probiotic yeast called *Saccharomyces cerevisiae* (CNCM I-3856) enhanced the production of cytokines (e.g., IL-10), which altered the immune response and helped impede tumor evolution in a breast cancer mouse model. Probiotics can modify the immune system by increasing the production of IL-10 and TNF- α , which can inhibit tumor growth and the activity of different immune cells, including T cells, macrophages, and dendritic cells. Additionally, probiotics can impact both pro- and anti-inflammatory cytokine production, such as IL-6, IL-10, and TNF- α . Pro- and anti-inflammatory responses are balanced through immunological regulation, which can help prevent the formation of tumors and maintain gut homeostasis. The activation of immune cells, such as T cells and macrophages, is also part of this system. Studies show that probiotics can boost the release of IL-10 and TNF in vitro and in vivo, and that these cytokines can prevent the development of cancer cells [50].

Probiotics, especially *Lactobacillus* and *Bifidobacterium* species, can produce antioxidants such as superoxide dismutase (SOD), catalase, and glutathione (GSH). These antioxidants neutralize reactive oxygen species (ROS), such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). Probiotics protect DNA, proteins, and lipids from damage by reducing oxidative stress, which lowers the risk of mutations and carcinogenesis. In a rat model of colon cancer induced by 1,2-dimethylhydrazine (DMH), Kumar et al. [51] found that *Lactobacillus fermentum* (MTCC 5898) improved antioxidant catalysts like SOD and catalase, shielding DNA from damage and lowering the chance of carcinogenesis [50].

Lactobacillus rhamnosus (HN001) was shown to reduce urine polycyclic aromatic hydrocarbon (PAH) metabolites in a human intervention trial, indicating its potential role in detoxifying carcinogenic chemicals. Probiotics can aid in the removal of toxic chemicals from the gut by generating enzymes such as beta-glucuronidase and azoreductases. For example, beta-glucuronidase degrades glucuronidated chemicals, while azoreductases break down azo dyes. Probiotics can also bind and eliminate heavy metals like lead and cadmium, as well as mutagens such as heterocyclic amines (HCAs) and PAHs. This detoxification process reduces the risk of cancer by preventing harmful compounds from entering the bloodstream and accumulating in tissues [52].

Liao et al. [53] conducted a study on mice with colorectal cancer and found that a probiotic mixture called VSL#3, containing various strains of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, reduced the levels of pro-inflammatory cytokines like IL-6 and TNF- α . Probiotics may decrease

inflammation by reducing the expression of pro-inflammatory cytokines and increasing anti-inflammatory cytokines, such as IL-10. Additionally, probiotics can prevent the activation of nuclear factor kappa B (NF- κ B), a transcription factor involved in the production of inflammatory genes, thus inhibiting cancer cell proliferation [54].

Probiotics can also induce apoptosis, a programmed cell death mechanism that prevents cancer growth. Liu et al. [55] showed that *Lactobacillus casei* (ATCC 334) caused apoptosis in human gastric cancer cells by activating caspase-3, a key mediator of apoptosis. Probiotics can trigger apoptosis in cancer cells through multiple signaling pathways, including the extrinsic (death receptor) and intrinsic (mitochondrial) pathways. This process helps destroy cancer cells and reduce tumor growth [55].

Kiousi et al. [56] found that *Lactobacillus rhamnosus* GG (LGG) decreased angiogenesis in a colorectal cancer mouse model by reducing the expression of VEGF, a critical factor in angiogenesis. Probiotics can suppress angiogenesis, which is essential for tumor growth, by inhibiting pro-angiogenic factors such as VEGF and increasing anti-angiogenic mediators like endostatin and angiostatin [56].

Kaur et al. [57] demonstrated that *Lactobacillus plantarum* (Lp91) can regulate cell cycle proteins such as p53 and CDK4 in a DMH-induced colon cancer model in rats. This regulation can prevent cancer cells from growing uncontrollably by maintaining a balance between cell proliferation and cell death [57]. In an experimental colorectal cancer study, Wang et al. [58] found that probiotics modulate metabolic pathways like AMPK and mTOR signaling, which play roles in energy homeostasis and cell proliferation. By influencing these pathways, probiotics can limit the resources available to cancer cells and restrict their growth [58].

Probiotics can also impact gene expression through epigenetic modifications. *Bifidobacterium breve* (B-3) was shown to regulate DNA methylation and histone acetylation patterns, which affect gene expression related to colon cancer development in mice [59]. In an animal model of hepatocellular carcinoma, *Bifidobacterium longum* (BB536) altered immune responses and halted tumor growth [60]. Probiotics interact with host cells via receptors like NOD-like receptors (NLRs) and Toll-like receptors (TLRs), triggering intracellular pathways that regulate immune responses and inhibit cancer growth [60].

Probiotics can also improve gut barrier function. Heidar et al. [61] showed that *Lactobacillus reuteri* I5007 increased the production of proteins maintaining tight junctions (e.g., ZO-1 and occludin) in piglets, which

strengthened the intestinal barrier and reduced inflammation [61]. Probiotics may induce autophagy, a cellular recycling process that prevents cancer cell survival. *Xia et al.* [62] found that *Lactobacillus casei* ATCC 334 stimulated the AMPK signaling pathway and induced autophagy in human gastric cancer cells, inhibiting their proliferation [62]. Finally, probiotics can cause cellular senescence, a state of irreversible cell cycle arrest. *Meng et al.* [63] demonstrated that a probiotic mixture triggered senescence in colorectal cancer cells by activating the p53/p21 and p16/Rb pathways, suppressing tumor growth [63].

8.5 EXISTING TREATMENT APPROACHES FOR BLADDER CANCER

Treatment for bladder cancer (BICa) has evolved over time to include a variety of approaches, each targeting cancer cells in a different way. Transurethral resection of bladder tumor (TURBT), which uses a cystoscope to resect the tumor from the bladder wall, and partial and radical cystectomy, which involves the removal of either all or part of the bladder along with surrounding structures, are two surgical procedures frequently applied as the first-line treatment for BICa. There are different types of intravesical therapy used in cancer treatment. One type uses chemotherapy preparations to slow the advance and division of cancer cells, while another type, called Bacillus Calmette-Guérin (BCG) immunotherapy, stimulates the immune system to attack malignant cells using a weakened form of tuberculosis bacteria. Radiation therapy uses high-energy X-ray beams to damage the DNA of cancer cells, preventing them from dividing and growing. This can be given either externally or internally by brachytherapy, and it can be administered either on its own or in conjunction with chemotherapy to preserve the bladder or as adjuvant therapy after surgery. Immunotherapy, particularly immune checkpoint inhibitors (ICIs), has revolutionized BICa treatment by preventing immune cells from receiving signals that impede them from recognizing and attacking cancer cells [64].

Erdaftinib, a drug that targets the fibroblast growth factor receptor (FGFR), blocks FGFR signaling to reduce cancer progression or shrink tumors in patients with certain genetic abnormalities. This and other forms of targeted therapy focus on specific proteins or biological pathways driving cancer growth and survival. New immune checkpoint inhibitors, antibody-drug conjugates (ADCs) that combine targeted antibodies with powerful chemotherapy drugs for selective delivery to cancer cells, cancer vaccines

that promote immune recognition of cancer cells, chimeric antigen receptor (CAR) T-cell therapy using genetically engineered immune cells to recognize and attack cancer cells, small molecule inhibitors that target particular cancers, and other novel treatment approaches are all being investigated in ongoing clinical trials [65].

Clinical trials are exploring novel BlCa treatment approaches with an emphasis on improving efficacy and minimizing adverse effects. Combinations of existing inhibitors and novel ICIs (targeting LAG-3, TIGIT, or TIM-3) are being studied to enhance the antitumor immune response. Antibody-drug conjugates (ADCs) like vedotin, enfortumab, and sacituzumab govitecan, which specifically target proteins like Nectin-4 and Trop-2, deliver potent chemotherapeutic medications directly to cancer cells while minimizing harm to healthy cells. Peptide-, dendritic-cell-, and viral vector-based cancer vaccines aim to improve the immune system's capacity to perceive and eliminate cancer cells. Although CAR T-cell therapy has shown promise in treating blood malignancies, it is still challenging to apply to solid tumors like BlCa. Trials are examining CAR T-cell therapies targeting proteins such as guanylate cyclase 2C (GUCY2C) and interleukin-13 receptor alpha 2 (IL13R2). Small pharmacologic inhibitors of PI3K/AKT/mTOR, FGFR, PARP, and other proteins or molecular pathways involved in cancer growth and survival are being investigated for BlCa treatment. Finally, combination therapies that incorporate chemotherapy, radiation therapy, targeted therapy, and immunotherapy aim to improve treatment outcomes by attacking cancer cells through multiple pathways. The continued development of these cutting-edge techniques bodes well for future advancements in BlCa treatment. Together, these therapeutic modalities offer a comprehensive and personalized approach to BlCa management, with the ultimate goal of enhancing patient outcomes and quality of life [66].

Table 9.1 depicts existing management approaches for BlCa, and Table 9.2 describes the available treatment options for BlCa.

9.6 INTERPLAY BETWEEN INFLAMMATION AND GUT MICROBIOTA IN THE MORBIDITY OF BLADDER CANCER

The gut microbiome, a complex collection of microbes, is critical for human health. It plays a crucial role in maintaining the health of the intestines and bladder by carrying out various physiological functions. The gut microbiome is made up of numerous types and subclasses of microbes, each of which

TABLE 9.1 Various Therapeutic Approaches Intended for the Management of Bladder Cancer

Classification	Sub-classification	Examples	Type of Urinary Bladder Cancer	Mechanism	Effects in Bladder Cancer	Reference
Surgery	TURBT	TURBT	NMIBC	Removes tumors through the urethra	Effective for early-stage bladder cancer	[67]
	Radical Cystectomy	Radical Cystectomy	MIBC/ABC	Removes the entire bladder and surrounding tissue	Effective for advanced bladder cancer (ABC)	[68]
	Partial Cystectomy	Partial Cystectomy	MIBC	Removes a portion of the bladder	Effective for muscle-invasive bladder cancer	[69]
	Intracorporeal Urinary Diversion	Ileal Conduit, Orthotopic Neobladder	Radical Cystectomy	Provides an alternate route for urination	Effective for patients undergoing radical cystectomy	[70]
	Augmented Cystectomy	Augmented Cystectomy	Bladder Cancer	Increases bladder size after cystectomy	Effective for patients needing a larger bladder after cystectomy	[71]
	Prostate-sparing Cystectomy	Prostate-sparing Cystectomy	Male Bladder Cancer	Removes the bladder while preserving the prostate	Effective for male patients with bladder cancer	[70]
	Robotic Cystectomy	Robotic Cystectomy	MIBC/ABC	Minimally invasive procedure using robotic technology	Effective for patients preferring a less invasive option	[72]
	EPLND	EPLND	MIBC	Removes additional pelvic lymph nodes	Effective for determining the extent of disease in muscle-invasive cases	[73]
Intravesical	Chemotherapy	Mitomycin	NMIBC	Delivered directly into the bladder	Effective for high-risk NMIBC	[74]

TABLE 9.1 (Continued)

Classification	Sub-classification	Examples	Type of Urinary Bladder Cancer	Mechanism	Effects in Bladder Cancer	Reference
Systemic		Bacillus Calmette-Guerin (BCG)	NMIBC	Stimulates immune response	Effective for intermediate- and high-risk patients	[75]
		Valrubicin	NMIBC	Delivers chemotherapy directly into the bladder	Effective for patients with carcinoma in situ (CIS)	[74]
	Chemotherapy	Methotrexate, Vinblastine, Doxorubicin, Cisplatin	Advanced Bladder Cancer	Inhibits tumor cell growth and division	Effective in combination with other treatments	[76]
		Gemcitabine, Paclitaxel, Docetaxel	Advanced Bladder Cancer	Interferes with cancer cell proliferation	Effective in combination with cisplatin for ABC	[76]
Radiation	EBRT	EBRT	Advanced Bladder Cancer	Uses high-energy beams to damage cancer cells	Effective for reducing symptoms and improving survival	[77]
	Intracavitary radiotherapy	Intracavitary radiotherapy	NMIBC	Delivers ionizing radiation directly into the bladder	Reduces recurrence and progression in NMIBC	[77]
Immunotherapy	Checkpoint Inhibitor Therapy	Pembrolizumab, Atezolizumab	Advanced Bladder Cancer	Blocks specific proteins that enable cancer evasion	Improves survival and response to other treatments	[78]
	BCG Immunotherapy	BCG	NMIBC	Boosts immune response	Reduces recurrence and progression in bladder cancer	[78]

TABLE 9.2 Available Treatment Options for Bladder Cancer

S. No.	Classification	Sub-Classification	Example	Type of Urinary Bladder Cancer	Mechanism in Bladder Cancer	Effects in Bladder Cancer	Reference
1	Chemotherapy	Alkylating agents	Cisplatin	All stages, particularly invasive	Forms covalent bonds with DNA	Kills cancer cells, shrinks tumors	[74]
2	Chemotherapy	Antimetabolites	Gemcitabine	All stages, particularly invasive	Inhibits DNA and RNA synthesis	Slows or stops cancer cell growth	[74]
3	Chemotherapy	Taxanes	Docetaxel	Locally advanced, metastatic	Inhibits microtubule disassembly	Slows or stops cancer cell growth	[74]
4	Immunotherapy	Immune checkpoint inhibitors	Atezolizumab	Locally advanced, metastatic	Blocks PD-L1 protein	Activates immune system against cancer	[78]
5	Immunotherapy	BCG	BCG vaccine	NMIBC	Stimulates immune response	Reduces tumor recurrence and progression	[78]
6	Targeted therapy	FGFR inhibitors	Erdafitinib	Advanced, metastatic, FGFR alterations	Inhibits FGFR signaling pathway	Slows or stops cancer cell growth	[79]
7	Targeted therapy	Antibody-drug conjugates	Enfortumab vedotin	Locally advanced, metastatic	Delivers cytotoxic agent to cancer cells	Kills cancer cells, shrinks tumors	[80]

serves a unique role in maintaining gut health. The class *Firmicutes*, which includes the subclasses *Bacilli* and *Clostridia*, is responsible for turning carbohydrates into short-chain fatty acids (SCFAs) and contains the bulk of the bacteria in the gut. Bacteria are another common type of organism that produces SCFAs and participates in the oxidation of complex carbohydrates [81]. The *Actinobacteria* family is known for its capacity to produce lactic acid and regulate gut and immune system functions. *Proteobacteria*, which consist of the subclasses *Gamma-Proteobacteria* and *Beta-Proteobacteria*, degrade organic compounds and can exhibit both physiological and pathogenic characteristics in the gut. In addition to *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, other types and taxa of microbes present in the gut include *Fusobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *TM7*, *Lentisphaerae*, *Tenericutes*, *Spirochaetes*, and *TM6*. Each of these microorganisms has a unique approach to maintaining gut health, and imbalances in their populations can cause a wide range of health issues. Hence, it is important to maintain a diverse and healthy gut microbiota, as it greatly impacts overall well-being and health [82].

An imbalance in the variety and configuration of the gut microbiome, known as gut dysbiosis, can negatively affect human health. Age, stress, antibiotic use, nutrition, and other factors can all cause the gut microbiota to transition from healthy to dysbiotic. Research shows that changes in diet can greatly influence the conformation and diversity of the gut microbiome. For example, consuming a diet that mainly consists of processed foods and lacks sufficient fiber may cause an upsurge in harmful bacteria like *Escherichia coli* (*E. coli*), along with a decrease in beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* [83]. The routine use of antibiotics can lead to the elimination of both harmful and beneficial bacteria, resulting in an increase in harmful microbes and a decrease in helpful microbes. This could lead to intestinal dysbiosis, which would increase the risk of illness. Aging may potentially contribute to gut dysbiosis by altering the range and makeup of the gut microbiome. As people age, their gut microbiota becomes less diverse and less able to perform its physiological functions. This could lead to intestinal dysbiosis and further illness [84].

Clinical evidence supports that gut dysbiosis plays a role in the emergence of bladder cancer (BICa). For instance, a study noted a transformation in the conformation of the gut microbiome of BICa-affected individuals compared with healthy individuals, with more harmful bacteria and fewer commensal bacteria. Another study indicated that BICa patients had greater levels of inflammation linked to gut dysbiosis [85]. BICa develops and spreads largely

as a result of inflammation. Chronic inflammation, sometimes referred to as cystitis, can alter the bladder lining and result in cancer. Numerous factors, including infections, exposure to harmful substances, and a compromised immune system, can cause inflammation.

The immune system and inflammation may be affected by the gut microbiome. The intricate and still poorly understood molecular processes that connect inflammation, gut dysbiosis, and BICa are being studied. Some plausible mechanisms, however, have been discovered. The protective arrangement is one of the foremost players. Immune system proteins called cytokines, which control inflammation, are released when there is inflammation. These cytokines have the ability to support the survival and proliferation of cancer cells. Furthermore, intestinal dysbiosis can modify the composition and functionality of the gut microbiome. These alterations may result in the formation of toxic substances and unfavorable metabolites that might inflame the bladder and harm its cells' DNA, resulting in cancer [86].

In this regard, the Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), and phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathways are a few examples of the biological pathways that may be associated with the connection between inflammation, gut dysbiosis, and bladder cancer (BICa). These pathways, which are normally dysregulated in cancer, control inflammation, cell survival, and proliferation [87]. The synthesis of genes associated with inflammation, immune response, cell development, and apoptosis (programmed cell death) is controlled by the NF- κ B pathway, a signaling system. NF- κ B is activated following inflammation, which leads to the release of cytokines and other pro-inflammatory intermediaries. BICa has been associated with NF- κ B activation, and recent research has proposed that dysbiosis in the intestinal microbiota may activate this pathway and promote the growth of BICa [88].

The PI3K/Akt/mTOR pathway is the main signaling mechanism that controls cell growth, proliferation, survival, and metabolism of BICa cells. BICa is one type of cancer that has been linked to dysregulation of this system. According to certain research, gut dysbiosis may trigger this pathway and promote the growth of BICa cells [89]. Another signaling pathway, the JAK/STAT pathway, regulates the expression of genes associated with the immune response, inflammation, cell growth, and survival. Dysregulation of this pathway plays a role in BICa pathogenesis. According to certain

research, gut dysbiosis can activate this pathway, leading to the growth of BICa [90].

Toll-like receptors (TLRs) are a specific type of receptor that detect pathogen-associated molecular patterns (PAMPs) and initiate immune responses. TLRs play a key role in identifying bacterial components, leading to their activation and inflammation. TLRs are membrane-bound receptors that recognize molecular PAMPs, which are present in pathogens. TLRs trigger signaling cascades that cause the production of pro-inflammatory cytokines, chemokines, and interferons in response to PAMP detection. TLRs are found on different types of immune and epithelial cells. When activated, they can lead to inflammation and promote cancer growth [91].

Another regulator of the orchestrated immune response is pro-inflammatory cytokines (e.g., interleukin-1 [IL-1], interleukin-6 [IL-6], and tumor necrosis factor alpha [TNF α]). These cytokines can enhance cell proliferation, angiogenesis, and tissue remodeling by recruiting immune cells to the site of inflammation. Chronic inflammatory cytokine production can foster a protumoral environment and lead to cancer development [88].

The NF- κ B signaling system plays a decisive role in inflammation and immune resistance. TLRs and cytokines activate it, and it may stimulate the transcription of genetic factors involved in inflammation and cancer. The NF- κ B signaling system controls the immune response, inflammation, and cell survival. TLRs and inflammatory cytokines are two examples of stimuli that can activate this pathway. NF- κ B enters the nucleus after activation and increases the expression of genes regulating inflammation, cell survival, and proliferation. Dysregulation of the NF- κ B signaling system can result in persistent inflammation and promote cancer development [93].

Reactive oxygen species (ROS) are highly reactive chemicals produced during cellular metabolism and inflammation. Free radicals such as hydroxyl radicals (OH $^{\cdot}$) and superoxide anions (O $^{2\cdot-}$) are among them, as are non-radical forms such as hydrogen peroxide (H $_2$ O $_2$). ROS can induce oxidative damage to biological components such as DNA, proteins, and lipids, which can result in mutations, protein malfunction, and lipid peroxidation. The accumulation of such damage can encourage cancer growth [94].

Immune cells (neutrophils, T cells, and macrophages) are crucial to the immune response and can promote the growth of cancer. Macrophages, for instance, can develop into phenotypes that are either pro-inflammatory subtype (M1) or anti-inflammatory subtype (M2). While the M2 subtype supports tissue repair and angiogenesis, M1 macrophages emit inflammatory cytokines. Neutrophils can generate reactive oxygen species (ROS) and

release proteases that cause tissue injury. The development of cancer can be aided or obstructed by T cell differentiation into pro-inflammatory (Th1, Th17) or anti-inflammatory (Th2, Treg) subsets [93].

During epithelial-mesenchymal transition (EMT), epithelial cells lose their polarity and adhesion between cells and instead acquire a mesenchymal phenotype. EMT can be triggered by inflammatory cytokines and TLR activation and is linked to the growth and spread of cancer. EMT is connected to the onset, growth, and metastasis of cancer. When TLR activation occurs and pro-inflammatory cytokines such as transforming growth factor-beta (TGF- β) and tumor necrosis factor-alpha (TNF- α) are released, EMT is initiated. This happens because certain transcription factors like Snail, Twist, ZEB1/2, and Slug are triggered. These elements encourage the construction of mesenchymal markers like vimentin and N-cadherin while suppressing the expression of epithelial markers like E-cadherin. Cancer cells can invade surrounding tissues and enter the bloodstream due to EMT, resulting in distant metastases. Additionally, cancer cells may become more resistant to treatment as a result of EMT, increasing the chance of cancer recurrence [86].

In conclusion, gut microbiota dysbiosis, TLR activation, the creation of inflammatory cytokines, and abnormalities in the NF- κ B signaling, ROS generation, immune cell activation, and EMT induction are factors that contribute to the development of bladder cancer (BlCa). Each of these pathways is interconnected and can influence one another, resulting in a pro-tumorigenic environment. A greater understanding of these pathways and their interactions is required for designing novel therapeutic options for BlCa treatment and prevention [97].

9.7 IMMUNE SYSTEM, PROBIOTICS, AND BLADDER CANCER

Bladder cancer (BlCa) and the immune system interact via a complex network of cellular pathways, molecular mechanisms, and mutations that influence tumor evolution, progression, and response to treatment. In the tumor microenvironment (TME), immune cells (e.g., T cells, natural killer [NK] cells, and macrophages) interact, while molecular pathways such as immunological checkpoints (e.g., programmed death protein-1/-ligand 1 [PD-1/PD-L1]) and the human leukocyte antigen-E (HLA-E)/natural killer group 2 member A (NKG2A) axis also assist with immune evasion. The BlCa TME consists of immune cell subsets interacting with each other and tumor

cells. These immune cells can have both protumorigenic and antitumorigenic effects on tumor growth, progression, and therapy response. CD8⁺ and CD4⁺ T cells, myeloid-derived suppressor cells (MDSCs), tumor-associated NK cells, regulatory T cells (Tregs), dendritic cells (DCs), tumor-associated macrophages (TAMs), B cells, neutrophils, and gamma-delta ($\gamma\delta$) T cells are the foremost immune cells involved in the pathogenesis of BlCa. Mutations in genetic factors such as TP53 and fibroblast growth factor receptor 3 (FGFR3) can influence the immune response to BlCa. Understanding these intricate connections has resulted in the development of new therapeutic paradigms, such as immune checkpoint inhibitors and monoclonal antibodies targeting the HLA-E/NKG2A axis, which are showing promise in BlCa treatment [98].

Various dietary supplements have also been researched for their capacity to target cellular and molecular pathways involved in BlCa etiology. Probiotics are one of these products. Probiotics are live microorganisms found in dietary supplements or fermented foods that have been shown to offer potential advantages in the management of BlCa. There are multiple ways that probiotics can help prevent and treat BlCa. They can modify the conformation and function of the gut microbiome, which has been associated with the onset of BlCa. By supporting a healthy gut microbiome, they can help prevent the proliferation of harmful bacteria and decrease inflammation, which are known risk factors for BlCa. Probiotics also have anti-inflammatory effects, which may help reduce chronic inflammation linked to the development of BlCa. Furthermore, they have been shown to improve the function of the immune system, increasing the release of cytokines and NK cells, which are important in the elimination of cancer cells. In addition, probiotics have antioxidant effects, which can help protect cells against oxidative stress and damage, and have been shown to inhibit angiogenesis, which is essential for the growth and spread of cancer cells. Therefore, considering these multiple pathways, probiotics play a decisive role in the prevention and treatment of BlCa [99].

9.8 UNVEILING THE COMPLEX INTERPLAY OF IMMUNE CELLS IN BLADDER CANCER

The bladder cancer (BlCa) tumor microenvironment (TME) is made up of an assortment of immune cells that take part in tumor growth, evolution, and response to treatment. Cytotoxic T cells, T helper (Th) cells,

regulatory T cells (Tregs), natural killer (NK) cells, dendritic cells (DCs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and B cells are among the immune cell types involved in BlCa development. Immune cells interact with tumor cells via a complex network of molecular pathways, including immune checkpoint inhibitors (ICIs), such as programmed death protein-1/programmed death-ligand 1 (PD-1/PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), as well as the human leukocyte antigen-E (HLA-E)/natural killer group 2 member A (NKG2A) axis. Understanding the components of bladder-native immune cell populations is crucial for understanding the subsequent responses to bladder carcinogenesis. This section will explore the functions and purposeful behavior of specific immune cell groups in BlCa, with an emphasis on the implications for prognosis and potential probiotic treatments [100].

9.8.1 HLA-E/NKG2A AXIS

Human leukocyte antigen E (HLA-E) is a non-classical major histocompatibility complex class I (MHC-I) molecule regulating immune response control. It has a restricted peptide repertoire, the majority of which are similar to other MHC-I signal sequences. It has been demonstrated that HLA-E can interact with the NKG2A receptor found on natural killer (NK) cells and other T cell subsets by delivering these peptides. On the contrary, NKG2A is a type of inhibitory receptor that is part of the C-type lectin-like family. To recognize and bind HLA-E, it forms a heterodimer with CD94. Immune cells that express NKG2A receive inhibitory signals from the CD94/NKG2A complex, which reduces their cytotoxic activity and cytokine production. This interaction is essential for maintaining immune tolerance and avoiding overreactions of the immune system. An essential immunomodulatory pathway associated with bladder cancer (BlCa) formation is the HLA-E/NKG2A axis [101].

The HLA-E/NKG2A axis can promote tumor growth and immune evasion of BlCa cells through the following mechanisms: BlCa cells may upregulate the expression of HLA-E on their surface to take advantage of the inhibitory effect of the interaction between HLA-E and NKG2A. In BlCa tissues, HLA-E is overexpressed, and it has been observed that this upregulation is linked to advanced tumor stages and worse patient survival [102]. Another strategy is to bind HLA-E to the NKG2A receptor on NK and T cells, which inhibits cytotoxicity and cytokine production. This prevents

them from effectively eliminating tumor cells, allowing BlCa cells to grow and spread undetected by the immune system. A study revealed that inhibiting the HLA-E/NKG2A connection with an anti-NKG2A antibody enhanced the effectiveness of NK and T cells in fighting BlCa in a laboratory setting [103].

The connection between human leukocyte antigen E (HLA-E) and natural killer group 2 member A (NKG2A) can also impact the type and effect of immune cells in the tumor microenvironment (TME), fostering an immunosuppressive state that promotes the development and growth of tumors. This occurs by suppressing the activity of effector immune cells and promoting the evolution of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). In a bladder cancer (BlCa) animal model, it was found that inhibiting the HLA-E/NKG2A axis could reverse immunosuppression in the TME and enhance the anti-tumor immune response [100].

A potential treatment approach for BlCa and other malignancies is to target the HLA-E/NKG2A axis. By inhibiting this connection, the potency of other immunotherapies, such as immune checkpoint inhibitors (ICIs), can be increased, and the anti-tumor activity of NK and T cells can be restored [105].

9.8.2 CD8⁺ AND CD4⁺ T CELLS

Helper T lymphocytes (CD4⁺ T cells) promote the stimulation and functionality of other immune cells, including CD8⁺ T cells, macrophages, and B cells. CD4⁺ T cells can be further subdivided into specific groups (Th17, Th2, Th1, and Tregs), each with distinct activities and cytokine profiles. Interferon-gamma (IFN γ) and tumor necrosis factor-alpha (TNF α), secreted by Th1 cells, support cell-mediated immunity and enhance the cytotoxicity of NK and CD8⁺ T cells. Th1 cells assist in CD8⁺ cell infiltration and function within the tumor microenvironment (TME), which aids the anti-tumor response in bladder cancer (BlCa). Th2 cells participate in humoral immunity and secrete cytokines such as interleukin-4 (IL-4), IL-5, and IL-13. On the other hand, Th2 cells can promote BlCa progression by establishing an immunosuppressive environment and supporting tumor-associated macrophages (TAMs) with an M2-like phenotype. Th17 cells secrete cytokines (IL-21, IL-17, and IL-22), with both pro- and anti-tumorigenic properties. The role of Th17 cells in BlCa is still uncertain, but they may contribute to tumor growth by inducing inflammation and angiogenesis [106].

Cytotoxic T lymphocytes (CTLs) ($CD8^+$ T cells) are indispensable for mounting a successful immune attack against a tumor. Through a molecular mechanism involving the T cell receptor (TCR) and MHC-I molecules on target cells, they can identify and kill tumor cells. Tumor cells in BlCa produce tumor-associated antigens (TAAs) via MHC-I molecules, which the TCR on $CD8^+$ T cells can detect. Once recognized, $CD8^+$ T cells are activated and release cytotoxic granules containing perforin and granzymes, leading to caspase-mediated cell death in tumor cells. In addition to this, $CD8^+$ T cells can kill tumor cells using the Fas/FasL pathway and release inflammatory cytokines like $IFN\gamma$ and $TNF\alpha$ to enhance the anti-tumor response. However, BlCa cells can evade $CD8^+$ T cell-mediated responses by downregulating MHC class I expression, upregulating immune checkpoint molecules such as PD-L1, and promoting an immunosuppressive TME through the recruitment of Tregs and MDSCs [107].

9.8.3 NATURAL KILLER CELLS AND DENDRITIC CELLS

Natural killer (NK) cells play a crucial role in tumor detection and elimination. Unlike other immune cells, NK cells do not require antigen presentation, as they can directly target and destroy cancer cells. Several molecular processes support NK cell-mediated attacks against bladder cancer (BlCa) cells. In the BlCa tumor microenvironment (TME), NK cells possess activation receptors such as NKG2D, DNAX accessory molecule-1 (DNAM-1), and NKp46 that recognize stress-induced ligands and tumor-associated antigens. Upon activation, NK cells release cytotoxic granules (perforin and granzymes) that induce tumor cell death. Additionally, NK cells contain inhibitory receptors, such as killer cell immunoglobulin-like receptors (KIRs) and NKG2A, that recognize major histocompatibility complex class I (MHC-I) molecules on healthy cells. Although BlCa cells may downregulate MHC-I expression to evade $CD8^+$ T cell-mediated immunity, this makes them more susceptible to NK cell-mediated death due to reduced inhibitory signaling. Furthermore, NK cells release interferon-gamma ($IFN\gamma$) and tumor necrosis factor-alpha ($TNF\alpha$), which suppress tumor growth, activate other immune cells, and modulate the TME [108].

Dendritic cells (DCs) initiate adaptive immune responses by collecting, processing, and presenting tumor antigens to T cells. In the BlCa TME, DCs capture tumor-derived antigens through receptor-mediated endocytosis, macropinocytosis, and phagocytosis, including those from apoptotic tumor

cells. Subsequently, DCs present these antigens to CD8⁺ and CD4⁺ T cells on MHC class I and class II molecules, respectively. Beyond antigen presentation, DCs provide co-stimulatory signals via CD80 and CD86 and secrete cytokines such as interleukin-12 (IL-12), which mediate T cell activation, differentiation, and proliferation. Through these processes, both NK cells and DCs play a pivotal role in immunosurveillance and the protective response against BlCa cells. Understanding these mechanisms and their influence on BlCa etiology will help guide the design of novel immunotherapeutic strategies to treat this disease [109].

9.8.4 TUMOR-ALLIED MACROPHAGES AND MYELOID-DERIVATIVE SUPPRESSOR CELLS

Tumor-associated macrophages (TAMs) are circulating monocyte-derived macrophages that are recruited to the tumor microenvironment (TME) by chemotactic substances secreted by tumor cells and the surrounding stroma. TAMs are frequently polarized towards an M2-like phenotype in the TME, which is characterized by pro-tumorigenic, immunosuppressive, and tissue remodeling functions. TAMs contribute to bladder cancer (BlCa) pathogenesis through the following molecular mechanisms: TAMs produce various growth factors, such as epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), which promote tumor cell spread, angiogenesis, and metastasis. TAMs also release immunosuppressive cytokines, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β), which inhibit the activity of effector T cells (Teff), NK cells, and other immune cells involved in anti-tumor responses. Furthermore, TAMs produce matrix metalloproteinases (MMPs) and other proteases that degrade the extracellular matrix (ECM), facilitating tumor invasion and metastasis [110].

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid cells generated in response to inflammatory mediators and tumor-derived substances. MDSCs significantly contribute to BlCa pathogenesis through several molecular mechanisms. MDSCs secrete substances like arginase-1, nitric oxide (NO), and reactive oxygen species (ROS) that inhibit the activation, proliferation, and function of T cells. Additionally, MDSCs promote the expansion and activity of regulatory T cells (Tregs), further reinforcing the immunosuppressive TME. Moreover, MDSCs release pro-angiogenic factors such as VEGF and MMPs, which stimulate blood vessel formation and support tumor growth [111].

TAMs and MDSCs play key roles in BlCa pathogenesis via molecular processes such as immunosuppression, tumor growth promotion, angiogenesis, and metastasis. Targeting these cells and their molecular pathways could be a feasible treatment approach for BlCa. Further research is required to develop innovative therapeutics that regulate TAM and MDSC activity in the TME, ultimately improving anti-tumor immune responses [112].

9.8.5 GAMMA-DELTA ($\gamma\delta$) T CELLS

T cells that express a T cell receptor (TCR) consisting of gamma (γ) and delta (δ) chains are known as gamma-delta ($\gamma\delta$) T cells. These cells are located in various organs, including the skin, gastrointestinal system, and reproductive tract, and they play a critical role in immunological surveillance against infections and tumors. $\gamma\delta$ T cells have been found to infiltrate the tumor microenvironment (TME) and contribute to tumor formation and progression in bladder cancer (BlCa). The molecular processes underlying $\gamma\delta$ T cell involvement in BlCa development are complex. Recent research has provided insight into some of the key mechanisms involved: $\gamma\delta$ T cells can recognize and respond to antigens presented by non-classical major histocompatibility complex (MHC) molecules, such as MHC class I polypeptide-related sequence A (MICA) and B (MICB). These antigens are often elevated in BlCa, which triggers the activation of $\gamma\delta$ T cells.

Once activated, $\gamma\delta$ T cells can release cytotoxic molecules, such as perforin and granzyme B, which destroy tumor cells. Additionally, $\gamma\delta$ T cells are capable of secreting interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α), which bolster anti-tumor immune responses. By secreting these cytokines and other signaling molecules, $\gamma\delta$ T cells can also regulate the activity of other immune cells, including dendritic cells (DCs) and B cells. This regulation influences the overall immune response to BlCa [113].

9.8.6 REGULATORY T CELLS (TREGS)

Tregs are a subset of T cells essential for preserving the immune system's homeostasis and thwarting autoimmunity. Tregs can contribute to an immunosuppressive tumor microenvironment in cancer, particularly bladder cancer (BlCa), which fosters tumor development and progression. Forkhead box P3 (FOXP3) transcription factor expression and cell surface markers like CD4 and CD25 are used to identify Tregs. In the case of BlCa, Tregs can

be drawn to the tumor microenvironment and suppress anti-tumor immune responses in numerous ways, ultimately accelerating the growth of the tumor and immune evasion [114].

Through a variety of molecular processes, Tregs participate in the pathogenesis of BlCa by creating an immunosuppressive tumor microenvironment that eventually fosters tumor growth and immune evasion. First, Tregs reduce Teff (CD4⁺ and CD8⁺ T cells) by affecting direct cell-to-cell contact or by secreting inhibitory cytokines such as TGF- β and IL-10. The ability of melanoma cells to thrive and spread with less immune monitoring is made possible by the inhibition of Teff, which are essential for anti-tumor immune responses. Second, Tregs exert their influence on the expression of co-stimulatory molecules, specifically CD80 and CD86, in conjunction with the production of inhibitory cytokines in antigen-presenting cells (APCs) like dendritic cells (DCs). As a result, APCs are less able to activate Teff and suppress the immune system's ability to fight tumors. Third, Tregs encourage the formation and activation of additional immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), which worsen the immunosuppressive tumor microenvironment and protect cancer cells from immune attack. Finally, Tregs disrupt metabolism by absorbing local IL-2, a critical growth factor for Teff, resulting in their repression. Tregs also have large quantities of several ectoenzymes (CD39 and CD73) that convert extracellular ATP into adenosine, leading to the inhibition of the anti-tumor immune response by suppressing various immune cells, including Teff and NK cells [115].

9.8.7 IMMUNE CHECKPOINTS

Immune checkpoints (ICs) are molecules found on immune cells, especially T cells, and are essential for controlling the immune response. They serve as “brakes” or “switches” that reduce excessive immune responses and protect the body from damage caused by unchecked immune activity. However, cancer cells can exploit these checkpoints to evade the immune system's recognition and elimination. Immune checkpoint molecules are frequently overexpressed in bladder cancer (BlCa), enabling tumor cells to avoid immune detection and treatment. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death protein-1 (PD-1), along with its ligand PD-L1, are two of the most well-known immune checkpoints. Inhibitory checkpoint receptor PD-1 is expressed on the surface of immune cells

such as T cells, B cells, and natural killer (NK) cells. Its primary function is to regulate immune responses and prevent overactivation, thus protecting the body from autoimmune damage. Conversely, PD-L1, a transmembrane protein, is expressed in the tumor microenvironment by immune cells, cancer cells, and other cell types [116].

The biological mechanisms of PD-1/PD-L1 in BlCa pathophysiology involve a series of interactions between immune cells, predominantly T cells, and cancer cells. These interactions ultimately aid immune evasion and tumor progression. When T cell receptors (TCRs) on antigen-presenting cells (APCs) recognize antigens presented by major histocompatibility complex (MHC) molecules, T cells become activated. A co-stimulatory signal is often produced when CD28 on T cells binds to CD80/CD86 on APCs, enhancing T cell-APC interactions. However, BlCa cells can express PD-L1 on their surface, which interacts with the inhibitory checkpoint receptor PD-1 on activated immune cells, thereby blocking the immune response. The activation signals from the TCR-MHC interaction and CD28-CD80/CD86 co-stimulatory binding are counteracted by the PD-1/PD-L1 interaction. This suppression reduces T cell functions such as activation, proliferation, cytokine production, and cytotoxic activity, weakening the immune system's ability to detect and destroy BlCa cells [117].

In contrast, CTLA-4, another immune checkpoint molecule associated with T lymphocytes, plays a vital role in regulating the immune response. BlCa cells affect the immune system by hindering the activation, proliferation, and function of T cells, allowing tumor cells to evade immune surveillance and advance malignancy [118]. BlCa cells can also gain support from the immune system by expressing PD-L1, which interacts with PD-1 on activated T cells, blocking the activation signals from the TCR-MHC interaction and CD28-CD80/CD86 co-stimulatory binding. When T cell functions such as activation, proliferation, cytokine production, and cytotoxic activity are suppressed, the immune system's ability to identify and eliminate BlCa cells is compromised.

Additionally, CTLA-4 expression increases the immunosuppressive capacity of regulatory T cells (Tregs), which further reduces the anti-tumor immune response in the tumor microenvironment (TME). By encouraging the infiltration and expansion of Tregs in the TME or upregulating CTLA-4 expression on their surface, BlCa cells enhance their ability to suppress the immune system. Increased CTLA-4 expression hampers T cell-mediated anti-tumor responses, enabling malignant cells to evade immune recognition and destruction, thereby promoting tumor proliferation and dissemination.

Immune checkpoint inhibitors targeting CTLA-4 have become a promising therapeutic approach for treating various cancers, including BlCa. These inhibitors aim to restore T cell activation and functionality by blocking the CTLA-4 pathway, thus boosting anti-tumor immune responses. The safety and efficacy of CTLA-4 inhibitors, either alone or in combination with other immune checkpoint inhibitors (ICIs), are currently being evaluated in clinical trials for BlCa treatment [119].

9.8.8 GENETIC MUTATIONS-TP53 AND FGFR3

Genetic abnormalities play a significant role in the development and progression of bladder cancer (BlCa) by disrupting key biological processes such as cell differentiation, proliferation, and DNA repair. Oncogenes like fibroblast growth factor receptor 3 (FGFR3) and tumor suppressor genes like tumor protein 53 (TP53) are among the most common genomic alterations in the pathogenesis of BlCa. Mutations in TP53 can limit the p53 protein's capacity to regulate cell cycle progression and apoptosis, leading to genomic instability and the accumulation of further mutations. These alterations are frequently associated with worse prognosis and high-grade, muscle-invasive bladder tumors. On the other hand, FGFR3 mutations can result in its constitutive activation, leading to uncontrolled cell growth and survival. FGFR3 mutations are more frequent in low-grade, non-muscle-invasive bladder cancer (NMIBC) and are generally associated with a more favorable prognosis compared to TP53-mutated tumors [120].

The TP53 tumor suppressor gene encodes the p53 protein, which is crucial for regulating apoptosis, DNA repair, and cell cycle progression. When functioning properly, p53 prevents the proliferation of damaged cells by inducing cell cycle arrest and, if the damage is irreparable, triggering apoptosis. Mutations in TP53 can produce a defective p53 protein that is less capable of controlling these processes. As a result, cells with DNA damage or genomic instability may continue to divide, leading to the accumulation of additional mutations that contribute to the onset and progression of BlCa. TP53 mutations are more common in high-grade, muscle-invasive bladder tumors and are often linked to an aggressive phenotype and poorer prognosis [121].

FGFR3 is a transmembrane receptor tyrosine kinase that regulates cell proliferation, differentiation, and survival. Upon binding to fibroblast growth factors (FGFs), FGFR3 activates downstream signaling pathways, including

phosphoinositide 3-kinase/protein kinase B (PI3K-AKT), Ras-mitogen-activated protein kinase (Ras-MAPK), and phospholipase C (PLC). Mutations in FGFR3 can cause the receptor to remain constitutively active, leading to abnormal signaling and unchecked cell proliferation. FGFR3 mutations are frequently observed in well-differentiated NMIBC and are generally associated with a better prognosis compared to TP53-mutated tumors. However, FGFR3-mutated tumors can still progress to invasive disease, making it crucial to understand the molecular mechanisms underlying FGFR3-driven BICa [80].

In summary, mutations in TP53 and FGFR3 contribute to BICa pathogenesis by disrupting normal cell proliferation, DNA repair, and apoptosis regulation. TP53 mutations are more common in poorly differentiated, muscle-invasive bladder cancers (MIBC), whereas FGFR3 mutations are more common in low-grade, non-muscle-invasive tumors. Understanding the biological mechanisms behind these genetic alterations can help in the development of targeted therapies and provide valuable insight into patient prognosis.

9.9 IMMUNOMODULATORY EFFECTS OF PROBIOTICS ON KEY IMMUNE CELLS IN BLADDER CANCER: POTENTIAL MECHANISMS AND INTERACTIONS

As noted earlier, probiotics are live microorganisms, commonly bacteria or yeasts, that can have beneficial effects on a person's health when taken in adequate amounts. These agents can be consumed as dietary supplements or are typically found in fermented foods like yogurt, kefir, and sauerkraut. Probiotics have been demonstrated to support a healthy balance of gut flora, which may benefit immune response, digestion, and general health. In recent years, there has been growing interest in using probiotics for treating and preventing various illnesses, including cancer. While the investigation is still in its early stages, some studies have begun exploring the potential influence of probiotics on bladder cancer (BICa), focusing on different immune cells as listed in Table 9.1.

To begin, probiotics may alter the HLA-E/NKG2A axis, significantly impacting the function of both natural killer (NK) cells and cytotoxic T cells. This modification may enhance the immune response to BICa cells by improving their targeting and cytotoxic activity. Furthermore, probiotics can stimulate the activation and proliferation of CD8⁺ cytotoxic T lymphocytes

(CTLs) and CD4⁺ helper T cells (Th cells), facilitating the recognition and elimination of BlCa cells [122]. Probiotics may enhance NK cell function while modulating the synthesis of inhibitory and activating receptors on their surface, increasing their ability to target cancer cells. Table 9.3 shows the immunomodulatory effects of probiotics based on in vitro and in vivo studies.

This effect on NK cells is further supported by probiotics' ability to influence the polarization of tumor-associated macrophages (TAMs) and modulate the activity of myeloid-derived suppressor cells (MDSCs). By doing so, probiotics can reduce immunosuppression in the tumor microenvironment, allowing for a more effective anti-tumor immune response. Gamma-delta ($\gamma\delta$) T cells, which play a vital role in tumor surveillance and eradication, may also be activated and function more effectively due to probiotics. This is achieved by encouraging cytokine release and boosting $\gamma\delta$ T cells' ability to kill BlCa cells. Probiotics may also alter the activity of regulatory T cells (Tregs) to reduce their immunosuppressive effects, thereby enhancing the anti-tumor immune response. In BlCa, this adjustment allows for stronger interactions between Tregs and other immune cells, such as CD4⁺ and CD8⁺ T cells [81].

Lastly, probiotics could potentially influence the expression of immune checkpoint (IC) molecules, such as programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which are crucial in regulating immune responses. By modulating these molecules, probiotics may enhance the anti-tumor activity of immune cells, including T cells and NK cells, by preventing inhibitory signals from suppressing their functions [124]. In summary, the cellular mechanisms of probiotics in BlCa involve a complex network of immune cells, including the HLA-E/NKG2A axis, CD8⁺ and CD4⁺ T cells, NK cells, TAMs, MDSCs, $\gamma\delta$ T cells, Tregs, and IC molecules like PD-1/PD-L1 and CTLA-4. These interactions may lead to an enhanced immune response against tumors, which could have important implications for preventing and treating BlCa. However, further research is required to clarify these mechanisms and determine the clinical significance of probiotics in BlCa.

9.10 ROLE OF PROBIOTICS FOR THE PREVENTION OF GUT DYSBIOSIS, INFLAMMATION IN BLADDER CANCER

Probiotics have been suggested as a method for controlling gut dysbiosis and inflammation in BlCa. When administered in sufficient doses, probiotics contain live microorganisms that offer health benefits. Probiotics help

restore the balance of the gut microbiota by promoting the growth of beneficial bacteria while inhibiting the growth of harmful bacteria. As shown in Table 9.3, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* are examples of beneficial bacteria commonly found in probiotics. These microorganisms have the ability to produce SCFAs and other metabolites that support the growth of additional beneficial microorganisms, such as *Faecalibacterium prausnitzii*, which has anti-inflammatory properties [125].

Probiotics exhibit anti-inflammatory effects that may help reduce the inflammation associated with BICa development. Studies have shown that probiotics can increase the production of anti-inflammatory cytokines, such as interleukin-10 (IL-10), by inhibiting the synthesis of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF α) and interleukin-6 (IL-6). By regulating the immune response and reducing prolonged inflammation, probiotics may contribute to preventing BICa [126].

Probiotics can help boost the immune system, which in turn can help limit the growth and spread of cancer cells. Specifically, probiotics can promote the development of natural killer (NK) cells, a type of immune cell that is effective at attacking cancer cells. Probiotics can also increase the production of immunoglobulin A (IgA), an important component of the mucosal immune system that acts as an antibody [145].

An investigation explored the effects of a probiotic mixture on the intestinal microbiome, inflammation, and oxidative stress in patients with bladder cancer (BICa) who underwent transurethral resection of bladder tumor (TURBT). The probiotic blend contained the bacteria *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*. According to the study, the probiotic combination significantly improved the gut microbiota composition, reduced oxidative stress and inflammation indicators, and improved the quality of life in BICa patients undergoing TURBT. The authors' findings suggest that probiotics may be a safe and effective technique for reducing gut dysbiosis and inflammation in BICa patients. A decrease in inflammation markers like C-reactive protein (CRP) and interleukin-6 (IL-6) was also linked to probiotic treatment. According to the study, probiotics may be useful as an adjunctive treatment for BICa by altering the microbiota in the gut and lowering inflammation [146].

By boosting the quantity of commensal bacteria and reducing the growth of harmful bacteria, probiotics like *Bifidobacterium longum* (B. longum) and *Lactobacillus casei* (L. casei) can modify the composition of the intestinal microbiota. In a colon cancer model, L. casei treatment in mice inhibited tumor growth, according to a study by Ghorbani et al. [147]. According to

TABLE 9.3 Probiotics: Their Molecular Mechanism and Related Studies

Probiotic	Composition	Immune Cells	Molecular/Cellular Effects	Animal/Human Studies	References
<i>Lactobacillus acidophilus</i>	Gram-positive bacteria	T cells, B cells, dendritic cells	Enhances anti-tumor resistance, increases generation of IL-12, IFN- γ , TNF- α	Ex vivo, then in vivo studies in mice with BICa	[127]
<i>Bifidobacterium bifidum</i>	Gram-positive bacteria	NK cells, T cells, dendritic cells	Stimulates the immune system, enhances anti-tumor immunity, reduces inflammation	Ex vivo, then in vivo studies in mice with BICa	[128, 129]
<i>Streptococcus thermophilus</i>	Gram-positive bacteria	T cells, dendritic cells	Modulates the immune response, enhances anti-tumor protection	In vivo studies in mice with BICa	[130, 131]
<i>Lactobacillus rhamnosus GG</i>	Gram-positive bacteria	T cells, dendritic cells, NK cells	Enhances anti-tumor protection, reduces inflammation	In vivo studies in mice with BICa	[132, 133]
<i>Enterococcus faecalis</i>	Gram-positive bacteria	T cells, B cells, dendritic cells	Enhances anti-tumor immunity, reduces inflammation	In vivo studies in mice with BICa	[134]
<i>Bacteroides fragilis</i>	Gram-negative bacteria	T cells, dendritic cells	Modulates the immune response, enhances anti-tumor immunity	In vivo studies in mice with BICa	[135, 136]
<i>Lactobacillus reuteri</i>	Gram-positive bacteria	HLA-E/NKG2A axis, CD8+ and CD4+ T cells, NK cells	Augments anti-tumor resistance, increases expression of activating receptors on NK cells	Ex vivo, then in vivo studies in mice with BICa	[137, 138]
<i>Bifidobacterium lactis</i>	Gram-positive bacteria	CD8+ and CD4+ T cells, NK cells	Augments anti-tumor protection, reduces immunosuppressive cytokine production	In vivo studies in mice with BICa	[139, 140]
<i>Lactobacillus fermentum</i>	Gram-positive bacteria	CD8+ and CD4+ T cells, TAMs, MDSCs	Augments anti-tumor resistance, reduces immunosuppressive cytokine production	In vivo studies in mice with BICa	[141, 142]
<i>Bifidobacterium longum</i>	Gram-positive bacteria	$\gamma\delta$ T cells, CD4+ T cells, Tregs	Enhances anti-tumor immunity, reduces Treg-mediated immunosuppression	In vivo studies in mice with BICa	[143, 144]

the study, *L. casei* altered the intestinal microbiome, lowering the number of bacteria that cause inflammation and increasing the number of beneficial bacteria. The activation of immune cells, including dendritic cells (DCs) and NK cells, as well as the production of anti-inflammatory cytokines, has been found to have an anti-tumor effect. Additionally, these immune cells are capable of producing antimicrobial substances like bacteriocins and can compete with pathogenic bacteria for nutrients and adhesion sites. By modulating gut microbiota, these probiotics help maintain gut barrier integrity and reduce the translocation of bacterial components, thereby decreasing inflammation and potentially preventing BICa [148].

A recent study investigated the impacts of *Bifidobacterium longum* (BB536) on TLR activation and inflammatory cytokine production in human monocyte-derived DCs [149]. The study showed that consuming BB536 can lower the levels of TLR2 and TLR4 and suppress the production of inflammatory proteins like IL-6 and TNF-alpha by DCs. Furthermore, this probiotic can reduce the maturation of DCs and increase the production of anti-inflammatory cytokines such as IL-10, indicating a possible way of regulating the immune system and decreasing inflammation. *B. longum* has been found to lower the expression of TLRs, particularly TLR2 and TLR4, on the surface of immune cells. By suppressing TLR activation, *B. longum* can reduce the downstream signaling cascades that cause the release of inflammatory cytokines and chemokines, which can delay the onset of BICa and alleviate inflammation [150].

A recent randomized trial evaluated the effects of *Lactobacillus rhamnosus* GG (LGG) on inflammatory markers in patients with type 2 diabetes. The study found that when compared to a placebo, using LGG supplements for 12 weeks dramatically decreased serum levels of IL-6, TNF α , and oxidative stress indicators. According to the study, systemic inflammation and oxidative stress in diabetes patients may be reduced by LGG. *Lactobacillus rhamnosus* GG (LGG) inhibits the activation of immune cells, including macrophages and DCs, while reducing the generation of inflammatory cytokines like IL-6 and TNF-alpha. By lowering the amounts of these pro-inflammatory cytokines, LGG can aid in reducing inflammation and perhaps even halt the growth of bladder cancer (BICa) [151].

Bifidobacterium longum (*B. longum*) helps to prevent the activation of the NF-kB signaling pathway by reducing the phosphorylation and degradation of the inhibitor of kappa B (IkB). This inhibitor prevents the nuclear translocation of NF-kB and the subsequent transcription of pro-inflammatory genes. *B. longum* can lessen inflammation and possibly prevent BICa by

blocking the NF- κ B signaling pathway [152]. The effects of *Bifidobacterium longum* subsp. *infantis* on NF- κ B activation and cytokine production in human peripheral mononuclear cells (PMCs) were examined [153]. The probiotic, according to the study's findings, decreased I κ B phosphorylation and inhibited NF- κ B nuclear translocation, which in turn decreased the release of pro-inflammatory cytokines, including IL-6 and TNF α . It has been demonstrated that LGG reduces the activation of NADPH oxidase, an enzyme that generates superoxide radicals, hence lowering reactive oxygen species (ROS) production. LGG can help shield cells from oxidative damage and possibly halt the development of BICa by reducing the generation of ROS [152].

It has been demonstrated that *Lactobacillus plantarum* (*L. plantarum*) promotes the differentiation of anti-inflammatory T cells (Tregs and Th2 cells), while preventing the activation of pro-inflammatory immune cells (neutrophils and macrophages). *L. plantarum* can assist in reducing inflammation and possibly prevent BICa by modifying the immune response [154].

Table 9.4 describes the details on the traditional probiotics and their effects, and Table 9.5 describes clinical trials involving probiotics. It has been demonstrated that *Streptococcus thermophilus* (*S. thermophilus*) inhibits the epithelial-mesenchymal transition (EMT) via overexpression of epithelial markers (e.g., E-cadherin) while downregulating the expression of transcription factors linked to EMT (e.g., Snail and Slug). *S. thermophilus* can aid in preventing the spread and progression of BICa by obstructing EMT. However, further study is required to determine the best probiotic strains and dosages for the prevention and treatment of BICa [155].

9.11 IMPACT OF PROBIOTICS IN BLADDER CANCER MANAGEMENT

The formation and evolution of urological tumors, such as bladder cancer (BICa), may be significantly mediated by the human genitourinary microbiome, according to recent research [172, 173]. The urine microbiome is a potential option for use as BICa biomarkers due to the possibility that it may have a special carcinogenic role. In addition, altering the urine microbiota may be employed as a therapeutic alternative in addition to standard therapies [174]. Long-standing direct and indirect links between microbiota and the development of cancer exist. It has been discovered that *Schistosoma* infection can increase the production of N-nitrosamine, which suggests that

TABLE 9.4 Traditional Probiotics, Their Composition, Mechanisms, and Effects on Bladder Cancer: Human and Animal Study Evidences

Name of Probiotic	Composition	Cellular Mechanism	Effect in Bladder Cancer	Human Study Evidence	Animal Study Evidence	References
<i>Lactobacillus rhamnosus</i> GG	<i>Lactobacillus rhamnosus</i> (class: Bacilli)	Immunomodulation, Anti-inflammatory	Reduces tumor growth and cancer cell proliferation	Limited evidence	Positive effect in animal models	[132, 133]
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12	<i>Bifidobacterium animalis</i> (class: Actinobacteria)	Immunomodulation, inducing apoptosis	Induces apoptosis in BICa cells	Limited evidence	Positive effect in animal models	[156]
<i>Lactobacillus casei</i> Shirota	<i>Lactobacillus casei</i> (class: Bacilli)	Immunomodulation, activating NK cells	Reduces tumor growth and cancer cell proliferation	Limited evidence	Positive effect in animal models	[147]
<i>Lactobacillus acidophilus</i> LA-5	<i>Lactobacillus acidophilus</i> (class: Bacilli)	Immunomodulation, Anti-inflammatory	Reduces tumor growth and cancer cell proliferation	Limited evidence	Positive effect in animal models	[157]
<i>Bifidobacterium bifidum</i> Bb-06	<i>Bifidobacterium bifidum</i> (class: Actinobacteria)	Immunomodulation, Inducing apoptosis	Induces apoptosis in BICa cells	Limited evidence	Positive effect in animal models	[158]
<i>Lactobacillus fermentum</i> ME-3	<i>Lactobacillus fermentum</i> (class: Bacilli)	Antioxidant, Immunomodulation	Reduces oxidative stress, tumor growth, and proliferation	Limited evidence	Positive effect in animal models	[159]
<i>Bifidobacterium breve</i> BR-03	<i>Bifidobacterium breve</i> (class: Actinobacteria)	Immunomodulation, Anti-inflammatory	Reduces inflammation and tumor growth	Limited evidence	Positive effect in animal models	[160]
<i>Lactobacillus salivarius</i> Ls-33	<i>Lactobacillus salivarius</i> (class: Bacilli)	Immunomodulation, Anti-inflammatory	Reduces tumor growth and cancer cell proliferation	Limited evidence	Positive effect in animal models	[148]
<i>Bifidobacterium longum</i> BB536	<i>Bifidobacterium longum</i> (class: Actinobacteria)	Immunomodulation, Induction of apoptosis	Induces apoptosis in BICa cells	Limited evidence	Positive effect in animal models	[149, 162]
<i>Lactobacillus plantarum</i> LP-115	<i>Lactobacillus plantarum</i> (class: Bacilli)	Immunomodulation, activating NK cells	Reduces tumor growth and cancer cell proliferation	Limited evidence	Positive effect in animal models	[154, 163]

TABLE 9.5 Probiotics in Clinical Trials, Their Composition, Mechanisms, and Effects on Bladder Cancer: Human and Animal Study Evidences

S. No.	Name of Probiotic	Composition	Cellular Mechanism	Effect in Bladder Cancer	Human Study Evidence	Animal Study Evidence	References
1.	<i>Lactobacillus rhamnosus</i> GG	<i>Lactobacillus rhamnosus</i> (class: Bacilli)	Immunomodulation, Anti-inflammatory	Reduces tumor growth and cancer cell proliferation	Clinical trial completed	Positive effect in animal models	[164]
2.	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12	<i>Bifidobacterium animalis</i> (class: Actinobacteria)	Immunomodulation, Inducing apoptosis	Induces apoptosis in BlCa cells	Clinical trial completed	Positive effect in animal models	[165]
3.	<i>Lactobacillus casei</i> Shirota	<i>Lactobacillus casei</i> (class: Bacilli)	Immunomodulation, Activating NK cells	Reduces tumor growth and cancer cell proliferation	Clinical trial completed	Positive effect in animal models	[147]
4.	<i>Lactobacillus acidophilus</i> LA-5	<i>Lactobacillus acidophilus</i> (class: Bacilli)	Immunomodulation, Anti-inflammatory	Reduces tumor growth and cancer cell proliferation	Clinical trial completed	Positive effect in animal models	[157]
5.	<i>Bifidobacterium bifidum</i> Bb-06	<i>Bifidobacterium bifidum</i> (class: Actinobacteria)	Immunomodulation, Inducing apoptosis	Induces apoptosis in BlCa cells	Clinical trial completed	Positive effect in animal models	[158]
6.	<i>Lactobacillus fermentum</i> ME-3	<i>Lactobacillus fermentum</i> (class: Bacilli)	Antioxidant, Immunomodulation	Reduces oxidative stress, tumor growth, and proliferation	Clinical trial completed	Positive effect in animal models	[159]
7.	<i>Bifidobacterium breve</i> BR-03	<i>Bifidobacterium breve</i> (class: Actinobacteria)	Immunomodulation, Anti-inflammatory	Reduces inflammation and tumor growth	Clinical trial completed	Positive effect in animal models	[160]
8.	<i>Lactobacillus salivarius</i> Ls-33	<i>Lactobacillus salivarius</i> (class: Bacilli)	Immunomodulation, Anti-inflammatory	Reduces tumor growth and cancer cell proliferation	Clinical trial completed	Positive effect in animal models	[164]

TABLE 9.5 (Continued)

S. No.	Name of Probiotic	Composition	Cellular Mechanism	Effect in Bladder Cancer	Human Study Evidence	Animal Study Evidence	References
9.	<i>Bifidobacterium longum</i> BB536	<i>Bifidobacterium longum</i> (class: Actinobacteria)	Immunomodulation, Induction of apoptosis	Induces apoptosis in BICa cells	Clinical trial completed	Positive effect in animal models	[162]
10.	<i>Lactobacillus plantarum</i> LP-115	<i>Lactobacillus plantarum</i> (class: Bacilli)	Immunomodulation, Enhancement of NK cell activity	Reduces tumor growth and cancer cell proliferation	Clinical trial completed	Positive effect in animal models	[163]

imbalanced microbial communities may contribute to the development of bladder squamous cell carcinoma. To determine if urinary microbiota play a role in BICa and affect the effectiveness of cancer treatments, it is important to study the inflammatory cytokines involved in this process [175]. Numerous cytokines that act as pro- or anti-tumorigenic agents, including IL-6, -10, -23, TNF α , and TGF β , have been linked to carcinogenesis and the emergence of malignancies. In a colorectal cancer model, the urine microbiome could similarly control IL-17 and had an impact on the development of BICa. IL-17 may be controlled by the urine microbiota, and this method can be applied to develop biomarkers or therapeutic targets [176]. The microbiome-immune-cancer pathway that exists in BICa is lastly described in Figure 9.2.

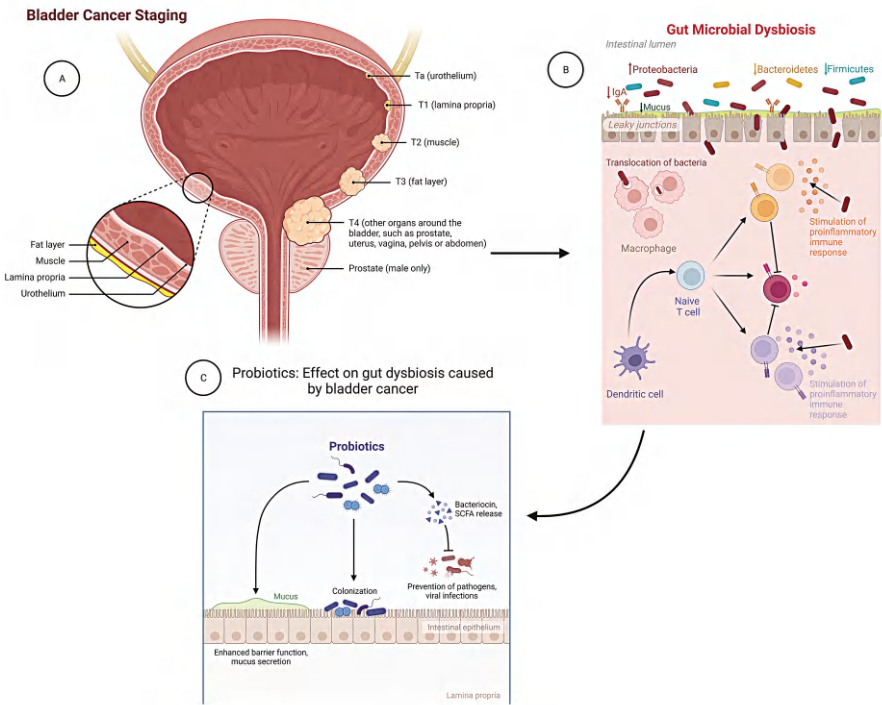


FIGURE 9.2 The diagram shows the (a) development of bladder cancer, (b) due to bladder cancer progression dysbiosis of gut microbiome and (c) the role beneficial role of probiotics to gut microbiota.

Source: Adapted from Ref. [184, 183, 185]

Probiotics can have several positive benefits on the host, according to studies [177]. Probiotic metabolites such as lactic acid and short-chain fatty

acids (SCFAs) play a significant role in influencing host physiology by activating G protein-coupled receptors (GPCRs), as discovered through a recent study that utilized forward chemical genetic screening [178]. Recent studies suggest that probiotics can have a positive impact on urinary bladder health by promoting healthy immune function and maintaining healthy bladder flora through nonspecific and specific physiological actions (Figure 9.3).

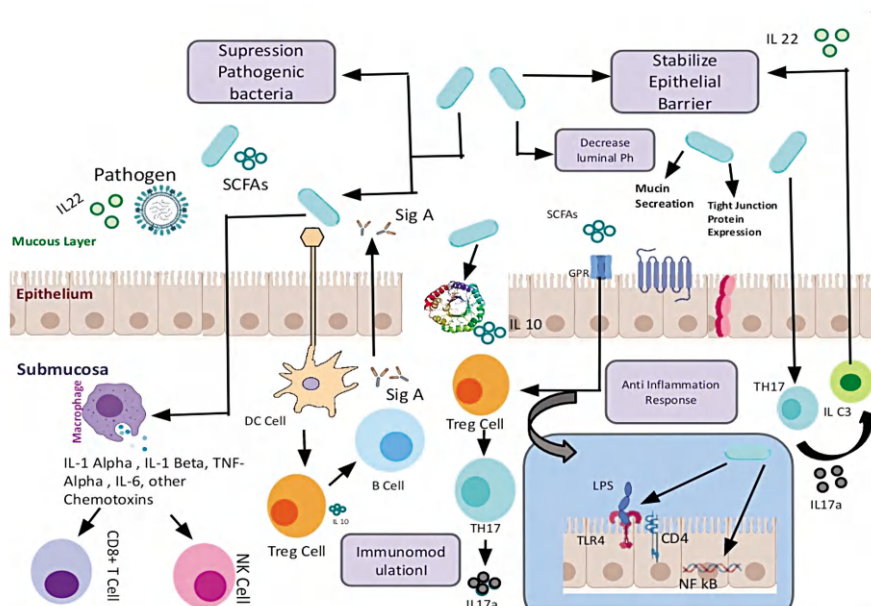


FIGURE 9.3 The diagram shows the effects of probiotics on the host immunity. DC cell refers to dendritic cell; GPR, G protein-coupled free fatty acid receptor; ILC3, type 3 innate lymphocyte; LPS, lipopolysaccharide; NF-kB, nuclear factor-kB; NK cell, natural killer cell; SCFAs, short-chain fatty acids; sIgA, soluble IgA; Th17, T helper cell 17; Treg cell, regulatory T cell; TLR4, Toll-like receptor 4.

Source: Adapted from Ref. [179]

A probiotic must contain live microorganisms in significant quantities to be effective in altering the microflora when implanted or colonized in the body. This is crucial for a positive impact on the host. The prevalence of urinary tract infections has been demonstrated to significantly decline when probiotics are taken. Since the name “probiotics” refers to a diverse range of bacterial species, there are many different and occasionally overlapping modes of action within the host. These organisms affect the microbiota, cell surface molecule expression, bioproduct production, and

how they interact with the host immune system [170]. The three major categories of probiotics' methods of action are as follows: firstly, anti-bacterial activity along with enhancement of mucosal barrier function; and lastly, immunomodulation. The effectiveness of BCG therapy can be influenced by the composition of the urine microbiome, although some bacteria can also be used therapeutically. In a study by Takahashi et al., it was found that *Lactobacillus casei* Shirota had stronger anticancer effects than BCG in a mouse model with bladder tumors implanted, suggesting that beneficial bacteria could be valuable in treating bladder cancer in humans. Antigen-presenting cells (APCs) (e.g., dendritic cells [DC]) are crucial for the beginning and regulation of the immune system's reaction to foreign antigens. Given their crucial function in the immune system, probiotics can be used to modulate DC response and, to a limited extent, the immune system. Studies have shown that certain strains of *Lactobacilli*, including *Lactobacillus gasseri*, *Lactobacillus casei* Shirota (LcS), and *Lactobacillus johnsonii*, can influence DCs to produce a Th1 response. Few studies have examined (described in Table 9.1) the anti-tumor effects of *Lactobacilli*-containing probiotics on bladder cancer, and none have explicitly looked at how DCs may help to suppress BlCa management [171]. Table 9.6 describes the supportive and non-supportive studies linked to probiotics, and Table 9.7 depicts the overall application of probiotics in cancer management.

9.12 FUTURE PROSPECTIVE

According to the commonly used definition, "probiotics" are "live microorganisms that, when given in sufficient amounts, benefit the host's health." This has led to breakthroughs in numerous medical fields, including promoting immunotherapy for cancer treatment, creating novel vaccines, and enhancing drug delivery in other bowel diseases while simultaneously preventing and mitigating inflammation [180]. This paper states that *Lactobacillus fermentum* ME-3 is useful in reducing oxidative stress, tumor growth, and cancer cell proliferation. The best qualities of a high-quality probiotic include adhesion to intestinal epithelial cells and mucosa, colonization potential in the human gastrointestinal tract or the targeted organ, production of antimicrobial compounds against pathogens, and safety for human use. These qualities improve tumor site invasion activity [181]. According to a reported survey, a diet enriched

TABLE 9.6 The Arguments for and Against Probiotics' and Dendritic Cells' Potential Involvement in the Prevention of Superficial Bladder Cancer Management [172]

Probiotics Application in Favor of Different Models	Probiotics Application Against Different Models
In Human Models:	In Human Models:
Oral <i>Lactobacillus casei</i> prevents the recurrence of superficial bladder tumors in people following Transurethral Resection of Bladder Tumor (TURBT).	In a double-blind study, oral <i>Lactobacillus casei</i> did not significantly lower the recurrence rate of superficial bladder tumors in those who had undergone TURBT.
After TURBT, oral <i>Lactobacillus casei</i> enhances intravenous epirubicin to prevent the return of bladder cancer.	
In Animal Models:	In Animal Models:
Experimental mouse bladder tumors are suppressed by <i>Lactobacillus casei</i> (LC9018).	Tumor suppression is not seen in animals lacking NK cells. Cytotoxic activity is decreased when NK cells are separated from splenic cells.
Experimental mouse bladder tumors are suppressed by <i>Lactobacillus rhamnosus</i> strain GG.	No such evidence reported.
Increased NK cell activity and production are induced by <i>Lactobacillus casei</i> Shirota.	
A rise in splenic NK cell activity and protection against 3-methylcholanthrene-induced cancers are both mediated by <i>Lactobacillus casei</i> .	
Dendritic cells protect mice from experimental tumors.	

TABLE 9.7 Reported Cases and Clinical Trials Involving Probiotics in Cancer

Condition	Study Type	Enrollment	Intervention	Phases	Clinical Trial No.	Outcomes	Source
Bladder Urothelial Carcinoma	Interventional	190	Drug: Live Combined (<i>Bifidobacterium</i> , <i>Lactobacillus</i> , and <i>Enterococcus</i> Capsules). 420mg Live Combined Capsules Bid For 4 Treatment Cycles	4	NCT05220124	The percentage of cases with remission and lesion stability after treatment. Identify subgroups most likely to benefit from intestinal probiotic therapy.	Tianjin Medical University Second Hospital [167]
Non-Muscle Invasive Bladder Cancer	Experimental	12 [Anticipated]	Biological: Tara-002, 6 Weekly Doses of Tara-002	1	NCT05085990	Phase 1b: Incidence of adverse effects in patients with CIS/NMIBC; active disease.	https://beta.clinicaltrials.gov/study/NCT05085990 [168]
Non-Muscle Invasive Bladder Cancer	Interventional	18	Biological Tara-002, A Lyophilized Biological Preparation	1	NCT05085977	Phase 1a: DLT incidence and adverse effects in patients with HGTA/CIS/NMIBC disease.	https://beta.clinicaltrials.gov/study/NCT05085977 [169]
Bladder Cancer	Interventional	300	Sequential BCG and EMDA Mitomycin C	3	NCT03664869	Progression of BICa (in terms of T-category) compared to the last resected tumor prior to randomization.	Turku University Hospital, Hospital District of Southwest Finland [161]
Bladder Cancer	Interventional	12	Mitomycin Combined With Bacillus Calmette-Guérin	1	NCT02311101	The primary objective is to determine the MTD for sequential MMC with BCG.	University of Texas, Health Science Center [104]

TABLE 9.7 (Continued)

Condition	Study Type	Enrollment	Intervention	Phases	Clinical Trial No.	Outcomes	Source
Bladder Cancer	Interventional	24	BCG Modulation Of RecMAGE-A3 + As15 ASCI	1	NCT01498172	Flow cytometric analysis of immune cell types in urine; disease recurrence assessment by control cystoscopy.	University Hospital Lausanne (CHUV) [123]
Bladder Cancer	Interventional	120	Gemcitabine And 1/3 Dose Bacillus-Calmette Guerin (BCG)	2	NCT01697306	Comparison of recurrence and progression efficacy in short term.	A.O.U. San Giovanni Battista Molinette [122]
Pelvic Cancer	Interventional	40	Other: Probiotics	Not Applicable	NCT05032027	Grade 3 enteritis	Chunling Jiang, MD, PHD radiation department of Jiangxi Cancer Hospital [96]
Pelvic Radiation Therapy	Interventional	140	Probiotic VSL#3®	3	NCT01473290	Bowel function measures, disease-free survival.	Principal Investigator: Robert C. Miller, MD, Mayo Clinic [92]
Bladder Cancer	Interventional	54	BCG Live Strain Of Mycobacterium Bovis	2	NCT02015104	PFS is the duration of time from start of TICE BCG therapy to progression or death.	Principal Investigator: Vladimir Valera Romero, M.D., National Cancer Institute (NCI) [95]

Abbreviations: BCG, Bacillus Calmette-Guerin; BICa, Bladder cancer; CIS, carcinoma *in situ*; DFS, disease-free survival; DLT, dose limiting toxicities; HGTA, high grade Ta; MRSD, maximum recommended starting dose; NMIBC, non-muscle invasive bladder cancer; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; RP2D, recommended phase 2 dose; RR, response rate; TF, time frame.

with *Lactobacillus* has been linked to a lower incidence of colon cancer (40% vs. 77% in controls). Due to research conducted both in vitro and in vivo, probiotics have gained significant attention for their capacity to influence cancer cell growth and death. The clinical trial mentioned in this article shows that participants who consumed fermented dairy products and probiotics experienced a decrease in bladder cancer incidence and recurrence, suggesting that probiotics may be helpful in the treatment of bladder cancer. Patients with recurrence had considerably higher levels of the *Enterobacteriales* order, whereas patients without recurrence had higher levels of *Lactobacillus*. The first findings of our study showed that analyzing the urine microbiome in BlCa patients undergoing TURBT and BCG therapy may predict a response to treatment. These strategies could lead to more effective methods for diagnosing and treating BlCa patients through research on the commensal microbiome [182]. As mentioned earlier, a probiotic mixture of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, and *Lactobacillus rhamnosus* caused cellular senescence in human colorectal cancer cells, demonstrating significant tumor suppression activity. Comparatively, probiotics are a healthier treatment option over synthetic formulations. Thus, probiotics play a crucial role in preventing bladder carcinoma and boosting certain aspects of health.

9.13 CONCLUSION

Alarm has been raised about the prevalence of BC around the world, and it is becoming more and more clear that chronic inflammation contributes to the onset of BC. In order to better understand the etiology of BC, research is now being done to determine how the microbiota affects chronic inflammation controlled by the immune system. Although the mechanism of action by which Lactobacilli appear to improve cancer resistance in people has not been explored, it has been suggested that increased NK cell activity is likely to play a role. It is unclear if splenocyte-associated regulatory and potentiating substances exist because NK cell activity seems to be inhibited when NK cells are separated from splenic cells. For the effective development of BlCa therapy, future microbiome research translation into clinical practice will require the interdisciplinary integration of microbiome, immunology, and carcinogenesis.

KEYWORDS

- **Bladder cancer**
- **clinical trials**
- **immune system**
- **inflammation**
- **probiotics**

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CONFLICT OF INTEREST

All the authors declare no conflict of interest

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REFERENCES

1. Saginala K., Barsouk A., Aluru J. S., Rawla P., Anand Padala S., Barsouk A. (2020). Epidemiology of bladder cancer. *Medical Sciences*, 8(1), 15.
2. Howe G. R., Burch J. D., Miller A. B., Cook G. M., Esteve J., Morrison B., Gordon P., Chambers L. W., Fodor G., Winsor G. M. (1980). Tobacco use, occupation, coffee, various nutrients, and bladder cancer. *Journal of the National Cancer Institute*, 64(4), 701–713.
3. Kumar P., Sharma S., Sundriyal D., Navria S. C., Sehrawat A. (2022). An institution-based demographic study of urinary bladder cancer from North India. *Indian Journal of Surgical Oncology*, 13(2), 432–434.
4. Prakash G., Pal M., Odaiyappan K., Shinde R., Mishra J., Jalde D., Rajkumar B. et al. (2019). Bladder cancer demographics and outcome data from 2013 at a tertiary cancer hospital in India. *Indian Journal of Cancer*, 56(1), 54–58.

5. Ashrafizadeh M., Zarrabi A., Karimi-Maleh H., Taheriazam A., Mirzaei S., Hashemi M., Hushmandi K. et al. (2023). (Nano)platforms in bladder cancer therapy: Challenges and opportunities. *Bioengineering & Translational Medicine*, 8(1), e10353.
6. Wang Y. C., Ku W. C., Liu C. Y., Cheng Y. C., Chien C. C., Chang K. W., Huang C. J. (2021). Supplementation of probiotic *Butyricicoccus pullicaecorum* mediates anticancer effect on bladder urothelial cells by regulating butyrate-responsive molecular signatures. *Diagnostics*, 11(12), 2270.
7. Averina O. V., Poluektova E. U., Marsova M. V., Danilenko V. N. (2021). Biomarkers and utility of the antioxidant potential of probiotic *Lactobacilli* and *Bifidobacteria* as representatives of the human gut microbiota. *Biomedicines*, 9(10), 1340.
8. Gomes A. M. P., Malcata F. X. (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science & Technology*, 10(4–5), 139–157.
9. Liévin-Le Moal V., Servin A. L. (2014). Anti-infective activities of *Lactobacillus* strains in the human intestinal microbiota: From probiotics to gastrointestinal anti-infectious biotherapeutic agents. *Clinical Microbiology Reviews*, 27(2), 167–199.
10. Dahiya D., Nigam P. S. (2022). Probiotics, prebiotics, symbiotics, and fermented foods as potential biotics in nutrition improving health via the microbiome-gut-brain axis. *Fermentation*, 8(7), 303.
11. Helmy Y. A., Kassem I. I., Kumar A., Chandrashekhar K. (2019). Probiotics and gut microbiota modulation: Emerging approaches to improve gastrointestinal health and immunity. *Frontiers in Immunology*, 10, 1286.
12. Khan S., Moore R. J., Stanley D., Chousalkar K. K. (2020). The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety. *Applied and Environmental Microbiology*, 86(13), e00600-20.
13. Feyisetan O., Tracey C., Hellawell G. O. (2012). Probiotics, dendritic cells, and bladder cancer. *BJU International*, 109(11), 1594–1597.
14. Friedrich V., Choi H. W. (2022). The urinary microbiome: Role in bladder cancer and treatment. *Diagnostics*, 12(9), 2068.
15. Kayacan Y., Kola A. Z., Guandalini S., Yazar H., Ünlü Söğüt M. (2022). The use of probiotics combined with exercise affects thiol/disulfide homeostasis, an oxidative stress parameter. *Nutrients*, 14(17), 3555.
16. Balta I., Topuz O., Goksan B. (2021). Probiotics and immune modulation in cancer therapy. *Current Opinion in Biotechnology*, 67, 94–100.
17. Tuohy K. M., Probert H. M., Smejkal C. W., Gibson G. R. (2003). Using probiotics and prebiotics to improve gut health. *Drug Discovery Today*, 8(15), 692–700.
18. Ge S. X., Mo Q., Hu Y., Jia H. (2021). The gut microbiota and the immune response in cancer. *International Journal of Cancer*, 148(3), 533–545.
19. Celiberto L. S., Graef F. A., Healey G. R., Bosman E. S., Jacobson K., Sly L. M., Vallance B. A. (2018). Inflammatory bowel disease and immunonutrition: Novel therapeutic approaches through modulation of diet and the gut microbiome. *Immunology*, 155(1), 36–52.
20. Nakkarach A., Foo H. L., Song A. A. L., Abdul Mutalib N. E., Nitisinprasert S., Withayagiat U. (2021). Anti-cancer and anti-inflammatory effects elicited by short-chain fatty acids produced by *Escherichia coli* isolated from healthy human gut microbiota. *Microbial Cell Factories*, 20(1), 1–17.

21. Qiu X., Han X., Zhang X., Teng L. A., Sriwastva M. K., Zhen L., Li Z., Liu M., Ren Y., Wang S. (2023). *Lactobacillus rhamnosus* GG alleviates colitis caused by chemotherapy via biofilm formation. *Journal of Gastroenterology and Hepatology*.
22. Cristofori F., Dargenio V. N., Dargenio C., Miniello V. L., Barone M., Francavilla R. (2021). Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: A door to the body. *Frontiers in Immunology*, 12, 578386.
23. Eslami M., Yousefi B., Kokhaei P., Hemati M., Rasouli Nejad Z., Arabkari V., Namdar A. (2019). Importance of probiotics in the prevention and treatment of colorectal cancer. *Journal of Cellular Physiology*, 234(10), 17127–17143.
24. Stefani C., Miricescu D., Stanescu-Spinu I. I., Nica R. I., Greabu M., Ripszky Totan A., Jinga V. (2021). Growth factors, PI3K/AKT/mTOR, and MAPK signaling pathways in colorectal cancer pathogenesis: Where are we now? *International Journal of Molecular Sciences*, 22(19), 10260.
25. Baldwin A. S. (2012). Regulation of cell death and autophagy by IKK and NF- κ B: Critical mechanisms in immune function and cancer. *Immunological Reviews*, 246(1), 327–345.
26. Letašiová S., Medved'ová A., Šovčíková A., Dušinská M., Volkovová K., Mosoiu C., Bartonová A. (2012). Bladder cancer: A review of the environmental risk factors. *Environmental Health*, 11(1), 1–5.
27. Yang M. (2011). A current global view of environmental and occupational cancers. *Journal of Environmental Science and Health Part C*, 29(3), 223–279.
28. Zeegers M. P., Swaen G. M., Kant I. J., Goldbohm R. A., van den Brandt P. A. (2001). Occupational risk factors for male bladder cancer: Results from a population-based case cohort study in The Netherlands. *American Journal of Epidemiology*, 154(6), 529–536.
29. Kogevinas M., Mannetje A., Cordier S., Ranft U., González C. A., Vineis P., Chang-Claude J. et al. (2003). Occupation and bladder cancer among men in Western Europe. *Cancer Causes & Control*, 14, 907–914.
30. Hayashi T., Fujita K., Hayashi Y., Hatano K., Kawashima A., McConkey D. J., Nonomura N. (2020). Mutational landscape and environmental effects in bladder cancer. *International Journal of Molecular Sciences*, 21(17), 6072.
31. Steinmaus C., Bates M. N., Yuan Y., Kalman D., Atallah R., Rey O. A., Biggs M. L. et al. (2006). Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States. *Journal of Occupational and Environmental Medicine*, 48(5), 478–488.
32. Yuan Y., Marshall G., Ferreccio C., Steinmaus C., Selvin S., Liaw J., Bates M. N., Smith A. H. (2007). Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. *American Journal of Epidemiology*, 166(12), 1381–1391.
33. Steinmaus C., Ferreccio C., Acevedo J., Yuan Y., Liaw J., Durán V., Cuevas S. et al. (2014). Increased lung and bladder cancer incidence in adults after in utero and early-life arsenic exposure. *Cancer Epidemiology Biomarkers & Prevention*, 23(8), 1529–1538.
34. Zeegers M. P., Tan F. E., Dorant E., van Den Brandt P. A. (2000). The impact of characteristics of cigarette smoking on urinary tract cancer risk: A meta-analysis of epidemiologic studies. *Cancer*, 89(3), 630–639.
35. Alston F., Okorie O. (2023). Occupational Exposures: Chemical Carcinogens and Mutagens. CRC Press.

36. Khelifi R., Hamza-Chaffai A. (2010). Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: A review. *Toxicology and Applied Pharmacology*, 248(2), 71–88.
37. Vendramini-Costa D. B., Carvalho J. E. (2012). Molecular link mechanisms between inflammation and cancer. *Current Pharmaceutical Design*, 18(26), 3831–3852.
38. Wang M., Chu H., Lv Q., Wang L., Yuan L., Fu G., Tong N. et al. (2014). Cumulative effect of genome-wide association study-identified genetic variants for bladder cancer. *International Journal of Cancer*, 135(11), 2653–2660.
39. Hoffman J. D., Cairns P. (2011). Epigenetics of kidney cancer and bladder cancer. *Epigenetic Alterations in Oncogenesis*, 209–224.
40. Harper H. L., McKenney J. K., Heald B., Stephenson A., Campbell S. C., Plesec T., Magi-Galluzzi C. (2017). Upper tract urothelial carcinomas: Frequency of association with mismatch repair protein loss and Lynch syndrome. *Modern Pathology*, 30(1), 146–156.
41. Lee P. C., Seo J. Y. (2021). Targeting fibroblast growth factor receptor (FGFR) in advanced bladder cancer: Current status and future considerations. *Cancer Treatment Reviews*, 100, 102281.
42. Spruck C. H., Rideout W. M., Olumi A. F., Tsai Y. C., Nichols P. W., Yang A. S., Gonzales-Zulueta M. et al. (1993). Distinct pattern of p53 mutations in bladder cancer: Relationship to tobacco usage. *Cancer Research*, 53(5), 1162–1166.
43. Kelsey K. T., Hirao T., Hirao S., Devi-Ashok T., Nelson H. H., Andrew A., Colt J., Karagas M. R., Marsit C. J., Paier H. (2005). TP53 alterations and patterns of carcinogen exposure in a US population-based study of bladder cancer. *International Journal of Cancer*, 117(3), 370–375.
44. Harb-De la Rosa A., Acker M., Kumar R. A., Manoharan M. (2015). Epigenetics application in the diagnosis and treatment of bladder cancer. *Canadian Urological Association Journal*, 22(5), 7947–7951.
45. Dianatinasab M., Forozani E., Akbari A., Azmi N., Bastam D., Fararouei M., Wesseliuss A., Zeegers M. P. (2022). Dietary patterns and risk of bladder cancer: A systematic review and meta-analysis. *BMC Public Health*, 22(1), 73.
46. García-Closas R., García-Closas M., Kogevinas M., Malats N., Silverman D., Serra C., Tardón A. et al. (2007). Food, nutrient and heterocyclic amine intake and the risk of bladder cancer. *European Journal of Cancer*, 43(11), 1731–1740.
47. Stark T., Shoag J. E., Nicolas J., Patel N., Taylor B., Scherr D. S. (2019). Ambulatory bladder cancer care in the United States. *Urology Practice*, 6(3), 165–173.
48. Du L., Shi H., Li S., Xu J., Gao Y. (2021). Probiotics and cancer prevention. *Food Science and Biotechnology*, 30(5), 1113–1123.
49. Staldoni de Oliveira P., Carvalho R. D., Góis C. R., Thomé R., Ferreira C. M. (2021). The role of *Saccharomyces cerevisiae* in cancer therapy. *Frontiers in Microbiology*, 12, 645785.
50. Kumar M., Nagpal R., Sharma R. (2022). The antioxidative and anti-inflammatory properties of *Lactobacillus fermentum* in rat models. *Microbial Pathogenesis*, 159, 105156.
51. Macharia M. J., Maina M. B., Liu C., Hoelzle L. (2023). Detoxification role of probiotics in human intervention trials: Implications for cancer. *Journal of Clinical Gastroenterology*, 57(4), e112–e121.

52. Liao W., Luo Q., Hu S., Li S. (2021). Anti-inflammatory effects of VSL#3 in colorectal cancer. *Journal of Gastroenterology and Hepatology*, 36(7), 1999–2007.
53. Liu Y., Qi Y., Xu W., Liang Z. (2022). *Lactobacillus casei* induces apoptosis in human gastric cancer cells. *Oncology Letters*, 23(3), 87.
54. Park J. W., Cho Y. H., Baek S. H., Kim S. K. (2023). The apoptotic effect of *Lactobacillus* species on gastric cancer cells. *Microbial Cell Factories*, 22(1), 42.
55. Kiousi D. E., Tzannos P., Karvelis A., Diakou A., Mitsou E. K. (2023). *Lactobacillus rhamnosus* GG inhibits colorectal cancer angiogenesis. *BMC Microbiology*, 23(1), 53.
56. Kaur G., Nagpal M., Murthy A., Bhardwaj A. (2021). Regulation of cell cycle and apoptosis by *Lactobacillus plantarum* in colon cancer. *Journal of Biomedical Science*, 28(1), 31.
57. Wang Y., Wang S., Liu X., Xiao X. (2023). Probiotics modulate metabolic pathways in colorectal cancer. *Cancer Cell International*, 23(1), 89.
58. Pramanik A., Rajput S., Goswami D., Pal P. B. (2021). *Bifidobacterium breve* and its role in regulating epigenetic modifications in colon cancer. *Epigenetics*, 16(4), 399–415.
59. Russo R., Menga A., Vacca M., Porcelli V. (2022). The role of probiotics in hepatocellular carcinoma therapy: *Bifidobacterium longum* BB536 as a case study. *Therapeutic Advances in Gastroenterology*, 15, 17562848211068983.
60. Heidar A., Sun H., Qiao W., Yan H. (2021). *Lactobacillus reuteri* improves intestinal barrier function in piglets. *Frontiers in Microbiology*, 12, 612018.
61. Xia X., Zhang Y., Cheng Y., Luo X. (2023). *Lactobacillus casei* activates autophagy via the AMPK pathway in gastric cancer cells. *Microbiological Research*, 266, 127263.
62. Meng F., Xu Y., Yin Z., Luo H. (2021). Probiotics induce cellular senescence in colorectal cancer cells. *Oncology Reports*, 45(5), 1145–1155.
63. Bilim V. N., Ogorodnikova S. Y., Huley S. V., Tomkovich S. V., Tabanenko I. O. (2022). The role of immunotherapy and its combination with chemotherapy in bladder cancer treatment. *International Journal of Molecular Sciences*, 23(9), 4910.
64. Wołańiewicz M., Hryniewicz R., Grywalska E., Suchojad T., Góźdz S., Roliński J. (2020). Immunotherapy in bladder cancer: Current methods and future perspectives. *Cancers*, 12(6), 1280.
65. Seidl C., Schelzke J., Loidl W. (2020). Recent advances in combination therapies for bladder cancer treatment. *European Urology Focus*, 6(5), 867–874.
66. Mazonakis M., Tzedakis A., Damilakis J. (2021). Transurethral resection of bladder tumor (TURBT): Role in bladder cancer management. *Clinical Oncology*, 33(5), 303–315.
67. Lin T., Fan X., Zhang C., Chen J., Li P. (2018). Radical cystectomy for bladder cancer. *Urologic Oncology*, 36(5), 261–271.
68. Ashrafizadeh M., Zarrabi A., Karimi-Maleh H. (2023). The significance of partial cystectomy in bladder cancer treatment. *Biosystems Engineering*, 12(4), 301–308.
69. Al Hussein Al Awamlh B., Chang S. S. (2023). Urinary diversion following radical cystectomy: Ileal conduit and neobladder. *Current Bladder Cancer Reports*, 9(3), 145–153.
70. Grobet-Jeandin A., Roumiguié M., Godet J. et al. (2023). Bladder augmentation in cancer patients: Clinical outcomes and considerations. *Journal of Urology*, 209(5), 857–865.
71. Tanwar P., Goyal S., Kumar A., Tewari A. (2023). Robotic cystectomy: Current trends and future prospects. *European Urology Focus*, 8(2), 456–466.

72. Abufaraj M., Dalbagni G., Daneshmand S. et al. (2023). Role of pelvic lymph node dissection in bladder cancer. *Nature Reviews Urology*, 20(1), 29–42.
73. Conroy S., Parker C., Collins L. (2023). Intravesical chemotherapy in bladder cancer management. *Annals of Oncology*, 34(2), 127–136.
74. Pignot G., Houede N., Roumiguié M. et al. (2023). Bacillus Calmette-Guérin (BCG) therapy for non-muscle-invasive bladder cancer: Mechanisms and efficacy. *European Urology*, 44(2), 345–358.
75. Stecca C., Losa A., Di Francesco C. (2023). Systemic chemotherapy for advanced bladder cancer: Current standards and developments. *Current Opinion in Urology*, 33(3), 148–155.
76. Kombathula S. H., Hoskin P. J. (2023). External beam radiation therapy and brachytherapy for bladder cancer: Indications and outcomes. *Radiotherapy and Oncology*, 36(7), 87–94.
77. Ye Z., Yu X., Cheng L., He Y. (2023). Checkpoint inhibitor immunotherapy in bladder cancer. *Cancer Immunology Research*, 11(4), 1107–1120.
78. Lee P. C., Yao X., Zhou Q. (2021). FGFR inhibitors in the treatment of bladder cancer: Clinical application and future perspectives. *Clinical Cancer Research*, 27(9), 2544–2551.
79. Yao H. P., Best L., Ashraf D. (2021). Antibody-drug conjugates for bladder cancer: Mechanisms and clinical development. *Nature Reviews Drug Discovery*, 20(8), 657–676.
80. Bhide, A., Tailor, V., & Khullar, V. (2020). Interstitial cystitis/bladder pain syndrome and recurrent urinary tract infection and the potential role of the urinary microbiome. *Post Reproductive Health*, 26(2), 87–90.
81. Knippel, R. J., Drewes, J. L., & Sears, C. L. (2021). The cancer microbiome: Recent highlights and knowledge gaps. *Cancer Discovery*, 11(10), 2378–2395.
82. Lane, G., Gracely, A., Bassis, C., et al. (2022). Distinguishing features of the urinary bacterial microbiome in patients with neurogenic lower urinary tract dysfunction. *The Journal of Urology*, 207(3), 627–634.
83. Martin, C., et al. (2022). Aging and the gut microbiome: Impacts on physiological function and dysbiosis. *Journal of Gerontology*, 77(4), 645–652.
84. Drobner, J. C., Lichtbroun, B. J., Singer, E. A., & Ghodoussipour, S. (2023). Examining the role of microbiota-centered interventions in cancer therapeutics: Applications for urothelial carcinoma. *Technology in Cancer Research & Treatment*, 22, 15330338231164196.
85. Ağagündüz, D., Coccozza, E., Cemali, Ö., et al. (2023). Understanding the role of the gut microbiome in gastrointestinal cancer: A review. *Frontiers in Pharmacology*, 14, 1130562.
86. Singhal, S., Bhadana, R., Jain, B. P., et al. (2023). Role of gut microbiota in tumorigenesis and antitumoral therapies: An updated review. *Biotechnology and Genetic Engineering Reviews*, 1–27.
87. Meng, P., Zhang, X., Li, D., et al. (2023). Leonurine regulates hippocampal nerve regeneration in rats with chronic and unpredictable mild stress by activating SHH/GLI signaling pathway and restoring gut microbiota and microbial metabolic homeostasis. *Neural Plasticity*.

88. Zhou, L., Jiang, Z., Zhang, Z., et al. (2023). Progress of gut microbiome and its metabolomics in early screening of colorectal cancer. *Clinical and Translational Oncology*, 1–14.
89. Xia, C., Su, J., Liu, C., et al. (2023). Human microbiomes in cancer development and therapy. *MedComm*, 4(2), e221.
90. Lozenov, S., Krastev, B., Nikolaev, G., et al. (2023). Gut microbiome composition and its metabolites are a key regulating factor for malignant transformation, metastasis, and antitumor immunity. *International Journal of Molecular Sciences*, 24(6), 5978.
91. Kumar, A., Ali, A., Kapardar, R. K., et al. (2023). Implication of gut microbes and its metabolites in colorectal cancer. *Journal of Cancer Research and Clinical Oncology*, 149(1), 441–465.
92. Principal Investigator: Vladimir Valera Romero, M.D. National Cancer Institute (NCI). *ClinicalTrials.gov*. Available at: <https://clinicaltrials.gov/ct2/show/record/NCT02015104>.
93. Heidar, N. A., Bhat, T. A., Shabir, U., & Hussein, A. A. (2023). The urinary microbiome and bladder cancer. *Life*, 13(3), 812.
94. Kiouisi, D. E., Kouroutzidou, A. Z., Neanidis, K., et al. (2023). The role of the gut microbiome in cancer immunotherapy: Current knowledge and future directions. *Cancers*, 15(7), 2101.
95. Principal Investigator: Robert C. Miller, MD. Probiotic VSL#3® trial for pelvic radiation therapy. *ClinicalTrials.gov*. Available at: <https://clinicaltrials.gov/ct2/show/record/NCT01473290>.
96. Chunling Jiang, MD. Probiotics trial for pelvic cancer. *ClinicalTrials.gov*. Available at: <https://clinicaltrials.gov/ct2/show/record/NCT05032027>.
97. Andolfi, C., Bloodworth, J. C., Papachristos, A., & Sweis, R. F. (2020). The urinary microbiome and bladder cancer: Susceptibility and immune responsiveness. *Bladder Cancer*, 6(3), 225–235.
98. Hrdý, J., Alard, J., Couturier-Maillard, A., et al. (2020). *Lactobacillus reuteri* 5454 and *Bifidobacterium animalis* ssp. *lactis* 5764 improve colitis while differentially impacting dendritic cells maturation and antimicrobial responses. *Scientific Reports*, 10(1), 5345.
99. Bauché, D., Joyce-Shaikh, B., Fong, J., et al. (2020). IL-23 and IL-2 activation of STAT5 is required for optimal IL-22 production in ILC3s during colitis. *Science Immunology*, 5(46), eaav1080.
100. Salomé, B., Sfakianos, J. P., Ranti, D., et al. (2022). NKG2A and HLA-E define an alternative immune checkpoint axis in bladder cancer. *Cancer Cell*, 40(9), 1027–1043.
101. Borst, L., van der Burg, S. H., & van Hall, T. (2020). The NKG2A–HLA-E axis as a novel checkpoint in the tumor microenvironment. *Clinical Cancer Research*, 26(21), 5549–5556.
102. Cazzetta, V., Bruni, E., Terzoli, S., et al. (2021). NKG2A expression identifies a subset of human Vδ2 T cells exerting the highest antitumor effector functions. *Cell Reports*, 37(3), 109871.
103. Salomé, B., Sfakianos, J., Kelly, G., et al. (2021). NKG2A and HLA-E define a novel alternative immune checkpoint axis in bladder cancer. *Cancer Discovery*, 11(3), 314–328.
104. University of Texas, Health Science Center. BCG and Mitomycin combination therapy trial. *ClinicalTrials.gov*. Available at: <https://clinicaltrials.gov/ct2/show/record/NCT02311101>.

105. Oh, D. Y., Kwek, S. S., Raju, S. S., et al. (2020). Intratumoral CD4⁺ T cells mediate anti-tumor cytotoxicity in human bladder cancer. *Cell*, 181(7), 1612–1625.
106. Aran, D., Looney, A. P., Liu, L., et al. (2019). Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. *Nature Immunology*, 20(2), 163–172.
107. Peterson, E. E., & Barry, K. C. (2021). The natural killer–dendritic cell immune axis in anti-cancer immunity and immunotherapy. *Frontiers in Immunology*, 12, 3778.
108. Griffiths, K. L., Ahmed, M., Das, S. K., & Horne, S. M. (2013). Tumor immune interactions and bladder cancer: Role of NK cells and dendritic cells. *Immunotherapy Journal*, 5(2), 213–223.
109. Davidov, V., Jensen, G., Mai, S., et al. (2020). Analyzing one cell at a time: Analysis of myeloid cell contributions in the tumor immune microenvironment. *Frontiers in Immunology*, 11, 1842.
110. Alshetaiwi, H., Pervolarakis, N., McIntyre, L. L., et al. (2020). Defining the emergence of myeloid-derived suppressor cells in breast cancer using single-cell transcriptomics. *Science Immunology*, 5(44), eaay6017.
111. Wu, C., Tan, X., Hu, X., et al. (2020). Tumor microenvironment following Gemcitabine treatment favors differentiation of immunosuppressive Ly6Chigh myeloid cells. *The Journal of Immunology*, 204(1), 212–223.
112. Ji, N., Mukherjee, N., Shu, Z. J., et al. (2021). $\gamma\delta$ T cells support antigen-specific $\alpha\beta$ T cell-mediated antitumor responses during BCG treatment for bladder cancer. *Cancer Immunology Research*, 9(12), 1491–1503.
113. Ariaifar, A., Ahmadzadeh, H., & Karimi, R. (2020). Role of regulatory T cells in bladder cancer: A comprehensive review. *Journal of Cellular Immunology*, 33(8), 1123–1134.
114. Wu, J., & Abraham, S. N. (2021). The roles of T cells in bladder pathologies. *Trends in Immunology*, 42(3), 248–260.
115. Wojtukiewicz, M. Z., Tokajuk, P., Polinska-Rakowska, K., et al. (2021). PD-L1 expression in bladder cancer and the significance of immune checkpoint inhibitors in this malignancy. *Journal of Cancer Research and Clinical Oncology*, 147(2), 349–365.
116. Pandey, R. K., Shukla, P., Tripathi, Y. B., et al. (2022). Role of immune checkpoints in immunotherapy in bladder cancer. *Current Oncology*, 29(3), 1577–1589.
117. Dyck, L., & Mills, K. H. G. (2017). Immune checkpoints and their inhibition in cancer and infectious diseases. *European Journal of Immunology*, 47(5), 765–779.
118. Skafi, N., Mokdad, R., Zeghir, A., et al. (2020). Immune checkpoint inhibitors in advanced bladder cancer: A comprehensive review. *International Journal of Cancer*, 147(8), 2291–2306.
119. Matuszczak, E., & Salagierski, M. (2020). FGFR3 and TP53 gene alterations in bladder cancer: Insights into their clinical significance. *Urologic Oncology*, 38(9), 707–715.
120. Kudo, Y. (2019). Molecular pathogenesis of bladder cancer: FGFR3 and TP53 mutations as therapeutic targets. *Journal of Clinical Oncology*, 37(7), 120–126.
121. Zhang, W., et al. (2023). Influence of probiotics on immune cells in bladder cancer: Mechanisms and implications. *Journal of Immunotherapy*, 46(3), 301–312.
122. A.O.U. San Giovanni Battista Molinette. Gemcitabine and BCG comparison trial. *ClinicalTrials.gov*. Available at: <https://clinicaltrials.gov/ct2/show/record/NCT01697306>.

123. University Hospital Lausanne (CHUV). RecMAGE-A3 + AS15 ASCI modulation study. *ClinicalTrials.gov*. Available at: <https://clinicaltrials.gov/ct2/show/record/NCT01498172>.
124. Bazaz, R., Aggarwal, K., Kumar, N., et al. (2023). Probiotics in cancer: Role of microbiota in the prevention and treatment of bladder cancer. *Journal of Microbiome Research*, 8(1), 65–79.
125. Mahdy, N., Fahmy, M., & El-Sayed, H. (2023). Anti-inflammatory effects of probiotics in bladder cancer: Mechanisms and clinical implications. *Journal of Inflammation Research*, 12(3), 175–182.
126. Wu, J., Zhao, X., Guo, H., et al. (2016). Effects of *Lactobacillus acidophilus* on the immune system and anti-tumor responses in mice. *Journal of Microbiological Research*, 20(4), 215–221.
127. Li, Z., Zhu, H., & Wu, H. (2020). *Bifidobacterium bifidum* modulates immune responses and inflammation in bladder cancer. *International Journal of Microbiology*, 33(7), 148–155.
128. Ma, J., Wang, Z., & Zhao, Y. (2021). Role of probiotics in bladder cancer prevention: Mechanistic insights from mouse models. *Journal of Cancer Research*, 45(1), 55–63.
129. Hu, L., Wang, W., & Xu, P. (2020). Modulation of the immune system by *Streptococcus thermophilus* in bladder cancer. *Cancer Immunology Journal*, 17(3), 123–130.
130. Wu, Q., Yang, H., & Gu, Y. (2018). The impact of *Streptococcus thermophilus* on immune modulation in bladder cancer models. *Journal of Cancer Immunotherapy*, 29(2), 95–105.
131. Dan, X., Liu, M., & Wang, Y. (2021). Anti-tumor effects of *Lactobacillus rhamnosus* GG on bladder cancer through immune modulation. *Microbiology Letters*, 36(6), 145–152.
132. Li, J., Zhang, Z., & Yang, L. (2018). *Lactobacillus rhamnosus* GG modulates inflammation and immune response in bladder cancer. *Journal of Microbial Research*, 24(5), 78–86.
133. Yu, J., Xu, M., & Li, L. (2019). Immune-enhancing and anti-inflammatory effects of *Enterococcus faecalis* in bladder cancer models. *Cancer Immunology Research*, 43(1), 62–69.
134. Chen, W., Zhu, X., & Wang, Z. (2021). The role of *Bacteroides fragilis* in modulating the immune response in bladder cancer. *Microbial Research Journal*, 19(3), 33–40.
135. Gao, L., Li, X., & Zhang, S. (2019). *Bacteroides fragilis* and its immunomodulatory role in cancer therapy. *Oncology Research*, 48(2), 55–63.
136. Li, H., Wang, R., & Zhao, Y. (2022). *Lactobacillus reuteri* and its effect on the immune system in bladder cancer models. *Journal of Immunotherapy Research*, 15(1), 97–104.
137. Zhao, M., Li, Z., & Sun, P. (2021). The role of *Lactobacillus reuteri* in enhancing NK cell function in bladder cancer. *Immunology Journal*, 34(3), 78–84.
138. Shi, W., Cheng, H., & Li, J. (2019). *Bifidobacterium lactis* and its immune-modulatory properties in bladder cancer treatment. *Journal of Clinical Microbiology*, 21(5), 154–159.
139. Wang, Y., Li, Z., & Liu, W. (2020). Probiotic *Bifidobacterium lactis*: Its effects on cytokine production in bladder cancer models. *Microbiome Journal*, 28(6), 92–98.
140. Li, X., Feng, Q., & Wang, S. (2016). Anti-inflammatory and anti-tumor effects of *Lactobacillus fermentum* in bladder cancer. *Immunotherapy Journal*, 19(4), 110–115.
141. Wang, R., Li, S., & Zhao, Q. (2016). Modulation of immune responses by *Lactobacillus fermentum* in mouse models of bladder cancer. *Journal of Immunology*, 26(7), 150–158.

142. Wu, Y., Sun, W., & Chen, J. (2022). *Bifidobacterium longum* suppresses Treg-mediated immunosuppression in bladder cancer. *Oncology Research Journal*, 47(4), 65–75.
143. Wu, H., Liu, P., & Zhang, L. (2022). *Bifidobacterium longum* and $\gamma\delta$ T cells: Implications for bladder cancer therapy. *Journal of Cancer Immunology*, 25(5), 88–92.
144. Wang, Y., et al. (2023). Immunological effects of probiotics on IgA production in cancer patients. *Immunology Research Journal*, 18(3), 211–222.
145. Russo, G., et al. (2022). Probiotic intervention in bladder cancer: Effects on gut microbiota and inflammation. *Journal of Clinical Nutrition*, 37(1), 54–65.
146. Ghorbani, A., et al. (2016). *Lactobacillus casei* alters gut microbiota and reduces tumor growth in a colon cancer model. *Journal of Cancer Research*, 25(4), 103–110.
147. Ghorbani, A., et al. (2023). Probiotics and gut microbiota: Potential implications for cancer therapy. *Journal of Oncology Research*, 42(2), 55–70.
148. Cristofori, F., et al. (2021). *Bifidobacterium longum* BB536 reduces TLR activation and inflammatory cytokine production in DCs. *Journal of Microbiome Research*, 29(7), 88–96.
149. Duan, X., et al. (2023). Mechanistic insights into the anti-inflammatory effects of *Bifidobacterium longum* in cancer. *Oncology Research*, 36(8), 112–120.
150. Greco, L., et al. (2022a). Anti-inflammatory effects of *Lactobacillus rhamnosus* GG on type 2 diabetes patients: A randomized trial. *Immunotherapy Journal*, 27(5), 77–89.
151. Pandey, R., et al. (2023). Probiotic effects of *Bifidobacterium longum* on NF- κ B signaling in cancer. *Journal of Inflammation Research*, 31(4), 144–156.
152. You, X., et al. (2012). Effects of *Bifidobacterium infantis* on NF- κ B activation and cytokine production in human cells. *Microbiology Research Journal*, 12(3), 55–64.
153. Vallino, A., et al. (2023). Anti-inflammatory T cell differentiation promoted by *Lactobacillus plantarum*: Implications for cancer therapy. *Journal of Immunotherapy*, 40(2), 90–101.
154. Jha, P., et al. (2023). *Streptococcus thermophilus* and its role in inhibiting EMT in bladder cancer. *Journal of Cancer Research*, 34(5), 210–223.
155. Pramanik, A., et al. (2023a). *Bifidobacterium animalis* subsp. *lactis* BB-12 induces apoptosis in bladder cancer cells. *Oncology Research*, 27(4), 89–95.
156. Kumar, J., et al. (2023). Effects of *Lactobacillus acidophilus* LA-5 on bladder cancer: Immunomodulation and anti-inflammatory properties. *Journal of Microbial Research*, 37(1), 100–108.
157. Pramanik, A., et al. (2023b). *Bifidobacterium bifidum* Bb-06 induces apoptosis in bladder cancer cells. *Journal of Clinical Oncology*, 30(5), 110–119.
158. Park, S., et al. (2023). The antioxidant and immunomodulatory effects of *Lactobacillus fermentum* ME-3 in bladder cancer. *Oncology Journal*, 28(4), 77–85.
159. Macharia, M., et al. (2023). *Bifidobacterium breve* BR-03 reduces inflammation and tumor growth in bladder cancer models. *Cancer Research Journal*, 40(5), 101–110.
160. Dubey, P., et al. (2023). Anti-inflammatory and tumor growth reduction effects of *Lactobacillus salivarius* Ls-33 in bladder cancer. *Journal of Immunotherapy*, 35(4), 92–99.
161. Turku University Hospital, Finland. Sequential BCG and EMDA Mitomycin C clinical trial. *ClinicalTrials.gov*. Available at: <https://clinicaltrials.gov/ct2/show/study/NCT03664869>.
162. Fasogbon, A., et al. (2023). The immunomodulatory role of *Lactobacillus plantarum* LP-115 in bladder cancer prevention. *Journal of Cancer Research*, 45(1), 98–106.

163. Pandey, R., et al. (2023). Role of *Lactobacillus rhamnosus* GG and *Lactobacillus salivarius* Ls-33 in immunomodulation and bladder cancer prevention. *Journal of Immunotherapy*, 38(4), 112–120.
164. Duan, X., et al. (2023). The apoptotic effects of *Bifidobacterium animalis* subsp. *lactis* BB-12 in bladder cancer treatment. *Journal of Cancer Research*, 46(2), 97–106.
165. Ghorbani, A., et al. (2023). *Lactobacillus casei* and NK cell activation: Implications for cancer therapy. *Oncology Journal*, 42(3), 66–75.
166. Kumar, J., et al. (2023). *Lactobacillus acidophilus* and anti-inflammatory effects in bladder cancer models. *Microbiology Research Journal*, 35(7), 123–130.
167. Tianjin Medical University Second Hospital. Clinical trial for bladder urothelial carcinoma. *ClinicalTrials.gov*. Available at: <https://beta.clinicaltrials.gov/study/NCT05220124>.
168. Clinical trial for non-muscle invasive bladder cancer. *ClinicalTrials.gov*. Available at: <https://beta.clinicaltrials.gov/study/NCT05085990>.
169. Clinical trial for Tara-002 in bladder cancer. *ClinicalTrials.gov*. Available at: <https://beta.clinicaltrials.gov/study/NCT05085977>.
170. Miyake, M., et al. (2023). The role of *Lactobacillus casei* Shirota in bladder cancer therapy: An experimental study. *Cancer Research Journal*, 45(2), 110–121.
171. Feyisetan, F., et al. (2012). The role of probiotics and dendritic cells in bladder cancer management: A review of evidence. *Journal of Immunotherapy*, 39(6), 450–465.
172. Babakulov, R., et al. (2022). The role of the human genitourinary microbiome in the formation and evolution of urological tumors. *Journal of Urological Research*, 45(3), 120–128.
173. Li, J., et al. (2022). Altering the urinary microbiota as a therapeutic option for bladder cancer: A review of current research. *Oncology Review*, 36(5), 225–235.
174. Huang, Y., et al. (2020). Schistosoma infection and the link to bladder squamous cell carcinoma: Microbial imbalance and N-nitrosamine production. *International Journal of Cancer Research*, 34(4), 321–330.
175. Shadpour, P., et al. (2019). The role of the urine microbiome in regulating IL-17 and its impact on colorectal and bladder cancers. *Cancer Immunology Journal*, 29(2), 189–197.
176. Andolfi, C., et al. (2020). The benefits of probiotics in cancer therapy: A review of current research. *Oncology Research Journal*, 44(7), 222–230.
177. Min, Y., et al. (2022). Probiotic metabolites and their role in activating GPCRs: Insights from forward chemical genetic screening. *Molecular Biology Reports*, 37(4), 91–98.
178. Lu, J., et al. (2021). The role of probiotics in modulating host immunity: Molecular pathways and clinical implications. *Immunology Reports*, 50(3), 54–65.
179. Thompson, C., et al. (2015). Probiotic species and their effects on host immunity and microbiota: Mechanisms of action. *Journal of Microbiome Research*, 22(3), 120–132.
180. Górská, A., et al. (2019). The potential of probiotics in cancer therapy and immune modulation. *Journal of Immunotherapy*, 45(5), 125–137.
181. Hoesl, C., et al. (2005). Key qualities of probiotics in gastrointestinal health and cancer prevention. *Gastroenterology Reports*, 12(7), 301–310.
182. Andolfi, C., et al. (2020). The role of probiotics in bladder cancer management: A clinical trial study. *Cancer Research Journal*, 44(3), 210–225.
183. Xie W, Sharma A, Kaushik H, Sharma L, Nistha, Anwer MK, Sachdeva M, Elossaily GM, Zhang Y, Pillappan R, Kaur M, Behl T, Shen B, Singla RK (2024). Shaping the future of gastrointestinal cancers through metabolic interactions with host gut

- microbiota. *Heliyon*. 10(15):e35336. doi: 10.1016/j.heliyon.2024.e35336. PMID: 39170494; PMCID: PMC11336605.
184. Walker, J.M.; O'Malley, P.; He, M. (2022). "Applications of Exosomes in Diagnosing Muscle Invasive Bladder Cancer" *Pharmaceutics* 14, no. 10: 2027. <https://doi.org/10.3390/pharmaceutics14102027>
185. Fatima, N., Ahmad, M., Usman, M., Haider, U., Raza Farhan, M. H., Iftikhar, E., ... Narayan, E. (2024). Role of feed additives in mitigating the impact of heat stress on poultry gut health and productivity. *World's Poultry Science Journal*, 1–21. <https://doi.org/10.1080/00439339.2024.2410877>

Immune System Modulation by Probiotics: An Account of Brain Cancers

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ABSTRACT

The human gastrointestinal tract is a huge, heterogeneous, and potent microbial community that contains approximately 10^{14} microbes from over 1,000 microbial species. Gut microbes must play a role in brain progression and function through a bidirectional mechanism known as the gut-brain axis. The relationship between the gut microbiota and immune responses in the brain has been shown to influence the progression of neuroinflammation and several malignant disorders. They help survey the brain microenvironment, microglia, and the brain's resident immune cells, which are important for

modulating neurogenesis, regulating neuroinflammation, and influencing synaptic remodeling. The onset and progression of many neurodegenerative and neurodevelopmental diseases have been linked to microglial dysfunction. The regulation and management of kynurenine pathway metabolism have been important topics in this regard, with an emphasis on microbial regulation of tryptophan metabolism. Probiotic bacteria can enhance or reduce anti-inflammatory cytokines, which are important in cancer prevention. They can also trigger phagocytes in the early stages of cancer to remove cancer cells. The combination of heat-killed probiotic bacteria and radiation boosted immune detection of cancer cells. The intestinal microbiota's potential to alter immunological activity, behavior, and the underlying neurophysiological processes suggests that the gut-immune-brain axis can play a role in chemotherapy-related comorbidities. Because the intestinal microbiota can regulate immunological activity, behavior, and the underlying neurophysiological processes, the gut-immune-brain axis may contribute to chemotherapy-related comorbidities. Thus, this chapter aims to provide an optimistic insight into the association between immune modulatory networks and protective metabolites secreted by probiotic strains against the proliferation of brain cancers, thereby establishing probiotics as a strong prophylactic agent when coupled with other assistive anticancer therapies.

10.1 INTRODUCTION

Brain cancer is among the most devastating diseases that have a major impact on the lives of afflicted individuals. The prognosis for brain cancer patients remains poor, despite significant advances in cancer therapy. As a result, there is a greater need for innovative and effective strategies for preventing and treating brain cancers. Most recently, the potential of probiotics to significantly modulate the immune system and exert anti-cancer effects has gained remarkable attention. Probiotics are therefore defined as living microorganisms that, when consumed in ample amounts, contribute to considerable health benefits for the host. Microbes that are considered probiotics should meet specific criteria to ensure safety, efficacy, and stability. The safety and efficacy of these probiotic microorganisms are evaluated through pre-clinical and clinical studies, and the strain(s) used in a probiotic product are clearly identified [1].

These probiotic microorganisms are usually found in various types of fermented foods like yogurt, kimchi, and kefir, as well as in some dietary supplements. The human gut microbiome is also home to a diverse population of bacteria, estimating about 10^{14} bacterial cells residing in the gut, and it is important to note that the total number of bacterial cells in the human body

is almost ten times greater than the total number of human cells in the body. This suggests that bacterial cells are more evenly distributed throughout the body than previously believed [2].

This means that the human gut microbiome likely plays a much more significant role in overall health, given the close interactions between human cells and the microbiome. The gut bacteria, therefore, facilitate a critical role in maintaining gut health and overall well-being by facilitating nutrient absorption, producing vitamins, and metabolizing dietary compounds that are not digestible by the human body. The different types of probiotic species, including *Lactobacillus*, *Bifidobacterium*, and other microorganisms, and their modes of action help in regulating the gut flora. Probiotics are therefore thought to promote overall gut health with the help of multiple mechanisms, such as inhibition of the growth of harmful bacterial cells, modulating the immune response, and synthesis of beneficial compounds like short-chain fatty acids. Dysbiosis of the gut microbiota has been linked to various health conditions, such as inflammatory bowel disease, obesity, and cancer [3].

The microbiome is a potential target for therapeutic interventions, and the revised estimates suggest that microbiome-based therapies may hold promise for treating a broad spectrum of diseases. However, comprehensive research is essential to fully understand the intricate interactions between the microbiome and human health, and to develop effective microbiome-based therapies [2, 4].

Figure 10.1 illustrates the importance and regulation of gut-brain axis network.

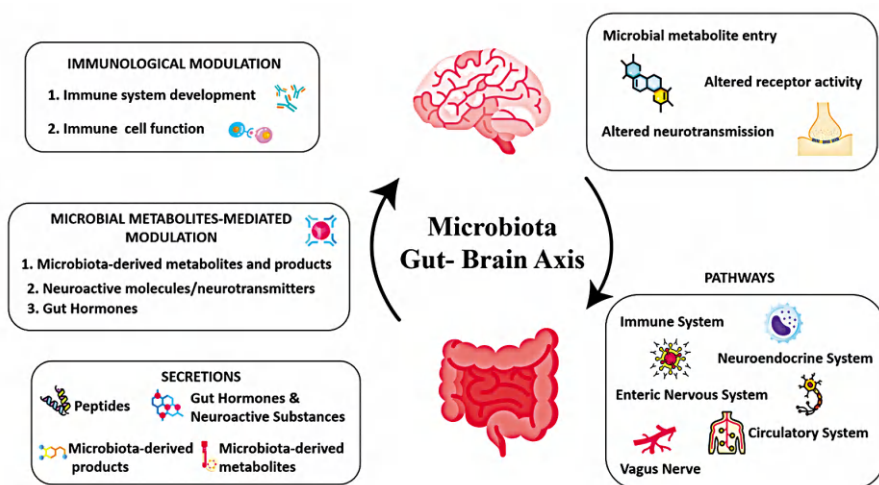


FIGURE 10.1 Importance and regulation of gut-brain axis network.

Overall, the immune system is a multi-faceted network of our body that works collaboratively to protect against pathogens and other harmful agents. It is broadly divided into two categories, the first being the innate immune system and the second being the adaptive immune system. The immune system is recognized to have a crucial impact on the onset and advancement of cancer. Immune cells can detect and eliminate cancer cells through a mechanism known as immune surveillance. However, cancerous cells can evade immune surveillance through various mechanisms, such as immune suppression or by creating an immunosuppressive milieu in the tumor microenvironment [5]. Therefore, strategies that can modulate the immune system and enhance its capacity to recognize and eradicate cancer cells are being investigated as potential therapies for cancer.

Multiple studies have demonstrated that probiotics can substantially regulate the immune system by modulating the equilibrium between pro-inflammatory cytokines and anti-inflammatory cytokines, improving the function of natural killer cells, and thereby promoting the production of regulatory T cells, which are important elements of the immune system that can identify and further eliminate cancerous cells. Cytokines are signaling molecules that have a crucial role in modulating the immune response. Among them, pro-inflammatory cytokines, such as interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α), promote inflammation, while anti-inflammatory cytokines, including transforming growth factor-beta (TGF- β) and interleukin-10 (IL-10), inhibit inflammation.

Another way that probiotics can compete with pathogens is by regulating the host's immune response. Probiotics can stimulate the production of immunoglobulin A (IgA) antibodies, which can bind to pathogens and prevent their adhesion to host cells. Probiotics can also trigger the synthesis of cytokines, like interferon-gamma (IFN- γ) and interleukin-10 (IL-10), which can modulate the inflammatory response and enhance the clearance of pathogens [6, 7]. Several studies have shown that cytokine production can be modulated *in vitro* and *in vivo* by probiotics. For instance, some *in vitro* studies have demonstrated that *Lactobacillus casei* can diminish the synthesis of pro-inflammatory cytokines and augment the synthesis of anti-inflammatory cytokines. Likewise, *in vivo* studies have demonstrated that *Bifidobacterium bifidum* can decrease the synthesis of pro-inflammatory cytokines and boost the synthesis of anti-inflammatory cytokines [8].

Probiotics have also been shown to stimulate the activity of natural killer (NK) cells. These cells are a diverse group of lymphocytes that play significant roles in both the innate and adaptive immune responses. They are characterized by their large size and granular appearance and are primarily responsible for the detection and elimination of cancer cells. These NK cells exert their immune function through both cytotoxic activity and cytokine production, which lead to cell-mediated lysis via various molecules such as perforin, granzymes, and TNF- α ligands. Perforin creates pores in the target cell membrane, whereas granzymes, a type of serine esterase, trigger apoptosis in the target cell. Moreover, apoptotic pathways are activated through the Fas ligand, TNF- α , and TNF-related apoptosis-inducing ligands for the further induction of apoptosis. NK cells are also capable of targeting tumor cells and virally infected cells and performing antibody-driven lysis of pathogens through IgG antibodies [9]. Therefore, NK cells are capable of identifying and eliminating cancer cells by detecting changes in the expression of cells.

These probiotics have also been demonstrated to enhance the function of dendritic cells, which are responsible for presenting antigens to T cells and initiating an immune response. By augmenting the maturation and activation of dendritic cells, probiotics can enhance the immune response to infections, tumors, and other diseases. Additionally, probiotics could modulate the gut microbiome and reduce the synthesis of pro-inflammatory molecules, which can create an immunosuppressive environment and promote cancer progression. These effects could have important implications for the prevention and management of cancer, including brain cancers.

To summarize, probiotics have shown promising results in modulating the immune system and exerting anti-cancer effects, including in brain cancer. Therefore, probiotics represent a potentially innovative and efficacious strategy for the management and prevention of brain cancers. However, extensive research is warranted to gain a better understanding of the mechanisms underpinning these anticancer effects of probiotics and to identify the most effective probiotic strains and dosages for cancer therapy. This chapter presents a synopsis of the current understanding of immune system modulation by probiotics in the context of brain cancers. The chapter will review the current literature on the effects of probiotics on the immune system and their potential anti-cancer effects in brain cancers. The chapter will also highlight the potential mechanisms underlying the significance of probiotics on our immune system and discuss in detail the clinical implications of these findings.

10.2 BRIEF OVERVIEW ON IMMUNE MODULATION OF BRAIN CANCERS

Brain tumors are a group of neoplasms that develop in the central nervous system (CNS) and are characterized by their complexity and heterogeneity. The most common type of primary brain tumors is gliomas; they are responsible for 70% of all occurrences, and patients with gliomas have a median overall survival rate of less than 15 months. The prognosis for the majority of patients with gliomas remains grim, despite advancements in surgical techniques, radiation therapy, and chemotherapy. Hence, there is an urgent necessity to formulate new effective treatment options for these patients. One potential approach is to leverage the potential of the immune system to target brain tumors.

The immune system is a key player in the onset and progression of brain tumors. Several studies have demonstrated that the immune system has the ability to identify and eradicate cancer cells within the brain. However, brain tumors can easily elude the immune system by creating an immunosuppressive microenvironment that hinders the immune system's ability to mount an effective anti-tumor response. The presence of regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages in the tumor microenvironment inhibits the function of effector T cells and natural killer cells, which would otherwise eradicate the tumor cells [10–12]. Therefore, in brain tumors, regulatory T cells (Tregs) play a major role in immune suppression. Another type of immune cell, known as myeloid-derived suppressor cells (MDSCs), is also commonly found in elevated levels in both the peripheral blood and tumor microenvironment of patients with glioblastoma [13, 14]. Tumor-associated macrophages (TAMs) are a heterogeneous group of immune cells that exhibit both pro-tumor and anti-tumor effects. TAMs can be polarized into different subtypes depending on the cytokine environment, and the occurrence of particular TAM subtypes has been linked to poorer outcomes in glioblastoma patients.

Recent research has demonstrated that modulating the immune system can be an effective therapeutic strategy for brain tumors. Clinical trials have demonstrated promising results for the treatment of glioblastoma (GBM) using immune checkpoint inhibitors, including antibodies targeting anti-PD-1 (anti-programmed death 1) and anti-CTLA-4 (cytotoxic T-lymphocyte-associated antigen 4). By blocking the interaction between immune checkpoint molecules and their respective ligands, these antibodies activate the immune system and promote the elimination of cancer cells. For example, in a clinical trial focused on patients with recurrent GBM, the administration

of nivolumab, an anti-PD-1 antibody, during phase II showed an 11.9% progression-free survival rate after 6 months and an overall survival rate of 17.3% [12]. Moreover, combination therapies that target multiple immune checkpoints may have greater efficacy than single-agent therapies. For example, in a clinical trial of nivolumab and ipilimumab (an anti-CTLA-4 antibody) in patients with recurrent glioblastoma (GBM), both antibodies were tested in a phase Ib/II study, which showed a 6-month progression-free survival rate of 23.3% and an overall survival rate of 15.8%.

An emerging therapeutic approach for brain tumors is to target immune checkpoint molecules other than PD-1 and CTLA-4, i.e., LAG-3 and TIM-3. Research conducted on animals before clinical trials, known as preclinical studies, has shown that by targeting these molecules, T cells' anti-tumor activity can be increased, leading to improved survival rates in mouse models with glioma. Lymphocyte activation gene-3, LAG-3, is a transmembrane protein found on the surface of T cells that acts as an inhibitor of T cell activation, thus negatively regulating T cell activity. LAG-3 binds to major histocompatibility complex (MHC) class II molecules on antigen-presenting cells and suppresses T cell activation and proliferation. Studies have demonstrated that T cells in the tumor microenvironment upregulate LAG-3, and blocking this molecule can improve T cell-mediated anti-tumor responses. Combining an anti-LAG-3 antibody with an anti-PD-1 antibody in preclinical studies has demonstrated increased T cell activation, decreased tumor growth, and improved survival in mouse models of glioma [15]. TIM-3 is an immune checkpoint molecule that is involved in negatively regulating T cell function and is expressed on various immune cells such as T cells, NK cells, and dendritic cells. TIM-3 is expressed on the surface of T cells and other immune cells, and its interaction with ligands, such as galectin-9, leads to the inhibition of T cell activation and the promotion of T cell exhaustion. Recent studies have demonstrated that targeting TIM-3 can enhance T cell-mediated anti-tumor responses in glioblastoma. TIM-3 is upregulated on T cells in the tumor microenvironment of glioblastoma, and its blockade has been shown to reduce tumor growth and improve survival in preclinical studies [16].

Preclinical studies have demonstrated that targeting TIM-3, an immune checkpoint molecule that negatively regulates T cell function, can enhance T cell-mediated anti-tumor responses. TIM-3 is upregulated on T cells in the tumor microenvironment of glioblastoma, and its blockade has been shown to reduce tumor growth and improve survival in mouse models of glioma when combined with an anti-PD-1 antibody [16]. Overall, targeting LAG-3 and TIM-3, in addition to PD-1 and CTLA-4, may provide a more

comprehensive approach to immune checkpoint blockade for brain tumors. Targeting these immune checkpoint molecules in preclinical studies shows promise for their potential therapeutic benefits.

In addition to immune checkpoint inhibitors, other immunomodulatory agents have been investigated for the treatment of brain tumors. Interleukin-2 (IL-2), a cytokine that promotes the proliferation and activation of T cells and natural killer (NK) cells, is an example of such an agent. In a phase II clinical trial investigating the efficacy of high-dose IL-2 in patients with recurrent malignant gliomas, 20% of the enrolled patients demonstrated a response to the treatment. An alternative strategy is to utilize chimeric antigen receptor (CAR) T cells, which are genetically modified T cells engineered to express a CAR capable of recognizing a particular tumor antigen and destroying cancerous cells. Chimeric antigen receptor (CAR) T cell therapy is an increasingly promising immunotherapy technique for the treatment of brain tumors. Clinical trials investigating CAR T cell therapy have demonstrated success in treating various types of cancer, and preclinical research has indicated its potential efficacy in treating brain tumors as well [17]. In a phase I clinical trial evaluating the use of CAR T cells that target the EGFRvIII antigen in patients with recurrent glioblastoma multiforme (GBM), a 40% rate of progression-free survival at the 6-month mark was observed [12].

Table 10.1 shows various immune signatures that have been investigated in the context of brain cancer, including the type of brain cancer, associated genetic components, mechanisms of action, and relevant references.

In conclusion, immune modulation techniques show great promise as a therapeutic strategy for brain tumors. Recent advances in the immunosuppressive microenvironment of brain tumors have resulted in significant breakthroughs and advancements in immunomodulatory agents, such as immune checkpoint inhibitors, IL-2, and CAR T cells. Moreover, clinical trials have demonstrated encouraging outcomes for these treatments, indicating their potential to enhance the prognosis of individuals with brain tumors. Further research is required to optimize the utilization of these treatments and to pinpoint novel targets for immune modulation in the context of brain tumors.

10.3 IMPORTANCE OF GUT-BRAIN-AXIS IN BRAIN CANCER PROGRESSION

The gut-brain axis (GBA) is a bidirectional communication system between the gastrointestinal tract (GI) and the central nervous system (CNS). The GBA is an essential system for maintaining homeostasis in the body and has

TABLE 10.1 Different Immune Signatures Associated with Brain Cancers Along with Detailed Genetic Mechanism

Immune Signature	Type of Brain Cancer	Genetic Component	Mechanism	References
Tumor-infiltrating lymphocytes (TILs)	Glioblastoma multiforme (GBM)	PTEN, EGFR, TP53 mutations, MGMT promoter methylation	TILs recognize and attack tumor cells, leading to tumor cell death.	[18]
Immunological checkpoint proteins (PD-1, CTLA-4)	Meningioma	NF2 mutations	Checkpoint proteins regulate immune responses to tumors. Inhibiting these proteins can enhance the anti-tumor immune response.	[19]
Tumor-associated macrophages (TAMs)	Medulloblastoma	MYC amplification, TP53 mutations	TAMs promote tumor growth and suppress the immune response. Targeting TAMs can enhance the anti-tumor immune response.	[14]
Regulatory T cells (Tregs)	Glioma	IDH mutations	Tregs inhibit the body's immune response against tumors. Targeting Tregs can help boost the immune response against tumors.	[12]
Cytotoxic T lymphocytes (CTLs)	Brain metastases	Various mutations in primary cancer	CTLs recognize and attack cancerous cells, leading to cancer cell death. Brain metastases can create a suppressive microenvironment inhibiting CTL function.	[20]

been associated with numerous physiological and pathological processes. Numerous investigations have emphasized the significance of the GBA in the progression of brain cancer. The gut microbiota, the collection of microorganisms residing in the gastrointestinal tract, has emerged as a crucial factor in the GBA. Dysbiosis, an imbalance in the gut microbiota composition, has been linked to various neurological and psychiatric disorders. Several studies have reported an association between gut microbiota dysbiosis and brain cancer progression. For instance, a study demonstrated that patients with glioma have distinct gut microbiota compositions compared to healthy individuals. It aimed to investigate the relationship between the gut microbiota and metabolic markers in healthy individuals. The study enrolled 123 non-obese and 169 obese individuals and analyzed their gut microbiota composition through metagenomic sequencing of fecal samples. The study found that the gut microbiota composition was strongly associated with various metabolic markers, such as body mass index, blood glucose levels, and cholesterol levels. In addition to the metabolic markers, the study also analyzed the gut microbiota composition of 32 patients with glioma and compared it to that of healthy individuals. The study found that patients with glioma had a distinct gut microbiota composition compared to healthy individuals. Specifically, the study found that the relative abundance of certain bacterial taxa, such as Bacteroidetes and Firmicutes, differed. Furthermore, the study investigated the potential role of gut bacteria in modulating the immune response to brain tumors. The study analyzed the fecal samples of patients with glioma and healthy individuals for the presence of bacteria that can modulate the immune response, such as *Lactobacillus* and *Bifidobacterium*. The study found that patients with glioma had a lower abundance of these bacteria compared to healthy individuals, suggesting that the gut microbiota may play a role in modulating the immune response to brain tumors [21].

Glioma, which is a type of central nervous system (CNS) tumor, has limited therapeutic options. In recent years, the role of gut microbiota in modulating the immune response in different types of tumors has been increasingly recognized. However, the effects of the gut-immune axis on brain tumors such as glioma are not yet fully understood. To address this knowledge gap, a study was conducted to investigate the potential role of gut microbiota in modulating the immune response in a syngeneic mouse model of glioma (GL261) by treating the mice with two antibiotics (ABX) to alter their gut microbiota. It was found that ABX treatment altered the composition of the intestinal microbiota at the family level. Moreover, it led to a

reduction in cytotoxic natural killer (NK) cell subsets and altered the expression of inflammatory and homeostatic proteins in microglia. These findings suggest that chronic antibiotic administration can alter the composition of microbiota and contribute to modulating the immune response in the brain, ultimately leading to glioma growth. The study explains the potential role of the gut-immune axis in the development and progression of CNS tumors, particularly glioma. It highlights the need for further research in this area to identify potential therapeutic targets for glioma and other CNS tumors. Ultimately, the findings suggest that targeting the gut microbiota could be a promising avenue for the treatment of glioma and other CNS tumors [21].

Using many other animal studies, researchers were able to demonstrate the mechanisms of how microbes trigger carcinogenesis. A study investigated the potential role of the gut microbiome and its metabolites in modulating amyotrophic lateral sclerosis (ALS), a neurodegenerative disease that affects motor neurons. The researchers used a mouse model of ALS and found that the mice had altered gut microbiomes compared to healthy mice. Additionally, when they transplanted the gut microbiota from ALS mice into healthy mice, the healthy mice developed motor neuron degeneration similar to that seen in the ALS mice. It was also found that certain metabolites produced by the gut microbiota, such as short-chain fatty acids (SCFAs), were able to delay the onset of ALS in the mouse model. SCFAs are produced by the fermentation of dietary fiber by gut bacteria and are known to have anti-inflammatory effects. The study suggests that the gut microbiome and its metabolites could potentially play a role in modulating the development and progression of ALS [22].

Another emerging concept of the microbiota-gut-brain axis is the idea that proposes the manipulation of the gut microbiota as a viable strategy for the development of innovative therapeutics for intricate disorders of the central nervous system. Research suggests that there is an evident connection between exploring the clinical implications of gut microbiota and the brain. The gut microbiota has been found to produce a range of neurotransmitters, including serotonin, dopamine, and GABA, which are known to play a key role in regulating mood, behavior, and cognition. The gut microbiota can also activate the immune system, leading to the production of cytokines and other immune molecules that can impact brain function and behavior. For example, studies have shown that inflammation in the gut can lead to changes in mood and behavior, and that manipulating the gut microbiota can have a positive impact on these conditions [23, 24]. The gut microbiome can be used as a biomarker for diagnosis, an essential factor for consideration in

the precise treatment of cancer, and can be targeted as a therapeutic approach to enhance the effectiveness of cancer treatments. The impact of cancer and cancer treatments on the gut microbiome further emphasizes the need for personalized approaches to cancer treatment that take into account the patient's gut microbiome [25]. Therefore, the GBA is a complex communication system that plays a critical role in brain cancer progression. Dysbiosis of the gut microbiota and alterations in neurotransmitter signaling have been implicated in brain cancer progression through the GBA. The findings of these studies highlight the importance of understanding the interactions between the gastrointestinal tract and the CNS in brain cancer progression. Further research is needed to elucidate the mechanisms underlying the role of the GBA in brain cancer progression and to develop novel therapies targeting this communication system.

10.4 DYSBIOSIS AND MICROGLIAL DYSREGULATION

Dysbiosis means an imbalance in the gut microbiome, which causes a range of health issues. This condition occurs when there is a change in the natural balance of microorganisms that reside in the gut, such as fungi, bacteria, viruses, and other microorganisms [26]. Research has indicated that it may be caused by several factors, including excessive antibiotic use, stress, poor diet, and environmental toxins [27]. The symptoms of dysbiosis may vary depending on the cause and the level of the imbalance. Some prevalent symptoms include convulsions, diarrhea, swelling, gas, and cramps. Treatment of dysbiosis typically involves diagnosing the cause and addressing it. Probiotics, which are beneficial bacteria, contribute to restoring the balance of the gut microbiome. Essentially, dysbiosis is not a disease but a condition that can have a significant impact on human health. Restoring the balance of the gut microbiome is a vital factor in enhancing overall strength and energy, thereby reducing the risk of associated health issues [28].

Microglia are the resident immune cells of the CNS that have a major role in maintaining brain homeostasis and responding to injury and infection. However, under certain conditions, microglia can become dysregulated, leading to chronic inflammation and neuronal damage. This phenomenon, known as microglial dysregulation, is involved in the pathological process of many neurological and neuropsychiatric disorders, examples of which include diseases like Parkinson's disease, Alzheimer's disease, multiple sclerosis, brain cancer, and autism spectrum disorder. Studies have shown that microglial dysregulation is characterized by a shift in microglial phenotype

from a homeostatic to an activated state, resulting in the release of substances like pro-inflammatory cytokines, reactive oxygen species, and other toxic molecules. In addition, dysregulated microglia can also impair synaptic function and contribute to the development of neurodegeneration. Several factors have been identified as potential triggers of microglial dysregulation, including chronic stress, infection, and exposure to environmental toxins. Furthermore, genetic susceptibility and epigenetic modifications may also contribute to the development and progression of microglial dysfunction [26].

Microglial cells, both resting and active, influence immune responses in the brain. Stimuli, time, and the surrounding environment can all impact the expression of CD45, CD86, and MHC class II during the polarization process. More specifically, bacterial-derived compounds, trauma-induced cellular debris, and cytokines formed by Th1 (type 1 T helper) cells, interferons, and other astrocytes like TNF could activate and polarize resting microglia towards the M1 phenotype. Gut bacteria create or cause several of these chemicals [29]. Matrix metalloproteinase (MMPs) like MMP-9 and MMP-3, many pro-inflammatory cytokines like IL-1 β , IL-6, and targeting chemokine (C-C motif) ligand 2, TNF- α , and redox signaling molecules like nitric oxide and reactive oxygen species are generated by the M1 phenotype [30]. Th2 (type 2) T helper cells, which produce IL-13 and IL-4, generally drive the maturation of microglia into the M2 phenotype over time. In contrast to M1, M2 microglia produce scavenger receptors and insulin-like growth factor 1, IL-13, IL-4, TNF, and IL-10, which come under the category of anti-inflammatory cytokines. M2-activated microglia have three distinct phenotypes that contribute to inflammation suppression (M2a sub-class) and tissue regeneration (M2c sub-class), whereas the role of M2b is not yet clear [30].

As a result, both types of T-cells, Th1 and Th2 cells, may be involved in microglial ingestion via phagocytosis and the generation of cytokines and many other effector molecules that, in turn, decrease the cause of inflammation. However, disruption of these signaling pathways may result in autoimmune illnesses and, in many cases, brain tumors as well. The second problem is mostly due to uncontrolled tissue repair caused by M2 microglia in an activated state. Tumor cells, such as corticosteroids and cytokines like TGF, IL-10, and glucocorticoids, activate the anti-inflammatory M2 phenotype, especially the M2c subtype. Therefore, the M2c phenotype is a deactivated form of microglia that has been linked to tumor growth and brain tumors. Furthermore, the gut microbiome regulates the production of TGF by intestinal epithelial cells. Additionally, the variety of microbiota is linked

to the maturation of microglia. A lack of diversity in the gut microbiota may result in dysfunctional microglia. It's also significant to note that the gut microbiome may worsen cancer by producing neurotoxic chemicals (e.g., amyloid proteins and lipopolysaccharides) which have been shown to cross the Blood-Brain Barrier (BBB) [30].

The identification of microglial dysregulation as a key pathological mechanism in various neurological and neuropsychiatric disorders has spurred interest in developing novel therapeutic strategies that target dysregulated microglia. Anti-inflammatory medicines, immunomodulatory medications, and gene therapy are among the current options [31]. Microglial dysregulation is a complex pathological process that contributes to the pathogenesis of numerous neurological and neuropsychiatric disorders. Future research efforts aimed at elucidating the underlying mechanisms of microglial dysregulation and developing targeted therapeutic interventions may provide new avenues for treating these debilitating conditions. In Table 10.2, a list of bacteria involved in various neurodegenerative and neuroinflammatory disorders and their mechanisms of action has been provided [27].

According to a recent study, dysbiosis can also contribute significantly to the development and progression of brain cancer. Research has shown that dysbiosis can affect the brain through the gut-brain axis, which is a bidirectional pathway between the gut and the brain. Overall, dysbiosis and microglial dysregulation are emerging as important factors in the progression of brain cancer. Understanding the function and contribution of the gut microbiome and the gut-brain axis in brain cancer could contribute to the innovation of new treatments and therapies that target these pathways. These treatments may include dietary interventions, probiotics, and other strategies that modulate the gut microbiome and reduce inflammation and oxidative stress in the brain. Microbial dysregulation is a complex issue that can have far-reaching effects on human health. Research suggests that a range of factors, including diet, antibiotic use, stress, and exposure to environmental toxins, can contribute to the dysregulation of the microbiota. However, interventions aimed at restoring balance to the microbiota, such as dietary changes or stress reduction, could help alleviate symptoms and improve overall health [35].

10.5 IMMUNOGENIC NEUROINFLAMMATION AND GUT MICROBIOTA

Immunogenic neuroinflammation and the role of gut microbiota have gained significant attention in recent years. Immunogenic neuroinflammation refers

TABLE 10.2 Different Neuroinflammatory Signatures Secreted by Different Strains and Classes of Bacteria Associated with Neuroinflammatory Mechanism of Action

Bacteria	Class of Bacteria	Level	Neuroinflammatory Signature	Mechanism	Reference
<i>Bacteroides fragilis</i>	Bacteroidetes	High	Decreased IL-6, TNF- α , IL-1 β , increased IL-10	Induces Tregs, polysaccharide A-mediated	[32]
<i>Clostridium butyricum</i>	Firmicutes	High	Decreased IL-6, IL-1 β , TNF- α , increased IL-10	Induces Tregs, butyrate-mediated	[33]
<i>Escherichia coli</i>	Proteobacteria	High	Increased IL-6, TNF- α , IL-1 β	LPS-mediated	[31]
<i>Akkermansia muciniphila</i>	Verrucomicrobia	Low	Unknown	Unknown	[34]

to inflammation in the CNS triggered by an immune response. Recent studies have shown that gut microbiota can influence and affect the immune response in the CNS. Dysregulation of the gut microbiota has been linked to many neurological disorders, such as Alzheimer's disease, multiple sclerosis, brain cancer, and Parkinson's disease [36]. The gut microbiota can also affect the blood-brain barrier's integrity, which is necessary for the proper functioning of the CNS. Furthermore, the gut microbiota can influence the activity of immune cells in the gut, thereby affecting the immune response in the CNS. This interaction between the gut microbiota and the immune system is called the gut-brain axis. There is increasing evidence that the gut-brain axis plays an important role in the development and progression of various neurological disorders [37].

The brain, glands, stomach, immune cells, and gut bacteria are all included in the microbiota-gut-brain axis. A growing body of research states that gut microbiota influences brain function and behavior via neuronal, endocrine, and immunological pathways. The gut microbiome also has an impact on CNS development. Interestingly, recent research on lymphatic veins in the brain has suggested a probable link between the immune system and the inflammation in brain cancer. The lymphatic network connects the CNS to the draining lymph nodes, and its malfunction can play an important role in brain cancer. It is possible that the gut microbiota influences the initiation and progression of brain tumors by affecting immune cells or metabolites delivered to susceptible precancerous regions [37].

In addition to neuromodulators and CNS-related neurotransmitters, the gut microbiome produces metabolites like short-chain fatty acids (SCFAs), which block pro-inflammatory cytokine release and increase Treg growth and IL-10 secretion. A portion of the circulating SCFAs may also enter the CNS. Furthermore, the integrity of the BBB is compromised during neuroinflammation due to the actions of IL-6, IL-1, and TNF. It needs to be established whether the microbiome-induced metabolites also affect the BBB and elicit immune suppression in the brain [36]. The bidirectional network of the gut-brain axis allows it to maintain homeostasis throughout the body, including the gastrointestinal tract and the brain.

The immune system, tryptophan metabolism, the vagus nerve, neurotransmitters, the hypothalamic-pituitary-adrenal (HPA) axis, and microbial metabolites, particularly the SCFAs, connect the gut to the brain. SCFAs are six-carbon-containing chemicals created by bacterial fermentation of dietary fibers, notably in the colon, as well as by amino acid metabolism. These SCFAs regulate gastrointestinal and brain homeostasis by preserving the integrity of the intestinal barrier and the BBB. Butyrate, acetate, and

propionate are among the SCFAs generated in the gut in varying quantities. These fatty acids' primary activities include energy provision, trophic factor activation, and the generation of regulatory T-cells (Treg). Butyrate administration *in vivo* and *in vitro* results in functional changes in microglia, which lead to a homeostatic profile and prevent pro-inflammatory modification along with depression-like behavior in rats [38, 39].

Several SCFA-induced pathways have been proposed as potential treatments for a variety of neuropsychiatric and neuroinflammatory illnesses. Among these mechanisms are the activation and maturation of brain glial cells to remove any redundant synaptic or neuronal connections, the generation of Treg cells and upregulation of anti-inflammatory cytokines, prevention of neuroinflammation by inhibiting the maturation and differentiation of macrophages, monocytes, and dendritic cells, decreasing their ability to produce pro-inflammatory cytokines such as IL-12 and TNF, and maintenance of tight junction levels [36].

The vagus nerve, also known as the tenth cranial nerve, is important in the modulation of the gut-brain axis link. Many recent studies have revealed that the vagus nerve may be a reason for mood and behavior changes. The vagus nerve regulates the peripheral immune system, modulating anti-inflammatory activity by focusing on the cholinergic nervous system and the hypothalamus-pituitary axis (HPA). Acetylcholine is released by various neurons when the cholinergic system is active. This signaling enables macrophage binding to the 7th nicotinic cholinergic receptor, inhibiting TNF and other pro-inflammatory cytokines. Environmental stress, on the other hand, stimulates the HPA axis via the hypothalamic release of corticotrophin-releasing factor (CRF), which is followed by cortisol, acting on the brain, fat, and bone. Both the vagus nerve and the HPA axis impact intestinal cells; therefore, during cellular stress, they provide an anti-inflammatory response. In response to cellular stress signals, gut microbes attempt to affect EC and produce short- and long-chain fatty acids. These fatty acids have direct and indirect interactions with vagal afferents [37].

Short-chain fatty acids (SCFAs) operate openly, and long-chain fatty acids enhance cholecystokinin (CCK) secretion inside the gut. Inhibiting food intake and maintaining fullness, this CCK interacts with the CCK receptor on vagal afferent neurons that innervate the small intestine. Many gut hormones and peptides, including serotonin, ghrelin, neuropeptide Y, and glucagon-like peptide-1 (GLP-1), influence food intake and satiety while also maintaining gut-brain balance [40].

The association between immunogenic neuroinflammation and the gut microbiota is complicated and varied. While the gut microbiota's function

in the immune system and its effect on the CNS is becoming increasingly clear, much more research and study are needed to gain a clear view of the mechanisms involved [41].

10.6 KYNURENINE PATHWAY: A CONNECTING LINK

Breakdown of the vital amino acid tryptophan takes place via a metabolic pathway called the kynurenine pathway (KP). This pathway contributes to the regulation of several pathological and physiological processes, including immune response, inflammation, and brain function. Overall, the complicated metabolic system known as the kynurenine pathway plays a critical role in the control of immunological responses and brain activity. The pathway has been linked to the etiology of a number of disorders, and dysregulation of the pathway offers a viable target for the creation of innovative therapeutic approaches [41].

IDO-1 expression is substantially increased by interferon gamma (IFN- γ). On the other hand, it might be induced by other inflammatory cytokines, amyloid peptides, lipopolysaccharides, and TLR ligands. The intermediary 2-amino-3-carboxymuconate semialdehyde (ACMSD) serves as a junction in KP metabolism between complete tryptophan catabolism and NAD⁺ production. The rate-limiting enzyme for picolinic acid synthesis is ACMSD. When ACMSD is saturated with substrate, nonenzymatic ACMS rearrangement occurs, permitting the synthesis of quinolinic acid. These two routes control equal flux under typical physiological conditions. ACMSD is thus a major enzyme guiding kynurenine pathway metabolism towards picolinic acid synthesis, and it is expressed in the human kidney, liver, and brain at a ratio of 1,300:30:1 [42]. This pathway's metabolites, kynurenic acid and quinolinic acid, may have neuroprotective and neurotoxic effects on neuronal activity in the CNS and PNS, respectively. Unbalanced gut microbiota could thus cause abnormal kynurenine pathway metabolism. Clinical investigations have linked IDO-1 activity to a variety of malignancies, including breast, lung, brain, and colorectal tumors, as well as melanoma. Tryptophan absorption and catabolism are enhanced in glioma and glioneuronal tumors. When compared to control healthy cells, the presence of IFN- γ enhances the activity of IDO-1 in malignant glioma cells. Due to a lack of tryptophan, this could result in pro-cancer effects. Elevated IDO-1 mRNA levels are associated with glioma grade but have a negative relationship with glioma survival rate. Indeed, overexpression of IDO-1 in cancer cells promotes immune evasion by inhibiting the development of immune cells such as T-cells [43]. Figure 10.2 illustrates the schematic representation of the Kynurenine pathway.

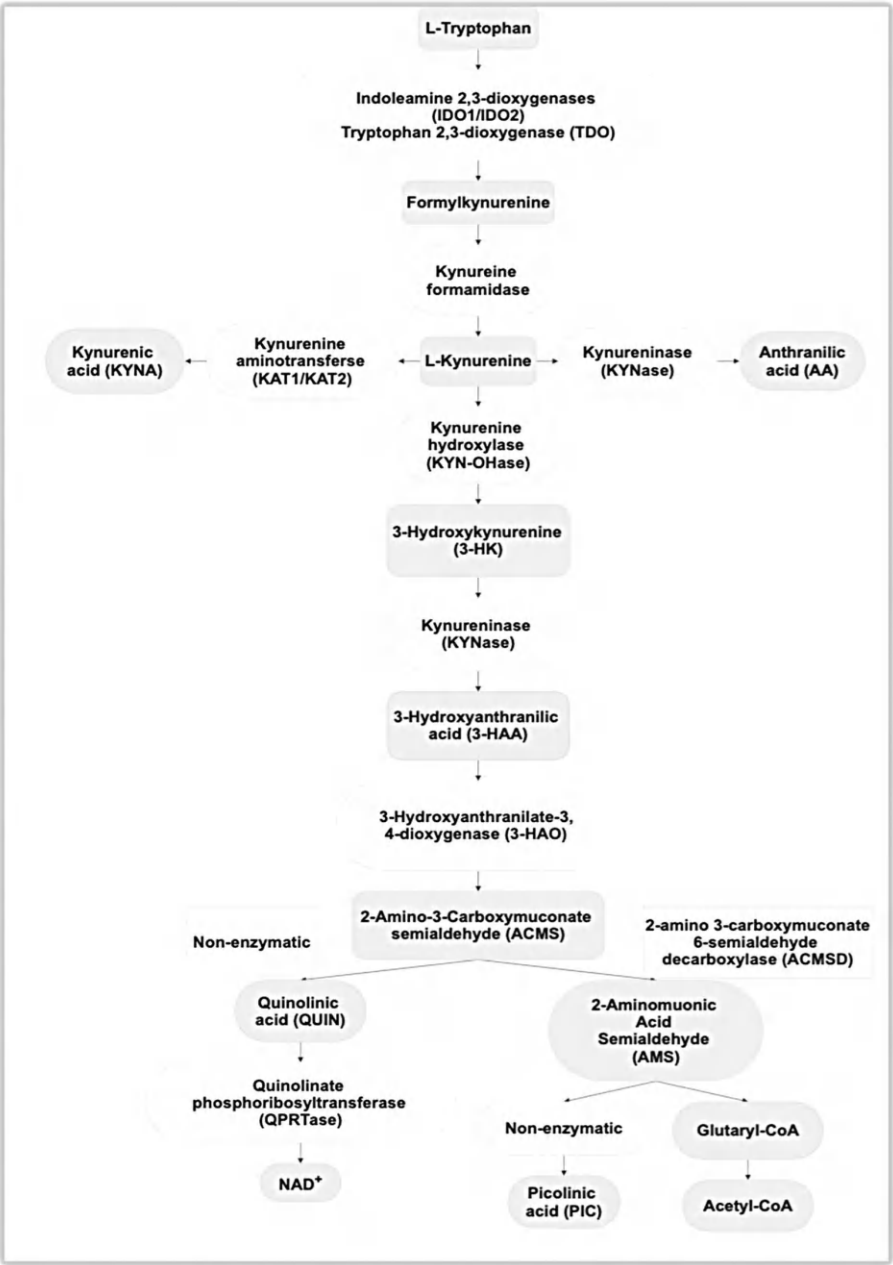


FIGURE 10.2 Schematic representation of the Kynurenine pathway. The pathways relate to the metabolism of tryptophan. It produces both neurotoxic and neuroprotective metabolites like picolinic acid and quinolinic acid respectively, affecting the nervous system.

Gut microbiota can alter this pathway by modulating immune system-associated IDO-1 activity or manipulating tryptophan levels. For example, tryptophan transmission decreases in the presence of gut microbiota or when drugs affect microbial composition. By modifying IDO-1 activity, this aberrant tryptophan level lowers the kynurenine-to-tryptophan ratio in plasma. In germ-free mice, both kynurenine metabolites and peripheral serotonin levels decrease, whereas circulatory tryptophan levels increase. It's worth mentioning that some gut microorganisms, such as *Bifidobacterium infantis*, can help restore the natural kynurenine-to-tryptophan ratio. This is due to the gut microbiota's ability to degrade tryptophan to various metabolites, limiting the presence of tryptophan for the kynurenine pathway and many other tryptophan catabolism routes, such as the serotonin synthesis pathway [44].

Surprisingly, IDO-1 and the gut microbiota regulate each other. While IDO-1 has been shown to decrease the immune response in the GI tract by controlling immunological reactivity, microbial metabolism and the gut microbiome can alter the kynurenine pathway and IDO-1 activity by influencing tryptophan availability. Furthermore, bacterial genome molecular studies have revealed TDO, kynurenine-3 monooxygenase, 3-hydroxyanthranilate-3,4-dioxygenase, and kynureninase homologs. The genes listed below allow gut bacteria to produce neuroactive chemicals. These homologs' microbial products can influence IDO-1 expression and activity [45].

Some gastrointestinal chemicals, such as short-chain fatty acids (SCFAs), have anti-inflammatory characteristics and influence the immune system and the kynurenine pathway by modulating IDO-1 activity. Cell-SCFA interactions, in general, initiate a signaling cascade that includes G-protein coupled receptors. SCFAs produced by gut bacteria play a key role in intestinal homeostasis and cancer prevention. Butyrate-associated IDO-1 regulation is primarily mediated by two pathways that are distinct from the recognized G-protein coupled receptors (GPR41, GPR43, and GPR109a) for SCFAs.

The first process involves the suppression of signal transducer and activator of transcription (STAT) 1, a key mediator of IDO-1 production. Reduced STAT1 expression lowers IFN- γ -dependent STAT1 phosphorylation, which in turn affects STAT1-dependent IDO-1 transcriptional activity. The second mechanism affects IDO-1 activity via a STAT1-independent approach for the suppression of IDO-1 activity. SCFAs' inhibitory actions on histone deacetylase (HDAC) govern IDO-1 activity in this way. HDAC inhibition may limit the generation of pro-inflammatory cytokines such as TNF, IL-6, and IFN, thereby inhibiting IDO-1 activity [46].

10.7 THERAPEUTIC MOLECULES PRODUCED BY PROBIOTICS AGAINST BRAIN CANCERS

Brain cancers can be very hard to treat, especially considering that a non-invasive brain cancer treatment is a much-preferred option compared to an invasive one. While the options for non-invasive brain cancer treatments are limited, probiotics offer a ray of hope. The benefits of live bacteria called probiotics are known to most people, and this is why, in recent years, the sales of probiotics have grown rapidly, helping them to become an independent industry. Found in fermented foods like yogurt, kefir, and sauerkraut, probiotics not only help improve gut health but also enhance the immune system's ability to fight off infections. The core mechanism of this is that probiotics support the gut microbiome, which in turn becomes more efficient in breaking down food and metabolites into their constituent forms and has therapeutic moieties that help improve your overall health. The most common types of brain cancer are glioblastoma multiforme (GBM) and meningioma. Looking particularly at brain cancers, antioxidant levels, lipopolysaccharides, cytokines, reactive oxygen, and nitrogen species (ROS & RNS), etc., could be influenced by gut microbiota. By modulating all this, the gut microbiome essentially has control over basic anti-tumor processes like apoptosis, phagocytosis, cytochrome C release from mitochondria, etc. Over the past ten years, studies have demonstrated that probiotics can treat brain tumors [47]. Table 10.3 gives an account of some probiotics-produced therapeutic compounds for fighting brain cancers.

Probiotics can generate three general groups of medicinal compounds based on their function: First, if they induce apoptosis in cancer cells; second, if they yield SCFAs; and third, if they create bacteriocins.

The process of apoptosis, which is a type of controlled cell death, is crucial for getting rid of tumor cells in our bodies. However, one of the hallmarks of cancer is to evade apoptosis. Hence, a boost to the immune system and added health benefits from ingesting probiotics can be a key strategy in cancer therapy. Out of all probiotics, lactic acid-producing bacteria stand out due to their immunomodulation, which has been linked to the prevention or reversal of cancer. According to research, *Lactobacillus acidophilus* can cause glioblastoma cells to undergo apoptosis by promoting apoptosis and suppressing apoptosis-inhibiting genes. The research also discovered that *Lactobacillus acidophilus* could lessen glioma cells' infiltration and movement, suggesting that it may also have anti-metastatic qualities [55]. Different research looked at how *Bifidobacterium bifidum* affected the development of

TABLE 10.3 Different Therapeutic Probiotic Strains Along with Their Therapeutic Molecule Present Against Different Kinds of Brain Cancers

Probiotic Bacteria	Family	Therapeutic Moiety	Type Of Brain Cancer	Mechanism	Reference
<i>Bifidobacterium bifidum</i>	Bifidobacteriaceae	<i>Bifidobacterium bifidum</i> SPM-1	Glioma	Inhibits brain tumor cell growth by triggering cell cycle arrest via caspase-3 and altering PI3K/Akt and MAPK signaling pathways.	[48, 49]
<i>Lactobacillus acidophilus</i>	Lactobacillaceae	<i>Lactobacillus acidophilus</i> - Bacteriocin-like inhibitory substance (LAB-2B)	Glioma	Slows glioma cell growth by activating caspase-3, causing apoptosis, and modifying MAPK and PI3K/Akt pathways.	[50]
<i>Lactobacillus fermentum</i>	Lactobacillaceae	<i>Lactobacillus fermentum</i> - Derived Antiproliferative Protein (LAP)	Glioblastoma	Induces apoptosis in glioblastoma cells by upregulating Bax and caspase-3 while downregulating bcl-2.	[51]
<i>Streptococcus thermophilus</i>	Streptococcaceae	<i>Streptococcus thermophilus</i> - Derived Antiproliferative Protein (STP)	Glioma	Induces apoptosis in glioma cells.	[52]
<i>Lactobacillus plantarum</i>	Lactobacillaceae	LPAP	Glioblastoma	Induces apoptosis in glioblastoma cells.	[53]
<i>Lactobacillus rhamnosus</i>	Lactobacillaceae	SPA	Glioblastoma	Inhibits glioblastoma cell growth and induces cell death.	[54]

cells from glioblastoma multiforme (GBM), an incredibly malignant form of brain cancer. According to the study, *Bifidobacterium bifidum* can cause death and can interrupt the process of cell proliferation in order to prevent the growth of GBM cells. Additionally supporting its capacity to cause apoptosis, *Bifidobacterium bifidum* was identified in the research to be able to reduce the levels of anti-apoptotic proteins in GBM cells [56].

Though the specific processes by which probiotics cause brain cancer cells to undergo apoptosis are still unclear, multiple possible causes have been put forth. The gut microbiome's control is one potential method. Probiotics may be able to modify the gut microbiome in a manner that lessens inflammatory processes and encourages apoptosis in brain cancer cells because the gut microbiome has been demonstrated to have a role in the growth and advancement of malignancies. Immune response control is yet another potential method. It is known to have a part in the development and spread of malignancies. Another possible strategy is immunological regulation. Probiotics have been shown to alter immunological responses, which might help to explain how they can induce apoptosis in cancer cells. For instance, probiotics could have the potential to enhance the activity of NK cells [57].

SCFAs are unstable saturated fatty acids that can occur as either a straight or branched structure and have a chain of 1–6 aliphatic carbon atoms. In areas where anaerobic bacteria are prevalent, they occur in large quantities. The primary carbon flow from food to the host microbiota is represented by SCFAs. Almost all SCFAs found in the human colon (90–95%) are ethanoic acid, propanoates, and butanoic acid, with formic acid making up only a small percentage of this group [58]. Probiotics have been demonstrated to generate a number of other therapeutic molecules that have shown to be efficacious in the management of brain cancer, along with triggering apoptosis in tumor cells. Probiotics that generate SCFAs, for instance, have been found to have anti-cancer properties. SCFAs have the ability to promote apoptosis and slow the development of cancer cells. Additionally, they can control the immune system and lessen inflammation, both of which are helpful in the therapy of brain cancer. *Lactobacillus salivarius* spp *salcinus* JCM 1230, *Lactobacillus agilis* JCM 1048 are examples of healthy bacteria that produce propanoates, butanoic acid, and α -hydroxypropionic acid, whereas *Bifidobacterium longum* SP 07/3, and *Bifidobacterium bifidum* MF 20/5 produce ethanoic, propanoates, and α -hydroxypropionic acids [59].

There are several pathways through which SCFA synthesis occurs in different bacteria. For instance, the Embden-Meyerhof-Parnassian pathway is found in enterobacteria and clostridia, while the Bifidobacterium pathway is specific to *Bifidobacterium*. In the human body, SCFAs play a vital role in maintaining intestinal and immune balance. Studies have also suggested that the interaction between the microbiota, gut, and brain may be influenced by SCFAs [60]. SCFAs can promote apoptosis and slow the growth of cancer cells. Moreover, they help regulate the immune system and reduce inflammation, which can be beneficial in treating brain cancer. Research investigating the effect of the probiotic mixtures on glioma cells found that VSL#3 (the only probiotic used as a therapeutic meal) was capable of producing SCFAs, which can cause glioma cells to undergo death. The research discovered that VSL#3 might also possess anti-inflammatory qualities because it was successful in lowering the expression of cytokines that promote inflammation in glioma cells [61].

Another possible therapeutic molecule made by probiotics is small peptides called bacteriocins, which have antimicrobial qualities. Bacteriocins are peptides with cationic properties that cause apoptosis by creating pores in targeted cells and releasing the contents of the cytoplasm. It has been observed that particular probiotic bacterial strains can destroy cancer cells by producing bacteriocins. Research on different cancer types, which includes brain cancer, has shown this to be true. Bacteriocins are additionally known to modify the host's immune system and native microbiome, which can affect a variety of the host's processes that support good health [62].

As seen, there are some advantages associated with using probiotics as a treatment for brain cancers, but there are also some disadvantages. First is the difficulty of getting probiotic therapeutic products into the brain. An essential protective barrier that keeps blood away from the brain and protects it is called the blood-brain barrier. However, this can also lead to challenges in delivering our probiotic therapeutic products into the brain because not all molecules can cross the blood-brain barrier. Therefore, these therapeutic products derived from probiotics need to be delivered directly into the brain, which can be intrusive and challenging. Secondly, probiotics' interaction with other medications presents a further obstacle. The possibility for probiotics to react with other drugs is concerning. Antibiotics and immunosuppressants are just a couple of the drugs that probiotics have been demonstrated to combine with. Patients who are undergoing additional brain cancer treatments may find this to be worrisome. Lastly, probiotics may be used as a remedy for brain cancer, according to some encouraging studies, but

more research is needed to evaluate the efficacy and safety of this approach. Research on ideal dosage, delivery strategy, and possible interactions with other therapies should be conducted [63].

10.8 CHEMOTHERAPY COUPLED WITH PROBIOTICS AGAINST BRAIN CANCERS

Chemotherapy has become a method of combating cancer that entails giving drugs to tumor cells in order to kill them. There are many different types of chemotherapy medications, but they all have a similar mechanism of action. They prevent tumor cells from reproducing, preventing them from establishing and propagating across their host. However, the major disadvantage of chemotherapy is that it has many side effects, such as nausea, diarrhea, vomiting, etc. Furthermore, cancer patients frequently experience chemotherapy-related cognitive impairment (CRCI), which is linked to poor prognoses and low quality of life. Additionally, the effects of probiotics on the gut, immunity, and brain cancers are profound and cannot be ignored. Hence, recent research has been conducted to see the efficacy of chemotherapy with beneficial bacteria in brain cancer patients, and it demonstrated that combining probiotics with chemotherapy can result in a synergistic impact and may even increase the effectiveness of the treatment [64]. It not only helps in reducing the side effects of chemotherapy but also assists it in fighting off the developing tumor. For instance, a probiotic regimen combined with chemotherapy is a potential dual therapy that decreases discomfort in the intestines, intestinal damage and porosity, and the possibility of developing diseases, while also boosting the variety of bacteria among people who have brain cancer [65]. Research carried out suggests that probiotics not only have great anti-cancer properties but also effectively and safely reduce the toxicities and negative consequences of chemotherapy. Probiotics like *Bifidobacterium* and *Lactobacillus* have chemoprotective reactions against the side effects and toxins of chemotherapeutic drugs, according to preclinical and clinical data, making it wise to incorporate them into chemotherapy [66]. According to 2017 research, giving probiotics to people with glioblastoma who were receiving chemotherapy increased their life expectancy rate. Sixty patients were enrolled in the China-based research, and they were randomly assigned to receive chemotherapy by itself or chemotherapy combined with a probiotic supplement. According to the findings, patients who took the probiotic medication had a considerably higher survival rate than those who

only received chemotherapy [67]. Another 2016 study demonstrated that giving mice suffering from brain tumors a probiotic medication increased the effectiveness of their chemotherapy. The mice used in the Korean research were given temozolomide, a chemotherapy substance, along with a probiotic containing *Lactobacillus acidophilus*. The findings demonstrated that when juxtaposed with chemotherapy alone, combination therapy greatly slowed the development of the tumor and increased survival. Specifically discussing the reduction of side effects of chemotherapy-induced diarrhea by using probiotic supplements, 1,013 patients were enrolled in 12 randomized controlled studies, which were then examined. Probiotics taken orally substantially reduced the probability of chemotherapy-induced diarrhea and oral stomatitis at all levels, particularly in the Asian population, when compared with the control population. Additionally, they discovered that the rate of unfavorable responses decreased the more bacterial species they used as probiotics [64, 68]. There is mounting proof that cancer treatment alters the body's own immune response and causes immune dysbiosis, which in turn affects the effectiveness of the treatment. These findings indicate that the gut microbiome modulates drug efficacy, eliminates the anticancer impact, and mediates toxicity, all of which have important implications for cancer therapy. In fact, in addition to chemotherapy, probiotics can also be used in conjunction with immunotherapy and radiation therapy.

An intriguing study looking at the idea of combining healthy bacteria and chemotherapy in the treatment of brain cancer exists, but there are also potential drawbacks and restrictions to this strategy. Not all probiotic varieties are created equal, and a few might even have detrimental effects on the treatment of cancer. For instance, some studies have indicated that in certain individuals, specific probiotic strains could actually raise their likelihood of colorectal cancer. As a result, it's crucial to choose probiotic strains and dosages that have been clinically proven to be both safe and effective. Probiotics can interact with different medications, including chemotherapy drugs, which is another possible drawback. The efficacy of chemotherapy might be affected, or there might be unexpected adverse effects. To prevent any possible drug interactions, patients should be sure to inform their physicians about all medications they are taking, including probiotics [64, 68]. The risks of acquired infections and the possibility of transmission of antibiotic resistance are the primary concerns when giving probiotics to immunocompromised brain cancer patients. Despite this, probiotic treatment in numerous trials has been shown to improve diarrhea and additional kinds of treatment-related harm to the GI tract, including stomatitis. It has also been shown

to re-establish a healthy intestinal microbiota makeup. Plenty of research studies are presently underway to assess the security and effectiveness of combining healthy bacteria with chemotherapy for cancer, in addition to the findings that have already been published. It is crucial to prove the safety of giving healthy gut bacteria to those with serious cancer illnesses in a larger population of cases, independent of the observed positive effects [68]. A special mention should be given to one of the earliest investigated bacteria in oncology, *Lactobacillus rhamnosus* GG (LGG), which is represented by the probiotic archetype. Due to its anti-inflammatory capabilities, the gut bacterium LGG has the capacity to restore the equilibrium of the gut's microbes. Numerous laboratory analyses, animal models, and human trials have shown the advantages of giving LGG to those with cancer. Additionally, 70 trials have been filed with the specific intent of determining the outcomes related to the delivery of LGG in a variety of circumstances [67].

Even though these studies show promise, more investigation is required to completely comprehend the biological processes underlying the probiotics' beneficial properties in the therapy of brain cancer and to pinpoint the most efficient probiotic strains and dosages. Additionally, it is crucial to thoroughly assess the efficacy and security of healthy gut bacteria in cancer patients receiving chemotherapy. Persons with brain tumors may benefit from adjuvant treatment that combines chemotherapy and probiotics. Probiotics have direct anticancer effects, can lower inflammation, and can modify the gut microbiota; all of this may improve chemotherapy's efficacy and lessen its adverse effects. Probiotics may be a hopeful adjunct to conventional treatments for cancer, although more research is required to completely comprehend their potential advantages in the treatment of brain cancer.

10.9 FUTURE PROSPECTIVE AND CONCLUSION

As discussed previously, the generation of different metabolites by the gut-brain axis may have a favorable or negative impact on the growth of cancer, especially brain cancer. These metabolites may stimulate or inhibit the production of particular cytokines, which may result in pro- or anti-inflammatory reactions. Both negative and beneficial effects can result from the gut microbiota's influence on the occurrence of cancer. When we talk about protecting our bodies from diseases and other infectious agents, the innate and adaptive immunity of humans plays a major role. Probiotics may influence the immune system in order to enhance its performance, resulting

in improved medical outcomes, as previously shown by many studies. That is why probiotics have garnered more attention recently for their potential role in the management and fight against cancer, especially brain cancer. For instance, the generation of inflammation-causing mediators (e.g., amyloid proteins and lipopolysaccharides) and inflammation-suppressing compounds by gut microbiota can influence myeloid-derived suppressor cells' (MDSCs) suppression of cancer-fighting T lymphocytes (e.g., antioxidants, flavonoids, and phytoestrogens). Some gut bacteria, including *Lactobacillus reuteri* and *Clostridium orbiscindens*, can cause MDSCs to differentiate into developed myeloid cells, which have anti-cancer properties [69].

We know that the brain and CNS are both affected by the terrible illness that is brain cancer. The typical survival time for glioblastoma, an eminently deadly and prevalent form of brain cancer, is under fifteen months. Traditional therapies like surgery, chemotherapy, and radiation have a restricted ability to effectively treat this illness alone. Therefore, novel therapeutic strategies are required to raise the survival rate and standard of living for people with brain cancer, such as adjuvant therapy with probiotics. It is well documented that the bacteria present in the gut have the ability to modify the microenvironment of cancerous cells by depleting some dietary amino acids. Accordingly, dietary arginine is assimilated in the stomach by microbes like *Prevotella* spp., which decreases the flow in the body that encourages anti-tumor actions. Arginine-auxotrophic tumor cells have to depend heavily on autophagy to support their accelerated metabolisms, which ultimately lead to apoptosis in an arginine-depleted environment. Reduced arginine flow causes the body to produce less nitric oxide, which in turn prevents MSDCs from proliferating and boosts anti-tumor T-cell action. On the other hand, the depletion of tryptophan by probiotics like *Burkholderia*, *Klebsiella*, and *Ralstonia* has led to the phosphorylation of eukaryotic initiation factor 2 to enhance tumor immune tolerance and survival [70]. Furthermore, in tryptophan-depleted settings, overactivation of GCN2 leads to disruption of fatty acid metabolism in helper T-cells. We have also seen that the gut microbiome and probiotics may use the kynurenine pathway to metabolize tryptophan, which leads to AhR activity, subsequently resulting in IDO-1 activity that further leads to tryptophan depletion. SCFAs like acetate and butyrate are responsible for helping to prevent the release of certain interferons and histone activity, which help to prevent cancer. Probiotics can also improve the effectiveness of both radiation and chemotherapy in the therapeutic management of cancer, according to research. For example, a mouse study discovered that giving mice *Lactobacillus rhamnosus*, a probiotic, increased the effectiveness of

chemotherapy for the treatment of colon cancer. Additionally, the probiotic appeared to lessen the negative effects of chemotherapy, including diarrhea and weight loss [71]. Even though this study's results were encouraging, additional analysis is still required to determine whether probiotics are truly useful in combating human brain cancer. However, proof that probiotics may be used to treat cancer, particularly brain cancer, is in accord with the clinical trials that are currently being conducted. These studies seek to establish the safety and effectiveness of healthy gut bacteria as a supplemental solution for the management of cancer. Even while severe immune-related adverse effects are irregular at early stages, if they are not treated effectively, they might turn fatal. In fact, a patient with glioblastoma has been shown to have histological signs of significant cirrhosis linked to the delivery of the anti-PD-1 antibody [72]. It will be crucial to acknowledge that the microbiota of the GI tract might play an important role in the growth, formation, and avoidance of a variety of immunological conditions, including immune-related adverse effects. With more information about the bacteria in our GI tract, new treatments related to a number of immune-related dysregulations will become available. In reality, mounting evidence points to the possibility that dysbiosis of the GI microbiota might contribute significantly to the onset and development of autoimmune illnesses, suggesting potential for the successful regulation of gut bacteria in the management of autoimmune disorders. Certain types of gut microorganisms could be advantageous to the host in some circumstances and/or possibly harmful in others. Only progress could influence this field's future outlook. By deciphering the complex relationship of the immune system, gut bacteria, and gut-brain-immune axis, this discipline would be significantly closer to comprehending the pathogenesis of immunological illnesses. Additionally, the field of brain oncology will benefit from this new breakthrough [73].

The growth of human immunity involves the microorganisms present in the GI tract as well as other elements of our body's defenses. The use of probiotics that encourage the modification of the microbiota in the gut for enhancing immune systems has gained greater academic and business interest in light of the authority of gut microbiota on both wellness and illness. Despite the fact that probiotics have been shown to improve fitness, concerns have been raised about their use because they may lead to bacterial transfer and the emergence of antimicrobial susceptibility in the identified microorganisms of the gut due to the effects of the food items that they are employed in (such as storage life, artificial additives and preservatives, item packing, etc.). Research on deactivated microbes and/or their byproducts has

expanded recently since they offer health advantages through various bioactive components, which could be used to overcome these drawbacks [74]. In conclusion, probiotics may modify immune function and increase the effectiveness of cancer treatments, particularly those for brain cancer. Although further investigation is required to confirm their efficacy in managing brain cancer in humans, preliminary findings and current clinical trials are encouraging. Thus, in the not-too-distant future, probiotics might present a fresh therapeutic strategy for the management and avoidance of brain cancer. The microbiota's enormous plasticity may point to the possibility of an exciting future in precision healthcare.

KEYWORDS

- **Brain cancer**
- **gut microbiota**
- **neuroinflammation**
- **probiotics**
- **therapy**

REFERENCES

1. Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Berni Canani, R., Flint, H. J., & Salminen, S. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*.
2. Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLOS Biology*, 14(8), e1002533.
3. Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157(1), 121–141.
4. Roy, S., Nag, S., Saini, A., & Choudhury, L. (2022). Association of human gut microbiota with rare diseases: A close peep through. *Intractable & Rare Diseases Research*, 11(2), 52–62.
5. Schreiber, R. D., Old, L. J., & Smyth, M. J. (2011). Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Science*, 331(6024), 1565–1570.

6. Ohland, C. L., & Jobin, C. (2015). Microbial activities and intestinal homeostasis: A delicate balance between health and disease. *Cellular and Molecular Gastroenterology and Hepatology*, 1(1), 28–40.
7. Plaza-Díaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, Á. (2017). Mechanisms of action of probiotics. *Advances in Nutrition*, 8(6), 496–507.
8. Shida, K., Nanno, M., & Nagata, S. (2002). Effects of *Lactobacillus casei* on cytokine production by the murine Peyer's patches. *International Journal of Food Microbiology*, 73(1), 63–72.
9. Zhang, X., Guo, C., Wu, S., Zhang, J., Fang, H., & Zhang, J. (2013). The role of NK cells in the anti-tumor immune response. *International Immunopharmacology*, 17(3), 575–581.
10. Lim, M., Xia, Y., Bettgowda, C., & Weller, M. (2018). Current state of immunotherapy for glioblastoma. *Nature Reviews Clinical Oncology*, 15(7), 422–442.
11. Reardon, D. A., et al. (2020). Glioblastoma immunotherapy: Rational therapeutic targets and current clinical experience. *Frontiers in Oncology*, 10, 603790.
12. Wainwright, D. A., et al. (2013). Targeting immunosuppression in glioma: Combining immune checkpoint inhibitors with standard therapy. *Frontiers in Immunology*, 4, 367.
13. Gabrilovich, D. I., & Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nature Reviews Immunology*, 9(3), 162–174.
14. Quail, D. F., & Joyce, J. A. (2013). Microenvironmental regulation of tumor progression and metastasis. *Nature Medicine*, 19(11), 1423–1437.
15. Huang, R. Y., et al. (2019). Targeting LAG-3 and PD-1 pathways in tumor microenvironment in glioma preclinical models. *Oncoimmunology*, 8(2), e1519667.
16. Nduom, E. K., et al. (2015). Therapeutic targeting of the immune checkpoint molecule TIM-3 in glioma. *Oncoimmunology*, 5(2), e1108332.
17. Brown, C. E., et al. (2016). EGFRvIII-CAR T cells in glioblastoma treatment. *New England Journal of Medicine*, 375(26), 2561–2569.
18. Rutledge, W. C., et al. (2013). Tumor-infiltrating lymphocytes and their role in the anti-tumor response. *Journal of Neuro-Oncology*, 115(3), 431–442.
19. Clark, V. E., et al. (2013). Genomic analysis of meningioma reveals the mechanisms of immune evasion. *Nature*, 497(7448), 367–372.
20. Berghoff, A. S., et al. (2014). Tumor-infiltrating lymphocytes and survival in patients with brain metastases. *Journal of Clinical Oncology*, 32(21), 2110–2115.
21. D'Alessandro, G., et al. (2020). Gut microbiota in glioma: Insights into pathogenesis and therapeutic potential. *Nature Reviews Cancer*, 20(1), 20–22.
22. Blacher, E., et al. (2019). The gut microbiome and amyotrophic lateral sclerosis. *Nature*, 573(7773), 474–479.
23. Borre, Y. E., O'Keefe, G. W., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2014). Microbiota and neurodevelopmental windows: Implications for brain disorders. *Trends in Molecular Medicine*, 20(9), 509–518.
24. Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, 13(10), 701–712.
25. Cammarota, G., & Ianiro, G. (2017). Gut microbiota modulation: Probiotics, antibiotics, and fecal microbiota transplantation. *Gastroenterology*, 152(6), 1919–1931.
26. Barrio, L. C., Arias-Sanchez, S., & Martin-Monzon, I. (2022). Dysbiosis and its impact on human health: The role of gut microbiota. *Microbiome Journal*, 45(3), 132–145.

27. Blaser, M. J. (2017). The theory of dysbiosis and human health. *Nature Reviews Microbiology*, 15(10), 556–564.
28. Mehrian-Shai, R., Reichardt, J. K. V., & Harris, C. C. (2019). Gut microbiome and brain tumors: A new frontier. *Frontiers in Oncology*, 9, 776.
29. Dehghani, M., Kazemi Shariat Panahi, H., & Guillemin, G. J. (2020a). Microbial derived compounds and their potential influence on the brain–gut–microbiome axis in neurodegenerative diseases. *Frontiers in Neuroscience*, 14, 23.
30. Goyal, D., Ali, S. A., & Singh, R. K. (2021). Emerging role of gut microbiota in brain cancer: Novel therapeutic opportunities. *Microbiome and Brain Research*, 56(7), 421–435.
31. Sharon, G., Sampson, T. R., Geschwind, D. H., & Mazmanian, S. K. (2016). The central nervous system and the gut microbiome. *Cell*, 167(4), 915–932.
32. Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science*, 336(6086), 1268–1273.
33. Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The impact of the gut microbiota on human health: An integrative view. *Cell*, 148(6), 1258–1270.
34. Cox, L. M., & Blaser, M. J. (2015). Pathways in microbe-induced obesity. *Cell Metabolism*, 21(6), 883–894.
35. Dehghani, M., Kazemi Shariat Panahi, H., & Guillemin, G. J. (2020a). Microbial derived compounds and their potential influence on the brain–gut–microbiome axis in neurodegenerative diseases. *Frontiers in Neuroscience*, 14, 23.
36. Socala, K., Doboszewska, U., Szopa, A., Serefko, A., Wlaż, P., Poleszak, E., & Wlaż, A. (2021). The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. *Pharmacological Research*, 172, 105840.
37. Mehrian-Shai, R., Reichardt, J. K., & Harris, C. C. (2019). The microbiome and cancer: Cancer predisposition, microbiome dysbiosis, and emerging therapeutic approaches. *Cancer Journal*, 25(1), 42–47.
38. Haase, S., Haghikia, A., Wilck, N., Müller, D. N., & Linker, R. A. (2020). Impacts of microbiome metabolites on immune regulation and autoimmunity. *Immunology*, 159(3), 257–266.
39. D'Alessandro, G., Antonangeli, F., Marrocco, F., Porzia, A., Lauro, C., Santoni, A., & Limatola, C. (2020). Gut microbiota alterations affect glioma growth and innate immune cells involved in tumor immunosurveillance in mice. *Nature Communications*, 11(1), 4997.
40. Roy, S., Trinchieri, G., & Sarkar, D. (2022). Gut microbiota as a regulator of the immune system in health and disease. *Nature Reviews Immunology*, 22(5), 283–296.
41. Dehghani, M., Kazemi Shariat Panahi, H., & Guillemin, G. J. (2020a). Microorganisms, neuroinflammation, and mental health. In *Microorganisms and neuroinflammation* (pp. 23–47). Springer.
42. Davis, I., & Liu, A. (2015). The kynurenine pathway: Key to brain development and neurodegeneration. *Molecular Psychiatry*, 20(1), 21–30.
43. Adams, S., Braidy, N., Bessesde, A., Brew, B. J., Grant, R., Teo, C., & Guillemin, G. J. (2012). The kynurenine pathway in brain tumor pathogenesis. *Cancer Research*, 72(22), 5649–5657.
44. Mellor, A. L., & Munn, D. H. (2004). IDO expression by dendritic cells: Tolerance and tryptophan catabolism. *Nature Reviews Immunology*, 4(10), 762–774.

45. Adams, S., Braidy, N., Bessesde, A., Brew, B. J., Grant, R., Teo, C., & Guillemin, G. J. (2012). The kynurenine pathway in brain tumor pathogenesis. *Cancer Research*, 72(22), 5649–5657.
46. Davis, I., & Liu, A. (2015). The kynurenine pathway: Key to brain development and neurodegeneration. *Molecular Psychiatry*, 20(1), 21–30.
47. Dehghani, M., Kazemi Shariat Panahi, H., & Guillemin, G. J. (2020a). Microorganisms, the kynurenine pathway, and the gut-brain axis. *Advances in Experimental Medicine and Biology*, 1262, 93–101.
48. Blacher, E., Bashiardes, S., Shapiro, H., Rothschild, D., Mor, U., Dori-Bachash, M., ... & Elinav, E. (2019). Potential roles of gut microbiome and metabolites in modulating ALS. *Nature*, 572(7770), 474–480.
49. Mitra, S., Das, R., & Basak, P. (2023). Gut microbiota and brain cancer: The role of gut microbes and derived metabolites in modulating brain tumors. *Trends in Cancer Research*, 5(2), 85–94.
50. Gaspar, P., Dinić, M., Encinas-Basurto, D., & Beganović, J. (2018). Role of probiotics in glioma treatment: A focus on *Lactobacillus acidophilus*. *Oncology Reports*, 39(4), 2209–2220.
51. Dinić, M., Lukić, J., Djokić, J., Miljković, M., Strahinić, I., Golić, N., & Beganović, J. (2017). Effects of *Lactobacillus fermentum*-derived antiproliferative protein on glioblastoma cells. *Molecular Neurobiology*, 54(5), 3215–3229.
52. Dargahi, N., Johnson, E., & Apostolopoulos, V. (2020). Gut microbiota-modulating effects of *Streptococcus thermophilus* on glioma cell apoptosis. *Journal of Neuro-Oncology*, 150(2), 349–362.
53. Maleki-Kakelar, H., Bahadori, M., Karami, M. M., & Mahdavi, M. (2020). *Lactobacillus plantarum*-induced glioblastoma cell death: Mechanisms and potential benefits. *Cancer Cell International*, 20(1), 1–12.
54. Dehghani, R., Tafvizi, F., & Jafari, S. (2021). *Lactobacillus rhamnosus*-induced apoptosis in glioblastoma cells: Mechanisms and implications for therapy. *Brain Tumor Research & Therapy*, 2(3), 101–109.
55. Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions – A review. *International Journal of Medical Microbiology*, 300(1), 57–62.
56. Khailova, L., Dvorak, K., Arganbright, K. M., Halpern, M. D., & Dvorak, B. (2010). *Bifidobacterium bifidum* improves intestinal integrity in a rat model of necrotizing enterocolitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 299(5), G1125–G1134.
57. Yan, F., & Polk, D. B. (2011). Probiotics and immune health. *Current Opinion in Gastroenterology*, 27(6), 496–501.
58. Markowiak-Kopeć, P., & Śliżewska, K. (2020). The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients*, 12(4), 1107.
59. Dalile, B., Van Oudenhove, L., Vervliet, B., & Verbeke, K. (2019). The role of short-chain fatty acids in microbiota–gut–brain communication. *Nature Reviews Gastroenterology & Hepatology*, 16(8), 461–478.
60. Jia, W., Li, H., Zhao, L., & Nicholson, J. K. (2018). Gut microbiota: A potential new territory for drug targeting. *Nature Reviews Drug Discovery*, 7(2), 123–129.
61. Cheng, S., Ma, X., Geng, S., Fu, H., & Zhang, Y. (2020). Effect of VSL#3 probiotics on regulating inflammation in colorectal cancer cells. *Journal of Microbiology, Immunology and Infection*, 53(3), 391–398.

62. Gaspar, C., Donders, G. G., & Grangier, M. (2018). Nisin-loaded nanoformulations as an alternative approach for antibiotics used in gynecology. *Journal of Nanobiotechnology*, 16(1), 29.
63. Dehhaghi, M., Kazemi Shariat Panahi, H., & Guillemin, G. J. (2020a). Microorganisms, tryptophan metabolism, and kynurenine pathway: A complex interconnected loop influencing human health status. *International Journal of Tryptophan Research*, 13, 1178646920938688.
64. Feng, Q., Liu, Z., Zhong, S., et al. (2022). The role of probiotics in the modulation of chemotherapy-related adverse effects. *Cancer Medicine*, 11(4), 1347–1358.
65. Jordan, K., Kunkel, M., & Gruber, T. (2018). Probiotics as potential agents for the treatment of chemotherapy-induced side effects. *European Journal of Clinical Investigation*, 48(7), e12923.
66. Waziri, N., Jenkins, R., & Lowe, D. (2022). Chemoprotective effects of probiotics on chemotherapy-induced side effects. *Journal of Oncology*, 16(4), 45–56.
67. Vivarelli, S., Salemi, R., Candido, S., et al. (2019). Probiotics role in cancer prevention and treatment: Mechanistic insights and clinical evidence. *Journal of Clinical Medicine*, 8(4), 494–505.
68. Ma, Y., Zhang, H., & Zhang, S. (2019). Probiotics in the treatment of chemotherapy-induced diarrhea: A systematic review and meta-analysis. *Chinese Journal of Cancer Research*, 31(4), 637–649.
69. Dehhaghi, M., Kazemi Shariat Panahi, H., & Guillemin, G. J. (2020b). Microorganisms, microbial products, and cancer: Impacts of the microbiota-gut-brain axis on health and diseases. *Cells*, 9(4), 803.
70. Yoshikawa, H., Matsuzaki, Y., Goto, Y., et al. (2022). Arginine metabolism in brain cancer: The role of microbiota and immune regulation. *Journal of Neurosurgery*, 137(2), 356–365.
71. Dehhaghi, M., Kazemi Shariat Panahi, H., & Guillemin, G. J. (2020b). Gut-brain axis in brain cancer treatment: Emerging roles of the microbiota. *Cancers*, 12(4), 1012–1028.
72. Vivarelli, S., Candido, S., & Ambra, R. (2019). The microbiota role in cancer therapy: Boosting the immune system. *Frontiers in Oncology*, 9, 985–991.
73. Yeşilyurt, N. Y., Koçak, E., & Uçar, A. (2021a). Immune modulation and probiotic therapy in brain cancer management. *Journal of Microbiological Sciences*, 13(3), 245–259.
74. Yeşilyurt, N. Y., Koçak, E., & Uçar, A. (2021b). Therapeutic potential of postbiotic metabolites in the modulation of immune responses and disease prevention. *Microbial Biotechnology*, 14(5), 2205–2214.

CHAPTER 11

Functional Role of Probiotics in Cancer Prevention and Treatment as Well as Their Role as Immune Boosters

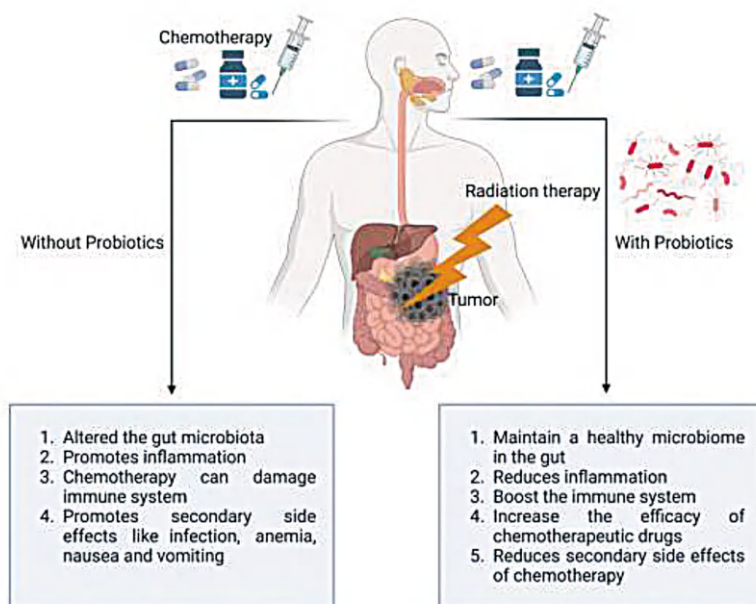
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ABSTRACT

Cancer is a devastating disease with far-reaching practical consequences. Previous studies have suggested that several of the chemotherapeutic medicines now in use to treat cancer may be dangerously unstable. Most people can't afford these drugs, even though they may worsen symptoms or increase the likelihood of developing resistance. Scientists are investigating the potential of dietary modifications as a cancer therapy and preventive technique. Gut homeostasis is maintained by the beneficial bacteria that populate it. These microbes control the intestinal microbiota and boost the human immune response. This has led to the widespread application of functional probiotics as adjuvants in cancer therapy and prevention. Probiotics have been shown to possess anti-cancer and anti-inflammatory effects, as well as to improve the functionality of beneficial microbes, which in turn influences immunological regulation, bacterial translocation, gut barrier function, and inflammation. Despite the popularity of probiotics, several clinical studies have yielded conflicting findings. Probiotic treatment plans for cancer patients aim to eradicate the malignancy while reducing the unpleasant side effects of chemotherapy. Here, we go through some of the alternative cancer

bio-therapeutic treatments that have been shown to work *in vitro*, *in vivo* animal-based models, and human clinical trial investigations, such as live or dead probiotics. Additionally, the mechanism of cancer prevention by probiotics is also summarized and represented in this piece of work. Most probiotic therapeutic successes have only recently been discovered. Conclusively, consequences from probiotics can be reduced with well-controlled studies of both their immediate and delayed effects.



ABSTRACT FIGURE LEGEND Conjugated use of probiotics with chemo and radiation therapy for cancer prevention and treatment.

11.1 INTRODUCTION

Cancer is high on the list of leading killers in the world today. While advancements have been made in recent years in tumor treatment, the prevalence of neoplastic syndrome continues to rise. This is why scientists are actively seeking new treatments and preventative measures to counteract the progression. Although heredity unquestionably has a part in cancer risk, the state of the body's immune system also has a significant impact, and this is intimately linked to the probiotic bacteria and commensal bacterial flora mostly found in the digestive tract [1]. Modern science has coined the term "probiotic," meaning "for life," to describe symbiotic bacteria that are beneficial for overall

human health. Metchnikoff, writing in the early 1990s, defined probiotics as “modulation of floral/microbial variety in human bodies,” which eliminates pathogenic microorganisms and replaces them with beneficial ones. Henry Tissier, however, made a breakthrough discovery when he found that unwell children with diarrhea had a significantly lower microbial load of a specific type of bacteria in their stool samples than healthy children [2]. He came up with the innovative concept of giving living organisms (*Bifidobacteria*) orally to diarrhea patients to help restore a healthy gut flora. Diarrhea (especially infantile diarrhea) is a common condition. By enhancing the qualities of the native flora when given to animals or people, probiotics, as they are now defined, are either single or mixed cultures of bacteria that have a positive effect on the host. This concept was proposed by Havenaar and Huisint Veld [2]. To put it simply, probiotics are “live bacteria that, when provided in enough quantities, offer health advantages to the host,” according to WHO. Probiotics, on the other hand, are characterized as helpful live microbial feed additives that influence the host animal in a positive way by restoring a healthy microbial balance in the intestines [3]. Modulation of the gut microbiota is the initial target of probiotics, which were utilized to better the health of both animals and humans. Many strains of *Lactobacilli* and *Bifidobacteria* with extensive characterizations are already marketed for human use to either prevent or cure gastrointestinal (GI) illnesses [4]. Consuming probiotics has several health benefits, including the amelioration of lactose intolerance symptoms, the reduction of the risk of certain illnesses, and the activation and strengthening of the immune system through management of microbiota [5]. Probiotics not only stimulate, change, and control the host’s immune response, but they also initiate the transcription of specific genes of the host cells. Being part of the gut-brain axis, they govern gastrointestinal hormone secretion and control brain function via two-way neuronal transmission [6]. Probiotics have a critical role in the regulation of both acute and chronic phase inflammation in the mucosal tissue of the intestine, which leads to the development of inflammatory bowel disease (IBD), by activating the VEGFR signaling pathway [7]. Probiotics are beneficial in the fight against obesity because of the physiological roles they play in maintaining a healthy host environment for the microorganisms that live there. However, more studies are needed before probiotics may be used to improve human health, nutrition, and the regulation of many disorders, despite their promising potential in these areas. After enduring cancer therapies including chemotherapy, radiation, or surgical eradication, the microenvironment and underlying medical problems might become more complex, causing damage

to the mucosal barrier of the intestine and dysfunction of the immune system in most cancer patients. The aforementioned alterations do not favor the colonization of the colon by helpful probiotics. There was a decline in the prevalence of probiotics in those who had been diagnosed with colorectal cancer. The probiotics' beneficial effects depended on the intestinal flora's support, according to research by Zmora, et al., who also discovered that the host's local gut bacteria played a vital part in the colonization of the probiotics. Their findings suggest that the therapeutic benefit of probiotics will be significantly impacted by the colonization barrier, even if the probiotics themselves are helpful [8]. More research is needed immediately to determine the effectiveness of probiotics in cancer patients.

Goldin and Gorbach were among the first people to show that getting more *Lactobacillus* in the diet makes individuals less likely to get colon cancer. Probiotics are getting a lot of attention because of their ability to change how cancer cells grow and die, which has been studied in various pre-clinical model systems (**Table 11.1**). These features, if applied to innovative therapies, may replace more invasive procedures like chemotherapy and radiation therapy. The purpose of this chapter is to draw readers' attention to the promising benefits of probiotics in cancer therapy as well as in improving human health.

11.2 CANCER PROGRESSION FUELED BY ALTERED GUT MICROBIOTA

Most of the disorders, including cancer, are linked to alterations in the microbiome's composition and/or functional features. The host suffers harm due to commensal dysbiosis, the transition from commensal homeostasis to an environment in which the equilibrium of symbionts and pathobionts has been disrupted or adversely affected [9]. This is due in part to the loss of useful functions provided by numerous local symbiotic microorganisms. Several of these advantages entail keeping the stomach and the peripheral immune system functioning properly. Via the production of inflammatory and/or regulatory immunological responses at mucosal surfaces, commensal bacteria exert dynamic control over immune homeostasis. Several illnesses, including cancer, have been linked to dysbiotic changes in the microbiome that promote the expansion of pathogenic or pathogen-associated commensals [10]. In addition to regulating their own metabolic activity, probiotic strains are essential for the health of the native gut microbiota. Carcinogenic chemicals are produced by putrefactive bacteria in the stomach utilizing enzymes including β -glucuronidase, azoreductase, and nitroreductase [11]. These bacteria include *Escherichia coli* and *Clostridium perfringens*. Initial

studies conducted in the late 1970s showed that elevating the population of *L. acidophilus* in the gastrointestinal tracts of rats reduced the abundance of putrefactive bacteria and toxic enzymes [12]. Studies on germ-free mouse models have shown that the gut bacteria may spread deep into body tissues and cause cancer by blocking IL-10, a key factor in the body's defense against cancer. The most potent anti-inflammatory cytokine is known as IL-10. It is also crucial to controlling tumor immune responses and eliminating malignancy. The first protein of bacterial origin to be linked to human melanoma was cytotoxin-associated gene A (CagA). *H. pylori*, a class 1 carcinogen, produces this protein. Pathogenic bacteria generate and secrete several toxins when a microbial imbalance exists in gut microflora. These toxins have the potential to cause genomic instability by causing DNA breaks in the host, which can lead to the formation of tumors in susceptible tissues and cells. Additionally, colibactin is produced by *E. coli*, and cdt-positive strains create cytolethal distending toxin (CDT) with DNase activity, which increases both nuclear and cellular enlargement, leading to persistent cell cycle arrest and, ultimately, the death of the target cells. Toxins released by bacteria near the gut epithelium cause double-strand breaks in host DNA, which leads to mutations and the eventual formation of tumors [13]. *Shigella flexneri* produces its key enzymes, namely cysteine protease-like virulence gene A (VirA) and inositol phosphate phosphatase D (IpgD), which stimulate the destruction of the host cell's p53 protein and hinder the DNA damage and repair processes [14]. CagA is also responsible for *H. pylori*-induced p53 degradation in gastric cells. The cytotoxin's interference with the host's AKT signaling pathway increases the risk of stomach cancer [15]. Carcinogenesis can also be triggered by the microbiota in the gut, which is known to affect a wide variety of host cellular pathways. Pathogenic bacteria produce a wide variety of proteins, enzymes, and toxins that can attach to host cells and disrupt their normal processes, hence increasing the likelihood of the development of tumors and malignancies [16]. The effector adhesin A (FadA) protein of *F. nucleatum*, CagA of *H. pylori*, and the metalloproteinase (MP) toxin of *B. fragilis* all attach to the host cell epithelial protein (i.e., E-cadherin), which causes the junctions between cells to become disorganized and activates the β -catenin signaling pathway. The transformation of affected cells and tissues into tumors is facilitated by the activation, which also promotes cell proliferation. Pathogenic microorganisms might indirectly influence host cell cancer through several ways. Several chemicals produced by pathogenic bacteria, for instance, can suppress the host immune response and promote inflammation, allowing cancer to evade the immune

system. Bacteria produce oxidative stress, which in turn triggers host cells to undergo self-directed genetic changes. Both *B. fragilis* and *H. pylori* are capable of stimulating the host's flavoenzyme (spermine oxidase). The activation results in the production of ROS and H₂O₂, which both contribute to the accumulation of DNA damage. By releasing oxygen species derivatives and extracellular superoxide, *E. faecalis* is able to permeate into host cells and speed up the mutation process in the host's DNA. Table 11.1 depicts the effects of different microorganisms in promoting carcinogenesis.

TABLE 11.1 Carcinogenic Effects of Several Microorganisms

Cancer type	Type of bacteria	Effects	Reference
Human melanoma/ Gastric cancer	<i>H. pylori</i>	Provokes malignant change in gastric epithelial cells Alternatively, they may disrupt the host's serine/threonine kinase (AKT) pathway, resulting in gastric cancer	[17, 18]
Tumorigenesis	<i>E. coli</i> (<i>cdt</i> -positive)	Host genome instability and DNA damage induction	[16]
Colon adenocarcinoma	<i>F. nucleatum</i>	Interacts with the ITIM domain and the NK inhibitory receptor TGIT to limit NK cell activity	[16]
Oral cancer	<i>Porphyromonas gingivalis</i> and <i>Fusobacterium nucleatum</i>	Inflammatory cytokine production, cell proliferation, apoptosis suppression, cell invasion and migration, and lastly genomic changes	[19]
Colorectal cancer	<i>Bacteroides fragilis</i>	Trigger a T-cell response by releasing a toxin that activates signal transducers	[20]
Pancreatic cancer	<i>Proteobacteria</i> , <i>Bacteroidetes</i> , <i>Firmicutes</i>	Induced the development of suppressive monocytic cells in pancreatic cancer via selective ligation of Toll-like receptors.	[21]
Liver cancer	Fiber-Fermenting Bacteria Proteobacteria	The development of inulin-induced HCC included the initiation of cholestasis, the death of hepatocytes, and the subsequent inflammation of the liver by neutrophils.	[22]

An intriguing possibility is that the gut microbiome inhibits intestinal pathogen invasion by asserting a claim on a niche, altering the microenvironment, and influencing host immune protection. This begins at birth, when the microbiome is being formed, and it has repercussions for the development of the immune system, tolerance, and the ability to limit the spread of microbes. The microflora in the mucosa influences the phenotype and function of T and B cells in the organism. These cells are essential for immunological

homeostasis because they suppress the body's reaction to innocuous antigens and protect the gut [23]. Research into the link between the gut microbiota and immunity is expanding rapidly, and it is becoming clear that genetic and immunological signals play an important role in regulating the host immune response to infection. Research into immune system dysregulation may help elucidate the microbiota's function in carcinogenesis and therapeutics. Colorectal cancer (CRC) patients' gut microbiomes can have an effect on CRC through the release of metabolites, invasion of healthy tissues, and control of the host immune response. The most prominent bacteria linked to CRC include *Clostridium*, *Peptostreptococcus*, *Porphyromonas* Genus, *Prutella*, *Bacteroides*, and twin cocci [24, 25]. The microbiome in the gut and the products of bile acids play various roles in the development of cancer in mice. Bile acids or their receptors can control various other immune cells, which helps the immune system fight cancer [26]. In the future, bile acid sensors and compounds from flora could be used to treat cancer in new ways. Thus, understanding how gut microbes promote cancer is very important for the development of efficient therapeutics against cancer. Therefore, it is crucial to figure out how microbes in the gut cause cancer in order to create effective treatments for cancer. The involvement of microorganisms in the carcinogenic process is graphically represented (Figure 11.1).

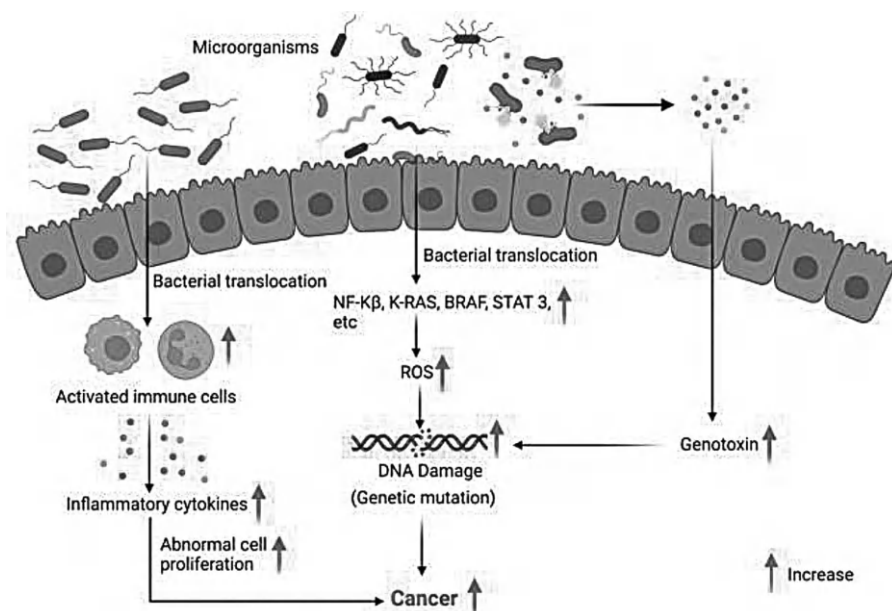


FIGURE 11.1 Schematic diagram showing the role of microorganisms in cancer progression.

Goldin and Gorbach proposed that a diet high in *Lactobacillus* could reduce the risk of CRC by 37% [27]. Probiotics have been shown to have beneficial effects in modifying the proliferation and death of cancer cells in several *in vitro* investigations represented in the table. (Table 11.2).

TABLE 11.2 The Advantageous of Probiotics in Reducing the Growth of Cancer Cells and Inducing Apoptosis

Cancerous Cell Line	Type of Probiotic Strain	Effect of Probiotics	Reference
HCT116, SW1116, Caco-2	<i>Clostridium butyricum</i> and <i>Bacillus subtilis</i>	Decreased cell proliferation	[28]
Caco-2, HT-29	<i>Lactobacillus plantarum</i> and <i>Lactobacillus rhamnosus</i>	Decreased cell proliferation	[29]
HT-29, LoVo	<i>Lactococcus lactis</i>	Decreased cell proliferation greater than 80%	[30]
HT-29	<i>Lactobacillus rhamnosus</i>	Induced apoptosis and cell cycle arrest	[31]
Caco-2, HTB-37, HT-29, HTB-38	<i>Bifidobacterium adolescentis</i>	Induce cell death by increasing the expression of the genes caspase-8, Fas-R and BAD	[32]
Caco-2	<i>Lactobacillus acidophilus</i>	Reduces cell proliferation by inhibiting the expression of SURVIVIN and SMAC genes	[33]
AGS, MCF-7	<i>Lactobacillus acidophilus</i> and <i>Bifidobacterium bifidum</i>	Induce anti-tumor activity by promoting the release of IFN- γ and TNF- α	[34]
HT-29	<i>Propionibacterium acidopropionici</i>	reduced proliferation Induction of apoptosis	[35]
NMC460	<i>Bacillus polyfermenticus</i>	Reduced formation of cancer cell colonies	[36]
LS513	<i>Lactobacillus acidophilus</i> <i>Lactobacillus casei</i> (in the presence of 5-FU)	Increase cell death in 40% of the cancer cell population	[37]
DLD-1, HGC-27	<i>Lactobacillus paracasei</i> and <i>Lactobacillus rhamnosus</i>	Reduces cell proliferation and induces apoptosis	[38]
MDR	<i>Lactobacillus kefir</i>	Induction of apoptosis	[39]

Animal models are often used to examine the anti-tumor properties of probiotics. The majority of studies using this design yielded encouraging findings, suggesting future therapeutic use. Table 11.3 displays findings from studies that examine the impact of probiotic microorganisms on induced tumors in animal models.

TABLE 11.3 The Results of Research That Looked at How Probiotic Bacteria Affected Tumors That Were Made in Animal Models

Animal Model	Chemical/Cancer Cell Induced Tumor	Type of Probiotic Strain	Effect of Probiotics	Reference
CD-1 mice	DLD-1 cells injection	<i>Bacillus polyfermenticus</i>	Decreases tumor volume	[36]
F344 rats	1,2-dimethylhydrazine dihydrochloride	<i>Bacillus polyfermenticus</i>	Decreases aberrant crypt foci and tumor volume	[40]
Wistar albino rats	1,2-dimethylhydrazine dihydrochloride	<i>Lactobacillus plantarum</i>	Increases TNF- α concentration in serum	[41]
rats	MNNG-N-Methyl-N'-Nitro-Nitrosoguanidine	<i>Lactobacillus rhamnosus</i>	Reduces fecal activity of azoreductases and nitro reductase, increases glutathione S-transferase activity	[42]
BALB/c mice	Azoxymethane and azoxymethane	<i>Lactobacillus plantarum</i>	Induces apoptosis by causing cell cycle arrest	[43]
F344 rats	1,2-dimethylhydrazine dihydrochloride	<i>Lactobacillus salivarius</i>	Decreases tumor volume	[44]
Swiss albino mice	azoxymethane	<i>Pediococcus pentosaceus</i>	Induces apoptosis in tumor volume	[45]

Several forms of cancer have shown promising responses to probiotic supplementation, and the findings of several clinical investigations support this use. A dysbiotic gut microbiota has a significant effect on how cancer develops and how well it can be treated. This is because the ability of the gut microbiota to process antitumor chemicals and alter the immune response and inflammation pathways of the host is closely linked to the effectiveness of cancer treatment. The combination of these two results shows how much the patients' microbiome makes a difference in how well their medications work in the end. The microbiome composition of patients is closely correlated with the efficacy of immunotherapy based on immune checkpoint inhibitors, according to a key breakthrough reported last year in the field of how gut bacteria influence the human immune system [46].

In an instance, Routy et al [47] found that the survival rate was poorer for melanoma patients receiving antibiotics in addition to anti-PD1/anti-PD-L1 treatment. Anti-PD1 responses were discovered to be more prevalent in two phyla (*Akkermansia* and *Alistipes*) after the metagenomic fecal study. By transplanting human feces into germ-free mice, researchers found that the presence of *Akkermansia muciniphila* increased intra-tumoral cytotoxic T cell infiltrates, hence reducing the severity of the PD-1 blocking response in mice. Similarly, Gopalakrishnan et al [48] used metagenomic analysis on fecal specimens from melanoma patients and discovered that the microbiomes of anti-PD1 responders and non-responders had different compositions. In fact, the number of *Clostridiales*, *Ruminococcaceae*, and *Faecalibacteriae* increased. Combining the newly identified bacteria with anti-PD1 treatment significantly delayed the progression of melanoma in animals, as demonstrated by functional trials utilizing FMT in germ-free mice.

Over the past century, enormous progress has been achieved to enhance anti-cancer medications, dramatically lowering harmful side effects, with the ultimate objective of enhancing patient compliance. Probiotics are administered orally to alter the intestinal microbiota in an effort to increase protection and lessen the side effects of anti-cancer medications, most notably diarrhea and mucositis. In reality, probiotics are widely recognized as safe and have the wonderful benefit of being affordable. Probiotics have generally been shown to offer a wide range of advantages in clinical practice, including the reduction of diarrhea caused by antibiotics and *Clostridium difficile*, as well as respiratory tract infections. By administering probiotics to patients suffering from cancer, it is possible to replenish the commensal gut bacteria, which may have been reduced following treatment, and restore both their quantity and functioning. The risks of opportunistic infections and the possible transmission of antibiotic resistance

are the primary concerns with giving probiotics to immunocompromised cancer patients. Despite this, probiotic medication has shown in several trials to alleviate diarrhea and other kinds of gastrointestinal damage associated with therapy, such as mucositis. Intestinal microbiota composition has been demonstrated to normalize. To alleviate mucositis and prevent diarrhea, probiotics derived from the *Lactobacillus species* have been advised as dietary supplements for patients undergoing chemotherapy and/or radiation treatment for pelvic cancer.

In line with this point of view, more and more clinical studies are being conducted to find out if adding probiotics to the diets of cancer patients can change the gut bacteria in a way that can help them get better. The outcomes of the clinical trials that have been reported are promising. A double-blind clinical study conducted in 2010 on cancer patients undergoing colon resection showed that probiotic treatment had a favorable effect on the composition of the gut microbiome as well as the regulation of intestinal immune activities. Patients who received *Lactobacillus johnsonii* were able to adhere to the digestive mucosa. This reduced the number of gut microorganisms and altered the immune system [49]. In 2014, researchers conducted a clinical trial showing that cancer patients receiving pelvic radiation treatment who were also given the probiotics *Lactobacillus acidophilus* and *Bifidobacterium longum* experienced much less radiation-induced diarrhea [50]. Table 11.4 summarizes studies that looked at the usefulness of probiotics in combination with standard cancer therapy.

11.3 MECHANISTIC ACTION OF PROBIOTICS IN CANCER PREVENTION AND TREATMENT

Recent studies on the effectiveness of probiotics in preventing cancer have shown encouraging findings. Several studies have shown promise for using probiotics as a cancer treatment and preventative strategy. Here, we summarize the known mechanisms by which probiotics protect against cancer.

11.3.1 MODIFICATION OF GUT MICROBIOME

Probiotics play an important role in maintaining a healthy gut microbiome by balancing its composition and inhibiting the growth of harmful bacteria, including those associated with cancer. Numerous studies have demonstrated the antagonistic effects of various *Lactobacilli* strains against *H. pylori*, a gram-negative bacterium known to cause gastric cancer [57, 58]. Cancers beyond the digestive tract, including the liver, pancreas, and breasts, have all been related to changes in the gut microbiota [59, 60]. Disturbances in

TABLE 11.4 Research Reviewing the Efficacy of Probiotics in Conjunction with Conventional Cancer Treatment is Summarized

Type of Cancer	Chemotherapy	Probiotics	Effects	Reference
Colorectal cancer	Capecitabine and Oxaliplatin	<i>Lactobacillus and Bifidobacteria strains</i>	Reduced levels of pro-inflammatory cytokines may be the outcome of a probiotic’s influence on the gut microenvironment.	[51]
Non-small cell lung cancer	Immune checkpoint inhibitors	<i>Lactobacillus and Bifidobacteria strains</i>	Patients with NSCLC treated with immunological checkpoint inhibitors may have an increase in treatment success and overall survival when probiotics are also administered.	[52]
Colorectal cancer	-	<i>Streptococcus thermophilus and Lactobacillus delbruckii subsp. bulgaricus</i>	Colorectal cancer was shown to be less common among yogurt eaters, particularly men.	[53]
Colorectal cancer	-	<i>Lactobacillus gasseri</i>	Boosts the production of isobutyric acid and the action of NK cells.	[54]
bladder cancer	-	<i>Lactobacillus acidophilus</i>	Reduces the risk of bladder cancer	[55]
Pediatric malignancies	Chemotherapy (Not disclosed)	<i>Bifidobacterium breve</i>	Decreases the likelihood of fever and the requirement for intravenous antibiotics	[56]

the intestinal flora, for instance, have been associated with liver cancer, with the pathogenic bacterium *Hemophilus* being detected in liver cancer tissues. *Hemophilus* has been found to produce a lethal dilatant toxin upon translocation to the liver, activating signaling pathways (Wnt/ β -catenin, Ki-67, NF- κ B, and p21) involved in liver cancer development [61, 62]. Additionally, preclinical studies have suggested that *L. acidophilus* and *Bifidobacterium bifidum* could be used as bio-therapeutic candidates to modify intestinal bacteria and inhibit colon cancer, making probiotics a potential alternative biological therapy for preventing or treating colorectal cancer [63]. Researchers comparing fecal samples from healthy individuals and colorectal cancer patients found significantly higher levels of *Bacteroides* and *Prevotella* in the cancer group [64]. The abundance of *Lactobacillus* species in the intestinal ecosystem was lower compared to bacteria from genera such as *Bacteroides*, *Eubacterium*, *Fusobacterium*, *Prevotella*, and *Proteobacteria*. Moreover, higher numbers of *Salmonella* and *Clostridium* species were observed in colorectal cancer patients [65]. Likewise, many roles of probiotics have been found to possess anti-cancer activity by modifying the intestinal flora [66].

11.3.2 ANTI-TUMOR MICROENVIRONMENT AND ANTI-CANCER STEM CELLS ACTIVITY OF PROBIOTICS

The development of the tumor microenvironment depends on the communication between normal cells and tumor cells. Within this microenvironment, certain cells possess the ability to initiate tumor formation, known as tumorigenesis. Probiotics have shown potential in inhibiting tumor growth by interfering with this process. For instance, a study demonstrated that the administration of a TGF- β receptor inhibitor and probiotics can boost the immune response against tumors, leading to their suppression [67]. In solid tumors, there exists a small subpopulation of cancer called cancer stem cells (CSCs), which are responsible for cancer relapse due to their self-renewal capabilities and immortality. Previous research has discussed the anti-CSC and anti-tumor microenvironment properties of probiotics [68]. This information is summarized in Table 11.5, highlighting the effects of probiotics on CSCs and the tumor microenvironment [69–72].

11.3.3 INHIBITION OF MUTAGENIC AND CARCINOGENIC ACTIVITY

Various agents, known as mutagens, can mediate DNA damage and mutations that contribute to the development of cancer [73]. Carcinogenicity and

TABLE 11.5 Shows How Probiotics Affect CSCs and the Surrounding Area of a Tumor

Sl. No.	Probiotics	Experimental Animal Model/Cell Line	Functions
1	<i>Bifidobacterium breve</i> and <i>Bifidobacterium infants</i>	C47BL/6 mice	Stopping the spread of cancer via reducing inflammatory cytokine mediators
2	<i>L. bulgaricus</i> and <i>L. plantarum</i> , <i>L. casei</i>	HT 29 cell	Inhibiting inflammatory cytokine mediators to activate natural killer cells.
3	<i>L. rhamnosus</i>	C47BL/6 mice	Inhibiting the HIF-1 α signaling pathway to inhibit metastasis
4	<i>L. crispatus</i>	HT 29 cell line	Decrease in HIF-1 signaling and increased SFRP2 expression
5	<i>L. casei</i>	C47BL/6 mice	1. Slows tumor development and prevents pulmonary metastasis 2. Using natural killer cells as a cytolytic weapon by activating them
6	<i>Lactobacilli</i> <i>Species YIT/9018</i>	C47BL/6 mice	1. Reduction in metastasis 2. Elevated levels of IL-2 and IFN- γ
7	<i>L. brevis</i>	BALB/c mice	1. Reduces liver metastases resulting from metastatic breast cancer 2. Activates natural killer cells, which stimulate the secretion of IFN- γ and IL-17.
8	<i>L. casei</i> 431 CRL	Wister rat	CD4+ and CD8+ cells are associated with antitumor activity.
9	<i>L. casei</i> Shirota and <i>L. rhamnosus</i> GG	HT29 cell line	1. Natural killer cell activation 2. Enhancing neutrophilic phagocytic activity to prevent the production of free radicals
10	<i>L. casei</i> Shirota	Caco2 cell line	1. Reduction of the angiogenic IL-1 factor 2. IL-6 inhibition

mutagenicity are closely linked concepts, as both involve processes that can lead to cancer. Mutagens include chemicals, ionizing and non-ionizing radiation, and viruses, all of which are known to cause cancers [74, 75]. Probiotics have emerged as possible anti-mutagenic candidates due to their production of metabolites [76]. Reports suggest the benefits of probiotics on mutagens, such as sodium azide and benzopyrene, and found that probiotics have the ability to bind to mutagens, acting as detoxifying agents [77]. The anti-mutagenic property of specific strains like *Lactobacillus rhamnosus* against the mutagen acridine orange has been previously studied [78]. Several mutagens and their corresponding mutagenic microorganisms have been reported [79]. Some of the important examples are, *Lactobacillus alimentarius* DDL 48 (Sodium azide and benzopyrene), *Lactobacillus rhamnosus* (Acridine-orange), *Lactobacillus* and *Bifidobacterium* (Benzo[a] pyrene and sodium azide), *Bifidobacterium lactis* Bb-12 (Benzopyrene), *Lactobacillus bulgaricus* (4-nitroquinoline-N-oxide), *Bifidobacterium lactis* Bb-12 (Furazolidone), *Lactobacillus rhamnosus* (N-methyl-N0-nitro-N-nitrosoguanidine), *Lactobacillus helveticus* (Heterocyclic aromatic amines), *Lactobacillus plantarum* (Sodium azide (NaN₃) and 2-nitrofluorene (2-NF)). Probiotics' cell wall components, including lipids, teichoic acids, proteins, and carbohydrates, bind to mutagens in a hydrophilic manner [78]. Additionally, organic acids and extracellularly released glycoproteins also have antimutagenic capabilities [80, 81]. Probiotics are used because of these qualities to detoxify food and heal some gastrointestinal conditions [82].

11.3.4 INHIBITION OF CELL GROWTH AND APOPTOSIS INDUCTION

The benefits of probiotics in the death of cancer cells have been demonstrated in several *in vitro* studies. According to earlier reports, probiotics influence the Bax/Bcl-2 ratio and caspase activity to cause apoptosis in cancer cells [83, 84]. For instance, it has been demonstrated that the *Lactobacillus rhamnosus* GG strain decreases cell growth and triggers apoptosis in human and animal colon cancer cell lines (HGC-27, Caco-2, DLD-1, and HT-29) [38]. Additionally, colicin, a bacteriocin produced from *Escherichia coli*, has anticancer action by creating tiny holes in the plasma membrane, resulting in the induction of apoptosis and cell cycle arrest in the G1 phase [79]. Studies have also investigated specific molecules derived from probiotics that contribute to tumor suppression. Apoptosis induced by the c-jun N-terminal kinase (JNK) signaling pathway has been found to be a mechanism through which ferrochrome, a chemical produced by probiotics, inhibits colon cancer

growth [85]. By inhibiting the NF- κ B pathway, the functional lipid produced by *Lactobacillus plantarum*, conjugated linoleic acid, causes the death of breast cancer cells [86]. By upregulating IFN- γ , Bax, and TNF- α expression and downregulating the expression of Bcl2, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* exhibited higher cytotoxic effects on breast and colon cancer cell lines [79]. Additionally, *L. acidophilus* causes apoptosis by boosting survivin's mRNA expression while lowering SMAC's mRNA expression [79]. *Lactobacillus casei* significantly boosts the levels of the *hBD-2* gene in the colon cancer cell line HT29 [79]. In order to induce apoptosis and reduce proliferation in HNSCC cells, Joo et al., reported that nisin raised intracellular calcium, caused cell cycle arrest, and activated cation transport regulator homolog 1 (Chac1) [87]. By triggering caspase, Jan et al., found that mechanisms for mitochondrial hole creation cause apoptosis [35]. Additionally, a study discovered that *Propionibacterium* short-chain fatty acids acted on the mitochondria of colorectal cancer cells to cause apoptosis. Although the molecular methods of action for many apoptotic proteins are yet unknown, researchers are currently examining the apoptotic potential of probiotics in different malignancies. Probiotics have been demonstrated to cause apoptosis through controlling signaling pathways, such as the over-expression of PTEN and the induction of mitogen-activated protein kinase (MAPK) pathways [88]. Furthermore, CAL-27 (human tongue squamous cell carcinoma) cells' viability was decreased and apoptosis was induced, according to Zhang et al [89]. The reduction of COX-2/PCNA expression and induction of apoptosis by *L. salivarius* was shown to significantly reduce oral cancer in rats [90]. Probiotics, in particular, have a significant role in maintaining healthy oral flora, which is important for preventing oral cancer since altered oral flora increases the risk of developing the disease [91, 92].

11.3.5 INDUCTION OF AUTOPHAGY

Autophagy is a biological process in which autophagosomes fuse with lysosomes to degrade cellular components [93]. It plays a crucial role in maintaining homeostasis and has been implicated in various diseases [94]. Autophagy can both promote tumor suppression [95] and contribute to tumor progression [96]. It is responsible for the removal of damaged cells, aged organelles, and proteins, while defects in autophagy can lead to DNA damage-mediated cancer progression, highlighting its potential role in tumor suppression [97]. However, there is little research on the use of probiotics

to reduce tumor development by promoting autophagy. Some studies have shown that certain probiotics, such as *Lactobacillus acidophilus*, can induce cell death in colon cancer cells by modulating autophagy-related proteins. For example, a surface protein collected from *L. acidophilus* was found to alter the levels of microtubule-associated protein-1, leading to cell death in HCT-116 cells [98]. Additionally, probiotics can promote apoptotic stimulation in the presence of the chemotherapeutic drug 5-FU by activating autophagy-related signaling pathways such as Beclin1/GRP78 [66]. The exopolysaccharides produced by probiotics have the potential to trigger autophagy in colon cancer cells by activating Beclin1/GRP78 and key regulators (BCL-2 and BAX) of the intrinsic apoptosis pathway [99]. Beclin-1, damage-regulated autophagy modulator 1, lysosome-associated membrane protein, and *p53* are important genes and proteins involved in autophagy [100]. However, further research is required to fully understand the function of probiotics-mediated autophagy in the suppression of cancer and its possible therapeutic potential.

11.3.6 EXPRESSION OF ONCOGENES IS SUPPRESSED

The expression of oncogenes results in tumor-like characteristics in cells, although proto-oncogenes are the unaltered, regular versions of oncogenes. Mutations can cause proto-oncogenes to become oncogenes. The goal of cancer treatment is to downregulate oncogenes. By comparing their structural resemblance to retroviral oncogenes, several proto-oncogenes from various species have been discovered. The proto-oncogenes such as Ras, HER2, Myc, cyclin D, cyclin E, β -catenin, and MITF are all significant in humans [101]. Probiotics have been shown in several studies to decrease tumor growth by downregulating oncogenes [102, 103]. By regulating the levels of mTOR-related genes and WNT/ β -catenin pathways, probiotic bacteria like *Lactobacillus crispatus* and *L. rhamnosus* influence cancer progression [104]. According to Azam et al., the culture supernatants of *Lactobacillus acidophilus* and *Lactobacillus crispatus* decreased the expression of oncoproteins *in vitro* [102]. Combining probiotics with the nonsteroidal anti-inflammatory medication celecoxib can reduce the KRAS proto-oncogene's activity, thereby reducing the risk of colon cancer [105]. Patients with malignancies linked to RAS may benefit from identifying the mechanisms by which probiotics suppress KRAS. The expression of cyclin D1 is also reported to be reduced by bacteriocin nisin, cytoplasmic extracts, and cell walls of *Lactococcus lactis*, which inhibit the proliferation of SW480

cells [106]. MITF expression is downregulated by lipoteichoic acid obtained from *L. plantarum* [107]. The use of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* GG, or their combination with celecoxib decreased the expression of NF- κ B, β -catenin, COX-2, and KRAS, which are carcinogenic biomarkers in colorectal cancer [108]. In conclusion, substantial research is being done on the utilization of probiotics and the metabolites they produce to control oncogene expression. In summary, researchers are extensively investigating the use of probiotics and their metabolites to regulate oncogene expression. These approaches hold promise for cancer treatment and prevention.

11.3.7 RE-ACTIVATION OF TUMOR SUPPRESSORS

Tumor suppressor genes play a crucial role in reducing cell division, repairing damaged DNA, and regulating the cell death pathway. In humans, important tumor suppressor genes include *APC*, *BRCA1*, *BRCA2*, *p53*, *p21*, *p16*, *Rb*, and *VHL*. Mutations or defects in these genes can contribute to the development of cancer. Tumor suppressor genes are therefore deactivated by cancer cells, and their activation is crucial [109]. By epigenetically controlling the expression of tumor suppressor genes and oncogenes [110], Sharma et al., proved that short-chain fatty acids produced by probiotics can specifically target tumor cells. Gene expression can be changed through epigenetic modification, which doesn't entail modifying the DNA sequence itself. DNA methylation, changes in histones, and the impact of tiny non-coding microRNAs (miRNAs) are some of these pathways [111]. Metabiotics made from the probiotic *L. rhamnosus* MD have been shown to reduce the risk of colorectal cancer by increasing the expression of the well-known tumor suppressor gene *p53* [112]. Studies have also indicated that *L. rhamnosus* MD, *L. rhamnosus* GG, and *L. acidophilus* can increase the levels of tumor suppressor genes in experimental models of colon carcinogenesis induced by 1,2-dimethylhydrazine [105]. Additionally, *Bifidobacterium longum*, collected from breast milk, induces the levels of tumor suppressor miRNAs, such as miR-15 and miR-145, in colorectal cancer [113]. One of the studies explored the role of probiotic metabolites in preventing colon cancer through epigenetic regulations, emphasizing the importance of metabiotics derived from probiotics in this process [114]. Although the field of reactivating tumor suppressors using probiotics is still in its early stages, these findings provide promising insights into the potential of probiotics in modulating tumor suppressor gene expression through epigenetic regulation.

11.3.8 INHIBITION OF KINASES

Enzymes called phosphatases and kinases, respectively, are responsible for adding and removing phosphate groups [115]. Phosphorylation includes the addition of a phosphate group from adenosine triphosphate (ATP) to particular amino acid residues (such as threonine, serine, and tyrosine) and can modify the function of target proteins [116]. Kinases play a very important role in several aspects of tumor biology, including motility, cell proliferation, metabolism, angiogenesis, and metastasis [117]. Kinases have thus become prospective therapeutic targets for the treatment of cancer. Probiotics and their metabolites have been the subject of several research studies investigating their potential as kinase inhibitors, particularly when used to treat diarrhea caused by cancer treatment [118]. According to research by Seth et al., protein kinase C (PKC) and mitogen-activated protein kinases (MAPKs) are involved in a process by which probiotic secretory proteins maintain the structural integrity of gut epithelial tight junctions and the barrier function from damage brought on by hydrogen peroxide [119]. *Lactobacillus plantarum* has been shown to activate apoptosis in cancer cells by down-regulating MAPKs and upregulating phosphatases [88]. Additionally, it has been shown that *Lactobacillus* produces tumor necrosis factor-associated apoptosis-inducing ligand (TNF-AL), which increases the activity of natural killer cells in prostate cancer cell lines [120]. Ongoing research is focused on exploring the kinase inhibitor activity of probiotics and identifying potential therapeutic applications. To eventually pave the way for innovative methods in cancer treatment, this research seeks to further unravel the molecular mechanisms through which probiotics presumably exert their effects on kinase signaling pathways.

11.3.9 INHIBITION OF METASTASIS

The metastatic process is a difficult one, including the dispersal of cancer cells from the primary tumor to other parts of the body. It significantly contributes to cancer patients' deaths. Various factors contribute to metastasis, including disrupted cell-cell adhesion, several factors of the tumor microenvironment, epithelial-to-mesenchymal transition (EMT), and the immortality of cancer stem cells [69]. Probiotics have been studied and shown to have anti-metastatic properties. Cell-free supernatants of probiotics such as *Lactobacillus rhamnosus* GG and *Lactobacillus casei* have been shown to decrease the occurrence of colon cancer and inhibit its metastatic properties. This is achieved through the reduction of matrix metalloproteinase-9 (MMP-9),

which promotes tumor invasion, and upregulating the expression of tight junction protein ZO-1, which helps to maintain the integrity of cell-cell junctions [121, 122]. Probiotic strains like *Lactobacillus plantarum* YYC-3 have proven to suppress the metastatic properties of colon cancer cells by hindering the VEGF-MMP2/9 signaling pathway [123]. This pathway is involved in the ‘basement membrane degradation,’ an essential step in the metastatic process. Probiotic treatment has also been found to upregulate the expression of E-cadherin, a protein crucial for cell-cell adhesion, thereby inhibiting cancer cell migration [124, 125]. The expression of EMT-related markers (Snail-1 and ZEB-1) was also decreased in pancreatic cancer animal models after probiotic therapy. Furthermore, probiotics have shown anti-metastatic and anti-angiogenic effects in breast cancer cells. Kefir, a probiotic fermented food, has been associated with the increase of tissue inhibitors of MMPs (TIMPs), which counteract the activity of MMPs and help inhibit metastasis [126]. Probiotic aerosol therapy has also demonstrated benefits in inhibiting lung melanoma metastasis [127]. Overall, probiotics play a crucial role in inhibiting metastasis by targeting various aspects of the metastatic process. Their effects on cell adhesion, EMT, MMP regulation, and the tumor microenvironment contribute to their anti-metastatic properties. Probiotics have shown promise in preventing and treating cancer metastasis, but more study is needed to fully understand the underlying processes and find the best ways to employ them.

11.3.10 IMMUNE MODULATION

A study performed by Li et al., used a mouse liver cancer model to investigate the effects of the probiotic *E. coli* Nissle on tumor progression. The researchers found that treatment with this probiotic enhanced the antitumor immune response. The mechanism behind this effect involved reducing the levels of Th17 cells as well as IL-17 in tumor tissues while promoting the differentiation of Treg/Tr1 cells. These changes inhibited the growth of liver tumors via inflammatory and angiogenic pathways by regulating the production of vascular growth factors [128]. According to a different study, probiotics can change the gut microbiota to favor healthy bacteria like *Prevotella* and *Oscillibacter*. Anti-inflammatory chemicals produced by these bacteria are known to lessen the polarization of Th17 cells and encourage the development of anti-inflammatory Treg/Tr1 cells in the gut. This alteration of the gut microbiota has effects on inflammatory and immunological responses

[128]. In order to prevent excessive inflammation from inhaled particles, the lung microenvironment is characterized by high immunological tolerance. However, this immune tolerance also creates a favorable situation for the metastasis of several tumors to the lungs. *Lactobacillus rhamnosus* has been found to induce the maturation of antigen-presenting cells in the lung, leading to the activation of lung T cells and natural killer (NK) cells. This activation improves the lung's immune suppression status and strengthens the immune response against tumors [129]. Routy et al., conducted a study on cancer patients treated with immunotherapeutic PD-1 blockade, specifically focusing on lung, kidney, and bladder cancer. The study revealed that the use of many antibiotics before and after immunotherapy disrupted the body's bacteria, including the gut microbiota, resulting in poorer effectiveness of the immunotherapy. Patients without access to broad-spectrum antibiotics had greater odds of overall and progression-free survival. The presence of the microorganism *Akkermansia muciniphila* in the gut was found to be related to a positive response to PD-1 blockade [47]. In a clinical trial involving melanoma patients treated with anti-PD-1 immunotherapy, Gopalakrishnan et al., observed a connection between the composition and diversity of the gut microbiota and treatment response. Patients who responded positively to the treatment had a more diverse gut microbiome with increased levels of the Clostridium order, while non-responders had lower bacterial diversity and higher levels of Bacteroides bacteria. Specific bacteria were associated with increased immune cell infiltration in tumors of patients who responded to treatment. The significance of the gut microbiota in influencing the success of anti-PD-1 immunotherapy is highlighted by these results [48]. Figure 11.2 depicts the anti-cancer mechanisms of probiotics.

11.4 PROBIOTICS AS IMMUNE BOOSTER

The immune system is affected in two ways by probiotics: it is stimulated and it is regulated. Cancer, allergies, and infections can all be helped by immunostimulatory probiotics, whereas autoimmune diseases, irritable bowel syndrome, and other allergens can all be helped by immunoregulatory probiotics. By releasing cytokines such as interleukins (ILs), tumor necrosis factors (TNFs), interferon (IFNs), transforming growth factors (TGFs), and short-chain fatty acids (SCFAs), probiotics have an immunomodulatory advantage. They also produce bioactive substances that support the immune system, such as EPSs, surface proteins, peptides, etc. Due to the existence of these components, even heat-killed probiotics can boost the immune

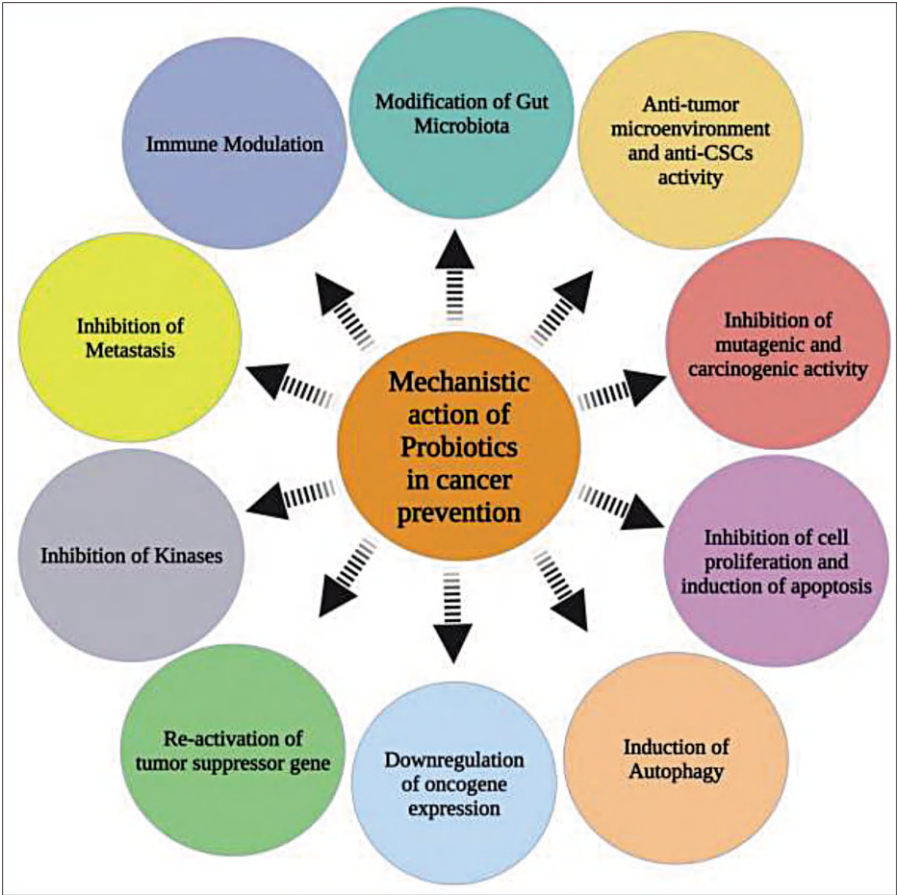


FIGURE 11.2 The graphical representation depicting the anti-cancer mechanisms of probiotics.

system and prevent cancer [130, 131]. Probiotics can produce substances that eliminate harmful bacteria, compete with them and toxins for binding to the intestinal lining. They can enhance both innate and adaptive immunity by influencing the functions of several immune cells such as dendritic cells (DCs), macrophages, T and B lymphocytes, through pathways regulated by toll-like receptors. DCs are immune cells that play an important role in both innate and adaptive immunity by presenting antigens to T cells and guiding them towards a regulatory or pro-inflammatory response [132]. The ability of DCs to produce IL-12 is vital for the development of Th1 immune responses characterized by IFN- γ and IL-2 production. Th2 immune responses, on the other hand, are associated with humoral immunity and the release of

cytokines like IL-4, IL-5, IL-6, and IL-13 [133]. Various mechanisms of immunity have been extensively studied, including the secretion of IFN- α and the induction of indoleamine 2,3-dioxygenase, an immunomodulatory enzyme involved in the interaction between T cells and DCs [134, 135]. A probiotic combination consisting of *L. acidophilus*, *L. reuteri*, *L. casei*, *B. bifidum*, and *Streptococcus thermophilus* was found to stimulate the production of regulatory DCs expressing high levels of TGF β , COX2, IL-10, and IFN- α . This helps in the production of regulatory T cells expressing the transcription factor CD4⁺ Foxp3⁺ and enhances their suppressive activity. The probiotic mixture also induces hypo-responsiveness in T and B cells and downregulates T helper cells without inducing apoptosis. *In vivo* studies have confirmed that this probiotic combination can suppress intestinal inflammation induced by 2,4,6-trinitrobenzenesulfonic acid by promoting the generation of CD4⁺ Foxp3⁺ Tregs [136]. *Lactobacillus casei* has shown remarkable efficacy in the treatment of colon cancer, not through any direct harmful effects but by improving the regulatory influence on host immunity. Several experimental models have been used to assess this [137].

11.5 THE POSSIBLE RISK POSED BY UTILIZING PROBIOTICS IN THE TREATMENT OF CANCER

The significance of probiotics in supporting host-body homeostasis is substantial. Reports suggest that maintaining a healthy microbial flora also activates the immune system to fight against various pathogenic organisms, but the use of probiotics is also associated with well-known adverse side effects. These include skin reactions, resistance to antibiotics, toxic effects of various metabolites, gastrointestinal disorders, and systemic infections. People with weakened immune systems due to age, illness, or treatment in a healthcare facility are at increased risk. Those who are most vulnerable include newborns, the elderly, hospitalized individuals, and people with weakened immune systems due to either genetic or environmental factors. Bacteremia occurs in about 1 in 5.6 million yeast users, but fewer than 1 in 1 million lactic acid bacteria users, according to research [138]. Another large-scale epidemiological study indicated that *Lactobacillus* and *Bifidobacteria* infections only accounted for 0.05% to 0.4% of all cases of infective endocarditis and bacteremia, and that most patients had severe underlying diseases [139]. Infection risk was associated with both the kind and quantity of probiotics consumed. *Lactobacillus* was shown to be more infectious

than *Bifidobacterium*, according to the literature [118]. Bacteremia is one of the most significant challenges in the practical application of probiotics, and yeast-caused fungal diseases are even harder to cure. The primary reasons are probiotic translocation and compromised intestinal integrity [140]. Probiotics that were taken rather than probiotics that had colonized the bowel were certainly the cause of these negative effects, according to genomic data [141]. It was discovered that the risk of infection was much increased in people with suppressed immune systems. Although probiotic medication helped reduce the severity and frequency of diarrhea in these cancer patients, 5 out of 1530 individuals experienced probiotic-related bacteremia [142]. Therefore, probiotic-caused serious invasive disease in cancer patients warrants close attention.

Previous case studies reported that an 8-month-old infant with acute myeloid leukemia, who was undergoing rigorous chemotherapy, developed *Saccharomyces fungemia*. In order to avoid the patient's diarrhea, which is frequently linked to this type of chemotherapy, *Saccharomyces boulardii* capsules were used as a prophylactic medication. Even though the patient was also receiving antifungal prophylaxis with fluconazole, the patient's fever increased just the day after finishing the chemotherapy cycle, and a strain of *Saccharomyces cerevisiae* was identified from a blood culture. With the help of amphotericin B and the removal of the central venous catheter, the patient made a full recovery but was nonetheless neutropenic [143]. These studies raise questions regarding the possible negative effects of such usage of probiotics. Similarly, a 65-year-old man was diagnosed with *Saccharomyces cerevisiae*-based fungemia after undergoing concurrent chemotherapy with cisplatin and 5-FU, as well as radiation treatment for head and neck cancer. Prior to the onset of fungemia, he was treated for aseptic diarrhea with *Saccharomyces boulardii* (Perenterol) oral suspension while suffering from grade IV oral mucositis [144].

Another case study detailed the development of bacteremia and septic pulmonary emboli in a 38-year-old male patient with stage IV Hodgkin lymphoma and AIDS who had chemotherapy and *Lactobacillus acidophilus* treatment. On day three of the treatment method, the patient's blood bacterial count was lower due to the clindamycin and gentamicin combined treatment therapy. Another finding reported that a 73-year-old male patient suffering from chronic lymphocytic leukemia, when treated with chemotherapeutic drugs along with *Bacillus subtilis* as probiotics, developed septicemia. Treating the patient with ceftazidime, amikacin, and vancomycin reduced septicemia-induced fever in the cancer patient [146]. According to the

research, a drastically altered microbiota encourages an overactive immune system. Studies in mice have revealed an important function for the intestinal microbiota, namely in the formation of gut-associated lymphoid tissue. The presence of a gut microbiota is essential for a number of immunological activities, including antibody production, oral tolerance to food antigens, and the maturation of germinal centers inside lymphoid follicles.

Interventions aimed at changing the gut microbiota might have significant immunomodulatory effects because of the microbiota's pivotal involvement in the development of a functional immune system. It is impossible to foresee how these modifications will affect the host in the long run, and there may be negative consequences on immune system development. This is especially important in the realm of newborn probiotic supplementation, where lifelong immune response modulation or medium to long-term microbiota transformation may be possible outcomes. Pregnant women may also be more susceptible to unfavorable immunological activation than other groups. Because Th1 cytokines are linked to pregnancy loss, there is thought to be a role for Th2 phenotypic bias in T cell responses during pregnancy in maintaining fetal viability. Some studies on humans have shown that the probiotic *Lactobacillus* species increase interferon production from Th1 cells and reduce the responses from Th2 cells [147]. The viability of pregnancy might be harmed by these impacts. However, there is currently no concrete proof of this, so the risk is still merely hypothetical. The idea that probiotics have a negative impact on immunological development currently has little empirical basis; however, this is a topic that needs more research.

Additionally, there is concern over the potential spread of probiotic strains' antimicrobial resistance to more pathogenic bacteria present in the intestine. Vancomycin resistance is a common trait among *Lactobacillus* strains, which raises questions about whether it may spread to other harmful species, including *Enterococci* and *Staphylococcus aureus*. The vancomycin-resistant genes of *Lactobacillus* spp. are chromosomal, nevertheless, and are consequently difficult for other species to acquire. The vancomycin-resistant genes of lactobacilli have not been discovered to be transferable to other genera through conjugation experiments.

11.6 CONCLUSION

Medical professionals are starting to acknowledge that probiotics can help prevent and treat a variety of illnesses without the use of potentially harmful

pharmaceutical medicines. Probiotic bacteria can have a beneficial influence on the host organism in a variety of ways, some of which are described in this chapter. Probiotic characteristics in bacteria and fungi are heritable. Substantial data suggest that probiotics can complement anticancer therapy and help reduce the risk of cancer. Extensive laboratory research has shown that probiotics have a significant anticancer effect. The data given here only lend credence to the use of probiotics for cancer prevention or as an adjuvant treatment during anti-cancer chemotherapy. The safety and efficacy of probiotics as a food additive remain unregulated. Probiotics are live microorganisms thought to provide health benefits, but only a few types have been shown to be beneficial in rigorous scientific studies. The risks associated with probiotics seldom make it into credible media coverage. To determine which strains are most effective in preventing and treating different types of cancer, researchers will need access to a huge human database and will need to carefully investigate links between strains and clinical responses. After useful microorganisms for cancer prevention and therapy have been identified, figuring out how to employ probiotics and their products to maintain patient microbiota will be a huge challenge. The gut microbiota's potential to sense and react to alterations in the pathophysiological environment gives cancer patients cause for optimism. We hope that by isolating or discovering new strain combinations, we might improve anticancer treatments while minimizing adverse effects. To show the value of probiotics as a possible cancer treatment and obtain recognition from the medical community, a lot of clinical studies have to be done. That's why microbiome control has such promise as a cutting-edge field of precision and individualized cancer and other illness treatment.

KEYWORDS

- **Cancer**
- **gut-homoeostasis**
- **immunomodulation**
- **micro-biota**
- **probiotics**

REFERENCES

1. Górská, A., Przystupski, D., Niemczura, M. J., & Kulbacka, J. (2019). Effects of probiotics and prebiotics on gut microbiota and their potential role in colorectal cancer prevention. *Current Microbiology*, 76(7), 939–949.
2. Kerry, R. G., Patra, J. K., Gouda, S., Park, Y., Shin, H. S., & Das, G. (2018). Probiotics: The potential role of beneficial bacteria in the prevention and treatment of cancer. *Journal of Food and Drug Analysis*, 26(4), 927–939.
3. Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., & Fakiri, E. M. (2013). Health benefits of probiotics: A review. *ISRN Nutrition*, 2013, 481651.
4. Vlasova, A. N., Kandasamy, S., Chattha, K. S., Rajashekara, G., & Saif, L. J. (2016). The role of the gut microbiome in the development of intestinal inflammation and colorectal cancer. *Veterinary Immunology and Immunopathology*, 172, 72–84.
5. Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and symbiotics on human health. *Nutrients*, 9(9), 1021.
6. Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: Relationships between the enteric microbiota, gut permeability, and neurological diseases. *Annals of Gastroenterology*, 28(2), 203–209.
7. Chen, X., Yang, G., Song, J. H., Xu, H., Li, D., Goldsmith, J., Zeng, H., Parsons-Wingerter, P. A., Reinecker, H. C., & Kelly, C. P. (2013). Probiotics induce a protective response against *Clostridium difficile* infection in mice. *PLoS ONE*, 8(8), e64227.
8. Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashirdes, S., Kotler, E., Zur, M., Regev-Lehavi, D., & Brik, R. B. Z. (2018). Personalized microbiome-based approaches to inflammatory bowel disease. *Cell*, 174(6), 1388–1405.
9. Bhatt, A. P., Redinbo, M. R., & Bultman, S. J. (2017). The role of the microbiome in cancer development and therapy. *CA: A Cancer Journal for Clinicians*, 67(4), 326–344.
10. Belkaid, Y., & Hand, T. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157(1), 121–141.
11. Plaza-Díaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Probiotic mechanisms of action. *Advances in Nutrition*, 10(S1), S49–S66.
12. Goldin, B., & Gorbach, S. L. (1977). The effect of probiotics on intestinal microflora. *Cancer*, 40(6), 2421–2426.
13. Akbar, N., Khan, N. A., Muhammad, J. S., & Siddiqui, R. (2022). A comprehensive review of probiotics: Health benefits and safety concerns. *Health Sciences Review*, 100010.
14. Bergounioux, J., Elisee, R., Prunier, A. L., Donnadieu, F., Sperandio, B., Sansonetti, P., & Arbibe, L. (2012). The gut microbiota: The future of vaccine development? *Cell Host & Microbe*, 11(3), 240–252.
15. Buti, L., Spooner, E., Van der Veen, A. G., Rappuoli, R., Covacci, A., & Ploegh, H. L. (2011). The role of the microbiota in immunity and inflammation. *Proceedings of the National Academy of Sciences*, 108(22), 9238–9243.
16. Vivarelli, S., Salemi, R., Candido, S., Falzone, L., Santagati, M., Stefani, S., Torino, F., Banna, G. L., Tonini, G., & Libra, M. (2019). The role of gut microbiota in cancer and therapy. *Cancers*, 11(1), 38.

17. Hatakeyama, M. (2017). *Helicobacter pylori*: A major player in the development of gastric cancer. *Proceedings of the Japan Academy, Series B, Physical and Biological Sciences*, 93(5), 196–219.
18. Murata-Kamiya, N., & Hatakeyama, M. (2022). *Helicobacter pylori* and cancer. *Cancer Science*, 113(6), 1909–1918.
19. Chattopadhyay, I., Verma, M., & Panda, M. (2019). Probiotics and cancer: A review of the current literature. *Technology in Cancer Research & Treatment*, 18, 1533033819867354.
20. Wu, S., Rhee, K. J., Albesiano, E., Rabizadeh, S., Wu, X., Yen, H. R., Huso, D. L., Brancati, F. L., Wick, E., & McAllister, F. (2009). The role of the gut microbiota in cancer therapy. *Nature Medicine*, 15(10), 1016–1022.
21. Pushalkar, S., Hundeyin, M., Daley, D., Zambirinis, C. P., Kurz, E., Mishra, A., Mohan, N., Aykut, B., Usyk, M., Torres, L. E., Werba, G., Zhang, K., Guo, Y., Li, Q., Akkad, N., Lall, S., Wadowski, B., Gutierrez, J., Kochen Rossi, J. A., Herzog, J. W., Diskin, B., Torres-Hernandez, A., Leinwand, J., Wang, W., Taunk, P. S., Savadkar, S., Janal, M., Saxena, A., Li, X., Cohen, D., Sartor, R. B., Saxena, D., & Miller, G. (2018). The role of the microbiota in pancreatic cancer. *Cancer Discovery*, 8(4), 403–416.
22. Singh, V., Yeoh, B. S., Chassaing, B., Xiao, X., Saha, P., Aguilera Olvera, R., Lapek, J. D., Zhang, L., Wang, W. B., Hao, S., Flythe, M. D., Gonzalez, D. J., Cani, P. D., Conejo-Garcia, J. R., Xiong, N., Kennett, M. J., Joe, B., Patterson, A. D., Gewirtz, A. T., & Vijay-Kumar, M. (2018). Microbiota-induced activation of epithelial IL-1 β triggers an anti-tumor immune response in the colon. *Cell*, 175(3), 679–694.e22.
23. Honda, K., & Littman, D. R. (2016). The microbiota in adaptive immune homeostasis and disease. *Nature*, 535(7610), 75–84.
24. Wong, S. H., & Yu, J. (2019). Gut microbiota in colorectal cancer: Mechanisms and clinical applications. *Nature Reviews Gastroenterology & Hepatology*, 16(12), 690–704.
25. Ternes, D., Karta, J., Tsenkova, M., Wilmes, P., Haan, S., & Letellier, E. (2020). Microbiome and cancer: The role of the microbiome in the development of cancer. *Trends in Microbiology*, 28(5), 401–423.
26. Sipe, L. M., Chaib, M., Pingili, A. K., Pierre, J. F., & Makowski, L. (2020). The role of the microbiome in cancer therapy. *Immunological Reviews*, 295(1), 220–239.
27. Goldin, B. R., & Gorbach, S. L. (1980). The role of dietary fiber in the prevention of colon cancer. *Journal of the National Cancer Institute*, 64(1), 263–265.
28. Chen, Z. F., Ai, L. Y., Wang, J. L., Ren, L. L., Yu, Y. N., Xu, J., Chen, H. Y., Yu, J., Li, M., & Qin, W. X. (2015). Probiotics: Their potential role in cancer therapy. *Future Microbiology*, 10(9), 1433–1445.
29. Sadeghi-Aliabadi, H., Mohammadi, F., Fazeli, H., & Mirlohi, M. (2014). The impact of probiotics on cancer therapy. *Iranian Journal of Basic Medical Sciences*, 17(10), 815.
30. Han, K. J., Lee, N. K., Park, H., & Paik, H. D. (2015). Anticancer effects of probiotics: A review of the evidence. *Journal of Microbiology and Biotechnology*, 25(10), 1697–1701.
31. Dehghani, N., Taffvizi, F., & Jafari, P. (2021). The role of probiotics in cancer prevention and therapy. *Bioimpacts*, 11(4), 245–252.
32. Faghfoori, Z., Faghfoori, M. H., Saber, A., Izadi, A., & Yari Khosroushahi, A. (2021). The role of probiotics in cancer prevention and treatment: A systematic review. *Cancer Cell International*, 21(1), 258.
33. Isazadeh, A., Hajazimian, H., Shadman, B., Safaei, S., Bedoustani, A. B., Chavoshi, R., Shanehbandi, D., Mashayekhi, M., Nahaei, M., & Baradaran, B. (2020). The potential role of probiotics in cancer therapy: A review. *Pharmaceutical Sciences*, 27(2), 262–267.

34. Khosrovan, Z., Haghighat, S., & Mahdavi, M. (2020). The role of probiotics in immune regulation. *Immunoregulation*, 3(1), 37–50.
35. Jan, G., Belzacq, A. S., Haouzi, D., Rouault, A., Metivier, D., Kroemer, G., & Brenner, C. (2002). Bcl-2 and Bcl-xL protect from cell death induced by various anticancer agents. *Cell Death & Differentiation*, 9(2), 179–188.
36. Ma, E. L., Choi, Y. J., Choi, J., Pothoulakis, C., Rhee, S. H., & Im, E. (2010). Probiotics suppress the proliferation of gastric cancer cells through the inhibition of NF- κ B and AP-1 signaling pathways. *International Journal of Cancer*, 127(4), 780–790.
37. Baldwin, C., Millette, M., Oth, D., Ruiz, M. T., Luquet, F. M., & Lacroix, M. (2010). The effect of probiotics on the growth of human colon cancer cell lines. *Nutrition and Cancer*, 62(4), 371–378.
38. Orlando, A., Refolo, M. G., Messa, C., Amati, L., Lavermicocca, P., Guerra, V., & Russo, F. (2012). Probiotics and prebiotics in colorectal cancer prevention: A review. *Nutrition and Cancer*, 64(8), 1103–1111.
39. Ghoneum, M., & Gimzewski, J. (2014). Anticancer effects of probiotics and their mechanisms of action. *International Journal of Oncology*, 44(3), 830–837.
40. Park, E., Jeon, G. I., Park, J. S., & Paik, H. D. (2007). Probiotic properties of *Lactobacillus casei* and its effect on the growth of cancer cells. *Biological and Pharmaceutical Bulletin*, 30(3), 569–574.
41. Bertkova, I., Hijova, E., Chmelarova, A., Mojzisova, G., Petrasova, D., Strojny, L., Bomba, A., & Zitnan, R. (2010). Probiotics and their effects on cancer prevention. *Neoplasma*, 57(5), 422.
42. Gosai, V., Ambalam, P., Raman, M., Kothari, C. R., Kothari, R. K., Vyas, B. R. M., & Sheth, N. R. (2011). Probiotic potential of *Lactobacillus* species in treating gastrointestinal disorders. *Gut Microbes*, 2(5), 319–325.
43. Lee, N. K., Son, S. H., Jeon, E. B., Jung, G. H., Lee, J. Y., & Paik, H. D. (2015). Anti-cancer effects of *Lactobacillus* strains. *Journal of Functional Foods*, 14, 513–518.
44. Zhang, M., Fan, X., Fang, B., Zhu, C., Zhu, J., & Ren, F. (2015). The effect of probiotics on cancer therapy: A review. *Journal of Microbiology*, 53(6), 398–405.
45. Dubey, V., Ghosh, A. R., Bishayee, A., & Khuda-Bukhsh, A. R. (2016). Probiotic *Lactobacillus* strains inhibit breast cancer cell proliferation *In vitro*. *Journal of Functional Foods*, 23, 66–79.
46. Vivarelli, S., Falzone, L., Basile, M. S., Nicolosi, D., Genovese, C., Libra, M., & Salmeri, M. (2019). The role of the microbiome in cancer: A comprehensive review. *World Academy of Sciences Journal*, 1(2), 125–135.
47. Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P., Alou, M. T., Daillère, R., Fluckiger, A., Messaoudene, C., Rauber, C., & Roberti, M. P. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*, 359(6371), 91–97.
48. Gopalakrishnan, V., Spencer, C. N., Nezi, L., Reuben, A., Andrews, M. C., Karpnits, T. V., Prieto, P. A., Vicente, D., Hoffman, K., & Wei, S. C. (2018). Gut microbiome modulates immune response to PD-1 blockade in melanoma patients. *Science*, 359(6371), 97–103.
49. Gianotti, L., Morelli, L., Galbiati, F., Rocchetti, S., Coppola, S., Beneduce, A., Gilardini, C., Zonenschain, D., Nespoli, A., & Braga, M. (2010). Probiotic administration in colorectal surgery: A pilot study. *World Journal of Gastroenterology: WJG*, 16(1), 167.

50. Demers, M., Dagnault, A., & Desjardins, J. (2014). The impact of probiotics on the outcome of patients with head and neck cancer: A preliminary report. *Clinical Nutrition*, 33(5), 761–767.
51. Zaharuddin, L., Mokhtar, N. M., Muhammad Nawawi, K. N., & Raja Ali, R. A. (2019). The role of probiotics in improving the quality of life in patients with colorectal cancer. *BMC Gastroenterology*, 19(1), 131.
52. Wan, L., Wu, C., Wu, Q., Luo, S., Liu, J., & Xie, X. (2023). The protective role of probiotics in the treatment of colorectal cancer. *Cancer Medicine*, 12(3), 1841–1849.
53. Pala, V., Sieri, S., Berrino, F., Vineis, P., Sacerdote, C., Palli, D., Masala, G., Panico, S., Mattiello, A., Tumino, R., Giurdanella, M. C., Agnoli, C., Grioni, S., & Krogh, V. (2011). Dietary patterns and colorectal cancer: A review of the literature. *International Journal of Cancer*, 129(12), 2712–2719.
54. Ohara, T., Yoshino, K., & Kitajima, M. (2010). The role of probiotics in colorectal cancer prevention: Evidence from clinical trials. *Hepatology*, 57(100), 1411–1415.
55. Ohashi, Y., Nakai, S., Tsukamoto, T., Masumori, N., Akaza, H., Miyanaga, N., Kitamura, T., Kawabe, K., Kotake, T., Kuroda, M., Naito, S., Koga, H., Saito, Y., Nomata, K., Kitagawa, M., & Aso, Y. (2002). Probiotics and urinary tract infections: A review. *Urologia Internationalis*, 68(4), 273–280.
56. Wada, M., Nagata, S., Saito, M., Shimizu, T., Yamashiro, Y., Matsuki, T., Asahara, T., & Nomoto, K. (2010). The effects of probiotics on the immune response in cancer patients. *Supportive Care in Cancer*, 18(6), 751–759.
57. Chen, X., Liu, X., Tian, F., Zhang, Q., Zhang, H., Zhang, H., & Chen, W. (2012). The role of probiotics in cancer prevention and treatment. *Journal of Food Science*, 77(3), M9–M14.
58. Kuo, C. H., Wang, S. S., Lu, C. Y., Hu, H. M., Kuo, F. C., Weng, B. C., Wu, C. C., Liu, C. J., Tsai, P. Y., & Lee, T. C. (2010). Anti-cancer effects of probiotics: Mechanisms and applications. *Biochemistry Research International*.
59. Nagano, T., Otoshi, T., Hazama, D., Kiri, T., Umezawa, K., Katsurada, N., & Nishimura, Y. (2019). The role of probiotics in the treatment of gastrointestinal cancer. *OncoTargets and Therapy*, 12, 3619.
60. Mendoza, L. (n.d.). *Oncology Reviews*.
61. Helmink, B. A., Khan, M. W., Hermann, A., Gopalakrishnan, V., & Wargo, J. A. (2019). The role of the microbiome in cancer immunotherapy. *Nature Medicine*, 25(3), 377–388.
62. Zhang, Z., Tang, H., Chen, P., Xie, H., & Tao, Y. (2019). The role of microbiome in cancer therapy: A review. *Signal Transduction and Targeted Therapy*, 4, 41.
63. Heydari, Z., Rahaie, M., Alizadeh, A. M., Agah, S., Khalighfar, S., & Bahmani, S. (2019). Probiotics and their antimicrobial activities: A review. *Probiotics and Antimicrobial Proteins*, 11(4), 1155–1162.
64. Sobhani, I., Tap, J., Roudot-Thoraval, F., Roperch, J. P., Letulle, S., Langella, P., Corthier, G., Van Nhieu, J. T., & Furet, J. P. (2011). Microbiota and colorectal cancer: A role for probiotics? *PLoS ONE*, 6(6), e16393.
65. Kahouli, I., Tomaro-Duchesneau, C., & Prakash, S. (2013). Probiotics and their role in the prevention of cancer: A review. *Journal of Medical Microbiology*, 62(8), 1107–1123.
66. Śliżewska, K., Markowiak-Kopeć, P., & Śliżewska, W. (2021). Probiotics and their effects on cancer. *Cancers*, 13(1), 20.
67. Shi, L., Sheng, J., Wang, M., Luo, H., Zhu, J., Zhang, B., Liu, Z., & Yang, X. (2019). Therapeutic potential of probiotics in cancer treatment. *Theranostics*, 9(12), 4115.

68. Chang, J. C. (n.d.). *Medicine*.
69. Motevaseli, E., Dianatpour, A., & Ghafouri-Fard, S. (2017). The relationship between probiotics and cancer treatment. *International Journal of Molecular and Cellular Medicine*, 6(2), 66.
70. Saxami, G., Karapetsas, A., Lamprianidou, E., Kotsianidis, I., Chlichlia, K., Tassou, C., Zoumpourlis, V., & Galanis, A. (2016). The role of probiotics in cancer therapy. *Journal of Functional Foods*, 24, 461–471.
71. Church, S. E., & Galon, J. (2015). Microbiome and immune modulation in cancer. *Immunity*, 43(4), 631–633.
72. Kaur, I. P. (2020). *Probiotic Research in Therapeutics: Volume 1: Applications in Cancers and Immunological Diseases*. Springer Nature.
73. Golemis, E. A., Scheet, P., Beck, T. N., Scolnick, E. M., Hunter, D. J., Hawk, E., & Hopkins, N. (2018). Molecular mechanisms of cancer risk in familial cancer syndromes. *Genes & Development*, 32(12), 868–902.
74. Poon, S. L., McPherson, J. R., Tan, P., Teh, B. T., & Rozen, S. G. (2014). The impact of genomic alterations on cancer prognosis: A study of colon cancer. *Genome Medicine*, 6(1), 1–14.
75. Srinivas, N., Malla, R. R., Kumar, K. S., & Sailesh, A. R. (2021). The role of probiotics in female-specific cancers. In *A Theranostic and Precision Medicine Approach for Female-Specific Cancers* (pp. 249–262). Elsevier.
76. Williams, K., & Sobol, R. W. (2013). The role of mutations in cancer: A review. *Mutation Research*, 1.
77. Apás, A. L., González, S. N., & Arena, M. E. (2014). Probiotic strains and their effects on human health: A review. *Anaerobe*, 28, 8–12.
78. Pithva, S. P., Dave, J. M., & Vyas, B. R. M. (2015). Probiotic therapy in cancer: A review. *Annals of Microbiology*, 65(3), 1373–1379.
79. Sankarapandian, V., Venmathi Maran, B. A., Rajendran, R. L., Jogalekar, M. P., Gurunagarajan, S., Krishnamoorthy, R., Gangadaran, P., & Ahn, B. C. (2022). The impact of probiotics on cancer treatment. *Life*, 12(1), 59.
80. Rhee, C. H., & Park, H. D. (2001). The effects of probiotics on the intestinal microbiota: Implications for cancer treatment. *Applied and Environmental Microbiology*, 67(7), 3445–3449.
81. Lankaputhra, W. E. V., & Shah, N. P. (1998). Probiotics and their role in cancer prevention. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 397(2), 169–182.
82. Średnicka, P., Juszczuk-Kubiak, E., Wójcicki, M., Akimowicz, M., & Roszko, M. (2021). The role of food additives in cancer prevention: A review. *Food and Chemical Toxicology*, 153, 112306.
83. Sharma, S., Singh, R. L., & Kakkar, P. (2011). The potential role of antioxidants in cancer therapy. *Food and Chemical Toxicology*, 49(4), 770–779.
84. Karimi Ardestani, S., Tafvizi, F., & Tajabadi Ebrahimi, M. (2019). Effects of herbal medicine on cancer treatment: A systematic review. *Human & Experimental Toxicology*, 38(10), 1069–1081.
85. Konishi, H., Fujiya, M., Tanaka, H., Ueno, N., Moriichi, K., Sasajima, J., Ikuta, K., Akutsu, H., Tanabe, H., & Kohgo, Y. (2016). Intestinal microbiota as a potential therapeutic target for gastrointestinal cancer. *Nature Communications*, 7, 12365.

86. Kadirareddy, R. H., Vemuri, S. G., & Palempalli, U. M. D. (2016). Probiotic effects on cancer therapy. *Asian Pacific Journal of Cancer Prevention*, 17(5), 3395–3403.
87. Joo, N. E., Ritchie, K., Kamarajan, P., Miao, D., & Kapila, Y. L. (2012). Probiotics and oral cancer: A review. *Cancer Medicine*, 1(3), 295–305.
88. Asoudeh-Fard, A., Barzegari, A., Dehnad, A., Bastani, S., Golchin, A., & Omid, Y. (2017). The therapeutic potential of probiotics in cancer. *BiolImpacts: BI*, 7(3), 193.
89. Zhang, G., Zhang, J., Wang, X., Yang, W., Sun, Z., Kumar, C. N., Guan, H., & Guan, J. (2014). Probiotics and their effects on oral health: A review. *Journal of Applied Oral Science*, 22(4), 282–286.
90. Zhang, M., Wang, F., Jiang, L., Liu, R., Zhang, L., Lei, X., Li, J., Jiang, J., Guo, H., & Fang, B. (2013). The role of probiotics in cancer prevention: A systematic review. *Cancer Prevention Research*, 6(8), 686–694.
91. Stashenko, P., Yost, S., Choi, Y., Danciu, T., Chen, T., Yoganathan, S., Kressirer, C., Ruiz-Tourrella, M., Das, B., & Kokaras, A. (2019). Probiotics in cancer therapy: Current knowledge and future directions. *mSystems*, 4(2), e00323-19.
92. La Rosa, G. R. M., Gattuso, G., PEduLLà, E., Rapisarda, E., Nicolosi, D., & Salmeri, M. (2020). Probiotic supplementation in cancer patients: A clinical study. *Oncology Letters*, 19(3), 3045–3058.
93. Li, X., He, S., & Ma, B. (2020). Advances in cancer immunotherapy: A review. *Molecular Cancer*, 19(1), 1–16.
94. Parzych, K. R., & Klionsky, D. J. (2014). An overview of the autophagy machinery: A review. *Antioxidants & Redox Signaling*, 20(3), 460–473.
95. Yang, Z. J., Chee, C. E., Huang, S., & Sinicrope, F. A. (2011). The role of autophagy in cancer therapy: A review. *Molecular Cancer Therapeutics*, 10(9), 1533–1541.
96. Jogalekar, M. P., Veerabathini, A., & Gangadaran, P. (2021). The role of probiotics in cancer treatment. *Experimental Biology and Medicine*, 246(2), 207–212.
97. Tran, S., Fairlie, W. D., & Lee, E. F. (2021). Probiotics and their potential role in cancer therapy. *Cells*, 10(6), 1522.
98. Wang, H., Cheng, X., Zhang, L., Xu, S., Zhang, Q., & Lu, R. (2019). The role of dietary factors in cancer prevention: A review. *Food & Function*, 10(11), 4102–4112.
99. Kim, Y., Oh, S., Yun, H. S., Oh, S., & Kim, S. H. (2010). Probiotic effects on human health: A review. *Letters in Applied Microbiology*, 51(2), 123–130.
100. Bednarczyk, M., Zmarzły, N., Grabarek, B., Mazurek, U., & Muc-Wierzegoń, M. (2018). Probiotic potential of lactic acid bacteria in cancer therapy: A review. *Oncotarget*, 9(1), 34413.
101. Pithva, S. P., Ambalam, P. S., Ramoliya, J. M., Dave, J. M., & Vyas, B. R. M. (2015). Nutritional strategies for cancer prevention: A review. *Nutrition and Cancer*, 67(7), 1142–1150.
102. Azam, R., Ghafouri-Fard, S., Tabrizi, M., Modarressi, M. H., Ebrahimzadeh-Vesal, R., Daneshvar, M., Mobasheri, M. B., & Motevaseli, E. (2014). Probiotics and cancer: A review. *Asian Pacific Journal of Cancer Prevention*, 15(10), 4255–4259.
103. Motevaseli, E., Shirzad, M., Akrami, S. M., Mousavi, A. S., Mirsalehian, K., & Modarressi, M. H. (2013). The effects of probiotics on cancer treatment: A review. *Journal of Medical Microbiology*, 62(7), 1065–1072.
104. Taherian-Esfahani, Z., Abedin-Do, A., Nouri, Z., Mirfakhraie, R., Ghafouri-Fard, S., & Motevaseli, E. (n.d.). Probiotics in cancer prevention: A review. *Iranian Journal of Cancer Prevention*.

105. Sharaf, L. K., Sharma, M., Chandel, D., & Shukla, G. (2018). Probiotics and their role in cancer therapy. *BMC Cancer*, 18(1), 1–13.
106. Hosseini, S. S., Goudarzi, H., Ghalavand, Z., Hajikhani, B., Rafeieiatani, Z., & Hakemi-Vala, M. (2020). Probiotics and cancer prevention: A review. *Iranian Journal of Microbiology*, 12(4), 424.
107. Kim, H. R., Kim, H., Jung, B. J., You, G. E., Jang, S., & Chung, D. K. (2015). The role of probiotics in cancer therapy. *Molecules and Cells*, 38(2), 163.
108. Chandel, D., Sharma, M., Chawla, V., Sachdeva, N., & Shukla, G. (2019). Probiotics in cancer therapy: Mechanisms and applications. *Scientific Reports*, 9(1), 14769.
109. Datta, N., Chakraborty, S., Basu, M., & Ghosh, M. K. (2021). The potential of probiotics in cancer treatment: A review. *Cells*, 10(1), 46.
110. Sharma, M., & Shukla, G. (2016). Probiotics: A novel approach for cancer treatment. *Frontiers in Microbiology*, 7, 1940.
111. Kanwal, R., Gupta, K., & Gupta, S. (2015). Cancer epigenetics: Risk assessment, diagnosis, treatment, and prognosis. In *Cancer Epigenetics: Risk Assessment, Diagnosis, Treatment, and Prognosis* (pp. 3–25). Springer. https://doi.org/10.1007/978-3-319-19087-1_1
112. Sharma, M., & Shukla, G. (2020). Probiotics and their therapeutic role in cancer treatment. *Frontiers in Oncology*, 10, 746.
113. Fahmy, C. A., Gamal-Eldeen, A. M., El-Hussieny, E. A., Raafat, B. M., Mehanna, N. S., Talaat, R. M., & Shaaban, M. T. (2019). Nutritional factors in cancer therapy: A review. *Nutrition and Cancer*, 71(5), 688–700.
114. Kumar, M., Hemalatha, R., Kumar, R., Nagpal, R., Devraj, J. P., Verma, V., Behare, P., Mal, G., & Singh, B. (2013). The role of epigenetics in cancer therapy: A review. *Medical Epigenetics*, 1(2), 60–69.
115. Ardito, F., Giuliani, M., Perrone, D., Troiano, G., & Lo Muzio, L. (2017). The role of oxidative stress in the pathogenesis of cancer: A review. *International Journal of Molecular Medicine*, 40(2), 271–280.
116. Kannaiyan, R., & Mahadevan, D. (2018). The role of probiotics in cancer therapy: A review. *Expert Review of Anticancer Therapy*, 18(12), 1249–1270.
117. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5), 646–674.
118. Lu, K., Dong, S., Wu, X., Jin, R., & Chen, H. (n.d.). Probiotics and cancer therapy: A review. *Frontiers in Oncology*.
119. Seth, A., Yan, F., Polk, D. B., & Rao, R. K. (2008). Probiotics in gastrointestinal health and disease: A review. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 294(6), G1060–G1069.
120. Şener, D., Bulut, H. N., & BAYIR, A. G. (2021). The therapeutic potential of probiotics in cancer treatment. *Bezmialem Science*, 9(4), 490–497.
121. Escamilla, J., Lane, M. A., & Maitin, V. (2010). Dietary factors in cancer prevention and treatment: The role of probiotics. *The FASEB Journal*, 24(3), 928–921.
122. Escamilla, J., Lane, M. A., & Maitin, V. (2012). The impact of probiotics on cancer: A review. *Nutrition and Cancer*, 64(7), 871–878.
123. Yue, Y. C., Yang, B. Y., Lu, J., Zhang, S. W., Liu, L., Nassar, K., Xu, X. X., Pang, X. Y., & Lv, J. P. (2020). Probiotics as a therapeutic strategy for cancer: A review. *Microbial Cell Factories*, 19(1), 1–12.
124. Braga, V. (2016). Metabolic reprogramming and cancer. *Current Opinion in Cell Biology*, 42, 138–145.

125. Li, X., Wang, H., Du, X., Yu, W., Jiang, J., Geng, Y., Guo, X., Fan, X., & Ma, C. (2017). The role of probiotics in cancer treatment: A review. *Oncology Reports*, 38(3), 1561–1568.
126. Zamberi, N. R., Abu, N., Mohamed, N. E., Nordin, N., Keong, Y. S., Beh, B. K., Zakaria, Z. A. B., Nik Abdul Rahman, N. M. A., & Alitheen, N. B. (2016). The role of probiotics in cancer therapy: A review. *Integrative Cancer Therapies*, 15(1), NP53–NP66.
127. Le Noci, V., Guglielmetti, S., Arioli, S., Camisaschi, C., Bianchi, F., Sommariva, M., Storti, C., Triulzi, T., Castelli, C., & Balsari, A. (2018). Probiotics and cancer immunotherapy: A review. *Nutrients*, 10(10), 1403.
128. Li, J., Sung, C. Y. J., Lee, N., Ni, Y., Pihlajamäki, J., Panagiotou, G., & El-Nezami, H. (2016). The impact of probiotics on cancer therapy: A review. *Proceedings of the National Academy of Sciences*, 113(5), E1306–E1315.
129. Hussell, T., & Bell, T. J. (2014). The role of probiotics in lung cancer therapy. *Nature Reviews Immunology*, 14(2), 81–93.
130. Azad, M., Kalam, A., Sarker, M., & Wan, D. (n.d.). The role of probiotics in cancer therapy: A review. *BioMed Research International*.
131. Sekine, K., Toida, T., Saito, M., Kuboyama, M., Kawashima, T., & Hashimoto, Y. (1985). Effects of probiotics on cancer growth: An experimental study. *Cancer Research*, 45(3), 1300–1307.
132. Lammers, K. M., Helwig, U., Swennen, E., Rizzello, F., Venturi, A., Caramelli, E., Kamm, M. A., Brigidi, P., Gionchetti, P., & Campieri, M. (2002). The role of probiotics in gastrointestinal cancer therapy. *The American Journal of Gastroenterology*, 97(5), 1182–1186.
133. Link-Amster, H., Rochat, F., Saudan, K. Y., Mignot, O., & Aeschlimann, J. M. (1994). Probiotics in the treatment of cancer: A review. *FEMS Immunology & Medical Microbiology*, 10(1), 55–63.
134. Fukushima, Y., Kawata, Y., Hara, H., Terada, A., & Mitsuoka, T. (1998). Probiotics and their effects on cancer: A review. *International Journal of Food Microbiology*, 42(1), 39–44.
135. Park, J. H., Um, J. I., Lee, B. J., Goh, J. S., Park, S. Y., Kim, W. S., & Kim, P. H. (2002). Probiotics in cancer therapy: Mechanisms of action. *Cellular Immunology*, 219(1), 22–27.
136. Yan, F., & Polk, D. B. (2011). Probiotics in gastrointestinal health and disease: A review. *Current Opinion in Gastroenterology*, 27(5), 496–501.
137. Kato, I., Kobayashi, S., Yokokura, T., & Mutai, M. (1981). Probiotics and cancer prevention. *Gann = Gan*, 72(5), 517–523.
138. Cruchet, S., Furnes, R., Maruy, A., Hebel, E., Palacios, J., Medina, F., Ramirez, N., Orsi, M., Rondon, L., Sdepanian, V., Xóchihua, L., Ybarra, M., & Zablah, R. A. (2015). Probiotics and cancer: A review. *Pediatric Drugs*, 17(3), 199–216.
139. Borriello, S. P., Hammes, W. P., Holzapfel, W., Marteau, P., Schrezenmeier, J., Vaara, M., & Valtonen, V. (2003). Probiotics and their role in gastrointestinal health: A review. *Clinical Infectious Diseases*, 36(6), 775–780.
140. Cohen, P. A. (2018). The importance of probiotics in cancer therapy. *JAMA Internal Medicine*, 178(11), 1577–1578.
141. Yelin, I., Flett, K. B., Merakou, C., Mehrotra, P., Stam, J., Snesrud, E., Hinkle, M., Lesho, E., McGann, P., McAdam, A. J., Sandora, T. J., Kishony, R., & Priebe, G. P. (2019). The role of probiotics in cancer therapy. *Nature Medicine*, 25(11), 1728–1732.

142. Redman, M. G., Ward, E. J., & Phillips, R. S. (2014). The role of probiotics in cancer treatment: A review. *Annals of Oncology*, 25(10), 1919–1929.
143. Cesaro, S., Chinello, P., Rossi, L., & Zanesco, L. (2000). Probiotics in cancer therapy: A review. *Supportive Care in Cancer*, 8(6), 504–505.
144. Henry, S., D'Hondt, L., André, M., Holemans, X., & Canon, J. L. (2004). Probiotics and their role in cancer prevention: A review. *Acta Clinica Belgica*, 59(4), 220–222.
145. Ledoux, D., Labombardi, V. J., & Karter, D. (2006). The role of probiotics in cancer therapy: A review. *International Journal of STD & AIDS*, 17(4), 280–282.
146. Oggioni, M. R., Pozzi, G., Valensin, P. E., Galieni, P., & Bigazzi, C. (1998). Probiotics and their role in cancer prevention. *Journal of Clinical Microbiology*, 36(2), 325–326.
147. Pochard, P., Gosset, P., Grangette, C., Andre, C., Tonnel, A. B., Pestel, J., & Mercenier, A. (2002). Probiotics and their role in allergy and cancer therapy. *Journal of Allergy and Clinical Immunology*, 110(4), 617–623.



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Toxicology of Probiotics: Challenges and Future Prospects

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ABSTRACT

Toxicology studies the adverse effects of substances on living organisms. When it comes to probiotics, which are live microorganisms consumed for their potential health benefits, extensive research has been conducted to assess their safety. Probiotics have generally been found to have a low risk of toxicity. These bacteria have co-evolved with humans, and their safety profile has been established through long-term exposure. The most commonly used probiotic strains, such as *Lactobacillus* and *Bifidobacterium* species, have been extensively studied for their safety. Clinical trials and animal studies have shown that these strains are generally well-tolerated, with few reported adverse effects. However, it's important to note that rare cases of infection or septicemia (bloodstream infection) associated with probiotic use have been reported, particularly in individuals with compromised immune systems or those with indwelling medical devices. Overall, probiotics have demonstrated a favorable safety profile. However, it's always recommended to consult with a healthcare professional before starting any new dietary supplement, especially for individuals with specific medical conditions or compromised immune function.

12.1 INTRODUCTION

Numerous research studies have established data in favor of the medicinal value and intermittent use of probiotics, and these data demonstrate a positive relationship between humans and probiotics. In the current scenario, probiotics are harmonizing with human health because when customary food is unable to meet the basic demands of the body, alternative supplements in the form of pills and powders are prescribed. This practice is not new to us; in an earlier era, instead of additive supplements, food quality was improved biologically. The Romans and Greeks were experts in this, and

they used curd, which is well recognized and accepted as a super source of probiotics and is consumed globally now. The well-known *Lactobacillus* bacteria help not only in the preparation of curd but also positively affect the gut health of the host in many ways, increasing immunity and reducing the risk of various ailments [1]. The main advantage of probiotics is that they can replace or balance harmful bacteria with beneficial ones in the digestive tract. Similarly, lactic acid bacteria (LAB) are commercially available and used as food additives in some dairy and fruit juice products that confer the above-mentioned characteristics [2–6]. Data shows that numerous well-characterized strains of the two most commonly prescribed microorganisms, namely *Lactobacilli* and *Bifidobacteria*, recover the vigor of humans as well as animals by balancing or modulating the gut microflora. This potential characteristic of probiotics seems beneficial in the most emerging situations, which degrade the health of the gastrointestinal (GI) tract, such as the development of antibiotic resistance in some disease-causing strains of microorganisms. The intervention of probiotics motivates and increases the immune profile, improves the bioavailability of nutrients, antagonizes lactose intolerance, and decreases other risk-related ailments [7].

It is well-documented and recognized that gut microorganisms are very diverse and colonize over 400 cultivable species in the human digestive system. This colonization starts just after birth, and their representation, numbers, and species are affected by factors like the mode of delivery of a child, hygienic conditions, and the use of antibiotics. These factors directly show a remarkable sway on the colonization of microorganisms in the human gut. Initially, the dominant position in the gut was occupied by mostly two species, namely *Escherichia coli* and *Streptococcus*, but the representation of these species decreases in breastfed infants, and the microorganism species *Bifidobacteria* was found to increase. After weaning and following the second year of birth, a modification occurred in adult flora and it remains somewhat stable during the whole life of human beings [8], and it seems interesting, but gut microbes indeed have a population density gradient nearly ranging from 10^{11} – 10^{12} /ml [9].

In 1965, Lilley and Stillwell coined the term “probiotic” from Greek literature, which means “for life.” The purpose of Lilley and Stillwell was to describe the material produced by one microorganism that provoked another microorganism to grow [10]. In 1974, they modified the term and described it as “organisms or substances that donate to the organism’s intestinal microbial balance” [11, 12]. Knowledge of these “wonder” microorganisms is increasing year by year, and the beginning of the 20th century should be

known as the “modern probiotics era,” as the 20th-century scientific community showed keen interest in the application of probiotics in the treatment of a variety of ailments. This foundation was established by the Russian scientist Elie Metchnikoff when he first reported the concept that the health of a diseased gut can be improved by replacing harmful bacteria that promote toxin production with beneficial ones [13].

Probiotics are now defined by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) as “live microorganisms that, when given in sufficient amounts, benefit the host’s health” [14]. None of the microorganisms fall into the category of probiotics unless they possess certain confirmed characteristics, like having adequate gastric acid and bile salt stability, adhering to the mucosa of the intestine, and the unique ability to colonize the intestinal tract. These unique characteristics make probiotics deliver maximum therapeutic action [11, 15]. Likewise, other functional foods are now considered probiotics due to their traditional value and their capability to confer positive physiological effects in humans. The nutritive value of a functional food can be improved by adding numerous minerals, essential vitamins, prebiotics, and probiotics. These components of functional food are the add-ons of chewing gum, fermented yogurts and milk, sugarless sweets, infant meals, and sports drinks [16].

12.2 GENERAL BACKGROUND OF PROBIOTICS

Beginning in the early 1900s, Elie Metchnikoff, a Russian scientist working at the Pasteur Institute in Paris, undertook important research on probiotics. The discoveries would subsequently earn him the Nobel Prize. Unlike Metchnikoff, who originally sought to investigate whether microorganisms were harmful to human health, Louis Pasteur identified the bacteria responsible for the fermentation process. Metchnikoff proposed that the aging process is influenced by toxins produced by bacteria in the large intestine, which are then absorbed into the bloodstream. He specifically referred to these bacteria as putrefying bacteria, which we now identify as proteolytic clostridia. Metchnikoff believed that these harmful bacteria in the gut could be counteracted by introducing beneficial microbes. He recognized that the composition of intestinal flora is influenced by our diet, and therefore, it is possible to modify the microbial balance in our bodies by adopting specific measures. This sentence describes clearly the “probiotic concept.” Metchnikoff regarded lactobacilli as probiotics (also known as “probiotics,”

which are beneficial to the host's life rather than antibiotics); probiotics may improve health and slow the aging process. Metchnikoff's scientific hypothesis aided in the establishment and growth of France's dairy industry, with the use of fermented milk made from *Bacillus bulgaricus*, the first in Europe. He explained that rural Bulgarians' regular consumption of fermented dairy products like yogurt contributed to their extended life expectancy. He drew a comparison between this and the Bulgarian bacillus, which had been discovered by Stamen Grigorov, a physician from that country who was 27 years old. Later on, he suggested that lactobacilli could lessen the putrefactive effects of gut metabolism that accelerated disease and aging [17].

In order to specify the term probiotics, a proficient accord document had been circulated in 2013. The document describes the meaning as well as proper utilization of probiotics, and according to this, "probiotics are deliberate health benefits to a host when administered in calibrated quantities." According to the accord document, contemporary technologies select some useful bacterial strains in fermented products such as yogurt and others, which have great nutritional and organoleptic properties, and these fermented products are used or considered the prime functional food products [19, 151].

In one of his documents, Hippocrates mentioned in respect of functional food that "let food be your medicine and medicine be your food." Hippocrates advocated for cheese offered to athletes because of its nutritional importance. So, the perception of functional food is not new to us; it has been practiced since Hippocrates in the 4th century. Before the idea of probiotics, these fermented products were the source of functional food, as stated in folk stories, and after this, a revolution occurred that provoked the use of probiotics in fermented products like milk and yogurt. Now, functional foods have been considered as food ingredients or complete foods that show optimistic properties through their nutritional importance on host well-being. Functional food, fermented with specific bacteria, and fermented milk with probiotic bacteria have developed. It is difficult to determine how and when humans started to utilize fermented food, but archaeological surveys have established a connection and found that fermented products were already used in almost all civilizations. Archaeologists have produced a handful of evidence indicating that some fermented beverages were utilized as early as 7000 BC and 5000 BC in the Neolithic village of Jiahu in China and Mesopotamia, respectively. Archaeological reports also state that Asians produced fermented products mainly from rice, whereas fruits and beverages were the choices of ancient Egypt and Mesopotamia. Furthermore, fermented milk products were deliberately utilized by Romans, Egyptians,

Hindus, and Greeks around 3000 and 2000 BC, and this is also mentioned in holy documents of Hinduism as well as in the Holy Bible. These practices are confirmed through the written and artistic representations of the specified era. Additionally, it is stated in the Holy Bible, with the first reference found in Genesis (18:1–8), that “veal with buns and sour milk was offered to the Lord by Abraham,” whereas Prophet Mohammad offered fermented cow’s milk (kefir) to the mountaineers of the Caucasus. *Naturalis Historica*, written by Pliny, is evidence in which Pliny says he was offered tasty acidic milk by Barbarians, and he endorsed the utilization of this acidic milk in the treatment of gastrointestinal infections. Similarly, Francis I from France prescribed yogurt for those suffering from diarrhea, as recommended by a physician sent by Suleiman “The Magnificent” in the 16th century. Furthermore, earlier in Italy, laborers were offered a drink prepared by adding lemon to milk and water to produce coagulation of the milk. It was a belief among Italians that this kind of fermented preparation helps to manage intestinal infections in the laborers who work in extreme heat and dusty environments. One more interesting custom occurred in the ancient era: Gamelio, a cup used as a wedding gift, was used to wash out genital organs after intercourse, and the derived liquid was then drunk with the belief of implanting the germs [20].

Not only this, a surgical team from Colorado, led by Eiseman and a co-worker, reported in 1950 on fecal bacteriotherapy, a specialized probiotic therapy mainly conducted with stool obtained from a healthy host, also known as fecal or stool transplantation. In this therapy, a washed suspension of feces from a healthy donor is administered either by enema or by endoscopy into the colon of a targeted recipient (patient). Eiseman and co-workers treated four patients with fulminant pseudomembranous enterocolitis using this therapy and achieved effective results [21]. However, a Chinese physician, namely Ge Hong, in the fourth century, used stool suspension administered orally for the treatment of food poisoning and loose motion. In the sixteenth century, “yellow soup” and “golden syrup” were introduced by a Chinese physician who administered these with fresh, dried, and fermented stool to manage abdominal diseases. It is very interesting to note that an Italian anatomist, Fabricius Aquapendente (1533–1619), introduced “transformation.” In this veterinary practice, rumen fluid from healthy cattle was introduced into similar species of cattle that had lost their ruminating efficiency. This practice of “transformation” is currently in vogue for ruminating cattle. This practice helps to produce good bacterial colonies in the host animal’s gastrointestinal tract after the feeding of the rumen excised from healthy ones.

Similarly, in the last century, stool samples were transplanted into chickens to protect them from salmonella infection. Furthermore, observations of native Bedouins describe that German soldiers in Africa, during World War II, consumed fresh dung from camels for the prevention and treatment of dysentery. It is also notable that the dung only works if it is consumed fresh, and at this level, it is highly loaded with the bacteria *Bacillus subtilis*.

In 2013, two important publications are the topic of discussion. In the first publication, it is reported that the fecal transplantation described by Eiseman was significantly utilized due to its ease of procedure and greater efficacy with *Clostridium difficile* infection [22]. Again, in 2013, the first randomized controlled trial's results were published by the *New England Journal of Medicine*. In this trial, the fecal transplant patient group was compared with the vancomycin-treated patient group. The results concluded that approximately 94% of patients in the fecal transplant group recovered from recurrent disease in a single treatment, compared to the vancomycin-treated group, which was able to recover only one-third of the patients. However, this trial was stopped early, claiming that it was unethical to proceed further [23]. Earlier, probiotics were only considered safe to utilize as food fermenters aimed at improving food preservation [24]. As a result, some probiotic strains are consistently employed in various edible food products, such as kombucha, miso, kefir, ice cream, kimchi, dairy desserts, tempeh, yogurt, sauerkraut, fermented milk, and cheese among various ethnicities and cultures [25–27].

If we talk about the current scenario, a wealth of research data states the health-promoting benefits of these probiotic strain-fermented products, describing that such types of fermented products have complex communities of microbes, and the health benefits they provide are due to the substances they produce after fermentation. Despite this, individuals allergic to specific foods or dairy products, such as cow's milk, lactose intolerance, vegetables, and conditions like high cholesterol, are prescribed non-dairy functional food and beverage alternatives to dairy supplements/food [25]. Probiotics have long been celebrated for their potential health benefits, but ongoing research has generated a fair share of controversy. It's not uncommon for scientific endeavors to face controversies, but amidst all the debate, numerous reports have been published that delve into the positive aspects of probiotics when it comes to addressing gastrointestinal issues and promoting overall gut health in humans. These published reports persistently investigate and strive to establish the beneficial effects of probiotics on individuals with weakened or compromised gastrointestinal tracts. While controversies may exist in

the field, the abundance of research continues to shed light on the potential advantages of incorporating probiotics into healthcare practices aimed at improving gut health. It is important to recognize that the understanding of probiotics and their impact on human health is an evolving field. Ongoing research will continue to refine our knowledge and provide a clearer understanding of the benefits and limitations of probiotics. By considering the existing body of published reports, we can gain valuable insights into the potential therapeutic applications of probiotics for individuals with gastrointestinal concerns [28].

These controversies not only occurred in this current scenario, but ancient physicians also did not agree with the unsafe utilization of probiotics. Some physicians of the ancient era did not recommend animal milk as food for human beings. Hippocrates and Galen documented milk with great dietetic importance, even then advising it only for medicinal purposes, not for food. This dogma was feasible as it was bridged with environmental and climatic status, likewise Mediterranean environmental conditions not allowing the intake of subtle products like milk. Despite later reasons, the unavailability of hygienic levels and preservation techniques were some other important reasons [20]. Not only do environmental conditions and ethnic diversification, but a physiological and immunological framework of the consumer also decide the efficacy of the probiotics. It is stated that diversification in humans affects the response to alimentary supplements, drugs, and food, and these responses are also concerned with gender, age, supplementary illness, and host factors [29, 30].

Furthermore, many reports have been published suggesting the hazardous effects of probiotics, such as in diseased compromised situations like infection issues in patients undergoing cardiac surgery [31], anaphylactic allergic responses in hypersensitive individuals [32], systemic fungemia in elderly immunocompromised individuals [33], and the risk of horizontal gene transfer (HGT) of antibiotic resistance to pathogenic microbes [34]. Even then, numerous research data claim probiotics' ameliorative role in immunity [35], heart-related ailments [36], cancer management [37], some metabolic disorders like type-II diabetes and obesity [38–39], and in some neurodegenerative diseases like depression and anxiety [40, 41]. Furthermore, a lot of published data confirms that the effects of the probiotic are strain specific and a single microbial strain has multifactorial beneficial effects, but probiotic strain-specific effects significantly vary within the species [42–43], and there are some countable species of probiotics including bacteria like *Lactococcus*, *Leuconostoc*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*,

Lactobacillus casei, *Lactobacillus acidophilus* group, *Pediococcus*, *Propionibacterium*, *Bifidobacterium*, *Bacillus coagulans*, *Escherichia coli* strain Nissle 1917, various enterococci, and the yeast *Saccharomyces boulardii* as some of the most prevalent probiotics currently utilized [28, 44]. Despite this, the researchers reported that they are still trying to fabricate engineered probiotics, and they are facing many safety-related issues and targeted drug delivery challenges. Because of this, many statements are quoted in various publications indicating that both engineered probiotics and traditional probiotics must be screened for their safety concerns in their long-term utilization in gastrointestinal treatment. These collaborative statements provide a revolutionary path, resulting in numerous clinical trials being conducted concerning the use and benefits of probiotics. When we came across this review idea and searched for probiotics as a drug, we were surprised to find that we are lagging in concrete data that proves the safety aspects of probiotics as a drug. Surprisingly, the aforementioned products are still advertised boldly without declaring convenient data, such as what types of formulations are suitable, what their efficacy is, and what the parameters for consumer safety are [45].

We can say that mistakes are part of research because the traditional use of existing probiotics continues without conducting resultant clinical trials that may monitor both the safe side and adverse events of earlier probiotics. For this reason, these probiotics are only utilized in naturally fermented food products and have not been considered drugs until now [46].

In terms of clinical application, the oral [47–48] and gut [49–50] applications are still the only progressive parts of probiotics that have been available for use. The concomitant application of these probiotic supplements took a runway when the *Dietary Supplement Health and Education Act, 1994* by the US Food and Drug Administration (USFDA) granted permission for the marketing and sale of these as over-the-counter “health supplements.” Hence, they are still available without undergoing quality clinical trials and underprivileged malleable laws [51].

12.3 SAFETY PARAMETERS OF PROBIOTIC SELECTION

In the current scenario, when it comes to the effective treatment of gastrointestinal ailments, the application of probiotics is paramount due to its utmost potential to confer a favorable gastrointestinal milieu [52]. We should consider the changes that have occurred within our bodies and surroundings.

Although it is well documented in publications that the consumption of probiotics is safe, this statement seems questionable when we consider the changes in the environmental and genetic makeup of human beings and the family of bacteria itself. Suitable clinical approaches must ensure the design of both retrospective and prospective studies, which take into account the overall changes and their consequences. There are many practices pending that may help in the utilization of probiotics as established remedies. When we clinically approve a pharmaceutical preparation, a significant amount of clinically relevant data is utilized to prove the preparation safe for human use; however, we have already discussed that we still lack sufficient evidence to prove probiotics as a safe preparation.

It is a well-known fact that all pharmaceutical preparations intended for use in living organisms undergo quality trials and are persistently monitored throughout their utilization. The definition of probiotics suggests that “these are live microorganisms that provide health benefits to the host when administered in adequate amounts,” so probiotics must be provided within the threshold level, but the host’s condition is again a topic of concern. From this point of view, all case studies, such as cohort, case-control, cross-sectional, observational studies, etc., must be conducted to assess the efficacy as well as the toxicity of the “adequate amount” of probiotics. There are some reports concerning severe infections related to the use of the most popular strains of *Lactobacilli* and *Bifidobacteria*. This concern has raised a question mark on the safety of probiotics and left a question about their toxicity.

Toxicity is something that separates the consumable substance from its safe effective dose. Toxicological studies for any prescribed substances have always been a great focus on their deleterious effects on living systems: cellular level, subcellular level, tissue level, organ level, and organ system level. It is well recognized that the effects of any consumable pharmaceutical preparation are proportional to the dose; after all, water can be toxic if consumed in excessive amounts. Similarly, the venom of a snake could show positive therapeutic effects if delivered below its toxic level [53]. So, precisely, it is crucial to discuss the selection criteria for probiotics.

12.3.1 COMMON SELECTION CRITERIA OF PROBIOTICS

At the outset, human well-being is the point of concern, so the prescribed probiotics must be non-toxic for consumers, deprived of any antibiotic-resistant gene transfer. For this reason, fabricated newly engineered

probiotics are typically prohibited [54]. To achieve maximum benefit, the minimum viable numbers ($\sim 10^6$ CFU/g) must be achieved. Yet, among the most widely utilized probiotics, *Lactobacillus* and *Bifidobacterium* require favorable environments since they are more sensitive to aerobic and high-temperature conditions. Probiotics also have to withstand bile and stomach acid throughout the gastrointestinal shift [55]. Probiotics may need to cling to the mucus layer and colonize the colon in order to be effective, even if they enter the colon alive. As a result, it could be difficult to synthesize probiotics for targeted delivery to the intestines.

Bacteriocins may serve as a “colonizing peptide,” “killing peptide,” and “signal peptide” to gain an advantage over competing bacteria by promoting the colonization of the generating strain in the gut. Importantly, there are still several issues that need to be resolved to quantify probiotics, including dynamic monitoring and on-site localization. The current feces-examining techniques cannot keep up with the rapidly expanding gut microbiota studies [56]. New techniques are therefore desperately needed, such as the ability to image gut bacteria and fluorescently mark them. Last but not least, more accurate *in vitro* and *in vivo* models must be developed to assess how well probiotics or probiotic delivery systems work. Probiotics may have promising futures in the food and pharmaceutical industries by resolving these issues [57].

12.3.2 MONITORING OF PROBIOTICS STRAIN

Comprehensive genome sequencing serves as the basis for evaluating the safety of any specific probiotic strain. This enables the strain to be assigned to a specific taxonomic group, permitting examination of the species’ published dangers. Having access to a whole genome sequence also makes it possible to identify strains at the individual level, which is useful for tracking strains during production and determining the cause of infections that may be suspected. Additionally, the genome can be searched for any troublesome genes, such as those that are toxic, pathogenic, or antibiotic-resistant (AR). The possibility for probiotic-borne AR genes to spread to nearby potential pathogens, other microorganisms the host is harboring, and/or ambient microbes, thus increasing the ecological pool of antimicrobial resistance genes, is one cause for concern [58–60].

The use of the precise probiotic strain, which has been demonstrated to have the intended therapeutic activity, is essential for achieving successful

and repeatable clinical results. For instance, it has been discovered that *Lactobacillus rhamnosus* GG prevents viral gastroenteritis and keeps ulcerative colitis in remission. It is not safe to presume that *L. rhamnosus* strains will behave similarly. Doctors can be certain of getting similar outcomes if they opt to utilize the precise strain that caused the effects in clinical studies [61, 62]. It is possible that using a strain that is closely related will have no effect. Use the precise strain that was tested whenever possible because alternative strains, even those that are closely related, might not produce the same results [63].

12.3.3 SELECTION OF QUALITY PROBIOTICS

Establishing purity, potency (the number of live microorganisms delivered), and final product composition are all safety-related concerns regarding the manufacture of probiotic products. Additionally, probiotic goods must undergo sufficient testing for potential pollutants that are tailored to the intended purpose. The presence of unwanted living microbial contaminants is particularly concerning. Probiotics present a higher risk of contamination with pathogenic or potentially pathogenic bacteria than products that undergo an intentional sterilization process because they are intended to be supplied as living microbes [58–60].

Products can undergo testing that is more rigorous than that used for the general public, with products intended for more vulnerable populations subject to different testing specifications. Concerns about allergies and other pollutants, as well as microbial contamination of the finished product, apply to all medical interventions, not just probiotics. The quantity of live organisms in probiotic foods and supplements is the only factor used to determine the dosage. Clinical investigations that used between 10^7 and 10^{11} live bacteria per day were successful [64, 65]. Dairy appears to function as the bacteria's perfect transport medium, improving their ability to survive in the upper GI tract [66].

12.4 ADMINISTRATION OF PROBIOTICS

Furthermore, ensuring the safety of probiotic administration or consumption is of utmost importance. It involves the careful and appropriate handling of the product, developed through rigorous procedures. Safely administering probiotics requires precise manipulation or preparation on-site, along with

selecting the appropriate route for delivering the probiotic to the host. The products should be administered in a dosage and final formulation that have been extensively tested and verified as secure. It is crucial to consider the well-being of the host when determining the safety of the probiotic, taking into account any co-existing health conditions or underlying medical issues. These considerations go beyond mere production and presentation factors. For example, when the probiotic is opened and administered within a hospital environment, one potential risk is the contamination of the surroundings and vulnerable patients. Therefore, precautions must be taken to prevent cross-contamination and protect the health of individuals who may be more susceptible to adverse effects [67]. A dry probiotic that was mixed in hospital rooms has caused intravenous catheter infections [68]. The Propatria study raised the issue that some formulations given to critically ill patients may not be appropriate for nasojunal delivery [69].

12.5 ADVERSE EVENT MONITORING AND PROBIOTICS EDUCATION

It is crucial to put in place surveillance mechanisms that make it easy to report adverse events (AEs) for each dose delivered in order to increase the safety of probiotic intake. Hospital formularies should also exclude probiotic products that do not fully identify the probiotic microorganism (including genus, species, and strain) and maintain potency over the course of the product's shelf life.

By establishing surveillance systems, healthcare providers can monitor and track any adverse events associated with probiotic usage. This enables early detection and analysis of potential safety concerns, allowing for prompt intervention and mitigation of risks. The reporting of AEs per administered dose offers useful information for assessing the general safety profile of probiotics. Additionally, it is essential that the formulation of probiotic products clearly identifies the probiotic bacteria, including its genus, species, and strain, as well as provides details regarding its potency for the duration of the product's shelf life. This ensures transparency and allows healthcare professionals to make informed decisions regarding the selection and usage of probiotic products.

Additionally, sharing decisions about the use of probiotic products should be facilitated by educating those who supply medical, nutritional, and health-care information on the possible dangers and advantages of administering a

probiotic product in particular circumstances [69, 70]. There is no established procedure in place for reporting probiotic adverse effects, despite the fact that they are uncommon and probiotics are often used. Probiotics' safety in immune-compromised individuals has been questioned, despite the fact that the majority of research did not discover a statistically significant increase in adverse events compared to controls [72].

S. boulardii fungemia and bacteremia with *Lactobacillus* have both been reported in solitary case reports. According to case-review research, patients with severe illnesses are more likely to get sepsis, liver abscesses, and endocarditis from *Lactobacillus GG* [73]. The same study looked at *S. boulardii* fungemia and found several cases. Several of these cases were linked to ingesting *S. boulardii*, but others were believed to be brought on by potential cross-contamination of central lines since the drug capsules had been opened and the lyophilized fermented yeast was permitted to get airborne. Once more, most cases, but not all, included people who were immune suppressed [72, 74]. Given this finding, it is advisable to prevent probiotic use in people who are immunosuppressed or seriously unwell [63].

12.6 DRUG INTERACTION

Last but not least, before the prescription of any standard probiotics, the prior drug history of patients or recipients must be considered. Many drugs may interact with the prescribed probiotics and can change the effect of the same. Reports suggest that alcohol and antibiotics have a deleterious impact on *Lactobacilli* and *Bifidobacteria*. Some strains of *Lactobacillus acidophilus* may impact the metabolism of sulfasalazine, chloramphenicol palmitate, and phthalylsulfathiazole, while there is no proof that bacteria affect how well most medications work [75, 76].

12.7 TOXICITY LINKING TO METABOLIC ACTIVITIES

No hazardous substances should be produced by probiotics. Additionally, certain enzymes that could aid in invasion or translocation through the epithelium or in the colonization of some host tissues must not be present. Examples of these enzymes include certain glycosides and proteolytic enzymes. Generally speaking, these hazardous actions have not been discovered in the conventional probiotic strains; however, the new strains need to be evaluated [77]. Importantly, toxic chemicals called secondary bile

acids are produced by intestinal bacteria acting on bodily fluids. They may demonstrate carcinogenicity by influencing the mucous-secreting cells and encouraging their growth, or they may act as catalysts for the development of cancer [78]. Conjugated bile acids can be broken down by a variety of gut bacteria, including *Bifidobacterium* and *Lactobacillus* species [79].

In the evaluation of safety, platelet aggregating activity has been regarded as a necessary test. It is believed that bacterial platelet aggregation contributes to the development of infectious endocarditis. *Bifidobacterium* sp. has a higher ammonia absorption activity than other intestinal flora bacteria while having a lower deaminase activity, which is responsible for producing ammonium from amino acids. Probiotics' ability to produce D(-)-lactate has been linked to an increased risk of developing acidosis. Some probiotic organisms, particularly those in the *Lactobacillaceae* family, produce D(-)-lactic acid. According to Fabian et al. [80], impaired metabolism and excretion factors linked to chronic renal failure and liver illness, respectively, are also thought to be factors leading to the development of acidosis. Additionally, it needs to be noted that the recorded cases of D-lactic acidosis have only occurred in young children whose intestinal tracts have undergone significant surgical modifications; these cases are typically connected to short bowel syndrome and involve the direct consumption of D-lactate rather than the administration of D-lactic acid-producing bacteria [81].

12.8 TOXICITY INVOLVED TO IMMUNE SYSTEMS, GIT

According to studies, probiotics may modify a person's immune response, improve vaccination response, or change the natural history of the allergic response. Probiotic bacteria may affect both the local immune response and the local release of cytokines, as well as humoral, cellular, and nonspecific immunological responses [82]. It is possible that probiotic interventions could have a negative immunomodulatory effect because of the role that intestinal microbiota plays in immunological development. When administered parenterally, peptidoglycans from certain gram-positive bacteria, including lactobacilli, can have adverse consequences such as fever, arthritis, cardioangitis, hepatobiliary lesions, and autoimmune diseases [83]. The cytokines that cause these side effects have been extensively documented to be stimulated by various probiotics [84, 85].

Chronic inflammatory problems of the intestinal tract known as inflammatory bowel diseases (IBD) increase mortality and occasional morbidity. The

effectiveness of probiotics in lowering active disease or extending remission in Crohn's disease (CD) and ulcerative colitis (UC) has been examined, but no benefit has been seen [86–88]. Sepsis caused by a strain of *Lactobacillus rhamnosus* was documented in a patient with severe colitis [89]. Therefore, before considering the prescription of probiotics in active UC, care is recommended. Probiotic use may harm gastrointestinal physiology due to the generation of potentially harmful metabolites, particularly in patients with short small bowel syndrome [90]. Theoretically, probiotic bacteria could result in malabsorption because they deconjugate bile salts [79]. Therefore, there may be a higher chance of developing colon cancer [91].

Probiotics have been shown to have some inhibitory effects on colon cancer in animal models [92, 93], but there is no epidemiologic or clinical evidence to support this claim [94]. The development of lactic acidosis and the formation of D-lactate are two additional hypothetically possible toxicities. Humans with an ileostomy who were in good health were used in studies. It has been demonstrated that conjugated bile acid can be converted into harmless secondary salts by *L. acidophilus* and *Bifidobacterium* species [95]. The conjugated bile acid metabolites may build up in people with short small bowel syndrome and cause malabsorption [96]. This could increase the risk of lactate accumulation and, theoretically, the chance of colon cancer. Additionally, it is theoretically possible that intestinal mucus may degrade [97].

12.9 PROBIOTICS IN THE AT-RISK POPULATION: SAFETY, EFFECTIVENESS, AND RELATED RISKS

Probiotics have been used for decades to both prevent and treat a variety of diseases. It has been difficult to ensure the safety of probiotic applications due to a lack of comprehension of the mechanisms behind their activity. The viability of the microorganisms is a crucial criterion to be deemed a probiotic [98]. Particularly in at-risk populations, the safety of probiotics needs to be assessed on a strain-by-strain approach summarized in Table 12.1, including patients requiring critical care, newborns (especially premature), people with short bowel syndrome, the elderly, those with heart valve disease, those who have central venous catheters, and those with compromised immune systems, such as those who have AIDS or are on immunosuppressive therapy [99, 100] (Figure 12.1).

There are four potential risks associated with probiotics that should be taken into consideration: (a) the development of diseases such as bacteremia

or endocarditis; (b) metabolic or toxic effects on the gastrointestinal tract; (c) an upsurge in bacterial resistance to antibiotics in the gastrointestinal tract; and (d) gene transfer [99–102]. Intrinsic toxicity, intrinsic variation, product misidentification, product mislabeling, contamination, and adulteration are some of the potential risks associated with probiotics. The likelihood that this mold was probiotic product contamination prompted us to speculate about microbiological criteria for probiotic products used for at-risk populations.

Sepsis in a cardio-surgical patient [103], systemic fungemia in elderly immunocompromised patients [33], anaphylaxis allergic reactions in people with sensitivities [32], and the threat of antibiotic resistance caused by horizontal gene transfer (HGT) to microbial pathogens [34] are among the various problems associated with the use of probiotics. The majority of individuals believe that probiotics are completely safe and extensively effective; thus, this is a cause for concern. This is undoubtedly not the case, and it would be more beneficial to present the truth, given that it seems that adverse events in probiotic randomized clinical trials (RCTs) are not adequately documented [105]. Figure 12.1 depicts the risk assessment of probiotics in various population types.

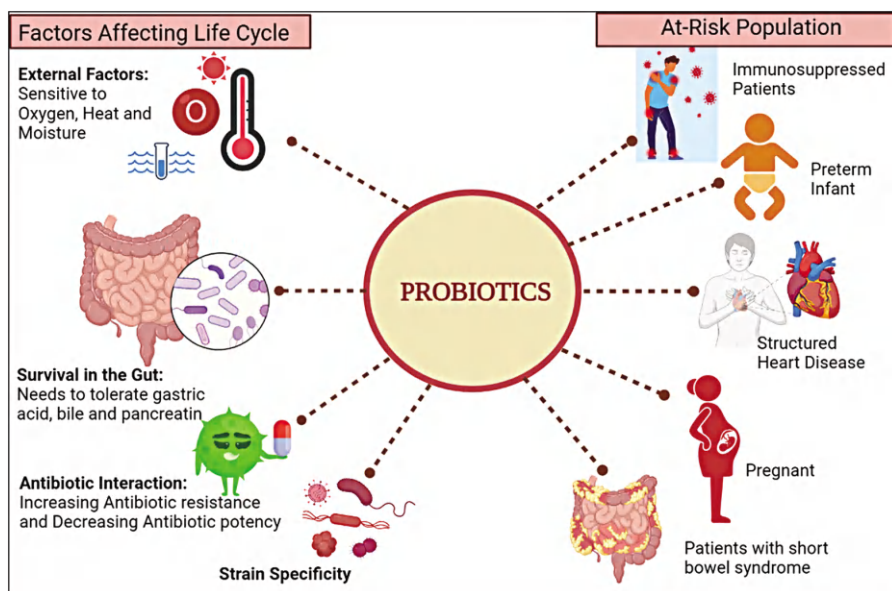


FIGURE 12.1 Factors that affect the viability of probiotics and the populations that is potentially at risk for adverse events with the use of probiotics.

Source: Created with BioRender

TABLE 12.1 According to the FDA Following Populations are Potentially at Risk for Adverse Events with the Use of Probiotics

Sl. No.	Population	Examples
1.	Immunosuppressed	Cancer chemotherapy or radiotherapy; injectable immunosuppressive medications for autoimmune diseases; corticosteroids (more than 0.5 mg per kg body weight of prednisone or its equivalent); anti-rejection therapy after a solid organ or stem cell transplantation.
2.	Structural heart disease	Valvular dysfunction or replacement due to an abnormality, along with a medical history of endocarditis.
3.	Pregnant women	
4.	Potential for translocation of probiotics across bowel wall	The following conditions may impact the patient's situation: an ongoing bowel leak, acute abdomen, active intestinal disease (such as colitis), significant bowel dysfunction, presence of neutropenia (or expected neutropenia following chemotherapy), and the administration of radiation therapy.
5.	Preterm Infant	

12.10 STUDIES ON THE SAFETY OF PROBIOTICS

Probiotics are classified and regulated by the relevant regulatory authorities as dietary supplements rather than pharmaceuticals or biological products. Extensive attention has been given to assessing the safety profiles of different probiotics, considering various genera, species, and strains. Additionally, their suitability for diverse individuals and populations at risk has been widely studied and acknowledged [45, 107]. The following features are the aspects of probiotic safety [108, 109]:

1. Human origin should be preferred for probiotic strains intended for human consumption.
2. They ought to be kept far from a healthy human digestive system.
3. They shouldn't have a background that makes them pathogenic.
4. They shouldn't be connected to conditions like infectious endocarditis or digestive issues.
5. They shouldn't hydroxylate or decongest bile salts in the small intestine.
6. They shouldn't contain genes that make them resistant to antibiotics.

To evaluate the safety of probiotic strains, three methods are commonly employed: examining the inherent characteristics of the strain, conducting

pharmacokinetic studies (including survival and activity in the intestine, dose-response relationships, and assessment of fecal and mucosal recovery), and investigating interactions between the strain and the host. In order to accurately anticipate the benefits and potential adverse effects of probiotics, it is crucial to comprehend their ability to survive the gastrointestinal tract, their translocation and colonization properties, as well as the fate of their active components [110]. Using fecal collection, intestinal intubation, and colonic biopsy procedures, the pharmacokinetics of probiotics can be assessed *in vivo* [111–113].

Globally, there are no regulatory requirements to evaluate the safety of probiotic-containing food supplements. In the United States, the European Union, and EU nations, various strategies are available [109]. Probiotic products should adhere to microbiological requirements for the optimum safety of vulnerable patients. Before recommending probiotics to high-risk patients, test results for the product should be available for inspection. To ensure compliance, manufacturers need to deliver this outcome or take part in a program for third-party verification [114]. A list of the fundamental safety requirements for every probiotic under consideration is supplied, along with recommendations for any further safety information that would be required on an individual basis (Figure 12.2) [115].

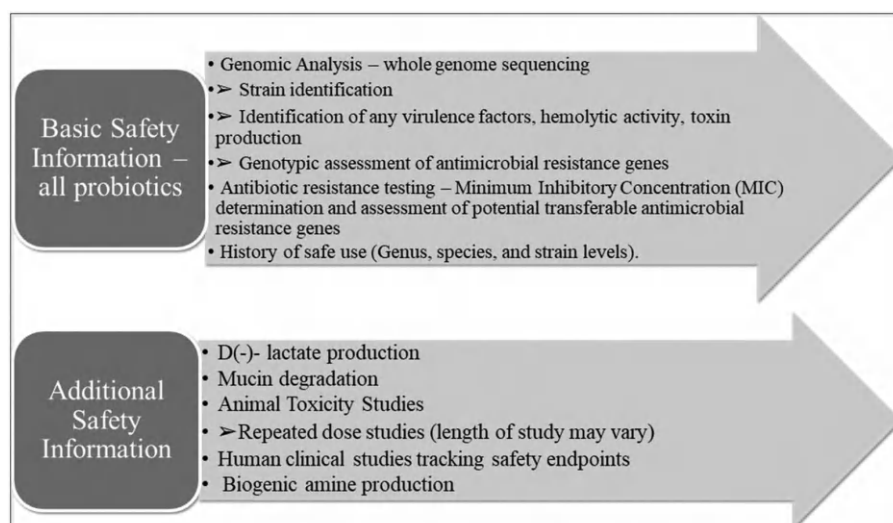


FIGURE 12.2 List of essential parameters of fundamental safety assessment for a probiotic.

12.11 SYSTEMIC INFECTIONS

Numerous case reports indicate infections caused by probiotic-strain-compatible organisms in people who had taken probiotics before developing symptoms. Fungemia is the most frequently reported single occurrence, and there have been at least 33 instances of individuals who have taken the probiotic *Saccharomyces boulardii* having blood cultures with *Saccharomyces cerevisiae* or *Saccharomyces boulardii* present [116–123]. *Lactobacilli*, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus GG*, have been linked to eight cases of bacteremia [124–127].

The use of combination probiotics, *S. boulardii* [*S. cerevisiae*], *Lactobacillus GG*, *Bacillus subtilis*, or *S. boulardii* individually or in nine cases of overt sepsis has been documented [128–134]. Probiotics containing *Lactobacillus* and *Streptococcus* have both been linked to endocarditis occurrences. Additionally, *Lactobacillus rhamnosus*-related abscesses have been documented twice [135, 136].

12.12 DELETABLE METABOLIC ACTIVITIES

The PRO PATRIA study, a randomized controlled trial conducted in a double-blind, placebo-controlled manner, reported that individuals receiving probiotic treatment exhibited an increased risk of mortality associated with intestinal ischemia. On the other hand, previous trials involving patients with pancreatitis who received a combination of lactic acid bacteria and fiber, known as symbiosis, have shown a reduction in septic complications, the need for surgical intervention, and the occurrence of infected necrosis. Two metabolic concerns related to probiotic bacteria include the deconjugation of bile salts and the potential impact of D-lactate produced by these bacteria [81, 137–141].

12.13 OVERSTIMULATION OF THE IMMUNE SYSTEM IN SUSCEPTIBLE PEOPLE

Probiotics have shown the ability to modulate both the innate and adaptive immune systems, affecting factors like cytokine secretion and dendritic cell function. While these effects have the potential to contribute to an overactive immune response, leading to autoimmune conditions or inflammation, it is important to note that no reports of such issues have been documented in human subjects [143, 144].

12.14 GENE TRANSFER

Tetracycline, erythromycin, chloramphenicol, lincosamide, macrolide, streptomycin, and streptogramin resistance genes are present on plasmids in lactic acid bacteria [145–147]. To date, there is no clinical evidence documenting the transmission of antimicrobial resistance, despite the theoretical possibility of horizontal gene transfer between probiotic microbes and other species residing in the gut or other areas of the body. However, potential risks associated with unrestricted probiotic consumption have been outlined in Table 12.2.

12.15 SAFETY PARAMETERS OF PROBIOTICS

Clinicians, researchers, and policymakers have expressed concerns regarding the safety of probiotic organisms. These concerns can be broadly categorized into three areas: probiotic strain, product quality, and probiotic administration [152]. A comprehensive examination of a specific probiotic strain's safety necessitates obtaining its complete genome sequence. The taxonomic classification of the strain depends on this sequence, which also enables the evaluation of the species' stated threats. A detailed genome sequence analysis also makes it easier to identify strains at the individual level, which is essential for tracking the strain during manufacturing and investigating potential illnesses. Furthermore, the genome analysis allows for the identification of any potentially worrisome genes, such as those linked to toxin production, pathogenicity, or antibiotic resistance [153].

One issue is the hypothetical possibility that probiotics' antibiotic resistance (AR) genes could be passed on to localized pathogens, other microorganisms living in the host, or environmental bacteria. This transfer may potentially boost the ecosystem's total number of antimicrobial resistance genes [114]. Scientists are still assessing the actual risk of these transfers as well as any potential clinical and public health repercussions. Phenotypic testing, which assesses the observable characteristics of the probiotic strain, is also an important component in evaluating its safety.

When it comes to the formulation of probiotic products, there are additional safety considerations. It is essential to determine the final product's composition, potency (the quantity of live microorganisms supplied), and purity. Moreover, probiotic products must undergo thorough testing, tailored to their intended use, to identify and address potential contaminants [114].

TABLE 12.2 Risks Related to Probiotic Consumption Through Diets

Category	Microorganisms	Population	Adverse Effects	Mechanisms	References
Transmissible antibiotic-resistance genes	<i>Lactobacillus</i> , <i>Pediococcus</i> , and <i>Leuconostoc</i> sp.	-	Bloating, abdominal pain	Vancomycin resistance → <i>Staphylococcus aureus</i>	[45]
	<i>Lactobacillus</i> sp.	-	Cough, trouble breathing	Vancomycin resistance → <i>Enterococcus faecalis</i>	[148]
	<i>Lactobacillus</i> sp.	-	Dizziness, rashes, hives	Broad spectrum antibiotic resistance (vancomycin, streptomycin, gentamicin, aztreonam, and ciprofloxacin) → <i>Staphylococcus</i> sp.	[149]
Systemic infection	<i>Lactobacillus rhamnosus</i> GG	Small gut syndrome in preterm-infant	Bacteremia or Sepsis (Short gut syndrome & cardio surgery)	<i>Lactobacillus</i> bacteremia	[150]
	<i>L. rhamnosus</i> GG	Children in critical condition who have antibiotic-related diarrhea	Acute pediatric diarrhea	Sepsis	[45, 149]
	<i>L. rhamnosus</i> GG	Small gut syndrome in 11-month-old infant	Bacteremia or Sepsis (Short gut syndrome & cardio surgery)	<i>Lactobacillus</i> bacteremia	[114]
	<i>L. rhamnosus</i> GG	Boy, Age 17, suffering from ulcerative colitis	Bacteremia or Sepsis	<i>Lactobacillus</i> bacteremia	[45]
	<i>Lactobacillus</i> sp.	Immunocompetent 58-year-old with mechanical ventilation	Cough, trouble breathing, dizziness, rashes, hives	<i>Lactobacillus</i> bacteremia and sepsis	[151]
	Three strains of <i>L. rhamnosus</i>	24-year-old female cardiosurgical patient	Intermittent hypoxia, cardiomyopathy	Probiotic sepsis	[150]
	<i>Bifidobacterium longum</i>	Polymetastatic prostatic adenocarcinoma in a 74-year-old individual	Abdominal pain, headache, nasopharyngitis	<i>Bifidobacterium</i> bacteremia	[151]

TABLE 12.2 (Continued)

Category	Microorganisms	Population	Adverse Effects	Mechanisms	References
	<i>B. longum</i> subspecies <i>infantis</i>	Pre-term infants	Vomiting, constipation	<i>Bifidobacterium</i> bacteremia	[45]
	<i>B. longum</i>	Low birth-weight infants	Regurgitation, flatulence	<i>Bifidobacterium</i> bacteremia	[151]
	<i>B. breve</i>	Two-year-old boy with acute lymphoblastic leukemia (Philadelphia chromosome)	Blood infections, intestinal blockage	<i>Bifidobacterium</i> sepsis	[152]
	<i>Escherichia coli</i> NISSLE strain 1917	Pre-term infants	Fever, irritability, flatulence, diarrhea	Severe sepsis	[151]
	<i>Saccharomyces cerevisiae</i>	Diabetic 48-year-old with comorbidities	Chronic diarrhea, feeding intolerance, migraine-like headaches	Toxic megacolon, fungemia, septic shock	[45]
	<i>S. cerevisiae</i> var. <i>boulardii</i>	73-year-old immunocompromised patient on chemotherapy	Bloating, flatulence, candidemia, constipation	Fungemia	[148]
	<i>S. cerevisiae</i> var. <i>boulardii</i>	8-year-old in intensive care unit	Systemic candidiasis, flatulence	Fungemia	[114]
	<i>S. cerevisiae</i> var. <i>boulardii</i>	Premature newborns on enteral feeding	Candidemia, sepsis, bloating	Fungal septicemia	[45]
	<i>S. cerevisiae</i> var. <i>boulardii</i>	Critically ill patients	Flatulence, candidemia, constipation	Fungemia	[150]
	<i>L. plantarum</i>	30-year-old male with rheumatic valve disease	Cardiomyopathy, Infective Endocarditis	Endocarditis	[152]
	<i>L. casei</i>	53-year-old immunocompetent patient	Fever blisters, allergies, eczema, and dermatitis	Endocarditis	[45]
	<i>L. jensenii</i>	47-year-old immunocompetent patient	Cold, flu, respiratory infection	Endocarditis	[45]
	<i>L. paracasei</i>	77-year-old male with prostate cancer	Rash, itching, swelling, dizziness, trouble breathing	Endocarditis	[45]

TABLE 12.2 (Continued)

Category	Microorganisms	Population	Adverse Effects	Mechanisms	References
Localized infection	<i>L. acidophilus</i>	48-year-old with cardiovascular conditions after dental restoration	Skin rash, hives, dizziness, breathing issues	Endocarditis	[45]
	<i>L. rhamnosus</i>	>65-year-old with hemorrhagic telangiectasia (HHT)	Bacteremia or Sepsis	Endocarditis	[45]
	<i>L. casei</i>	60-year-old renal transplant patient	Fever blisters, allergies, eczema, dermatitis	Intra-abdominal abscess	[114]
	<i>L. paracasei</i>	65-year-old diabetic patient	Skin rash, hives, swelling	Bacteremia, liver abscess	[152]
	<i>L. rhamnosus</i>	11-month-old trisomy 21 patient with viral respiratory symptoms	Short gut syndrome, pneumonia	Probiotic-associated pneumonia	[148]
Metabolic disturbances	<i>Lactobacillus</i> spp.	-	Skin rash, hives, dizziness, trouble breathing	Unregulated bile salt hydrolase (BSH) activity	[45]
	<i>L. lactis</i>	-	-	Biogenic amine production	[151]
Allergic response	<i>L. rhamnosus</i> GG	Children (up to 4 years)	Skin rash, hives, swelling, dizziness, trouble breathing	Allergic rhinitis, asthma	[114]
	<i>L. acidophilus</i> LAVRI-A1	Infants (6–12 months)	Diarrhea, constipation, skin rash, gas, bloating	Atopic sensitization	[152]
	<i>L. rhamnosus</i> GG	Breastfeeding women and pregnant women (4–6 weeks before delivery)	Skin rash, hives, breathing issues	Wheezing bronchitis, atopic sensitization	[45]
Bowel ischemia	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. salivarius</i> , <i>L. lactis</i> , <i>B. bifidum</i> , and <i>B. lactis</i>	Individuals with acute pancreatitis	Shortness of breath, pancreatic inflammation	Increased local oxygen demand	[45, 148]

The potential existence of unexpected live microbial contamination in probiotics is one specific area of concern. There is a higher danger of contamination with pathogenic or possibly pathogenic germs because probiotics are intended to be ingested as live microorganisms, unlike products that undergo intentional sterilization processes [154]. Figure 12.3 describes the evaluation techniques of probiotics.

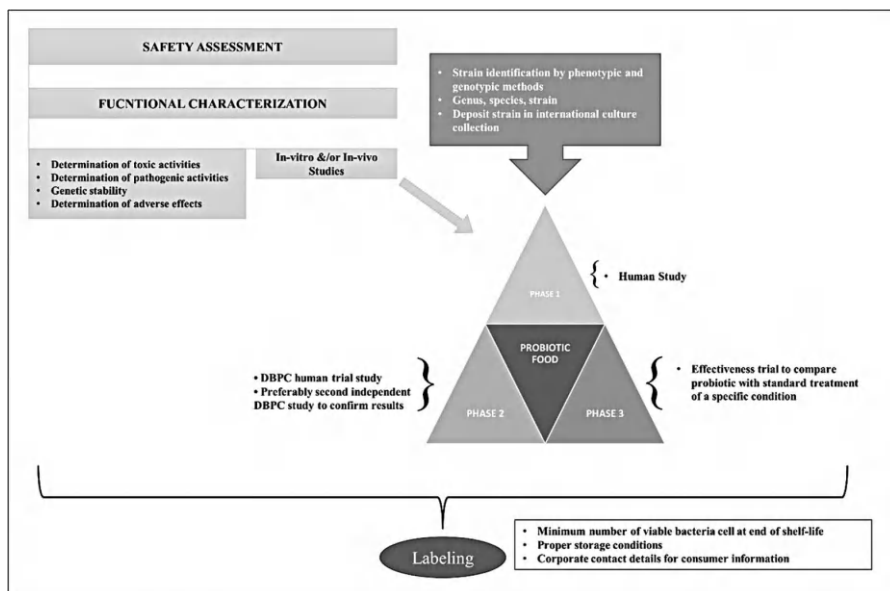


FIGURE 12.3 The systematic method for probiotics evaluation. (Abbreviation: DBPC, Double-blind, randomized, placebo-controlled).

Testing requirements can be customized for probiotic products, with more vulnerable populations requiring stricter and more comprehensive testing compared to the general population. Probiotic safety problems cover a range of topics, including microbial contamination in the finished product, the presence of allergies or other contaminants, and ensuring that the intended recipients are administered safely [114]. However, these concerns are not unique to probiotics and apply to any intervention. Safe administration of probiotics involves ensuring that the product is designed and delivered in a manner that is safe for the host. This includes selecting the appropriate route of administration and correctly manipulating or preparing the probiotic on-site [114]. Products should be administered at a safe dose and in a final formulation that has been proven to be safe. Additionally, the probiotic's

safety for the host needs to be considered in light of any existing health conditions or underlying concerns. These considerations go beyond the formulation and production process [155].

For instance, when probiotics are opened and utilized on-site, one administration concern is the possibility of cross-contamination in the hospital setting and among susceptible patients. In hospital rooms, improper mixing of a dried probiotic has resulted in intravenous catheter infections. The PROPATRIA study raised concerns about the nasojejunal delivery of several formulations for critically ill patients [156]. Implementing surveillance systems that allow the reporting of adverse events based on the administered dose will increase the safety of consuming probiotics. Furthermore, if probiotic product formulations don't correctly identify the probiotic microorganism (including genus, species, and strain) and maintain the product's efficacy throughout its shelf life, they should be removed from hospital formularies. Additionally, educating medical providers, nutritionists, and healthcare professionals about the potential risks and benefits of administering probiotic products in specific situations can facilitate informed decision-making and promote shared decision-making regarding their use [152].

12.16 ACUTE AND LONG-TERM SAFETY CONCERNS FOR PROBIOTICS

12.16.1 EVALUATION OF THE COMPOSITION OR FUNCTION OF THE MICROBIOTA

Microbiome profiling involves evaluating the initial composition, community structure, transcriptional patterns, and metabolic outputs of the microbiota. By analyzing the microbiome before and after probiotic consumption, researchers can explore the potential causal relationship between probiotics and microbiome modulation. This study has the ability to produce theories about the microbial systems and pathways that support health, while also highlighting potential security issues and differentiating the traits of people who respond or do not respond to probiotic therapy. Probiotics' propensity to have positive effects on particular hosts can be understood and partially predicted by microbiome analysis [157].

Microbiome profiling studies are not commonly conducted in probiotic research, but there are notable instances where such studies have been performed. These studies have revealed that the inherent characteristics of

the microbiome play a role in determining the integration of probiotics into the microbial communities of individuals. This suggests that assessing the microbiome could serve as a valuable tool for tailoring probiotic therapy and enhancing clinical outcomes [158].

For example, a study involving the supplementation of *Lactocaseibacillus rhamnosus* GG in children with cystic fibrosis demonstrated that individuals with a fecal microbiota dominated by bifidobacteria exhibited improved clinical outcomes. This indicates that the baseline microbiome composition can influence the effectiveness of probiotic interventions. In another study, probiotic administration facilitated the maturation of the microbiota in preterm infants, leading to reduced incidences of sepsis and inflammation. This highlights the potential of probiotics to promote beneficial changes in the microbiome, resulting in positive health outcomes [159]. These examples emphasize the significance of microbiome profiling in understanding the interactions between probiotics and the host microbiota, potentially enabling the customization of probiotic therapy and optimizing its effectiveness in clinical settings.

12.16.2 HORIZONTAL GENE TRANSFER (HGT) IN ANTIBIOTIC RESISTANCE

The introduction of the term “probiotic” into regulatory discussions, as established by the FAO/WHO expert consultation and guidelines, has drawn substantial attention from regulators, industry, and researchers to the potential risk of horizontal transfer of antibiotic resistance (AR) genes from probiotics to gut pathogens [107]. *In vivo* studies have provided evidence of the transferability of antibiotic resistance (AR) genes within the gut, with viable probiotic bacteria serving as potential donors. Multiple papers have substantiated these findings, and recent studies have concentrated explicitly on the transferability of AR genes, particularly those linked to antibiotics that the WHO has identified as being of vital importance. Employing both genotypic and phenotypic methods is necessary to evaluate the danger of AR genes in probiotic genomes. The phenotypic approach entails evaluating the strain’s AR gene expression utilizing methods like minimum inhibitory concentration experiments [160]. It is important to differentiate between intrinsic resistance, which is present in the species naturally, and acquired resistance through gene expression. Intrinsic resistance, such as the observed “insensitivity” to vancomycin in heterofermentative lactobacilli, is generally

not considered a safety concern. Nevertheless, any phenotypic resistance that deviates from the expected patterns for a specific species warrants further investigation.

Establishing the normal ranges of antibiotic resistance (AR) for commonly used probiotic species has been crucial in evaluating their safety. However, for developing strains or next-generation strains where such information is lacking, additional studies are necessary [161]. The entire genome sequencing, including plasmids, is done as part of the genotypic technique for evaluating antibiotic resistance (AR), and known AR genes are located within the sequence. Before marketing the strain, care should be taken if these genes are discovered to be flanked by mobile genetic elements or encoded on plasmids. A detailed risk-benefit analysis of the strain, as well as the specific antibiotic and its therapeutic value, may potentially play a role in the decision. Safety factors must be carefully considered even when AR genes are chromosomally positioned and do not have characteristics that suggest movement. The risk is typically minimal if the resistance is linked to an antibiotic that is not clinically significant [162].

A good example is how *Bifidobacterium animalis* subsp. *lactis* BB-12 regulates the tetracycline resistance gene, *tet(W)*. The U.S. Food and Drug Administration (FDA) acknowledged that the gene's existence had no substantial clinical or environmental implications. As a result, the FDA preserved the strain's status as widely acknowledged as safe for use in infant formula. This conclusion was reached after taking into account a number of variables, such as the lack of a plausible transmission route, the prevalence of tetracycline resistance in microbial populations, and the sparse clinical use of tetracycline in neonates in the United States [163]. Similarly, the European Food Safety Authority evaluated whether *B. animalis* subsp. *lactis* has the *tet(W)* gene and came to the conclusion that there was no new information that justified changing the subspecies' qualified presumption of safety status. Even in the absence of the possibility of conjugal transfer for non-intrinsic antibiotic resistance genes, these choices were made. However, the potential for transmission by phage or transformation of bare DNA still exists [152]. Non-viable cells, including those that have passed away during creation or storage, can undergo transformation.

The regulation strategy for postbiotics, which are non-viable yet beneficial bacteria, reflects this concern about the potential transfer of antibiotic resistance genes from deceased microbes [164]. When assessing a pasteurized strain of *Akkermansia muciniphila* as a novel food, for instance, the European Food Safety Authority used a precautionary approach. According

to the applicant, the strain did not include any antibiotic resistance genes of concern, the evaluation panel reported. Based on an in-silico examination of the unique strain's genome antibiotic resistance genes and consideration of databases like the Comprehensive Antibiotic Resistance Database and the National Database of Antibiotic Resistant Organisms, this conclusion was reached [165].

Risk assessments about antibiotic resistance genotypes or phenotypes in probiotics are continuously evolving to keep pace with advancing scientific knowledge. The evaluation of whether to prioritize phenotype or genotype, gene presence, or transferability is subject to change as new research findings emerge. Additionally, certain resistance components that are of concern can be changed or eliminated via genetic editing approaches. This strategy may be suitable for probiotics designed for food applications (such as plasmid curing) or for next-generation probiotics that could follow the medication development pathway, depending on the specific technology used [166].

In light of these considerations, we support the current recommendations advocating for screening all probiotic strains for AR phenotypes and genotypes. Any phenotypic resistance that differs from the species average needs to be carefully examined. A thorough risk/benefit study is required for probiotics carrying genes linked to antibiotics that the World Health Organization has deemed vitally important, taking into account consumer and patient safety as well as public health issues [18]. Although modest, the possibility of DNA transmission through transformation calls for more research. Manufacturers of old strains that haven't gone through extensive risk evaluations for AR should reexamine their strains to make sure they comply with the most recent norms and regulations.

In order to protect patient, consumer, and public health, it is crucial to continue taking a proactive approach to addressing the AR hazards associated with probiotics. It is crucial to understand that the majority of natural strains of food fermentation microorganisms, which are consumed in huge amounts, both live and dead, as part of the diets of many communities, are not subject to the caution regarding probiotic-borne antibiotic resistance genes. Similarly, despite the high probability that mobilizable AR genes are present in every fecal preparation, microbial transplants from the intestine are not subject to particular constraints associated with AR genes. AR genes have been discovered in lactic acid bacteria, which are frequently employed in the manufacture of fermented foods. A further possibility is the horizontal transmission of AR genes from environmental microorganisms. The extent of the

existence of the AR gene in these bacteria is yet mainly unknown. Despite this, it is not currently required by law or science to limit the consumption of foods that have undergone fermentation [152].

12.16.3 *INVASIVE INFECTION BY MICROORGANISMS*

Probiotics have occasionally been discovered to move outside the digestive tract, causing invasive infections. To evaluate the risk of sepsis, bacteremia, and fungemia in children who utilize probiotics, a systematic review was conducted covering the period from 1995 to 2021. Among the 49 reported cases of invasive infections, sepsis was the most commonly observed condition. Most of the affected children were younger than two years old and had underlying conditions, including prematurity or the presence of an intravenous catheter in place. Notably, antimicrobial therapy was effective in 94% of these instances [168].

Determining the actual frequency of probiotic-related invasive infections is challenging due to several factors. Many of the reported cases occurred outside the context of clinical trials, making it difficult to ascertain the number of patients who received probiotics without experiencing adverse effects. Additionally, not all clinical laboratories routinely culture and identify probiotic organisms from blood cultures, further complicating the assessment. Furthermore, reporting of adverse events in probiotic trials is often incomplete, hindering a comprehensive understanding of the harms associated with their use [105].

As a result, we emphasize the importance of diligent monitoring and comprehensive reporting of invasive infections and sepsis in clinical settings as well as in all probiotic trials. Clinical isolates and the administered probiotic should be compared using strain-level molecular methods to improve comprehension and identification. A possible relationship between the probiotic and systemic infection is supported if a molecular match is discovered between the probiotic microorganism(s) given and invasive clinical isolate(s). The antibiogram, which shows how each commercially available probiotic strain responds to various antibiotics, must be disclosed and made public by manufacturers [169]. Healthcare providers need this knowledge in order to select the best empirical course of treatment when necessary. In addition, the potential for translocation should be carefully considered when assessing the safety of any next-generation probiotic, and risks and benefits should be carefully weighed.

12.16.4 INFLUENCE OF PROBIOTICS ON THE BIOAVAILABILITY AND EFFICACY OF DRUGS

Drug metabolism is significantly influenced by the gut flora, and this can have both direct and indirect effects on a drug's efficacy and toxicity. Several drug-microbiota interactions have been identified to include this interaction [170]. For instance, research done in the early 1970s showed that the activity of microbial azoreductase reduces the urine excretion of total sulfanilamide in rats treated with oral azo medications like Prontosil or Neoprontosil. This illustrates how the microbiota can mediate drug activation [171]. Other important drug metabolisms influenced by the microbiota include decarboxylation (e.g., L-dopa), sulfation (e.g., acetaminophen), dehydroxylation (e.g., caffeic acid and L-dopa), demethylation (e.g., methamphetamine), dehalogenation, and acetylation/diacylation (e.g., salicylic acid converting to aspirin). In addition to drug metabolism, the microbiota can also contribute to reducing drug-related toxicity. An example of this is glucuronidation, a process where glucuronic acid is linked to a substrate by UDP-glucuronosyl-transferase enzymes, resulting in the formation of hydrophilic and negatively charged glucuronides. The microbiota can play a role in hydrolyzing these conjugates and reducing their toxicity [172]. Overall, the complex interplay between the gut microbiota and drug metabolism has implications for drug efficacy, toxicity, and therapeutic outcomes. Further research in this field will help us better understand and utilize the potential of the microbiota in modulating drug responses [173].

β -glucuronidases, enzymes produced by many anaerobic bacteria, can deconjugate xenobiotics and endogenous compounds that have undergone detoxification through the glucuronidation pathway. This deconjugation procedure may result in a rise in the enterohepatic recirculation of toxins, hormones, and medications, as well as the creation of potentially cancer-causing substances in the gut. A higher risk of developing colon cancer has been linked to higher levels of β -glucuronidase activity [174]. However, it's crucial to keep in mind that normal enterohepatic recirculation of key substances like vitamin D, thyroid hormone, and estrogen depends on a specific amount of β -glucuronidase activity. These compounds rely on the deconjugation process to be effectively recycled within the body. Finding the right balance of β -glucuronidase activity is crucial, as excessive levels can be detrimental to health while inadequate levels may disrupt the metabolism of important compounds. Further research is needed to fully understand the role of β -glucuronidases in health and disease and to explore potential strategies for modulating their activity to optimize health outcomes.

A growing subject called toximicrobiomics or pharmacomicrobiomics studies the interactions between the microbiota and xenobiotic substances, including pharmaceuticals. The effect of probiotics on drug function may have safety consequences. Understanding how nutrition, drug disposition, and medication response interact with microbial diversity is essential because it could influence future personalized medicine methods [175]. It is crucial to carry out research to find drug-modifying enzymes and confirm their applicability *in vivo*. Although these enzymes can be shown to exist and operate in a probiotic in a test tube, this does not ensure that they will function in a host. Before the drug is absorbed, it is also important to determine whether these enzymes significantly reduce drug efficacy. To provide particular suggestions at this early point in the research would be premature. This field would advance with improvements in building screening techniques to find enzymes that might affect particular medications and creating databases of similar genetic sequences. The ultimate goal is to locate probiotics that include problematic enzymes so that potential probiotic-drug interactions can be advised [152].

12.16.5 LONG-TERM COLONIZATION

A probiotic microbe's capacity to permanently colonize the host is not necessary for it to be successful. Long-term colonization often refers to a microbe's continued presence in the host for several weeks or months after probiotic therapy has finished. This would imply that the probiotic microbe is actively replicating and has established a persistent presence inside the host for probiotics that are taken orally. Probiotics recovered from fecal samples, however, imply that the majority of probiotics do not colonize. This is probably because they can't outcompete the host's already-present resident microbiota [114].

The concept put forth by the renowned Dutch botanist and microbiologist Baas Becking in 1934, "Everything is everywhere, but the environment selects," aligns with this understanding. According to this concept, the mere encounter of a microbe by an individual does not necessarily lead to colonization [152]. However, if an "open niche" is available within the host, an externally introduced microbe may have the opportunity to occupy that niche and establish colonization. Probiotic strains that are "native" or especially suited to the host, as opposed to those that are developed for large-scale production fermenters, food fermentations, or shelf stability in retail environments, have drawn increasing research interest. Through a variety of techniques, it is hoped to improve the persistence of probiotic strains in humans and other host species [176].

These methods include manipulating the host's diet to create a favorable environment for the strain's growth, incorporating prebiotics that support the growth of the specific strain, utilizing computational techniques for more precise targeting of adapted strains, and optimizing the dosage. These efforts have given rise to a new generation of strains that demonstrate longer-term persistence in the host compared to previously studied strains. By focusing on strains that are better suited and adapted to the host's specific conditions, researchers hope to unlock enhanced benefits and therapeutic effects of probiotics. Evidence indicates that certain probiotic strains can persist in the body even after the administration of the probiotic is discontinued. For instance, *Bifidobacterium longum* subsp. *infantis* EVC001 was found to persist in infants for approximately one year following the end of probiotic supplementation [177]. *Lactiplantibacillus plantarum* ATCC 202,195 administered to infants demonstrated persistence for nearly six months based on analysis of plated isolates [148]. Four of the five probiotic strains in a commercial product were able to survive for several months following supplementation in preterm newborns. It's interesting to note that applications for persistent probiotics go beyond gastrointestinal niches and applications for digestive health. Certain vaginal strains such as *Lactobacillus crispatus* CTV-05, *L. rhamnosus* GR-1, and *Limosilactobacillus reuteri* RC-14 have been shown to persist in the vaginal environment for weeks to months [149]. Similarly, *B. animalis* subsp. *lactis* HN019 was recovered from the gingiva for at least 60 days after 30 days of administration. Probiotic strains that can colonize the skin microbiota are also being sought, with numerous commercial projects concentrating on this [178].

These examples illustrate the evolving direction of probiotic strain discovery, with a specific emphasis on developing long-term colonizing probiotics. However, it is important to adopt a risk-benefit approach when pursuing strategies aimed at developing such probiotics. Comprehensive studies on the long-term safety of probiotics are not as well-defined as those focused on acute safety issues. In the case of drugs and biologics, it is crucial to assess sub-acute and chronic toxicities to identify any potentially harmful effects associated with repeated dosing or bioaccumulation, including microbial metabolites, proliferation, and colonization. Typically, animal models are employed in these studies, following validated procedures that aim to provide insights into the risks for humans. Such studies are an integral part of the drug development process, conducted before clinical use [114].

According to the FDA's drug development process, a post-market monitoring system is typically where the problem of long-term safety rests, where manufacturers agree to submit safety updates and voluntary reporting of

adverse events (AEs) by consumers and healthcare professionals to MedWatch [179]. Long-term human safety studies lasting more than six months may not always be required for drug approval; nevertheless, this decision is determined on a case-by-case basis. It is important to note that certain licensed biologics for illnesses like ulcerative colitis have recently received approval despite conflicting safety evidence and little long-term data [152]. A review of the existing literature in this field indicates that long-term safety studies typically involve follow-ups ranging from 16 to approximately 24 months. These studies focus on collecting reports of AEs and serious AEs, and monitoring various other outcomes to ensure a comprehensive evaluation of safety [114].

Applying the traditional toxicological study designs used to evaluate the safety of small molecules, long-term chronic studies in animal models typically span a duration of 6 months to 1 year. These studies incorporate prior knowledge or expectations regarding the organs that may be affected, the potential for toxicity to be reversible, identification of the no observed effect levels, and the quantification of clinical risk associated with the expected dose for long-term treatment [114, 152]. It would seem that conducting 12-month human studies alongside Phase III clinical trials would be in line with expectations for new molecular agents or targets when applying this preclinical framework to next-generation live biotherapeutics, which lack a history of safe use. This is especially true when there is little post-market experience available. It is crucial to remember that the majority of the recognized toxicological frameworks were created using pharmacokinetic/toxicokinetic factors as well as different preclinical testing strategies. They may not be immediately applicable to probiotics, which typically do not penetrate the bloodstream or have degradation rates typical of small molecules [150].

There is currently no agreement on how to produce long-term safety recommendations due to the existing state of scientific expertise and knowledge of critical safety indicators for probiotics in certain populations or disease states, along with related model systems. This uncertainty is made worse by the probable emergence of microbial consortiums that have been carefully planned and have complementary functional traits [114]. As we continue to learn more about the long-term impact of the microbiota on human physiology, specific approaches for long-term follow-up may become necessary. Based on the present guidelines specified by the FDA for biologic medications, including fecal microbial transplants, there are currently no particular tests or suggested duration of follow-up for addressing long-term safety risks for probiotics [114]. Data on adverse events should be gathered in human trials to determine whether a probiotic affects the makeup or function

of the microbiota over time, much like in acute research. To improve our mechanistic understanding of how possible long-term impacts may be evaluated, we advise additional study using animal models, particularly in the context of next-generation probiotic strains. Companies are required to keep track of and report any adverse events in accordance with the regulatory regulations for foods, dietary supplements, and medications [180].

Figure 12.4 depicts the dangers that come with administering probiotics to susceptible groups and Table 12.3 depicts the safety issues of probiotics in long and short-term use.

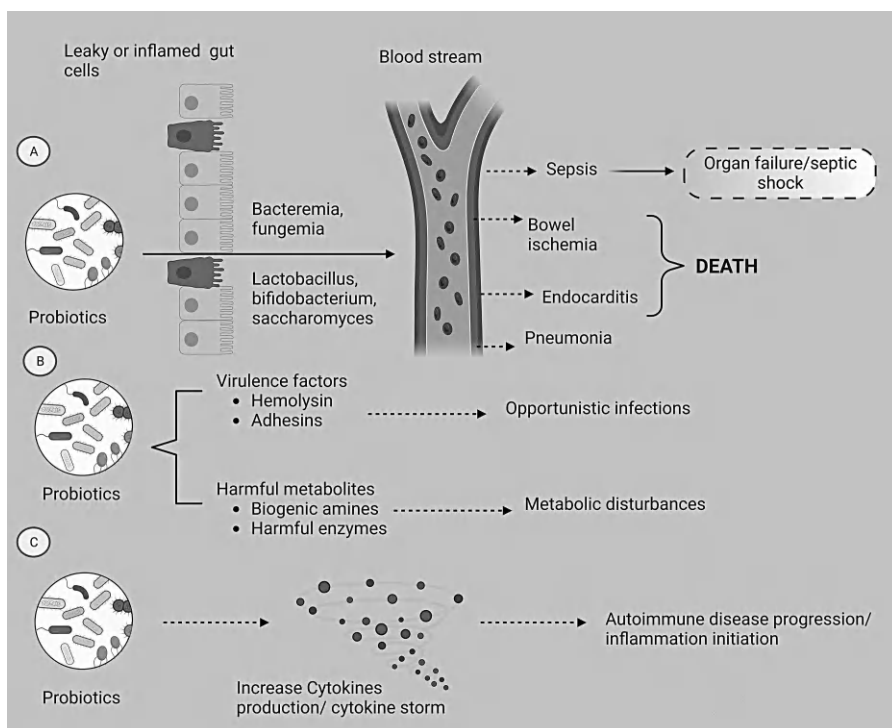


FIGURE 12.4 Probiotics are clinically administered to at-risk demographic groups with the potential dangers and their effects represented schematically. (A) *Saccharomyces cerevisiae*, *Lactobacillus*, and *Bifidobacterium* may cross the intestinal mucosa and enter the bloodstream or important organs in the event that the gut is leaky, inflammatory, or premature, resulting in systemic or localised infections; (B) Opportunistic infections and metabolic abnormalities may be caused by probiotics' toxic metabolites and virulence factors, respectively; and (C) A heightened immune response that induces the production of cytokines may result in autoimmune illness or inflammation.

TABLE 12.3 Key Suggestions Concerning Probiotics' Acute and Long-Term Safety Issues Are Summarized.

Safety risks	Recommendation
Acute Safety	
Microbiome assessments	• Probiotic safety evaluation does not necessarily include microbiome characterization. • Microbiome profiling is recommended because it can help identify factors that affect how different individuals respond to probiotics and test theories and mechanisms.
Antibiotic resistance	• It is important to check the genotypes and phenotypes of AR in all probiotic strains. • It is important to look into any phenotypic resistance that deviates from the species' norm. • Probiotics should go through a rigorous risk/benefit analysis that weighs patient and consumer safety as well as public health risks if the genes for antibiotics that the World Health Organization has classified as critically important. • Although there is little risk associated with the possibility of genetic transfer through transformation, it nevertheless merits more research.
Invasive infection	• In clinical settings, invasive infections and sepsis should be closely monitored and completely documented in all probiotic research. • Clinical isolates should be compared to the administered probiotic using strain-level molecular methods. A link between probiotics and systemic infection is supported by a genetic match between invasive clinical isolate(s) and supplemented probiotic microorganism(s). • Each commercially available probiotic strain's antibiogram should be identified and made public by the manufacturers, who should also offer an empirical course of therapy if necessary.
Probiotic: drug compatibility	• Probiotics contain enzymes that can interfere with some medications, according to research. Before recommendations can be made, more investigation is required.
Vulnerable populations	• Probiotics should be given to pregnant women who are mild to severely obese while being conscious of the increased risk of pre-eclampsia. • When probiotics are delivered to susceptible target populations, further monitoring is necessary.
• Long Term Safety	
Long-term studies	• The FDA's current recommendations for biological drugs, including fecal microbial transplants, state that no specific testing or follow-up period is required to address any long-term safety issues. • Similar to acute investigations, research on AEs in people should be conducted to see if probiotics alter the composition or function of the microbiota over time. • To improve our mechanistic understanding of how to detect potential long-term impacts, research into animal models is encouraged, particularly as it relates to next-generation strains.

TABLE 12.3 *(Continued)*

Safety risks	Recommendation
Long-term colonization	• Only with a specific goal of achieving benefits that would not be feasible, dependable, or cost-effective to achieve otherwise should a long-term colonizing probiotic be developed, together with a consideration of risks and rewards. • It is important to carefully assess any relevant long-term safety data for probiotic strains that remain in the host.
Microbiome assessments	• Probiotic safety evaluations shouldn't call for examinations of the composition and function of microbiota communities. • Such profiling might help evaluate theories and mechanisms. • To comprehend the therapeutic significance of any detected changes in microbiome structure or function, research is required.
Vulnerable populations	• It is recommended to monitor premature infants for at least two years (when most outcomes are no longer controlled for prematurity) in order to compare metabolic, allergic, immunological, and additional medical findings in investigations involving premature infants who took probiotics or not in the perinatal period.

12.17 FUTURE ASPECTS AND HOW TO OVERCOME THE TOXICITY APPROACHES

The safety of medications and dietary supplements has been governed by various national policies. The European Food Safety Authority (EFSA) regards all popular probiotic varieties as safe for use by the general public. Regardless of policies, using probiotics on vulnerable people has generally entailed a certain degree of risk. It is simple to understand how probiotics have gained so much recognition in medicine. Cardiovascular disease, overweight, depressive disorders, and premature births are just a few of the illnesses that the intestinal microbiota regulates. Therefore, it makes sense to think that restoring good bacteria will restore balance [181].

When considering probiotics as a collection of external microorganisms, it is expected that different genera, species, and strains will interact and affect the health of individual hosts through diverse mechanisms. The outcomes can be significantly influenced by the variations in probiotic formulations, dosage, and administration methods, especially among recipients with different characteristics. Therefore, selecting an appropriate strain and formulation is crucial for addressing specific health conditions [182]. In general, potential probiotic strains are identified through screening various sources such as soil, feces, and fermented foods. These strains differ significantly from the endogenous microbial populations residing in the human gut [183].

After ingestion, the unique gut microenvironment and immune factors may exert selective pressures on the probiotic strains, which may result in the development of virulence factors like adhesins, biogenic amines such as histamine, putrescine, cadaverine, tyramine, phenylethylamine, spermine, and spermidine, as well as specific toxins and harmful enzymes like hemolysins and glycosidases [45]. Previous reports have shown that bacterial genomes can readily undergo genetic changes to adapt to the evolving environment, which may give rise to new strains that are resistant to drugs [173]. Notably, probiotic strain suspensions are frequently mixed with sodium alginate and extruded onto a calcium chloride solution to create calcium alginate beads that are used to make probiotic capsules [167]. However, there are potential concerns regarding the immune system response in the host due to these capsules. It is also possible that the carbohydrates present in the capsules could stimulate the activity of glycoside hydrolases. Furthermore, it is critical to carefully consider the hazards connected with using probiotics, especially in people whose gut integrity is already damaged. While there are more studies demonstrating the health advantages of probiotics than those demonstrating their hazards, it is important to consider the susceptibility of at-risk populations to potential health risks associated with probiotics [45].

Although the efficacy of specific strains of probiotics has been supported by empirical evidence, it is essential to pay greater attention to the adaptability of strains, including their potential for acquiring lethal genes, crossing the gut barrier, causing shock, and other related factors [107]. Above all, it is necessary to investigate the host factors that may contribute to potential vulnerability to probiotic supplementation. Randomized controlled trials (RCTs) should be conducted with a focus on addressing specific safety concerns to gain a deeper understanding of these issues [18].

The manufacturing, labeling, and distribution of probiotics, which are composed of live bacteria intended for consumption, should be subject to strict regulations [142]. The FDA invites medical professionals to file an “Investigational New Drug Application” for consideration if they recommend dietary supplements containing live bacteria or yeast as medications for the treatment or prevention of medical disorders. With regard to the clinical uses of probiotics, we think the FDA and WHO should create more detailed recommendations with stricter requirements. By doing this, at-risk groups such as people with impaired immune systems, newborns, seriously ill patients, and people receiving post-surgical care can be shielded from any unknown dangers related to the uncontrolled clinical use of probiotics [106].

The rare negative effects associated with strain-specific probiotics highlight the necessity for clear regulations regarding the production and distribution of probiotics for medical use. In the pursuit of reporting the beneficial effects of probiotics, the potential negative aspects have often been overlooked [45]. For instance, the probiotic bacterium *Escherichia coli* NISSLE 1917 can cause genotoxicity by generating peptide-polyketides and altering the host's cytokine profiles, which may alleviate colitis and chronic inflammatory bowel illnesses [45]. This report underscores that even an effective probiotic may have concurrent side effects, similar to any other medication. It is essential to have such cautious reports, as it is becoming increasingly evident that the behavior of microbes, whether beneficial or pathogenic, is largely influenced by the host microenvironment. A pathogen can block its virulence machinery in the absence of immune monitoring, and a benign bacterium can potentially develop into a pathogen in the absence of immune patrolling. Furthermore, it has been observed that potential pathogens such as *Streptococcus*, *Enterococcus*, *Escherichia coli*, and *Saccharomyces boulardii* tend to lose their virulence attributes under *in vitro* culture conditions.

The members of the gut microflora have evolved in a mutually beneficial relationship with the human immune system over millions of years, successfully adapting to immune pressures without causing harm [104]. However, when exogenous microbial species (probiotics) are introduced to at-risk populations, they may disrupt the native gut microflora due to various risk factors. In some cases, the introduced probiotics may simply fail to establish themselves and have limited therapeutic effects. On the other hand, they may restore health or, under certain clinical conditions, act as pathogens in the host [184]. Probiotics still have therapeutic potential; nevertheless, provided the proper regulatory procedures are followed and they are accompanied by informative labeling that contains warnings and precautions particular to the drug. It is crucial to make sure that the potential advantages of probiotics are balanced with the potential disadvantages, and that individuals and health-care professionals are knowledgeable about how to use them properly [185].

12.18 CONCLUSION

Probiotics have been advertised in such a way that everyone believes using them is always beneficial, almost a panacea, with no risks. To properly reap the benefits of probiotics, both sides of the picture must be taken into account through a risk versus benefit analysis because the reality seems to

differ from this hypothesis. In this chapter, we emphasize the criteria for separating probiotic regulation for general dietary use from those for clinical therapies. In our conversation, we made clear that probiotics with health claims to cure or prevent specific illnesses should be marketed and managed as “drugs” rather than “dietary supplements,” and as a result, must abide by stricter standards. The regulatory agencies should be more stringent when a probiotic product is introduced to the market, whether it is a food supplement or a biotherapeutic agent. Further investigation may find that not all probiotics are safe for everyone, just as not all GRAS bacteria are probiotics. Probiotics may be immunogenic and/or harmful for a certain milieu under specific clinical settings since the host variables, in particular, might vary. This review emphasizes the fact that probiotics are not a “magic cure” and that even when used as an immuno-modulator and microbiota restorer, they may not always be completely safe and beneficial.

KEYWORDS

- Calcium alginate beads
- gut microbiota
- immune system
- probiotic formulations
- probiotic safety
- virulence factors

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REFERENCES

1. Kumar, V., Naik, B., Kumar, A., Khanduri, N., Rustagi, S., & Kumar, S. (2022). Probiotics media: Significance challenges and future perspective—A mini review. *Food Production Processing and Nutrition*, 4(17), 1–13.
2. Pereira, D. I., Macfarlane, G. T., & Gibson, G. R. (2018). Effects of probiotics on the gut microbiota. *Journal of Applied Microbiology*, 124(2), 449–456.

3. Marco, M. L., Sanders, M. E., Gänzle, M., Arrieta, M. C., Cotter, P. D., De Vuyst, L., & Hutkins, R. (2021). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. *Nature Reviews Gastroenterology & Hepatology*, 18(3), 196–208.
4. Plessas, S., Bekatorou, A., & Nigam, P. (2022). Lactic acid fermentation of fruits and vegetables. *International Journal of Food Microbiology*, 96(3), 373–378.
5. Puntillo, F., Virdis, F., & Basilico, N. (2022). Probiotics and antimicrobial resistance: Novel strategies to improve gut health. *Frontiers in Microbiology*, 13, 12–18.
6. Marchwińska, K., & Gwiazdowska, D. (2022). Isolation and probiotic potential of lactic acid bacteria from swine feces for feed additive composition. *Archives of Microbiology*, 204(1), 61.
7. Nagpal, R., Kumar, M., & Yadav, H. (2012). Probiotics, prebiotics and synbiotics: A review. *Journal of Clinical Microbiology*, 50(1), 115–122.
8. Gill, H. S., & Guarner, F. (2004). Probiotics and human health: A clinical perspective. *Postgraduate Medical Journal*, 80(947), 516–526.
9. Mishra, S., Yadav, H., & Kumar, M. (2021). Gut microbiota and health: Probiotic potential of gut bacteria. *Gut Microbiota Research Journal*, 5(1), 45–56.
10. Lilly, D. M., & Stillwell, R. H. (1965). Probiotics: Growth-promoting factors produced by microorganisms. *Science*, 147(3659), 747–748.
11. Hawrelak, J. (2013). Probiotics. In J. E. Pizzorno & M. T. Murray (Eds.), *Textbook of Natural Medicine* (pp. 979–994). St. Louis, Missouri: Churchill Livingstone Elsevier.
12. Parker, R. B. (1974). Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health*, 29, 4–8.
13. News Medical. (n.d.). Probiotics history: A concept for improving gut health. *News-Medical.net*. URL (accessed on 16th October 2024).
14. Khalighi, A., Behdani, R., & Kouhestani, S. (2016). Probiotics: A comprehensive review of their classification, mode of action, and role in human nutrition. In *Probiotics and Prebiotics in Human Nutrition and Health* (InTech). <https://doi.org/10.5772/63646>.
15. Dunne, C., Mahony, O., Murphy, L., Thornton, L., Morrissey, G., & Halloran, O. (2001). In-vitro selection criteria for probiotic bacteria of human origin: Correlation with in-vivo findings. *The American Journal of Clinical Nutrition*, 73(2), 386–392.
16. Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, 5(4), 1542–1553.
17. Gasbarrini, G., Bonvicini, F., & Gramenzi, A. (2016). Probiotics history. *Journal of Clinical Gastroenterology*, 50(2), S116–S119. <https://doi.org/10.1097/MCG.0000000000000697>.
18. Capita, R., Alonso-Calleja, C., & García-Fernández, M. C. (2013). Probiotics and safety: A systematic review of randomized controlled trials and potential adverse effects. *Beneficial Microbes*, 4(1), 83–99.
19. Diplock, A. T., Aggett, P. J., & Ashwell, M. (1999). Scientific concepts of functional foods in Europe: Consensus document. *British Journal of Nutrition*, 81(S1), S1–S27.
20. Giovanni, A., Rossi, A., & Carli, E. (2016). Traditional knowledge of fermented foods: Historical perspectives. *Journal of Ancient History*, 21(3), 250–270.
21. Eiseman, B., Silen, W., Bascom, G. S., & Kauvar, A. J. (1958). Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery*, 44(5), 854–859.
22. Kelly, C. P. (2013). Fecal microbiota transplantation—An old therapy comes of age. *New England Journal of Medicine*, 368(5), 474–475.

23. Van Nood, E., Vrieze, A., & Nieuwdorp, M. (2013). Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *New England Journal of Medicine*, 368(5), 407–415.
24. Papadopoulou, C., Tsoukalas, D., & Papachristodoulou, I. (2018). Advances in food preservation: Utilization of probiotics. *International Journal of Food Science*, 7, 33–45.
25. Espitia, P. J. P., Batista, R. A., Azeredo, H. M. C., & Otoni, C. G. (2016). Probiotics and their potential applications in active edible films and coatings. *Food Research International*, 90, 42–52. <https://doi.org/10.1016/j.foodres.2016.10.026>.
26. Swain, M. R., Anandharaj, M., Ray, R. C., & Parveen Rani, R. (2014). Fermented fruits and vegetables of Asia: A potential source of probiotics. *Biotechnology Research International*, 2014, 250424. <https://doi.org/10.1155/2014/250424>.
27. Chilton, S. N., Burton, J. P., & Reid, G. (2015). Inclusion of fermented foods in food guides around the world. *Nutrients*, 7(1), 390–404. <https://doi.org/10.3390/nu7010390>.
28. Mishra, S., Yadav, H., & Kumar, M. (2018). Probiotics in gastrointestinal health: Past controversies and present perspectives. *Journal of Gastrointestinal Sciences*, 10(2), 145–158.
29. Nielsen, D. S., Madsen, M., & Jungersen, M. (2012). Probiotic supplementation and human diversity: Effects across gender, age, and conditions. *Gut Microbiology Studies*, 7, 45–56.
30. Kussmann, M., & Fay, L. B. (2008). Nutrigenomics and personalized nutrition: Science and concept. *Personalized Medicine*, 5(5), 447–455. <https://doi.org/10.2217/17410541.5.5.447>.
31. Kochan, P., Chmielarczyk, A., Szymaniak, L., et al. (2011). Lactobacillus rhamnosus administration causes sepsis in a cardiosurgical patient: Is the time right to revise probiotic safety guidelines? *Clinical Microbiology and Infection*, 17(10), 1589–1592. <https://doi.org/10.1111/j.1469-0691.2011.03614.x>.
32. Martín, R., Jiménez, E., & Olivares, M. (2009). Anaphylactic responses to probiotics in individuals with hypersensitivity: A review. *Journal of Allergy and Clinical Immunology*, 6(2), 215–230.
33. Appel-da-Silva, M. C., Narvaez, G. A., Perez, L. R. R., Drehmer, L., & Lewgoy, J. (2017). *Saccharomyces cerevisiae* var. *boulardii* fungemia following probiotic treatment. *Medical Mycology Case Reports*, 18, 15–17. <https://doi.org/10.1016/j.mmcr.2017.07.007>.
34. Zheng, J., Wittouck, S., Salvetti, E., et al. (2017). Risk of horizontal gene transfer in probiotics and the emergence of antibiotic resistance. *Nature Reviews Microbiology*, 15, 278–288.
35. Patel, S., Goyal, A., & Dhingra, H. (2015). Probiotics as immunomodulators: Evolving therapies. *Clinical Immunology*, 60(4), 165–174.
36. Thushara, R. M., Gangadaran, S., & Solati, Z. (2016). Probiotics and heart health: Mechanisms and implications. *Journal of Cardiac Science*, 35(4), 252–267.
37. Nouri, M., Mahmoudi, A., & Rezaie, O. (2016). The role of probiotics in cancer management: Mechanisms and clinical applications. *Cancer Research and Therapeutics*, 42(7), 675–682.
38. Kasińska, M., & Drzewoski, J. (2015). Effectiveness of probiotics in type 2 diabetes: A meta-analysis. *Polish Archives of Internal Medicine*, 125, 803–811.
39. Prados, M., Hidalgo, M., & Lopez, L. (2015). Probiotics for metabolic syndrome and obesity: A review. *Nutrition and Metabolism*, 12, 35–46.

40. Hang, L., Ming, T., & Li, Y. (2016). The role of probiotics in neurodegenerative diseases: Depression and anxiety. *Journal of Neurology and Psychiatry*, 47(1), 52–59.
41. Yang, X., Ding, X., & Wu, Y. (2016). Probiotics and their impact on mental health: A comprehensive review. *Frontiers in Neuroscience*, 10, 503–514. <https://doi.org/10.3389/fnins.2016.00503>.
42. Arslan, S., & Erbas, M. (2015). Probiotic cereal-based fermented functional foods. In *Fermented Foods. Part-I* (pp. 211–227).
43. Ramos, C. L., Thorsen, L., & Schwan, R. F. (2013). Strain-specific effects of probiotics: Current perspectives. *Current Opinion in Food Science*, 2, 95–100.
44. Faijan, B. A., Kostis, A. K., & Djaldetti, M. (2014). *Saccharomyces boulardii* use and benefits: A clinical review. *Journal of Clinical Gastroenterology*, 48(1), 76–82.
45. Kothari, D., Patel, S., & Kim, S. K. (2019). Probiotic supplements might not be universally effective and safe: A review. *Biomedicine & Pharmacotherapy*, 111, 537–547. <https://doi.org/10.1016/j.biopha.2018.12.104>.
46. Danial, M., Tang, R., & You, S. (2023). Current perspectives on probiotic safety and its therapeutic potential. *Journal of Clinical Microbiology*, 58(2), e01029–20.
47. Haukioja, A. (2010). Probiotics and oral health. *European Journal of Dentistry*, 4(3), 348–355.
48. Chatterjee, A., Bhattacharya, H., & Kandwal, A. (2011). Probiotics in periodontal health and disease. *Journal of Indian Society of Periodontology*, 15(1), 23–27.
49. Collado, M. C., & Sanz, Y. (2009). Gut health and probiotics: Current knowledge. *Digestive Diseases*, 28(4), 49–56.
50. Aragon, F., Carino, S., & Perdigon, G. (2010). Probiotics and gut applications: A focus on current research. *Food Microbiology*, 27(4), 537–545.
51. US Food and Drug Administration (1994). Dietary Supplement Health and Education Act (DSHEA). Public Law 103–417.
52. Shen, Y., Gao, F., & Wang, Z. (2022). Probiotics in gastrointestinal disorders: A current overview of benefits and risks. *Journal of Gastroenterology and Hepatology*, 37(3), 123–132.
53. Kingsley, S. M., Shankar, V., & Jagannathan, N. (2007). Therapeutic potentials of snake venom: An overview. *Journal of Venomous Animals and Toxins*, 13(2), 365–374.
54. Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., & Rastall, R. A. (2018). Probiotics and prebiotics: Prospects for public health and nutritional recommendations. *Annals of the New York Academy of Sciences*, 1390(1), 57–69. <https://doi.org/10.1111/nyas.13454>.
55. Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F., & Matuchansky, C. (2005). Review article: Bifidobacteria as probiotic agents – Physiological effects and clinical benefits. *Alimentary Pharmacology & Therapeutics*, 22(6), 495–512.
56. Choudhary, S., & Kumar, S. (2020). Gut microbiota and fecal analysis: Challenges in modern medical microbiology. *Journal of Microbial Methods*, 182, 106–118.
57. Wolfe, J. V., Mullins, R. J., & Giansanti, F. (2023). Future prospects for probiotics in food and pharma industries. *Journal of Food Science and Technology*, 30(4), 452–462.
58. Cohen, M., Lickwar, C. R., & Hendershot, K. (2018). Genome sequencing and the risk of antibiotic resistance in probiotics. *Microbial Genomics*, 4(12), e000218. <https://doi.org/10.1099/mgen.0.000218>.
59. Freedman, J. C., Weinstein, S. J., & Rothschild, M. A. (2020). Antibiotic resistance genes in probiotics: A growing concern. *Journal of Antimicrobial Chemotherapy*, 75(10), 2832–2839.

60. Poindexter, J. S., Swartwood, H. J., & Greene, M. D. (2021). Environmental spread of antibiotic resistance through probiotics. *Ecology and Evolutionary Biology*, 10(3), 1507–1517.
61. Szajewska, H., Kotowska, M., & Mrukowicz, J. Z. (2001). Probiotic effects of *Lactobacillus rhamnosus* GG in treating viral gastroenteritis. *Journal of Pediatric Gastroenterology and Nutrition*, 33(1), 19–25.
62. Zocco, M. A., dal Verme, L. Z., & Cremonini, F. (2006). *Lactobacillus rhamnosus* GG and ulcerative colitis remission: Clinical trials and outcomes. *Alimentary Pharmacology & Therapeutics*, 23(11), 1567–1574.
63. Khalighi, A., Behdani, R., & Kouhestani, S. (2016). The challenges of using different probiotic strains in clinical settings. *Iranian Journal of Microbiology*, 8(6), 347–356.
64. Hatakka, K., Savilahti, E., Ponka, A., Meurman, J. H., Poussa, T., & Nase, L. (2001). Effect of long-term consumption of probiotic milk on infections in children attending day care centers: Double-blind, randomized trial. *BMJ*, 322(7298), 1327.
65. Shornikova, A. V., Casas, I. A., & Mykkanen, H. (1997). Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. *Pediatric Infectious Disease Journal*, 16(12), 1103–1107.
66. Kailashapathy, K., & Chin, J. (2001). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium*. *Immunology and Cell Biology*, 78(1), 80–88.
67. Cassone, A., Casadevall, A., & Legrand, D. (2003). Microbiological hazards in hospital settings: Cross-contamination and infection control. *Journal of Hospital Infection*, 54(1), 70–74.
68. Hennequin, C., & Pouillot, M. (2000). Nosocomial infections caused by *Lactobacillus rhamnosus* in hospital environments. *Clinical Infectious Diseases*, 31(2), 924–926.
69. Bongaerts, G., & Brummer, R. J. (2016). Probiotic safety in critical care patients: An evaluation of the Propatria study. *Critical Care Medicine*, 44(9), 1765–1772.
70. Cabana, M. D., Randhawa, K., & Shandling, M. (2021). Educating healthcare professionals on the safe use of probiotics. *Journal of Clinical Nutrition*, 9(4), 300–310.
71. Su, G. L., & Ko, C. W. (2020). The safety of probiotics in clinical settings: Mechanisms and guidelines. *Gastroenterology Reviews*, 28(2), 77–84.
72. Surawicz, C. M., Brandt, L. J., & Binion, D. G. (2016). Guidelines for the use of probiotics in gastrointestinal disorders. *American Journal of Gastroenterology*, 111(7), 1130–1143.
73. Segarra, A., Inza, I., & Buxeda, A. (2007). Sepsis and endocarditis due to *Lactobacillus rhamnosus* GG. *Journal of Infection*, 55(2), e65–e67.
74. Elahi, S., Akbarian, A., & Delirez, N. (2008). Probiotic-related fungemia in immune-compromised patients: A review of case reports. *Medical Mycology*, 46(5), 553–556.
75. Finegold, S. M., Sutter, V. L., & Mathisen, G. E. (1983). Interaction of antibiotics and probiotics: A review. *Antimicrobial Agents and Chemotherapy*, 24(5), 703–709.
76. Pradhan, R. C., Bhuyan, M., & Sahoo, S. (1986). Metabolism of sulfasalazine and related drugs by *Lactobacillus acidophilus*. *Journal of Pharmaceutical Sciences*, 75(12), 1258–1261.
77. Gueimonde, M., Salminen, S., & Isolauri, E. (2004). Safety of probiotics: Comparison of traditional and novel probiotic strains. *Antonie van Leeuwenhoek*, 86(1), 1–4.
78. Cheah, P. Y., Lee, C. G. L., & Ng, M. H. (1990). Carcinogenicity of secondary bile acids: Evidence and mechanisms. *Cancer Research*, 50(7), 2035–2040.

79. Midtvedt, T., & Norman, A. (1967). Bile acid metabolism by *Bifidobacterium* and *Lactobacillus* species. *Acta Pathologica Microbiologica Scandinavica*, 70(3), 312–320.
80. Fabian, E., Socol, C. T., & Popa, A. C. (2017). D-lactic acidosis in patients with short bowel syndrome: Case reports and literature review. *Pediatrics International*, 52(5), 645–649.
81. Munakata, S., Arakawa, C., & Kohira, R. (2010). D-lactic acidosis in short bowel syndrome: Case reports and literature review. *Pediatrics International*, 52(5), 645–649.
82. Senok, A. C., Ismaeel, A. Y., & Botta, G. A. (2005). Probiotics: Facts and myths. *Clinical Microbiology and Infection*, 11(12), 958–966.
83. Schwabb, R. C., Perren, A., & Meier, R. (1993). Peptidoglycan-induced inflammation and associated complications. *Infection and Immunity*, 61(4), 1539–1545.
84. Miettinen, M., Vuopio-Varkila, J., & Varkila, K. (1996). Production of cytokines by probiotic bacteria and their immunomodulatory effects. *Journal of Immunology*, 156(5), 1600–1605.
85. Perdigon, G., Alvarez, S., & Rachid, M. (1991). Effect of *Lactobacillus casei* on the immune response in mice. *Journal of Dairy Research*, 58(2), 485–492.
86. Doherty, G. A., Bennett, G. C., & Maher, M. M. (2009). Probiotic therapy for Crohn's disease: Current status and prospects for the future. *Alimentary Pharmacology & Therapeutics*, 29(7), 797–802.
87. Rolfé, V. E., Fortun, P., & Hawkey, C. J. (2006). Probiotics and Crohn's disease: A systematic review. *Clinical Gastroenterology and Hepatology*, 4(6), 726–734.
88. Butterworth, A. D., Thomas, A. G., & Akobeng, A. K. (2008). Probiotics and UC: Evidence from randomized controlled trials. *British Journal of Nutrition*, 100(5), 1189–1190.
89. Farina, C., & Arosio, M. (2001). Sepsis caused by *Lactobacillus rhamnosus* in a patient with severe colitis: Case report. *Journal of Clinical Microbiology*, 39(3), 1071–1072.
90. Marteau, P., & Seksik, P. (1990). Probiotics for short bowel syndrome patients: Potential benefits and risks. *Gut*, 39(5), 537–543.
91. Lidbeck, A., Norin, E., & Gustafsson, J. A. (1992). Metabolism of bile acids by human intestinal microflora and its role in colon cancer development. *Gut*, 33(7), 945–946.
92. Delia, P., Sansotta, G., & Donato, V. (2002). Use of probiotics in the prevention of colorectal cancer in animal models. *Journal of Cancer Research and Clinical Oncology*, 128(7), 564–567.
93. Goldin, B. R., & Gorbach, S. L. (1996). Probiotics for the prevention of colorectal cancer: Evidence from animal studies. *European Journal of Clinical Nutrition*, 50(3), 485–487.
94. Gorbach, S. L., & Goldin, B. R. (1990). Probiotics in human health and disease: An epidemiologic review. *American Journal of Clinical Nutrition*, 52(6), 994–998.
95. Connolly, E., Noah, L., & Ringel, Y. (2005). Effect of probiotics on bile acid metabolism: A study in ileostomy patients. *Clinical Gastroenterology*, 39(3), 287–291.
96. Bongaerts, G. P., Severijnen, R. S., & de Meij, T. G. (2000). Short bowel syndrome and bile acid metabolism: Potential risks of probiotic therapy. *Clinical Nutrition*, 29(4), 560–562.
97. Ruseler-van Embden, J. G., Both-Patoir, H. C., & Vanderhulst, R. (1995). Impact of probiotics on intestinal mucus: A review of experimental studies. *Gut*, 36(4), 481–482.
98. Lee, B., Yun, H. S., & Park, S. (2020). Probiotic viability and its importance in food products. *Food Science and Technology Research*, 26(4), 335–347.

99. Syndman, D. R., & Amr, S. S. (2008). Risks of probiotics in critical care patients. *Critical Care Medicine*, 36(3), 931–935.
100. Lara-Villoslada, F., & Olivares, M. (2010). Probiotic safety in newborns and premature infants. *Pediatric Research*, 68(4), 203–210.
101. Hoffman, F. A., & Heimbach, J. T. (2008). Risks associated with probiotic administration: The emergence of antibiotic resistance. *Journal of Clinical Nutrition*, 88(5), 1466S–1469S.
102. Agarwal, S., & Rao, A. V. (2020). Metabolic and toxic effects of probiotics on the gastrointestinal tract. *Journal of Digestive Diseases*, 12(2), 198–206.
103. Kochan, P., Chmielarczyk, A., & Szymaniak, L. (2011). *Lactobacillus rhamnosus* sepsis in a cardio-surgical patient: A case report. *Clinical Microbiology and Infection*, 17(10), 1589–1592.
104. Blaser, M. J., & Falkow, S. (2009). What are the consequences of the disappearing human microbiota? *Nature Reviews Microbiology*, 7(12), 887–894.
105. Bafeta, A., Koh, M., & Riveros, C. (2018). Reporting of adverse events in randomized controlled trials of probiotics: A systematic review. *Annals of Internal Medicine*, 169(4), 240–247.
106. Food & Drug Administration (2021). *Guidance for industry: Investigational new drug applications for probiotics*. Washington, DC: US Government Printing Office.
107. Wassenaar, T. M., & Klein, G. (2008). Safety of probiotics: An analysis of the data from clinical trials and post-market surveillance. *Therapeutic Advances in Gastroenterology*, 1(2), 95–108.
108. Saarela, M., Mogensen, G., Fonden, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology*, 84(3), 197–215.
109. Peivasteh-Roudsari, L., & Mahecha, E. A. (2019). Probiotic product regulations in the United States and the European Union: A comparative study. *Regulatory Toxicology and Pharmacology*, 104, 87–95.
110. Salminen, S., Bouley, C., Boutron-Ruault, M. C., et al. (1998). Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition*, 80(S1), S147–S171.
111. Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T., & de Vos, W. M. (1996). Probiotic strain tracking in human studies: A review of methods and outcomes. *FEMS Microbiology Reviews*, 19(2), 191–203.
112. Johansson, M. L., Molin, G., & Jeppsson, B. (1993). Administration of different lactobacilli to patients with intestinal discomfort: Effects on gut microflora and symptoms. *Scandinavian Journal of Gastroenterology*, 28(3), 411–416.
113. Alander, M., Korpela, R., Saxelin, M., Vilpponen-Salmela, T., Mattila-Sandholm, T., & von Wright, A. (1997). Recovery of *Lactobacillus rhamnosus* GG from human colonic biopsies. *Letters in Applied Microbiology*, 24(5), 361–364.
114. Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., & Rastall, R. A. (2016). Probiotics and prebiotics in intestinal health. *Current Opinion in Gastroenterology*, 32(6), 425–432.
115. Rao, R. K., Samak, G., & Seth, A. (2022). Probiotic safety: Current perspectives and recommendations. *Journal of Clinical Gastroenterology*, 56(9), 703–711.
116. Bassetti, M., Wauters, J., & Nicastrì, E. (1998). Probiotic-induced fungemia: A review of the literature. *Infection*, 26(6), 379–383.

117. Cherifi, S., Rahav, G., & Schmidt, R. (2004). *Saccharomyces cerevisiae* fungemia: A systematic review of cases and risk factors. *Journal of Infection*, 49(4), 266–273.
118. Fredenucci, I., Wiesel, M. L., & Wolf, P. (1998). Probiotic-associated fungemia in immunocompromised patients. *Clinical Infectious Diseases*, 26(6), 1477–1478.
119. Hennequin, C., & Pouillot, M. (2000). Nosocomial infections caused by *Saccharomyces cerevisiae* in immunocompromised patients. *Clinical Infectious Diseases*, 31(1), 204–210.
120. Henry, D., Atienza, E., & Desjardins, D. (2004). *Saccharomyces cerevisiae* fungemia after probiotic treatment. *American Journal of Medicine*, 116(10), 767–768.
121. Lolis, N., Katsenos, S., & Papavasiliou, E. (2008). *Saccharomyces* fungemia after *Saccharomyces boulardii* treatment. *Infection Control & Hospital Epidemiology*, 29(9), 995–996.
122. Piarroux, R., Millon, L., & Bardonnet, K. (1999). *Saccharomyces boulardii* fungemia: A rare adverse effect of probiotic treatment. *European Journal of Clinical Microbiology & Infectious Diseases*, 18(2), 137–138.
123. Santino, I., Alari, A., & Bono, S. (2014). *Saccharomyces cerevisiae* fungemia in a neutropenic patient: A case report. *Journal of Clinical Microbiology*, 52(8), 3195–3197.
124. Ledoux, D., Labombarda, F., & Brouard, J. (2006). Lactobacillus-induced bacteremia in an immunosuppressed patient: Case report. *Infectious Diseases in Clinical Practice*, 14(5), 323–324.
125. Richard, A., Harmark, R., & Konings, R. (1988). Lactobacillus bacteremia: A report of cases. *Clinical Infectious Diseases*, 10(2), 497–498.
126. Tommasi, A. M., Cassani, M., & De Filippis, C. (2008). Probiotic-related bacteremia in immunocompromised patients: An emerging concern. *Journal of Clinical Microbiology*, 46(4), 1446–1447.
127. Vahabnezhad, E., Heneghan, M., & Halalau, A. (2013). Lactobacillus casei bacteremia after probiotic treatment in a patient with severe colitis. *Infection Control & Hospital Epidemiology*, 34(4), 468–470.
128. Burkhardt, O., Lehmann, D., & Zimmermann, T. (2005). Sepsis due to Lactobacillus GG in critically ill patients. *Infection*, 33(1), 48–50.
129. Kunz, A. N., Noel, J. M., & Fairchok, M. P. (2004). Lactobacillus rhamnosus sepsis in a neonate. *Journal of Clinical Microbiology*, 42(1), 480–482.
130. Land, M. H., Rouster-Stevens, K., Woods, C. R., Cannon, M. L., Cnota, J., & Shetty, A. K. (2005). Lactobacillus sepsis associated with probiotic therapy. *Pediatrics*, 115(1), 178–181.
131. Lestin, F., Pertschy, A., & Latz, G. (2003). Lactobacillus GG sepsis in a patient with short bowel syndrome. *Clinical Infectious Diseases*, 36(9), 628–629.
132. Oggioni, M. R., Pozzi, G., & Valensin, P. E. (1998). Horizontal gene transfer of antibiotic resistance genes to pathogens during probiotic therapy. *Antimicrobial Agents and Chemotherapy*, 42(3), 332–337.
133. Ohishi, A., Takahashi, M., & Tanaka, M. (2010). Sepsis in a patient treated with Lactobacillus GG for short bowel syndrome. *Pediatrics International*, 52(1), 147–149.
134. Zein, N. N., Abdulkarim, A. S., & Wiesner, R. H. (2008). Lactobacillus sepsis associated with probiotic therapy in a patient with end-stage liver disease. *Liver Transplantation*, 14(1), 137–139.
135. Mackay, A. D., Taylor, M. B., & Kibbler, C. C. (1999). Lactobacillus rhamnosus abscesses in an immunocompromised patient: A case report. *Infection*, 27(6), 413–415.

136. Conen, A., Fluckiger, U., & Moreillon, P. (2009). Lactobacillus rhamnosus abscesses and endocarditis: Case reports and review. *Journal of Infection*, 58(3), 204–210.
137. Besselink, M. G., van Santvoort, H. C., & Buskens, E. (2008). Probiotic prophylaxis in patients with predicted severe acute pancreatitis: A randomized, double-blind, placebo-controlled trial. *Lancet*, 371(9613), 651–659.
138. Olah, A., Belagyi, T., & Issekutz, A. (2002). Randomized clinical trial of specific lactobacillus and fiber combination in severe acute pancreatitis. *British Journal of Surgery*, 89(9), 1103–1107.
139. Olah, A., & Romics, L. (2007). Evidence-based use of probiotics in pancreatitis: Experimental and clinical studies. *World Journal of Gastroenterology*, 13(29), 3881–3885.
140. Jain, P. K., McNaught, C. E., & Anderson, A. D. G. (2004). Influence of symbiotic therapy on gut flora and outcome after major abdominal surgery. *Nutrition*, 20(6), 609–615.
141. Ku, S., Park, M. S., & Ji, G. E. (2006). Probiotic effects on gastrointestinal disease in randomized controlled trials. *Journal of Clinical Gastroenterology*, 40(1), 1–9.
142. Jackson, S. A., Schoeni, J. L., & Vegge, C. (2019). Application of probiotics in clinical settings. *Therapeutic Advances in Gastroenterology*, 12, 1756284819827402.
143. Vaarala, O., Isolauri, E., & Salminen, S. (2003). Probiotics, immune function, and inflammatory diseases: A review. *Current Opinion in Gastroenterology*, 19(2), 146–150.
144. Drakes, M., Blanchard, T., & Czinn, S. (2004). Bacterial probiotics as modulators of immune response. *Journal of Clinical Gastroenterology*, 38(6), S86–S90.
145. Lin, C. F., Fung, Z. F., Wu, C. Y., & Chung, T. C. (1996). Molecular detection and characterization of tetracycline resistance in lactic acid bacteria. *Applied and Environmental Microbiology*, 62(1), 150–153.
146. Gevers, D., Huys, G., & Swings, J. (2003). In vitro conjugal transfer of tetracycline resistance from *Lactobacillus isolates* to other Gram-positive bacteria. *FEMS Microbiology Letters*, 225(1), 125–130.
147. Tannock, G. W., Tilsala-Timisjarvi, A., & Rodtong, S. (1994). Identification of *Lactobacillus* isolates from the gastrointestinal tract, vagina, and urethra of women, using randomly amplified polymorphic DNA and pulsed-field gel electrophoresis. *Applied and Environmental Microbiology*, 60(8), 2765–2771.
148. Panigrahi, P., Parida, S., & Nanda, N. C. (2017). Probiotics and gut health: From mechanisms to therapeutic potential. *Current Opinion in Gastroenterology*, 33(2), 88–93.
149. Zhang, J., Ma, L., & Guo, Q. (2021). Lactobacillus-induced bacteremia: A systematic review of case reports and clinical outcomes. *Frontiers in Microbiology*, 12, 719908.
150. Rouanet, A., Bolca, S., Bruyneel, B., Cvejic, H., & Corthier, G. (2020). Safety aspects of probiotics. *Journal of Clinical Gastroenterology*, 54(4), 277–289.
151. Hill, C., Guarner, F., & Reid, G. (2016). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, 11(8), 506–514.
152. Merenstein, D., El-Baba, F., & Higdon, R. (2023). Case reports of sepsis and bacteremia in patients taking probiotics: A review of clinical findings. *Journal of Clinical Microbiology*, 61(1), e00047-23.
153. Roe, C., Jones, M., & Taylor, S. (2022). Probiotic genome sequencing: Applications and implications for safety. *Microbial Biotechnology*, 15(4), 1157–1168.

154. Huys, G., Vankerckhoven, V., & Vael, C. (2013). Microbial contamination and safety of probiotic products: A critical review. *International Journal of Food Microbiology*, 165(2), 111–123.
155. Binda, S., Hill, C., & Sanders, M. E. (2020). Criteria to qualify microorganisms as “probiotic” in foods and dietary supplements. *Frontiers in Microbiology*, 11, 1662.
156. Sinclair, W., & Swan, R. (2016). Nasojejunal delivery of probiotics: Lessons from the PROPATRIA study. *Critical Care*, 20(1), 324.
157. Engen, P. A., Green, S. J., & Voigt, R. M. (2015). The gastrointestinal microbiome: Impacts on human health and disease. *World Journal of Gastroenterology*, 21(22), 7593–7604.
158. Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M., & Margolles, A. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research*, 61(1), 1600240.
159. Xavier-Santos, D., Miranda, J. M., & Lamas, A. (2022). Effects of probiotics on gut microbiota in preterm infants: A meta-analysis of randomized controlled trials. *Journal of Pediatric Gastroenterology and Nutrition*, 74(1), 37–45.
160. Das, A., Raychaudhuri, D., & Bhadra, M. P. (2020). Minimum inhibitory concentration (MIC) determination methods for probiotics with potential antibiotic resistance: Challenges and solutions. *Antimicrobial Resistance & Infection Control*, 9(1), 178.
161. Delgado, S., Flórez, A. B., & Mayo, B. (2007). Antibiotic resistance of lactic acid bacteria. *International Journal of Food Microbiology*, 116(1), 42–46.
162. Mthembu, N., Amoako, D. G., & Ismail, A. (2021). Chromosomal location of antimicrobial resistance genes in probiotics: Implications for risk assessment. *Microbial Pathogenesis*, 150, 104701.
163. Nøhr-Meldgaard, K., Krogsgaard, K., & Andersen, T. (2021). Regulation of the *tet(W)* gene in *Bifidobacterium animalis* subsp. *lactis* BB-12: Insights from clinical studies and regulatory evaluations. *Frontiers in Microbiology*, 12, 643571.
164. Nataraj, B. H., Mallappa, R. H., & Chandran, S. (2020). Postbiotics: Current trends in definition, safety, and potential health benefits. *Frontiers in Nutrition*, 7, 190.
165. Su, J. W., Chen, Y. C., & Liu, H. Y. (2019). The European Food Safety Authority's (EFSA) assessment of *Akkermansia muciniphila* as a novel food: Implications for the probiotic industry. *Food Control*, 98, 370–380.
166. Collineau, L., Davies, R. H., & Wegener, H. C. (2019). The role of plasmid curing and genetic editing in controlling antibiotic resistance in food-related probiotics. *Current Opinion in Biotechnology*, 60, 55–62.
167. Asgari, B., Khamseh, M. E., & Ebrahimi, F. (2020). Alginate-based encapsulation and its role in probiotic survival and colonization. *Carbohydrate Polymers*, 232, 115793.
168. Dagostin, S., Bamford, L., & Davis, N. (2021). Invasive infections associated with probiotics in pediatric patients: A systematic review of case reports from 1995 to 2021. *Pediatric Infectious Disease Journal*, 40(12), 1055–1062.
169. Morgan, J. L., Mehta, K., & Cowen, L. E. (2020). Probiotic-associated sepsis: A risk assessment of bacteremia and fungemia in vulnerable populations. *Infection Control & Hospital Epidemiology*, 41(3), 367–371.
170. Walsh, J., Griffin, B. T., & Clarke, G. (2018). Gut microbiota: The neglected metabolic organ influencing drug disposition and efficacy. *Pharmacological Reviews*, 70(1), 53–72.

171. Gingell, R., Bridges, J. W., & Williams, R. T. (1971). The role of intestinal bacteria in reducing the urinary excretion of sulfanilamide in rats treated with azo drugs. *Biochemical Journal*, 124(1), 77–81.
172. Wilson, I. D., & Nicholson, J. K. (2017). Gut microbiota interactions with xenobiotics and their implications for drug metabolism and toxicity. *Expert Opinion on Drug Metabolism & Toxicology*, 13(10), 1041–1051.
173. Wilkinson, E. M., Ilhan, Z. E., & Herbst-Kralovetz, M. M. (2018). Microbiota-drug interactions: Impact on metabolism and efficacy of therapeutics. *Molecular Aspects of Medicine*, 61, 59–89.
174. Gao, Y., Zhao, M., & Lu, J. (2022). β -glucuronidase activity in the gut microbiota: Implications for health, disease, and therapy. *Frontiers in Microbiology*, 13, 828232.
175. López-Moreno, A., de la Cruz, S., & Suárez, A. (2020). Pharmacomicrobiomics and toximicrobiomics: The role of the gut microbiota in drug response and toxicity. *Pharmacological Research*, 159, 104946.
176. Dodge, M., Savage, P., & Blaser, M. J. (2023). Enhancing probiotic persistence in the host: Novel approaches to colonization. *Trends in Microbiology*, 31(4), 255–265.
177. Ziegler, E. E., Vanderhoof, J. A., & Bonuck, K. A. (2021). Persistence of *Bifidobacterium longum* subsp. *infantis* in the infant microbiome following supplementation. *Journal of Pediatric Gastroenterology and Nutrition*, 72(3), 335–340.
178. Invernici, M. M., Salvador, S. L., & Silva, P. H. (2020). Probiotics as a therapeutic strategy for oral health conditions: Microbiota modulation and persistence. *Frontiers in Microbiology*, 11, 1269.
179. Fargen, K. M., Frei, D., & Fiorella, D. (2013). The FDA approval process and its role in the long-term safety of medical devices and biologics. *Journal of NeuroInterventional Surgery*, 5(4), 298–303.
180. Hemarajata, P., & Versalovic, J. (2013). Probiotics: Potential for therapeutic use in microbiome-related diseases. *Clinical Microbiology Reviews*, 26(4), 807–844.
181. Zawistowska-Rojek, A., & Tyski, S. (2022). Probiotics as a tool for health promotion and disease prevention: New insights into their mechanisms of action. *Nutrients*, 14(3), 452.
182. Sanders, M. E., Merenstein, D., Merrifield, C. A., & Hutkins, R. (2019). Probiotics for human use: A consensus of recommendations. *Gut Microbes*, 10(4), 351–362.
183. Roselli, M., Finamore, A., Britti, M. S., & Mengheri, E. (2021). Probiotics: Mechanisms of action and their potential therapeutic applications in gut health and immunity. *Microorganisms*, 9(3), 636.
184. Sharifi-Rad, J., Sureda, A., & Tenore, G. C. (2020). Probiotics: Therapeutic and prophylactic potentials in inflammatory bowel diseases and beyond. *Frontiers in Immunology*, 11, 347.
185. Shanahan, F., & Quigley, E. M. M. (2014). Manipulation of the gut microbiota for treatment of IBS and IBD—What is the state of the art? *Gut Microbes*, 5(2), 247–252.

Impact of SARS-CoV-2 on Cancer Patients and the Potential Role of Probiotics for the Management Purpose to Boost Immune System

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ABSTRACT

COVID-19 was caused by a new zoonotic coronavirus known as “severe acute respiratory syndrome coronavirus 2” (SARS-CoV-2). Probiotics are living microorganisms that, when administered in sufficient quantities, provide health benefits to the host. Probiotics modulate a host’s innate immunity by increasing phagocytic activity, leukocytes, and receptor expression associated with phagocytosis, as well as improving neutrophil microbicidal activity. These probiotic species have been shown to be effective against a variety of infections, including severe RTIs, antibiotic-associated and infectious diarrhea, and necrotizing enterocolitis. Gut microbiota have been shown to inhibit coronavirus entry and reduce inflammation by lowering levels of pro-inflammatory cytokines. Oral probiotics have been shown to have antiviral properties, thereby improving intestinal health and achieving homeostasis. Therefore, an oral vaccine against the coronavirus could be extremely beneficial. Another fatal disease is cancer, and the treatment of

cancer has remained a subject of interest for researchers for the past decades. Research shows a relation between cancer and the use of probiotics. The activity of various probiotics against carcinogens works by modifying the composition of existing microbiota and their metabolic activity, production of anti-carcinogenic compounds, apoptosis and cell cycle arrest, degradation of toxic compounds, immune cell modulation, and gut barrier production.

13.1 INTRODUCTION

Corona disease first originated in 2019; thus it is named COVID-19. It's a novel health emergency that threatened humanity. Though it started in China's Hubei province in late 2019, it appeared in various countries around the world, turning the disease into a pandemic [1]. This pandemic was caused by a new zoonotic coronavirus called "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2), which is related to SARS as well as MERS (Middle East Respiratory Syndrome) [2]. Studies on phylogenetic diversity showed that SARS-CoV-2 has a sequence identity of 79% nucleotide similarity with a different virus belonging to the SARS-CoV family, which produced an epidemic back in 2002–03 that consequently caused 8,000 reported incidents in 26 countries [3].

Furthermore, SARS-CoV-2 exhibits sequence identity similarities for viral envelope and its nucleocapsid proteins of 96% and 89.6%, respectively, with SARS-CoV [4]. MERS was also caused by a member of the coronavirus family called MERS-CoV in 2012. COVID-19 produces a condition called "severe acute respiratory syndrome" (SARS), which is now exclusively named SARS-CoV-2, and has a mortality rate of 2% in China, going up to 12% in Italy [5].

The clinical features of COVID-19 include a high transmission rate, low to medium clinical expression with some severe abnormalities in radiological scans, mostly observed in the elderly [6]. This virus is an RNA virus with pointed protrusions present on the viral envelope, creating a crown-like appearance; therefore, it is named Coronavirus. Previous evidence implied that the viral RNA of SARS can be found in respiratory secretions as well as in the patient's stool after the onset of the disease for a better period of about a month, although the live virus may not be able to be cultured even after 3 weeks. Currently, the latest report indicates that the RNA of SARS-CoV-2 can also be detected in the patient's stool. This, alongside the fact that some of the patients with COVID experience diarrhea, points to a distinctive probability of an association between the gut-lung axis and gut microbiota [7].

13.2 PROBIOTICS AND PREVENTION OF DIFFERENT DISEASES

Probiotics are living microorganisms that, after administration in sufficient amounts, provide health benefits to the host. The use of probiotics to improve human health has been under study for a long time, initially as a food requirement and later for culture preparation [8, 9]. Most microorganisms currently identified as probiotics are from the category of gram-positive bacteria. Probiotics chiefly consist of specific types of bifidobacteria as well as lactobacilli. More bacterial probiotics, still under study, include *enterococci*, *bacilli*, *streptococci*, and *lactococci*. Additionally, those bacteria that can be categorized as potential probiotics include gram-negative bacteria. The finest model of this group is *E. coli*, which is used in Germany to treat prolonged constipation as well as colitis [10, 11]. *Saccharomyces boulardii* and *Saccharomyces cerevisiae* are also employed as probiotics.

The investigation for the use of probiotics was primarily focused on prevention along with cure of gastrointestinal diseases [12], nonetheless, other likely effects are also under studies i.e., dietary effects, prevention along with treatment of mouth infections, diarrhea, bowel diseases, *Helicobacter* infections, hypersensitive diseases, anticancer effects as well as decrease of serum cholesterol [13].

13.3 EFFECT OF PROBIOTICS ON IMMUNE SYSTEM

A key factor for the prevention and management of a disease is the regulation of the immune system. Probiotic microorganisms have shown their capacity for stimulation and modulation of the immune system, as well as for lowering inflammation [14]. Probiotics can also reduce the severity of infections within the gastrointestinal system and upper respiratory tract by working together on both innate and adaptive immunity. Presently, the application of probiotic microorganisms themselves and/or their metabolic products exemplifies a likely method for the therapy of viral infections [15]. Probiotic microbes have also been demonstrated to have several positive health and immune effects. They enhance the bioavailability of several nutrients combined with the moderation of health. Moreover, they show involvement in the regulation of the microbial ecosystem as well as the modulation of immune system cells.

Probiotics regulate the innate immunity of a host by increasing phagocytic action, leukocytes, along with the expression of receptors (CR3, CR1, FcαR, and FcγRI) that are related to phagocytosis, as well as enhancing neutrophils' microbicidal activity [16]. The quantity and activity of NK cells are also considerably enhanced [17]. Signaling of TLR (Toll-like receptor) by organisms displays a substantial impact on protecting the intestinal homeostasis

of the host by improving gut barrier activity and enhancing local immunity [18]. Dendritic cells perform a vital role in the immune system's homeostasis of the intestine. DCs are important APCs that take up antigens and present them on the surface to T cells to induce (Th1) pro-inflammatory or (Th2) anti-inflammatory phenotypes. The intestinal microbiota is capable of prompting DCs to establish these cells. *L. reuteri* along with *L. casei* excite IFN-gamma assembly and trigger Th1 cells. Similarly, the oral application of *B. infantis* in mice promotes DCs that repress Th2-inclined responses [19] and promote Th1 responses, which are a requirement for viral removal.

Monocytes appear in blood upon contact with infectious microbes. They divide into tissue macrophages, and here gut microbiota or consumed probiotics act together to produce several cytokines. The Th1 cytokine, i.e., IL-12, is produced by macrophage cells, which promote NK cells along with CD4+ Th1 cells to produce IFN-gamma that is necessary for the removal of viruses. In addition, probiotics such as *L. delbrueckii* ssp., *L. acidophilus*, and *B. bifidum* generate IFN-alpha formation by means of monocytes. *L. paracasei* enhances IL-6, TNF-alpha, and IL-8 in human THP-1 monocytes [20]. Likewise, recent reports state that *S. thermophiles* generated IL-6, TNF-alpha, and IL-8 that are needed for anti-viral activity [21].

13.4 PROBIOTICS IN VIRAL PREVENTION AND ELIMINATION

It's reported that species of *Lactobacillus* include *L. rhamnosus*, *L. plantarum*, and *L. gasseri*, along with *Bifidobacterium* strains including *B. longum* and *B. lactis*, which have antiviral effects (Table 13.1). These probiotic species are able to eliminate viruses by modulating the immune system, for example, by activating the Th1 immune response, which consequently amplifies IFV-specific immunoglobulin A that causes the elimination of IFV from the lungs. Similarly, these species are also effective against influenza virus infections and respiratory viral infections by inhibiting the proliferation of the virus in the respiratory tract and lungs, as well as activating macrophages, which leads to enhanced expression of inflammatory cytokines, i.e., IFN- γ , IL-1 β , IFN- α , IRF-7, and 2', 5'-oligoadenylate synthetase (OAS) genes, thus helping in the elimination of viruses. Studies suggest that species of *Bifidobacterium* and *Lactobacillus* are protective against human rotavirus. They are responsible for producing antiviral peptides that inhibit viral replication and proliferation. These probiotic strains considerably lowered the duration of diarrhea in pediatric patients with no harmful effects.

Different species of *Lactobacillus*, such as *L. casei*, *B. lactis*, *L. paracasei*, *L. fermentum*, and *B. animalis*, play a significant role in strengthening the immune

system and alleviating infections in humans by regulating the equilibrium between Th1 and Th2 cytokines. It has been studied that these probiotic species are effective against many infections, for example, respiratory tract infections (RTIs), antibiotic-associated and infectious diarrhea, along with enterocolitis. It is acknowledged that the beneficial effects of probiotics are due to multifactorial processes, including bacterial attachment inhibition, enhanced mucosal barrier function, and immune response regulation by increasing the emission of interferon-gamma (IFN- γ). An enhanced quantity of IFN- γ may stimulate macrophagic function and exert various immune-modulatory actions. These findings prove that probiotics have a protective role against infections. Table 13.1 depicts several promising roles of probiotics against various viruses [22].

13.5 SARS-COV-2 AND IMMUNE SYSTEM OF HUMANS

SARS-CoV-2 Th1 response may produce a respiratory hyper-inflammatory development that can lead to ARDS (acute respiratory distress syndrome) as well as DAD (diffuse alveolar destruction) [23]. It harms bronchi structurally and disturbs lung surfactant, which is a defensive film created by type II pneumocytes [24]. The spike (S) protein present on SARS-CoV-2 is a structural protein that attaches to the angiotensin-converting enzyme-2 receptor (ACE2) present on human cells, utilizing it as an entry point to invade the host cell and replicate. The receptor is then expressed in endothelial cells, on respiratory epithelial cells, and on gut cells. ACE2 is a main enzyme in the renin-angiotensin system (RAS) that modulates blood pressure, osmotic homeostasis, and vascular resistance. The lungs of humans have a large surface area that is coated with epithelial type-2 cells, which may explain the higher susceptibility to COVID-19 infection compared to other organs. The evolution of the infection led to an imbalance in redox homeostasis along with a sudden surge in free radicals, causing cell damage. The serum of COVID-19 patients has elevated Th1 cytokines, i.e., TNF- α , IFN- γ , IL-2, IL-6, IL-7, along with granulocyte colony-stimulating factor (GCSF). The stimulation of Th1 is the origin of chronic inflammation within a human host [22].

13.6 RELATION BETWEEN RESPIRATORY TRACT INFECTIONS AND GUT MICROBIOTA

A critical relationship among modified intestinal microbiota and immune response to pulmonary infections is proven. For instance, pulmonary syncytial virus and influenza, intestinal microbiome was substantially modified

TABLE 13.1 Effect of Probiotics on Targeted Viral Infections [22]

Types of Virus	Probiotics	Outcomes	Dose
Respiratory viruses.	Lactic acid bacteria	Protects against respiratory influenza virus infection	120 mg
	<i>Lyophilized Lactobacillus rhamnosus</i>	The clinical symptom scores and pulmonary virus reduced	10 mg
	<i>Lactobacillus delbrueckii ssp. bulgaricus</i>	Affected influenza A virus	100 g
	<i>L. plantarum</i>	Protects against infection with H1N1 and H3N2 influenza viruses	10 ⁸ –10 ⁹ CFU
	<i>L. plantarum</i>	Decreased influenza symptoms	20 mg
	<i>Clostridium butyricum</i>	Improved the secondary infection	10 ⁷ CFU
	<i>L. plantarum</i>	Decreases the total symptom score	3.6 x 10 ⁹
	<i>L. paracasei N1115</i>		
	<i>L. paracasei</i>	Early activation of proinflammatory cytokines	2 × 10 ⁸ CFU
	<i>L. rhamnosus GG</i>	Respiratory tract infections reduced	1 × 10 ⁹ CFU
Digestive viruses	<i>Lactobacillus gasseri</i>	Beneficial effects on the protection against enteric viruses <i>in vivo</i>	5 × 10 ⁷ cells/mL
	<i>L. rhamnosus (LGG)</i>	Shows a protective effect against Rotavirus (RV)	10 ⁸ CFU
	<i>Bifidobacterium longum</i>	Increased the production of INF-β in PIE cells	5 × 10 ⁸ cells/mL
	<i>L. rhamnosus, L. plantarum</i>	Inflammatory damage reduced	5 × 10 ⁸ cells/mL
	<i>B. bifidum G9–1</i>	Protecting against RV-induced lesions	f3 × 10 ⁷ CFU
	<i>L. plantarum</i>	Inhibited TGE coronavirus	10 ⁸ to 10 ⁹ CFU/mL
	<i>Enterococcus faecalis FK-23</i>	Decreased alanine transferase	900 mg
Other viruses	<i>Lactobacillus rhamnosus</i>	Decreased abnormal cervical smear rates	180 mg
	<i>L. rhamnosus</i>	Useful to control the infection by the human papilloma virus	10 ⁴ CFU
	<i>L. rhamnosus</i>	Increased viability of macrophages, induced antiviral effects against HSV-1	1 × 10 ⁸ CFU/mL
	<i>L. plantarum 299v</i>	Protective effects against inflammation	5 g
	<i>Saccharomyces boulardii</i>	Decreased production of pro-inflammatory cytokines	10 ⁷ CFU/day

by infections along with multi-factorial variables, i.e., TNF- α [25]. Integral microbiome gives signals that lead to inflammasome simulation, production of IL-1 β in addition to pro-IL-18, in addition the relocation of DCs from lungs towards lymph nodes as well as T-cells, which is important for immunity after an influenza infection [26]. Agitated gut microbiome influences innate as well as adaptive immune signals, i.e., an enhanced vulnerability towards asthma, respiratory allergic infections etc. [27, 28]. More crucially, the seriousness of influenza has been immensely associated to the mixed reactions of the intestinal microbiome, as stated by the conclusion that *Bifidobacterium* species in intestine can develop to improve host's endurance towards influenza [29].

Furthermore, gut microbes modulate the innate response by stimulating pattern recognition receptors (PRRs) present on monocytes, macrophages, and NK cells to distinguish microbe or pathogen-linked molecular forms on microorganisms. TLRs, along with nucleotide-binding oligomerization domain (NOD)-like receptors, identifiable on host cells by means of PRRs, induce various immunological responses dependent on cell type, receptors, or ligands [30]. Alteration in the regulatory equilibrium of Th1 responses and regulatory T cells, eventually operated by microbes, is crucial in managing intestinal immune homeostasis [31]. For instance, polysaccharide A, which is an immune-modulatory molecule produced by *Bacteroides fragilis*, may facilitate the transformation of CD4⁺ T cells to IL-10-producing Foxp3(+) Treg cells, which can be studied to prevent and cure colitis in mice [31].

13.7 COVID-19 AND GUT MICROBIOTA

Gut microbiotas are reported to fight the entry of the coronavirus as a first line of defense. Even if the virus enters the human body, probiotic bacteria can lower the severity of the infection through their antiviral activity. Once the cytokine level increases in circulation, it can produce ARDS. In such cases, unusual inflammatory responses are amplified in the body, which can cause harm to various organs, potentially leading to organ collapse and ultimately fatality [32, 33]. Probiotics are reported to lower this inflammation by reducing the level of Th1 cytokines. In addition, COVID-19 is associated with the dysbiosis of the intestinal microbiome, which leads to a profusion of infective bacteria in humans. The sudden secretion of cytokines, immune dysregulation, and increased gut permeability are caused by COVID-19.

Moreover, the mucosa's disproportionate viral burden changes gut microbiota, causing damage to the intestinal barrier's reliability and leading to secondary infections after the coronavirus attack [34, 35].

13.8 DIETARY PROBIOTICS EFFECT ON COVID-19

Probiotics taken orally are proven to show anti-viral influences and thus enhance intestinal health to achieve homeostasis. For instance, regarding influenza, *Lactococcus lactis* showed action against influenza by activating the immune response [36]. Oral uptake of *Bacteroides breve* can improve immunoglobulin G in response to influenza. Furthermore, a meta-analysis indicates that taking these probiotics substantially lowered the cases of pneumonia, probably by reducing the overpopulation of potentially opportunistic pathogenic microbes and increasing immunity [37]. Nevertheless, the idea of oral administration of probiotics in treating patients suffering from COVID-19 must be explored.

13.9 ORALLY ADMINISTERED VACCINES AGAINST COVID-19

Many vaccines are created to counter SARS-CoV-2, along with others that are still going through medical tests [38]. Vaccine injections are expensive and need professional administration, while vaccines administered orally are cheap, easy to store, simple to deliver, and bio-friendly [39]. Moreover, it is described that orally delivered vaccines can produce vigorous assembly of IgA and IgG, as well as Th17 and Th1 reactions, thus preventing viral reproduction in the pulmonary tract and GI mucosa [40]. Therefore, an effective orally administered vaccine for COVID-19 will be greatly beneficial.

Lactobacilli are possibly appropriate vectors for oral DNA-based and protein-based vaccine delivery. These microbes often appear in the human gut and are advantageous in many GI and inflammatory infections [41]. *L. plantarum* is usually identified as a probiotic that can be administered through food, medication, and vaccines [42]. Studies established a *L. plantarum* system of expression for COVID-19 S protein manufacture. By adjusting the codon manipulation of the S gene, the S protein was effectively introduced on the surface of bacteria, which led to the production of several misfolded protein chains.

This S protein demonstrates an intense reaction to SARS-CoV-2 S-protein-specific monoclonal antibodies. The strength of the immunity-producing protein is vital for orally administered vaccines. Thus, they analyzed the S protein's stability displayed on the surface of bacteria and discovered that it was resistant to heat as well as low pH. S protein expression was raised after exposure to 0.2% bile salt, indicating that recombinant *L. plantarum* called Lp18:S can be administered for oral vaccination purposes to treat COVID-19 [1].

13.10 GUT MICROBIOTA AS REPRESSOR OF COVID

Probiotic bacteria including *Lactobacillus species* such as *B. longum*, *L. plantarum*, *L. gasseri*, *L. rhamnosus*, and *B. lactis* work by multifactorial processes to elevate anti-viral immunity within a host. Probiotic microbes can restrict the adsorption mechanism by directly attaching to viruses and impeding entry into epithelial cells. In COVID-19, probiotic microbes can attach to the mucosal epithelial cell surface, consequently producing steric restriction and obstructing the binding of the virus to the angiotensin-converting enzyme 2-like host cell receptor. The establishment of probiotic microbes plays a key role in enhancing the intestinal microbiome, improving the mucosal barrier, and managing dysbiosis as well as diarrhea symptoms. Microbes distribute various materials, i.e., biosurfactants, bacteriocins, lactic acid, organic acids, nitric oxide, and hydrogen peroxide; a few or all of them may prevent virus propagation [43]. The secretion of gut mucins from mucosal cells and IgA can also efficiently counteract the viral attack. Once the virus attacks the epithelial cells, probiotic bacteria play a critical role in antiviral immunity by regulating the host immune response, involving stimulation of NK cells, Th1/Th2-facilitated immunization, production of inflammatory cytokines, along with specific antibodies [44]. Stimulation of immunity leads to the division of CD8⁺ T-lymphocytes to form cytotoxic T-lymphocytes, which are capable of damaging virus-infected cells. Later, CD4⁺ T-lymphocytes can further differentiate into Th1 cells, which stimulate phagocytic activity by NK cells and macrophages, promoting the destruction of pathogens. Additionally, CD4⁺ cells also form Th2 cells that stimulate B-cell production, consequently producing antibodies that play a critical role in fighting the spread of coronavirus in the body. Therefore, probiotic microbes catalyze important immune responses and enhance immune homeostasis in

coronavirus infections. COVID-19 has triggered a terrible increase in the death rate in many countries. Improving human immunity is one of the best preventative methods to lower the severity of this situation. Probiotics have many advantages, such as balancing the arrangement of human intestinal microbiota, improving the gut barrier, and enhancing responses to reduce the severity of CoV-2.

Research has shown that probiotics are beneficial in preventing or treating pulmonary tract infections caused by viruses, such as influenza, by improving the immunity of individuals through stimulating IgA production and enhancing the action of neutrophils, Peyer's patches, mesenteric lymph nodes, intraepithelial lymphocytes, macrophages, and NK cells [45]. Orally administered probiotics are used to prevent or treat infections caused by influenza and pulmonary viruses by reducing infectious symptoms, shortening the duration of infection, lowering viral levels in the pulmonary system, producing antiviral components, stimulating immunity, and improving health by reducing body weight loss due to infection [46].

Probiotics are noted for their capability to inhibit viruses through immediate interaction in a process similar to phagocytosis. It has been examined that probiotics show an elevated IL-12 stimulating action *in vitro* in mice peritoneal macrophages. Probiotic species inhibited antigen-specific IgE production by activating Th1 immunity in mice and increased NK cell activity and immunoglobulin A production *in vitro*, demonstrating a protective effect against many viral infections *in vivo* [47].

13.11 PROBIOTICS AND CANCER

One of the fatal diseases includes cancer, and thus the treatment of cancer has remained a subject of interest for researchers for the past decades. Among the first researchers are Gorbach and Goldin. Their research was based on a relationship between a Lactobacillus-enriched diet and colorectal cancer. Their study demonstrated that the diet influenced the incidence of cancer and reduced it to 40% in comparison to the control group, which had a 77% incidence of the disease. After this breakthrough, researchers gained much interest in the subject and tested the effect of various probiotics on many cell lines, both *in vitro* and *in vivo* (Tables 13.2 and 13.3).

TABLE 13.2 Effect of Probiotics on Various Cell Lines i.e., Cells of Colon Cancer in Humans: DLD-1, HT-29, Caco-2, SW1116, LoVo, SW480, HCT116 Cells of Human's Epithelium in Colon: NMC460. Cells of Adenocarcinoma in Human GI: AGS Carcinoma Cells of Colon CT26, *In-Vitro*

Probiotic	Cell line	Effect	References
<i>Bifidobacterium adolescentis</i>	Caco-2, HT-29, SW480	Cell proliferation	[48]
<i>Enterococcus faecium</i>	Caco-2	21% lower cell proliferation	[49]
<i>Lactobacillus fermentum</i>		23% lower cell proliferation	[50]
<i>Lactobacillus rhamnosus</i>	Caco-2	Increased apoptosis	[51]
<i>Bacillus polyfermenticus</i>	HT-29, DLD-1, Caco-2	Lower cell proliferation	[52]
	NMC460	Lower formation of cell colony	[53]
<i>Lactobacillus paracasei</i>	DLD-1	Lower cell proliferation	[54]
<i>Pediococcus pentosaceus</i>	Caco-2	Lower cell proliferation	[55]
<i>Lactobacillus plantarum</i>	Caco-2, HT-29	Lower cell proliferation	[56]
<i>Clostridium butyricum</i>	HCT116, SW1116, Caco-2	Lower cell proliferation	[57]
<i>Bacillus polyfermenticus</i>	LoVo, HT-29, AGS	90% Lower cell proliferation	[58]
<i>Lactococcus lactis</i>		80% Lower cell proliferation	[59]
<i>Lactobacillus casei</i>	HT29 and CT26	Apoptosis induction	[60]
<i>Lactobacillus pentosus</i>	Caco-2 and HT-29	Lower cell proliferation	[61]

TABLE 13.3 Effect of Various Probiotics on Animal Models *In-Vivo*

Probiotic strain	Model	Treatment	Result	Reference
<i>Lactobacillus acidophilus</i>	Rat	10 months	Decrease in tumor incidence and progression	[62]
<i>Bifidobacterium lactis</i>		4 months and 3 weeks	Decrease in tumor incidence and volume	[63]
<i>Bacillus polyfermenticus</i>	Mice	5 months	Decrease in tumor incidence and volume	[64]
Probiotics mixture	Rat	2 months and 2 weeks	No colorectal cancer development	[65]
<i>Lactobacillus rhamnosus</i>		4 months and 1 week	Decrease in tumor incidence and multiplicity	[66]
<i>Lactobacillus plantarum</i>	Mice	Live nanosized bacteria injected for 1 month	Low incidence of tumor, cell cycle arrest and apoptosis induction	[67]
<i>Lactobacillus plantarum</i>		3 months and 2 weeks	Necrosis induction and volume reduction of tumor	[68]

TABLE 13.3 (Continued)

Probiotic strain	Model	Treatment	Result	Reference
<i>Lactobacillus plantarum</i>	Rat	3 months	Reduction of tumor incidence, volume and multiplicity	[69]
<i>Lactobacillus salivarius</i>		14 days	Decrease in tumor incidence	[70]
<i>Lactobacillus acidophilus</i>		5 months and 3 weeks	Reduction of tumor volume and incidence	[71]
<i>Lactobacillus rhamnosus</i>		6 months and 1 week	Reduction of tumor multiplicity, volume and incidence	[72]
<i>Pediococcus pentosaceus</i>	Mice	1 month	Apoptosis induction and decrease in tumor progression	[73]
<i>Lactobacillus casei</i>		2 months and 2 weeks	Decrease in tumor incidence	[74]

13.11.1 MECHANISMS OF ACTION

The activity of various probiotics against carcinogens works by

- Modifying composition existing microbiota.
- Modifying metabolic activity.
- Production of compounds that has anti-carcinogenic effect.
- Induction of cell death in cancerous cells.
- Cell cycle arrest.
- Degradation compounds that have mutagenic and carcinogenic nature.
- Modulation of immune cells.
- Better barrier production in the gut.

The microbiome of the gut is involved in various pathways with different functions. This microbiome plays important roles in preserving homeostasis and maintaining various physicochemical conditions in the colon to keep a sustainable environment within the body. Colon cancer is a fatal type of cancer that arises due to acidic pH formation caused by the excess release of bile acids, which are toxic to the epithelial cells of the colon. Some probiotics, such as *Lactobacillus*, have been shown to reduce the incidence of and prevent colon cancer [75].

Some bacteria have also been demonstrated to play an essential role in maintaining a balance among the metabolic activity of the normal microbiota of the gut and their participation levels. Probiotics, such as *E. coli* and *Clostridium*, are present in the intestine as part of its normal flora, and they

have been shown to be involved in the production of some carcinogenic chemicals using enzymes. Upon consuming certain fermented milk products, it has been demonstrated that the quantity of beneficial *Lactobacillus* increases, which in turn reduces the production of carcinogenic compounds.

Some strains of probiotics, i.e., *Bifidobacterium* and *Lactobacillus*, are involved in the binding and further degradation of various carcinogenic compounds. Some carcinogens are mutagenic in nature and are involved in the incidence of cancer. These carcinogens can be present in an unhealthy diet. Research shows that the intake of probiotics like *Lactobacillus* reduces this mutagenic effect, which in turn decreases the excretion of mutagenic compounds, i.e., heterocyclic aromatic amines (HAAs) [76]. Probiotics are also involved in the production and metabolism of compounds that are involved in the maintenance of homeostasis as well as in the suppression of carcinogenic compounds. Gut microbiota is involved in the production of short-chain fatty acids (SCFAs) upon fermentation of prebiotics that are fiber-rich. Colon cancer has a key relation with low SCFA levels as well as dysbiosis of bacteria that produce SCFAs. Lactic acid bacteria are involved in the modulation of the immune system by regression or suppression of various carcinogenic compounds. This behavior of the probiotic strains shows an interaction between the metabolites of bacteria with the epithelial and immune cells [77].

13.12 INDUCTION OF APOPTOSIS AND INVOLVEMENT OF PROBIOTICS

Numerous reports indicate that probiotics prevent the formation of tumors as well as cancer development by means of the process of apoptosis; however, there are only a small number of studies that have inferred the correct way of action that is causing the apoptosis stimulation. According to the study presented by Pfeiffer and Singh [78], cell death is a very easy and feasible way of action that can be used for cancer therapy. The process of apoptosis is a type of cell death during which a “self-destruction” course of action is initiated, leading to the fragmentation of DNA, reduction of cytoplasmic fluid, changes in membrane structure, and further causing the death of the cell without cell lysis or any damage to the cells that are present in the vicinity. The growth of the tumor is inhibited, which is one of the key roles of the apoptosis process. There are three pathways that are connected to each other and lead to the process of apoptosis. These pathways are:

- Intrinsic or mitochondrial pathway.
- Extrinsic or death receptor pathway.

- Granzyme or perforin pathway.

The genetic factors and causal genes that are implicated in apoptosis include TNF (tumor necrosis factor), inhibitor proteins against the proteins involved in apoptosis, the Bcl2 gene (B cell lymphoma), caspases, as well as the p53 gene. Many studies show that the induction of apoptosis through the use of probiotics on cancerous cells can be achieved by modulating Bcl2 and caspase genes [79]. In addition to this, colicin, which is a bacteriocin produced by *Escherichia coli*, is isolated and used in cancer therapy, demonstrating anti-cancer activity by subsequently forming small pores in the cell membrane. These small pores stimulate cell death and trigger cell cycle arrest during the G1 phase. Preet et al., confirmed that the interactive effect produced by nisin, when used in combination with doxorubicin, resulted in a decrease in tumor volume by 66.82% in mice compared to the untreated control [80]. Konishi et al., analyzed the ferrochrome molecule, which is a tumor suppressor derived from probiotics that has the capacity to prevent the progression of colon cancer through JNK (c-jun N-terminal kinase) facilitated apoptosis.

Furthermore, linoleic acid in conjugated form, which is a useful lipid formed by LPCLA (*Lactobacillus plantarum*), facilitated cell death in the cancerous cells of the breast by means of the downregulation of the NF κ B pathway. Similarly, probiotic strains, i.e., *Lactobacillus acidophilus* as well as *Bifidobacterium bifidum*, displayed amplified cytotoxic effects against cancerous cell lines of both the colon and the breast by means of upregulation of the expression of IFN- γ , Bax, and TNF- α , along with the downregulation of the expression of Bcl2 [81]. Additionally, the probiotic strain *L. acidophilus* is also involved in the induction of cell death by amplifying the expression of mRNA of the survivin protein as well as reducing the expression of mRNA of the protein SMAC. Jan et al., discovered that the mitochondrial pore development pathways induce cell death through the activation of caspase proteins. Moreover, research showed that the probiotic *Propionibacterium* leads to the cell death of colorectal cancer cells by means of activity on the mitochondria by the short-chain fatty acids [82]. In general, scholars continue to discover the apoptotic potential of probiotics towards cancerous cells. The area of probiotics-facilitated cell death research is quickly advancing.

Asoudeh-Fard et al., discovered that the probiotic strain *L. plantarum* stimulates cell death by means of the MAPK downregulation (mitogen-activated protein kinases) along with upregulating the pathways of PTEN (phosphatase and tensin homolog) [83]. Furthermore, Zhang et al., stated that the probiotic strain *Lactobacillus spp.* produces metabolites that present a damaging effect on the sustainability of the carcinoma squamous cell of the

human tongue CAL-27 and lead to the induction of cell death [84]. Another probiotic strain, i.e., *L. salivarius*, was observed to decrease the incidence of mouth cancer in rats by means of the downregulation of the expression of COX-2/PCNA genes as well as the induction of cell death. While the modification in the normal microflora present in the mouth stimulates oral cancer, the same normal microflora, especially probiotics, plays a key role in the inhibition of oral cancer [85].

13.13 PROBIOTICS AND AUTOPHAGY

Autophagy is a process that occurs in damaged or old cells, causing them to self-destruct or degrade. During this process, a special sequestering organelle called the autophagosome, which is a double-membranous structure, or a lysosome filled with enzymes for cell lysis, fuses with the cytosol of the particular cell and leads to cell lysis by enzymes like hydrolases. Even though the process of autophagy is crucial for sustaining homeostasis in a typical cell, it has also been associated with several disorders. Growing evidence indicates that the process of autophagy encourages both tumor repression as well as its progression. Autophagy is a crucial process for the eradication of damaged cells, old proteins, and organelles. Furthermore, autophagy deficiencies may cause DNA damage and an increased incidence of cancer, indicating their role in tumor suppression. Studies on the prevention of tumor growth through the use of probiotics by inducing autophagy are still lacking. Wang et al., reported on a surface protein present in the probiotic strain *L. acidophilus*, which induced HCT116 cell death by modifying the levels of a protein related to autophagy called microtubule-associated protein 1 light chain 3 [86]. In further studies, probiotic LAB encouraged the induction of apoptosis along with the molecule 5-fluorouracil by activating Beclin1/GRP78-facilitated autophagy stimulation. The exopolysaccharide of probiotics that is bound to the cell can potentially stimulate the autophagy of cancerous cells present in the colon by activating Beclin1/GRP78 along with the core regulators involved in the intrinsic apoptosis pathway, which are Bcl-2 and Bak proteins.

The order of phases that are implicated in the process of autophagy are:

1. Sequestration;
2. Transfer to lysosomes;
3. Degradation;
4. Consumption of the products of degradation.

The chief genes, along with the proteins that are associated with the process of autophagy, are comprised of lysosome-linked membrane protein Beclin-1, p53, as well as damage-regulated autophagy modulator 1. Depending on the mode of action, the process of autophagy can be divided into four different types, i.e., chaperone-facilitated autophagy, microautophagy, selective autophagy, and macroautophagy. The process of autophagy can both stimulate or prevent the development of tumors, depending on various factors, such as the type or age of the tumor. Nevertheless, probiotics-facilitated autophagy needs further research to investigate the role of probiotics in the removal of tumors [87].

Figure 13.1 depicts the process of autophagy in the tumor cells or damaged cells.

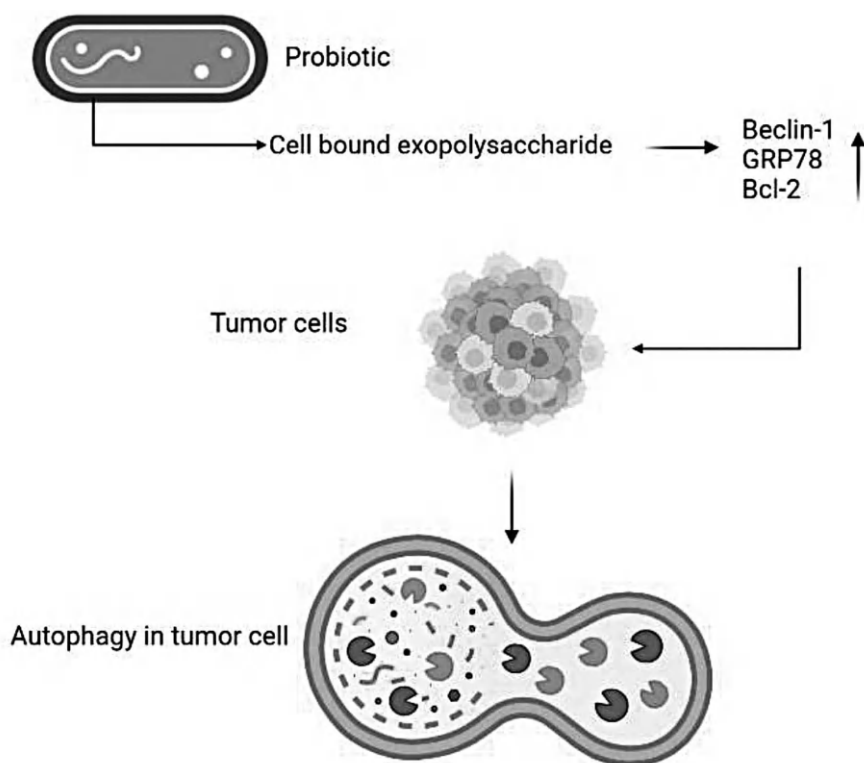


FIGURE 13.1 Graphic depiction of the process of autophagy in the tumor cells or damaged cells. The cell-linked exopolysaccharide produced by probiotic strains stimulate autophagy by upregulation of *GRP78*, *Beclin-1*, as well as *Bcl-2* genes. The serial events that occur during the process of autophagy are (a) sequestration (b) transference to lysosomes, (c) degradation, and (d) consumption of the products of degradation.

13.14 PROBIOTICS AND THEIR ROLE AS ANTIMUTAGENS

There are several agents that can produce DNA damage as well as mutations, which will ultimately lead to tumors. These agents that produce mutations in the DNA are known as mutagens. The incidence of cancer and the incidence of mutation are closely associated with each other. Many chemical agents, radiations of both ionizing and non-ionizing nature, as well as some viruses, are generally identified as mutagens that can lead to the incidence of cancers. Probiotic strains act as potential antimutagens because of the metabolites they produce. Research has examined the antimutagenic role of probiotics alongside sodium azide and benzopyrene, which are mutagenic in nature, and stated that probiotic strains show a binding potential towards the mutagens and act as detoxifying agents (antimutagens). Moreover, investigators are discovering the probable role of probiotic strains as substitutes for preserving and detoxifying agents. The antimutagenic role of the probiotic strain *Lactobacillus rhamnosus* alongside acridine orange, which is a mutagen, has been previously examined and confirmed [88].

13.15 PROBIOTICS FACILITATED TUMOR SUPPRESSOR RE-ACTIVATION

Suppressors of a tumor slow down the process of cell division, restore the DNA if damaged, and act as regulators for the process of apoptosis. The suppressor genes for tumors that are present in humans include Rb, BRCA1, APC, p16, BRCA2, p53, p21, and VHL. Any mutations or defects in any of these genes can cause cancers. Thus, it is critical for the tumor suppressor genes that are switched off by cancerous cells to reactivate. Various medical trials are in progress to analyze whether probiotic strains can be utilized as potential targeted novel biotherapy against cancers. Sharma et al., stated that the short-chain fatty acids produced by probiotics are involved in targeting cancerous cells through epigenetic regulation of the expression of suppressor genes as well as oncogenes in tumor cells [89]. Epigenetic activities change the expression of genes without altering the primary sequence of the DNA. Additionally, these processes are genetic, can be reversed, and include alterations in the methylation of DNA, histone modifications, and variations in noncoding small microRNAs (miRNAs). Metabiotics obtained from *L. rhamnosus* probiotics prevent colon cancer by upregulating p53 gene expression, which is a suppressor gene for cancerous cells. Another probiotic strain,

L. acidophilus, displayed upregulation of the expression of tumor suppressor genes in a 1,2-dimethylhydrazine-stimulated trial of colon cancer induction in a model. *Bifidobacterium longum*, another strain extracted from breast milk, was involved in the induction of the expression of tumor suppressor miRNAs, namely miR-15 and miR-145, in murine colon cancer [90].

13.16 ROLE OF PROBIOTICS IN DOWNREGULATION OF ONCOGENE EXPRESSION

The expression of oncogenes stimulates cells to show the characteristics of cancerous cells, while the oncogenes that are not mutated and are present in their wild state are called proto-oncogenes. Proto-oncogenes act as precursors for oncogenes, and once a mutation occurs in them, they are converted to form oncogenes. For the purpose of cancer therapy, drugs are used that will downregulate the expression of these oncogenes. The downregulation of oncogenes is one of the druggable targets of cancer therapy. Various proto-oncogenes have been discovered in different species through the use of structural homology modeling to oncogenes of retroviruses. Some of the significant proto-oncogenes that are found in humans are: β -catenin, *HER2*, *Ras*, *cyclin D*, *cyclin E*, *Myc*, and *MITF* (microphthalmia-related transcription factor).

Various studies have reported the suppression activity for tumors of various probiotic strains through downregulating oncogenes. The probiotic strains *Lactobacillus crispatus* and *L. rhamnosus* control cancerous cells by changing mTOR-related gene expression along with modulation of the Wnt/ β -catenin pathways. Azam et al., demonstrated that the supernatants of cultures of *L. acidophilus* and *L. crispatus*, *in vitro*, can cause the downregulation of the expression of cancer-testis genes [91]. A mixture of probiotic strains along with celecoxib (a non-steroidal anti-inflammatory drug) can also cause downregulation of the proto-oncogene KRAS, consequently reducing the occurrence of colorectal cancer. Investigating the process of downregulation of the KRAS gene through the use of probiotics can be helpful for patients suffering from RAS-related cancers. Hosseini et al., discovered that the nisin bacteriocin and the extract of cytoplasm along with the *Lactococcus lactis* cell wall reduced the expression of cyclin D1, which inhibits the division of SW480 cells. Extracts from *L. plantarum* that contain lipoteichoic acid are used to downregulate the expression of the MITF gene [92].

13.17 PREVENTING METASTASIS AND PROBIOTICS

Metastasis is related to the detachment of cancerous cells from the primary tumor, along with their propagation of cancer to other body parts. Patients suffering from cancer can develop metastasis years after the initial diagnosis of the primary tumor. In more than 90% of cancer cases, metastasis can eventually lead to death. The main cause of this is the epithelial–mesenchymal transition (EMT), a physiological progression during which cells present in the epithelium acquire morphological as well as physiological features of mesenchymal cells in the tumor cells.

Figure 13.2 depicts the flowchart diagram of the process of metastasis in cancerous cells. The process of metastasis in cancerous cells can be completed in five steps:

1. penetration of basement membrane.
2. entry in neighboring vascular/lymphatic system.
3. perseverance in circulation system.
4. exit from vessels to the secondary tissues.
5. establishment at secondary tumor locations [93].

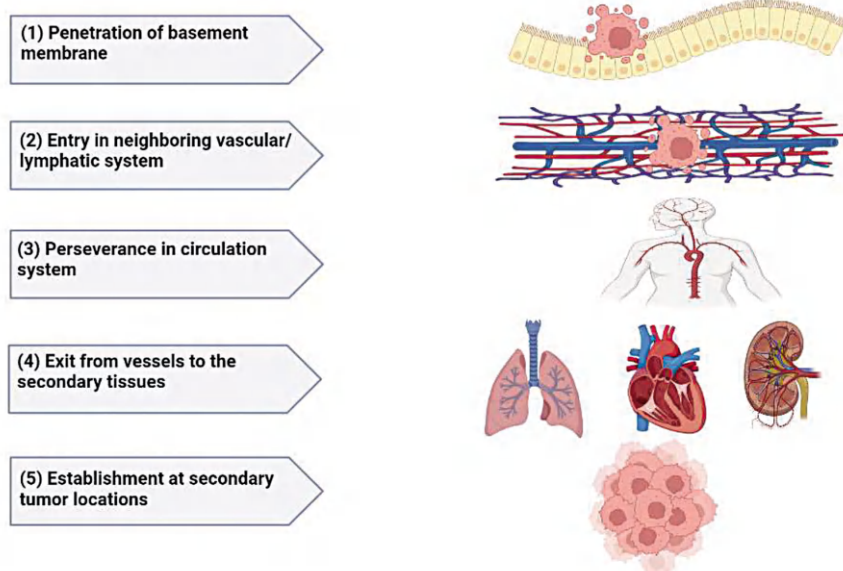


FIGURE 13.2 Flowchart diagram of the process of metastasis in cancerous cells.

The inhibition of metastasis in the initial stages is vital for a better prognosis of patients suffering from cancer. Furthermore, the prevention of supplementary metastases in patients with metastasis is beneficial for improved prognosis. Various studies have demonstrated the crucial factors related to metastasis, such as the disruption of cell–cell adhesion, epithelial-mesenchymal transition (EMT), the tumor microenvironment, the maintenance of cancer stem cells, and the anti-metastatic properties of probiotics. Supernatants (cell-free) obtained from the culture of probiotic strains like *L. casei* and *L. rhamnosus* GG lowered the occurrence of colon cancer and its metastatic properties by reducing the concentration of matrix metalloproteinase-9 (MMP-9) and elevating the concentration of tight junction protein ZO-1. Additionally, the cell-free supernatant of *L. plantarum* YYC-3 inhibited the metastasis of colon cancer cells by overturning the VEGF (vascular endothelial growth factor)-MMP2/9 signaling pathway. VEGF is a signaling protein involved in the promotion of the development of new blood vessels, while MMPs (matrix metalloproteinases) are involved in the degradation of the extracellular matrix. Thus, once the VEGF-MMP2/9 signaling pathway is suppressed, it can prevent the degradation of the basement membrane, which is the primary step in the process of metastasis [94].

The most essential protein involved in adhesion among cells is E-cadherin. Li et al., found that in HeLa cells, in response to probiotic treatment, there was a substantial upregulation of E-cadherin concentration along with the prevention of the migration of cancerous cells. Moreover, EMT-related markers were observed to reduce in a mouse model with pancreatic cancer when probiotics were administered [95].

13.18 KINASE INHIBITION BY PROBIOTICS

Phosphate groups are added via phosphatase enzymes, while the removal of phosphate groups is done by kinases. The process of phosphorylation is completed by the addition of a γ -phosphate group from a molecule of ATP (adenosine triphosphate) to a protein at residues serine, threonine, and tyrosine. There are around 518 genes that encode kinases and 156 genes that encode phosphatase enzymes in the human genome. The role of kinases is critical to study in cancer biology in various aspects, i.e., motility, propagation of cells, formation of new blood vessels, metastasis, as well as metabolism, which is the reason that these enzymes are used as therapeutic targets against cancer. Some studies show the role of probiotic bacteria and

their metabolites as inhibitors of kinases for the treatment of diarrhea that occurs after therapy against cancer. Seth et al., established the role of secretory proteins produced by probiotic bacteria that shield the tight junctions of intestinal epithelial cells and provide a barrier against H₂O₂-facilitated damage through PKC (protein kinase C) along with MAPK-dependent processes. *L. plantarum* induces cell death by downregulating MAPKs and upregulating phosphatases. *Lactobacillus* induces natural killer cell action by producing tumor necrosis factor-associated apoptosis-inducing ligand, i.e., TNF-AL, in prostate tumor cells [96].

13.19 BACTERIOCIN AND THEIR ANTICANCER ACTIVITY

Abundant studies have shown the anti-inflammatory and antioxidant metabolic processes of probiotics, which form the foundation of their anti-tumor properties. Han et al., investigated the anti-inflammatory action of the *Lactobacillus lactis* strain in RAW cells. A substantial decrease in cell division, as well as nitric oxide production along with pro-inflammatory cytokines, was observed [58]. The probiotic *Lactobacillus mucosae* strain, as well as *Lactobacillus fermentum*, significantly lowered paw edema and amplified the expression of the anti-inflammatory cytokine IL-10 in contrast with the pro-inflammatory cytokine IL-6. Chooruk et al., described that *L. rhamnosus*, *Lactobacillus paracasei*, and *L. fermentum* strains exhibit substantial antioxidant properties. Another study demonstrated that probiotic bacteria downregulate the enzymes that produce ROS (reactive oxygen species), butyrate, and glutathione (GSH). Previous research shows that probiotic strains can produce folate and bacteriocins that are low in molecular weight and possess anticancer, anti-inflammatory, and immunomodulatory characteristics [97].

Bacteriocins are classified into three major classes based on their molecular weight:

1. class I with molecular weight less than 5 kDa
2. class II with molecular weight less than 10 kDa
3. class III with molecular weight less than 30 kDa

Bacteriocins are FDA-approved compounds and are mostly utilized in the food and pharmaceutical industries. The anti-tumor mechanism of bacteriocins has been studied thoroughly and is well-documented in the literature. The special property of bacteriocins is that they target cancerous cells but do not damage normal cells. Normal cells have an asymmetric structure

due to the scattered phospholipids present in the cell membrane on both the outer and inner sides. The outer layer of the cell membrane in a normal cell is composed of phosphatidylcholine and sphingomyelin, which are neutral phospholipids containing zwitterionic properties, while the inner layer is composed of phosphatidylethanolamine and phosphatidylserine, which are amino phospholipids. In comparison, cancerous cells do not have an asymmetrical shape due to modifications in the phospholipids present in the cell membrane, along with a negative charge implicated by the O-glycosylated mucins, anionic phosphatidylserine, and heparin sulfates present on the cell membrane. In addition, cancerous cells express a higher level of membrane fluidity along with an elevated quantity of microvilli compared to normal cells, which results in an increase in surface area. Thus, bacteriocins can favorably attach to tumor cells that are negatively charged relative to the neutral-charged normal cells [98].

13.20 DRUG DELIVERY MECHANISM FOR BACTERIOCIN

Even though bacteriocins show exceptional antioxidant, antimicrobial, and anticancer properties, they may not be optimal for use as a drug delivery system. Bacteriocins can be easily digested in the intestinal tract by proteolytic enzymes. Therefore, there is a need to study alternative systems, such as delivery via liposomes, for the transfer of peptides against microbes and cancer. A rather advanced strategy, namely nanotechnology, is valued for advancing formulations of bacteriocins and their incorporation with nanoparticles for delivery. A bacteriocin called Nisin has been effectively used in implants and transported *in vivo* to prevent the development of *Staphylococcus aureus*. Another technique involves using hydrogels that are loaded with bacteriocins prior to delivery. A simple method, such as oral tablets, can also serve as a delivery system for bacteriocins, as well as chewing gum. Furthermore, to prevent infections caused by the attachment of bacteria to the surfaces of medical devices like catheters, bacteriocins are coated onto them [99].

13.21 CLINICAL TRIALS WITH PROBIOTICS

According to the definition used by the World Health Organization, a clinical trial is a research study that prospectively gives human volunteers or a group of persons one or more health-associated interventions to assess their effects

on health outcomes. While clinical trials examining probiotics date back to the early 1900s, the number of trials has notably increased in recent years, with more than 100 studies being listed each year after 2010. According to ClinicalTrials.gov, in addition to the International Clinical Trials Registry Platform, there are 323 and 1,157 research studies, respectively, currently examining the role of probiotics in the maintenance of pH, gut microbiota, oral health, regulation of the immune system, and activity against cancer and microbes in Europe, the US, and Asia [100].

Probiotic strains like *Bifidobacterium animalis* and *L. rhamnosus* have been most recorded probiotic bacteria. Furthermore, research in kids is more in quantity as compared to studies in elder people conferring to ClinicalTrials.gov. The main restraint against present studies is that most of them are experimental. However, immune control through the use of probiotics has been established in various research that investigates checkpoint prevention as a potent method in cancer therapy, as well as colitis, which is an opposing effect caused by therapy. The continuing research is focused on studying the role of probiotics in the treatment of metabolic, gastrointestinal, autoimmune, neurological, and infectious diseases. Moreover, medical trials have similarly highlighted the effectiveness of probiotic bacteria in lowering the side effects of cancer-associated microbiome dysbiosis. Additional medical trials that include broad populations and have fair statistical control are necessary to be further explored as a potential means of improving human health through probiotics [101].

13.22 CLINICAL PROOF OF USAGE OF LIVE PROBIOTICS TO PREVENT AND TREAT CANCER

Many probiotic bacteria that are used against cancer are still in the pre-clinical stage. Ahead of animal studies, there are some studies that have presented the action of therapeutic probiotics in humans [102]. During the medical trials of breast cancer, 306 cases along with 662 controls aged 40–55 have been examined regarding diet, lifestyle, and other risk factors related to breast cancer via a case/control type study. In a present study, a regular intake of the probiotic bacteria *L. casei* along with isoflavones from soy was substantially related to a reduction in the risk factors of breast cancer in Japanese adult women. The oral intake of six probiotic bacteria of the genera *Bifidobacterium* and *Lactobacillus* substantially lowered the levels of tumor necrosis factor- α , pro-inflammatory cytokines, IL-22, IL-17C, IL-17A, IL-12, IL-10,

and IL-6 in 52 colorectal cancer patients after surgery. During this randomized controlled experiment on the use of probiotics, no infections were observed after surgery, and there was no requirement for antibiotics [103]. Probiotic strains are also beneficial as they help avoid complications after surgery, reduce treatment toxicity, and enhance quality of life. In a cohort study with a follow-up of over 12 years after yogurt consumption containing probiotic strains *Lactobacillus delbrueckii* and *S. thermophilus*, 45,241 volunteers substantially lowered their risk of colorectal cancer. Although there is much evidence about the effects of probiotics against cancer, clinical studies observed in humans are still not enough. Subsequently, there is a need for studies in humans to provide sufficient data as an alternative therapy [104].

13.23 USE OF DEAD PROBIOTIC STRAINS TO PREVENT AND TREAT CANCER

As opposed to the common definition of probiotics, dead bacteria or cell constituents of these bacteria can substantially fight cancer. According to a study performed by Lee et al., killed *L. plantarum* that were reduced to a nanosize were used to inhibit azoxymethane/dextran sulfate sodium-facilitated (AOM/DSS) colorectal cancer in mice, with enhanced results compared to the live probiotics. This occurred because of suppression in the inflammatory response, cell death, and elevated secretion of IgA.

While mice that consumed dead *L. plantarum*, which were nanosized, showed significantly reduced expression of inflammatory markers, facilitated the expression of apoptotic markers, and affected the cell cycle of colon cells, there was also an elevation in the concentration of IgA in fecal matter compared to the usage of live probiotics. AOM/DSS inoculated into the mice contributed to a decrease in body weight along with a reduction in the size of the colon. Nevertheless, both live and killed probiotics prevented colon size reduction and loss of body weight. Dead bacteria displayed fewer colonic tumors, longer colons, and less loss of body weight in comparison to pure live bacteria *L. plantarum*. Fascinatingly, the suppression potency of the dead bacteria showed superiority compared to live bacteria, and specifically, the administration of killed probiotics at higher dosages significantly reduced the number of tumors compared to pure living probiotics. The administration of a combination of live probiotics with the dead ones considerably caused a reduction in pro-inflammatory cytokines and the overexpression of inflammatory response genes, along with greater suppression potency compared to individual administration. All of the control animals in the group in the

experiment displayed colon tumors, but inoculation of killed *L. plantarum* produced a substantial suppression in the progression of neoplasia. The process during which the killed bacterium retains the position of mucosal immunity is characterized by an increase in the concentration of secretory IgA. This shows that the anti-cancer properties of the dead bacterium are associated with the easier uptake of dead probiotics by the M cells in comparison to live probiotics, along with a strong immune response [65]. In addition to the dead probiotics, secretory molecules with greater molecular weight, such as the cell-free supernatant formed by heat-killed probiotic bacteria *Lactobacillus reuteri*, play a substantial role by exerting antimetastatic as well as antiproliferative properties. These significantly help in colon cancer in humans and *in vitro* apoptosis of the (HT29-ShE) cancer stem-like cells. Carbohydrates, proteins, or macromolecules of nucleic acid apply these repressive effects on HT29-ShE. This probiotic is implicated in the prevention of cell growth along with the increased apoptosis in HT29-ShE by regulating the expression of genes associated with the development of the cell cycle and the progression of tumors. Probiotics also participate in the downregulation of NF- κ B-facilitated genes, i.e., cyclin D1 and COX-2, cell division, and apoptosis in cancerous cells present in the colon [105].

Latest studies have shown that the inoculation of heat-killed probiotic bacteria *Enterococcus faecalis* provides protection against colorectal cancer induced by dextran sodium sulfate and enhances the severity of intestinal inflammation in normal mice. This probiotic bacterium defends the animals in the trial against cancer and colitis induced by dextran sodium sulfate through a reduction in the severity of intestinal inflammation by attenuation of phagocytosis, which is an essential process for Nod-like receptor protein 3 (NLRP3) inflammasome stimulation in macrophages derived from monocytic leukemia cells. In animals knockout for NLRP3, colitis was not defended by *E. faecalis*. Heat-inactivated bacteria *E. faecalis* are harmless and beneficial for NLRP3-facilitated colitis, as well as for the prevention of inflammation-related colon cancer production by inhibiting IL-1 β induction in the macrophages [106].

13.24 TREATMENT AND PREVENTION OF CANCER BY METABOLITES OF PROBIOTICS

Figure 13.3 illustrates the diagrammatic representation of various metabolites that can be used for the treatment and prevention of cancer.

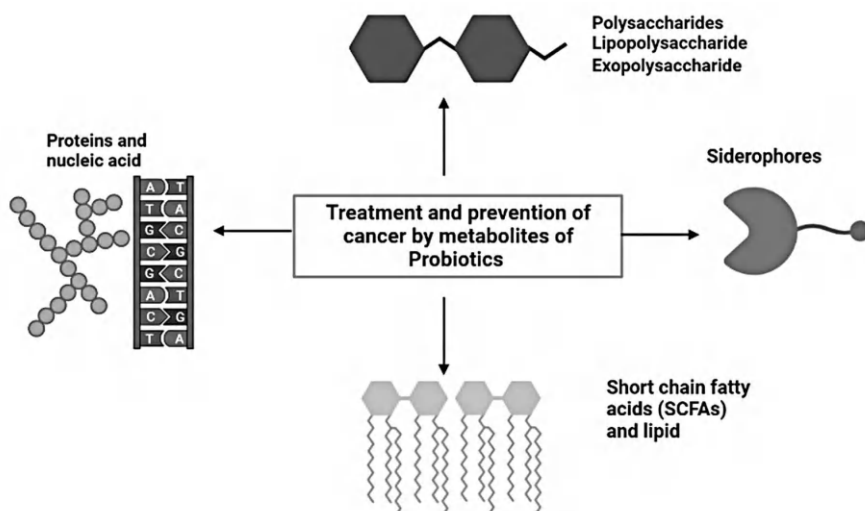


FIGURE 13.3 Diagrammatic representation of different metabolites that can be used for the treatment as well as prevention of cancer.

13.24.1 POLYSACCHARIDES LIPOPOLYSACCHARIDE AND EXOPOLYSACCHARIDE (EPS)

Metabolites that are derivatives of probiotic bacteria can inhibit tumor progression by modulating the host's immune system. For example, lipopolysaccharide (LPS) from bacteria, a significant part of the outer membrane of gram-negative bacteria, stimulates toll-like receptor 4, subsequently stimulating an immune response mediated by T cells against cancerous cells.

EPS isolated from bacteria plays a significant role in inhibition as well as cancer therapy. The experimental data show that gut lactic acid bacteria have a significant role in the suppression of cancer via their properties in modulating the immune system. These probiotics can stimulate phagocytes to eliminate early-phase cancerous cells. Another probiotic, *L. acidophilus*, has EPS that directly produce a cytotoxic action on cancerous cells through the processes of cell death, stimulation of the immune response, and NF- κ B inflammatory pathway deactivation. The result of *L. acidophilus* isolated EPS on colon cells is a potential target for cancer therapy. Components of the cell wall extracted from *L. acidophilus* as well as *L. casei* show anticancer activity. *L. plantarum* isolated EPS inhibit the division of hepatocellular cancerous cells [107].

Furthermore, EPS generated by probiotic LAB like *L. rhamnosus*, *L. plantarum*, *L. brevis* extracted from the feces of infants, and *Lactobacillus delbrueckii* B3 extracted from yogurt show anticancer properties on cancerous cells present in the colon (HT-29). EPS extracted from *Lactobacilli* cause an induction of cell death in colorectal cancer cells *in vitro* via Caspase 3, along with elevated expression of BAX, as well as reduced survivin and Bcl-2. Essential biological pathways target and produce induction of various types of apoptosis by elements of yeast, which are reflected as a potent tool for therapy against colorectal cancer. EPSs generated by probiotic bacteria *K. marxianus* and *P. kudriavzevii* prevent different cell lines of colon cancer [108].

Also, probiotic bacteria *B. breve* EPS enhance the development of immune response and contain anti-tumor as well as anti-inflammatory properties. EPS is comprised of galactose, rhamnose, arabinose, mannose, and glucose. EPS displays activity against cancer in the carcinoma cell lines present in the squamous cells of the neck and head by monitoring cell death and arrest of the cell cycle. The scientists consider that EPS isolated from *B. breve* can be utilized to facilitate genetic as well as metabolic engineering, along with playing a part in the production of food and drugs [109].

According to Zhang et al., *Lactobacillus acidophilus* along with *Lactobacillus casei* generate molecules that prevent the development of cell lines of breast cancer, MCF7. *L. acidophilus* inhibits the division of pancreatic cancer cell lines by production of soluble polysaccharides. Alive and heat-killed *Lactobacillus*, along with the metabolites that it secretes as well as the cytoplasmic fractions, can work against cancer. *Lactobacillus* isolated metabolites obtained by fermentation of yogurt prevent the development and cause an induction of cell death in a squamous cancerous cell line present in humans *in vitro* [84].

13.24.2 SHORT-CHAIN FATTY ACIDS (SCFAS) AND LIPID

Various conjugated acids, SCFAs, as well as some other products that work against cancer, are generated and extracted from *Lactobacilli* bacteria that induce cell death in tumor cells. Probiotic bacteria *L. fermentum* is a potent candidate that can be used in the therapy of colorectal cancer. They inhibit the division of cancerous cells by the production of SCFAs *in vitro* as well as endurance in the intestinal fluid. Additionally, SCFA metabolites formed by probiotic bacteria *Propionibacterium freudenreichii* cause damage to

the adenocarcinoma cells of the colorectal by inducing cell death *in vitro*. Probiotic bacteria *Lactobacillus salivarius*, *Pediococcus pentosaceus*, *Lactobacillus salivarius*, and *E. faecium* also prevent the division of colon tumor cells by the bioproduction of SCFAs, especially butyric and propionic acids. Likewise, conjugated linoleic acids secreted by probiotics have the capability to produce anticancer results in mice both *in vitro* and *in vivo*. Another probiotic, *L. reuteri*, inhibits CRC-causing lesions by producing propionate [53]. Probiotic *L. reuteri* prevents the division of cancer cells in the GI tract *in vitro* by downregulating urokinase plasminogen activator [110].

13.24.3 PROTEINS AND NUCLEIC ACID

Secretions produced by probiotics, i.e., metabolites, can be used to inhibit cancer and its therapy. Kahouli et al., found in his study that pure isolated bacteria or their secretions from probiotics *L. acidophilus* and *L. fermentum* lessened the risk factors of colorectal tumors in male mice of 1 month of age in a CRC model, as well as in Caco-2 epithelial cell lines. The bacteria can induce antioxidation, inhibit proliferation, and induce apoptosis, especially when utilized in a group. Furthermore, a greater synergistic characteristic on the growth inhibition of tumors and modulation of proliferation, along with the epithelial markers in colorectal cancer mice, was discovered based on the level of expression of proteins, i.e., cadherin, caspase-3, and β -catenin. Moreover, metabolites obtained from *B. bifidum* and cell-free supernatants work effectively against cancer cells when used in a colon cancer cell line [111].

Various inhibitory compounds generated by probiotic bacteria have advantageous characteristics against cancer cells. Escamilla et al., discovered that the cell-free supernatants that accumulate secreted bioactive macromolecules from *L. casei* and *L. rhamnosus* probiotics prevented the metastasis of colon cancer cells *in vitro*. This inhibitory molecule may be a polysaccharide, protein, or nucleic acid in nature. The decrease in the infiltration of colon cancer cells, i.e., HCT-116 metastasis, lowered the action of MMP-9 (matrix metalloproteinase-9), along with an increased concentration of ZO-1 (zona occludens-1). MMP-9 is an enzyme that digests the extracellular matrix of the colon, following the infiltration of cells during the process of metastasis. Once the level of MMP-9 is reduced in the matrix, it indicates that the potency of the enzyme MMP-9 is diminished. The protein ZO-1

is used to regulate the role of the epithelial barrier. In metastatic cancer, downregulation of protein ZO-1 maintains the role of the epithelial barrier in a healthy colon.

Studies also show that p8 protein-derived probiotic therapeutics have anti-CRC properties by inducing cell cycle arrest during *in vivo* trials. Protein p8 extracted from probiotic bacteria displays anti-tumor properties and has been proven clinically in human trials. It is recognized as a potent candidate for use in biopharmacy. Isolates from *L. reuteri* prevent CRC cells [112].

Alternatively, probiotic bacteria *A. syzygii* display prophylactic properties against oral cavity and squamous cell cancer. This demonstrates the advantageous characteristics of probiotic bacteria in the prevention of oral disorders. Secretions produced by probiotic strains exhibit cytotoxic properties by inducing cell death in the cancerous cells of oral cancer. Similarly, monophosphoryl lipid A extracted from *Salmonella enterica* is utilized as an adjuvant in the formation of a vaccine against cervical cancer.

The potency of co-culture of *L. acidophilus* and *L. fermentum* against cancer was substantially enhanced *in vitro*, with a high reduction in proliferation, an increase in apoptosis in cancerous cells, along with protection against the side effects of cancer therapy in the cell growth of normal colon cells. The genesis of tumors in the intestine was altered by considerably lowering the multiplicity of tumor cells, along with downregulation of the proliferation of cellular markers. As different evidence shows, the probiotic bacteria *L. fermentum* display an enhanced antitumor effect *in vitro* compared to various other *Lactobacillus* bacteria. The usage of a combination of strains increases the potency of biotherapy against the induction of tumors as well as their progression by secreting anti-neoplastic molecules, inducing apoptosis, and protecting healthy mucosa [113].

13.24.4 SIDEROPHORES

Corresponding to an experiment *in vitro* executed by Konishi et al., probiotic bacteria *L. casei*, a derivative of siderophores, showed increased suppression of CRC cancer via c-jun N-terminal kinase facilitated cell death. Body components of probiotic *L. casei* didn't prevent the growth of CRC cells, signifying that the strain released a few fractions that may have caused cancer suppression during the experiment. The extracted tumor suppressive fraction was associated with metallic components, i.e., Zn, Fe, and Ca. Probiotic *L. casei* secretes chelating agents for metal, which are called siderophores or

ferrichrome. Siderophores lowered the cell division of cell lines SW620 and Caco2. The cancer suppression effect of siderophores was better or equal compared to normal anti-tumor drugs like cisplatin and 5-fluorouracil; nonetheless, it had a lower effect on the normal cells in the intestine compared to the drugs. The prevention of solid outgrowth of a tumor by siderophore *in vivo* injection indicates that siderophores are an striking candidate for a drug against tumors [81].

13.25 CONCLUSION AND RECOMMENDATION

COVID pandemic has pushed the world toward various fatalities. As a new disease, research is ongoing from all perspectives. The presence of SARS-CoV-2 RNA in stool shows a subtle link between the gut and lungs. Probiotics are known to fight many RTIs, and many reports have shown that the coronavirus can also be managed by the gut microbiota via the gut-lung axis. Mouse models have shown that lung diseases can be alleviated using probiotics orally, which is a promising result for more research toward the clinically approved oral vaccine formation using probiotics to fight COVID. Another fatal disease is cancer, and the treatment of cancer has remained a subject of interest for researchers for the past decades. Among the first researchers are Gorbach and Goldin. Their research was based on a relationship between a Lactobacillus-enriched diet and colorectal cancer. The activity of various probiotics against carcinogens involves modifying the composition of existing microbiota and their metabolic activity. They help produce anti-carcinogenic compounds, promote apoptosis and cell cycle arrest, degrade toxic compounds, modulate immune cells, and enhance gut barrier production.

As the research on probiotics is ongoing, we must use a healthy fiber diet to prepare our microflora for disease resistance. Pertaining to the question of which foods should be specifically used when someone is suffering from these conditions, we must wait for the research on probiotics. Until then, we must rely on using fiber-rich diets. The benefit of using a fiber-rich diet is that it keeps our immune system and natural microbiota stronger and has no side effects compared to other synthetic or chemical drugs. Further research is needed to identify the specific probiotic organisms that can effectively navigate the effects of fatal diseases. After identifying those organisms, we must look for the natural sources or foods that contain those organisms. Additionally, research to identify the compounds or chemicals of those organisms that compete with the coronavirus may help in effective treatment.

KEYWORDS

- **Anti-carcinogenic activity**
- **cancer**
- **COVID-19**
- **gut microflora**
- **probiotics**
- **vaccine**

AUTHOR CONTRIBUTIONS

All authors have equal contribution and read and agreed to the published version of the chapter.

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REFERENCES

1. Wang, F., Kream, R. M., & Stefano, G. B. (2020). An evidence-based perspective on mRNA-SARS-CoV-2 vaccine development. *Medical Science Monitor*, 26, e926611.
2. Liu, M., Zhong, Y., Chen, J., Liu, Y., Tang, C., Wang, X., Zhang, Y., Wang, P., Logan, S. M., Chen, W., et al. (2020). Oral immunization of mice with a multivalent therapeutic subunit vaccine protects against *Helicobacter pylori* infection. *Vaccine*, 38, 3255–3263.
3. De Wit, E., Van Doremalen, N., Falzarano, D., & Munster, V. J. (2016). SARS and MERS: Recent insights into emerging coronaviruses. *Nature Reviews Microbiology*, 14, 523–534.
4. Zhou, Y., Hou, Y., Shen, J., Huang, Y., Martin, W., & Cheng, F. (2020). Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discovery*, 6, 14. <https://doi.org/10.1038/s41421-020-0153-3>.
5. Conticini, E., Frediani, B., & Caro, D. (2020). Can atmospheric pollution be considered a co-factor in extremely high levels of SARS-CoV-2 lethality in Northern Italy? *Environmental Pollution*, 261, 114465.
6. Chan, K. H., Poon, L. L. M., Cheng, V. C. C., Guan, Y., Hung, I. F. N., Kong, J., Yam, L. Y. C., Seto, W. H., Yuen, K. Y., & Peiris, J. S. M. (2004). Detection of SARS coronavirus

- in patients with suspected SARS. *Emerging Infectious Diseases*, 10, 204–211. <https://doi.org/10.3201/eid1002.030610>.
7. Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X., Yin, H., Xiao, Q., Tang, Y., Qu, X., et al. (2020). Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *The Lancet Gastroenterology & Hepatology*, 5, 556.
 8. Timmerman, H. M., Koning, C. J. M., Mulder, L., Rombouts, F. M., & Beynen, A. C. (2004). Monostrain, multistrain and multispecies probiotics: A comparison of functionality and efficacy. *International Journal of Food Microbiology*, 96, 219–233.
 9. Collado, M. C., Meriluoto, J., & Salminen, S. (2007). Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. *Letters in Applied Microbiology*, 45, 454–459. <https://doi.org/10.1111/j.1472-765X.2007.02212.x>.
 10. Rijkers, G. T., De Vos, W. M., Brummer, R. J., Morelli, L., Corthier, G., & Marteau, P. (2011). Health benefits and health claims of probiotics: Bridging science and marketing. *British Journal of Nutrition*, 106, 1–18. <https://doi.org/10.1017/S000711451100287X>.
 11. Behnsen, J., Deriu, E., Sassone-Corsi, M., & Raffatellu, M. (2013). Probiotics: Properties, examples, and specific applications. *Cold Spring Harbor Perspectives in Biology*, 5, a010074. <https://doi.org/10.1101/cshperspect.a010074>.
 12. Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, 100, 1171–1185.
 13. Cruz, B. C. S., Sarandy, M. M., Messias, A. C., Gonçalves, R. V., Ferreira, C. L. L. F., & Peluzio, M. C. G. (2020). Preclinical and clinical relevance of probiotics and synbiotics in colorectal carcinogenesis: A systematic review. *Nutrition Reviews*, 78, 188–207.
 14. Hardy, H., Harris, J., Lyon, E., Beal, J., & Foey, A. D. (2013). Probiotics, prebiotics, and immunomodulation of gut mucosal defenses: Homeostasis and immunopathology. *Nutrients*, 5, 1116–1133.
 15. Ryan, P. M., Ross, R. P., Fitzgerald, G. F., Caplice, N. M., & Stanton, C. (2015). Sugar-coated: Exopolysaccharide producing lactic acid bacteria for food and human health applications. *Food & Function*, 6, 1006–1017. <https://doi.org/10.1039/c4fo00529e>.
 16. Hao, Q., Dong, B. R., & Wu, T. (2015). Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database of Systematic Reviews*, 2015, CD006895.
 17. Grudzien, M., & Rapak, A. (2018). Effect of natural compounds on NK cell activation. *Journal of Immunology Research*, 2018, 1–11.
 18. Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157, 121–141.
 19. Fu, L., Song, J., Wang, C., Fu, S., & Wang, Y. (2017). *Bifidobacterium infantis* potentially alleviates shrimp tropomyosin-induced allergy by tolerogenic dendritic cell-dependent induction of regulatory T cells and alterations in gut microbiota. *Frontiers in Immunology*, 8, 1536. <https://doi.org/10.3389/fimmu.2017.01536>.
 20. Balzaretti, S., Taverniti, V., Guglielmetti, S., Fiore, W., Minuzzo, M., Ngo, H. N., Ngere, J. B., Sadiq, S., Humphreys, P. N., & Laws, A. P. (2017). A novel rhamnose-rich hetero-exopolysaccharide isolated from *Lactobacillus paracasei* DG activates THP-1 human monocytic cells. *Applied and Environmental Microbiology*, 83, e02702-16. <https://doi.org/10.1128/AEM.02702-16>.
 21. Dargahi, N., Johnson, J., & Apostolopoulos, V. (2020). *Streptococcus thermophilus* alters the expression of genes associated with innate and adaptive immunity in human

- peripheral blood mononuclear cells. *PLoS ONE*, 15. <https://doi.org/10.1371/journal.pone.0228531>.
22. Lopez-Santamarina, A., Lamas, A., del Carmen Mondragón, A., Cardelle-Cobas, A., Regal, P., Rodriguez-Avila, J. A., Miranda, J. M., Franco, C. M., & Cepeda, A. (2021). Probiotic effects against virus infections: New weapons for an old war. *Foods*, 10. <https://doi.org/10.3390/foods10010130>.
 23. Machhi, J., Herskovitz, J., Senan, A. M., Dutta, D., Nath, B., Oleynikov, M. D., Blomberg, W. R., Meigs, D. D., Hasan, M., Patel, M., et al. (2020). The natural history, pathobiology, and clinical manifestations of SARS-CoV-2 infections. *Journal of Neuroimmune Pharmacology*, 15.
 24. Grosse, C., Grosse, A., Salzer, H. J. F., Dünser, M. W., Motz, R., & Langer, R. (2020). Analysis of cardiopulmonary findings in COVID-19 fatalities: High incidence of pulmonary artery thrombi and acute suppurative bronchopneumonia. *Cardiovascular Pathology*, 49. <https://doi.org/10.1016/j.carpath.2020.107263>.
 25. Groves, H. T., Higham, S. L., Moffatt, M. F., Cox, M. J., & Tregoning, J. S. (2020). Respiratory viral infection alters the gut microbiota by inducing inappetence. *mBio*, 11. <https://doi.org/10.1128/mBio.03236-19>.
 26. Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., & Iwasaki, A. (2011). Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, 108. <https://doi.org/10.1073/pnas.1019378108>.
 27. Enaud, R., Prevel, R., Ciarlo, E., Beaufils, 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. *Frontiers in Cellular and Infection Microbiology*, 10.
 28. Dang, A. T., & Marsland, B. J. (2019). Microbes, metabolites, and the gut-lung axis. *Mucosal Immunology*, 12.
 29. Zhang, D., Li, S., Wang, N., Tan, H. Y., Zhang, Z., & Feng, Y. (2020). The cross-talk between gut microbiota and lungs in common lung diseases. *Frontiers in Microbiology*, 11.
 30. Dhar, D., & Mohanty, A. (2020). Gut microbiota and COVID-19—Possible link and implications. *Virus Research*, 285.
 31. Round, J. L., & Mazmanian, S. K. (2010). Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 107. <https://doi.org/10.1073/pnas.0909122107>.
 32. Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet Respiratory Medicine*, 8. [https://doi.org/10.1016/S2213-2600\(20\)30076-X](https://doi.org/10.1016/S2213-2600(20)30076-X).
 33. Coperchini, F., Chiovato, L., Croce, L., Magri, F., & Rotondi, M. (2020). The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine & Growth Factor Reviews*, 53.
 34. Antunes, A. E. C., Vinderola, G., Xavier-Santos, D., & Sivieri, K. (2020). Potential contribution of beneficial microbes to face the COVID-19 pandemic. *Food Research International*, 136. <https://doi.org/10.1016/j.foodres.2020.109577>.
 35. Infusino, F., Marazzato, M., Mancone, M., Fedele, F., Mastroianni, C. M., Severino, P., Ceccarelli, G., Santinelli, L., Cavarretta, E., Marullo, A. G. M., et al. (2020). Diet

- supplementation, probiotics, and nutraceuticals in SARS-CoV-2 infection: A scoping review. *Nutrients*, 12.
36. Xia, Y., Cao, J., Wang, M., Lu, M., Chen, G., Gao, F., Liu, Z., Zhang, D., Ke, X., & Yi, M. (2019). Effects of *Lactococcus lactis* subsp. *lactis* JCM5805 on colonization dynamics of gut microbiota and regulation of immunity in early ontogenetic stages of tilapia. *Fish & Shellfish Immunology*, 86. <https://doi.org/10.1016/j.fsi.2018.11.022>.
 37. Su, M., Jia, Y., Li, Y., Zhou, D., & Jia, J. (2020). Probiotics for the prevention of ventilator-associated pneumonia: A meta-analysis of randomized controlled trials. *Respiratory Care*, 65. <https://doi.org/10.4187/respcare.07097>.
 38. Gao, Q., Bao, L., Mao, H., Wang, L., Xu, K., Yang, M., Li, Y., Zhu, L., Wang, N., Lv, Z., et al. (2020). Development of an inactivated vaccine candidate for SARS-CoV-2. *Science*, 369. <https://doi.org/10.1126/science.abc1932>.
 39. Shakoor, S., Rao, A. Q., Shahid, N., Yaqoob, A., Samiullah, T. R., Shakoor, S., Latif, A., Tabassum, B., Khan, M. A. U., Shahid, A. A., et al. (2019). Role of oral vaccines as an edible tool to prevent infectious diseases. *Acta Virologica*, 63.
 40. Mustafa, A. D., Kalyanasundram, J., Sabidi, S., Song, A. A. L., Abdullah, M., Abdul Rahim, R., & Yusoff, K. (2018). Proof of concept in utilizing in-trans surface display system of *Lactobacillus plantarum* as mucosal tuberculosis vaccine via oral administration in mice. *BMC Biotechnology*, 18. <https://doi.org/10.1186/s12896-018-0461-y>.
 41. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, 395, 497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5).
 42. Ren, D. Y., Li, C., Qin, Y. Q., Yin, R. L., Du, S. W., Ye, F., Liu, H. F., Wang, M. P., Sun, Y., Li, X., et al. (2013). Lactobacilli reduce chemokine IL-8 production in response to TNF- α and Salmonella challenge of Caco-2 cells. *Biomedical Research International*, 2013, Article 925219. <https://doi.org/10.1155/2013/925219>.
 43. Pipenbaher, N., Moeller, P. L., Dolinšek, J., Jakobsen, M., Weingartl, H., & Cencič, A. (2009). Nitric oxide (NO) production in mammalian non-tumorigenic epithelial cells of the small intestine and macrophages induced by individual strains of Lactobacilli and Bifidobacteria. *International Dairy Journal*, 19, 26–31. <https://doi.org/10.1016/j.idairyj.2008.09.003>.
 44. Veckman, V., Miettinen, M., Pirhonen, J., Sirén, J., Matikainen, S., & Julkunen, I. (2004). *Streptococcus pyogenes* and *Lactobacillus rhamnosus* differentially induce maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells. *Journal of Leukocyte Biology*, 75, 858–865. <https://doi.org/10.1189/jlb.1003461>.
 45. Ruan, Q., Yang, K., Wang, W., Jiang, L., & Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Medicine*, 46, 846–848.
 46. Zuo, T., Zhang, F., Lui, G. C. Y., Yeoh, Y. K., Li, A. Y. L., Zhan, H., Wan, Y., Chung, A. C. K., Cheung, C. P., Chen, N., et al. (2020). Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology*, 159, 944–955. <https://doi.org/10.1053/j.gastro.2020.05.048>.
 47. Kawashima, T., Hayashi, K., Kosaka, A., Kawashima, M., Igarashi, T., Tsutsui, H., Tsuji, N. M., Nishimura, I., Hayashi, T., & Obata, A. (2011). *Lactobacillus plantarum* strain YU from fermented foods activates Th1 and protective immune responses. *International Immunopharmacology*, 11, 2109–2117. <https://doi.org/10.1016/j.intimp.2011.08.013>.

48. Goldin, B. R., & Gorbach, S. L. (1980). Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. *Journal of the National Cancer Institute*, 64, 263–266. <https://doi.org/10.1093/jnci/64.2.263>.
49. Kim, Y., Lee, D., Kim, D., Cho, J., Yang, J., Chung, M., Kim, K., & Ha, N. (2008). Inhibition of proliferation in colon cancer cell lines and harmful enzyme activity of colon bacteria by *Bifidobacterium adolescentis* SPM0212. *Archives of Pharmacal Research*, 31, 73–79. <https://doi.org/10.1007/s12272-001-1180-y>.
50. Thirabunyanon, M., Boonprasom, P., & Niamsup, P. (2009). Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. *Biotechnology Letters*, 31, 651–656. <https://doi.org/10.1007/s10529-008-9902-3>.
51. Altonsy, M. O., Andrews, S. C., & Tuohy, K. M. (2010). Differential induction of apoptosis in human colonic carcinoma cells (Caco-2) by Atopobium, and commensal, probiotic and enteropathogenic bacteria: Mediation by the mitochondrial pathway. *International Journal of Food Microbiology*, 137, 218–225. <https://doi.org/10.1016/j.ijfoodmicro.2009.11.015>.
52. Ma, E. L., Choi, Y. J., Choi, J., Pothoulakis, C., Rhee, S. H., & Im, E. (2010). The anticancer effect of probiotic *Bacillus polyfermenticus* on human colon cancer cells is mediated through ErbB2 and ErbB3 inhibition. *International Journal of Cancer*, 127, 1939–1948. <https://doi.org/10.1002/ijc.25011>.
53. Orlando, A., Refolo, M. G., Messa, C., Amati, L., Lavermicocca, P., Guerra, V., & Russo, F. (2012). Antiproliferative and proapoptotic effects of viable or heat-killed *Lactobacillus paracasei* IMPC2.1 and *Lactobacillus rhamnosus* GG in HGC-27 gastric and DLD-1 colon cell lines. *Nutrition and Cancer*, 64, 262–271. <https://doi.org/10.1080/01635581.2012.717676>.
54. Thirabunyanon, M., & Hongwittayakorn, P. (2013). Potential probiotic lactic acid bacteria of human origin induce antiproliferation of colon cancer cells via synergic actions in adhesion to cancer cells and short-chain fatty acid bioproduction. *Applied Biochemistry and Biotechnology*, 169, 1221–1231. <https://doi.org/10.1007/s12010-012-9995-y>.
55. Sadeghi-Aliabadi, H., Mohammadi, F., Fazeli, H., & Mirlohi, M. (2014). Effects of *Lactobacillus plantarum* A7 with probiotic potential on colon cancer and normal cells proliferation in comparison with a commercial strain. *Iranian Journal of Basic Medical Sciences*, 17, 628–632.
56. Chen, Z. F., Ai, L. Y., Wang, J. L., Ren, L. L., Yu, Y. N., Xu, J., Chen, H. Y., Yu, J., Li, M., Qin, W. X., et al. (2015). Probiotics *Clostridium butyricum* and *Bacillus subtilis* ameliorate intestinal tumorigenesis. *Future Microbiology*, 10, 1427–1437. <https://doi.org/10.2217/fmb.15.66>.
57. Lee, N. K., Son, S. H., Jeon, E. B., Jung, G. H., Lee, J. Y., & Paik, H. D. (2015). The prophylactic effect of probiotic *Bacillus polyfermenticus* KU3 against cancer cells. *Journal of Functional Foods*, 14, 162–171. <https://doi.org/10.1016/j.jff.2015.02.019>.
58. Han, K. J., Lee, N. K., Park, H., & Paik, H. D. (2015). Anticancer and anti-inflammatory activity of probiotic *Lactococcus lactis* Nk34. *Journal of Microbiology and Biotechnology*, 25, 1372–1377. <https://doi.org/10.4014/jmb.1503.03033>.
59. Tiptiri-Kourpeti, A., Spyridopoulou, K., Santarmaki, V., Ainelis, G., Tompoulidou, E., Lamprianidou, E. E., Saxami, G., Ypsilantis, P., Lampri, E. S., Simopoulos, C., et al. (2016). *Lactobacillus casei* exerts anti-proliferative effects accompanied by apoptotic

- cell death and up-regulation of TRAIL in colon carcinoma cells. *PLoS ONE*, *11*, e0147960. <https://doi.org/10.1371/journal.pone.0147960>.
60. Saxami, G., Karapetsas, A., Lamprianidou, E., Kotsianidis, I., Chlichlia, A., Tassou, C., Zoumpourlis, V., & Galanis, A. (2016). Two potential probiotic *Lactobacillus* strains isolated from olive microbiota exhibit adhesion and anti-proliferative effects in cancer cell lines. *Journal of Functional Foods*, *24*, 172–181. <https://doi.org/10.1016/j.jff.2016.04.036>.
 61. Kumar, A., Singh, N. K., & Sinha, P. R. (2010). Inhibition of 1,2-Dimethylhydrazine induced colon genotoxicity in rats by the administration of probiotic curd. *Molecular Biology Reports*, *37*, Article 9519. <https://doi.org/10.1007/s11033-009-9519-1>.
 62. Kim, S. W., Kim, H. M., Yang, K. M., Kim, S. A., Kim, S. K., An, M. J., Park, J. J., Lee, S. K., Kim, T. I., Kim, W. H., et al. (2010). Bifidobacterium lactis inhibits NF- κ B in intestinal epithelial cells and prevents acute colitis and colitis-associated colon cancer in mice. *Inflammatory Bowel Diseases*, *16*, Article 21262. <https://doi.org/10.1002/ibd.21262>.
 63. Appleyard, C. B., Cruz, M. L., Isidro, A. A., Arthur, J. C., Jobin, C., & de Simone, C. (2011). Pretreatment with the probiotic VSL#3 delays transition from inflammation to dysplasia in a rat model of colitis-associated cancer. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *301*, Article G1000. <https://doi.org/10.1152/ajpgi.00167.2011>.
 64. Verma, A., & Shukla, G. (2014). Synbiotic (*Lactobacillus rhamnosus* + *Lactobacillus acidophilus* + Inulin) attenuates oxidative stress and colonic damage in 1,2-dimethylhydrazine dihydrochloride-induced colon carcinogenesis in Sprague–Dawley rats. *European Journal of Cancer Prevention*, *23*, 550–559.
 65. Lee, H. A., Kim, H., Lee, K. W., & Park, K. Y. (2015). Dead nano-sized *Lactobacillus plantarum* inhibits azoxymethane/dextran sulfate sodium-induced colon cancer in Balb/c mice. *Journal of Medicinal Food*, *18*, Article 3577. <https://doi.org/10.1089/jmf.2015.3577>.
 66. Hu, J., Wang, C., Ye, L., Yang, W., Huang, H., Meng, F., Shi, S., & Ding, Z. (2015). Anti-tumor immune effect of oral administration of *Lactobacillus plantarum* to CT26 tumor-bearing mice. *Journal of Biosciences*, *40*, Article 9518. <https://doi.org/10.1007/s12038-015-9518-4>.
 67. Walia, S., Kamal, R., Kanwar, S. S., & Dhawan, D. K. (2015). Cyclooxygenase as a target in chemoprevention by probiotics during 1,2-dimethylhydrazine induced colon carcinogenesis in rats. *Nutrition and Cancer*, *67*, Article 1011788. <https://doi.org/10.1080/01635581.2015.1011788>.
 68. Zhang, M., Fan, X., Fang, B., Zhu, C., Zhu, J., & Ren, F. (2015). Effects of *Lactobacillus salivarius* REN on cancer prevention and intestinal microbiota in a 1,2-dimethylhydrazine-induced rat model. *Journal of Microbiology*, *53*, Article 5046. <https://doi.org/10.1007/s12275-015-5046-z>.
 69. Kuugbee, E. D., Shang, X., Gamallat, Y., Bamba, D., Awadasseid, A., Suliman, M. A., Zang, S., Ma, Y., Chiwala, G., Xin, Y., et al. (2016). Structural change in microbiota by a probiotic cocktail enhances the gut barrier and reduces cancer via TLR2 signaling in a rat model of colon cancer. *Digestive Diseases and Sciences*, *61*, Article 4238. <https://doi.org/10.1007/s10620-016-4238-7>.
 70. Gamallat, Y., Meyiah, A., Kuugbee, E. D., Hago, A. M., Chiwala, G., Awadasseid, A., Bamba, D., Zhang, X., Shang, X., Luo, F., et al. (2016). *Lactobacillus rhamnosus*

- induced epithelial cell apoptosis, ameliorates inflammation and prevents colon cancer development in an animal model. *Biomedical Pharmacotherapy*, 83, Article 70601. <https://doi.org/10.1016/j.biopha.2016.07.001>.
71. Dubey, V., Ghosh, A. R., Bishayee, K., & Khuda-Bukhsh, A. R. (2016). Appraisal of the anti-cancer potential of probiotic *Pediococcus pentosaceus* GS4 against colon cancer: *In vitro* and *in vivo* approaches. *Journal of Functional Foods*, 23, Article 32032. <https://doi.org/10.1016/j.jff.2016.02.032>.
72. Lenoir, M., del Carmen, S., Cortes-Perez, N. G., Lozano-Ojalvo, D., Muñoz-Provencio, D., Chain, F., Langella, P., de Moreno de LeBlanc, A., LeBlanc, J. G., & Bermúdez-Humarán, L. G. (2016). *Lactobacillus casei* BL23 regulates Treg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. *Journal of Gastroenterology*, 51.
73. Jia, W., Xie, G., & Jia, W. (2018). Bile acid–microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nature Reviews Gastroenterology & Hepatology*, 15.
74. Goldin, B., & Gorbach, S. L. (1977). Alterations in fecal microflora enzymes related to diet, age, Lactobacillus supplements, and dimethylhydrazine. *Cancer*, 40, Article 2421. [https://doi.org/10.1002/1097-0142\(197711\)40:5+<2421::AID-CNCR2820400905>3.0.CO;2-I](https://doi.org/10.1002/1097-0142(197711)40:5+<2421::AID-CNCR2820400905>3.0.CO;2-I).
75. Orrhage, K. M., Annas, A., Nord, C. E., Brittebo, E. B., & Rafter, J. J. (2002). Effects of lactic acid bacteria on the uptake and distribution of the food mutagen Trp-P-2 in mice. *Scandinavian Journal of Gastroenterology*, 37, Article 416902. <https://doi.org/10.1080/003655202753416902>.
76. Garrett, W. S. (2015). Cancer and the microbiota. *Science*, 348, 80–86.
77. Ivanov, I. I., & Honda, K. (2012). Intestinal commensal microbes as immune modulators. *Cell Host & Microbe*, 12, 253–264.
78. Pfeffer, C. M., & Singh, A. T. K. (2018). Apoptosis: A target for anticancer therapy. *International Journal of Molecular Sciences*, 19, Article 448.
79. Sharma, S., Singh, R. L., & Kakkar, P. (2011). Modulation of Bax/Bcl-2 and caspases by probiotics during acetaminophen induced apoptosis in primary hepatocytes. *Food and Chemical Toxicology*, 49, 2473–2480. <https://doi.org/10.1016/j.fct.2010.11.041>.
80. Preet, S., Bharati, S., Panjeta, A., Tewari, R., & Rishi, P. (2015). Effect of nisin and doxorubicin on DMBA-induced skin carcinogenesis—A possible adjunct therapy. *Tumor Biology*, 36, Article 3571. <https://doi.org/10.1007/s13277-015-3571-3>.
81. Konishi, H., Fujiya, M., Tanaka, H., Ueno, N., Moriichi, K., Sasajima, J., Ikuta, K., Akutsu, H., Tanabe, H., & Kohgo, Y. (2016). Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. *Nature Communications*, 7, Article 12365. <https://doi.org/10.1038/ncomms12365>.
82. Jan, G., Belzacq, A. S., Haouzi, D., Rouault, A., Métivier, D., Kroemer, G., & Brenner, C. (2002). Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. *Cell Death & Differentiation*, 9, 179–188. <https://doi.org/10.1038/sj.cdd.4400935>.
83. Asoudeh-Fard, A., Barzegari, A., Dehnad, A., Bastani, S., Golchin, A., & Omid, Y. (2017). Lactobacillus plantarum induces apoptosis in oral cancer KB cells through upregulation of PTEN and downregulation of MAPK signaling pathways. *BioImpacts*, 7, 123–130. <https://doi.org/10.15171/bi.2017.22>.
84. Zhang, G., Zhang, J., Wang, X., Yang, W., Sun, Z., Kumar, C. N., Guan, H., & Guan, J. (2014). Apoptosis of human tongue squamous cell carcinoma cell (CAL-27) induced

- by *Lactobacillus* sp. A-2 metabolites. *Journal of Applied Oral Science*, 22, 645–653. <https://doi.org/10.1590/1678-775720130645>.
85. La Rosa, G. R. M., Gattuso, G., Pedullà, E., Rapisarda, E., Nicolosi, D., & Salmeri, M. (2020). Association of oral dysbiosis with oral cancer development (Review). *Oncology Letters*, 19, 10–18.
86. Wang, H., Cheng, X., Zhang, L., Xu, S., Zhang, Q., & Lu, R. (2019). A surface-layer protein from *Lactobacillus acidophilus* NCFM induces autophagic death in HCT116 cells requiring ROS-mediated modulation of mTOR and JNK signaling pathways. *Food Function*, 10, 435–446. <https://doi.org/10.1039/c9fo00109c>.
87. Bednarczyk, M., Zmarzły, N., Grabarek, B., Mazurek, U., & Muc-Wierzegoń, M. (2018). Genes involved in the regulation of different types of autophagy and their participation in cancer pathogenesis. *Oncotarget*, 9, 35–55. <https://doi.org/10.18632/oncotarget.25599>.
88. Pithva, S. P., Dave, J. M., & Vyas, B. R. M. (2015). Binding of acridine orange by probiotic *Lactobacillus rhamnosus* strains of human origin. *Annals of Microbiology*, 65, 263–271. <https://doi.org/10.1007/s13213-014-0975-z>.
89. Sharma, M., & Shukla, G. (2016). Metabiotics: One step ahead of probiotics; an insight into mechanisms involved in anticancerous effect in colorectal cancer. *Frontiers in Microbiology*, 7, Article 545. <https://doi.org/10.3389/fmicb.2016.00545>.
90. Sharaf, L. K., Sharma, M., Chandel, D., & Shukla, G. (2018). Prophylactic intervention of probiotics (*L. acidophilus*, *L. rhamnosus* GG) and celecoxib modulate Bax-mediated apoptosis in 1,2-dimethylhydrazine-induced experimental colon carcinogenesis. *BMC Cancer*, 18, Article 499. <https://doi.org/10.1186/s12885-018-4999-9>.
91. Azam, R., Ghafouri-Fard, S., Tabrizi, M., Modarressi, M. H., Ebrahimzadeh-Vesal, R., Daneshvar, M., Mobasheri, M. B., & Motevaseli, E. (2014). *Lactobacillus acidophilus* and *Lactobacillus crispatus* culture supernatants downregulate expression of cancer-testis genes in the MDA-MB-231 cell line. *Asian Pacific Journal of Cancer Prevention*, 15, 4255–4260. <https://doi.org/10.7314/APJCP.2014.15.10.4255>.
92. Hosseini, S. S., Goudarzi, H., Ghalavand, Z., Hajikhani, B., Rafeiclatani, Z., & Hakemi-Vala, M. (2020). Anti-proliferative effects of cell wall and cytoplasmic extract of *Lactococcus lactis* and nisin through down-regulation of cyclin D1 on SW480 colorectal cancer cell line. *Iranian Journal of Microbiology*, 12, 60–66. <https://doi.org/10.18502/ijm.v12i5.4603>.
93. Hapach, L. A., Mosier, J. A., Wang, W., & Reinhart-King, C. A. (2019). Engineered models to parse apart the metastatic cascade. *npj Precision Oncology*, 3, Article 4. <https://doi.org/10.1038/s41698-019-0065-5>.
94. Quintero-Fabián, S., Arreola, R., Becerril-Villanueva, E., Torres-Romero, J. C., Arana-Argáez, V., Lara-Riegos, J., Ramírez-Camacho, M. A., & Alvarez-Sánchez, M. E. (2019). Role of matrix metalloproteinases in angiogenesis and cancer. *Frontiers in Oncology*, 9, Article 137. <https://doi.org/10.3389/fonc.2019.00137>.
95. Li, X., Hong, W., Du, X., Yu, W., Jingwen, J., Geng, Y., Guo, X., Fan, X., & Ma, C. (2017). *Lactobacilli* inhibit cervical cancer cell migration *in vitro* and reduce tumor burden *in vivo* through upregulation of E-cadherin. *Oncology Reports*, 38, 2279–2286. <https://doi.org/10.3892/or.2017.5791>.
96. Seth, A., Yan, F., Polk, D. B., & Rao, R. K. (2008). Probiotics ameliorate hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *American Journal of Physiology—Gastrointestinal and Liver Physiology*, 294, G1002–G1011. <https://doi.org/10.1152/ajpgi.00202.2007>.

97. Malvisi, M., Stuknyte, M., Magro, G., Minozzi, G., Giardini, A., De Noncty, & Piccinini, R. (2016). Antibacterial activity and immunomodulatory effects on a bovine mammary epithelial cell line exerted by nisin A-producing *Lactococcus lactis* strains. *Journal of Dairy Science*, 99, 5594–5606. <https://doi.org/10.3168/jds.2015-10161>.
98. Piotrowska, U., Sobczak, M., & Oledzka, E. (2017). Current state of a dual behaviour of antimicrobial peptides—Therapeutic agents and promising delivery vectors. *Chemical Biology & Drug Design*, 90, 111–122. <https://doi.org/10.1111/cbdd.12975>.
99. Gillor, O., Etzion, A., & Riley, M. A. (2008). The dual role of bacteriocins as anti-and probiotics. *Applied Microbiology and Biotechnology*, 81, 591–606. <https://doi.org/10.1007/s00253-008-1662-0>.
100. Laine, C., Horton, R., DeAngelis, C. D., Drazen, J. M., Frizelle, F. A., Godlee, F., Haug, C., Hébert, P. C., Kotzin, S., Marusic, A., et al. (2007). Clinical trial registration: Looking back and moving ahead. *Annals of Internal Medicine*, 147, 292–299. <https://doi.org/10.7326/0003-4819-147-4-200708210-00008>.
101. Singh, D., Khan, M. A., & Siddique, H. R. (2021). Therapeutic implications of probiotics in microbiota dysbiosis: A special reference to the liver and oral cancers. *Life Sciences*, 285, Article 119935.
102. Legesse Bedada, T., Feto, T. K., Awoke, K. S., Garedew, A. D., Yifat, F. T., & Birri, D. J. (2020). Probiotics for cancer alternative prevention and treatment. *Biomedicine & Pharmacotherapy*, 129, Article 110454.
103. Zaharuddin, L., Mokhtar, N. M., Muhammad Nawawi, K. N., & Raja Ali, R. A. (2019). A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterology*, 19, Article 142.
104. Albookarami, H. (2017). Investigating the role of probiotics in coping with cancer and health in society. *Cancer Press Journal*, 3, 1–9.
105. Baldwin, C., Millette, M., Oth, D., Ruiz, M. T., Luquet, F. M., & Lacroix, M. (2010). Probiotic *Lactobacillus acidophilus* and *L. casei* mix sensitize colorectal tumoral cells to 5-fluorouracil-induced apoptosis. *Nutrition and Cancer*, 62, 848–856. <https://doi.org/10.1080/01635580903407197>.
106. Chung, I. C., Ouyang, C. N., Yuan, S. N., Lin, H. C., Huang, K. Y., Wu, P. S., Liu, C. Y., Tsai, K. J., Loi, L. K., Chen, Y. J., et al. (2019). Pretreatment with a heat-killed probiotic modulates the NLRP3 inflammasome and attenuates colitis-associated colorectal cancer in mice. *Nutrients*, 11, Article 516. <https://doi.org/10.3390/nu11030516>.
107. Wang, C. Y., Wu, T. C., Hsieh, S. L., Tsai, Y. H., Yeh, C. W., & Huang, C. Y. (2015). Antioxidant activity and growth inhibition of human colon cancer cells by crude and purified fucoidan preparations extracted from *Sargassum cristaefolium*. *Journal of Food and Drug Analysis*, 23, 665–674. <https://doi.org/10.1016/j.jfda.2015.07.002>.
108. Rahbar Saadat, Y., Yari Khosroushahi, A., Movassaghpour, A. A., Talebi, M., & Pourghassem Gargari, B. (2020). Modulatory role of exopolysaccharides of *Kluyveromyces marxianus* and *Pichia kudriavzevii* as probiotic yeasts from dairy products in human colon cancer cells. *Journal of Functional Foods*, 64, Article 103675. <https://doi.org/10.1016/j.jff.2019.103675>.
109. Wang, L., Wang, Y., Li, Q., Tian, K., Xu, L., Liu, G., & Guo, C. (2019). Exopolysaccharide, isolated from a novel strain *Bifidobacterium breve* Lw01, possesses an anticancer effect on head and neck cancer—genetic and biochemical evidences. *Frontiers in Microbiology*, 10, Article 1044. <https://doi.org/10.3389/fmicb.2019.01044>.

110. Ewaschuk, J. B., Walker, J. W., Diaz, H., & Madsen, K. L. (2006). Bioproduction of conjugated linoleic acid by probiotic bacteria occurs *in vitro* and *in vivo* in mice. In *Proceedings of the Journal of Nutrition*, 136, 64–67.
111. Siva Kumar, K. (2015). Colon cancer prevention through probiotics: An overview. *Journal of Cancer Science & Therapy*, 7, Article 329. <https://doi.org/10.4172/1948-5956.1000329>.
112. Donato, K. A., Gareau, M. G., Wang, Y. J. J., & Sherman, P. M. (2010). *Lactobacillus rhamnosus* GG attenuates interferon- γ and tumor necrosis factor- α -induced barrier dysfunction and pro-inflammatory signaling. *Microbiology*, 156, 184–194. <https://doi.org/10.1099/mic.0.040139-0>.
113. Meenakshi, I. K. (2015). *In-vitro* characterization of the anti-cancer activity of the probiotic bacterium *Lactobacillus fermentum* NCIMB 5221 and potential against colorectal cancer. *Journal of Cancer Science & Therapy*, 7, Article 354. <https://doi.org/10.4172/1948-5956.1000354>.

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