

Cancer Drug Discovery and Development

Neeraj Mishra
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Role of Gut Microbiota and Postbiotics for Colorectal Cancer

Advancing Therapeutic Strategies

 Humana Press

Cancer Drug Discovery and Development

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Editors

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Preface

The “Role of Gut Microbiota and Postbiotics for Colorectal Cancer: Advancing Therapeutic Strategies” is a dynamically advancing domain at the convergence of microbiology, oncology, and precision medicine. This book examines the complex interrelationships among gut microbiota, postbiotic metabolites, and the development of colorectal cancer (CRC), illuminating the ways in which microbial dysbiosis, immunological regulation, and bioactive chemicals derived from microbe’s impact carcinogenesis and treatment results. As interest in microbiome-based therapies grows, a critical inquiry arises: Can postbiotics provide innovative approaches for the prevention and management of colorectal cancer?

This book connects fundamental microbiome science with translational and clinical applications through a multidisciplinary approach. The text commences with an extensive examination of gut microbiota composition, diversity, and function, highlighting their significance in the development and advancement of CRC. The effects of postbiotics, which are bioactive metabolites made by gut bacteria, on inflammation, oxidative stress, and the dynamics of the tumour microenvironment are covered in detail in later chapters. The therapeutic potential of microbiota-targeted approaches, such as probiotics, prebiotics, postbiotics, and faecal microbiota transplantation, is rigorously evaluated.

This book primarily concentrates on the emerging domain of microbiome-based precision medicine, which seeks to individualise colorectal cancer treatment using microbiota-focused diagnostics and therapies. This work emphasises the transformative potential of gut microbiota manipulation in colorectal cancer therapy by integrating advanced research with clinical views.

We express our profound appreciation to our colleagues, mentors, and contributors for their vital insights, and to our families for their steadfast support. Gratitude is extended to the editorial staff at Springer Nature, Humana Press for enabling this

undertaking. We anticipate this book will be a significant resource for researchers, doctors, and healthcare professionals dedicated to enhancing microbiome-based approaches in oncology.

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Chapter 1

Gut Microbiota: Role in Health and Disease



Msoumeh Bagheri, Azadeh Zahmatkesh, Abrar Hussain, Tahreen Taj, Sumel Ashique, and Naheed Mojjani

Abstract The gut of humans harbors a multifarious and dynamic population of microorganisms, known as gut microbiota. This microbial ecosystem is crucial for preserving human health through diverse functions, including nutrient metabolism, immune system development, and protection against pathogens. Prebiotics and probiotics are known to preserve a healthy microbiome. The community of microbes inhabiting the digestive tract is recognized as a metabolic powerhouse actively influencing the host's overall metabolism, contributing significantly to the digestion of food, nutrient absorption, and the production of essential metabolites. The microbiota of the gut has a multifaceted role in health and illness, and any imbalance or changes in the gut composition could contribute to disease pathogenesis. A balanced gut microbiota, its specific microbial compositions, and functional activities are referred to as eubiosis or equilibrium. While eubiosis is associated with overall well-being and health, any disruptions in this equilibrium (termed dysbiosis) caused by biotic or abiotic factors can alternatively lead to a number of chronic illnesses, comprising inflammatory bowel disease, type 2 diabetes, obesity, cardiovascular diseases (CVDs), and neurodevelopmental disorders. Understanding the intricate association existing among the gut microbiota and the host health paves the way for innovative diagnostic and therapeutic strategies targeting this critical microbial

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ecosystem. Recent advancements in artificial intelligence (AI) have revolutionized the study and application of gut microbiota. In this chapter, we describe the key roles of gut microbiota, emphasizing the role of eubiosis and dysbiosis in health and illness.

Keywords Gut microbiota · Microbiome · Dysbiosis · Probiotics · Metabolic diseases · Artificial intelligence

1.1 Introduction

The human gut, once considered a simple digestive tract, is now recognized as a complex ecosystem teeming with trillions of microorganisms, together known as the gut microbiota [25]. This intricate group of bacteria, fungi, viruses, and archaea plays a major role in protecting human health, influencing a myriad of physiological functions from digestion and immunity to metabolism and brain function [50]. With the expansion of research studies, the complex interaction between gut microbiota and human health has become increasingly evident, highlighting its vital role in both preserving homeostasis and contributing to the development of various diseases [57]. The equilibrium within the microbiota of the gut is vital for maintaining human well-being, and any imbalance or disruptions caused in this delicate equilibrium, termed as dysbiosis, can have profound consequences for host physiology and disease susceptibility [2]. The most important factors that could manipulate the gut microbiota include dietary regimens and supplements like prebiotics, probiotics, and synbiotic [49]. Dysbiosis refers to the alterations in the composition, diversity, and purpose of gut microbiota, leading to detrimental shifts in microbial populations and metabolic activities [33]. Numerous issues can contribute to dysbiosis including diet, age, genetics, drug and medication intake, physical and physiological stress, smoking and alcohol use, and other ecological influences. Targeting dysbiosis has become a key focus in developing new beneficial approaches for various illnesses. Interventions like dietary modifications, probiotics, prebiotics, synbiotic, fecal microbiota relocation, and even targeted antibiotics are investigated to refurbish gut microbiome balance and improve health outcomes [57]. A number of investigations supported the idea that, in modulating immunity, gut microbiota plays an important role in weight management, energy homeostasis, and disorders related to obesity [4]. Nevertheless, gut microbiota and its metabolic products are reportedly connected to a number of diseases like alcoholic and nonalcoholic fatty hepatic disease (NAFLD), inflammatory bowel disease (IBD), hepatocellular carcinoma, cardiovascular diseases (CVDs), chronic kidney disease (CKD), and cirrhosis [47, 68].

More recently, the emergence of AI is rapidly transforming gut microbiota research by offering powerful tools for data analysis and interpretation. Machine learning algorithms can analyze vast datasets of microbiome sequencing data, identifying patterns and associations between specific microbial communities and health

outcomes [56]. By integrating individual data on microbiome composition, diet, and health history, AI models could offer a profounder vision into interactions among the gut microbiome and the host and highlight how they contribute to various disease processes and how a person might respond to different therapeutic interventions [6, 32]. This personalized approach significantly progresses treatment consequences and revolutionizes gut health management. The current review seeks to discover the complex relationship among the gut microbiota and host health, highlighting its diverse functions, the consequences of dysbiosis on disease development, and the potential therapeutic approaches of the burgeoning field of AI.

1.2 Defining Microbiome and Microbiota

The terms “microbiome” and “microbiota” are frequently used as synonyms. The groups of microbes dwelling in the human body are specifically termed the human microbiota. This term encompasses the community of microorganisms that exists within a precise environment, like the saliva, oral cavity, skin, conjunctiva, respiratory system, and the genitourinary and gastrointestinal (GI) tracts. Conversely, microbiome is a broader term encompassing not just the organisms but also their genetic material, metabolic products, and interactions with their environment.

1.3 Composition of Gut Microbiota

The gut microbiota plays an important role in balancing human health, influencing various bodily functions and disease processes. The composition of this microbial community varies considerably between individuals, influenced by factors like diet, daily habits, and environmental exposures [38]. The vast array of microbial populations is essential for preserving a balanced gut ecology and encouraging optimal health. Gut microbiota is highly diverse and is made up of bacteria, yeasts, and viruses. A healthy community of microbiota frequently exhibits great taxonomic diversity, great microbial gene productivity, and constant core microbiota. In total, 10^{14} bacteria represent the gut microbiome, and 10^{11} bacteria flow every day from the pharynx to the stomach. The composition of gut microbiota differs across diverse regions of the gastrointestinal tract. For instance, *Proteobacteria*, including *Enterobacteriaceae*, are present in the small intestine but absent in the colon. Conversely, *Bacteroidetes*, for example, *Bacteroidaceae*, *Prevotellaceae*, and *Rikenellaceae*, are predominantly seen in the colon. These differences are mainly attributed to varying environments; the small intestine has a rapid transit time and high concentration of bile, while the colon properties include slower flow rates, further neutral pH, and larger populations of microorganisms, particularly anaerobes.

Additionally, gut microbiota changes with age, and microbial diversity rises from childhood to adulthood and subsequently declines after the age of 70. Usually,

the initial 3 years of life are essential for the growth of the adult microbiome, which is influenced by conversions like weaning, introducing solid food, and most prominently, discontinuing breastfeeding. Aerobic bacteria like *Staphylococcus* and *Enterococcus* are supremely widespread at birth. Far along, anaerobes surrender, with *Bacteroidetes* and *Firmicutes* being the most common. Prior to the formation of comparatively stable gut microbiota constituents, children's microbiota is largely dominated by *Akkermansia muciniphila*, *Bacteroides*, *Veillonella*, *Clostridium coccooides* spp., and *Clostridium botulinum* spp. At around the age of three, children's gut microbiota starts resembling that of adults, with *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* becoming the major microbial phyla. As individuals age, dietary changes and alterations in the immune system can significantly influence the gut microbiota composition. Notably, older adults often show a decline in *Bifidobacterium* as well as a growth in *Clostridium* and *Proteobacteria*. The decline in anaerobic *Bifidobacterium* is thought to be linked to a deteriorating inflammatory status, given its role in immune system stimulation [28].

1.4 Function of Gut Microbiota

The gastrointestinal (GI) tract of humans, different from other regions of the body, contains an extensive microbial community, estimated to comprise around 100 trillion microorganisms [16]. Recent research has emphasized the vital role of human microbiota in nutrient extraction, metabolic processes, and immune system functionality. The microbiota affects various biological processes through multiple mechanisms. It is indispensable for deriving energy and nutrients from food, which is related to its diverse array of metabolic genes that enable the production of unique enzymes and biochemical pathways [28]. Moreover, the gut microbial community is known to be essential for bioactive compound synthesis such as vitamins, amino acids, and lipids [34]. The human microbiota supports the immune system by producing antimicrobial compounds that defend against external pathogens. Additionally, it plays a crucial role in the development of the intestinal mucosa and stimulates the host's immune responses [9, 28].

1.5 Role of Gut Microbiota in Health

The immune systems of mammals are a multifarious linkage of innate and adaptive components present in all tissues, crucial for defending against harmful external agents and maintaining homeostasis. Mammals and their symbiotic microbes have coevolved in mutually useful ways (perspective et al. 2007). Different investigations have revealed that immune receptors such as Toll-like receptors (TLRs) undergo purifying selection as a dominant force, with signatures of positive selection in

altered species. A highly specific location was identified in cell-surface TLRs rather than endosomal TLRs, suggesting less conserved features that enable rapid evasion of bacterial PAMPs recognized by cell-surface TLRs. This positive selection mediated by pathogens has shaped the diversity in mammalian TLRs [7]. This close association necessitates effective host immunity to prevent excessive resource exploitation by microbiota while allowing tolerance to harmless stimuli [14, 40]. The gut microbiome disruptions due to issues like the use of antibiotics and changes in dietary or geographical shifts lead to the systemic spread of commensal microorganisms, improved susceptibility to pathogens, and irregular immune responses. Moreover, connections among the microbiome and immunity are linked to various noncommunicable gastrointestinal diseases such as celiac disease [61], inflammatory bowel disease (IBD) [66], and extraintestinal issues ranging from rheumatoid arthritis [41] and metabolic diseases [8] to neurodegenerative ailments [43] and cancers [27]. The association among the gut microbiota and host immunity is intricate, lively, and influenced by context [27]. The ways in which microbiota exerts its positive or negative effects are not fully understood; nonetheless, they involve the synthesis of signaling molecules and the capability of mucosal immune cells and intestinal epithelial cells to distinguish bacterial epitopes [53]. The intestinal mucosa has the major surface area exposed to external antigens; it is found that the gut microbiota plays a major role in developing mucosal immunity. The dense layer of microbiota on the mucosa typically represents the majority of antigens presented to resident immune cells, which activate pattern recognition receptors like NOD-like receptors (NLRs) and TLRs in intestinal epithelial cells [48]. This system includes gut-associated lymphoid tissue (GALT), such as lymphoid aggregates in the large intestine, Peyer's patches (PPs), small intestinal lymphoid tissue (SILT) in the small intestine, and immune cells scattered throughout the lamina propria of the gastrointestinal (GI) tract. These immune cells connect through a broader immune system through local mesenteric lymph nodes (MLNs). Additionally, the intestinal epithelium contributes to immune responses by triggering foreign antigens through TLRs and NLRs [48].

The immune system of mucosa must balance two seemingly contradictory roles: it must tolerate the microbiota to avoid triggering an excessive systemic immune response while also controlling the microbiota to prevent its evolution and movement to other parts of the body. The gut microbiota is considered pivotal in succeeding in these functions within the GI tract mucosal immune system. The microbiome has several vital purposes. In healthy persons, it protects against pathogenic organisms, which can lead to infections. For example, microbiota creates short-chain fatty acids (SCFAs) by fermenting multifarious plant carbohydrates, supplying energy for colonocytes to support their variation and redevelopment [10]. Additionally, microbiota synthesizes important vitamins and amino acids, controls fat metabolism [5, 23], and creates numerous small molecules that interrelate through the host environment. In turn, the microbiome has a significant role in shaping the immune system and promoting anti-inflammatory responses [10, 24] (Fig. 1.1).

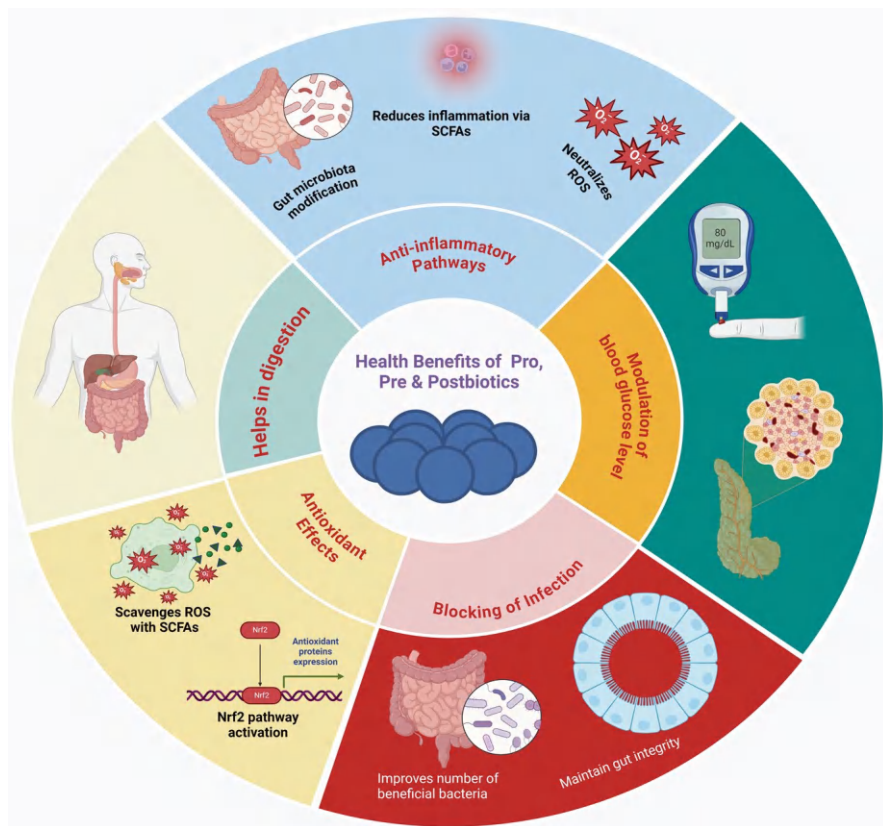


Fig. 1.1 Health benefits of different “biotics”

1.6 Role of Gut Microbiota in Diseases

The gut microbiota comprises all microbial species that reside in the lining of the gastrointestinal tract and make a microenvironment in the gut [11]. Approximately 10^{14} different types of microbes are present, which are constituted of thousands of different species. The combined genome burden of these microorganisms is many times more than the human genome itself [12, 31]. This holistic microenvironment has an intricate balance of versatile microorganisms and generally shows a state of eubiosis [46]. There is also a system called quorum sensing, which helps these microorganisms communicate with each other. The microbial crosstalk in the gut is facilitated by small peptides called autoinducers. Any disparity in the gut microbiota can lead to the devastation of the normal functioning of these microbes and thus produce dysbiosis [30, 36]. The diverse gut microflora impacts various bodily functions, including the production of essential compounds and the regulation of multiple physiological mechanisms. For instance, the gut–brain axis, the bidirectional

communication method associating the gastrointestinal system and the brain, facilitated by different neuronal, humoral, and immunological factors, can affect brain functions. Moods, behaviors, cognitions, etc. are a few glimpses of the gut–brain axis functioning. Likewise, many important metabolites such as dopamine, short-chain fatty acids, and choline are released by the gut microorganisms, which leads to the performance of different functions [18, 20, 31, 52]. Taking into account all these aspects of the gut microbiota, it is easy to estimate the potential role of these microorganisms in sicknesses, particularly those that are directly associated with the GI tract, like digestive and metabolic disorders. The gut microbiota's role in different diseases is explored and documented [11, 15, 20, 46, 52]. It is clear that these microbes have used different mechanisms to cause the diseases and also help prevent or treat the disease. The significant performance of beneficial microorganisms (probiotics) in the inhibition as well as management of sicknesses serves as a remarkable illustration. Gut microorganisms, as described, produce different metabolites, which directly or indirectly influence various metabolic and physiological functions [46]. Different pathways and mechanisms are explored that are followed to help in the prevention and treatment of these disorders. For instance, gut restoration, pathogen killing, nutrient depletions, eradication of pathogens, etc. are some routes that are followed to trace these diseases. Although the complexity and intricate balance of the gut microbiome can create a hurdle in treating them in a controlled way, there are still plenty of examples that indicate the potential role of these microbial groups residing in the gut in illness management.

1.7 Role of Gut Microbiota in Metabolic Disorders

Besides other diseases, metabolic disorders affect a huge population of all ages, genders, and locations around the globe. The word metabolic syndrome initially appeared in the 1970s in association with atherosclerosis [15]. A significant contribution was made in the early 1990s when an insulin-resistance disease was identified. Both individually and globally, efforts were made to highlight the different aspects of metabolic syndrome by different organizations and prevention centers [15]. Substantial work has been done so far, but there are many aspects that still need to be explored for a comprehensive understanding of the role of gut microbiota in the prevention, management, and treatment of metabolic illnesses. The complexity of genetic as well as environmental factors, diet plans, and even the lifestyle of an individual greatly affect the potential effect of gut microorganisms on metabolic disorders. Initially, the gut microorganisms' role in these sicknesses was ignored, but later, it was recognized that these microbes have a potential contribution [15]. The gut-center theory of metabolic syndrome was developed in 2007 after a series of experiments on the rodent models. The outcome of the results signifies the role of bacterial lipopolysaccharide (LPS) in causing low-grade inflammation. The effect of LPS on toll-like receptors (TLRs) was also identified, which attracted researchers around the globe, and hence, the field was explored [15]. Similarly, it

was identified that genetically, there are variations in the gut microbial configurations of obese and nonobese mice [29, 35, 64].

How gut microbiome regulates metabolic disorders still needs to be explored in detail. However, diet harvesting, energy consumption, carbohydrate metabolism, etc. are a few options that are followed [35]. Gut permeability is another avenue that affects metabolic disorders. Gut microflora changes intestinal permeability and triggers low-grade inflammation. Likewise, the microbial metabolites in the gut also play a potential role in metabolic sicknesses [21, 29, 35, 64]. Obesity, currently affecting a large population of Westerners, is vastly under the impact of the gut microbiota. A study conducted on human volunteers who were fed a plant-based diet and an animal-origin diet, reflecting the decrease in fiber-fermenting bacteria in the animal-fed group, indicated the impact of diet on gut microbiota and its effect on the body [15]. Likewise, gut microbial metabolites were shown to be responsible for the prevention of dyslipidemia (an irregular quantity of lipids in the blood) [11, 21, 51]. It was recognized that short-chain fatty acids, microbial lipopolysaccharides, peptidoglycans, etc. are considered foreign particles, and the immune system can eradicate them, and thus, they help lower cholesterol in the blood [12, 15].

1.8 Modulation of Gut Microbiota by Probiotics and Prebiotics

Diet, as a highly effective resource for changing the microbiome, can improve well-being and reduce healthcare expenses [45]. Of these, prebiotic and probiotic boosters preserve a healthy microbiome [19]. The gut hosts a diverse and lively microbiome that is continually influenced by numerous external issues like diet, antibiotics, infections, and foreign substances [37, 60]. Both digestible and indigestible carbohydrates, proteins, fats, polyphenols, prebiotics, probiotics, and synbiotic can modify the microbiota and impact the host's immune and metabolic responses [55]. In 2017, the definition of prebiotics was modernized to describe them as “a substrate that is selectively utilized by host microorganisms, providing a health benefit.” Initially, prebiotics were studied for their effects on enhancing probiotics. Common examples of prebiotics include inulin, fructooligosaccharides (FOS), lactulose, and galactooligosaccharides (GOS). They primarily help modulate the growth of *Bifidobacterium* and *Lactobacillus* strains, which create lactic acid and acetate, thus supporting host health through fermentation [3]. Investigations exposed that prebiotics promote the selective growth of probiotics in the gut, aiding in immune regulation and pathogen prevention [42]. Although some foundational studies indicate that prebiotics may inhibit pathogen colonization by mimicking microvilli glycoconjugates [22] and directly influencing gut immunity [54], their clinical application remains a topic of debate.

There is a significant connection between the gut microbiome, overall health, and diet, suggesting that dietary modifications can improve health by modulating

the microbiota. Over the past decade, extensive research into the connections among the microbiomes and the immune system has improved the knowledge of their molecular foundations and highlighted their role in various immune-related diseases. This knowledge is driving the expansion of microbiome-focused therapeutic approaches for immune-mediated conditions, such as fecal microbiome transplantation (FMT), effectively used for *Clostridium difficile* infections, being measured as a management option for patients with dysbiosis related to immune disorders. However, there is no agreement on what defines a “healthy” microbiome, and the effectiveness of FMT for conditions like inflammatory bowel disease (IBD) is still being studied. Challenges such as optimizing fecal processing and ensuring patient safety remain [67].

Traditional probiotics have limited efficacy in promoting health, leading to an interest in “next-generation probiotics” or carefully formulated microbial consortia as promising alternatives [58]. Additionally, new techniques are being developed to edit the microbiome more precisely [69], such as using bacteriophage therapy to selectively eliminate certain harmful bacteria [62]. Altering nutrient availability through diet could also be a viable strategy for modifying the microbiome, given its strong correlation with dietary habits. Exploring personalized diets or specific dietary manipulations may provide insights into treating immune-related diseases and their effects on immune responses [65].

Moreover, the metabolites created by the microbiome, which are abundant in the gut and bloodstream, present opportunities for modulating these bioactive compounds (known as “postbiotics”) [39]. Their supplementation or targeted inhibition in specific immune settings could lead to novel microbiome-directed management. Chemical genetic screening of gut metabolites may help identify those crucial for host physiology or linked to immune disorders [13]. Overall, advancing microbiome-based therapies requires a deeper knowledge of the complex connections among the microbiomes and immunity. Successfully translating these treatments into clinical practice will depend on rigorous, standardized preclinical and clinical studies.

1.9 AI and Gut Microbiota

To date, a number of methodologies have been implied by researchers to discover and know the multifarious associations and links that exist among gut microbiota and host health. Nevertheless, the complex nature and assortment of gut microbiota present significant obstacles in deciphering its functions and interactions. In contrast, conventional methods used for microbiome analysis have proven not only to be time-consuming but also to possess limitations when it comes to analyzing extensive datasets [26]. A considerable fraction of the human microbiota, estimated at around 70%, is uncultivable, which poses challenges for traditional microbiological techniques. However, the advent of novel molecular profiling tools, including genomic next-generation sequencing (NGS) and metagenomic shotgun

sequencing, has underscored the importance of microbiota in both health and illnesses. Despite the progress in multi-omics technologies in the research of microbiomes, the analytical competencies in these areas remain constrained. The extensive quantities of data produced by these methodologies necessitate bioinformatic approaches, making analysis and interpretation a complex endeavor. In this context, artificial intelligence is regarded as a crucial asset in gut microbiota research, facilitating the examination of extensive microbiome datasets. Machine learning algorithms are adept at uncovering patterns and correlations within intricate datasets, thereby offering important understandings of the composition and functionality of the gut microbiota. For instance, deep learning methodologies have been employed to forecast disease states based on microbiome profiles, thereby aiding in early diagnosis and intervention [17]. However, with the emergence of artificial intelligence (AI) technologies, comprising machine learning (ML) and deep learning (DL), the healthcare landscape has been transformed, offering the potential to enhance and progress the worth of life [32]. These advancements in AI methodologies, such as ML and DL, undoubtedly create new avenues to overcome the significant encounters that have previously obstructed the assessment of the microbiome's intricate diversity [59]. With its advanced computational capabilities, AI aids in processing and interpreting massive datasets, allowing for the rapid and accurate detection of microbial diversity and its influence on human health and metabolic processes.

However, AI continues to encounter several limitations in the evaluation of gut microbiota. These include the inherently noisy characteristics of gut microbiota data, its limited availability, high dimensionality, complex compositional structures, and significant diversity. Such factors create obstacles for AI algorithms, which depend on extensive amounts of clear and consistent data [1, 44]. The information obtained from gut microbiota studies often contains inaccuracies or inconsistencies, complicating the analytical process. It is important to report concerns associated with data privacy, ethical concerns, and the standardization of methodologies to fully harness the potential of AI in this field [63]. Additionally, data sourced from various platforms and methodologies may introduce biases and additional variables. To overcome these difficulties, a multidisciplinary method, pioneering data explanation approaches, and the expansion of further robust AI models are required. Utilizing data from multicenter cohorts could help mitigate some of these issues [32].

Despite the limitations and challenges, computational methods such as ML and DL have the potential to connect the gap in the understanding of microbiomes and their direct health interferences. Within the field of gut microbiota and microbiome, AI offers robust tools for data analysis, personalized treatment development, and improved diagnostics, empowering scientists to decipher the intricacies and complexities of the gut microbiome, resulting in a profounder comprehension of its effects on health and illnesses.

1.10 Conclusion

The gut microbiota constitutes a fundamental component of human physiology, significantly influencing both health and illnesses. Exploring complex interactions that exist among the microbial community and the host presents promising avenues for developing innovative therapeutic interventions and the enhancement of overall well-being. Ongoing research is pivotal for a deeper understanding of the gut microbiota's intricacies, discovering new therapeutic targets, and utilizing its potential for a healthier future. Artificial intelligence is transforming the domain of gut microbiota and probiotics by offering advanced tools for data analysis, the creation of personalized treatment plans, and enhanced diagnostic capabilities. The advent of AI facilitates the exploration of the complex microbial assortment residing in the host gut, contributing to a profounder comprehension of its effect and influence in health and diseases. Nevertheless, challenges, for example, data privacy, ethical issues, and the necessity for standardized methodologies, must be explained to fully harness AI's potential in this area. Continued collaboration among AI specialists, microbiologists, and healthcare practitioners will be vital in leveraging AI's capabilities for the advancement of human health.

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Chapter 2

Introduction to Gut Microbiota and Its Role in Health and Diseases



Somya Sharma, Md Sadique Hussain, Yumna Khan, and Mudasir Maqbool

Abstract The gut microbiota plays a crucial role in maintaining human health and is implicated in a wide range of diseases. This chapter begins by providing an overview of the gut microbiota, highlighting its diverse microbial communities and their essential functions in digestion, immune regulation, and homeostasis. The interplay between gut microbiota and neurodegenerative diseases (NDs) is explored, with a focus on Parkinson's disease (PD) and Alzheimer's disease (AD), underscoring how microbial imbalances may contribute to the pathogenesis of these disorders. Next, the relationship between gut microbiota and cardiovascular diseases (CVDs), including hypertension and atherosclerosis (AS), is discussed, demonstrating how microbial metabolites affect cardiovascular health. The chapter also examines the role of gut microbiota in metabolic diseases, such as obesity, diabetes, and nonalcoholic fatty liver disease (NAFLD), detailing how dysbiosis influences metabolic pathways. Furthermore, the gut microbiota's involvement in gastrointestinal (GI) diseases, including inflammatory bowel disease (IBD) and colorectal cancer (CRC), is reviewed. The chapter concludes by discussing future research directions and potential therapeutic strategies targeting the gut microbiota to improve disease outcomes.

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Keywords Cardiovascular health · Gut microbiome · Health · Neurodegenerative diseases · Obesity

2.1 Introduction

The complex, dynamic, and regionally varied ecosystem known as the human gut microbiome (GM) is home to a broad variety of microorganisms, such as viruses, bacteria, fungi, and archaea. Both the microorganisms and their human host can communicate with one another [1]. Ten times more genetic material than the human genome is included in the collection of genes that are common to all intestinal bacteria. In some respects, it is also regarded as the “essential organ” of the human body [2]. As the largest microenvironment in humans, the GM works in unison with its host to maintain normal physiological processes in a state of dynamic equilibrium. *Bacteroides*, *Proteus*, *Firmicutes*, and *Actinomyces* are the four main groups that make up GM [3, 4]. One of the most important markers for determining GM dysfunction is the *Firmicutes*-to-*Bacteroidetes* ratio. Furthermore, the location, homogeneity, and variety of GM serve as significant indicators of the makeup of the gut flora [5]. The gut flora will alter in composition and function as soon as the GM problem arises, and it may even be the underlying cause of some diseases. This is the result of several complex regulatory processes [6, 7]. GM research has advanced quickly in recent years because of the fast expansion of technologies including high-throughput sequencing, genomics, molecular biology, and bioinformatic analysis [8]. Numerous studies have shown that GM and its metabolites have a notable effect on preserving intestinal equilibrium in the host and affecting the start of a variety of illnesses, including metabolic diseases, neurological diseases,

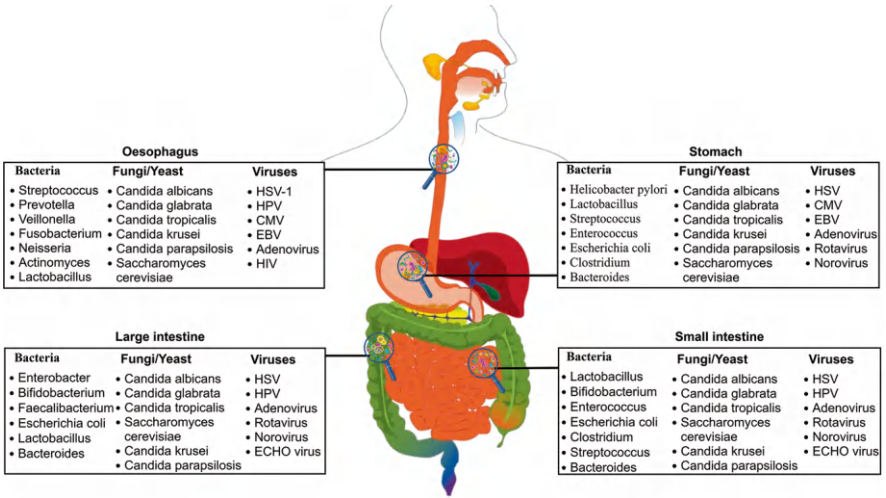


Fig. 2.1 Gut microbiota composition along the gastrointestinal tract, highlighting region-specific bacteria, fungi/yeast, and viruses in the esophagus, stomach, small intestine, and large intestine

cardiovascular diseases (CVDs), and gastrointestinal (GI) problems (Fig. 2.1). The host's health will be impacted by the imbalance of GM in a number of ways, including basophil activator (BA), choline, the gastrointestinal–cerebral axis, and short-chain fatty acids (SCFAs) [9, 10]. It is yet unknown, however, just how GM affects human health. This article's first part summarizes the most recent research on the relationship between GM and human disorders, as well as how such relationships are managed [11, 12]. The features of altered GM linked with several conditions are then described, as well as possible mechanisms involving microbial metabolism and byproducts that may be in charge of the onset and growth of illnesses, thus, providing a possible center of attention for sickness management [13].

2.2 Pathology of Gut Microbiota Dysbiosis in Humans

As a consequence of the dysbiosis of the GM, infection is among the most prevalent disorders. Numerous investigations have demonstrated the close correlation between disease and dysbiosis of the microbial community. Infectious diseases and their management have a substantial influence on the makeup of the human microbiota, therefore dictating the course of the illness. Gastrointestinal (GI) invasion of pathogens triggers a strong inflammatory reaction, which is subsequently followed by the movement of intestinal bacteria [14, 15]. Acute infection is linked to both bacterial and viral pathogens [16]. It illustrates the defensive function of the GM against human diseases. For instance, the composition of the GM in individuals with *Clostridium difficile* infection is markedly changed. Moreover, the human immunodeficiency virus and hepatitis B virus are also linked to the disruption of GM [17]. Figure 2.2 depicts the condition of eubiosis and dysbiosis linked with several illnesses.

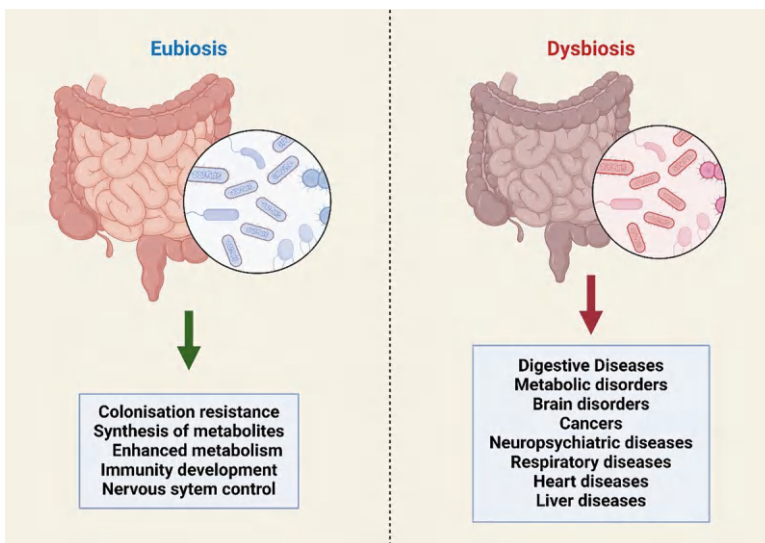


Fig. 2.2 Gut microbiota in health and diseases

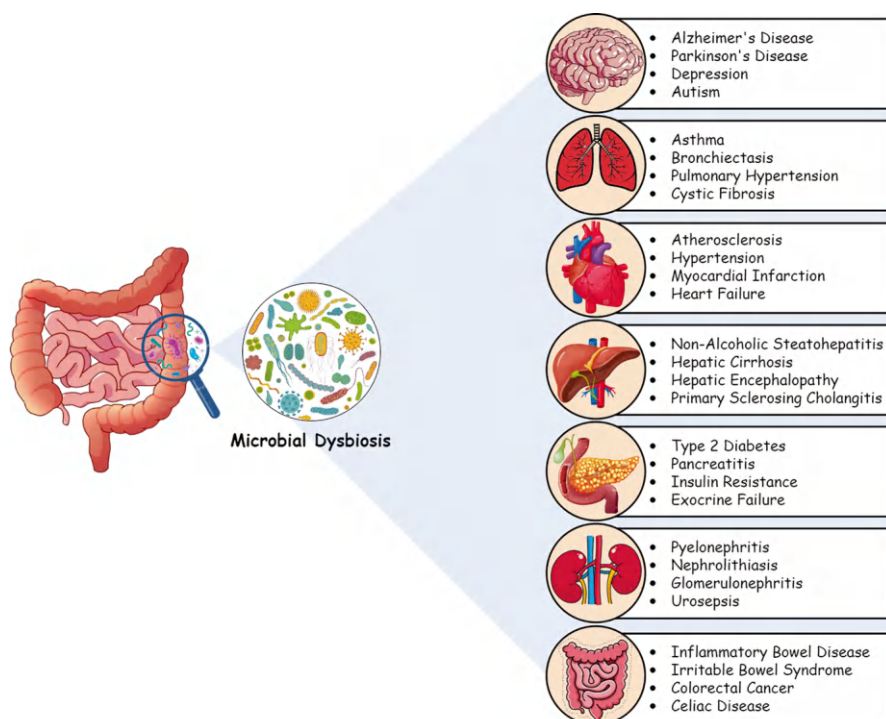


Fig. 2.3 Organ-specific diseases linked to gut dysbiosis

Gut dysbiosis is increasingly recognized as a significant factor in the pathogenesis of various organ-specific diseases (Fig. 2.3). The interplay between the gut microbiome and systemic health emphasizes the need for further research into microbiome modulation as a potential therapeutic strategy for preventing and managing these conditions. Understanding these relationships may pave the way for innovative treatments targeting the microbiome to improve health outcomes across multiple organ systems.

2.3 Gut Microbiota and Neurodegenerative Diseases (NDs)

In the medical world, there has been a lot of attention lately to the connection between GM and NDs. GM may have an impact on the immune system, hormone control, and nervous system communication via the gut–brain axis, according to an increasing amount of research [18]. This may result in the onset and exacerbation of illnesses that impact the central nervous system (CNS), including Parkinson's disease (PD) and Alzheimer's disease (AD) [19, 20].

2.3.1 *Parkinson's Disease*

PD is a prevalent ND that affects the CNS. Its pathogenic features include abnormal synuclein aggregation in Lewy bodies and dopaminergic neuron degeneration and death in the substantia nigra [21, 22]. Numerous studies suggest that there are substantial differences in the GM composition between those with PD and those in good health, notwithstanding the wide range of findings. A number of studies have shown a decrease in the amount of *Brautella*, *Prevotella*, and *Faecococcus* in the GM of patients with PD, whereas *Bifidobacterium*, *Pasteurella*, and *Enterococcus* were found to be much more prevalent [23, 24]. Although *Bifidobacterium* was shown to be more common in people with PD, a subsequent decline in *Bifidobacterium* may indicate whether or not PD will advance. This suggests that the existence of such alterations, such as an increase in *Bifidobacterium* in people with PD, may represent a defense process against the worsening of NDs [25]. It suggests that a probiotic treatment with *Bifidobacterium* may stop PD from progressing into more severe stages [26, 27]. The quantity of *Lactobacillus* present in individuals with PD, however, differed greatly across studies. The results are not entirely unexpected, given that a number of variables, including eating choices, age, geography, body mass index, and ethnicity, have an influence on genetic variation [28]. The composition of *Lactobacillus* varies greatly throughout nations because of the differences in dietary patterns. Even within a single nation, there are variations in *Lactobacillus* strains across areas, such as southeast and southwest China [29]. This discrepancy might be due to variations in regional eating traditions. Furthermore, as *Lactobacillus* may increase irritable bowel syndrome (IBS) symptoms that are prevalent in diarrhea and decrease those that are predominant in constipation, the rise in *Lactobacillus* may be related to the frequent constipation that PD patients experience [30, 31]. The intestinal–cerebral axis is a two-way communication channel between the gut bacteria and CNS that regulates brain growth, aging, and neuronal function. The interaction between the GM and brain axis occurs via three basic routes [32]. Substance cues come first. Microbes may affect the neurological system directly or indirectly by the production of other metabolites and SCFAs. They may also have an effect on the neuroendocrine system; regulate gamma-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), and other neurotransmitter levels; and directly affect the neurological system [33, 34]. Brain pathways are involved in this regulation. Bacteria and their metabolites may impact the vagal and intestinal nerve networks, which, in turn, can impact the brain and behavior. The immune system is the last system in place [35]. The two main mediating mechanisms are microglia and cytokines in the systemic circulation, which is a measure of inflammation. These pathways might be involved in the development of nervous system problems, such as NDs [36, 37].

Many investigations have shown that the intestinal–cerebral axis has a role in PD. PD patients may have neurons with an excess of microglia activation and an accumulation of alpha-synuclein due to altered gut flora (Table 2.1) [38]. Mice with non-eradicated microbial communities showed greater alpha-synuclein

Table 2.1 Concise overview of the associations between specific gut microbes, altered gut flora, and their implications for Parkinson's disease, encompassing neurological impact, mediating systems, and potential interventions

Gut microbe	Parkinson's link	Neurological impact	Mediating system	Implications for PD
<i>Bifidobacterium</i>	Increase, potential defense	Direct/indirect effects	Substance cues	Potential probiotic intervention
<i>Lactobacillus</i>	Varied, influenced by factors	Influence on IBS	Gut–brain pathways	Association with constipation
Microbial flora	Altered in PD patients	Impact on alpha-synuclein	Immune system	Significance in Parkinson's pathology
Short-chain fatty acids	Reduction in PD pathogenesis	Regulation of ghrelin	Gut–brain axis	Implications for Parkinson's disease
Neurotransmitters	Altered levels in PD	Impact on neuroendocrine system	Gut–brain communication	Implications for Parkinson's symptoms
Pro-inflammatory mediators	Potential involvement in PD	Influence on inflammation	Immune response	Connection to neurodegenerative process

aggregations in their brains than germ-free animals without gut flora, according to an investigation using mice overexpressing α -synuclein. In the same research, fecal microflora from PD patients was transferred into microorganism-free mice, exacerbating the motor symptoms brought on by α -synuclein, rather than using healthy donor feces [39, 40]. Recent research indicates that a significant part of the pathogenesis of PD may include the reduction of ghrelin and SCFAs that occurs in PD patients as a consequence of GM change. Despite inconsistent results among studies, several of them show a significant difference in the GM makeup between those with PD and those who are healthy [41]. In PD, microbes may affect the neurological system directly or indirectly via chemical signals, neuronal connections, or the immune system. Therefore, past research suggests that intestinal lesions associated with the beginning of PD may be significantly influenced by GM [42, 43].

2.3.2 Alzheimer's Disease

The most common ND affecting the CNS is AD. AD is characterized by hyperphosphorylation of tau, beta-amyloid ($A\beta$) protein accumulation, beta-amyloid plaques, and neurofibrillary tangles; these factors set off an inflammatory cascade that eventually results in irreversible brain damage due to neuronal death or necrosis [44]. Similarities exist between the beginning of PD and AD, including intestinal bacteria and an unbalanced gut–brain axis. A growing amount of research has shown a link

Table 2.2 Comprehensive overview of Alzheimer’s disease (AD), highlighting the key features, impact of gut microbiota, microbial infections, and therapeutic implications, aiding in understanding and potential treatment strategies

Alzheimer’s features	Gut microbiota impact	Microbial infections in AD	Therapeutic implications
Amyloid, tau, neurofibrillary tangles	Dysbiosis, inflammation	Herpes simplex virus type 1 role	Gut flora control for treatment
Similarities with PD	Altered gut–brain axis	Beta-amyloid plaques in AD	Prebiotics, GV-971, neuroprotection
Fecal flora diversity	GM influence on AD	AD pathogenesis and gut–brain axis	Phenylalanine, isoleucine effects
Early AD detection	Inflammatory bacterial changes	Beta-amyloid plaques in AD	GM dysbiosis, neuroinflammation
AD and GM connection	GM dysbiosis effects	Aging and gut flora imbalance	Therapeutic approaches for AD

between AD and GM (Table 2.2) [45, 46]. Clinical data suggests that GM has a role in the early pathophysiology of AD in humans. When compared to those in excellent health, the diversity of fecal flora was much lower, and the changes in intestinal flora at the stage of moderate cognitive impairment were shown to be comparable to those in the early stages of AD [47]. This suggests that dysbacteriosis may aid in the early detection of AD. Several research have shown evidence of a close connection between AD and inflammation caused by GM. The imbalance in GM seen in AD individuals may lead to a decline in pro-inflammatory bacteria such as *Shigella* and *Escherichia* and an increase in anti-inflammatory bacteria [48]. Pro-inflammatory compounds generated by various gut bacterial taxa have the capacity to increase peripheral immune cell invasion into the CNS, as well as contribute to systemic and plasma inflammation. Mice with AD have altered GM makeup, reduced epithelial barrier integrity, and prolonged inflammation in the gut and throughout the body, in contrast to wild-type mice in normal health [49, 50].

In recent times, there has been a proposition indicating that microbial infection accelerates the progression of AD [51]. Abbott and colleagues have reported the presence of herpes simplex virus type 1 infection within the brains of individuals afflicted with AD. Subsequent investigations have revealed that beta-amyloid (A β) plaques serve as a form of defensive protein, potentially directly enveloping and enclosing the virus that penetrates the brain. These so-called protective proteins accumulate in the brain, become difficult to get rid of, and eventually cause significant damage to neural tissue [52, 53]. This study raises the possibility that microbial infections are the underlying causes of AD. One of the main pathological features of AD, apart from microbial infection, is the buildup of beta-amyloid protein in the brain, which causes plaques and subsequently nerve damage [54]. Growing evidence has shown that in models of AD, GM affects the degree of beta-amyloid protein pathology and cognitive decline. For example, compared to mice grown normally, APPPS1 animals maintained in a germ-free environment showed a substantial decline in both neuronal beta-amyloid protein buildup and Iba-1-positive microglia, which ultimately resulted in an overall reduction in neuroinflammation

[55]. Similar effects were shown when the GM of healthy mice was added to models of AD, which decreased glial activation, cognitive loss, and the formation of neurofibrillary tangles and beta-amyloid protein plaques. One possible cause of AD is an imbalance in gut flora brought on by aging [56]. This imbalance may initiate and exacerbate AD by activating the CCAAT/enhancer-binding protein beta/asparagine endopeptidase pathway in the stomach and brain. Further analysis has demonstrated that the prebiotic R13 may prevent this process by encouraging certain bacteria and reducing amyloid plaques in the digestive system [57, 58]. Furthermore, recent research found that the changed GM composition causes a buildup of phenylalanine and isoleucine in the blood, which promotes the maturation and proliferation of T helper 1 cells that support inflammation. Additionally, GV-971 reduced neuroinflammation, enhanced cognitive impairment, and blocked peripheral phenylalanine and isoleucine accumulation, according to experimental validation in the same study [59]. AD patients' pathogenesis includes a disturbance in the gut–brain axis, which is the first step toward intestinal microbiota involvement. A growing body of research has shown a clear link between AD and inflammation caused by GM [60]. The aforementioned studies unequivocally demonstrate that GM dysbiosis may alter the dynamic balance of GM and result in neuroinflammation during AD, indicating that this may represent a unique therapeutic approach for AD. Treatment for neurological diseases may include controlling gut flora [61–63].

2.4 Gut Microbiota and Cardiovascular Diseases

Atherosclerosis (AS), hypertension, and heart failure are examples of CVDs. Globally, CVDs are the leading causes of mortality [64]. A growing body of research has shown that GM and its metabolic byproducts interact with the host in several ways, influencing the growth and progression of CVDs [65]. Numerous studies have connected the metabolic byproducts of the GM—which include basophil activator, SCFAs, and trimethylamine oxide (TMAO)—to CVDs [66, 67].

2.4.1 Hypertension

One of the most common risk factors for CVDs is high blood pressure. Changes in gut bacterial compositions, gut function, and the gut–brain system relationship are all associated with hypertension [68]. Microbial diversity, abundance, and homogeneity were all significantly lower in hypertensive people, whereas the ratio of *Firmicutes*-to-*Bacteroidetes* was noticeably greater. Blood pressure is mostly regulated by changes in the gut flora, and the production of GM byproducts may be a key mechanism behind these changes [69]. The concentration of circulating TMAO in serum or plasma has been shown to have a significant dose-dependent link with both the risk of hypertension and CV metabolic risk markers such as high-density

lipoprotein (HDL) cholesterol. This conclusion was drawn from a meta-analysis of eighteen observational studies [70, 71].

Genetic modification has implications that extend far beyond intestinal health. The blood-brain barrier may be crossed by metabolites from the GM, which can then alter brain function by entering the circulation. The disturbance of SCFAs' balance brought on by GM dysbiosis may increase the generation of 5-hydroxytryptamine (5-HT) by intestinal chromaffin cells [72]. This compound may interact with the 5-HT₃ receptor on the intestinal vagus nerve, hence impairing sensory activation of the vagus nerve from the gut to the brain. Vasoconstriction may result from the release of 5-HT into the circulation. 5-HT and SCFA may affect blood pressure via circulation due to the blood-brain barrier being compromised by high blood pressure [73, 74]. Moreover, the production of hydrogen sulfide (H₂S) by genetically modified crops has a detrimental effect on human health. It has been shown that H₂S can control a number of physiological functions, such as angiogenesis, hypotension, and vasorelaxation [75]. The absence of H₂S occurs prior to the onset of hypertension. Exogenous H₂S donors may prevent hypertension in spontaneously hypertensive rats, suggesting a significant role for H₂S deficiency in blood pressure control. Via several methods, such as the suppression of oxidative stress or inflammation and the induction of vascular smooth muscle relaxation via ion channel mediation, H₂S may be able to lower blood pressure [76].

A growing body of research indicates that intestinal dysbacteriosis might be related to vascular permeability, blood pressure, and the control of inflammatory processes. Through the induction of inflammation and vascular immune cell infiltration, GM exacerbates the hypertension and vascular dysfunction caused by angiotensin II [77]. Ex vivo studies have shown that the addition of human feces elevated the mice's blood pressure, pointing to a possible link between genetically modified organisms (GMO) and the onset of hypertension. According to a different study, administering antibiotics to rats lowers the *Firmicutes*-to-*Bacteroidetes* ratio, which, in turn, lowers raised blood pressure and helps balance the dysbiotic hypertension gene [78]. Furthermore, eradicating the whole gut flora with a cocktail of antibiotics markedly decreased the frequency of hypertension-associated aneurysms. This suggests that the immune system and inflammation are regulated by the GM, which serves in the pathogenesis of aneurysms [79]. A high-polyphenol diet has been shown in a randomized controlled experiment to significantly increase intestinal permeability in older adults. It also led to a drop in blood pressure and an elevation in the percentage of butyrate- and cellulose-producing bacteria in the GM [80]. The GM's diversity, abundance, and homogeneity significantly decreased in those with high blood pressure diagnoses. Genetic modification affects hypertension by changing the way the digestive system is controlled and how its metabolites are produced [81]. These results demonstrate a strong correlation between GM and hypertension, suggesting that bringing GM back to balance might be a useful strategy for controlling high blood pressure [82].

2.4.2 Atherosclerosis

AS is the primary cause of cerebral infarction, coronary heart disease, and peripheral vascular disease, accounting for around 50% of all CVDs. Atherosclerotic plaques were shown to include both live oral bacteria and bacterial DNA, suggesting a possible role for GMO in the onset and progression of AS [83, 84]. These results point to a possible link between CVDs and genetic engineering (GE). There is a discernible difference in the makeup of GM between healthy persons and those who have AS, according to a metagenome-wide association research [85]. Beneficial bacterial growth was restricted by the considerably higher numbers of *Enterobacteriaceae* and *Enterobacter aerogenes* in patient samples with AS than in control samples [86]. Numerous research on humans and animals have shown that dysbiosis in the gut linked to AS may also increase intestinal permeability, which, in turn, raises the quantity of lipopolysaccharides (LPS) taken up into circulation. Thus, the formation of atherosclerotic plaques may be influenced by an infection, either direct or indirect, of the wall cells of the artery [87].

Furthermore, the development of AS may be influenced by the impacts of GM and its metabolites. Acetate, propionate, and butyrate are a few of the SCFAs produced by human genetic modification via the fermentation of food fibers [88]. Two instances of G-protein-coupled receptors that are triggered by signaling molecules known as SCFAs are GPR41 and Olfr78. These receptors have the power to elevate the production of peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), which may lower blood pressure and prevent AS [89]. Basophil activator have antiatherogenic properties and are a result of the metabolism of cholesterol. One of the main kinds of substances that genetically alters is a basophil activator (BA) [90]. It works by activating the farnesoid X receptor (FXR), which suppresses the expression of inflammatory markers on monocytes, macrophages, and dendritic cells, thus preventing the development of AS. Another important GM molecule linked to the advancement of AS is TMAO [91]. According to epidemiological studies, TMAO is a new marker that may be used to assess the risk of CVDs. Research has shown that there is a positive correlation between high levels of TMAO and AS [92]. Choline, betaine, and carnitine were degraded by the GM to produce trimethylamine [93]. This was then transferred to the liver, where flavin monooxygenase converted it into TMAO. Higher level of TMAO accelerates the migration of macrophages and their conversion into foam cells, which, in turn, stimulate the production of pro-inflammatory cytokines and AS [94]. Moreover, TMAO causes oxidative stress and reduces vascular reactivity, impairing endothelial function and eventually advancing AS. Moreover, TMAO may activate platelets, which promote thrombosis and the rupture of atherosclerotic plaque [95]. Recently, Nemet et al. observed phenylalanine as phenylacetylglutamine (PAGln) by non-targeted metabonomics. The metabolite PAGln, which is generated by the GM, has the capability to elevate characteristics linked to platelet activation and thrombosis potential. PAGln triggers physiological effects downstream of its interaction with G-protein-coupled receptors. Subsequent research showed that PAGln raises the risk of thrombosis by acting via a G-protein-coupled receptor [96].

People with AS have a genetic composition that is quite different from that of healthy people. Following the identification of bacterial DNA in atherosclerotic plaques, other investigations revealed a link between the GM and the development of AS [97]. AS may be impacted by the growth of gut microbes and the chemicals they produce. Some alkaloids have been transformed by microorganisms into dangerous compounds that encourage the synthesis of inflammatory cytokines, which, in turn, stimulate inflammation and hasten the onset of AS [98]. Taken together, these studies demonstrate that GMO and their offspring possess the capacity to regulate the onset and advancement of AS via many mechanisms. Reducing GM may be one way to address CVDs [99].

2.5 Gut Microbiota and Metabolic Diseases

Although both hereditary and environmental variables impact the development of metabolic diseases, in people with comparable caloric consumption and genetic profiles, the makeup of the GM is linked to an increased vulnerability to metabolic disorders [100]. An increasing body of recent research indicates that aberrations in GM are closely related to a number of metabolic illnesses, including diabetes, obesity, and nonalcoholic fatty liver disease (NAFLD) [101, 102].

2.5.1 Obesity

Over 1.9 billion people are overweight, and over 650 million people are obese. Public health is severely hampered by excess weight and its metabolic effects. Studies reveal that GMO have a significant impact on the rise in obesity [103]. Less diversity and richness in the GM of obese individuals is suggestive of GM dysbiosis, which is associated with obesity. *Bacteroides*, *Faecalibacterium prausnitzii*, and *Akkermansia muciniphila* were less common, but the *Firmicutes* phylum showed a significant increase in frequency [104]. In 2006, Turnbaugh and colleagues discovered variations in the intestinal microecology between individuals classified as lean and those classified as obese. Additionally, they observed that germ-free mice receiving gut microbiota transplants from obese mice exhibited increased fat accumulation and weight gain compared to those receiving transplants from lean mice. These findings imply that dysbiosis of gut microbiota might play a role in the development of obesity [105]. An examination of metagenome-wide association conducted on a Chinese teenage population that was both lean and obese showed a significant reduction in the prevalence of *Bacteroides thetaiotaomicron* in obese people. A negative association between this and blood glutamate levels was discovered [106]. Animal studies revealed that *B. thetaiotaomicron* gavage reduced blood concentrations of branched-chain amino acids and alleviated diet-induced obesity and weight gain in mice [107]. Moreover, two additional advantages of bariatric

surgery point to targeting GM as a viable therapy for obesity: lowering the quantity of *B. thetaiotaomicron* and altering the GM in ways that may last for 10 years [108].

Furthermore, nondigestible carbohydrates may be fermented by the GM, which yields significant metabolites such as succinates and SCFAs. Recent research found that these byproducts are significantly involved in the development of obesity and the issues that accompany it [109]. SCFAs increase energy expenditure and decrease appetite in order to preserve energy homeostasis and delay the development of obesity [110]. Increased sensations of fullness and reduced food intake are linked to enteroendocrine L-cell-secreted GLP-1 and PYY [111]. Through the receptors GPR41 and GPR43, SCFAs modify immune cell activity, thus controlling GLP-1 and PYY. Moreover, by augmenting lipid oxidation, SCFAs may promote the upregulation of heat-generating proteins (PPAR α , PGC1 α , and UCP1) as well as proteins associated with lipid oxidation (CPT-I and UCP2) [112]. By doing this, you will consume more calories and prevent obesity. The phylum *Succinicella* produces succinates via GM. It is possible for bacteria to increase intestinal gluconeogenesis because of their ability to attach to the GPR91 receptor [113]. This might then initiate a gut–brain glucose pathway that affects body mass and fat tissue [114]. In an investigation involving experimental animals, De Vadder and colleagues uncovered the significance of succinate, an essential molecule synthesized internally by *Prevotella*, in the modulation of glucose metabolism. Following oligofructose supplementation, mice showed an increase in cecal succinate content. This resulted in the stimulation of gluconeogenesis and prevented the mice from developing fat and impaired glucose tolerance [115]. Obese individuals often have abnormalities in their gut flora, including decreased intestinal flora richness and variety [116]. Some goods induce obesity by elevating energy consumption and promoting fat oxidation; other products made from GMO-indigestible carbohydrates prevent obesity by boosting calorie intake and lowering hunger [117]. In an effort to prevent and treat obesity, the aforementioned studies have shown the role that GMO and their byproducts play in the beginning and progression of the disease.

2.5.2 Diabetes

An extensive metabolic disease featuring high blood glucose levels is called diabetes. Gestational diabetes mellitus (GDM), type 2 diabetes mellitus (T2DM), and type 1 diabetes mellitus (T1DM) are all included. An increasing body of research indicates that GMO increase the chance of developing diabetes [118, 119]. T1DM is one kind of diabetes that requires insulin. Although the precise etiology of T1DM is still unknown, it is known that both environmental and genetic factors are involved in the disease's formation [120]. When combined, GMO pose a serious risk to the environment. Numerous studies have shown that individuals with T1DM have a very different GM than healthy individuals [121]. Patients with T1DM have lower levels of GM variety, as seen by lower concentrations of *Clostridium* and *Prevotella*. T1DM may develop spontaneously in diabetic mice that are not fat [122]. It is possible to increase

inflammation in the pancreas of antidiabetic mice by introducing the fecal microbiota of nonobese diabetic mice into these animals. This suggests that GM has little effect on the pathogenic process of T1DM [123, 124]. Insulin is not necessary for T2DM; instead, it is characterized by decreased insulin output and resistance to insulin. Patients with T2DM exhibited a considerable decrease in GM, according to Swedish cohort research [125]. When compared to healthy persons, the quantity of *Dallella* in the stomachs of those with T2DM is greater than that of *Bifidobacteria* and *Akkermansia* [126]. According to epidemiological research, those who have undergone a colectomy are more likely to develop T2DM than those who have not [127]. This finding suggests that glucose metabolism may be regulated by the GM and hormone synthesis at the distal end of the colon. In a large prospective cohort study that looked at the richness and diversity of GM in several subcategories, a significant negative correlation was discovered between the baseline g-linolenic acid level and the diversity and composition of GM during the follow-up period [128]. Furthermore, a significant link was discovered between the risk of T2DM and an increase in g-linolenic acid levels [129]. The findings suggest that GM influences the interaction between g-linolenic acid and T2DM. The findings mentioned earlier point to a link between T2DM and GM alterations [130]. Pregnancy complications are prevalent, and there is a higher chance of adverse pregnancy outcomes in women with GDM. Several investigations have shown that gut microbial problems in GDM patients are characterized by an increase in *Rumenococcus*, *Desulfovibrio*, *Enterobacter*, *Bacteroides*, and *Prevotella*, along with a decrease in *Bifidobacterium* and *Fischeri* [131]. It is interesting that alterations in the GM signals may still be seen 8 months after conception. GM dysbiosis coexists with obesity, reduced glucose tolerance, and inflammation in patients with GDM [132]. The organization of metagenomic linkage groups in people with GDM is different from that in the control group, according to a metagenomic investigation [133]. Furthermore, a functional analysis shows that in comparison to the microbial community in control subjects, the microbial community in patients with GDM has higher levels of membrane transport, phosphotransferase system (PTS), LPS, energy metabolism pathways, and PTS [134]. Moreover, there is a connection between PTS and LPS signaling and the level of glucose tolerance [135]. Furthermore, the most recent comprehensive investigation revealed that probiotic supplementation may control GM to stop GDM [136]. Numerous studies have connected GMO byproducts to the development and progression of diabetes. Disruptors of intestinal epithelial tight junctions, such as LPS, flagellin, lipoteichoic acid, and peptidoglycan, may cause inflammation by transmitting signals related to TLR2 and TLR4. Insulin resistance and inflammation may both rise in response to elevated levels of branched-chain amino acids and TMAO [137]. Increased glucagon-like peptide-2 production may result in inflammation, endotoxemia, and impaired intestinal barrier function when SCFA levels are raised. By secreting GLP-1 and PYY, intestinal passage and insulin insensitivity may be inhibited. Intestinal cells produce GLP-1 when basophil activator (BA) connect to the TGR5 signaling receptor, improving glucose absorption and insulin sensitivity [138]. A team of Chinese researchers has made significant strides in understanding how small-molecule metabolites regulate blood glucose levels [139]. It has been discovered that hyocholic acid (HCA) may

successfully cure T2DM by regulating the blood glucose basophil activator [140]. By inducing intestinal endocrine cells to produce and release GLP-1, HCA regulates blood glucose levels by inhibiting the FXR signal and activating TGR5. A clinical data analysis also showed an inverse correlation between blood glucose levels and diabetes and serum HCA [141]. With less emphasis on basophil activator, HCA is expected to become a viable treatment drug for diabetes. Additionally, it may be used in combination with other hypoglycemic medications for optimal clinical efficacy [142]. Moreover, because intestinal flora controls the metabolism of cholic acid, research and development projects aimed at developing new drugs will concentrate on determining how to increase cholic acid levels in diabetics by influencing their GM. However, enteric neurons allow the GM to independently regulate blood glucose levels. The distribution of GM throughout the digestive system is not uniform [143]. Innate enteric-associated neurons control intestinal motility and secretion by keeping an eye on dietary cues and signals from commensal microorganisms [144]. Research has shown that gut bacteria control cocaine- and amphetamine-regulated transcript (CART) neurons, which control blood glucose levels, as opposed to the CNS. Moreover, studies have shown that GM is essential for the function of intrinsic enteric-associated neurons in different regions of the mouse gut [145]. Environmental factors and genetics work together to influence T1DM. Patients with T1DM and healthy individuals have very different GM. Patients with T1DM have less genetic diversity [146]. Transplanting the feces of healthy individuals might 1 day lead to a novel approach to the treatment of T1DM. Additionally, GM uses intestinal neurons to sense nutritional cues and signals from commensal microorganisms to regulate secretory processes and intestinal motility [147]. This allows GM to independently manage blood glucose levels. The peripheral neural circuit's finding provides new insights into the roles of GM in metabolism, tissue physiology, immunology, and nervous system activities. In the future, controlling GM or their species' chemical production may be part of treating diabetes [148].

2.6 Gut Microbiota and Liver Diseases

Over 66% of the blood allocated to the liver originates from the gut via the portal venous network. Consequently, the liver is subjected to many microbes and metabolites [149]. A rising body of research is linking the gut microbiota to the development of various liver disorders.

2.6.1 *Alcoholic Liver Disease (ALD)*

ALD encompasses a range of impairments associated with prolonged and severe alcohol use. Research conducted by Bode et al. in 1984 revealed that individuals with persistent alcohol misuse had an excessive proliferation of microorganisms in

their small intestines [150]. One theory of disease development is that the disruption of gut permeability caused by alcohol results in the widespread distribution of LPS throughout the body [151]. Changes in the GM in individuals with alcoholism seem to be associated with elevated levels of LPS in the blood [152]. The compromised intestinal epithelial barrier may manifest prior to these changes in GM. The impact of microbial activity on bile acid metabolism may also be associated with ALD tissue damage [153].

2.6.2 *Cirrhosis*

Cirrhosis is the permanent tissue damage of the liver that results from certain chronic liver disorders. Analysis of the European Metagenomics of the Human Intestinal Tract (MetaHIT) study revealed significant variations in the quantity of gut bacterial genes between individuals with cirrhosis and those without the disease. The majority of these species originated from the buccal region [154], indicating that liver cirrhosis may be associated with the migration of oral microbiota into the lower GI tract. Previous research indicates variations in GM in individuals with cirrhosis. Specifically, at the phylum stage, there was a decrease in the percentage of *Bacteroidota*, whereas *Pseudomonadota* and *Fusobacteria* were discovered to have increased [155]. Investigators applied an index measuring GM dysbiosis in individuals with cirrhosis and discovered a correlation between the level of dysbiosis and both endotoxin levels and the increasing severity of the illness [156].

2.6.3 *Hepatic Encephalopathy (HE)*

The GM may also play a role in the occurrence of HE, which is a decline in neurological function (leading to a loss of consciousness or coma) caused by liver damage. GM is believed to impact the progression of HE by generating ammonia, which may trigger a widespread inflammatory reaction that affects the brain [157]. An investigation conducted on an animal model of cirrhosis has shown that alterations in GM are responsible for the neuroinflammatory and systemic inflammatory reactions that occur as a consequence of the illness [158]. The use of antibiotics is a conventional approach for the treatment of HE. Whether administered with or without lactulose, rifaximin demonstrated superior effectiveness compared to lactulose alone in treating those suffering from HE [159]. It was also more successful than a placebo in sustaining remission from HE [160]. This provides more evidence of the significance of GM in HE.

2.6.4 Nonalcoholic Fatty Liver Disease

Hepatocellular carcinoma and liver cirrhosis are possible outcomes of untreated NAFLD. It is a severe, ongoing illness. Alongside the obesity crisis, NAFLD is becoming more common [161]. Environmental and genetic factors may have an impact on the complicated disorder known as NAFLD. Although there is still much to learn about the molecular processes behind the development of NAFLD, investigations have demonstrated a strong association between GM and the illness [162].

In order to prevent intestinal microorganisms from infiltrating the liver, the integrity of the gut barrier must be maintained. In people with NAFLD, intestinal permeability was higher than in healthy persons, and this was associated with the degree of hepatic steatosis, as per a meta-analysis [163]. Furthermore, NAFLD patients' GM is not like that of healthy individuals. *Rumenococcus*, *Prevotella*, and *Flavobacterium* were less common than *Lactobacillus*, *Dorea*, and *Streptococcus* in the intestines of NAFLD patients [164]. Because of the increased lipid content in their livers, mice who received liver transplants containing the feces of NAFLD patients developed hepatic steatosis [165]. This suggests that alterations in the gut flora might be one of the primary causes of NAFLD [166]. GMO, often referred to as byproducts of saccharolytic and proteolytic fermentation, may influence the liver–gut relationship via a variety of pathways and eventually engage in the growth of NAFLD. SCFAs have a beneficial effect on the liver's function and metabolic processes [167]. In particular, butyrate promotes the production of mucosal secretions and tight junction proteins, which shields the liver from harmful substances and strengthens the intestinal barrier. Some of the possible underlying processes include decreased expression of tumor necrosis factor, increased glycogen storage, decreased activity of liver fatty acid synthase, and enhanced hepatic lipid oxidation via the AMPK–acetyl–CoA carboxylase pathway [168]. Moreover, by controlling BA metabolism and FXR signaling pathways, the GM may prevent the production of hepatic lipids. Studies indicate that glycine and the beginning of NAFLD may be directly related [169]. Test rat NAFLD is significantly reduced by glycine via changes in blood metabolite concentrations, GM composition, fat oxidation, and glutathione generation [170]. Moreover, during the late fed phase, hepatic lipogenesis is suppressed by fibroblast growth factor-15/19, a gastrointestinal hormone generated by bile acid [171]. Fibroblast growth factor-15/19 suppresses hepatic lipogenesis by binding to its downstream nuclear receptor, small heterodimer partner, via an epigenetic mechanism. Therefore, DNA methyltransferase-3a (DNMT3a) recruits lipogenic gene sites, such as fatty acid synthase. This research might provide fresh insights into the management of metabolic disorders [172]. Furthermore, endogenous ethanol generated by GM may cause direct harm to liver cells, hastening the development of NAFLD. Because endogenous ethanol may alter the function of the gut barrier and increase peripheral cell activation of the nuclear factor- κ B pathway, it can produce elevated portal endotoxemia [173]. Studies like the one cited above have shown how important the GM and liver are to the development and course of NAFLD. NAFLD patients have distinct GM compared to healthy

individuals, in addition to genetic differences [174]. The gut–liver axis may be impacted by certain metabolites from the GM in a number of ways, which may contribute to the development of NAFLD [175].

2.7 Gut Microbiota and Gastrointestinal Diseases

During a protracted evolutionary process, interactions and limitations with the host are how intestinal flora manages intestinal homeostasis. Additionally, it constantly adapts to both the process of natural selection and individual acclimatization [176]. Microbes in the intestines help break down food remnants that the host is unable to metabolize, process nutrients in the gastrointestinal tract (GIT), and take up nutrients from the host. A growing amount of research is connecting disorders of the digestive tract such as colorectal cancer (CRC) and inflammatory bowel disease (IBD) to GM dysbiosis [177].

2.7.1 Inflammatory Bowel Disease

IBD, which includes Crohn's disease and ulcerative colitis, is caused by immune system dysregulation that results in microbial dysbiosis and intestinal inflammation [178]. Many pieces of evidence suggested that GMO may have a role in promoting intestinal inflammation [179]. Reduced GM diversity, one of the primary indicators of dysbiosis, is often seen in patients with IBD. In contrast to individuals without health complications, individuals with IBD exhibit a notable decline in gut microbiota diversity. This reduction is characterized by a significant decrease in *Firmicutes* and a concurrent increase in *Enterobacter* and *Proteobacteria* [180]. Furthermore, alterations in intestinal fungi have been observed in patients with IBD. Patients with Crohn's disease had higher concentrations of *Aspergillus albicans*, *Candida albicans*, and *Cryptococcus neoformans* in their colons, ilea, and feces, suggesting a significant increase in the variety of fungi present. More evidence confirming the role of GM in the development of IBD has come from investigations conducted on experimental animals [181]. After ingesting the gut bacteria from mice with IBD, animals without germs showed clinical signs of the condition [182]. Similarly, Eftychi et al. demonstrated that a mix of specific symbiotic flora can stimulate colitis in mice with impaired intestinal barrier functioning. According to the findings above, some gut microorganisms may be associated with inflammatory enteritis; however, further research is required to pinpoint the precise microbial community in question [183]. Furthermore, the substances that GM generates have an impact on the course of IBD. One of the many impacts that SCFAs have on mucosal immunity is that they promote B cell proliferation, producing IL-18 and maintaining the integrity of the mucosa by activating the inflammasome. By directly activating FXR, which has anti-inflammatory properties and offers defense against chemically

induced colitis, BAs have an immunomodulatory impact [184]. GM synthesizes indoles from tryptophan, which modulate mucosal immunity by activating polycyclic aromatic hydrocarbon receptors. Furthermore, by using epigenetics, gut microorganisms may control how host cells behave. The most current research indicates that by encouraging the development of intestinal epithelial cells and the repair of intestinal lesions, the GM metabolite inositol triphosphate may exacerbate IBD [185]. Additionally, it has the ability to reverse the suppressive impact of butyric acid on histone deacetylase 3 and activate protein deacetylase 3. The aforementioned research suggests that GMO may induce or exacerbate IBD; the precise mechanism behind this effect remains unknown. Therefore, in order to develop a new treatment approach for IBD, additional study on the relationship between GM and IBD is required [186].

2.7.2 Colorectal Cancer

CRC is among the most common cancers in the digestive tract [187]. GM dysbiosis and CRC have been linked in studies. The intestinal microbiota of individuals with CRC exhibits dysbiosis, characterized by a decrease in helpful gut bacteria and an increase in dangerous bacteria [188]. The pathogenic bacteria release toxins that harm intestinal lining cells and cause a protracted inflammatory response, both of which are factors in the progression of CRC. Studies conducted in vitro and in vivo have shed further light on the process by which GM promotes the development and metastasis of cancers [189]. Tsoi H et al. observed that *Peptostreptococcus anaerobius* (Pa) is substantially enriched in feces samples and colon cancer tissues of individuals with CRC, which can induce the abnormal growth of colon cells. Experiments on cells and animals provided further details on the mechanism by which Pa develops CRC. Researchers found that Pa may bind to the PCWBR2 surface protein on CRC cells, which would then cause the PI3K-Akt-NF κ B signaling cascade to be triggered, which would eventually cause CRC [190]. Studies conducted by Garrett WS and colleagues have revealed that *Fusobacterium nucleatum* possesses the ability to attach to cadherin and the inhibitory immune receptor T cell immunoreceptor with Ig and ITIM domains (TIGIT) on the surface of CRC cells via adhesin FadA and Fap2, respectively. This interaction activates the oncogenic Wnt/beta-catenin signaling pathway while simultaneously suppressing the activity of tumor-infiltrating lymphocytes and natural killer cells. Consequently, this mechanism contributes to the advancement of CRC. Furthermore, studies have shown that GM may change the way many host genes work biologically [191]. The p53 gene alterations in the animal model of CRC affect distinct areas of the gut in different ways. The p53 mutant has the predicted carcinogenic impact in the distal gut, but it may greatly lower the occurrence and development of cancers in the proximal intestine [192]. Additional investigation revealed that the metabolite gallic acid, which is generated by gut bacteria, has the ability to change the tumor-suppressive qualities of mutant p53 into carcinogenic ones [193]. It suggests that altered cancer genes may interact

with GM. According to a recently released study, the gastrointestinal tract's microbiota may produce memory T cells that are unique to symbiotic relationships and show cross-reactivity with tumor antigens [194]. Additionally, certain *Enterococci* phages make use of this molecular pathway to boost antitumor immune treatment efficacy. According to the previously cited research, GMO may cause cancer directly or indirectly by suppressing the immune system or causing inflammation. Moreover, GM infections are a significant contributor to the incidence of CRC [195].

2.8 Modifications of the Gut Microbiota

The GM is essential for maintaining metabolic balance and may influence the general wellness and conditions of the individual. Hence, it is advisable to address and avoid these disorders by adjusting the makeup and variety of GM via dietary modifications. There are many methodologies at one's disposal, such as the use of antibiotics, the use of probiotics, the incorporation of prebiotics as food supplements, dietary modifications, and fecal microbiota transplantation (FMT). Among these approaches, nutrition is presumably the most widespread but one that is sometimes overlooked as a regulator of the GM. The inability to acknowledge its influence adds complexity to the analysis of several microbial investigations. The consequences of medicines on the GM are considerably more intricate than first believed. While antibiotics may help save lives, their influence on colonies of beneficial microbes can have therapeutic significance [196]. The notion of prebiotics was first proposed in 1995 by Glenn Gibson and Marcel Roberfroid. Prebiotics refer to non-digestible oligosaccharides, both in general and specifically fructooligosaccharides. They are indigestible dietary components that favorably impact the host by specifically promoting the development and/or function of one or a small number of varieties of bacteria that exist in the intestines to enhance the well-being of the host. By growing the population of certain bacteria, the use of prebiotics greatly alters the makeup of the GM. Prebiotics promote the proliferation of naturally occurring *Bifidobacteria*, following a brief time of consumption, starting to dominate in human excrement. Probably via fermented goods, prebiotics regulate the breakdown of lipids [197].

Not all probiotics need colonization of the digestive system to exhibit their benefits. While certain probiotics such as *Bifidobacterium longum* integrate into the human GM, others like *Lactobacillus casei* exhibit their benefits informally and transiently by modifying or altering the present microbial population as they circulate [198]. Probiotics refer to microbial nutritional products that have a positive impact on the host by enhancing the harmony of GM. Consequently, they are being employed to alter the makeup of the GM. Nevertheless, these alterations might be temporary, thereby restricting the establishment of foreign microorganisms. Furthermore, probiotics not only enhance cognitive function but also regulate stress reactions, thereby earning them the designation of psychobiotics (living organisms, when consumed in sufficient quantities, provide a health advantage in individuals

with mental disorders) [199]. For instance, preclinical assessment in rats indicates that specifically, particular psychobiotics have antidepressant or anxiolytic properties. Potential hazards linked to probiotic microorganisms include probiotic sepsis, compromise of the probiotic item, and diminished effectiveness resulting from reduced survival. The occurrence of probiotic sepsis is quite uncommon across a range of commercial formulations. The oral consumption of probiotics and prebiotics has been frequently employed to change the composition of GM. Practically all prebiotics and probiotics may be ingested without any adverse reactions. In a small number of instances, however, adverse consequences may manifest as stomach pain, bloating, and gas. The notion of synbiotics, which involves a blend of probiotics and prebiotics, is suggested to describe certain colonic meals that include valuable nutritional qualities, making them possible choices for categorization as functional food components that promote health [197].

Numerous investigations indicate the significance of FMT as an efficacious therapy for recurring *Clostridium difficile* infection in cases where antibiotic therapy is ineffective. FMT is the process of transferring the feces of a healthy donor into the GIT of an ill individual to replenish beneficial bacteria in the GM. Colonoscopy is a common technique for doing this procedure, while other procedures such as retention enema or oral capsule ingestion are also available. Constipation is a prevalent symptom among persons with PD. Huang et al. documented the first use of FMT for the treatment of PD sufferers presenting with tremors and constipation. The FMT procedure successfully alleviated the neurological and GI symptoms (such as tremors and constipation) in the individual with PD, indicating that the restoration of GM may have a beneficial impact on therapy [200]. Moreover, there is less knowledge of the function of FMT in AD. One study found that FMT therapy enhanced cognitive impairments and decreased the accumulation of A β plaques in the brains of transgenic mice. The observed enhancements were related to a reduction in the phosphorylation of tau protein and the concentrations of A β 40 and A β 42. Additionally, the alterations in GM and SCFAs after FMT was reversed suggested that FMT might be a promising therapeutic approach for AD [201]. FMT and pre- and probiotics are being explored as promising therapeutic approaches for the management of NDs. Nevertheless, the availability of clinical data is limited, and more study is required to substantiate its application.

2.9 Conclusion and Future Perspective

Because GMO are closely related to human health and illnesses, they provide enormous prospects for disease prevention, diagnosis, and therapy. This article highlights the pathophysiology and mechanism of GMO in multisystem illnesses as an area of ongoing research. In recent years, there has been a growing concern in using metagenomic investigation to investigate the relationship between gut microorganisms and illnesses. Obtaining high-quality reference genome collections might substantially elevate the accuracy and clarity of metagenomic studies and provide the

groundwork for closely examining the connection between the GM that lives in the human gut and phenotype. The most recent studies found that the human GM is a gene collection consisting of 200,000 genomes and 171 million protein sequences. The fact that 71% of the strains among 4644 species are uncultivable and that strain diversity varies greatly across continents indicates that most microorganisms still need experimental study. However, there are still a lot of unanswered questions about the connection between illnesses and gut microorganisms. First, opinions on what constitutes “good” gut flora vary. How may GM disorders be clinically described and robust microbiota screening approaches created for precise diagnosis to identify individuals with intestinal microbial problems? Second, the exact mechanism by which the gut bacterial issue relates to the disease is yet unknown. Moreover, it is unclear whether a single strain of bacteria or the whole GM is to blame for the illness. The exact mechanism by which GMO cause and progress through illness is yet unknown. Moreover, may the gut microorganisms that aid in medicinal treatment be used for illness prevention and enhancing general immune function? Finally, since host immune systems, gut bacteria, and illnesses are all intricately linked, a patient may need a variety of GM therapies. Currently, there are few practical approaches for treating clinical disorders by regulating gut flora. Therefore, for forward-looking studies requiring large sample numbers, it is crucial to include state-of-the-art techniques using approaches like metagenomics, transcriptomics, proteomics, and metabolomics. Individually customized GMO may be used in the future in precision medicine to treat and prevent certain illnesses. Focusing on the composition and metabolic functions of GM may be a novel approach to illness prevention and treatment. To sum up, there are many prospects for fundamental and translational research as well as epidemiological and clinical assessments that may help us better comprehend this intricate gut ecology. This necessitates more meticulous high-throughput sequencing, multi-omics analysis, dynamic long-term monitoring of alterations in gut flora and the onset of disease, and rational experiment design. We must look more closely at specific strains to identify potentially harmful bacteria that are conditionally associated with illness. It may realize the promise of precision medicine and provide fresh perspectives on how to treat illnesses.

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Chapter 3

Molecular Mechanisms of Postbiotics in Colorectal Cancer



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Abstract Numerous research studies have linked colorectal cancer (CRC), the third-highest-recurring cancer in the world, to microbiota in the gut, a complex system of bacterial communities essential to metabolic regulation, immune system control management, and the maintenance of intestinal homeostasis. A deficiency in the microbiota that is present in the gut has been strongly connected to the development of CRC through chronic swelling, inflammation, immune resistance, and metabolic dysregulation. Because of their stability and safety relative to live probiotics, postbiotics—defined as nonviable microbial cells, their components, or metabolic by-products—have become attractive therapeutic agents in CRC treatment. These bioactive substances change important molecular pathways to have anticancer properties. Recently discovered short-chain fatty acids (SCFAs) such as butyrate block the histone deacetylases (HDACs), which leads to activated tumor suppressor genes as well as the death of CRC cells. In addition, postbiotics can interact with G-protein-coupled receptors (GPCRs), for example, GPR41 and GPR43, reducing inflammation

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as well as increasing the immune response. They can also raise the health of your gut through the growth of beneficial bacteria and prevent pathogenic organisms such as *Fusobacterium nucleatum* and *necrophorum* both known as contributors to CRC. Through stimulating tight junction proteins, they also improve the intestinal barrier and prevent the transmission of harmful bacteria and contaminants that could cause carcinogenesis. This chapter explores the cellular mechanisms underlying the role of postbiotics in the management of colorectal cancer. It also outlines their ability to be a revolutionary therapy for the prevention and treatment of cancer, especially in those suffering from dysbiosis-related CRC.

Keywords Colorectal cancer (CRC) · Postbiotics · Gut microbiota · Short-chain fatty acids (SCFAs) · Gut dysbiosis

3.1 Introduction

Colorectal cancer (CRC) constitutes a major world health issue, as it is the third-most diagnosed cancer and the leading cause of cancer-related deaths in the world [1]. CRC is multifactorial and entangles genes, environmental triggers, food habits, lifestyle, and other factors. Researchers have realized that gut microbiota plays an important role in the progression of CRC in the past couple of years [2]. The evidence is increasing to suggest that changes in the microbial structure, also known as dysbiosis, may contribute to the growth of tumors. The gut microbial community, consisting of microorganisms residing in the gastrointestinal tract (GIT), plays a crucial role in maintaining host health by regulating digestion and metabolic functions in addition to immune function and the health of the intestinal barrier [3]. The microbiome of the gut is a key factor that triggers the onset and development of CRC by multiple mechanisms that include persistent inflammation, genetic toxicities, and altered immune function. Particularly, the pathogens *Fusobacterium nucleatum* and *necrophorum* *Bacteroides fragilis* have been proven to contribute to CRC by causing inflammation, generating carcinogenic metabolites, and directly interfacing with epithelial cells of the host to promote tumorigenesis [4]. These results emphasize the importance of a well-balanced intestinal microbiota in the treatment of colorectal cancer and emphasize the potential benefits of microbiota-modulating treatments.

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In this regard, postbiotics are the treatment option. Postbiotics are bioactive substances created during fermentation by microbial cells that are not viable, which include short-chain fatty acids (SCFAs) and peptides. They also include polysaccharides, components of the cell wall, and other microbial metabolic compounds. Postbiotics focus on providing an alternative, particularly for those with immunodeficiency, because they do not carry the risk of infection in comparison with probiotics that require consumption [5]. Postbiotics have biological benefits by regulating inflammatory pathways, controlling apoptosis as well as the proliferation of cells, and enhancing the functioning of the intestinal barrier. The most extensively studied postbiotic is butyrate as an SCFA made by the diet fibers produced by the gut bacteria [6]. It has been demonstrated that butyrate can provide potent anticancer benefits in CRC by blocking the histone deacetylases (HDACs) and resulting in the activation of the tumor suppressor genes and the initiation of apoptosis within cancer cells. Moreover, bacteriocins and exopolysaccharides (EPSs) show how postbiotics' ability to stop tumor development and control the immune system supports their possible therapeutic value in CRC [7]. This chapter aims to provide an overview of postbiotics that influence CRC development and progression. Through a detailed examination of these mechanisms, we aim to highlight the potential of postbiotics as advanced therapeutic agents in the battle against CRC. This opens additional potential for prevention and strategies of treatment that tap into the capabilities of the gut microbiota.

3.2 Gut Microbiota and Colorectal Cancer

Incorporating billions of microorganisms such as archaea, bacteria, viruses, and fungi into the gut's microbiota is crucial to humans' health, specifically in the context of the metabolic processes, regulation of immunology, and defense against pathogens. The microbiota in the gut is led by three major phyla, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*. Each group plays a role in the functions of the microbiome in the gut, like digestion, the production of vital vitamins, and the modulation of immune response [8]. However, if the delicate balance of these groups is disrupted, a condition referred to as dysbiosis may develop, resulting in a decline in beneficial microbiome diversity and the growth of pathogenic bacteria. This is strongly linked to numerous chronic illnesses, such as CRC. Microbiome imbalances in the gut are associated with colorectal cancer. It is among the most prevalent and fatal cancers that we face. If the gut does not have an appropriate number of bacteria, it can cause bad bacteria, like *Escherichia coli*, *Fusobacterium nucleatum*, and *Bacteroides fragilis*. Bacteria play a role in the development and onset of colorectal cancer (CRC) through various mechanisms. For instance, in colon epithelial cells, *Fusobacterium nucleatum* can contribute to cancer progression by inducing immune evasion and promoting inflammation [9]. These bacteria have been concerned with the initiation and progression of CRC through several mechanisms. In the case of colon epithelial cells, for example,

Fusobacterium nucleatum is known to promote cancer through interactions resulting in inflammation and immune evasion [10]. Cell proliferation and survival depend on activating the β -catenin signaling system, which this bacterium does by binding to E-cadherin on the surface of colorectal cells using FadA adhesin. Apart from encouraging tumor development, this relationship generates an immunosuppressive milieu that lets cancer cells avoid immune detection. Important in the pathophysiology of CRC are also toxins produced by other bacteria such as *Bacteroides fragilis*, which can cause DNA damage and aggravate inflammation. The bacterial toxin's genotoxic activity, along with the chronic inflammatory state they cause, plays a role in the accumulation of mutations and the advancement of tumors in patients with CRC [11]. Furthermore, an imbalance in the gut microbiome could affect the efficacy of treatment for cancer by affecting the immune system's reaction and thereby reducing the sensitivity of chemotherapy and other immunotherapies. The relationship between the microbiome in the gut and cancer requires a complex set of communication pathways. The activation of the Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and STAT3 pathways due to the microbial metabolites as well as bacterial elements results in a pro-inflammatory condition that aids tumor development. The process is exacerbated due to dysbiosis, which breaks down the barrier between the intestines and permits bacterial compounds such as lipopolysaccharides (LPSs) to infiltrate the bloodstream, creating ongoing inflammation and promoting the proliferation of cancerous cells [11, 12]. The microbiota in the gut plays an important function in the development and progress of cancers such as colorectal cancer through its influence on DNA damage, inflammation, and immune system modulation. The possibility of improving the outcomes of patients is demonstrated in the knowledge of specific microbiomes and mechanisms involved in the pathogenesis of CRC and can be employed in the development of targeted therapies and preventive methods that include microbiota-mediated therapy.

3.2.1 Composition of Gut Microbiota

The term "gut microbiota" refers to the diverse community of microorganisms, including archaea, bacteria, protozoa, and fungi, that inhabit the GIT. They play a vital part in physiological processes like digestion metabolic processes, the regulation of immune systems, and protection against pathogens. The microbiota in the gut is mostly dominated by bacteria of the phyla *Firmicutes* and *Bacteroidetes* that together make up more than 90% of the population of bacterial species within the colon. The other significant phyla are *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*. Maintaining gut well-being and general health is affected by the different functions that each one of these groups plays [13, 14]. As an example, *Clostridia* species are among the forms that produce butyrate, a fatty acid with a short chain that is crucial for colon health. *Bacteroidetes* are a group of bacteria that is primarily represented by *Bacteroides* varieties and play an essential role in

maintaining intestinal barrier function and destruction of complex carbohydrates. *Actinobacteria* including ones like *Bifidobacterium* and *Bifidobacterium* are essential for the metabolization of oligosaccharides. They are major factors in the modulation of immune function by releasing vital vitamins such as B12 [15, 16]. Additionally, *Proteobacteria*, which include *Escherichia coli*, are less prevalent when healthy; however, they may be prevalent in conditions of dysbiosis that cause inflammation as well as the progression of diseases, such as CRC. Gut bacteria develop from birth, and how the baby is born plays a major factor in the formation of these. If a child is born vaginally, microbes such as *Lactobacillus* and *Bifidobacterium* generally begin to thrive in the gut from the moment they are born. These microbes can be beneficial for the growth of the immune system. Contrary to this, cesareans are often a trigger for the growth of skin-associated bacteria such as *Staphylococcus*, potentially increasing the likelihood of developing dysbiosis later in the course. The microbiota of the gut is constantly evolving and expanding during the first years of life and is affected by such factors as food habits (breastfeeding in contrast to formula feeding), antibiotic usage, and environmental exposures [17, 18].

Dysbiosis is the result of a disturbance of regular gut microbial balance and may result from a variety of factors that include poor diet and antibiotics, as well as infections and chronic stress. The imbalance could lead to the overgrowth of pathogenic microbes as well as a reduction in the diversity of microbes and the breakdown of the intestinal barrier. CRC development has been linked with dysbiosis. By stimulating the b-catenin signaling pathway within colon epithelial cells, which results in the growth of cells and a longer survival, *Fusobacterium nucleatum*, which is a pathogen found in a dysbiosis environment, encourages carcinogenesis of the colorectal [19]. In addition, dysbiosis could cause chronic inflammation, an established health risk for cancer, by altering the immune response and encouraging an inflammatory milieu. It is clear that the gut microbiota is an intricate and active ecosystem, which has a crucial role to play in the maintenance of the health of humans. Its composition and balance are essential for the proper functioning of our bodies as any disruption in the equilibrium can lead to the development of severe diseases, such as colorectal cancer. Knowing the complex relationships between intestinal microbiota and physiology is crucial for the development of targeted treatments to treat and prevent diseases.

3.2.2 Dysbiosis and Colorectal Cancer Pathogenesis

An imbalance in the natural health of the gut flora that results in an imbalance in the function and composition of microbes in the intestinal tract is known as dysbiosis. A loss of beneficial organisms, an overgrowth of possibly dangerous ones, or a decline in the diversity of microorganisms in the surroundings defines this syndrome. Dysbiosis can be caused by many things, such as using antibiotics, not eating well, being under a lot of stress, and getting diseases. This imbalance weakens the gut's barrier function, allows pathogens to reside in, and starts chronic

inflammation, all of which make diseases like CRC more likely to happen. When it comes to CRC, dysbiosis is a key part of how the disease starts [20, 21].

The microbiota of the gut in a state of disordered digestion can be increased by pathogenic microorganisms such as *Fusobacterium nucleatum*, *Escherichia coli*, and *Bacteroides fragilis*. *Fusobacterium nucleatum* is the most prominent concern with CRC due to its capacity to bind to and infiltrate epithelial and colorectal cells through FadA adhesin that is bound to the E-cadherin protein on the cell's surface. Contact with the cell triggers the signaling pathway b-catenin, stimulating cell proliferation and preventing the process of apoptosis [22]. *F. nucleatum* also creates an inflammatory environment by encouraging the release of inflammatory cytokines, which help the tumor grow and hide from the immune system. Enterotoxins like *B. fragilis* toxin (BFT) are made by other bacteria, like *Bacteroides fragilis*. These can damage the DNA of the host cells immediately, which can cause changes and help cancer grow. These bacteria damage the intestinal barrier and allow for the translocation of bacterial products such as lipopolysaccharides (LPSs) into the systemic circulation, prolonging chronic immunological activation and chronic inflammation, a cancer risk factor [23].

Gut microbiota dysbiosis, the imbalance in the microbial communities within the GIT, can also be attributed to several etiological factors, each contributing uniquely to this disruption (Table 3.1). All of these factors, including antibiotic use, dietary practices, environmental toxins, chronic stress, and infections, play a part in influencing the composition and function of the gut microbiota. The usage of antibiotics has a suggestive influence on dysbiosis. Antibiotics, although they are successful in eradicating harmful bacteria, also completely eradicate helpful gut bacteria, resulting in a reduction in the variety of microorganisms and an increase in the proliferation of resistant harmful strains [24, 25]. According to the research by Palreja et al. (2018), it has been proven that the gut microbiota composition can undergo long-term changes even after a brief course of antibiotics. Certain species may not show any improvement even 6 months after the treatment. This loss of variety can weaken the defense system of the gut, making it easier for pathogens to colonize and causing inflammation that can make people more likely to get colorectal cancer [26].

The way we eat also has a significant impact on the microbiota in our digestive tract. Dysbiosis can develop when the body's microbiome is imbalanced due to a diet heavy in processed foods and sugar and poor in fibers. The gut microbiota of children in rural Africa who ate a high-fiber diet and those of children in Europe who ate a Western diet were compared in a study [27]. The study found that African children have more good bacteria like *Prevotella*, which aids fiber digestion, in their gut microbiome [27]. Bacteroides, connected to a diet high in animal protein and fats, dominated the gut microbiota of European children, causing dysbiosis. Environmental contaminants, including pesticides, heavy metals, and pollutants, are also substantial aspects of dysbiosis. These substances can directly alter the gut microbiota by selecting resistant strains of bacteria while inhibiting the growth of beneficial microbes. In an animal model study, Claus et al. (2016) discovered that the pesticide chlorpyrifos drastically changed the gut microbiota by boosting the abundance of

Table 3.1 Etiological factors contributing to the pathogenesis of gut microbiota dysbiosis

Etiological factor	Type of gut microbe affected	Mechanism	Concerning pathology	Scientific evidence	Reference with DOI
Antibiotic use	<i>Bifidobacterium</i> , <i>Lactobacillus</i>	Broad-spectrum antibiotics reduce microbial diversity and promote resistant pathogens	<i>Clostridium difficile</i> infection (CDI), colorectal cancer (CRC)	The long-term use of antibiotics can significantly impact the gut microbiota, leading to dysbiosis, a condition characterized by an imbalance in the microbial community. This dysbiosis can weaken the gut's natural defenses, making the body more susceptible to infections and potentially increasing the risk of developing colorectal cancer (CRC).	[31]
High-fat diet	<i>Bacteroides</i> , <i>Firmicutes</i>	Diet-induced dysbiosis leads to an increase in pro-inflammatory microbes while decreasing the abundance of beneficial bacteria	Obesity, metabolic syndrome, CRC	High-fat diet shifts gut microbiota composition toward inflammation-promoting species, increasing CRC risk	[32]
Environmental toxins	<i>Proteobacteria</i> , <i>Firmicutes</i>	Pesticides and heavy metals disrupt gut microbiota balance by promoting resistant strains and inhibiting beneficial microbes	Inflammatory bowel disease (IBD), CRC	Chlorpyrifos pesticide exposure linked to altered gut microbiota and increased inflammatory markers	[33]
Chronic stress	<i>Lactobacillus</i> , <i>Bifidobacterium</i>	Stress-induced HPA axis activation increases cortisol, altering gut motility and permeability	Depression, anxiety, IBD, CRC	Stress leads to a reduction in beneficial bacteria, increased gut permeability, and inflammation	[34]
Poor diet (low fiber)	<i>Prevotella</i> , <i>Bacteroides</i>	Low-fiber diet reduces beneficial bacterial species, affecting gut barrier function	Constipation, CRC	A low-fiber diet is linked to reduced microbial diversity and increased colon cancer risk	[35]
Infections (e.g., <i>Clostridium difficile</i>)	<i>Clostridium difficile</i> , <i>Bacteroides</i>	Infection causes an imbalance in gut microbiota, leading to overgrowth of pathogenic bacteria and toxins	<i>Clostridium difficile</i> infection, CRC	<i>C. difficile</i> infection leads to severe dysbiosis, promoting chronic inflammation and CRC progression	[36]
Smoking	<i>Proteobacteria</i> , <i>Firmicutes</i>	Smoking alters gut microbiota composition, increasing pro-inflammatory species	IBD, CRC	Smoking is associated with increased <i>Proteobacteria</i> and inflammation, contributing to IBD and CRC	[37]
Alcohol consumption	<i>Bacteroides</i> , <i>Lactobacillus</i>	Alcohol disrupts gut barrier integrity and promotes the growth of harmful bacteria	Liver disease, CRC	Chronic alcohol intake increases gut permeability, allowing the translocation of endotoxins that promote liver disease and CRC	[38]

Proteobacteria and decreasing the variety of other microbes. *Proteobacteria* is a phylum that is commonly linked to inflammation and dysbiosis [28].

Dysbiosis may also result from chronic stress. The hypothalamic–pituitary–adrenal (HPA) axis is altered by stress, resulting in the release of cortisol. Cortisol can affect intestinal motility and permeability, leading to changes in the microbiota of your gut. The researchers found that stress in mice caused significant changes to the gut microbiota. These changes were characterized by a decrease in positive *Lactobacillus* species as well as a rise in pathogenic bacteria and a rise in intestinal permeability as well as inflammation. Additionally, infections can cause dysbiosis through the delicate alteration of intestinal bacteria [29]. Severe dysbiosis is induced by antibiotic use, which provides the environment for the pathogenic bacteria *Clostridium difficile* to overgrow. Buffie et al. (2015) emphasized how infections, such as *C. difficile*, can lead to an extensive drop in the diversity of gut bacteria. This condition of reduction in the diversity of gut microbiota not only augments the risk of CRC but also invites other pathogenic infections to occur. Antibiotics, food, environmental pollutants, chronic stress, and infections all contribute to gut microbiota dysbiosis [30]. All these variables play a role in disturbing the intricate harmony of the gut microbiome, which can result in various health issues, such as the development of CRC.

3.2.3 Interaction Between Microbiota and Host in Cancer Development

The interaction between gut microbiota and the host plays a crucial role in the development and progression of cancer. Increasing evidence highlights the significant impact of this interplay, particularly in colorectal cancer (CRC). Immune modulation is among the main ways that the microbiome of your gut influences cancer development. Researchers have discovered certain bacteria, such as *Fusobacterium nucleatum*, that are key actors in promoting the development of tumors in colorectal cancer. If *F. nucleatum* interacts with E-cadherin in colorectal epithelial cells, it activates the signaling pathway b-catenin and interacts with the host. The activation helps in the development of tumors by stimulating the growth and longevity of cancerous cells. *F. nucleatum* does not just hinder T cells as well as natural killer (NK) cells from carrying out their work, which is essential for the body's immune defense against cancer, but it also produces myeloid-derived suppressor cells (MDSCs) that make the surrounding environment less immune-friendly [39]. Moreover, dysbiosis can exacerbate inflammation, a well-established risk factor for cancer. For instance, *Bacteroides fragilis* produces a toxin called *B. fragilis* toxin (BFT), which induces DNA damage and promotes chronic inflammation in the gut mucosa. The incidence of inflammation not only enables the onset of tumor formation but also accelerates the progression of preexisting tumors. Chronic inflammation results in the generation of pro-inflammatory cytokines, including Interleukin 6 (IL-6) and Tumor necrosis factor-alpha (TNF- α), which can encourage the survival and growth of cancerous cells [40].

The gut microbiome can also alter the metabolism of the patient by affecting the progression of cancer. Microbiome metabolites, including SCFAs and bile acids that are secondary such as propionate and butyrate, are crucial for regulating the growth of cells, their death, and immune reactions. In particular, the bile acids generated by gut microbes can stimulate nuclear receptors such as the farnesoid-X receptor (FXR) as well as the G-protein-coupled bile acid receptor Takeda G protein-coupled receptor 5 (TGR5) and affect gene expression associated with cell growth and the functioning of the immune system. A lack of balance in these pathways due to the microbial population that is not healthy could lead to the development of an environment conducive to cancer, particularly within the colon. In addition, the gut microbiota can influence the efficacy of treatments for cancer [41, 42]. Studies have proven that the structure of the microbiome in your gut influences the way that patients respond to immunotherapies, for example, checkpoint inhibitors. For instance, certain bacterial species like *Bifidobacterium* have been associated with enhanced responses to PD-1 blockade in cancer treatment, while others, like *Akkermansia muciniphila*, have been associated with enhanced consequences in immunotherapy-treated patients. The research finding suggests that modifying the gut microbiota may be an option to increase the effectiveness of chemotherapy for cancer [43, 44].

The relationship between gut microbiota and the host has also been shown to be an important part of the development of GIT cancers, with specific bacteria driving tumor growth through several different molecular mechanisms. For instance, researchers have extensively studied the role of *Helicobacter pylori* in gastric cancer. Takahashi-Kanemitsu and his team conducted a study in 2020 that revealed that the CagA protein in *H. pylori* can inhibit the signaling pathways of gastric epithelial cells. This intrusion eventually results in chronic inflammation due to the elevated levels of IL-8 and stimulation of the NF- κ B pathway. This eventually encourages the development of the tumor [45]. In a 2024 study, Sadeghi and colleagues highlighted the role of *Escherichia coli* (pks+ strain) in CRC. They explored that the production of colibactin caused DNA double-strand breaks, which, in turn, led to chromosomal instability and mutagenesis through the Wnt/catenin signaling pathway [46]. Furthermore, researchers have broadly studied the role of *Enterococcus faecalis* in colon cancer. Huycke and Gaskin's 2019 study revealed that the bacterium's production of extracellular superoxide leads to oxidative DNA damage. This impairment triggers the DNA damage response (DDR) pathway, eventually leading to mutagenesis and the development of cancer. These studies also emphasize the significant role of gut microbiota dysbiosis in cancer expansion and highlight the possibility of microbiota-targeted therapies in cancer prevention and treatment [47].

3.3 What Are Postbiotics?

Elie Metchnikoff first recognized the positive effects of gut microbiota as early as the twentieth century. He laid the basis for the postbiotic era. There was a need for more secure and more reliable alternatives that could offer the same health benefits

but without the risk of live bacterial treatments, especially those with immunocompromised patients, prompting the shift from probiotics toward postbiotics [48]. Presently, postbiotics are viewed as an exciting alternative to probiotics. They offer stability and efficacy in a variety of therapies without the issues that are associated with live bacteria. In 2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) officially recognized the term “postbiotics,” which refers to living microbes, or their constituents, which give benefits to the health of the person taking them. This term covers a vast spectrum of bioactive substances, which include SCFAs like acetate, butyrate, and propionate and cell wall fragments of bacterial cells. These compounds are normally derived from probiotics because of their bodily process during the anaerobic digestion of food fibers as well as other substances [49]. Postbiotics are considered viable and safe over time because they do not pose the risk of infections, such as bacteremia, or any other infections associated with living microorganisms. Postbiotics’ therapeutic uses can be extensive and diverse. They have proven promising for the management and treatment of a variety of chronic illnesses because of their immunomodulatory antioxidant, anti-inflammatory, and anticancer effects. Butyrate, which is a form of short-chain fatty acids (SCFAs), has significant importance in the field of cancer research. The microbiota in the gut produces butyrate, which is a chemical that causes the death of cells programmed to die within cancerous colorectal cells. It also lowers inflammation and increases the quality of the intestinal barrier. It is believed that butyrate may serve as a beneficial supplementary treatment for colorectal cancer [50]. Postbiotics have also been studied by researchers for potential advantages in treating IBD, diabetes, hypertension, allergic reactions, and neurodegenerative disorders such as overweight, skin health, respiratory infections, and other autoimmune diseases, as well as cancer. The dysbiosis associated with CRC can be prevented due to the change in the microbiota of the gut and enhancement of the function of the gut barrier. In addition, by influencing gene expression as well as altering and enhancing immune responses, postbiotics like butyrate can provide an effective, noninvasive alternative to conventional treatments for cancer [51]. The growing data about the safety of postbiotics as well as their efficiency suggests that they could help in the treatment of the case of colorectal cancer. Gut health is a factor in cancer development as well as treatment results.

3.3.1 Types and Classification of Postbiotics

Recently, the International Scientific Association for Probiotics and Prebiotics (ISAPP) classified probiotics based on their health benefits and bioactivity. This categorized approach not only emphasizes the wide range of postbiotics but also their numerous roles in preserving and improving health [49]. The fermentation of dietary fibers by species of *Lactobacillus* and *Bifidobacterium* mainly produces short-chain fatty acids (SCFAs) like butyrate and propionate. These SCFAs are

crucial for gastrointestinal health and the prevention of colorectal cancer. To strengthen immune responses and create biofilms that defend against infections, bacteria like *Lactobacillus plantarum* and *Lactobacillus rhamnosus* release exopolysaccharides (EPSs). Teichoic acids, found in the cell walls of *Lactobacillus rhamnosus*, interact with immune receptors to reduce inflammation, while peptidoglycans from *Bifidobacterium* species act as potent immunomodulators, enhancing vaccine efficacy [51]. Certain compounds, such as nisin and reuterin, which are derived from *Lactococcus lactis* and *Lactobacillus reuteri*, respectively, possess potent antimicrobial properties. As a result, they have significant applications in the realms of food preservation and infection control. The cell-free supernatants (CFSs) of *Lactobacillus casei* contain a combination of organic acids and enzymes that are designed to help regulate digestive issues. Bone and cardiovascular health depend critically on vitamins, especially vitamin K2 from *Bacillus subtilis*. By killing dangerous bacteria, lactic acid from *Lactobacillus acidophilus* preserves gut and vaginal integrity. With uses in both food safety and environmental bioremediation, diacetyl generated by *Lactococcus lactis* and biosurfactants such as surfactin from *Bacillus subtilis* have antibacterial and biofilm-disrupting action [52, 53].

The prevention of infections is a critical aspect of vaginal health, which is facilitated by hydrogen peroxide produced by *Lactobacillus* spp. The potential of neurotransmitters such as gamma-aminobutyric acid (GABA), which are produced by *Lactobacillus brevis*, in regulating stress and anxiety is being investigated, underscoring the gut–brain axis [54]. Sphingolipids from *Bacteroides* spp. have the potential to be used therapeutically in neurodegenerative diseases and contribute to the integrity of cell membranes. Gut bacteria make urolithin A from ellagic acid, and it has been shown to activate mitophagy, which has antiaging properties. Conjugated linoleic acid (CLA) derived from *Lactobacillus reuteri* possesses remarkable properties that combat cancer and inflammation. This makes it a promising option for addressing obesity and improving metabolic health. Collectively, these postbiotics illustrate the profound impact of microbial metabolites on health, with applications ranging from gut health to cancer prevention and beyond [55, 56]. Since they can change the microbiome and help with a wide range of health problems, they form an important area of ongoing study in the health sciences. The uses of postbiotics as medicine are very broad and include a lot of different illnesses, mostly cancer. The inference of butyrate in CRC is broadly recognized, as it stimulates the differentiation of cells in the colon's epithelium, suppresses cell growth, and activates apoptosis. Recent research proposes that postbiotics may have advantageous effects on numerous forms of cancer, like breast, stomach, and liver malignancies. For example, propionate has been revealed to prevent the growth of breast cancer cells by ceasing the cell cycle by promoting apoptosis [57]. Bacteriocins, such as nisin, have been found to kill stomach cancer cells by causing membrane damage and commencing the cell death process. The fact that postbiotics can alter the gut microbiota, decrease inflammation, and boost immune responses lends credence to their promise as a cancer treatment [58].

3.3.2 Mechanisms of Action of Postbiotics

Modes of action of postbiotics are closely entwined with cellular and molecular pathways, thereby indirectly altering physiological processes like cancer treatment and immunological regulation (Table 3.2). At the cellular level, postbiotics like SCFAs such as propionate, acetate, and butyrate play an important role. SCFAs, which are produced by gut microbiota such as *Bifidobacterium* and *Lactobacillus* when they ferment food fibers, have a substantial impact on host cells. Butyrate, for example, inhibits histone deacetylases (HDACs), leading to hyperacetylation of histones and the activation of tumor suppressor genes like p21. Apoptosis is induced by this process, which protects the barrier of the gut and reduces the inflammation in the CRC. On a molecular level, postbiotics engage with various receptors and signaling pathways to exert their effects [59, 60].

Postbiotics, like butyrate, have an impact on the signaling pathway NF- κ B, which is the key controller of inflammatory reactions. Butyrate blocks NF- κ B activity by blocking the phosphorylation and degrading of I κ B α . The result is a decrease in the pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6. This is accomplished through the inhibition of these cytokines those are responsible for inflammation in CRC [61]. Postbiotics can also trigger G-protein-coupled receptors (GPCRs) such as GPR41 and GPR43. These are then stimulated by short-chain fatty acids (SCFAs) like propionate. Propionate-induced stimulation of the receptors led to the inhibition of the mitogen-activated protein kinase (MAPK) pathway, primarily in reducing the phosphorylation levels of ERK1/2 as well as p38 MAPK, which was demonstrated in a study with an animal model of IBD (IBD). This resulted in a decrease in inflammation through the downregulation of inflammatory cytokines as well as chemical mediators, resulting in improved stability of the intestinal barrier. Propionate's potential as a therapeutic agent in reducing inflammatory bowel disease (IBD) was established in the study that showed a decrease in the amount of infiltrating the inflammatory cells as well as improved gut well-being [62].

The bonding of postbiotics such as peptidoglycans and lipoteichoic acids with toll-like receptors (TLRs) on immune cells forms another important molecular mechanism. Embedded in bacterial cell walls, these elements activate the MyD88-dependent pathway, generating IL-12 and consequent Th1 cell differentiation—processes essential for antitumor immunity [63]. In gastric cancer models, treatment with these postbiotics resulted in reduced tumor growth and enhanced immune cell infiltration into the tumor microenvironment, demonstrating their potential in immunotherapy for gastric cancer. Postbiotics also play a significant role in enhancing gut barrier function through the upregulation of tight junction proteins like claudins, occludins, and zonula occludens-1 (ZO-1). When it comes to colorectal cancer, this function is crucial because a good gut barrier stops toxins and harmful bacteria from entering the body, which could cause inflammation and cancer [64].

Lower levels of the circulating endotoxins and pro-inflammatory cytokines evidenced the reduction in systemic inflammation and reduced gut permeability

Table 3.2 Summary of postbiotics, their sources, mechanisms of action, and therapeutic outcomes in cancer pathologies

Type of postbiotic	Source name	Exact composition	Targeted cancer pathology	Mechanism of action	Therapeutic outcome	References
Butyrate	<i>Bifidobacterium</i> , <i>Lactobacillus</i>	Short-chain fatty acid (SCFA)	Colorectal cancer (CRC)	Inhibits HDACs, activates tumor suppressor genes, suppresses NF-κB pathway	Induces apoptosis, reduces cell proliferation	[67]
Propionate	<i>Bacteroides fragilis</i>	SCFA	Inflammatory bowel disease	Activates GPR41/43, suppresses MAPK pathway	Reduces inflammation, improves gut barrier integrity	[68]
Peptidoglycan	<i>Lactobacillus rhamnosus</i>	Bacterial cell wall component	Gastric cancer	Activates TLRs, stimulates IL-12 production, enhances Th1 differentiation	Reduces tumor growth, increases immune cell infiltration	[69]
Lipoteichoic acid	<i>Staphylococcus aureus</i>	Bacterial cell wall teichoic acids	Gastric cancer	Activates TLR2, MyD88-dependent pathway	Reduces tumor progression, enhances antitumor immunity	[70]
Exopolysaccharides	<i>Lactobacillus helveticus</i>	Extracellular polysaccharides	Colorectal cancer (CRC)	Upregulates tight junction proteins (claudins, occludins, ZO-1)	Enhances gut barrier, delays CRC progression, reduces metastasis	[71]
Nisin	<i>Lactococcus lactis</i>	Bacteriocin	Head and neck cancer	Disrupts cell membrane integrity, induces oxidative stress	Inhibits tumor growth, promotes apoptosis	[72]
Lactate	<i>Lactobacillus reuteri</i>	Organic acid (lactic acid)	Cervical cancer	Inhibits cell proliferation, induces p53-dependent apoptosis	Reduces tumor size, induces apoptosis	[73]
Teichoic acid	<i>Staphylococcus aureus</i>	Bacterial cell wall teichoic acids	Melanoma	Activates TLR2, enhances NK cell activity	Suppresses tumor growth, enhances immune response	[74]
Reuterin	<i>Lactobacillus reuteri</i>	Glycerol-derived antimicrobial compound	Pancreatic cancer	Induces oxidative stress, disrupts redox balance	Inhibits tumor cell proliferation, promotes cell death	[75]
Valeric acid	<i>Clostridium difficile</i>	SCFA	Colorectal cancer (CRC)	Inhibits HDACs, modulates Wnt signaling pathway	Promotes differentiation, reduces tumorigenicity	[76]

demonstrated by a study on exopolysaccharides (EPSs) from *Lactobacillus helveticus*. Metastasis was reduced and CRC progression was delayed by the EPS treatment, proving it to be a potential therapy for cancer prevention [65]. Among other complex and diverse methods, postbiotics enhance intestinal barrier function, interact with immunological receptors such as TLRs, and modulate critical signaling pathways such as NF- κ B and MAPK. These mechanisms collectively contribute to the therapeutic potential of postbiotics in preventing and treating various diseases, particularly cancers like colorectal and gastric cancer. Postbiotics are expected to be a potential weapon against cancer treatment and prevention by affecting these pathways along with microbiome regulation [66].

3.4 Molecular Mechanisms of Postbiotics in Colorectal Cancer

Postbiotics possess anti-inflammatory, immune-modulatory, antioxidant, antiproliferative, and proapoptotic activities, which contribute to their anticancer properties in colorectal cancer. Their actions modulate the composition of the gut microbiota, influencing signaling pathways, immune cells, and oxidative stress levels. Additionally, they promote apoptosis to enhance survival, offering therapeutic potential for treating colorectal cancer patients by inducing apoptosis (Fig. 3.1) [77].

3.4.1 Anti-inflammatory Pathways

Postbiotics play an essential part in treating colorectal cancer (CRC). The anti-inflammatory properties of these supplements stem from their alteration of the microbiota of the gut and the function of our immune system through the creation of healthier populations and the elimination of pathogens from the gut and surrounding. They may reduce inflammation by creating an environment that supports greater gut health. Short-chain fatty acids (SCFAs), specifically propionate and butyrate, contain anti-inflammatory properties that help control chronic inflammation in the gut by binding with GPCR41/43 receptors to promote intracellular signaling pathways such as PLC/MAPK that reduce inflammation while simultaneously decreasing tumor cell proliferation [78]. Control by these channels is crucial in maintaining intestinal homeostasis as well as decreasing the chances of CRC by mitigating chronic inflammation. Postbiotics contain metabolites with antioxidant effects that help alleviate oxidative stress in the intestinal mucosa and therefore help decrease inflammation. They do this by neutralizing reactive oxygen species (ROS) that trigger inflammatory responses or DNA damage that leads to cancerous cell formation if left uncontrolled, leading to further carcinogenesis if unchecked. Overall, postbiotic modulation of gut microbiota composition and action on GPCR-mediated pathways as well as direct antioxidant properties make up key

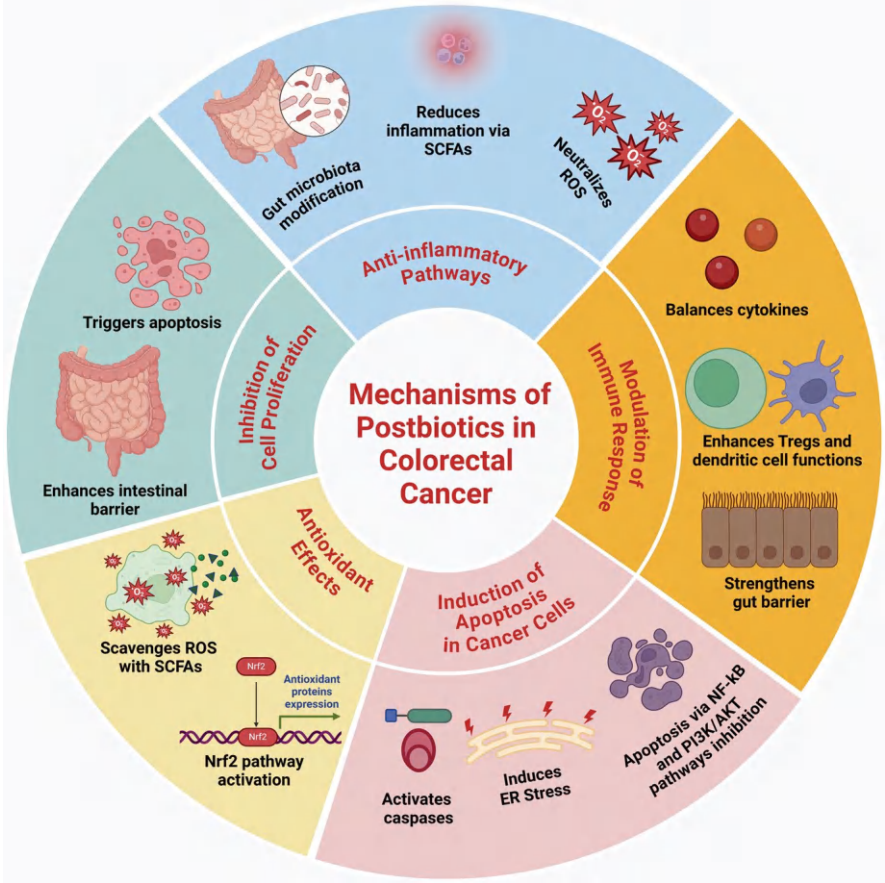


Fig. 3.1 Molecular mechanisms of postbiotics in colorectal cancer

anti-inflammatory mechanisms contributing to their therapeutic value in colorectal cancer treatment [79].

3.4.2 Modulation of Immune Response

The regulation of the immune system via postbiotics in the treatment of colorectal cancer (CRC) is a result of several important molecular mechanisms that contribute to the anticancer properties of these compounds. The effects of postbiotics could affect the production of cytokines. They constitute the key mediators in the immune system [78]. They regulate anti-inflammatory and pro-inflammatory cytokines. This helps reduce chronic inflammation, which can aid in the progress of CRC. Postbiotics such as short-chain fatty acids (SCFAs) benefit by increasing the creation of

anti-inflammatory cytokines such as TGF- β and IL-10. The payoff is a reduction of the inflammatory pathways that could cause tumors. Postbiotics can also decrease pro-inflammatory cytokines like TNF- α , INF- γ , and IL-1 β [80]. The decrease in pro-inflammatory signals can help reduce the chronic inflammatory conditions that are conducive to the development of CRC [76]. Postbiotics may alter the functions of many immune cells and boost antitumor immunity. Postbiotics may improve the function and differentiation of the T regulator cells (Tregs) that keep immune tolerance intact and limit the overactive immune response, which can cause inflammation. Dendritic cells (DCs) are the cells that play a crucial role in presenting antigens as well as initiating adaptive immune responses. Postbiotics can increase the development and function of dendritic cells. This can enhance the body's capability to fight cancerous cells. A short-chain fatty acid and other postbiotic substances can boost natural killer (NK) cell activation, resulting in greater recognition and elimination of tumor cells [77].

Postbiotics help maintain and repair the intestinal walls, which are crucial in ensuring that your immune system is operating correctly. The effects of postbiotics raise the levels of proteins that form tight junctions like claudin and occludin that help strengthen the gut barrier. They also block the spread of microbes and pollutants that may trigger an immune response that can cause inflammation. They also stimulate mucin production and create an outer layer of protection for the epithelium of the gut. It improves the functioning of the barrier. In addition, by promoting the growth of beneficial bacteria as well as suppressing the harmful ones, postbiotics can decrease the impact of antigens within the immune system. It can result in a healthy immune system that is less likely to result in inflammation and CRC [81].

Certain postbiotics can influence epigenetic pathways, such as DNA methylation as well as histone modification, that, in turn, influence gene expression implicated in inflammation and immune response. Postbiotics may trigger the process of apoptosis (programmed death of cells) on cancerous cells by mechanisms that involve caspases as well as mitochondrial membrane disruption that, in turn, may decrease the burden of tumors and alter the immune response to CRC [23]. Modulation of the immune system through postbiotics for colorectal cancer is an interdisciplinary strategy that involves the control of cytokine production, improvement of the immune system's function, improvement of gut barrier function, and inhibition of pathogenic microbes, as well as epigenetic control [23]. Together, these processes can be used for therapeutic effects in the treatment and prevention of CRC.

3.4.3 Antioxidant Effects

Postbiotics have a variety of antioxidants that are potent and help boost their anti-cancer effects in the case of colorectal cancer (CRC). The principal molecular cause of these benefits of antioxidants is specifically neutralizing and scavenging reactive oxygen species (ROS) that include superoxide anions, hydrogen peroxide, and the hydroxyl radicals caused by chemicals like short-chain fatty acids as well as

polyphenols [79]. Postbiotics raise the activity and expression of antioxidant enzymes produced by the body, including catalase, superoxide dismutase, and glutathione peroxidase. These are all essential in the detoxification of ROS. Furthermore, postbiotics are able to enhance the signaling pathway Nrf2 that acts as a key controller of the expression levels of antioxidant and cytoprotective genes that add protection from the damaging adverse effects of oxidative stress. In addition, by reducing the amount of ROS as well as strengthening antioxidant defenses, postbiotics may inhibit the transcription factors that are sensitive to redox like NF- κ B, which is involved in the creation of pro-inflammatory cytokines. This results in a stoppage of the cycle of inflammation, stress oxidation, and carcinogenesis in the normal environment that causes colorectal cancer. Postbiotics protect DNA from damage caused by oxidation. They could influence epigenetic functions, such as histone acetylation and DNA methylation that regulate how genes are released. They are involved in the antioxidant defense process and in controlling the cycle of cells [87]. The multifaceted postbiotic antioxidant properties are beneficial to keeping a healthy level of redox. They also reduce the pressure of oxidative aging and cancerous and inflammation-related conditions within the colon. These postbiotics also could be a factor in the treatment of colorectal cancer.

3.4.4 Inhibition of Cell Proliferation

The molecular mechanisms through which postbiotics reduce the proliferation of cancer cells in colorectal cancer are multifaceted. Postbiotics exert antiproliferative actions by a myriad of mechanisms, most notably those that involve the regulation of the gut microbiota as well as immunity system functions. A key aspect is their capability to reduce inflammation by reducing the activity of NF- κ B, vital to the progression of cancers such as colorectal cancer. This inhibition is a result of the inactivation of nuclear translocation by NF- κ B by preventing I κ B phosphorylation as well as its consequent proteasomal degradation [82].

Furthermore, postbiotics could trigger the process of apoptosis (programmed death of cells) in cancer cells. This further reduces the proliferation of cancer cells. The apoptotic effects are mediated through the activation of several ways to kill cells and the blocking of the antiapoptotic signaling within cancerous cells. Furthermore, postbiotics raise the health of the intestinal barrier, thus reducing exposure of the cells of the intestinal tract to carcinogenic substances and thereby reducing the risk of cancer growth. In addition, postbiotics have antioxidants that decrease oxidative stress, which is an essential aspect of the proliferation of cancer cells. In the aggregate, these properties highlight the benefits of postbiotics in the prevention and treatment of colorectal cancer because they are energetically stopping cancer cell growth using a broad-spectrum approach to biology [6, 83].

3.4.5 Induction of Apoptosis in Cancer Cells

Postbiotics have potent antiproliferative properties against cancerous cells of the colorectal tract through different molecular mechanisms, which primarily cause apoptosis. Postbiotics can activate caspases, which are the primary executioners of apoptosis. This is done through both the extrinsic (death receptor) and the intrinsic (mitochondrial) pathways. They can also trigger mitochondrial dysfunction that results in the generation of antiapoptotic compounds and the activation of mitochondrial pathways [76]. Additionally, postbiotics can cause endoplasmic-reticulum-related stress to enhance the creation of death receptors and generate reactive oxygen species, each of which is involved in the apoptosis induction inside cancer cells. They also inhibit death signaling pathways including NF- κ B and the PI3K/AKT pathways, which trigger DNA damage response, ultimately stimulating apoptosis through the p53 gene. All in all, the many procedures demonstrate the capability that postbiotics have to end the proliferation of cancerous cells within the colon, highlighting the possibilities for these substances as therapies for treating this disease [84].

3.5 Postbiotics and Gut Barrier Function

Postbiotics increase the health of the gut barrier in several ways: increasing the tight protein junctions, promoting mucus production, and thereby affecting the microbiome of your gut, as well as regulating the signals. The result is a stronger biological and functional barrier blocking the entrance of pathogens and carcinogens, which reduces the chance of developing colorectal cancer [85].

3.5.1 Enhancement of Epithelial Barrier Integrity

Postbiotics play a vital role in the strengthening of the intestinal epithelial barrier. This is essential to ensure the health of gut homeostasis as well as stop the development of cancers, such as colorectal cancer (CRC). One of the main ways through which postbiotics increase the barrier to gut health is by enhancing the production of proteins that comprise tight junctions. These are specialized proteins that seal the epithelial space that divides adjacent cells and create a physical barrier to stop the movement of potentially hazardous pathogens, agents, and inflammation-related mediators through the intestine lumen and then into the surrounding tissue [81]. Postbiotics, including short-chain fatty acids (SCFAs), along with a variety of components of bacteria have been proven to improve the amount and quantity of proteins that are associated with tight junctions. This includes claudins and occludins as well as the zonula occludens-1 (ZO-1). The strengthening of the tight junctions

helps increase overall health, and it improves the permeability of the intestinal barrier. Additionally, it helps block the circulation of harmful substances along with microbes, which can trigger inflammation that can trigger CRC development [86, 87].

Postbiotics raise the production of glycoproteins by mucin that create the mucus layer on the inside epithelium's outer surface. Mucus acts as a physical and chemical barrier that blocks harmful substances and pathogens, in addition to other hazardous chemicals, prior to their entry into epithelial cells. Postbiotics, particularly short-chain fatty acids (SCFAs), have been shown to increase the expression of mucin genes, such as MUC2 and MUC2Aq29, leading to the enhancement of the mucus layer and an improvement in gut barrier function [88].

Additionally, postbiotics can alter the structure and character of microbiota in the gut that is directly connected to the preservation of the intestinal barrier. Furthermore, by facilitating the growth of beneficial commensal bacteria and slowing the growth of pathogenic strains, we can keep a stable and healthy microbiome in the gut. This helps strengthen the structure and overall function of the intestinal barrier as having a healthy microbiome within the gut is essential for proper development and function of the epithelium within the intestinal tract [89]. The pathways that these can follow consist of the rise in tight junction proteins, stimulation of the production of mucin, and modulation of the gut microbiota. Postbiotics play an important role in enhancing the strength in the epithelial layer of the intestine. This improvement in barrier function can be crucial in limiting the spread of harmful chemicals, reducing inflammation, and in turn, reducing the likelihood of developing cancerous colorectal growth [90].

3.5.2 Prevention of Pathogen Invasion

Postbiotics play an essential part in maintaining the health of the intestinal barrier. It is crucial to prevent the invasion of carcinogens and pathogens, which can lead to the development of various cancers like colorectal cancer. Postbiotics enhance the connections between epithelial cells in the intestine by improving the effectiveness of their barrier function. This process stimulates the production of mucus, which serves as a protective layer, while also creating a physical barrier against harmful substances. They also have antimicrobial benefits that help maintain an optimal gut microbiome. They regulate the immune system by enhancing the lymphoid tissues that line the intestine; they also reduce inflammation pathways that could cause disruption to the barrier between the gut and the stomach, as well as encourage the development and expansion of epithelial cell lines. They additionally assure the continuous renewal of the linings of the intestinal tract. Additionally, they improve the intestinal barrier with their diverse strategies. They effectively block the entry of pathogens and carcinogens. They also aid in the fight against colon cancer [91].

3.5.3 Regulation of Tight Junction Proteins

Postbiotics play a crucial role in controlling the localization, expression, and activity of proteins that form tight junctions that are essential elements in the intestinal barrier. Postbiotics trigger the improvement of important tight junction proteins like occludin, claudins, and ZO proteins in the intestinal epithelial cells, thereby strengthening the barrier that physically separates cells and reducing intestinal permeability. They also increase the distribution and stability of these tight junction proteins in the apical part of the epithelium. This ensures that they are properly assembled and functioning in the complex [92]. In addition, postbiotics regulate the pathways of signaling involved in the regulation of tight junctions to prevent disruptions of tight junctions due to different factors like inflammatory cytokines and stress oxidation. Additionally, they improve cross-linking and interaction between different tight junction proteins. They enhance the overall quality and elasticity of the intestinal barrier. By utilizing the various mechanisms that control the dynamics that occur within tight junctions, postbiotics perform crucial roles in preserving the intestinal barrier. It aids in the prevention of the introduction of carcinogens and pathogens that can cause the development of cancers, such as colorectal cancer [90].

3.6 Postbiotics Modify Epigenetics

Epigenetics as a biological process is considered a major one that alters the genes' activity without changing the DNA sequence. In recent years, there has been much interest in postbiotic regulation of gene expression through epigenetic changes; however, how these microbial metabolites produced by gut microbiota influence host gene expression is relatively undocumented [93]. These mechanisms are involved in several biological processes, such as inflammation, immune response, metabolism, and behavior, but none of them alters the actual DNA code. They do not change the genes but alter how they act or function. As a deep dive into postbiotics, this study will focus on how postbiotics cause changes at the epigenetic levels, including histone modification, DNA methylation, and noncoding RNA. Each of these mechanisms plays a crucial role in regulating gene expression, and a detailed analysis of their complexities is outlined in the following sections [94].

3.6.1 Histone Modifications by Postbiotics

DNA is coiled around histones, which are structural, to produce nucleosomes. Postbiotics can modify histones by adding acetyl, methyl, phosphate, or ubiquitin groups, thereby altering chromatin accessibility and conformation. These modifications influence gene expression, histone acetylation, and the inhibition of histone deacetylases (HDACs) [95].

Mechanism Histone acetylation usually speeds up transcriptional activation because it lowers the positive charge of histones. This means that they have less attraction to the negative nucleic acid DNA, which means that chromatin is less crowded. The other target of postbiotic molecules are histone deacetylases (HDACs) and short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate. This action is carried out by the HDAC, which reduces the acetyl groups on the histone tail, and this condenses chromatin, thus repressing gene expression. Short-chain fatty acids (SCFAs) enhance histone acetylation by inhibiting histone deacetylases (HDACs), thereby promoting the expression of genes involved in the cell cycle, apoptosis, and anti-inflammatory responses [96].

Scholarly Evidence Investigations show that butyrate suppressed the activity of HDACs; thus, histones H3 and H4 became over-acetylated at specific promoters of genes such as p21, which is a cyclin-dependent kinase inhibitor, and FOXO3, which is a gene that responds to oxidative stress. As has been shown, this process promotes apoptosis and differentiation of colorectal cancer cells, and because of this, butyrate is considered a chemopreventive agent [97].

3.6.2 *Histone Methylation*

Mechanism On the other hand, when it comes to histone methylation, it has been discovered that what methylates the histone is the degree of methylation or mono, di, or tri-methylation and at which amino acid residue the methylation is to activate or repress transcription. The postbiotics anticipated in this perspective reportedly have the possibility of altering the histone methylation patterns by modulating the enzymatic actions of histone methyltransferases (HMTs) and histone demethylases (HDMs). For example, butyrate changes the pattern of the enzymes and participates in the histone methylation status and therefore can be stated to regulate it indirectly [98].

Scholarly Evidence In experimental conditions, exposure of cells to butyrate leads to changes in the histone methylation pattern of cells and has given rise to theories of H3K4me3 for active genes and H3K27me3 for inactive genes. Due to the duality of regulation, butyrate may specifically induce genes related to immune responses without compromising the layer of epithelial cells [99].

3.6.3 *DNA Methylation by Postbiotics*

Among all the types of epigenetic modifications that are linked to the repression of the gene transcription process, one is DNA methylation, which takes place in most cases at the CpG dimer within the promoter region of a gene. Postbiotics either

increase or decrease the expression of DNA methyltransferases (DNMTs) or regulate the access of methyl donors to change the DNA methylation status [100].

3.6.4 Methionine and Folate Pathways

Mechanism An essential operation in the methionine cycle makes folate with the help of bacteria in the human intestines and produces S-adenosylmethionine (SAM), which is a methyl donor and is used in DNA methylation. Similarly, postbiotics enhance the host's ability to DNA methylate, and by ensuring adequate SAM, postbiotics modulate methyl status across the entire genome [101].

Scholarly Evidence Research has demonstrated that the regulation of DNA methylation in host cells is unnaturally associated with the microbial synthesis of the vitamin folic acid. For example, in animal models, shifts in gut microbial composition in the production of folate are associated with alterations in the DNA methylation profile of the genes affecting proliferation and differentiation, including certain cancer and developmental disease genes [102].

3.6.5 SCFAs and DNMT Inhibition

Mechanism The interaction between SCFAs, which include butyrate, and DNMT involves direct binding of the SCFAs to the DNMTs, leading to hypomethylation of the DNA. This effect is of special importance because tumor suppressor gene silencing and the increased level of hypermethylation concern malignancy [103].

Scholarly Evidence Research has shown that butyrate-induced tumor cell differentiation is associated with cancer cell line, DNA global hypomethylation, and tumor suppressor genes such as RAR β 2 and p16. Based on these observations, butyrate may bring back a normal methylation profile and therefore normal cellular function to prevent the development of cancer [104].

3.6.6 Postbiotics Control Noncoding RNAs

LncRNA stands for long noncoding RNAs, which are involved in gene regulation, and have functional activities associated with them. Among them is microRNA, or miRNA. It was found that postbiotics can modulate the expression of noncoding RNAs (ncRNAs), which function to control both messenger RNA (mRNA) translation as well as stability [105].

3.6.7 *MicroRNA Modulation*

Mechanism One of these postbiotics, butyrate, can impact some miRNAs and thereby initiate the breakdown or repression of their targeting mRNA. For example, butyrate exerts an anti-inflammatory action by enhancing the level of miRNA, which is well-documented to suppress inflammatory genes [106].

Scholarly Evidence Butyrate therapy has been shown to increase the anti-inflammatory/immune-modulatory miR-21 and miR-146a in an in vitro study. These miRNAs provide insights into how butyrate targets the essential parts of one of the best-studied inflammatory pathways namely, the NF- κ B and its anti-inflammatory function at the epigenetic level [106].

3.6.8 *lncRNAs, or Long Noncoding RNAs*

Mechanism Recent studies have shown that long noncoding RNAs and chromatin-modifying complexes can modulate specific genes. It is feasible that postbiotics could alter chromatin structure and have access to the gene through the regulation of lncRNAs [107].

Scholarly Evidence More current investigations demonstrate that butyrate and other SCFAs modulate the regulation of lncRNAs that regulate inflammation and epithelial cell differentiation. For instance, regulation of inflammatory genes at the transcription level in the intestinal epithelial cells and alterations of chromatin structure have been attributed to butyrate-regulated lncRNA expression [108].

3.6.9 *Modulation of Epigenetic Enzyme Activity by Postbiotics*

Postbiotics can also modify the activity of epigenetic regulators, such as HDACs and DNA methyltransferases (DNMT), HMTs, or histone demethylases (HDMs), and SCFAs.

Mechanism The mechanism of butyrate in gene regulation is well-established to include the inhibition of HDACs. Second, butyrate inhibits HDAC activity to promote histone acetylation and the relaxed chromatin structure for increased gene transcription [109].

Scholarly Evidence The treatment of human colon cancer cell lines with butyrate results in a dose-dependent inhibition of HDAC activity and an increase in acetylation levels within histones H3 and H4. This is associated with the upregulation of cell cycle arrest and apoptosis genes like Bax and Bim [110].

3.6.10 Synergistic Effects of Postbiotics and Other Microbial Metabolites

Postbiotics often exhibit multiple effects on the epigenomic setup of the host cell due to the interaction with other microbial metabolites.

3.6.10.1 Synergistic Interactions

Mechanism An important step would be to explore whether polyphenol metabolites and SCFAs could enhance the effects of epigenetic modifications, thereby fortifying our hypothesis that these chemicals function in a concerted manner. Flavonoids are effective inhibitors of enzymes such as DNMTs. HDACs might be predominantly SCFAs and certain polyphenol metabolites such as quercetin and catechins. Together, these mechanisms are likely to underlie substantial changes in histone modification and DNA methylation [111].

Scholarly Evidence It has also been revealed that when the colon cancer cells are treated with polyphenols and butyrate at the same time, the substances have a greater capability in halting the development of the colon cancer cells than the two solitary substances. Significantly the raised levels of histone acetylation and decreased levels of DNA methylation, tumor suppressor genes are reactivated, and apoptosis is induced, altogether having a synergistic effect [112].

3.7 Challenges and Future Direction

3.7.1 Epigenetic Mechanisms' Complexity

The first challenge associated with the use of postbiotics for epigenetic regulation is the fact that epigenetic regulation is a very complex process. Several layers of regulations are employed in epigenetic changes such as DNA methylation, histone modifications like acetylation or methylation, and the action of noncoding RNAs capable of putting a gene into a state of inactivity or making it work. It is still unknown to what extent postbiotics interfere with these processes, especially given the fact that the given classification encompasses a very wide spectrum of substances. For instance, there is information that the SCFAs, such as butyrate, inhibit HDACs from keeping the chromatin structure open and enhance gene expression. However, the general impact of this relationship has not been fully understood up to now, and this knowledge gap applies to many forms of disease such as cancer, neurological disorders, or chronic inflammation. Hence, before they can be used confidently in clinical practice, more knowledge about how postbiotics modulate different epigenetic processes is required [113].

3.7.2 Different Postbiotic Profiles

The large fluctuation of postbiotic production and its effects greatly become a challenge in its use. The nature of Postbiotics, the probiotic strain, and the current structure of the host's microbiome all play a major part in establishing what they consist of. For this reason, the standardization of postbiotics for their medicinal benefits is not easy to implement. For instance, the same postbiotic might somatically influence epigenetic profiles in a manner that depends on the consumers' microbiome profile and genotypes. Notably, in scientific and therapeutic domains, where it is necessary to count on a high level of replicability and predictability of the chosen approach, that is not the case. Additionally, there is the question about the development of general therapeutic approaches since an impact will not be made with a simple postbiotic chemical compound on all people [114].

3.7.3 Lack of Human Clinical Research

To date, there is still a lack of postbiotics in clinical practice, despite preclinical studies showing positive results. Few human experiments have explored the potential epigenetic effects of postbiotics, with the majority of existing research utilizing in vivo or animal approaches. For instance, evidence from preclinical evidence shows that SCFAs such as butyrate may be able to alter immune responses and levels of inflammation by altering epigenetic markers, although such findings have not been well-supported by human clinical investigations. Due to this gap, there is a significant barrier to the clinical use of postbiotics because the safety and efficacy of these products as well as the long-term consequences of using these substances have not been investigated in human volunteers. They may therefore be advantageous for epigenetic modification even though human clinical trials are not exhaustive on postbiotics [115].

3.7.4 Safety and Legal Issues

Another challenge to postbiotics' application in epigenetic activities is the lack of sufficient progress in the regulation of postbiotics. These actions may be semi-conserved and affect key epigenetic modules when primed with postbiotics, possibly bearing long-term consequences, some of which may be trans-generational. For example, when passed on to the next generations, epigenetic changes in genes that are associated with immunological response, inflammatory response, or tumor suppression may have unforeseen consequences. Postbiotics remain safe up to the time that they are used for the recommended duration, and the side effects that may accrue from their use need to be regulated, especially since they are proven to have

an impact on key biological processes. However, the regulatory policies on postbiotics in therapeutic applications are still rather undefined, which complicates clinical research into postbiotics [116].

3.7.5 Target Specificity of Postbiotics

Another major problem is how to achieve the selectivity of the epigenetic effects of postbiotics. Many postbiotic substances may affect the various epigenetic processes; therefore, they may have undesirable side effects. For instance, a postbiotic intended to prevent tumor development by altering specific histone changes may affect several other genes whereby the intended result is not achieved due to the presence of undesired effects. It is virtually impossible to employ postbiotics in precision drugs when sharp interference is needed to avert adverse impact due to nonselective effects. Thus, there is a need to examine the specific molecular targets that postbiotics modify to establish how to ensure that they solely act on the stated epigenetic networks [94].

3.7.6 Future Directions

3.7.6.1 Development of Postbiotic Libraries

One of the possible developments of this study in the future can be creating a whole variety of postbiotics with different epigenetic profiles. Scientists would, in a short time period, be able to identify the use of each postbiotic in altering the specified epigenetic paths by using the postbiotic library. Inferring the impacts of various postbiotics in epigenetic regulation such as DNA methylation, histone modifications, and noncoding RNAs could be useful through a high-throughput screening assay. This strategy could help define new therapeutic postbiotics more quickly and bring postbiotic-based therapies to diseases including cancer and neurological disorders with referred epigenetic regulation faster [117].

3.7.6.2 Tailored Antibiotic Treatment

Advancements in genetics and microbiome science may pave the way for personalized postbiotic interventions. It may be possible to regulate the epigenetics associated with certain diseases after the microbiome, and genetic indicators indicate the most effective course of action in postbiotic treatment. For instance, a 45-year-old client with a family history of colorectal cancer could be prescribed a particular postbiotic that would alter her or his epigenetic markers that are associated with

colon tumor suppression. Variation in postbiotic production and effect has some implications, and one potential solution to these implications is personalized postbiotic therapy [118].

3.7.6.3 Discovery of Epigenetic Biomarkers

The successful application of postbiotics in therapeutic practice requires credible epigenetic biomarkers to be discovered. The identification of biomarkers would permit a more accurate control of the therapeutic process during the postbiotic treatment. For instance, it has been proposed that changes in DNA methylation could likely be used as an index to the capacity that a postbiotic has in impacting gene expression. This strategy would allow setting individual dose schedules based on the patients' response together with enhancing the efficiency of postbiotic treatment [119].

3.7.6.4 Combining Other Therapies

No studies have attempted to investigate postbiotics when administered in conjunction with other therapeutic approaches: immunotherapy, chemotherapy, or changes in diets. For instance, it is hypothesized and suggested that postbiotics could enhance the effectiveness of cancer therapy by changing the epigenome of tumor cells. Instead, they can be employed to decrease the side effects of chemotherapy since they will stimulate the genes responsible for inflammation and tissue repair. Such an integrative approach can reduce the side effects caused by treatment and could improve the patient results obtained [120].

3.7.6.5 Longitudinal Studies and Clinical Trials

More rigorous case studies, at least regarding RCTs and long-term investigations, are needed to uncover the postbiotic effects of therapy. Significant postbiotic effects on epigenetic regulation should be the primary focus of this research, both in the short term and the long term. Anticipated goals in the long term could comprise changes in the incidence or prevalence of a disease, disease relapse, or remission, while short-term results could comprise changes in the immune system and genes. It will be possible for researchers to assess the safety and efficacy of postbiotics in terms of clinical research and also find out how these agents influence epigenetic changes in the future once they get longitudinal information [115].

3.7.6.6 Advanced Delivery Systems

It may therefore be the case that postbiotics administered in different ways are more therapeutically effective ways. For instance, to increase the bioavailability of postbiotic chemicals and target specific tissues or cells, nanocarriers or encapsulation techniques can be employed. Since these epigenetic changes are often time-dependent, controlled-release formulations may reduce possible side effects and reduce the need for repeated administration by maintaining targeted epigenetic modulation for an extended period. Due to such developments in delivery technology, postbiotics could be easier to implement and also more effective in clinical applications in the future [121].

3.8 Conclusion

There is a great deal of therapeutic application of postbiotics, especially when it comes to ailments such as cancer, metabolic diseases, and immunological disorders. Postbiotics can be considered the prospective approach to noninvasive gene regulation, as it was proven that they can affect key processes, including histone modifications, DNA methylation, and the actions of noncoding RNAs. However, there are significant barriers that have to be addressed to ensure their further clinical development. Epigenetic control is not simple, and variations in the postbiotic production owing to the microbiome and genetic background of each patient make standardization of medicines challenging. Because of this variability, it is difficult to predict similar therapy effectiveness in other groups. Moreover, current data on the safety, efficacy, and versatility of postbiotics in epigenetic changes is limited because of minimal human intervention studies. To facilitate better and more efficient therapies in the following studies, further research should focus on customized health interventions, taking into consideration patients' genetic and microbial differences. It will therefore be crucial to identify reliable epigenetic markers for monitoring the therapeutic response to postbiotics and avoiding side effects. In addition, developing a better delivery system—for instance, the nanocarriers and encapsulation modalities—will play a vital role in enhancing the bioavailability and precision of postbiotics. In conclusion, postbiotics are still at the very beginning of their epigenetic potential, even though there are many challenging barriers on the way. This implies that postbiotics may revolutionize personalized medicine by offering safer and more efficacious medicines to different patients because of their epigenetic maps, the current science and clinical and regulatory challenges notwithstanding.

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Chapter 4

Postbiotics and Metabolic Signaling in Colorectal Cancer



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Abstract Postbiotics are nonliving products from microbes that show promise as helpful compounds for treating various diseases, including colorectal cancer (CRC). They are created during the fermentation process in the gut and include substances such as short-chain fatty acids (SCFAs), peptides, and lipopolysaccharides. In CRC, postbiotics can help fight cancer by influencing important metabolic pathways that affect cancer growth. Short-chain fatty acids (SCFAs), like butyrate, propionate, and acetate, serve a vital function in controlling cell growth, programmed cell death, and inflammation, all of which are connected to colorectal cancer development. For instance, butyrate can trigger cancer cell death while supporting the growth of healthy colon cells. Moreover, postbiotics can affect gene expression and immune responses, making the body less likely to develop cancer. Additionally, postbiotics

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can impact major metabolic pathways like Wnt/ β -catenin, PI3K/Akt, and mTOR, which are often disrupted in CRC. By targeting these pathways, postbiotics may slow down tumor growth and limit the spread of cancer. The relationship between postbiotics from gut bacteria and the body's metabolic signals presents a new way to prevent and treat CRC. More studies on specific postbiotic compounds and how they work could lead to new strategies for managing colorectal cancer.

Keywords Postbiotics · Colorectal cancer · Metabolic signaling · Short-chain fatty acids (SCFAs) · Wnt/ β -catenin pathway · Gut microbiota

4.1 Introduction

The gut microbiome is very important for our bodies. A healthy mix of bacteria, fungi, and viruses in the gut helps keep it balanced. Numerous methods exist to modify the gut flora, including dietary adjustments and probiotics, advanced probiotics, fecal microbiota transplants, various treatments, and postbiotics. Postbiotics are bioactive compounds made during fermentation. They come from the metabolic activities of microbes and help the host in both direct and indirect ways. The idea of postbiotics is that the good impacts of gut microbiota happen through the release of various metabolites [1]. It plays a vital role in many body functions like digestion, immune response, metabolism, disease development, detoxification, and the production of important substances like vitamins. The microbiota can also influence the gut-brain connection, affecting feelings like anxiety and mood. The beneficial bacteria in our gut can be grouped into various categories, including *Bacteroidetes* and *Firmicutes*, along with *Proteobacteria*, *Fusobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Spirochaetes*. Colorectal cancer (CRC) is the third-most prevalent cancer among men and the second among women worldwide. To effectively prevent CRC, a major lifestyle change is necessary. CRC is a complex disorder affecting the intestinal lining, including the colon, rectum, and anus. It involves an unbalanced immune response, the buildup of mutations in stem cells, damage to the

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intestinal barrier, and changes in gut bacteria, often seen as negative shifts in microbiota. About 90% of CRC risk is believed to come from lifestyle choices, mainly influenced by diet and environment. Factors like fiber intake, intake of red and processed meats, alcohol consumption, and insufficient levels of omega-3 fatty acids and vitamin D play a role. Additionally, obesity, inactivity, and smoking are important risk factors that can lead to changes in gut bacteria linked to CRC [2]. Metabiotics is a term that combines “metabolites” and “probiotics.” They are also called parabiotics and postbiotics. Metabiotics consist of parts from probiotic bacteria and their metabolites, which have a specific chemical structure. These components have shown effectiveness in preventing and treating CRC. Key elements of metabiotics include short-chain fatty acids, peptides, mucopeptides from peptidoglycan, enzymes, polysaccharides, vitamins, teichoic acids, proteins, and plasmalogens. Metabiotics enhance gastrointestinal (GI) health and facilitate the proliferation of advantageous bacteria, which helps lower the enzymes that turn pro-carcinogens into carcinogens. Some metabiotic components, especially SCFAs, can identify cancer cells and activate genes that are usually turned off [3]. Gut microbiota is connected to cancer development and the growth of colorectal tumors. Pathobionts, which are normally innocuous microorganisms in the gut that can become harmful, may create genotoxins. These substances can damage DNA and change the tumor microenvironment (TME), resulting in shifts in the levels of harmful bacteria in the colon. For example, *Escherichia coli* are common bacteria in the human gut, but certain strains can cause disease through various harmful factors [4]. Investigation of the relationship between nutrition and metabolic syndrome looks at more than just cutting calories and adding macronutrients; it also examines how natural bioactive ingredients can help manage body weight and improve sensitivity to insulin. Recent research has undertaken to explore how gut microflora affects the development of the metabolic syndrome. This is important because the gut microbiota interacts closely with the body, which is essential for maintaining gut health. Gut microbiota plays a vital role in managing metabolic processes that turn food into energy. This interaction between gut microflora and the body is facilitated by metabolites, known as postbiotics, which are created in the colon when nondigestible substances are fermented [5]. The gut microbiota constantly releases bioactive substances into the bowel. Some of these substances can enter the bloodstream and affect metabolism as specific ligands. Intestinal integrity is crucial in this process, as it helps prevent harmful bacterial by-products from entering the circulation. Probiotics are helpful microorganisms, while prebiotics are nondigestible food constituents that enhance the proliferation and function of specific bacteria in the gastrointestinal tract [6]. Ectopic fat tissue develops when there is too much fat stored in areas outside the normal fat reserves, which can raise the risk of CRC by affecting metabolism and inflammation. Fat tissue is spread across the body in various areas, each serving a specific purpose. Visceral fat, located mainly in the abdomen around organs like the liver, pancreas, and intestines, has been linked to a higher risk of CRC in multiple studies. In contrast to general obesity assessed by BMI, visceral obesity may present a heightened risk for colorectal cancer due to its impact on metabolism. This type of fat releases hormones like adipokines and cytokines,

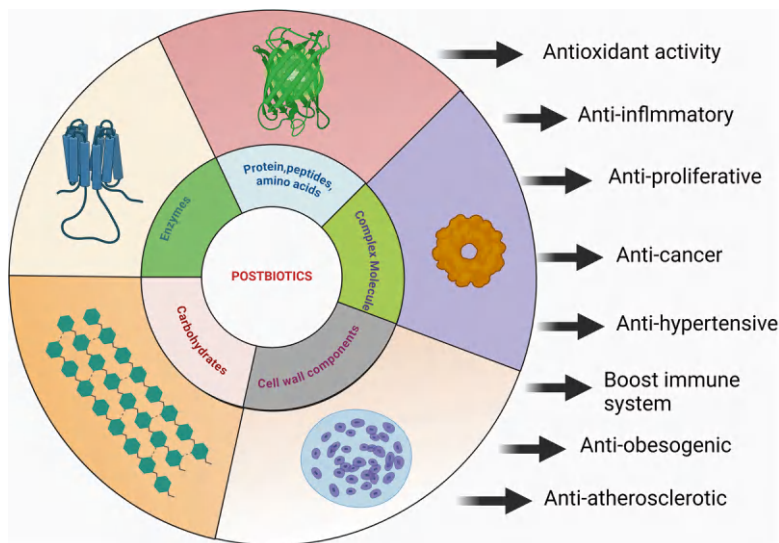


Fig. 4.1 Examples of postbiotics and their proposed activity in patients with colorectal cancer

which can lead to inflammation and insulin resistance, both of which are connected to the progression of CRC [7]. Dysbiosis and unusual metabolites can lead to the progression of CRC and affect treatment outcomes. Metabolites in the tumor environment may trigger chronic inflammation and tumor growth by activating immune cells. An imbalance between harmful bacteria and beneficial probiotics can disturb the balance in the intestines. Managing CRC includes various methods. Traditional treatments like endoscopy, surgery, chemotherapy, radiotherapy, and immunotherapy are common, but using microbes for management is a new and essential approach. The gut microbiota can help identify tumors and influence the effectiveness of CRC treatments and prevention. As we learn more about how gut bacteria play a role in cancer development and treatment outcomes, controlling and adjusting gut bacteria may become key strategies for preventing and treating CRC [8]. Figure 4.1 depicts several types of postbiotics and their potential health benefits.

4.2 Postbiotics Overview

Gut microbiota is crucial for protecting the GI system from harmful elements and pathogens. It helps maintain mucus layers and competes with harmful microbes for diets and space in the intestines. Microbial elements like lipopolysaccharides and peptidoglycans encourage mucus production and layer formation. Research indicates that the mucus layer in germ-free mice is significantly different from that in normally raised mice due to the lack of microbiota. Additionally, colon microbiota transforms dietary fiber into short-chain fatty acids that function as an energy source

for the host. Thus, a diet rich in fiber is crucial for colon health; without enough fiber, microbiota may start using mucin glycans for energy, leading to a significant reduction in the colonic mucus layer [9]. Different types of postbiotics can trigger cell death in CRC, slow down cell growth, and stop cell movement. They also help the immune system. Besides blocking cancer-causing signals, they support the health of the intestinal lining and work well with chemotherapy drugs. As a result, postbiotics and their active compounds offer many advantages, including benefits for gut health, immune support, and cancer-fighting properties, while also improving cancer treatments. These substances have been effective against various cancers, including colon, stomach, blood, breast, and cervical cancers. In clinical settings, using postbiotics with cancer treatments helps maintain good bacteria in patients, supports recovery after surgery, and reduces the risk of infections, leading to shorter hospital stays. Additionally, postbiotics can help lessen the side effects of standard cancer medications, like nausea and diarrhea [10]. Early life microbiota is influenced by factors like delivery method, birth timing, breastfeeding, formula feeding, and having pets. As people grow older, diet, medications like antibiotics and proton pump inhibitors (PPIs), stress, smoking, alcohol use, living environment (rural or urban), and physical activity also impact microbiota. The microbiota produces many enzymes that help digest nutrients not fully processed by the human digestive system. The gut microbiome has many genes for carbohydrate metabolism, including over 115 glycoside hydrolase families and more than 21 polysaccharide lyase families. It can also create various proteases. The microbiota alters substances absorbed by the host and makes new metabolites. Short-chain fatty acids (SCFAs) are mainly formed from the fermentation of undigested carbohydrates in the small intestine. SCFAs are the main carbon source of food for the host via the microbiome, with key SCFAs being formate, acetate, propionate, and butyrate. Amino acid fermentation also helps produce SCFAs, mainly acetate and propionate [11]. When we take prebiotics with probiotics, they provide energy for the live bacteria in our gut and help them grow. This mix of prebiotics and probiotics is called synbiotics. Synbiotics are characterized as a consortium of living microorganisms and substances that these bacteria use, which benefits the host's health. This mix helps increase the number of good bacteria in the colon and supports the survival and growth of new probiotic strains. Synbiotics can be classified as "synergistic," which helps new probiotics grow, or "complementary," which supports both existing and new probiotics. Research on synbiotics shows that they may help prevent cancer and mutations. The benefits of prebiotics come from encouraging good bacteria, producing short-chain fatty acids, changing gene activity, improving nutrient absorption, and affecting metabolism and immune response [12]. Probiotics often create biofilms to protect against harmful bacteria in the mouth. They occupy areas where cavity-causing bacteria could grow and compete with these pathogens for space. Some probiotics can also stop the growth of bacteria linked to gum disease. Other ways in which they work include competing for food and attachment spots, producing substances that kill bacteria or break down toxins, and helping regulate the immune system locally or throughout the body [13].

4.3 Metabolic Signs and Symptoms and Pathogenesis of CRC

CRC is associated with several metabolic alterations, reflecting the profound changes in cellular energy demands and signaling pathways during tumor development [14]. Key metabolic signs and symptoms include unexplained weight loss, fatigue, and altered bowel habits, which may be linked to the disruption in glucose metabolism, often referred to as the Warburg effect [15]. In CRC, cancer cells rely on aerobic glycolysis over oxidative phosphorylation, despite the availability of oxygen, resulting in increased glucose absorption and lactate synthesis [16]. This metabolic alteration facilitates swift cellular proliferation and sustenance in the hypoxic tumor microenvironment, contributing to systemic symptoms like cachexia and weakness [17]. Additionally, CRC is linked to metabolic syndrome, characterized by obesity, insulin resistance, and dyslipidemia, which further exacerbates the disease process by promoting chronic inflammation and enhancing tumorigenesis through insulin-like growth factors (IGFs) and inflammatory cytokines [18].

The pathogenesis of colorectal cancer involves complex interactions between genetic mutations and epigenetic changes that drive tumor initiation and recurrence [19]. Early in CRC development, mutations in the adenomatous polyposis coli (APC) gene result in the dysregulation of the Wnt/ β -catenin pathway, promoting uncontrolled cell proliferation [20]. As the disease progresses, mutations in key oncogenes including KRAS and tumor suppressor genes like TP53 further enhance malignancy, facilitating resistance to apoptosis and increased invasiveness. In parallel, metabolic reprogramming occurs, where cancer cells adopt a hyperactive glycolytic pathway to fuel biosynthetic processes necessary for rapid tumor growth [21, 22]. Moreover, metabolic enzymes such as lactate dehydrogenase and pyruvate kinase M2 (PKM2) are upregulated, reinforcing the metabolic phenotype of CRC. This metabolic dysfunction, coupled with inflammation, oxidative stress, and dysregulated immune responses, creates a pro-tumorigenic microenvironment that fosters CRC progression [23].

4.4 Signaling Pathways Linked to Colorectal Cancer

CRC is influenced by a multifaceted interaction of hereditary, epigenetic, and environmental factors that converge on several key signaling pathways [24]. The Wnt/ β -catenin pathway is pivotal in the onset and advancement of CRC [25]. Dysregulation of this pathway, often attributable to mutations in the APC gene or β -catenin itself, leads to uncontrolled cellular proliferation [26]. Mutant APC fails to form a destruction complex that degrades β -catenin, allowing it to accumulate in the nucleus and initiate transcription of target genes promoting tumorigenesis [20]. This aberrant signaling is further compounded by crosstalk with alternative pathways, such as the PI3K/Protein kinase B (AKT) and mitogen-activated protein kinase (MAPK)

pathways, both of which are frequently altered in CRC due to mutations in oncogenes like KRAS and PIK3CA or tumor suppressor genes such as PTEN [27].

Another important signaling pathway in CRC is the TGF- β /SMAD pathway, which typically functions as a tumor suppressor in normal epithelial cells [28]. In early CRC, activating mutations in SMAD4 or inactivating mutations in TGF- β receptors lead to the disruption of this pathway, contributing to enhanced tumor growth and metastasis [29]. Additionally, the Notch and Hedgehog signaling pathways are implicated in the maintenance of cancer stem cells and tumor heterogeneity [30]. Together, these dysregulated pathways promote cellular proliferation, resistance to apoptosis, angiogenesis, and metastasis, all of which are hallmark features of CRC [31, 32]. Understanding these routes has resulted in the advancement of targeted medicines aimed at inhibiting specific molecular drivers of colorectal cancer progression.

4.4.1 Metabolic Signaling Involved in Colorectal Cancer

With a 60% increase in incidence expected by 2030, colorectal cancer (CRC) ranks fourth in the world for cancer-related mortality and is the third-most prevalent cancer diagnosed internationally. Risk factors for colorectal cancer (CRC) include genetic, environmental, and lifestyle factors [33]. The majority of CRC instances are sporadic and progress gradually over several years through a gradual process referred to as the adenoma–carcinoma sequence while being heavily influenced by hereditary components. According to this hypothesis, CRCs develop and grow as a result of a series of progressive aggregation of mutations in the Wnt, EGFR, P53, and TGF- β signaling pathways. Seventy percent of colorectal adenomas have early APC gene mutations; most of these will develop into carcinomas as a result of activating mutations in KRAS succeeded by inactivating mutations in TP53 and SMAD4. A tiny percentage of spontaneous colorectal cancers do, however, arise from distinct molecular pathways, such as BRAF mutant activation or DNA mismatch repair gene inactivation, which are found in serrated lesions and microsatellite instability-high (MSI-H) CRCs, accordingly [34]. Comprehensive transcriptome investigations have unveiled a consensus molecular classification of colorectal cancer that includes four consensus molecular subtypes (CMSs) that are distinguished by their distinct clonal, stromal, and immunological dependencies, in addition to these genetic events. These CMSs forecast the outcome of targeted treatments and replicate the heterogeneity seen in CRC. Interestingly, these transcriptome and genetic investigations have yielded a wealth of information that has improved CRC diagnosis and treatment. Metastasis of CRC is a major cause of cancer-related fatalities; however, at later stages, the illness becomes challenging to manage and ultimately acquires resistance to the majority of combined therapy [35]. Given this, the prognosis for CRC patients is still poor, with a 60% 5-year survival rate and about 50% of patients dying from the disease even after curative resection. These unsatisfactory treatment results emphasize how important it is to comprehend the mechanisms behind the development, progression, and dissemination of colorectal cancer.

4.4.2 Different Types of Postbiotics and Their Mechanisms of Action

Bioactive materials called postbiotics are created during matrix fermentation and are beneficial. Postbiotics are in a delicate balance in human bodies, and excessive metabolites of any kind have the potential to impair or interfere with a variety of physiological functions [36]. Postbiotics, defined as the metabolic by-products or bioactive compounds produced by probiotic bacteria, have arisen as prospective therapeutic agents in CRC owing to their capacity to regulate gut flora and influence key cancer-related pathways [5, 37]. Key types of postbiotics include SCFAs like butyrate, acetate, and propionate; vitamins; cell lysates; bacterial cell wall components including peptidoglycans and lipoteichoic acids; and extracellular metabolites like bacteriocins [38]. Butyrate, the most well-studied SCFA, exerts antitumor effects through multiple mechanisms, including promoting apoptosis, inhibiting histone deacetylase (HDAC) activity, and inducing cell cycle arrest in colorectal cancer cells [39]. Bacteriocins, on the other hand, possess antimicrobial properties that help maintain gut homeostasis and prevent dysbiosis, a key factor linked to CRC development [40].

The mechanisms by which postbiotics demonstrate their anticancer properties are multifaceted [41]. SCFAs, particularly butyrate, modulate immune responses by enhancing the production of anti-inflammatory cytokines and inhibiting pro-inflammatory signaling pathways like NF- κ B, which play a critical role in colorectal carcinogenesis [42]. Furthermore, postbiotics can strengthen the intestinal barrier through the upregulation of tight junction proteins, thereby preventing microbial translocation and inflammation [43]. Through the modulation of gut microbiota combination, postbiotics also influence the production of secondary bile acids and other metabolites that are implicated in CRC progression [44]. These bioactive compounds target multiple pathways involved in tumorigenesis, such as apoptosis, cell proliferation, and immunity evasion, offering a promising avenue for CRC prevention and adjunctive therapy [45].

4.4.3 Postbiotics and Gut Microbiota: Correlation with Cancer

The human microbiota, sometimes known as another “organ” of the body, is becoming more and more important to the human digestive system, metabolic processes, immune function, and numerous other bodily functions. The bacteria, archaeabiota, virobiota, and mycobiota that make up the human microbiota interact with the host in a very intricate way [46]. In the human digestive tract, there are over 100 trillion microbes. In the colon, there are between 10^{11} and 10^{12} bacterial cells per milliliter, according to estimates. Postbiotics are created by inactivating microorganisms using a variety of methods, either separately or in combination, including solvent extraction, heat treatment, enzymatic procedures, chemical treatments, sonication,

and hyperbaric environments. The targeted postbiotics are separated from the resultant solutions using processes such as column purification, centrifugation, dialysis, and lyophilization. Cell constituents are regarded as a significant class of postbiotics. These could include the plasma membrane, organelles, flagella, pili, and capsules; surface layers (S-layers); teichoic acid and lipoteichoic acid; and the cell wall and its constituents (peptidoglycans) [47]. Other than mycoplasmas, peptidoglycans located in the cell walls of both gram-positive and gram-negative bacteria give their cells their distinctive form and mechanical protection. The term “peptidoglycan” refers to the combination of two primary constituents: short peptide chains composed of two to five amino acid residues and glycan strands composed of repeated disaccharide units [48]. The anionic teichoic acids seen in gram-positive bacteria belong to a broad family of glycopolymers with phosphodiester-linked polyol repeat units on their cell surfaces. Teichoic acids comprise wall teichoic acids that covalently bind to peptidoglycan and lipoteichoic acids that attach to the bacterial membrane through a glycolipid.

4.5 Mechanism of Postbiotics in CRC Inhibition

Postbiotics, the bioactive substances generated by probiotic bacteria during fermentation, play a significant role in inhibiting CRC through multiple mechanisms [49]. They exhibit anticancer properties by regulating gut microbiota, improving mucosal barrier function, and reducing inflammation, which is a key factor in CRC progression [50]. Short-chain fatty acids, particularly butyrate, are among the most effective postbiotics that modulate cell proliferation and death in colonocytes by inhibiting histone deacetylases (HDACs) and activating G-protein-coupled receptors (GPCRs) [51, 52]. This results in the suppression of pro-inflammatory cytokines, modulation of immune responses, and promotion of anti-tumorigenic pathways. Furthermore, postbiotics contribute to maintaining gut homeostasis by enhancing the synthesis of mucins and tight junction proteins, thereby preventing CRC initiation and progression [44].

4.6 Clinical Significance of Postbiotics in CRC

The clinical relevance of postbiotics in CRC lies in their ability to regulate epigenetic mechanisms and influence cellular signaling pathways critical to cancer progression. Among these, butyrate, a well-characterized postbiotic, acts by inhibiting histone deacetylases (HDACs), leading to tumor suppression and the promotion of cancer cell apoptosis [11, 44]. Furthermore, postbiotics target key oncogenic routes, including Wnt/ β -catenin, NF- κ B, and PI3K/Protein kinase B (AKT), all of which are involved in tumor development and progression. By downregulating these signaling pathways, postbiotics hold promise as adjunct therapies in the treatment of

CRC [53]. In addition, postbiotics, which are bioactive metabolites synthesized during the fermentation of prebiotics by probiotics, including SCFAs like butyrate, acetate, and propionate, have gained considerable interest due to their capacity to influence gut microbiota, enhance immune function, and exert anti-inflammatory and anticancer effects [54]. Butyrate specifically induces apoptosis, inhibits cancer cell proliferation, and enhances cellular differentiation by modulating paths such as Wnt/ β -catenin and NF- κ B [55]. Additionally, postbiotics strengthen gut barrier integrity and reduce oxidative stress, both of which are crucial in preventing CRC development. These emerging insights underscore the therapeutic potential of postbiotics as novel, microbiota-driven strategies for the management and avoidance of colorectal cancer [44].

4.6.1 Future Role of Postbiotics in Cancer Cell Cycle Regulation and Growth Inhibition

By regulating cell division, cancer cell cycle arrest inhibits cancer cell growth. Cells continue to cycle through the G1, S, G2, and M phases; DNA replication occurs during the S phase, and in the M phase, cell division takes place. G1 and G2 phases are used to confirm that replication and division have finished. The advancement of the cell cycle depends on the precise regulation of the interaction between cyclins and cyclin-dependent kinases (CDKs). Cell cycle regulation is governed by cyclin D/CDK4 during the G1 phase, cyclin A/CDK2 in the S phase, and the cyclin B/CDC2 complex in the G2/M phase, sequentially [56]. When applied to cancer cell lines, components of the cell wall, peptidoglycans, cytoplasmic extracts, and cell supernatants from various postbiotics exhibit antiproliferative effects. According to fluorescence-activated cell sorting (FACS) measurements, SCFAs generated in *Propionibacterium freudenreichii* led to the aggregation of the sub-G1 phase and the decrease of S and G2/M phases in human colorectal cancer RKO cells. According to reports, Species of *Listeria* (*L. spp.*) cell-free pentasaccharide stimulates apoptosis and prevents the advancement of the S-phase cell cycle in primary colon cells and human colorectal cancer HT-29 cells. The antiproliferative activity of nisin, the cytoplasmic extract of *Lactobacillus lactis ssp. lactis*, and the bacterial cell wall was demonstrated by T4056 [57] and was linked to a decrease in the presence of cyclin D1 in human colorectal SW480 cancer cells. It discovered that the suppression of the p53-p21-cyclin B1/Cdk1 signal route controlled the antiproliferation effect of probiotic-derived p8 protein, leading to in vitro growth arrest at the G2 phase of the cell cycle in human colorectal DLD-1 cells. It follows that postbiotics may prevent cancer cells from proliferating [58].

4.7 Future Role of Postbiotics

Future research is essential as postbiotics remain in their early stages of development. Standardization of various microorganism inactivation procedures and methods is necessary. It is necessary to specify the postbiotic product's composition [59]. The necessary techniques for quantifying postbiotics must be identified and created. The fact that toxic shock and sepsis can be brought on by the lipopolysaccharides found in gram-negative bacteria should not be disregarded. Contamination of the product must be taken seriously [60]. It is essential to compare the effects of postbiotics and probiotics. It should be mentioned that distinct postbiotics might be produced by various strains of the same bacteria. A postbiotic may consist of a complicated mixture; however, it is necessary to identify the component causing the positive effects. The necessary dosage needs to be ascertained [61]. It is necessary to assess bioavailability and bio-accessibility. It is necessary to decide how to assess the level of microbial cell damage during inactivation. Large-scale, randomized controlled clinical trials must be carried out to prove the therapeutic benefits of postbiotics [62]. It is worth looking into the possibility that postbiotic metabolites already in place could break the feedback loop and interfere with endogenous synthesis.

4.8 Safety Consideration of Postbiotics

Prior to talking about postbiotics' therapeutic advantages, the topic of postbiotic stability needs to be covered. Living bacteria are used in probiotic treatments, yet this can lead to issues because in vivo virulence factors and antibiotic-resistant genes can arise. Postbiotics can get around this issue, but it is particularly crucial for young children with developing immune systems and weak barriers. Supplementation with heat-killed *Lactobacillus acidophilus* LB decreased the duration of diarrhea and avoided laryngitis, pharyngitis, and diarrhea, according to seven randomized controlled trials (RCTs) involving 1740 children [63]. Only one RCT displayed dehydration linked to the administration of heat-killed *L. acidophilus* LB. It has been established that heat-inactivated *Lactobacillus acidophilus* LB may stimulate intestinal cell adhesion and pro-inflammatory chemokines, two immune-inflammatory pathways, in children. In a clinical trial including children aged 6–14, the impact of *Lactobacillus paracasei* CNCM I-1572 on intestinal microbiota was assessed. Consequently, it was noted that the gut microbiota's function was altered without having a negative impact [64]. Additionally, it was found that irritable bowel syndrome was relieved by heat-killed *Bifidobacterium bifidum* HI-MIMBb75 [65, 66]. In this clinical research, suspected abdominal discomfort was the most frequent adverse event, seen in two patients (<1%) in the *B. bifidum* HI-MIMBb75 cohort and one patient (<1%) in the placebo cohort. More research is needed to determine the effects and potential adverse effects of various postbiotics. Postbiotics

are far more advantageous for industrial production than probiotics since they are more user-friendly and compact, possess an extended shelf life, exhibit stability throughout a broad pH and temperature spectrum, and do not generate bio-amines. However, more research on the production processes, delivery methods, and safety standards for medications and functional foods is required in order to use postbiotics as probiotic alternatives.

4.9 Conclusion

In addition to destroying the immune system and intestinal barrier during oncogenesis, cancer also does so in tandem with anticancer therapy. The review's study shows how the metabolites found in postbiotics cause a biological response that protects the intestinal barrier and has positive health consequences. The utilization of postbiotics in cancer therapy and prevention is explained by a number of processes. Postbiotics suppress cancer cells' apoptosis, proliferation, and autophagy, as demonstrated by numerous research. Thus, the objective of this research is to develop postbiotics with fewer adverse effects and improved cancer therapy outcomes. To further explore the safety criteria, anticancer capabilities, stability throughout food manufacture, marketing, and gastrointestinal status of novel postbiotics, more metabolomic research is required. Clinical trials that are double-blind and randomized are also required to ascertain the right amount and frequency of supplementation for cancer patients. With the right postbiotics, anticancer treatment will be viable in the future.

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Chapter 5

Gut Microbiota and Colorectal Cancer Pathogenesis



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Abstract This chapter explores the complex relationship between the gut microbiota and the pathogenesis of colorectal cancer (CRC), highlighting the role of microbial dysbiosis in promoting inflammation, DNA damage, and immune dysregulation. It reviews key pro-carcinogenic bacteria, such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, and pks+ *Escherichia coli*, which contribute to tumor initiation and progression. The chapter also discusses the protective effects of beneficial microbes like *Lactobacillus* and *Bifidobacterium*, which help maintain gut health and inhibit carcinogenesis. Therapeutic strategies aimed at modulating the gut microbiota, including dietary interventions, probiotics, prebiotics, fecal microbiota transplantation (FMT), and postbiotics, are examined for their potential in CRC prevention and treatment. Finally, future directions in personalized microbiome-based therapies, diagnostic biomarkers, and the challenges facing clinical translation of gut microbiota research are addressed. The chapter emphasizes the growing importance of targeting the microbiome in CRC management to enhance prevention, diagnosis, and therapeutic outcomes.

Keywords Colorectal cancer · Gut microbiota · Dysbiosis · Probiotics · Fecal microbiota transplantation · Cancer biomarkers

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5.1 Introduction

Colorectal cancer (CRC) is very widespread worldwide, being the third-most-often diagnosed cancer and the second biggest cause of cancer-related deaths globally. The prevalence of colorectal cancer (CRC) has been consistently increasing, particularly in nations experiencing fast economic growth, where changes in lifestyle are playing a crucial role in this pattern. According to estimates, the worldwide incidence of colorectal cancer is projected to rise by 60% by the year 2030. This increase would result in almost 2.2 million new cases and 1.1 million deaths from the disease per year [1]. The etiology of CRC involves a complex interplay of genetic, environmental, and lifestyle variables. CRC typically develops by one of three pathways: the adenoma–carcinoma sequence, the serrated route, or the inflammatory pathway. Each pathway is linked to distinct molecular and histological features [2].

5.1.1 *Epidemiology and Global Burden of CRC*

Colorectal cancer has a similar impact on both men and women; however, males have a slightly greater occurrence rate. Historically, North America, Europe, and Oceania have consistently reported the highest rates of colorectal cancer (CRC). However, there has been a significant rise in CRC incidence in some areas of Asia, South America, and Africa as the people in these countries embrace Westernized lifestyles. Based on statistics, most occurrences of CRC are detected in individuals who are 50 years old or older. However, new research has shown a concerning rise in early-onset colorectal cancer, which is discovered before the age of 50. The mortality rate of colorectal cancer (CRC) differs worldwide due to variations in health-care systems, screening initiatives, and public knowledge. While mortality rates have decreased in high-income nations as a result of successful screening and early diagnosis, they are still increasing in low- and middle-income countries where colorectal cancer (CRC) screening is not easily available [4]. The year 2020 was projected to have over 1.9 million new cases of colorectal cancer and 930,000 fatalities. The greatest incidence rates were found in areas around Australia and New Zealand, as well as in European regions (40.6 per 100,000 men), while the lowest rates were found in many African regions and Southern Asia (4.4 per 100,000 females). There were also similar trends seen for death rates, with the greatest mortality rates being reported in Eastern Europe (20.2 per 100,000 men) and the lowest mortality rates being recorded in Southern Asia (2.5 per 100,000 females). By the year 2040, it is anticipated that the burden of colorectal cancer will grow to 3.2 million new cases and 1.6 million deaths, with the majority of cases being forecast to occur in countries with high or very high human development index (HDI) [5].

5.1.2 Key Factors Contributing to CRC: Genetic, Lifestyle, and Environmental Factors

- *Genetic Factors:* Approximately 5–10% of colorectal cancer (CRC) cases are hereditary, mostly linked to genetic abnormalities like Lynch syndrome (hereditary nonpolyposis colorectal cancer (HNPCC)) and familial adenomatous polyposis (FAP). These hereditary diseases greatly elevate the overall chance of acquiring colorectal cancer (CRC) during one's lifetime, and persons with these illnesses often experience the onset of cancer at a very early age. The majority of instances of colorectal cancer (CRC) are caused by spontaneous mutations in the adenomatous polyposis coli (APC) gene [3].
- *Diet:* Consuming diets that are heavy in red and processed meats, low in dietary fiber, and high in refined carbs and sugars has been linked to a higher likelihood of developing colorectal cancer (CRC). On the other hand, diets that include a high amount of fruits, vegetables, and whole grains have been shown to lower the incidence of colorectal cancer [6].
- *Physical Inactivity and Obesity:* Both sedentary behavior and obesity are acknowledged as risk factors for colorectal cancer (CRC). Obesity is associated with systemic inflammation and insulin resistance, both of which contribute to the development of a cancer-promoting environment [7].
- *Alcohol and Tobacco Use:* Excessive alcohol use and smoking are both separately linked to an increased likelihood of getting colorectal cancer (CRC). Both practices lead to the creation of cancer-causing chemicals that may harm the lining of the colon [1].

5.1.3 Gut Microbiota: Definition and Importance

The gut microbiota is a diverse population of microorganisms, including bacteria, viruses, fungi, and archaea, that live in the human gastrointestinal system. These bacteria are not only passive residents, but they play crucial roles in maintaining the health of the host. These roles include food metabolism, regulation of the immune system, and preserving the integrity of the gut barrier [8]. The gut microbiota has been shown to impact a range of physiological functions, such as digestion, vitamin production, and the elimination of toxic substances. Dysbiosis, which is the disturbance in this microbial environment, has been linked to several disorders such as inflammatory bowel disease (IBD), obesity, and CRC [9].

5.1.3.1 Overview of Gut Microbiota and Its Functional Role in Human Health

The gut microbiota has a role in several aspects of the host's well-being. Microorganisms in the gut metabolize complex carbohydrates into short-chain fatty acids (SCFAs), such as butyrate. These SCFAs provide energy for the cells in the

colon and also possess anti-inflammatory qualities. Furthermore, the gut microbiota plays a critical role in immune system regulation. For example, a number of bacterial species stimulate the production of anti-inflammatory cytokines, which help keep the immune system in balance and avoid overreactions [10]. An imbalance in the gut microbiota may disturb these essential functions, resulting in long-term inflammation, weakened immunological function, and a damaged intestinal barrier. All of these factors are involved in the development of colorectal cancer (CRC) [8].

5.1.3.2 Composition and Diversity of the Gut Microbiome

The gut microbiota is subject to constant change and is influenced by several variables including nutrition, age, genetics, and geographic location. The gut microbiome of a healthy human is mostly composed of two bacterial phyla: *Firmicutes* and *Bacteroidetes*, with minor proportions of *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. The bacteria carry out specific metabolic functions such as fermenting indigestible fibers, metabolizing bile acids, and producing vitamins such as vitamin K and biotin [7]. Microbial diversity, which refers to the variety of different bacterial species present, plays a crucial role in preserving the stability and functionality of the gut environment. There is a connection between a decrease in the variety of microorganisms and the development of certain long-lasting illnesses, such as colorectal cancer (CRC). Research has shown that individuals with colorectal cancer (CRC) often have a decrease in the variety of microorganisms present in their bodies, along with an excessive presence of potentially harmful bacteria, such as *Fusobacterium nucleatum* and *Bacteroides fragilis*. Additionally, there is a decrease in helpful microbes like *Lactobacillus* and *Bifidobacterium* [6].

5.2 Link Between Gut Microbiota and Colorectal Cancer

5.2.1 Microbiota Dysbiosis and CRC Risk

The disorder known as dysbiosis results from an unequal gut microbe distribution that reduces good bacteria and increases possibly dangerous or deadly ones. Supporting immunological activity and digestion and preserving the integrity of the epithelial barrier depend on homeostasis, which the bacteria in a healthy gut guarantee. Nevertheless, dysbiosis disrupts this balance, leading to inflammatory processes, immunological dysregulation, and metabolic alterations that provide a favorable environment for tumor growth in the colon. Gut dysbiosis is influenced by several variables such as dietary choices (such as high-fat or low-fiber diets), the use of antibiotics, changes in lifestyle, and genetic predispositions. Dysbiosis impacts gut health by elevating intestinal permeability, diminishing the generation of short-chain fatty acids (SCFAs), and fostering chronic inflammation, all of which contribute significantly to the development of colorectal cancer (CRC) [11, 12].

5.2.2 Evidence Supporting the Association Between Gut Microbiota Alterations and CRC Development

The correlation between an imbalance in gut microbiota and the development of colorectal cancer (CRC) has been well investigated. Multiple studies have shown that dysbiosis, which is defined by an excessive presence of bacteria that promote inflammation and a reduction in bacteria that provide benefits, is a distinguishing feature of colorectal cancer (CRC). For instance, there has been a continuous observation of elevated levels of species such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *Escherichia coli* in individuals with colorectal cancer (CRC). The bacteria have the ability to generate virulence factors and toxins that facilitate inflammation and DNA damage, which are crucial pathways in the development of colorectal cancer [13]. Moreover, research conducted using germ-free and antibiotic-treated animal models has shown that the existence of imbalanced microbiota might heighten the vulnerability to colorectal cancer (CRC). For example, when mouse models with imbalanced gut ecosystems are exposed to carcinogens, it may speed up the development of tumors. This demonstrates the direct involvement of microbiota in the process of cancer formation [14].

5.2.3 Role of Bacterial Toxins and Inflammatory Responses

Several bacterial species and their toxins have been implicated in CRC pathogenesis. The most well-characterized species include:

- *Fusobacterium nucleatum*: This particular microorganism that causes disease in the mouth is often present in larger quantities in colorectal cancer tissues. This substance has the ability to stick to the cells that line the colon, causing inflammation and helping cancer cells avoid the immune system. *Fusobacterium nucleatum* induces the production of pro-inflammatory cytokines, which may establish a microenvironment that promotes the formation of tumors [15].
- *Bacteroides fragilis*: Enterotoxigenic *Bacteroides fragilis* (ETBF) is a kind of bacteria that has been associated with colorectal cancer (CRC). ETBF produces a toxic substance called *Bacteroides fragilis* toxin (BFT), which causes an increase in the growth of cells in the lining of the colon, DNA damage, and long-lasting inflammation. Specifically, BFT has the ability to stimulate the activation of nuclear factor kappa B (NF- κ B), which is a transcription factor that has a role in both inflammation and oncogenesis [16].
- *Escherichia coli*: Certain strains of *E. coli*, particularly those with the pks pathogenicity island, generate colibactin, a genotoxin that induces DNA double-strand breaks in the cells of the host. The genotoxic action enhances the occurrence of mutations and instability in chromosomes, thus leading to the development of colorectal cancer (CRC). *E. coli* is also recognized for its ability to trigger inflammation and alter the intestinal barrier, which, in turn, promotes the development of cancer [17].

5.2.4 Mechanisms by Which Microbial Toxins Induce Inflammation and DNA Damage and Promote Tumorigenesis

Microbial toxins and inflammatory responses play critical roles in the initiation and progression of CRC. These processes are interconnected, as bacterial toxins can initiate inflammation, which, in turn, perpetuates DNA damage and tumorigenesis.

- **Inflammation:** Several bacteria linked with colorectal cancer, such as *Fusobacterium nucleatum* and *Bacteroides fragilis*, have the ability to cause long-lasting inflammation. *F. nucleatum* attaches to colonic cells by means of its FadA adhesion molecule, which triggers inflammatory signaling pathways including NF- κ B and enhances the production of cytokines such as interleukin-8 (IL-8). The presence of inflammation in the body creates an environment that supports the growth of tumors by increasing cell division and the formation of new blood vessels [18]. Bacterial toxins may cause inflammation, which can result in a breakdown of the protective layer of cells and an increase in the permeability of the gut. Bacteria and their metabolites may move over the epithelial barrier, worsening inflammation and encouraging the growth of tumors [19].
- **DNA Damage and Genotoxicity:** Specific gut bacteria generate genotoxins that directly harm the DNA of the host, resulting in genetic alterations and the development of cancer. Colibactin-producing *Escherichia coli*, for example, may induce double-strand breaks in the DNA of colonic epithelial cells. If these breaks are not adequately repaired, it might result in genomic instability, which is a characteristic feature of cancer. Furthermore, the toxin produced by *Bacteroides fragilis* may cause DNA damage by producing reactive oxygen species, thus exacerbating the development of colorectal cancer [17]. Reactive oxygen species, produced during chronic inflammation, are a significant contributing cause of DNA damage. Bacterial species often associated with colorectal cancer typically induce the production of reactive oxygen species, which may oxidize DNA bases and cause genetic changes. Oxidative stress has a crucial role in the initiation of colorectal cancer [20].
- **Tumor Microenvironment Modulation:** Pathogenic bacteria have the capacity to alter the tumor microenvironment to evade immune system recognition and promote tumor growth. *Fusobacterium nucleatum*, for example, lures myeloid-derived suppressor cells and impedes the function of natural killer (NK) cells, thus facilitating unrestricted growth of cancer cells. The imbalance of microbiota, known as dysbiosis, plays a crucial role in developing colorectal cancer by aiding the immune system evasion required for the progression of existing tumors [15].

5.3 Mechanisms of Gut Microbiota in Colorectal Cancer Pathogenesis

The gut microbiota, which consists of trillions of microorganisms, plays a significant role in colorectal cancer (CRC) pathogenesis. Through a variety of mechanisms—including inflammation, genotoxicity, and metabolic modulation—gut microbes contribute to cancer initiation, promotion, and progression (Table 5.1). In this section, we explore these mechanisms in detail.

5.3.1 Inflammation-Induced Tumorigenesis

Chronic inflammation is a well-recognized and documented condition that increases the chance of developing several types of cancer, including colorectal cancer. Inflammation in the colon may disturb the protective layer of cells, allowing germs to move across and causing an increase in the presence of immune cells. This sequence of events creates an environment that supports the growth of tumors, partly because of the ongoing production of pro-inflammatory cytokines. This, in turn, causes prolonged stress to cells and damage to DNA. Inflammatory bowel illnesses, such as Crohn’s disease and ulcerative colitis, provide us with clear illustrations of how long-lasting inflammation heightens the likelihood of colorectal cancer

Table 5.1 Mechanisms of gut microbiota in CRC pathogenesis

S. no.	Mechanism	Description	Key players
1	Inflammation-induced tumorigenesis	Chronic inflammation triggered by gut microbiota leads to immune dysregulation, promoting tumorigenesis through pathways such as NF-κB activation and cytokine release (e.g., IL-6, TNF-α)	<i>Fusobacterium nucleatum</i> , <i>Bacteroides fragilis</i>
2	Microbiota and genotoxic effects	Certain bacteria that induce DNA damage and mutations drive colorectal carcinogenesis	pks+ <i>Escherichia coli</i> , enterotoxigenic <i>Bacteroides fragilis</i>
3	Microbial metabolites and their impact on CRC	Microbial metabolites like short-chain fatty acids (SCFAs) have dual roles: butyrate promotes anti-inflammatory effects, while secondary bile acids (e.g., deoxycholic acid) have pro-carcinogenic properties	Butyrate, deoxycholic acid, hydrogen sulfide
4	Epigenetic regulation by microbiota	Gut microbiota influences epigenetic changes in CRC through histone modification, DNA methylation, and regulation of noncoding RNAs, impacting gene expression and tumor progression	Butyrate, <i>Fusobacterium nucleatum</i> , <i>Bacteroides</i> spp.

(CRC). Individuals diagnosed with inflammatory bowel disease (IBD) have a much greater likelihood of acquiring colorectal cancer (CRC) in comparison to the general population. This increased risk is attributed to the inflamed lining of their intestines, which produces a conducive environment for the occurrence of genetic mutations and the development of cancerous changes. Myeloid and lymphoid cells invade existing tumors, forming a cycle of interaction where “tumor-induced inflammation” continues to promote the survival and growth of cancer cells [21].

The presence of gut bacteria may have a substantial impact on the development of colorectal cancer (CRC) by triggering inflammatory reactions. One example is *Fusobacterium nucleatum*, which is a kind of bacteria often linked with colorectal cancer. It attaches to the cells on the surface of the colon via a chemical called FadA. This connection activates oncogenic pathways, such as β -catenin signaling, and induces the secretion of pro-inflammatory cytokines like IL-8 and tumor necrosis factor-alpha (TNF- α), which are known to cause cancer. Cytokines promote the formation of a tumor-friendly environment by increasing cell and blood vessel proliferation, thus accelerating tumor advancement [22]. Furthermore, bacteria such as *Escherichia coli* and *Bacteroides fragilis* possess the capacity to induce the production of reactive oxygen species (ROS) due to persistent inflammation. ROS, on the other hand, inflict damage on cellular DNA, leading to genetic defects that promote the growth of cancer. Microbial compounds, such as lipopolysaccharides (LPS), activate the Toll-like receptor 4 (TLR4) signaling pathway. This stimulus triggers the activation of the NF- κ B and MAPK pathways, which play a crucial role in regulating inflammation and tumor formation [23].

5.3.2 Microbiota and Genotoxic Effects

Specific gut microorganisms generate genotoxins that directly harm the DNA of the host cells, thus encouraging the occurrence of mutations and instability in the genome, which are characteristic signs of cancer formation. Colibactin, which is generated by some strains of *Escherichia coli* that have the pks pathogenicity island, is one of the most extensively researched microbial genotoxins. Colibactin causes DNA double-strand breaks, which, if not properly repaired, result in mutations and abnormalities in the chromosomes. Research has shown that colibactin-producing *E. coli* are more often found in the protective lining of the colon in individuals with colorectal cancer (CRC), indicating their significance in the development of CRC [24]. Another instance is the presence of enterotoxigenic *Bacteroides fragilis* (ETBF), a bacterium that generates the *Bacteroides fragilis* toxin (BFT). BFT causes DNA damage by generating reactive oxygen species (ROS) and triggers the activation of Wnt signaling, a critical pathway involved in cellular growth and the development of tumors. ETBF's continuous stimulation of this system causes inflammation and cellular instability, thus exacerbating the development of CRC

[16]. In addition to colibactin and BFT, there are additional microbial genotoxins that have been linked to the development of colorectal cancer. One example is the bacteria *Morganella morganii*, which is found in both individuals with inflammatory bowel disease (IBD) and colorectal cancer (CRC). This bacterium generates indolimines, a group of tiny molecules that are genotoxins. These genotoxins cause damage to the DNA and contribute to the development of tumors in animal models [24].

5.3.3 Microbial Metabolites and Their Impact on CRC

Short-chain fatty acids (SCFAs) are generated by the process of gut microorganisms fermenting food fiber. Short-chain fatty acids (SCFAs) have a wide range of impacts on the health of the colon and the risk of developing colorectal cancer. Specifically, butyrate is recognized for its defensive properties against CRC. It acts as the main energy source for colonocytes, stimulates anti-inflammatory reactions, and triggers programmed cell death in CRC cells by blocking histone deacetylases (HDACs) [25]. Nevertheless, short-chain fatty acids (SCFAs) might exhibit effects that vary depending on the situation. Although butyrate has tumor-suppressive characteristics, its concentration may decrease in persons with CRC due to a fall in the microbial populations responsible for its synthesis during dysbiosis. On the other hand, under some circumstances, short-chain fatty acids (SCFAs) such as acetate and propionate might contribute to the development of colorectal cancer (CRC) by acting as substances that bacteria use to undergo fermentation. This fermentation process results in the creation of detrimental metabolites such as hydrogen sulfide. The presence of two contrasting aspects underscores the complex nature of microbial metabolites in the development of colorectal cancer [9].

Additional microbial metabolites that have been linked to the development of colorectal cancer (CRC) include secondary bile acids, polyamines, and trimethylamine-N-oxide (TMAO). Gut bacteria convert main bile acids into secondary bile acids, including deoxycholic acid (DCA), which include pro-inflammatory and pro-carcinogenic properties. Higher concentrations of DCA have been linked to greater permeability in the intestines and DNA harm, which promotes the development of tumors [23]. Polyamines, such as spermidine and putrescine, have a role in the processes of cellular development and differentiation. The dysregulation of polyamine metabolism in colorectal cancer (CRC) is associated with the overexpression of genes, such as c-Myc, which controls polyamine production and promotes tumor development. Research has shown that bacteria such as *Bacteroides fragilis* have the ability to increase the activity of polyamine metabolism by means of spermine oxidase, which, in turn, enhances inflammation and the development of tumors [26].

5.3.4 *Epigenetic Regulation by Microbiota*

Cancer development is significantly influenced by epigenetic alterations, which are heritable variations in gene expression without corresponding changes in the DNA sequence. The gut microbiota influences epigenetic regulation in colorectal cancer (CRC) by metabolizing substances such as polyamines, bile acids, and short-chain fatty acids (SCFAs). Butyrate acts as an HDAC inhibitor in addition to providing colonocytes with energy. Because butyrate inhibits HDACs, histone acetylation is promoted. Tumor-suppressor genes are activated as a consequence of this process, which also stops the proliferation of cancer cells [25]. The gut microbiota also impacts the methylation patterns of DNA in the host genome. Anomalous DNA methylation is a distinctive characteristic of colorectal cancer (CRC), and an imbalance in the microbial community has been shown to impact the methylation state of crucial tumor-suppressor genes. One instance is the connection between *Fusobacterium nucleatum* and the excessive addition of methyl groups to genes responsible for DNA repair. This leads to an increase in genomic instability and the advancement of cancer [27]. Microbial metabolites might also impact the expression of noncoding RNA (ncRNA), which, in turn, controls gene expression in colorectal cancer (CRC). Specific gut bacteria influence the regulation of microRNA (miRNA) production, which may either stimulate or suppress the formation of tumors. For example, several microRNAs that are controlled by gut bacteria have been shown to decrease the activity of genes that are involved in programmed cell death and the regulation of the cell cycle, thereby enhancing the survival of colorectal cancer cells [25].

5.4 Specific Gut Microbes Associated with Colorectal Cancer

Colorectal cancer (CRC) is a multifactorial disease influenced by genetic, environmental, and microbial factors. The gut microbiota plays a critical role in modulating CRC risk by promoting or inhibiting tumorigenesis. Certain bacterial species are associated with pro-carcinogenic activities, while others exhibit anticarcinogenic properties. This section explores specific gut microbes and their mechanisms in CRC pathogenesis (Fig. 5.1).

5.4.1 *Pro-carcinogenic Bacteria*

5.4.1.1 *Fusobacterium nucleatum* and Its Association with Tumor Progression and Immunity Evasion

Fusobacterium nucleatum is a well-recognized bacteria that is strongly associated with the development of colorectal cancer. This microorganism is a kind of bacteria that lacks a certain type of stain and cannot survive in the presence of oxygen. It is often present in the mouth but has also been detected in the tumor tissues of patients

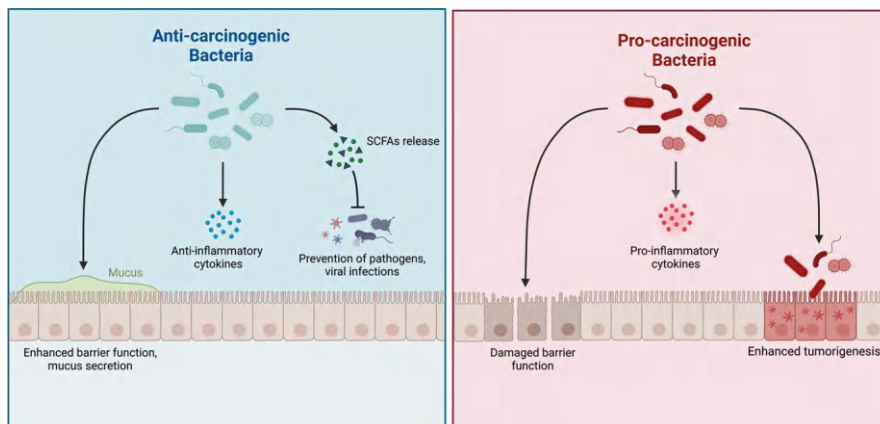


Fig. 5.1 Pro-carcinogenic and anticarcinogenic bacteria promoting or inhibiting tumorigenesis, respectively

with colorectal cancer. *Fusobacterium nucleatum* plays a role in the development of colorectal cancer (CRC) by many processes, such as evading the immune system, causing inflammation, and promoting tumor growth. *F. nucleatum*'s capacity to elude the immune system is a key factor in its promotion of carcinogenesis. The bacteria express FadA and Fap2 adhesins, which aid in the attachment of the bacterium to colonic epithelial cells. These adhesins stimulate cancer-causing signaling pathways, such as β -catenin, and hinder the body's immune response to tumors by attracting myeloid-derived suppressor cells (MDSCs), which reduce the function of cytotoxic T lymphocytes. The inhibition of the immune system enables the uncontrolled growth of cancer cells without being detected by the immune system [17].

In addition, *F. nucleatum* triggers long-lasting inflammation, which is a recognized contributing factor for colorectal cancer (CRC). The bacteria stimulate the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), by activating nuclear factor kappa B (NF- κ B) via Toll-like receptor 4 (TLR4) signaling. The presence of inflammation in the environment stimulates the growth of tumors by increasing the rate at which epithelial cells divide, survive, and develop new blood vessels [28]. Moreover, *F. nucleatum* has been linked to chemoresistance in CRC. Research has shown that the bacteria decreases the responsiveness of CRC cells to chemotherapy drugs like 5-fluorouracil, most likely by increasing the activity of genes that prevent cell death via the TLR4/ALPK1 pathway [29].

5.4.1.2 *Bacteroides fragilis* and Toxin-Mediated Pathways of Carcinogenesis

Bacteroides fragilis, namely its enterotoxigenic variant (ETBF), is a bacterium that has been associated with colorectal cancer. The ETBF bacterium releases the *Bacteroides fragilis* toxin (BFT), which is essential for encouraging the formation of tumors by causing inflammation and DNA damage. BFT causes disruption of the

colonic epithelial barrier, resulting in an increase in permeability. This allows for the transfer of germs and leads to the development of chronic inflammation [20]. BFT also triggers the Wnt/ β -catenin pathway, which is crucial for the growth and specialization of cells. This activation results in the excessive creation of reactive oxygen species (ROS), which induce DNA damage and mutations, thus promoting the onset and advancement of colorectal cancer. In addition, long-term exposure to BFT leads to continuous activation of nuclear factor kappa B (NF- κ B), which encourages the development of an inflammatory milieu that is favorable for the formation of tumors [17].

5.4.1.3 Role of *Escherichia coli* (Especially the pks+ Strain) in Promoting DNA Damage and Tumorigenesis

Colorectal cancer has been associated with certain strains of *Escherichia coli*, namely those that have the pks pathogenicity island. The *E. coli* strain pks+ generates colibactin, a genotoxin that causes DNA double-strand breaks in the cells of its host. Genotoxicity enhances chromosomal instability, which is a crucial factor in the development of tumors. Research has shown that pks+ *E. coli* is more often found in patients with colorectal cancer (CRC) compared to healthy persons, providing more evidence for its involvement in the development of cancer [30]. Evidence demonstrates that *E. coli* that produce colibactin are capable of triggering the DNA damage response, resulting in genetic abnormalities that facilitate the onset and advancement of cancer. In addition, *E. coli* may worsen inflammation, which, in turn, raises the risk of colorectal cancer by establishing an environment that promotes tumor growth [17].

5.4.2 Anticarcinogenic Bacteria

5.4.2.1 Beneficial Bacteria (e.g., *Lactobacillus* and *Bifidobacterium*) and Their Potential Protective Effects Against CRC

Certain kinds of gut bacteria have a preventive impact against colorectal cancer, whereas not all bacteria encourage its development. *Lactobacillus* and *Bifidobacterium* are two bacterial genera that are often linked to anticarcinogenic characteristics. Good microorganisms contribute to the maintenance of gut health by regulating the immune system, generating short-chain fatty acids (SCFAs), and suppressing the proliferation of harmful bacteria. *Lactobacillus* species are recognized for their capacity to generate lactic acid, which lowers the pH of the gastrointestinal tract, creating an inhospitable milieu for detrimental bacteria. Moreover, these bacteria have the ability to augment the function of the gut barrier by

stimulating the creation of mucins, which serve to safeguard the intestinal lining from harmful pathogens. *Lactobacillus* has shown the ability to regulate the immune system by stimulating the generation of anti-inflammatory cytokines like interleukin-10 (IL-10). This may effectively inhibit persistent inflammation and lower the likelihood of colorectal cancer (CRC) [31]. *Bifidobacterium* species, a different category of advantageous bacteria, generates acetate and butyrate, which are short-chain fatty acids (SCFAs) with qualities that combat inflammation and inhibit tumor growth. Butyrate, specifically, has been shown to impede histone deacetylase (HDAC), an enzyme implicated in the advancement of cancer. Butyrate enhances the expression of tumor-suppressor genes and triggers programmed cell death (apoptosis) in colorectal cancer cells by blocking HDAC activity [32].

5.4.2.2 Mechanisms of Protection (e.g., Modulation of Inflammation and Anticancer Metabolites)

Beneficial bacteria exert their benefits via many methods. One of the primary methods by which these bacteria defend against colorectal cancer is via the regulation of inflammation. Chronic inflammation significantly increases the likelihood of developing colorectal cancer (CRC). However, the presence of *Lactobacillus* and *Bifidobacterium* bacteria might mitigate this risk by stimulating anti-inflammatory processes and suppressing the production of pro-inflammatory cytokines. Another crucial pathway involves the synthesis of metabolites that have anticancer properties. As previously stated, short-chain fatty acids (SCFAs) like butyrate have a crucial function in suppressing the growth of cancer cells. Butyrate has the ability to trigger programmed cell death in cancer cells and also hinders the movement and infiltration of tumor cells, hence decreasing the likelihood of metastasis. Furthermore, research has shown that *Bifidobacterium*-produced acetate has the ability to strengthen the intestinal barrier, thereby inhibiting the movement of harmful bacteria and their toxins. This protective mechanism helps reduce the risk of inflammation and the development of tumors [31].

5.5 Gut Microbiota as Biomarkers and Diagnostic Tools

The gut microbiota has a crucial impact on the development of colorectal cancer (CRC), and their makeup and functioning have the potential to serve as biomarkers for early detection and diagnostic tools. The topic of microbiota-based diagnostics investigates the variations in microbial signatures between healthy persons and patients with colorectal cancer (CRC), with the goal of creating screening technologies that are noninvasive, affordable, and precise.

5.5.1 Microbiota Signatures in CRC

5.5.1.1 Differences in Microbial Composition Between Healthy Individuals and CRC Patients

Several studies have shown significant disparities in the makeup of gut microbiota between persons who are in good health and those who have colorectal cancer (CRC). These disparities often appear as dysbiosis, an interruption in the equilibrium between advantageous and detrimental bacterial species. CRC patients have an elevation in pro-inflammatory and possibly carcinogenic bacteria, including *Fusobacterium nucleatum*, *Bacteroides fragilis*, and pks+ *Escherichia coli*. Simultaneously, the levels of good bacteria, such as *Faecalibacterium prausnitzii*, *Lactobacillus*, and *Bifidobacterium*, are often reduced [33]. The rise of pathogenic bacteria such as *F. nucleatum* is especially significant since this microorganism is linked to immune system avoidance and the advancement of cancer. In addition, *Bacteroides fragilis* and its enterotoxigenic variant stimulate inflammation and cause DNA damage, thus playing a role in the development of cancer in the colon. Conversely, the reduction of helpful bacteria such as *Faecalibacterium* diminishes the body's ability to fight inflammation, making chronic inflammation worse and promoting the formation of tumors [34].

5.5.1.2 Potential of Microbial Markers for Early Detection and Prognosis of CRC

The unique microbial patterns seen in individuals with colorectal cancer (CRC) indicate that some bacteria or combinations of bacteria might potentially be used as dependable biomarkers for the early diagnosis and prognosis of CRC. Research has shown that microbiological indicators, such as *Fusobacterium nucleatum* and *Bacteroides fragilis*, are consistently higher in patients with colorectal cancer (CRC), suggesting their potential use as noninvasive screening tests. For example, increased concentrations of *F. nucleatum* in fecal samples have been associated with advanced stages of colorectal cancer (CRC) and a negative prognosis, emphasizing its usefulness not only as a diagnostic marker but also as a prognostic indication [35]. Stool samples obtained from patients with colorectal cancer (CRC) in a nested case-control study exhibited elevated levels of *F. nucleatum* and *clbA*+ bacteria. These bacteria are recognized for hosting the pks pathogenicity island, which is linked to DNA damage. When used together, these markers showed a sensitivity of 84.6%, making them a potential diagnostic approach for CRC [36].

5.5.2 *Fecal Microbiota Analysis and CRC Screening*

5.5.2.1 Current Advancements in Microbiota-Based Screening Tools

The development of advanced sequencing technologies and other molecular methods has made it easier to detect certain microbial patterns that are linked to colorectal cancer (CRC). An examination of the microbiota found in fecal samples, which entails examining the bacterial makeup, has become a viable method for screening for colorectal cancer (CRC). Fecal microbiota analysis offers a noninvasive alternative to conventional techniques such as colonoscopy or fecal occult blood tests (FOBTs) by identifying particular microbial patterns linked to malignant or precancerous areas. Recent research has investigated the use of fecal immunochemical tests (FITs) together with microbiota analysis to enhance the precision of diagnosing colorectal cancer (CRC) during screening. Studies have shown that FIT tubes, which are typically used for identifying hidden blood in feces, are effective in maintaining the microbial makeup of stool samples. Researchers may examine microbial communities and run routine FITs at the same time, which may enhance the sensitivity and specificity of CRC detection [37]. Research has shown that FIT tubes can maintain microbial fingerprints for a duration of 7 days, which makes them suitable for large-scale screening procedures. Crucially, the incorporation of microbial markers with FIT enhanced the rates of identifying advanced adenomas and early-stage CRC, which are vital for successful intervention [38].

5.5.2.2 Future Potential of Noninvasive Diagnostics Based on Gut Microbiota

As microbiome research progresses, the future of noninvasive diagnostics for colorectal cancer (CRC) is expected to focus on methods that are based on bacteria. Creating microbial panels or composite biomarkers that include various bacterial species is a potential strategy to improve the accuracy of colorectal cancer (CRC) diagnosis. These panels have the ability to differentiate between persons who are healthy, those who have precancerous adenomas, and those with colorectal cancer (CRC). This allows for early detection and reduces the need for intrusive operations. In a study that combined microbial and metabolomic analysis, researchers were able to identify a diagnostic panel of microbial species and blood metabolites that effectively differentiated between patients with colorectal cancer (CRC) and healthy individuals. The composite panel demonstrated a high level of accuracy for detecting colorectal cancer, as shown by its area under the curve (AUC) value of 0.994. This finding suggests that the panel has promising prospects for use in clinical settings [39, 40].

5.6 Modulation of Gut Microbiota as a Therapeutic Strategy for CRC

Since it is now well-known that the gut microbiota plays a significant role in the development of colorectal cancer (CRC), there is considerable interest in the manipulation of this microbial community as a therapeutic strategy for both CRC prevention and treatment. A balanced microbiota composition may help prevent the spread of cancer and improve the efficacy of treatment. A number of strategies, including dietary modifications, the use of probiotics, prebiotics, fecal microbiota transplantation (FMT), and postbiotics, have shown promise in this regard (Table 5.2).

Table 5.2 Overview of therapeutic strategies for CRC

Therapeutic intervention	Benefits	Mechanisms	Clinical applications
Dietary interventions (fiber-rich diets)	Promotes beneficial SCFA production, reduces inflammation, maintains gut integrity	Increases production of SCFAs (e.g., butyrate), reduces contact time between carcinogens and gut lining	Prevention of CRC, especially in high-risk populations
Probiotics	Restores microbial balance, reduces inflammation, enhances gut barrier function	Modulates immune responses, reduces inflammation, promotes anticarcinogenic microbial growth	Adjuvant therapy for CRC to reduce tumor progression and enhance treatment efficacy
Prebiotics	Promotes the growth of beneficial bacteria (e.g., <i>Lactobacillus</i> and <i>Bifidobacterium</i>), enhances SCFA production	Increases levels of SCFA-producing bacteria, enhances gut health by promoting beneficial microbial communities	Supplementary treatment to maintain gut health and reduce cancer risk
Fecal microbiota transplantation (FMT)	Restores healthy microbiota composition, reverses dysbiosis, enhances immune function	Transfers healthy microbiota from donor to recipient, reducing pathogenic bacterial load and enhancing immune responses	Potential therapy for CRC patients with dysbiosis, improving responses to immunotherapy and chemotherapy
Postbiotics	Anti-inflammatory and immunomodulatory effects without the risk of live bacterial infection	Postbiotics like butyrate inhibit histone deacetylase, promote apoptosis, and strengthen gut barrier function	Used as a complementary therapy in CRC, reducing inflammation and enhancing treatment outcomes

5.6.1 Dietary Interventions

5.6.1.1 Role of Fiber-Rich Diets in Maintaining a Healthy Microbiota and Reducing CRC Risk

Dietary fibers have a vital role in preserving gastrointestinal health and lowering the likelihood of colorectal cancer (CRC). Gut bacteria digest fiber, resulting in the synthesis of short-chain fatty acids (SCFAs), namely butyrate, which has anti-inflammatory and anticarcinogenic characteristics. Butyrate acts as the major fuel for colonocytes and has a crucial function in preserving the integrity of the gut barrier, decreasing inflammation, and triggering cell death in cancer cells [41]. Multiple epidemiological studies have consistently shown that communities that consume a significant amount of dietary fiber have a decreased incidence of colorectal cancer (CRC). A diet high in fiber stimulates the proliferation of good bacteria, such as *Bifidobacterium* and *Faecalibacterium*, while simultaneously decreasing the abundance of bad bacteria that cause inflammation. In addition, fiber aids in the regulation of bowel movements, reducing the duration of exposure between probable cancer-causing substances and the lining of the colon [42].

5.6.1.2 Impact of High-Fat, High-Protein, and Western Diets on CRC-Promoting Dysbiosis

Unlike diets that are high in fiber, Western diets, which are characterized by a high consumption of fat and protein, are significantly linked to dysbiosis that promotes colorectal cancer (CRC). According to research, eating a diet high in fat may alter the gut microbiota's composition, which can increase the amount of bacteria like *Bacteroides* and *Alistipes* that can cause cancer and create inflammation. Furthermore, a diet high in fat reduces the number of bacteria that create SCFAs, which weakens the intestinal barrier's integrity and fuels chronic inflammation. Both of these factors are important in the advancement of colorectal cancer (CRC) [42]. Dysbiosis has also been associated with high-protein diets, particularly ones that are abundant in red and processed meats. These diets stimulate the synthesis of detrimental metabolites, such as secondary bile acids and hydrogen sulfide, which have the potential to destroy DNA and facilitate the development of tumors. The detrimental effect of the Western diet on gut microbiota underscores the significance of dietary treatments in the prevention of colorectal cancer (CRC).

5.6.2 Probiotics and Prebiotics in CRC Prevention

5.6.2.1 Mechanisms of Probiotics in Preventing CRC

Probiotics are live microorganisms that, when consumed in adequate amounts, have positive effects on health. In the context of colorectal cancer, probiotics have shown anti-inflammatory qualities, enhanced the integrity of the gut barrier, and slowed the

growth of cancer. For instance, it has been shown that certain strains of *Lactobacillus* and *Bifidobacterium* bacteria control the immune response by inducing the production of anti-inflammatory cytokines and blocking pro-inflammatory pathways, such as NF- κ B activation [43]. In addition, probiotics improve the function of the gut barrier by stimulating the creation of mucins, which serve to protect the intestinal lining from harmful bacteria. By maintaining a healthy barrier in the gut, probiotics prevent the spread of bacteria and the subsequent activation of inflammatory pathways that lead to the development of colorectal cancer. Additionally, probiotics produce short-chain fatty acids (SCFAs) that directly inhibit the growth of tumor cells and cause programmed cell death (apoptosis) in order to prevent the development of cancer [44].

5.6.2.2 Role of Prebiotics in Promoting Beneficial Bacterial Growth

Prebiotics are indigestible fibers that especially promote the growth of beneficial bacteria, including *Lactobacillus* and *Bifidobacterium*. By encouraging the production of short-chain fatty acids (SCFAs), prebiotics reduce inflammation, maintain a healthy composition of the gut microbiota, and provide protection against colorectal cancer (CRC). In particular, research has shown that inulin, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS) limit the growth of harmful bacteria that may cause cancer while fostering the growth of bacteria that create short-chain fatty acids (SCFAs) [45]. Prebiotics also support the production of regulatory T cells and anti-inflammatory cytokines, which aid in the control of the immune system. A major factor in preventing the chronic inflammation that leads to colorectal cancer (CRC) development is immunomodulatory activity.

5.6.3 Fecal Microbiota Transplantation (FMT)

The goal of fecal microbiota transplantation (FMT) is to restore a healthy composition of gut microbiota by transplanting fecal matter from a healthy donor to a recipient. Fecal microbiota transplantation (FMT) has emerged as a promising therapeutic strategy for colorectal cancer (CRC) due to its potential to modulate immune function, balance gut microbiota, and prevent cancer from growing. Research is currently examining the potential applications of fecal microbiota transplantation (FMT) in the treatment of recurrent *Clostridium difficile* infections, including colorectal cancer (CRC) [45]. Studies indicate that FMT has the potential to enhance the effectiveness of cancer immunotherapy by boosting the immune response. Fecal microbiota transplantation (FMT) has shown the ability to enhance the population of advantageous bacteria, including *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*. These bacteria have been linked to better results in colorectal cancer (CRC) patients who are receiving immunotherapy. Moreover, FMT may help restore gut barrier function, reduce inflammation, and inhibit the growth of pro-carcinogenic bacteria [46]. Despite being in the early stages, clinical studies investigating the use

of FMT in CRC have shown encouraging preliminary findings. Research has shown that fecal microbiota transplantation (FMT) changed the makeup of the gut microbiota and increased the effectiveness of immune checkpoint inhibitors in patients with colorectal cancer (CRC). In addition, FMT has shown efficacy in alleviating chemotherapy-induced adverse effects, such as diarrhea, by reinstating equilibrium in the gut microbiota [45].

5.6.4 Postbiotics in CRC Therapy

Postbiotics refer to bioactive substances that are produced by the bacteria residing in the gut. These substances include short-chain fatty acids (SCFAs), peptides, and cell wall components. They provide various health advantages to the host. Postbiotics, unlike probiotics, are inanimate compounds that may have comparable therapeutic benefits without the potential hazards associated with live bacteria, such as infection or bacterial translocation [47]. Postbiotics in the treatment of colorectal cancer have shown the capacity to modulate the immune system by inducing the production of cytokines that inhibit inflammation and enhancing the activity of immune cells, such as macrophages and T cells. For example, butyrate, a well-recognized postbiotic, has anti-inflammatory and anticarcinogenic characteristics. It suppresses the NF- κ B pathway, decreases inflammation, and triggers apoptosis in cancer cells. Postbiotics also improve gut barrier integrity by stimulating the formation of mucins, which protect the intestinal lining from harmful bacteria and toxins [48].

5.7 Challenges and Future Directions in Gut Microbiota Research for CRC

Recent years have seen tremendous advancements in the research on gut microbiota and its involvement in colorectal cancer (CRC). Nevertheless, there are still several obstacles to overcome in comprehensively grasping the role of microbial communities in CRC pathogenesis as well as determining how they might be manipulated for therapeutic benefits. This section examines the primary obstacles, prospects for customized microbiome-centered treatments, research that may be applied to practical use, and the constraints of present research.

5.7.1 Complexity of the Microbiota–Host Interaction

A major obstacle in gut microbiota research lies in the complex nature of the interaction between the bacteria and the host. The gut microbiome consists of a varied collection of bacteria, viruses, fungi, and archaea. These microorganisms not only

interact with each other but also with the immune system, epithelial cells, and metabolism of the host. The complex network of interactions makes it challenging to pinpoint the exact bacteria or microbial metabolites that directly contribute to the development of colorectal cancer (CRC). The diversity of the gut microbiota is also impacted by other variables, including nutrition, genetics, antibiotic use, and lifestyle, which may differ significantly across people. The heterogeneity in data presents a difficulty in establishing consistent results across diverse groups. Furthermore, while specific bacteria like *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *Escherichia coli* have regularly been linked to colorectal cancer (CRC), our understanding of the whole microbial ecology and its role in cancer development is still limited [49].

Furthermore, the involvement of the human immune system in reacting to changes in microorganisms adds an additional level of complexity. The immune system may either facilitate or hinder the growth of tumors, depending on the circumstances. For instance, whereas inflammation caused by the microbiota might contribute to the development of cancer, certain immunomodulatory activities of beneficial bacteria may aid in inhibiting the formation of tumors. Comprehending this equilibrium is essential for the development of efficacious treatments [50].

5.7.2 Personalized Microbiome-Based Therapies

Due to the complicated and fluctuating nature of the gut microbiota, tailored medicines that use the microbiome are becoming a potential approach for treating colorectal cancer (CRC). The microbiota of each person is distinct, influenced by their genetic makeup, dietary choices, and surroundings. Personalized treatments seek to manipulate the gut microbiota in a manner that is customized to an individual's unique microbial makeup and illness condition. For instance, fecal microbiota transplantation (FMT) has shown potential in reestablishing a healthy microbial equilibrium in patients with colorectal cancer (CRC), especially when used in conjunction with immunotherapy. Nevertheless, the results of FMT might differ greatly across people, influenced by variables such as the microbiota of the donor and the initial microbial composition of the recipient [41].

Research is now being conducted on personalized probiotic medicines. These treatments include the administration of certain strains of bacteria, such as *Lactobacillus* or *Bifidobacterium*, which have been shown to have anti-inflammatory and anti-tumorigenic properties. Nevertheless, the difficulty is in determining the optimal assortment of bacterial strains for each patient, as people may exhibit distinct microbial imbalances. When designing personalized therapy, it is important to consider the patient's diet and other lifestyle characteristics, since they might affect how well microbial interventions work [51].

5.7.3 *Translational Research and Clinical Applications*

Translational research seeks to connect the divide between fundamental scientific knowledge and practical clinical applications, facilitating the use of discoveries from microbiome research in the prevention, diagnosis, and treatment of colorectal cancer (CRC). Various clinical uses have arisen, such as using microbial signatures as biomarkers for diagnosis and prognosis. Stool samples may be used to analyze the microbial composition, which can help identify colorectal cancer (CRC) at an early stage or predict how patients will respond to therapy. For instance, elevated levels of *Fusobacterium nucleatum* in fecal samples have been linked to a worse prognosis in individuals with colorectal cancer (CRC). Furthermore, some microbial indicators have been associated with improved reactions to immunotherapy, indicating that analyzing the microbiome might be used to customize treatment approaches [52].

Nevertheless, there are still obstacles to converting microbiome research into therapeutic use. A significant obstacle is the absence of standardized techniques for microbial profiling. Varied research often uses diverse sequencing technologies, sample-collecting methodologies, and data processing procedures, resulting in incongruities in findings. Establishing techniques for microbiome analysis is crucial to guaranteeing the repeatability and dependability of results in clinical contexts [53]. Another obstacle is in comprehending the mechanisms by which microbial therapies, such as probiotics or fecal microbiota transplantation (FMT), interact with other cancer treatments, such as chemotherapy or immunotherapy. Although several studies have shown that adjusting the microbiota may improve the effectiveness of these treatments, more study is required to establish the best time and dosage of microbial interventions when used with conventional medications [46].

5.7.4 *Limitations in Current Research*

Although there have been notable progressions, there are still several constraints in the field of gut microbiota research for colorectal cancer (CRC). An important constraint is the dependence on observational research, which may just illustrate connections between alterations in microorganisms and CRC but cannot show a cause-and-effect relationship. Although certain bacterial species have been linked to colorectal cancer (CRC), it remains uncertain if these bacteria are actively causing the development of cancer or just flourishing in the changed environment of a colon with tumors [54]. Another constraint arises from the challenge of accurately representing the complex nature of the human gut microbiome in animal-based research. Mice and other animal models often exhibit significantly distinct microbiomes in comparison to people, which poses a challenge in extrapolating results from pre-clinical investigations to human populations. In addition, several animal studies only concentrate on individual bacterial species, disregarding the wider microbial

ecology and its interactions with the host. Furthermore, there are obstacles associated with the safety and effectiveness of microbial therapies. Although FMT has shown potential in the treatment of certain illnesses, its long-term safety in individuals with CRC remains uncertain. Likewise, the utilization of live probiotics poses a potential danger of infection in persons with weakened immune systems. Further investigation is required to have a comprehensive understanding of the potential dangers and advantages of these treatments among various groups of patients [43].

5.8 Conclusion

In conclusion, the evidence linking gut microbiota to colorectal cancer (CRC) is substantial, highlighting its role in tumor initiation, progression, and therapeutic outcomes. Dysbiosis, characterized by an imbalance between beneficial and harmful bacterial species, has been shown to promote inflammation, DNA damage, and immune dysregulation, all of which contribute to CRC pathogenesis. Specific bacteria, such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *Escherichia coli*, have been identified as pro-carcinogenic agents, while beneficial bacteria like *Lactobacillus* and *Bifidobacterium* offer protective effects. The dynamic interaction between the gut microbiota and host immune system reveals how microbial communities influence cancer risk and progression. Looking ahead, targeting the gut microbiome presents a promising strategy for CRC prevention, diagnosis, and treatment. Dietary interventions, probiotics, prebiotics, fecal microbiota transplantation (FMT), and postbiotics offer novel approaches to modulate the microbiota and restore microbial balance, reducing CRC risk and enhancing treatment efficacy. Personalized microbiome-based therapies, tailored to an individual's specific microbial profile, hold significant potential for improving outcomes and minimizing adverse effects in CRC patients. Furthermore, leveraging microbial signatures as diagnostic biomarkers could enhance early detection efforts, making screening more effective and less invasive. As research continues to advance, understanding and manipulating the gut microbiome will likely become a cornerstone of CRC management, offering new avenues for prevention, early intervention, and improved patient outcomes.

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Chapter 6

Gut Microbiota in Colorectal Cancer: Fundamentals, Analytical Techniques, and Its Limitations



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Abstract One of the main causes of cancer-related death globally is colorectal cancer (CRC). Emerging research highlights the gut microbiota's important involvement in CRC aetiology, underscoring the complex interplay between host genetics, microbial dysbiosis, and tumour development. This chapter delves into the fundamental aspects of gut microbiota in CRC, exploring how microbial imbalances can promote or suppress tumorigenesis through mechanisms like inflammation, immune modulation, and production of carcinogenic metabolites. In addition to examining key microbial species implicated in CRC, such as *Fusobacterium nucleatum* and *Bacteroides fragilis*, the chapter outlines the most widely used analytical techniques for studying gut microbiota. These techniques include 16S ribosomal ribonucleic acid (rRNA) sequencing and metabolomics, which have enabled the identification of microbial signatures associated with cancer progression. Despite the advances in microbiome research, significant limitations persist. Furthermore, the lack of standardized protocols for microbiota analysis complicates reproducibility across studies. This chapter highlights these limitations and calls for the development of more refined techniques and integrative approaches that combine microbiome analysis with other omics technologies. In summary, comprehending the function of gut microbiota in colorectal cancer (CRC) is crucial for creating innovative approaches for diagnosis, prognosis, and treatment. This chapter delves into a detailed overview of the current knowledge, methodologies, and challenges in gut microbiota research in the context of colorectal cancer.

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6.1 Introduction

6.1.1 An Overview of the Gut Microbiota

The term “gut microbiota” describes the microbes that live in the gastrointestinal (GI) system of humans. This dynamic community is mostly made up of species from the prokaryotic domain, which includes bacteria and other unicellular organisms without specialized organelles [1]. Fungi, parasites, and archaea are also present to a lesser degree. Another component of this ecosystem is viruses. The gut microbiome is the collective name for the genetic and functional make-up of microbial species [2]. Humans can be categorized more broadly based on their gut microbial compositions, or enterotypes, which are identifiable patterns that are shared by certain groups. The human gut microbial species have been shown to have 3.3 million genes in a single individual [3]. The bulk of the gut microbiota is composed of strict anaerobes, which are two to three orders of magnitude more abundant than facultative anaerobes and aerobes [4]. The human gut microbiota is dominated by just two bacterial phyla—the *Firmicutes* and the *Bacteroidetes*—despite the identification of over 50 different bacterial phyla. *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Fusobacteria*, and *Cyanobacteria* are present in lesser quantities [5]. Although the number of bacterial species found in the human stomach varies greatly throughout research, it is usually agreed upon to be between 500 and 1000. However, a recent study involving several participants revealed that more than 35,000 different kinds of bacteria may make up the whole human gut microbiota [4]. Furthermore, there are about twice as many bacterial as human cells in the human body. Based on genetic similarities, bacteria may be categorized using phylogenetic nomenclature into several tiers of groupings known as taxonomic ranks [6]. Greater genetic sequence similarity between organisms belonging to the same lower taxonomic rank groupings indicates more recent evolutionary divergence [7]. This microbial community’s composition is host-specific, changing throughout the course of a person’s life and being altered by both endogenous and external factors. Its pivotal role in health and illness has been highlighted by the recent resurgence of interest in the composition and operation of this “organ.” Numerous facets of typical host physiology, including behaviour, stress response, and nutritional status, are closely influenced by the microbiota [8, 9]. Furthermore, they can impact both nearby and distant organ systems and be a primary or secondary cause of several illnesses. For there to be homeostasis at the intestinal mucosa and elsewhere, there must be a balance in the general make-up of the gut microbial population as well as the existence or non-existence of essential species that might elicit certain reactions [10]. The ways in which microbiota affect health are still mostly unclear, although

they involve the development of signalling molecules and the identification of bacterial epitopes by intestinal epithelial cells as well as mucosal immune cells. The development of gut microbiota modelling and analysis will increase our understanding of the role these microorganisms play in health and illness, enabling the personalization of current and future therapeutic and preventive strategies [11, 12].

6.1.2 *Significance of Gut Microbiota in Human Health*

The human gut is home to a rich microbial ecology that is made up of millions of bacteria. The gut microbiota has been described as a vital organ that connects with other organs in a variety of ways. To promote host–microbe interactions, the gut microbiota axis interacts with the neurological, endocrine, humoral, immunological, and metabolic pathways. Most human non-pathogenic gut microorganisms coexist symbiotically with their hosts and are frequently connected to the host's defences against pathogenic invasion. Thus, dysbiosis of the gut microbiota is linked to a range of human disorders, such as anxiety, depression, hypertension, cardiovascular disease, obesity, diabetes, inflammatory bowel disease (IBD), and cancer [13–15]. The mechanism leading to the disease development has a crucial correlation with gut microbiota, metabolic products, and host immune response in humans. The method by which gut microbiota exerts its beneficial or detrimental effects is still largely unknown. Nonetheless, a growing body of recent global clinical research is establishing a connection between certain microbial species and eubiosis in both health and illness [16]. Several studies involving populations have established the highly beneficial role that the human gut microbiota plays in healthy individuals. They have also highlighted the importance of understanding the composition and structure of this population's microbiome and the factors that influence it, such as age, location, systemic illnesses, and medications [17]. The phyla *Firmicutes*, *Bacteroides*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* account for the majority of the significant resident bacterial populations found in the gut microbiome. The first step in identifying the symbiotic interactions between intestinal bacteria and their hosts is identifying the balanced composition of the gut microbiota and alterations associated with illness. When the host is in good health, microorganisms live together in mutual association and influence the host's well-being by regulating nutritional metabolism [18], defending against pathogens, and communicating with immune cells to improve host immunity and physiology [19]. Due to the challenges in cultivating some bacteria, a number of *in vivo* and *ex vivo* studies have revealed an initial underestimation of the total number of microbial species in the gut. In addition to helping the body break down carbohydrates, regulating the gut microbiota, and defending against disease invasion, bacteria and *Protobacteria* also boost immunity. For the most part, the dietary substrates that microbes in the gut use to live are incompletely digested foods from the upper digestive system. Bacteria frequently resort to other energy sources when there are insufficient carbohydrates available for fermentation. This might lead to the

development of other metabolites that could be more dangerous for human health. Fermentation of saccharolytic bacteria typically yields advantageous by-products. Methane production from H_2 through bacterial metabolism depends on the human-associated archaea *Methanobrevibacter smithii*. It is an important and well-known archaeon in the gut microbiota [20]. While nutrition is widely believed to be important for promoting health and managing diseases, new studies have shown that diet also has a significant impact on the host's metabolism and the make-up of the gut microbiota. In turn, the gut environment can support the development, reproduction, and survival of the microbial population [21]. Carbohydrates are a vital and significant source of energy, and the gut bacteria provide them to the host throughout the fermentation step. The coexistence of the host and gut flora in balance is essential for human health; any disruption of this balance is linked to a variety of diseases, such as diabetes, obesity, hypertension, cardiovascular diseases, and inflammatory bowel disease (IBD) [22]. The study of the human microbiome is still in its early phases, but it aims to fill up knowledge gaps on the microbiome–host interaction, its role in disease aetiology, and its therapeutic value. Therefore, further in-depth research is needed to completely comprehend this fascinating yet enigmatic topic of study [23]. Human gut microbiota may also be involved in the production of essential vitamins, such as biotin, folate, and vitamin K, as well as the neutralization of carcinogens like pyrolysates. It has been demonstrated that a wide range of microbial substances that adhere to certain host membranes or nuclear receptors mostly affect host metabolism [24]. Gut microbiota is associated with most of the important processes involved in host physiology and maintenance, including nervous system development, intestinal development, appetite management, etc. [25].

6.1.3 Connection Between Gut Microbiota and Colorectal Cancer

The incidence of colorectal cancer (CRC) is predicted to rise gradually due to changes in Western food patterns over the world, adding two million additional cases by 2030. According to the report, around 90% of CRC cases are incidental, and the remaining 10% are brought on by exposure to particular environmental variables or genetic factors. Specifically, lifestyle variables that include obesity, poor fibre intake, alcohol consumption, smoking history, physical inactivity, and a Western diet are key contributors to colorectal cancer [26]. The alterations in the gut microbiota can be caused by the majority of these environmental variables. A person's vulnerability to colorectal cancer (CRC) or the progression of a tumour is influenced by changes in their gut microbiome, which have been linked to inflammation, DNA damage, and bacterial metabolites, according to a number of studies. Numerous research findings have indicated that the host's gut microbiota and colorectal cancer (CRC) development are closely related. Studies using high-throughput sequencing (HTS) to analyze the microbiome community in

tumour-formed and normal colon tissues have allowed for a better understanding of the differences in the gut microbiota between patients with colorectal cancer and healthy persons [27]. According to reports, people with colorectal cancer had a lower level of gut microbiota richness and variety. Researchers have hypothesized that the gut microbiome of patients with colorectal cancer (CRC) may be more affected by specific microbial groups than that of healthy individuals. In the gut microbiota of CRC patients, a total of 11 operational taxonomic units (OTUs) from the genera *Enterococcus*, *Escherichia/Shigella*, *Klebsiella*, *Streptococcus*, and *Peptostreptococcus* were found to be significantly more abundant, whereas five OTUs from the *Lachnospiraceae* family and *Roseburia* were found to be less abundant. Furthermore, a disturbance in the equilibrium of microorganisms was seen in colorectal cancer patients' gut microbiomes, leading to dysbiosis [28]. Increased intestinal permeability and gut microbial dysbiosis lead to colonic inflammation, which may accelerate the development of colorectal cancer (CRC). When comparing human CRC patients to healthy individuals, there is a notable rise in *Fusobacterium nucleatum* (*F. nucleatum*). Furthermore, the make-up of the microbiome differs in individuals with advanced adenoma (early-stage CRC) from those with definitive CRC (late-stage CRC) [29].

6.2 Fundamentals of Gut Microbiota

6.2.1 Composition and Diversity of Gut Microbiota

The human gastrointestinal (GI) tract, which is home to a vast and diverse microbial ecology, contains more than 100 trillion bacteria. One of the microbial habitats on Earth with the densest population is the colon; estimations of its bacterial cell density range from 10^{11} to 10^{12} per millilitre [30]. The human genome has about 23,000 genes, whereas the gut microbiome contains nearly three million genes that produce thousands of chemicals. The gut microbiota has long piqued the curiosity of scientists, but one of the main obstacles to pertinent study has been the cultivation of these microbes. We can refer to the host and the microorganisms living within it as “superorganisms” in light of the gut microbiota's traits, which include great variety, stability and durability, and symbiotic relationships. The gut microbiota is made up of several microorganism species, such as viruses, yeast, and bacteria [31]. Bacteria are categorized taxonomically based on their phyla, classes, orders, families, genera, and species. Out of almost 160 species, only a few phyla are represented. *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia* are the predominant microbial phyla found in the gut. Of these, 90% are found in the two phyla. More than 200 genera, including *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminococcus*, make up the *Firmicutes* phylum [32]. The *Clostridium* genera comprise 95% of the *Firmicutes* phylum. Among the *Bacteroidetes*, *Prevotella* and *Bacteroides* are two of the most prevalent

genera. The main representative of the somewhat rarer *Actinobacteria* phylum is the genus *Bifidobacterium*. The microbiota becomes more diverse as one ages, eventually reaching a stable adult composition dominated by three bacterial phyla [33].

6.2.2 Key Microbial Species and Their Roles in CRC

Published in 1967, a study demonstrated how gut microbiota mediated the carcinogenic effects of cycasin in germ-free mice. Many researchers have discovered a connection between the emergence of colorectal cancer and dysbiosis of the gut microbiota. There is growing evidence that changes to the gut microbiota impact the course of colorectal cancer. The term “gut dysbiosis” describes the functional and compositional changes brought on by an unbalanced population of opportunistic and symbiotic bacteria [34]. According to a 2017 study, giving germ-free, conventional mice faeces samples from CRC patients increased the risk of intestinal carcinogenesis. Scientific studies using germ-free mice and rats as models for colorectal cancer have found that these animals tend to develop fewer or smaller tumors compared to animals raised in normal conditions with exposure to bacteria and other microbes. This suggests that the presence of certain microorganisms in the gut may contribute to tumor growth in colorectal cancer. The microbiome exhibits modifications not just at the location of the tumour but also in neighbouring healthy tissues that share the same microbial make-up [35]. Hepatocellular carcinomas, pancreatic cancer, and breast cancer are among the various malignancies that have been connected to changes in the gut microbiota. Human studies have demonstrated that the gut microbiota of CRC patients is different from that of healthy individuals, exhibiting a greater number of pro-carcinogenic bacteria and a lower concentration of commensal bacteria. Research has also revealed variations in the mucosal and faecal microbiota of CRC patients [36]. Touchefeu et al. discovered that CRC patients had considerably lower levels of *F. prausnitzii*, *Eubacterium siraeum*, *Bacteroides eggerthii*, *Alistipes finegoldii*, and *Barnesiella intestinihominis* than controls. In faecal and mucosal samples, a number of researchers have attempted to delve deeply into the microbiome make-up linked to colorectal cancer. These investigations have discovered a change in the CRC microbiota’s worldwide make-up [37]. Numerous investigations have revealed that CRC has less bacterial diversity and more of some pro-tumorigenic microorganisms. *Fusobacterium nucleatum*, *E. coli*, *Bacteroides fragilis*, *Streptococcus bovis/gallolyticus*, *Clostridium septicum*, *Enterococcus faecalis*, and *Peptostreptococcus anaerobius* are among the bacterial species that are frequently linked to colorectal carcinogenesis. Seven bacterial species were found to be enriched in CRC in a core set of 536 faecal shotgun metagenomes, according to a meta-analysis. *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Parvimonas micra*, *Prevotella intermedia*, *Porphyromonas asaccharolytica*, *Alistipes finegoldii*, and *Thermanaerovibrio acidaminovorans* were among them [38]. 29 core species were found to be enriched in CRC in eight distinct geographic locations according to a 2019 study. According to Wang et al., the gut microbiota of CRC patients

contained significantly higher abundances of *Bacteroides fragilis*, *Enterococcus*, *Escherichia/Shigella*, *Klebsiella*, *Streptococcus*, and *Peptostreptococcus* compared to lower abundances of *Roseburia* and other butyrate-producing bacteria belonging to the *Lachnospiraceae* family. According to studies, Th17 cell response activation and DNA damage are the two ways that specific bacteria, such as *E. coli*, *Bacteroides fragilis*, and *Peptostreptococcus anaerobius*, are linked to colorectal carcinogenesis [39].

6.2.3 Mechanisms of Microbiota Interaction with the Host

Direct cell contacts and metabolites generated by the host, environment, or bacteria and absorbed by the bacteria both have an impact on microbiota interactions. They consist of butyrate, propionate, and acetate, which are short-chain fatty acids (SCFAs) [40]. Other examples include trimethylamine N-oxide, tryptophan, polyamines, metabolites of linoleic acid, vitamins, and secondary bile acids. These metabolites control the cell cycle, neural signalling, bile acid and cholesterol metabolism, immunological responses, and responses to antioxidants. Knowing the host–microbiota pathways and how they are modulated can help find tailored treatment targets for a variety of illnesses [41]. Complementary microorganisms, secretory immunoglobulin A, and antimicrobial peptides are found in the outer mucus layer. In a healthy condition, the epithelium layer and bacteria are kept apart by a physical barrier formed by the inner mucus layer. Colonocytes or enterocytes, goblet cells, enteroendocrine cells, Paneth cells, tuft cells, and M cells make up the epithelial layer [42]. These cells are joined by junctional complexes made of transmembrane proteins, namely claudin, occludin, and tricullulin. Dynamic interactions between bacteria that result in complex metabolic networks define the microbiota. Functional activities are influenced by interactions between microbes. Antagonistic interactions result from secreted compounds being absorbed by other microorganisms or environmental detoxification [43]. Whereas *Bacteroidetes*, one of the most prevalent phyla in the gut microbiota of people in developed nations, generally has antagonistic associations, some bacteria belonging to the phyla *Actinobacteria* and *Firmicutes* primarily have agonistic connections [44, 45]. Nutritional interactions may be divided into two groups: syntrophic metabolism, in which bacteria consume chemicals created by other bacteria, and nutrient competition, in which bacteria fight for scarce resources. Colonizing gnotobiotic mice with *Eubacterium rectale* and *B. thetaiotaomicron* can demonstrate syntrophic metabolism. When *B. thetaiotaomicron* is present, *E. rectale* up-regulates phosphoenolpyruvate carboxykinase, suggesting a carbon dioxide competition. Propionate concentrations are lower when *E. rectale* and *B. thetaiotaomicron* interact than when *B. thetaiotaomicron* acts alone. This is probably because *E. rectale* reduces carbon dioxide, which encourages *B. thetaiotaomicron* to make acetate instead of propionate. This makes it possible for *E. rectale* to produce more adenosine triphosphate (ATP) by converting acetate to butyrate [46–49].

6.2.4 Influence of Nutrition and Lifestyle on Gut Microbiota

One of the most potent factors influencing the activities of the gut microbiota is diet. The intricate interactions between the immune system and the intestinal barrier underscore the crucial role that nutrition plays in both the aetiology and management of many illnesses [50]. Long-term Western diet (WD) intake has a significant negative influence on gut flora compared to a Mediterranean diet (MD), which increases the risk of obesity and metabolic illnesses. Several studies revealed that the gut microbiota's diversity was declining and that its composition had changed significantly [51]. The abundances of *Firmicutes*, *Proteobacteria*, *Alistipes* species, *Bilophila* species, and *Enterobacteriaceae* may also rise as a result of WD. These bacterial groups and species have a significant role in dysbiosis and illness. Beneficial bacteria may be reduced in quantity by WD. In comparison to poorer MD adherence, better adherence to MD may be linked to improvements in the variety and richness of the gut microbiota, a decrease in the levels of *Escherichia coli*, a higher abundance of *Bifidobacteria*, a higher level of total SCFAs, and a lower ratio of *Firmicutes*-to-*Bacteroidetes* [52]. MD would be linked to more anal gas evacuations and increased bacterial richness in comparison to WD. However, in persons with significant beta diversity, these impacts could be less pronounced. In contrast to WD, MD includes less processed food, saturated fats, and some amino acids (L-carnitine and choline), favouring plant-based diets, consumption of mono- and polyunsaturated fats, and consumption of fibre. As a result, the pathogenic bacterial family *Proteobacteria* decreases and the variety of microorganisms increases [53]. Eating no meat or fish of any kind is part of the vegetarian diet. A vegan diet is a subset of vegetarianism that abstains from all animal products, including dairy, honey, and eggs. Plant-based diets take the role of animal-based diets, significantly boosting the consumption of resistant starch and insoluble fibre (cereals, legumes, and nuts) [54]. Vegetarian and vegan diets may be linked to increased *Bacteroides/Prevotella* ratios; increased abundances of *Bacteroides thetaiotaomicron*, *Clostridium clostridioforme*, *Klebsiella pneumoniae*, and *Faecalibacterium prausnitzii*; and decreased levels of *Bilophila wadsworthia* when compared to an omnivore diet. However, levels of methane and faecal SCFAs in omnivore and vegan/vegetarian diets did not substantially vary in other investigations [55]. According to other research, compared to an omnivorous diet, a vegan diet might significantly lower the abundance of potentially hazardous metabolites including aromatic compounds, branched chain amino acids (BCAAs), and lithocholic acid and boost the formation of SCFAs and their derivatives. A 3-month vegetarian diet or a short-term MD dietary intervention does not significantly alter the gut microbiota of the family [56]. At the genus level, MD may considerably alter the quantity of *Anaerostipes*, *Streptococcus*, and *Odoribacter*, whereas a vegetarian diet may enhance the number of *Enterorhabdus*, *Lachnoclostridium*, and *Parabacteroides*. In terms of SCFAs, a vegetarian diet lowers propionic acid synthesis, but an MD might

enhance it [57]. To clarify the advantages and disadvantages of adopting a vegan or vegetarian diet on intestinal health, further research is necessary given the body of current data and the complexity of assessing outcomes. It has been demonstrated that patients who follow a gluten-free diet (GFD), which entails removing all gluten-containing foods, may restore normal intestinal mucosa in both non-celiac gluten sensitivity and celiac disease patients [58]. GFD is a legitimate celiac disease treatment plan since it may help restore gluten tolerance when used with pre- and probiotics. Gluten-free diet (GFD) is being administered without a prescription more often in healthy patients; nonetheless, faecal samples from healthy individuals who followed a GFD for a month revealed an increase in *Enterobacteriaceae* and *Escherichia coli* abundances and a decrease in *Bifidobacterium*, *Clostridium lituseburense*, and *Faecalibacterium prausnitzii* levels. In individuals who have undergone a brief GFD (4 weeks), a reduction in the number of the families *Veillonellaceae*, *Bifidobacterium*, *B. longum*, and *Lactobacillus* is indicative of dysbiosis [59]. Different dietary types have different flora of microbes, which is illustrated in Table 6.1.

Table 6.1 Illustration of microbial diversity based on various diets with their significant characteristics

Dietary type	Dominant microbiota phyla	Microbial diversity	Characteristics	References
Western diet	<i>Bacteroidetes</i> , <i>Firmicutes</i>	Low-to-moderate	High fat and sugar intake, low fibre, promotes inflammation and reduced diversity	[60]
Mediterranean diet	<i>Firmicutes</i> , <i>Proteobacteria</i> , <i>Actinobacteria</i>	High	High intake of fruits, fibres, vegetables, healthy fats linked with higher diversity and inflammatory bacteria	[61]
Vegetarian diet	<i>Bacteroidetes</i> , <i>Firmicutes</i>	High	High fibre intake, promotes beneficial microbes such as <i>Prevotella</i> and <i>Bifidobacteria</i>	[62]
Ketogenic diet	<i>Firmicutes</i> , <i>Proteobacteria</i>	Moderate-to-low	High fat, low carbohydrate diet, decreases <i>Bifidobacteria</i> and increases <i>Firmicutes</i>	[63]
High-protein diet	<i>Firmicutes</i> , <i>Bacteroidetes</i>	Moderate	High protein, low fibre, increases bile tolerant bacteria, for example, <i>Alistipes</i> and <i>Bilophila</i>	[64]
High-fibre diet	<i>Bacteroidetes</i> , <i>Firmicutes</i>	Very high	Rich in fruits and legumes, increases beneficial microbes like <i>Bifidobacteria</i> and <i>Lactobacillus</i>	[65]
Traditional/rural diet	<i>Bacteroidetes</i> , <i>Firmicutes</i>	Very high	High in complex carbohydrates, foster diverse and resilient microbiota	[66]

6.3 Gut Microbiota and Colorectal Cancer

6.3.1 *Development of Colorectal Cancer*

Genetic changes in oncogenes and tumour suppressor genes occur as normal epithelium transitions to dysplastic tissue in the adenoma–carcinoma progression pathway, which leads to the development of colorectal cancer. Adenocarcinoma develops from normal glandular epithelium when genetic and epigenetic changes to the genome combine. The aetiology of colorectal cancer is linked to many genetic pathways, yet the molecular mechanism behind the disease's unregulated development still remains unclear [67–69]. The development of CRC is influenced by a multitude of molecular pathways, three of which are particularly important as a result of epigenetic and genetic changes: CpG island methylator phenotype (CIMP), microsatellite instability (MSI), and chromosomal instability (CIN). The CpG island methylator phenotype, chromosomal instability, and microsatellite instability are the three molecular pathways that have been found to indicate the progression of cancer. The rate at which a tumour grows and spreads can be influenced by various molecular pathways as each one has a unique epidemiology, mutational trial history, and immune response. Different methods may be used to treat the same illness [70, 71]. Elevated microsatellite changes at certain tetranucleotide repeats have been shown to modulate the three biological pathways, which is how a biomarker known as “elevated microsatellite alterations at selected tetranucleotide (EMAST)” predicts patient survival. Based on the previously known pathways, it is impossible to predict if individuals with sporadic colorectal cancer will have cancer or a metachronous adenoma. In order to identify and treat metachronous lesions in these individuals, clinical surveillance is essential. The prognosis, treatment response, and biological behaviour of colorectal cancer are very varied [72]. Based on the three mentioned molecular pathways of carcinogenesis, CIN, MSI, and CIMP, CRC has historically been classified into three categories. The majority of CRCs may be identified by their CIN, which accounts for about 80–85% of cases. Growth-promoting pathways are activated during chromosomal instability; at the same time, the apoptosis-related pathways are also inhibited, with the latter occurring more often. Because of a deactivation mutation in the APC gene that codes for the Wnt pathway effector adenomatous polyposis coli, these tumours first manifest as adenomatous polyps [73]. Additionally, the development of adenocarcinomas was facilitated by the activation of mutations in KRAS, which is in charge of receptor tyrosine kinase signalling, and the deactivation of mutations in SMAD4, which is in charge of the transforming growth factor beta (TGF β) and is crucial for the regulation of the cell cycle. The CIN pathway is free of mutations affecting BRAF, methylation, MLH1, or MSI. Furthermore, germline mutations in the MMR genes, such as MLH1, MSH2, MSH6, and PMS2, produce Lynch syndrome, the most prevalent type of hereditary colorectal cancer. It is thought that mutations in the MMR genes MLH1, MSH2, and MSH6 are caused by a deficiency in the MMR DNA. Dual somatic mutations in the MMR genes or biallelic hypermethylation of the MLH1

promoter may cause MLH1 deactivation, which results in MMR deficiency. It was noted that the patient with dMMR–MSI-H, a CRC with low MMR and high MSI, had a favourable prognosis but did not respond therapeutically to 5-fluorouracil [74–76]. Additionally, their MSI status had already been determined. CIMP is most likely to be detected in patients with colorectal cancer because of its strong correlation with the serrated pathway of colorectal carcinogenesis, hypermethylation of the MLH1 promoter, and associations with gender, age, and poor histology. However, it is challenging to transform this pathophysiological characteristic into a therapeutic strategy due to the absence of a clear diagnosis for CIMP-high. MLH1, MINT1, CACNA1G, and CDKN2A are the genes on which CIMP testing is most frequently performed. Gene changes have a role in the initiation and spread of cancer, starting with lesions in the polyp cancer formation sequence [77–79]. The primary histological lesion associated with the development of CRC is the aberrant crypt focus. Colorectal malignancies frequently have mutations in the tumour suppressor gene APC, and alterations can proliferate through dysplastic aberrant crypt foci. Inhibiting APC is a methodical strategy to initiate the series of events that lead to polyp cancer growth and activate the Wntless/Wnt pathway [80]. The Wnt signalling pathway facilitates the clonal growth of polyp cells that eventually lead to cancer through mutations in genes such as TP53 and KRAS. KRAS and TP53 mutations may potentially influence the transforming growth factor beta (TGFβ)-1-mediated cell signalling pathway, which would hasten the development of colorectal cancer. The development of colorectal cancer is associated with both genetic and epigenetic changes that cause the activation of oncogenes and inactivation of tumour suppressor genes. KRAS, a proto-oncogene that activates mitogen-activated protein kinase (MAPK) pathway, receives signals through the epidermal growth factor receptor (EGFR) through BRAF. Mutations in KRAS or BRAF, which trigger the MAPK signalling pathway, promote cell proliferation, and inhibit apoptosis, and is found in over half of colorectal cancers [81]. Additionally, CRC involves PI3K, Wnt–APC, CTNNB1, and TGFB1–SMAD signalling pathways, as well as the RAS–RAF pathway. The identification of frequently mutated genes in these pathways, driven by the study of epigenetics in cancer, has spurred extensive research into the biology of colorectal cancers [82].

6.3.2 The Influence of Gut Microbiota in the Development of Colorectal Cancer

There are several ways in which microorganisms are associated with the progression of colorectal cancer. The onset of inflammation is one step that is involved in this. The progression of colorectal cancer has been directly linked with inflammatory bowel disorders. Reactive oxygen species (ROS) and inflammatory cytokines produced by prolonged inflammation contribute to the development of a carcinogenic microenvironment [83]. In the same way that inflammation comes from dysbiosis, it is possible that inflammation itself causes microbiome dysbiosis. The CRC

and intestinal microbiota can communicate in two ways: either a dysbiotic microbiome promotes carcinogenesis or inflammation and tumours induce dysbiosis. Additionally, a pathogenic organism may identify a specific receptor on a host, allowing the host to actively “select” microbes. A host may be able to “select” germs in a passive manner by providing conditions that encourage the growth of particular harmful bacteria [84]. These “selections” have the potential to shift the odds in favour of inflammation development. The intestine is home to hundreds of microorganisms that work in symbiosis with intestinal cells to preserve the environment and the dynamic balance of the gut [85]. The intestinal flora will be out of balance if the equilibrium is lost, leading to a number of intestinal illnesses. Studies on the connection between gut microbes and colorectal cancer began in the 1960s. In this investigation, using both sterile and conventional rats for induction, it was discovered that *Cycas* causes cancer in conventional rats but not in sterile rats. This suggests that intestinal microbes are crucial to the carcinogenesis that *Cycas* mediates [86]. An unbalanced microbiome in the gut will result from modifications in the microbiota’s quantity and microecological structure. Pathogenic bacteria will proliferate the colon and probiotics will decrease once the intestinal microbiota is out of equilibrium. Intestinal epithelial cells are harmed by pathogenic bacteria, which also release a range of harmful substances and induce long-term inflammation [87]. A multitude of cytokines and chemokines are generated throughout the inflammatory process, activating signalling pathways linked to inflammation and ultimately causing colorectal cancer. Furthermore, epigenetic alteration gives host cells the ability to change the sequence and expression of genes. Through the interface of epigenetic alteration, microbes can dynamically interact with host genomes [88]. The genesis and progression of colorectal cancer are significantly influenced by epigenetics. Through pro-inflammatory cytokines, intestinal microbiota can cause mucosal lesions, which can worsen Crohn’s disease and inflammatory bowel disease. Cytokines released by inflammatory cells, including tumor necrosis factor (TNF- α), interleukin (IL)-6, IL-8, and IL-17, are crucial in the progression of colorectal cancer (CRC) [89]. Research has demonstrated that infection with *Enterococcus faecalis* can result in colitis and induce intestinal epithelial cells to release TGF- β , which activates the SMAD4 signalling pathway. Additionally, *E. faecalis* is capable of producing ROS and H₂O₂, which can damage DNA and activate Nuclear factor kappa B cells (NF- κ B) signalling pathway, causing inflammation and influencing the onset and progression of colorectal cancer. Simultaneously, that seems to be connected to the COX-2 bystander effect, wherein macrophages produce TNF- α , which results in chromosomal instability and cell transformation, ultimately causing colorectal cancer (CRC). Furthermore, by inducing an IL-8-mediated inflammatory response that damages colonic epithelial tissue, *Helicobacter pylori* (*H. pylori*) can contribute to the formation and incidence of colorectal cancer (CRC). Th17 cells secrete IL-17, a significant cytokine, and Th17 cell proliferation depends on STAT3 activity. A key factor in the diagnosis of colorectal cancer (CRC) is the intestinal flora. It can serve as a biomarker for colorectal cancer (CRC) diagnosis and prognosis. Using metagenomic analysis of faeces, several studies have identified features of intestinal microflora that might

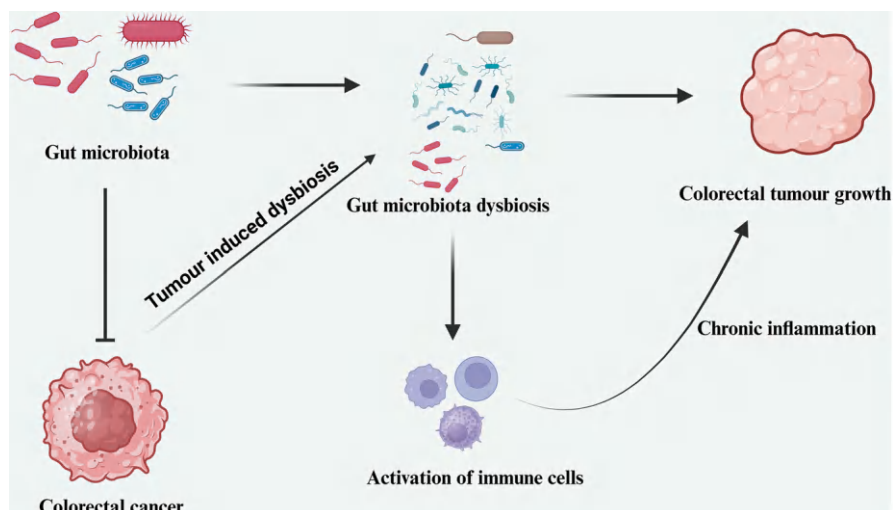


Fig. 6.1 Mechanisms of gut microbiomes in the development of colorectal cancer

predict colorectal cancer (CRC) [90]. Microorganisms linked to colorectal cancer (CRC) were found using metagenomic analysis of faeces from individuals from different nations and with varied living and eating habits and lifestyles [91]. These bacteria, including *Fusobacterium*, *Porphyromonas*, *Parvimonas*, etc., are used as indicators to identify colorectal cancer. They have also discovered two significant study findings:

1. The identification of *F. nucleatum*, a unique bacterium that is frequently found in the respiratory system and buccal cavity in colorectal cancer patients. Patients with colorectal cancer had a greater number of *F. nucleatum* than those in the general population.
2. The identification of a notable microbiome in the faeces of colorectal cancer patients and exploration of the connection between CRC and microbial enzyme genes.

Furthermore, evaluating gene alterations in stool samples can precisely represent the condition of the intestinal microbiota, which may offer crucial hints for the identification of illnesses and their causes and effects, as well as help with early cancer detection [92] (Fig. 6.1).

6.3.3 Dysbiosis and Its Implications for Colorectal Cancer

Microbial toxins are produced and secreted when pathogenic or excessively non-beneficial organisms colonize the gastrointestinal (GI) tract. As of right now, bacterial species have been found to produce three known genotoxins that cause strand

breaks in DNA: Gram-negative bacteria's cyto-lethal distending toxin (CDT), *Salmonella enterica* serovar *Typhi*'s (*S. Typhi*) typhoid toxin, and group B2 *E. coli*'s colibactin. Endotoxins, such as the *B. fragilis* toxin, are produced and secreted by *B. fragilis* and have the potential to harm DNA, resulting in mutations and the start of colon cancer. Through DNA damage and epithelial cell proliferation, strains secreting the *B. fragilis* toxin have accelerated the growth of tumours associated with colorectal cancer in mice models [93, 94]. ROS trigger the beginning of CRC by angiogenesis, apoptosis, DNA damage, epithelial-to-mesenchymal transition, and inflammation. ROS also cause mutations in a number of proteins known as proto-oncogenes, which control the cell cycle. These proteins include p53, Ras, and c-Myc, and when these mutations start, oncogene activation occurs. Enterotoxigenic *E. coli* that possesses genomic islands for polyketide synthesis (pks) produces colibactin, which disrupts the advancement of the cell cycle and damages the DNA. In test mice models, such pks + *E. coli* has stimulated colonorectal carcinogenesis [95, 96]. Pathogenic bacteria can also adhere to intestinal epithelial cells promoting cell division, growth, and hyperplasia. Roughly 80% of CRC instances occur randomly and in people who do not have any family or genetic predispositions. Chronic IBD, especially ulcerative colitis (UC); sedentary lifestyles; poor diets; and alcohol consumption are linked to sporadic colorectal cancer (CRC), which is not linked to any hereditary or family factors [96]. CRC incidence is linked to chronic inflammation, as seen in IBD patients. For instance, Crohn's illness in the ileocolic zone may raise the risk of colorectal cancer. Colitis and colorectal cancer are linked to enterotoxigenic *B. fragilis* (ETBF) strains. Interestingly, in clinical episodes of diarrhoea, sepsis, and extra-intestinal infections, *B. fragilis* is the most commonly identified opportunistic anaerobic pathogen [97]. Oncogenic activity is promoted in local cells by persistent infection, pathogenic virulence factors, and related inflammation. Having too many harmful fungi, such as *Malassezia restricta*, is linked to IBD and Crohn's disease. Because *Candida albicans* produces candidalysin, a cytosolic peptide toxin that is cytotoxic to epithelial cells and induces inflammation, the fungus is linked to inflammation [98]. Because ROS cause double-stranded breaks in DNA, the movement of immune cells and the resulting inflammatory response lead to inflammation. The interaction of the local microbiota with certain receptors on the immunological cell surface called Pattern recognition receptors (PRRs), which are triggered by microbial pathogen-associated molecular patterns (PAMPs), supports innate immunity [99]. These set off a series of events that result in the synthesis of chemokines, growth factors, and pro-inflammatory cytokines, which are essential in the integrity and repair of intestinal cells. Changes in these PRRs, like toll-like receptors (TLRs), can lead to dysbiosis changes, inflammasome activation, and exaggerated inflammatory responses—all of which are linked to the advancement of cancer [100]. Research has indicated a connection between the alterations in cytokine production and tumour growth in models of CRC linked with inflammation in TLR-deficient mice. It is widely recognized that the lipopolysaccharide (LPS) component of the gram-negative bacterial cell wall activates inflammasome, which, in turn, causes cytokines and interleukins to become active pro-inflammatory agents and cause inflammation [101]. Inflammasomes act as a link between the host

immune system and the microbiota by detecting pathogen-associated molecular patterns. Studies indicate the importance of the inflammasome–microbiota axis and inflammasome activity in a number of disease states, including colorectal cancer. In CRC metastases, LPS is linked to cell adhesion, cell disintegration, and cell invasion. Research has shown that in IBD animal models, LPS-associated inflammation enhances the expression of oncogenic genes and CRC cell proliferation. By breaking down the tight connections in the intestinal barrier [102], LPS also modifies its integrity. This can result in systemic inflammation, tumour development, CRC carcinogenesis, microbial product leakage into the circulation, and metabolic endotoxemia (ME). Compared to other *Enterobacteria*, *E. coli* species' LPS toxins have a higher potential for triggering the immune system. When *Fusobacterium*, *Clostridium*, *Prevotella*, *Desulfovibrio*, and *Enterococcus* are dysbiotically coexisting, inflammatory metabolites such as increased trimethylamine N-oxide (TMAO) and reduced SCFA levels can develop [103]. In addition to supporting intestinal integrity, SCFAs have been linked to an increase in anticancer activity, including CRC cell death. Moreover, SCFAs have roles in immunological control, anti-inflammatory actions, and host microbiota regulation. Changes in bile acid concentrations linked to intestinal inflammation, compromised activity of the farnesoid X receptor, and colorectal cancer (CRC) are caused by the gut microbiota, which controls bile acid metabolism and production. Commensal *Bifidobacterium* lowers the pH of the gut, stops pathogenic *E. coli* from growing, and blocks the genes that give pathogenic species their virulence. In individuals with sporadic CRC, mucosal biofilm production is linked to the disease and may have an effect on how familial CRC develops [104]. In fact, compared to 13% of healthy individuals, mucosal invasive biofilms have been seen in about 50% of CRC patients. Both healthy and sick people's intestines contain biofilms, which are communities of microbial cells suspended in an extracellular polymeric substance (EPS) matrix. *Veillonellaceae*, *Lachnospiraceae*, *Coriobacteriaceae*, *Bacteroidetes*, and *Firmicutes* species are found in the biofilms seen in CRC patients. CRC was formed in mice that were transplanted with tissues from CRC patients. Inflammatory interleukin 6 (IL-6) and signal transducer and activator of transcription (STAT) signalling are enhanced by the presence of biofilms. This leads to tight junction disruption, which facilitates tumour invasion and metastasis, as well as tumour proliferation and carcinogenesis [105]. Studies have shown that the mucosal biofilms of familial adenomatous polyposis (FAP) patients contain toxin-producing species like *B. fragilis* and pks + *E. coli*. Biofilms from CRC patients often contain *B. fragilis*, *E. coli*, and *F. nucleatum* and are polymicrobial in nature. Research indicates that the autophagy of CRC cells triggered by *F. nucleatum* through TLR pathways leads to resistance against fluorouracil and oxaliplatin. Further research on the precise molecular processes behind the microbiota-autophagy axis' apparent significance in the advancement of cancer is necessary to enhance anticancer therapy in clinical settings [106]. Microbial biofilms exhibit resistance to antibiotics and host immunity, leading to heightened inflammatory responses and cytokine release in resident species. These species may potentially generate genotoxic chemicals produced by bacteria [107].

6.4 Analytical Techniques for Studying Gut Microbiota

6.4.1 *Sampling Methods and Considerations*

The best sample technique would be non-intrusive, require minimal bowel preparation with a low risk of contamination, and gather gut microbiota from many premises. These days, most sequencing technologies rely on samples taken from intestinal fluid, mucosal biopsies, faeces, etc. Furthermore, there are several flaws in the sampling techniques used today. For instance, biopsies are intrusive for patients and unsuitable for healthy control, whereas faecal samples are only a stand-in for gut flora [108]. The limitations of the sampling techniques used today are unavoidable, even with great efforts on the part of researchers to acquire precise samples. It will be challenging to get reliable findings from erroneous samples. Because they are convenient and non-invasive, faeces are now the sample source of choice for most bacterial environment research. Still, even the lower digestive tract's microbial make-up, which is most similar to faeces, differs greatly from that of faeces [109]. The majority of the remaining sample techniques are intrusive and unfit for use on healthy individuals. Minimizing intrusiveness, ensuring there is no chance of cross-contamination while sampling at defined places, and limiting interference with the regular physiology of the gut are problems that need to be resolved in future sampling techniques. More accurate sampling techniques are thus required to guarantee the validity of research since the precision of the samples has a major influence on the usefulness of investigations of gut microbiota [110]. The following specifications should be taken into consideration while designing future ideal gut microbiota collection tools. Initially, the gadgets must have the ability to efficiently gather intestinal composition at a specific location and prevent cross-contamination of the samples. Also, devices have to be tiny in order to pass easily via the ileocecal valve and pylorus. Then, there is less psychological strain and pain during the sample procedure. The gadget must have a straightforward design that is simple to use. Future gut microbiota research will depend heavily on the development of more precise sample techniques, given the limitations of existing approaches [111]. The creation of swallowable technology appears to be the most practical way to satisfy these needs. In the future, doctors and researchers will be able to examine intestinal flora with specificity, site, and sensitivity, thanks to compact, autonomous, swallowable sampling devices. However, understanding the relationship between the flora and the host also requires an understanding of the spatial organization of intestinal flora. It appears impracticable to get samples with data on the spatial link between the bacteria and the gut for ethical grounds [112]. Alternatively, the creation of gut microbiomes in germ-free mice offers us another way around the challenges associated with sampling. The use of objective spatial macro-genomics in mice would significantly advance the knowledge of the spatial arrangement of the gut microbes, despite the fact that fluorescence imaging is not suitable for studying complicated microbiomes [113].

6.4.2 DNA Sequencing Technologies

The variety and abundance of the gut community and human microbiota create obstacles for researchers examining changes in community composition over time. For a century, the foundation of microbiology consisted of laborious cultivation-based procedures that only identified a limited variety of species existing within the human colon. A unique housekeeping gene in prokaryotes, the highly conserved 16S small subunit ribosomal gene, contains hypervariable sections from region V1 to V9 [114]. This gene may be utilized to identify microbial communities inside samples. Following polymerase chain reaction (PCR) amplification of a specific variable region using various primers, for the sequencing of 16S rRNA. The V4 region of the 16S rRNA gene is the gold standard for analyzing the human gut microbiome. Depending on the clades, sequencing, and analysis [115], a resolution at the strain, species, genus, or higher taxonomic levels can be produced. Whole genome sequencing (WGS) can provide details on the genes that the strains in the sample encode, or at least the most common ones [116]. This information may be used to reconstruct the potential metabolic capacity of a specific microbial habitat. WGS randomly breaks all of the DNA into small pieces several times in order to find the sequences of millions of fragments at once. They are then assembled into larger segments (contigs) that match chromosomal areas (potentially entire genomes for the most common species) by linking the overlapping ends. For the diagnosis of colorectal cancer (CRC), high-throughput “multi-omics” technologies provide non-invasive or minimally invasive techniques [117]. Proteomics, metabolomics, transcriptomics, genomics, and microbiomics are some of these technologies. Each method has a unique advantage when it comes to discovering novel cancer biomarkers for diagnosis. For instance, using genomics to evaluate the hereditary risk of the condition and the likelihood of colon cancer is a highly successful method. DNA sequences never precisely match phenotypes because of epigenetic, post-transcriptional, and post-translational modifications; as a result, their diagnostic value is constrained [118]. Transcriptomics and proteomics have significant therapeutic promise because they have a stronger connection to the physiological states of organisms. They are still unable to diagnose conditions as accurately and quickly as metabolomics, which enables a phenotypic description of the organism as well as its metabolic processes. Moreover, metabolomics offers a chance to assess the connection between the host and gut bacterial metabolites, a crucial phase in the development of colorectal cancer [119].

6.4.3 Metabolomics in Gut Microbiota Analysis

The diverse bacteria that comprise the gut microbes produce a large number of substances that are vital for the formation of metabolic signalling networks and the selection of microorganisms. Microbial activity is influenced by environmental

signals, which lead to the synthesis of several compounds that affect the host metabolome and well-being. In fact, profiling of metabolites linked with gut microbiomes may provide important information about how food and lifestyle choices are made. Metabolomics, meta-proteomics, and metagenomics are methods of meta-omics utilized to look into the control of the microbiomes [120]. Characterizing the products generated by the microbes in the intestine, profiling the metabolites, identifying and quantifying classes and compounds of interest, and determining the biochemical pathways of metabolites are all made possible by applying metabolic research to biofluids [121]. Nuclear magnetic resonance and mass spectrometry are the most common metabolite profiling techniques utilized in metabolomics research, and they differ in terms of quantification, sensitivity, and coverage. Apart from these two techniques, some other metabolomic techniques are used like gas chromatography coupled with mass spectrometry (MS), liquid chromatography mass spectrometry, and Fourier transform infrared spectroscopy. Furthermore, MS has a high level of sensitiveness and selectivity and a greater dynamic range. It also facilitates the identification of metabolites by enabling the determination of metabolite fingerprints for the creation of metabolome libraries. Targeted and untargeted metabolome analysis is now possible with MS-based metabolomics, which has grown to be a vital technique in metabolome study [122]. However, due to a variety of chemicals and differences in the characteristics of metabolites and metabolite concentration in samples, assessing the whole metabolome with a single technique is quite difficult. To cover the entire metabolome pathways in the various biofluids, different analytical techniques and computational approaches controlled by chemical and analytical parameters are needed, considering that microorganisms, plants, and animals have an undetermined number of predicted metabolites and derivatives. Additionally, selecting appropriate analytical platforms and preparing and collecting samples are crucial preconditions for the repeatability of sample manipulation [123, 124]. Furthermore, the samples' ongoing freezing and thawing during storage may alter their stability and composition, which might have an impact on the precision and accuracy of the findings. Finally, the fundamental elements of metabolomics research include proper study design, sample management, and platform set-up, backed by data processing, result integration, and biological interpretation [125].

6.4.4 Bioinformatic Tools and Data Analysis

The study of biological data via computer science and statistical techniques is known as bioinformatics, which has a number of benefits. Numerous types of data are produced by biological research, including transcriptomics, proteomics, and genome-sequencing data. With the use of computers and accompanying equipment, bioinformatic analysis is able to effectively process, analyze, and mine the information concealed in these massive amounts of data. In order to thoroughly analyze various biological information levels and uncover intricate biological processes and correlations [126], bioinformatics can integrate biological data from various sources

including genome, transcriptome, proteome, and metabolome data. Additionally, it may forecast biological issues including protein structure, gene regulatory networks, and gene function, which can help with experimental planning and biological research. In order to better understand how biological systems work, it may also simulate and mimic biological processes [127]. Additionally, it can lower research and development expenses, quicken the drug discovery process, and find potential biomarkers and therapeutic targets. The advantages of bioinformatic analysis include huge data processing that is efficient, multi-level data integration and analysis that is thorough, simulation studies, marker identification and identification of the target, sharing of data, and the provision of potent tools and methodologies for biological research [128]. The human body’s gut microbiota contains bacteria, fungi, viruses, and other microorganisms that create a microbial ecosystem. They may affect the immune system and metabolism of the host, which can influence the development of diabetes and insulin resistance. Microbiota species and metabolites linked to diabetes and insulin resistance are identified using whole genome sequencing, metabolomics, and 16S rRNA sequencing. Transgenic variety has little direct bearing on the aetiology of diabetes; instead, it usually relates to agricultural productivity and food safety [129]. Certain microbiota species and metabolites have been linked in several studies to insulin resistance and diabetes. For instance, there could be a connection between a bacterial species’ relative abundance and the risk of diabetes, and particular microbial metabolites might affect how well the host uses insulin [130]. To fully understand the specific mechanisms through which these bacteria influence diabetes and insulin resistance, more research is required, as the interaction between microbiota and diabetes is a complicated one. There are still issues and disagreements surrounding studies based on the variety of gut microbiomes in people with diabetes, including individual differences, environmental influences, and the influence of various conditions on study results. It also performs pathway analysis of metabolites that are differently expressed, offering a theoretical foundation for diagnosis and therapy [131] (Table 6.2)

Table 6.2 List of bioinformatic tools and their applications involved in the analysis of gut microbiota

Bioinformatic tools	Application	References
Gene sequencing of 16S rRNA	As compared to matching normal tissues, 16S rRNA gene sequences show a reduction in the number of bacterial genera engaged in bile secretion, terpenoid biosynthesis, and steroid production in tumours. These results are consistent with data from metabolomics study	[132]
Whole genome sequencing (WGS)	These days, methods such as whole genome sequencing (WGS) are employed to determine the human microbiome’s make-up	[133]
MetaPhlAn	MetaPhlAn2 software may be used to quantify the taxonomic composition of datasets	[134]
Machine learning and predictive modelling	The prognosis of patients with CRC can be estimated by the application of multi-omics machine learning techniques	[135]

Data analysis is a critical component in the context of today's world, as it allows for the development of a model that fits and infers causal correlations between biological data from several sources. In this pilot project, Bayesian networks were used in conjunction with the “intervention calculus when the DAG is absent” (IDA) technique to construct a graphical model that makes it easier to infer causal linkages from observational data. Previous applications of this technology have included determining the causal regulatory links between micro ribonucleic acid (miRNA) and messenger ribonucleic acid (mRNA) using observational data, among other situations [136].

6.5 Limitations of Current Analytical Techniques

6.5.1 Challenges in Sampling and Preservation

The sampling techniques utilized today will always have limitations even after all possible efforts by researchers. Getting trustworthy results from faulty samples is difficult. Using faecal samples not specifically collected for microbial profiling can present technical challenges such as insufficient sample quantity and inconsistent handling and storage methods. If the frequency of freeze–thaw cycles increases or if the freezing and storage time frames are extended, the biological information in the sample may become unstable [137]. Every attempt is made to lessen the influence of pre-experimental circumstances on the outcomes of studies and the efficacy of therapeutic care. Different preservation strategies are used based on the clinical requirements of various investigations [138].

6.5.2 Limitations of 16S rRNA Sequencing

The length of 16S rRNA is approximately 1550 bp. This gene sequencing involves one or more of the genes from various regions. Consequently, species resolution of some bacterial genera may not be possible with short sequencing reads from 16SNGS, which typically cover 300–500 bases, while next-generation sequencing (NGS) systems yield more accurate short reads. More sequencing of other genes in these bacteria will provide more precise species identification. When comparing *Enterobacteriaceae* species identification, the *dnaJ* sequence outperforms 16S rRNA [139]. The internal transcribed spacer (ITS) region and *recA* provide improved identification of *Burkholderia* sp. and *Mycobacterium* sp., respectively. Additionally, species of *Mycobacterium* can be recognized by their heat shock protein (hsp) sequence. Sabat et al. demonstrated enhanced identification of bacterial species. Church et al.'s latest review offers a thorough synopsis of the gene sequencing of 16S rRNA and its effectiveness in the identification of bacteria [140].

6.6 Future Directions and Innovations

6.6.1 *Advances in High-Throughput Sequencing*

High-throughput sequencing analyzes and pinpoints the different genes and characteristics that distinguish one disease from another. Compositional datasets are the core concept behind high-throughput data. Each sample in a compositional dataset has a predetermined total of values. Proportions are used to express this [141]. Numerous advancements in high-throughput sequencing mappers have been brought about by the growth of the numerous biological uses for this technology. There have been other sorts of specialized data introduced, such as bisulphite, RNA, ChIP, and miRNA. While some mappers from the past were designed entirely, some were modified to fit the most recent requirements. There is a growing need for mappers (like DynMap and GenomeMapper) that can efficiently match reads for different reference genomes due to the increase in re-sequencing studies [142]. Longer read durations, effective HTS process, and multi-reference alignments may all be improved. Many important HTS technologies have been helpful in determining the relation of microbiomes with cancer cells as well as the susceptibility or resistance to different therapies. Among the technologies are metagenomics, metabolomics, and 16S rRNA sequencing [143].

6.6.2 *Integration of Gut Microbiota with Multi-omics Approaches*

Understanding human gut microbiota's role in illnesses across various functional levels depends on multi-omics investigations of the microbiome. However, there are several obstacles in the way of integrating and analyzing such complicated datasets [144] (Fig. 6.2).

6.6.3 *Personalized Medicine and the Role of Gut Microbiota*

Recently, a lot of development has taken place in the healthcare system. Person-centred, individualized, stratified, and precision medicine have entered a new era, which is covered and celebrated by newspapers and specialist media outlets alike. It is true that both terms are often used interchangeably. The intention behind this lexical ambiguity is to mimic institutional and economic tactics and solutions in order to create a new paradigm for healthcare systems [145]. Genetic modification (GM) is a key component of this process that modifies the bioactivities, toxicities, and lifespans of medications [145]. Deeper knowledge of the relationship between the complexity and diversity of genetically modified organisms (GMOs) and their

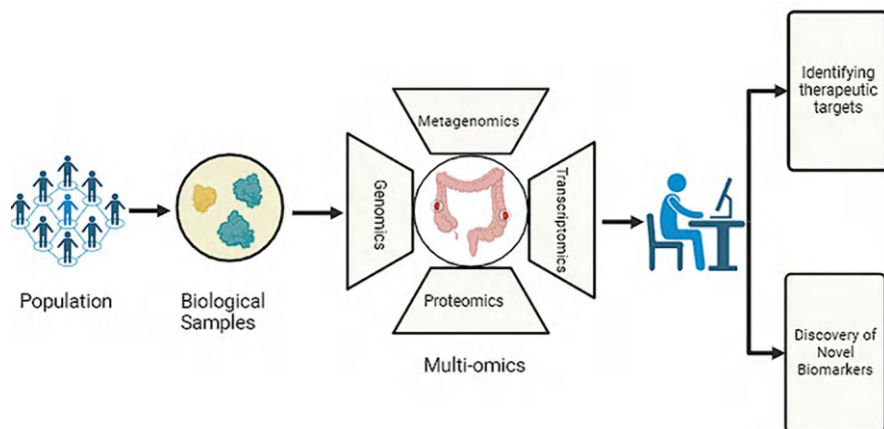


Fig. 6.2 Multi-omics approaches in gut microbiota

efficacy as a medication could guide customized nutrition and medical care, inform risk assessments for toxicology, improve drug research, and advance both clinical and pre-clinical research [146]. Changing the gut flora might prove to be a highly attractive approach of regulating medication safety and efficacy individually. Personalized medicine is becoming more and more dependent on the gut microbiota [147].

6.7 Novel Therapeutic Strategies Targeting Gut Microbiota

As novel cancer therapy methods, prebiotics, probiotics, and postbiotics are acknowledged on the basis of their distinct abilities to modify the gut microbiota, preserve the intestinal environment, and control cells of the immune system. A variety of drug delivery platforms that are prepared to handle the various difficulties involved in oral administration are produced using engineering tactics. Engineered drug delivery technologies, such as extracellular vesicles and polymeric, inorganic, and lipid-based biomaterials, are created to optimize the effectiveness of oral gut-targeted immunotherapy [148]. Specifically, targeted delivery, enhancing oral bioavailability and bioactivity, and protecting therapeutic cargo against various biological obstacles have all drawn significant interest to tailored drug delivery systems. The significant adaptability of using engineering techniques to manipulate materials at nano- and micro-scales and include various capabilities is another noteworthy benefit. In CRC cell lines, several postbiotics, such as lactate dehydrogenase obtained from *Lactobacillus* species, are reported to cause cell death and reduce invasions; however, the majority of these investigations are severely constrained by the absence of confirmation in various animal models [149]. According to reports, the supernatant of *Lactobacillus casei* ATCC334 had a strong tumoricidal impact.

Ferrichrome was shown to be the chemical that caused apoptosis through the JNK-DDIT3 signalling axis. Postbiotics that have been isolated have greater anticancer activity than traditional CRC medications and have less impact on normal intestinal epithelial cells, indicating the postbiotics' potential for therapeutic use [150]. There are several postbiotics that help in reducing the inflammation in the colon and repairing the integrity of the gut barrier. It has been found that p40, a soluble protein produced from *Lactobacillus rhamnosus* GG, inhibits cytokine-induced epithelium apoptosis, disrupts the gut barrier, and improves immunoglobulin release by the transactivation of the epidermal growth factor receptor (EGFR) [151]. Administering hydrogel-coated p40 in a targeted manner can effectively prevent and cure intestinal damage and inflammation while also enhancing the protective immune response and preventing p40 from degrading [152].

6.8 Conclusion

The gut microbiome has a major impact on colorectal cancer (CRC) growth and advancement. It serves as an essential intermediary for the interaction between host genetics, nutrition, and environmental variables. Clarifying the pathophysiology of colorectal cancer requires knowledge about the make-up and the role of gut microbiota. The chapter has highlighted the complex relationships between dysbiosis, inflammation, and carcinogenesis, demonstrating how specific microbial species, such as *Fusobacterium nucleatum* and *Bacteroides fragilis*, contribute to tumorigenesis through mechanisms like immune modulation, toxin production, and genomic instability. Various analytical techniques including 16S rRNA sequencing, metagenomics, and metabolomics have given a deep understanding of microbial diversity, metabolic pathways, and the microbial signature associated with CRC. However, these techniques face limitations such as low resolution, bias in sample preparation, and difficulty in distinguishing causality from correlation. In summary, even though a lot has been learned about the function of gut microbiota in colorectal cancer, further investigation is required to get past analytical constraints and consider the possible therapeutic benefits of microbiome modification. Personalized approaches that consider individual microbiome profiles, combined with advances in computational tools and multi-omics integration, hold promise for enhancing the precision of CRC diagnostics and treatment strategies.

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Chapter 7

Postbiotics: Bioactive Compounds and Their Effects



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Abstract Postbiotics are bioactive substances generated by bacteria during fermentation, providing unique health advantages beyond those of probiotics and prebiotics. These substances, including enzymes, peptides, short-chain fatty acids (SCFAs), bacteriocins, exopolysaccharides (EPSs), and vitamins, are essential for sustaining human and animal health. Postbiotics influence biological processes by mechanisms including gut microbiota modification, immunomodulation, and anti-inflammatory and antioxidant actions. They promote gastrointestinal (GI) health, regulate metabolism, increase immunological function, and have neuroprotective, antibacterial, and anticancer activities. Postbiotics are used in both the food and pharmaceutical industries, especially in fermented goods and nutritional supplements; nonetheless, issues concerning manufacturing, stability, and regulatory compliance persist. A recent study underscores their involvement with the gut–brain axis and possible synergistic effects with probiotics and prebiotics, paving the way for personalized nutrition and treatment. Moreover, postbiotics are progressively used in veterinary medicine for illness prevention and enhanced animal nutrition, particularly in poultry, aquaculture, and cattle. Technological developments are propelling breakthroughs in postbiotic manufacturing and delivery systems; nonetheless, future studies must tackle safety issues and develop definitive regulatory frameworks. Postbiotics, with their promised therapeutic potential, are poised to significantly influence future health policies, enhancing both preventive and personalized treatment.

Keywords Postbiotics · Bioactive substances · Gut microbiota · Short-chain fatty acids (SCFAs) · Probiotics · Immune function

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7.1 Introduction

7.1.1 Overview of Postbiotics

Postbiotics are characterized as nonviable bacterial metabolites or metabolic by-products derived from probiotic microorganisms that have beneficial effects on the host. In contrast to probiotics, which need live bacterial cells for efficacy, postbiotics include functional components such as cell-free supernatants, bacterial metabolites, and bacterial lysates. These chemicals have bioactive qualities that enhance gut health, modulate immune responses, and aid in disease prevention [2]. Recent studies emphasize their contribution to enhancing gastrointestinal (GI) functioning, providing an alternative to conventional probiotics in functional meals and therapies. The nonviable characteristics of postbiotics provide considerable stability benefits since they maintain efficacy in settings when probiotics may become nonviable, such as during industrial processing or gastrointestinal transit [4, 11, 28, 30].

Recent improvements highlight that postbiotics include bioactive metabolites such as short-chain fatty acids (SCFAs), peptides, and exopolysaccharides (EPSs) [2]. These components demonstrate antioxidant, anti-inflammatory, and antibacterial capabilities vital for enhancing human health and maintaining microbial equilibrium [44].

7.1.2 Differentiation Between Probiotics and Prebiotics

The names probiotics, prebiotics, and postbiotics are sometimes conflated; however, they denote distinct ideas. Probiotics are living bacteria that provide health advantages when ingested in sufficient quantities. Prevalent probiotic strains include *Lactobacillus* and *Bifidobacterium* species, which inhabit the gastrointestinal tract and influence the microbiota [16]. Probiotics, however, exhibit limits regarding stability during storage, processing, and gastrointestinal transit.

Conversely, prebiotics are indigestible food constituents that specifically promote the proliferation or activity of advantageous gut microbes, including dietary fibers and inulin [12]. They indirectly influence the host's health by acting as substrates for helpful microorganisms.

Postbiotics, however, do not need living bacteria or fermentation processes to provide advantages. They comprise metabolites or cellular constituents that have direct effects on the host's systems. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, generated by bacterial metabolism, are essential for mitigating inflammation and preserving intestinal homeostasis [35]. The difference is based on their method of action, with postbiotics functioning independently of live bacteria, providing more flexibility and stability than probiotics [10].

7.1.3 Importance of Postbiotics in Human and Animal Health

Postbiotics have considerable potential to enhance the health of both humans and animals owing to their diverse bioactive properties. In humans, postbiotics aid in preserving gut integrity, regulating immunological responses, and thwarting infections [38]. Probiotic strain metabolites may modulate intestinal permeability, thus decreasing the likelihood of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Moreover, postbiotics have the potential to improve metabolic health since short-chain fatty acids (SCFAs) affect insulin sensitivity and lipid metabolism [35, 47].

In animal health, postbiotics have arisen as substitutes for antibiotics in cattle nutrition. As limits on antibiotic usage in animal agriculture intensify, postbiotics provide a sustainable alternative by enhancing growth and avoiding illnesses without fostering antibiotic resistance [38]. In poultry and aquaculture, postbiotics promote gastrointestinal health, augment food assimilation, and bolster disease resistance, thus enhancing overall production and animal welfare.

Furthermore, postbiotics are under examination for their influence on mental health via the gut–brain axis. Research indicates that bacterial metabolites such as butyrate and serotonin precursors affect mood and cognitive abilities, presenting opportunities for addressing mental health illnesses through dietary treatments [8]. Their stability and simplicity of incorporation into food, supplements, and medications make postbiotics a viable asset for preventive healthcare. In summary, postbiotics signify a novel advancement in microbiome-based therapeutics, providing various health advantages for people and animals alike. Their stability, varied bioactive capabilities, and simplicity of application provide a distinct advantage over probiotics and prebiotics, establishing them as an essential element of future functional foods and therapies.

7.2 Postbiotics and Their Classification

7.2.1 Postbiotics

Postbiotics are bioactive substances generated by bacteria, particularly probiotics, following fermentation or metabolic activity [5]. They are nonviable bacterial metabolites, including enzymes, peptides, short-chain fatty acids (SCFAs), and polysaccharides, which may provide health advantages to the host [2]. In contrast to probiotics, which need living microbes, postbiotics retain their functionality even without live cells. This characteristic offers a significant benefit since postbiotics exhibit enhanced stability during storage and processing and remain unaffected by gastrointestinal circumstances that might diminish the efficiency of live microorganisms [36, 45].

The notion of postbiotics is advancing in microbiome research, with scholars highlighting their significance in enhancing gut health, regulating the immune system, and thwarting infections. Their capacity to work independently of live bacterial cultures makes them a desirable element in functional foods, medicines, and veterinary uses [1, 2].

7.2.2 *Types of Postbiotics*

7.2.2.1 Enzymes and Proteins

Postbiotic enzymes, including proteases and lipases, facilitate meal breakdown and improve nutrient absorption. These enzymes may regulate gut flora and enhance digestive health [33]. Proteases may mitigate inflammation by degrading inflammatory peptides in the gastrointestinal tract. Bacterial-produced antioxidant enzymes, including superoxide dismutase, are essential for neutralizing free radicals and mitigating oxidative stress.

7.2.2.2 Peptides and Bacteriocins

Bioactive peptides, such as bacteriocins, are antibacterial substances synthesized by probiotic bacteria. These peptides impede the proliferation of detrimental microorganisms by compromising their cell membranes, rendering them advantageous for food preservation and infection management [31]. Bacteriocins derived from *Lactobacillus* species have shown efficacy against gram-positive bacteria, aiding in the maintenance of microbial equilibrium in the gut.

7.2.2.3 Short-Chain Fatty Acids (SCFAs)

Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, are generated by the bacterial fermentation of dietary fibers. These fatty acids are crucial for preserving gut health and integrity [35]. Butyrate has anti-inflammatory properties and acts as the principal energy substrate for colonocytes, enhancing gut barrier integrity. Short-chain fatty acids (SCFAs) modulate metabolic pathways, enhancing insulin sensitivity and lipid metabolism while diminishing the risk of metabolic diseases [19].

7.2.2.4 Exopolysaccharides (EPSs)

Exopolysaccharides are extracellular polysaccharides produced by bacteria during fermentation. They facilitate biofilm development and provide structural stability to microbial populations. In the gastrointestinal tract, EPSs may enhance the

proliferation of beneficial bacteria and augment immunological responses by regulating cytokine production [33]. EPSs also has antioxidant and anticancer characteristics, making them a viable element in functional meals and nutraceuticals.

7.2.2.5 Cell Wall Components and Metabolites

Bacterial cell wall components, including peptidoglycan and lipoteichoic acid, contribute to immunomodulation via their interaction with receptors in the gut-associated lymphoid tissue (GALT). These interactions promote the synthesis of anti-inflammatory cytokines, augmenting immunological tolerance and diminishing inflammation [31]. Bacterial metabolites, including vitamins B and K, are crucial for metabolic activities and the maintenance of host health.

7.2.3 Criteria for the Classification and Identification of Postbiotics

7.2.3.1 Source and Composition

Postbiotics are categorized according to the microbial strains and fermentation substrates used in their manufacture. The specific probiotic bacteria, including *Lactobacillus* or *Bifidobacterium* species, dictate the characteristics of the bioactive substances [2]. Furthermore, the fermentation substrate, including fiber-rich meals or dairy products, affects the synthesis of certain SCFAs or exopolysaccharides [41].

7.2.3.2 Mechanism of Action

Postbiotics may also be categorized according to their method of action. Certain postbiotics, such as bacteriocins, directly suppress infections, while others, like SCFAs, exert indirect effects by modifying host metabolism or improving gut barrier integrity [35]. This functional categorization aids in finding appropriate postbiotics for certain therapeutic applications [20].

7.2.3.3 Stability and Delivery Method

The stability of postbiotics under several circumstances, including heat, pH, and storage, is a crucial determinant in their categorization. Postbiotics have greater stability than probiotics, enabling their incorporation into a broader array of items, including heat-processed meals [36]. The mode of distribution, including oral supplements or topical treatments, affects their categorization since various bioactive chemicals may target certain tissues or organs [23].

7.2.3.4 Functional Benefits

Postbiotics are categorized according to their functional advantages, such as immune modulation, metabolic control, and antibacterial properties. This categorization provides a framework for the selection of postbiotics with specific health advantages for use in functional foods, medicines, and veterinary products [22, 24, 33].

7.2.3.5 Safety and Regulatory Status

The safety of postbiotics is a fundamental requirement for their categorization. Postbiotics, lacking live microorganisms, are often regarded as safer than probiotics, especially for immunocompromised persons [36]. Regulatory bodies assess the formulation, manufacturing method, and safety information of postbiotics to ensure compliance with food and pharmaceutical standards [44].

Postbiotics, bioactive substances originating from probiotics, are a burgeoning field of study with considerable implications for human and animal health. Their categorization according to composition, mechanism of action, stability, and functional advantages offers a definitive foundation for their identification and use. The variety of postbiotic types, such as SCFAs, bacteriocins, enzymes, and polysaccharides, underscores their adaptability and efficacy in enhancing health outcomes. Future studies will probably concentrate on enhancing postbiotic production and investigating their therapeutic uses in personalized nutrition and medicine.

7.3 Mechanisms of Action of Postbiotics

7.3.1 Modulation of Gut Microbiota

Postbiotics are essential in regulating gut microbiota by affecting microbial makeup and activity. The metabolites generated during microbial fermentation, including short-chain fatty acids (SCFAs) and bacteriocins, play a crucial role in maintaining a healthy gut ecology [35]. Short-chain fatty acids (SCFAs), including butyrate, acetate, and propionate, promote the development of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* species while suppressing the growth of detrimental pathogens. This specific control maintains gut homeostasis, averting dysbiosis—a microbial imbalance linked to gastrointestinal diseases including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) [19, 25].

Postbiotics diminish the generation of hazardous compounds from pathogenic bacteria by modulating fermentation pathways. Bacteriocins generated by probiotic strains compromise the cell membranes of pathogenic bacteria, thus fostering a healthy microbial ecosystem [31]. Furthermore, postbiotic constituents such as exopolysaccharides (EPSs) promote the development of biofilms that safeguard beneficial bacteria, thus guaranteeing their viability and usefulness in the gastrointestinal tract [33]. These findings emphasize the potential of

postbiotics as therapeutic agents for reestablishing microbial equilibrium and avoiding gastrointestinal infections.

7.3.2 *Anti-inflammatory and Antioxidant Mechanisms*

Postbiotics significantly promote health by mitigating inflammation via many methods. Short-chain fatty acids (SCFAs), especially butyrate, modulate inflammatory responses by obstructing nuclear factor kappa B (NF- κ B) signaling pathways, which are recognized for promoting pro-inflammatory cytokine synthesis [35]. By modifying these pathways, postbiotics reduce the levels of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), alleviating chronic inflammation that may result in illnesses such as inflammatory bowel disease (IBD), obesity, and type 2 diabetes [18, 36].

Besides their anti-inflammatory actions, postbiotics demonstrate antioxidant characteristics by neutralizing reactive oxygen species (ROS). Bacterial metabolites, including superoxide dismutase (SOD) and glutathione, are recognized for their ability to scavenge free radicals, thereby averting oxidative damage to tissues and cells [31]. This antioxidant pathway is crucial for gastrointestinal health since oxidative stress may undermine intestinal barrier integrity and result in heightened permeability, often known as “leaky gut syndrome” [19]. Postbiotics, by their synergistic anti-inflammatory and antioxidant properties, aid in preserving a healthy gut milieu and mitigating the risk of systemic inflammation.

7.3.3 *Immunomodulation*

Postbiotics regulate the immune system by augmenting the host's immunological response and fostering immune tolerance. Postbiotics primarily exert their effects by engaging with receptors in the gut-associated lymphoid tissue (GALT), including toll-like receptors (TLRs) [36]. These interactions enhance the generation of regulatory T-cells (Tregs) and anti-inflammatory cytokines such as interleukin-10 (IL-10), facilitating immunological homeostasis. Furthermore, constituents such as peptidoglycan and lipoteichoic acid from bacterial cell walls function as immune stimulants, augmenting innate immune responses to infections [26, 33]. Postbiotics further affect the synthesis of immunoglobulin A (IgA), which is essential for mucosal immunity. Elevated IgA levels enhance the intestinal barrier and inhibit the adherence of pathogens to the gut lining [31]. Furthermore, some postbiotic peptides and metabolites have shown the ability to enhance the development of dendritic cells, thereby increasing antigen presentation and immune surveillance [35]. The immunomodulatory effects underscore the potential of postbiotics in the management of autoimmune illnesses and the enhancement of general immune function.

In summary, the mechanisms of action of postbiotics are varied and complex, including the modification of gut microbiota, anti-inflammatory and antioxidant

properties, and immunomodulation. These pathways make postbiotics interesting candidates for therapeutic applications in human and animal health. The capacity of postbiotics to function autonomously from live bacteria has considerable benefits, ensuring stability and effectiveness across various formulations and clinical environments [3].

7.4 Key Bioactive Compounds in Postbiotics

Short-chain fatty acids (SCFAs), bacteriocins, peptides, exopolysaccharides, vitamins, and enzymes are some of the main bioactive substances found in postbiotics that have positive health effects (Fig. 7.1). Acetate, propionate, butyrate, and other SCFAs are critical for gut health and metabolic control. Peptides and bacteriocins have antibacterial properties that help the immune system and suppress harmful germs. Exopolysaccharides have anti-inflammatory qualities and help maintain the integrity of the gut flora. Postbiotic vitamins and enzymes also help improve metabolic processes and food absorption. Together, these bioactive substances support digestive health, immunological regulation, and even neuroprotection, which makes postbiotics an important part of medical treatments.

7.4.1 Short-Chain Fatty Acids (SCFAs)

Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, are principal bioactive metabolites generated during bacterial fermentation of dietary fibers. These compounds are essential for sustaining gut health by providing an energy source for intestinal epithelial cells, especially colonocytes [19]. Butyrate fortifies

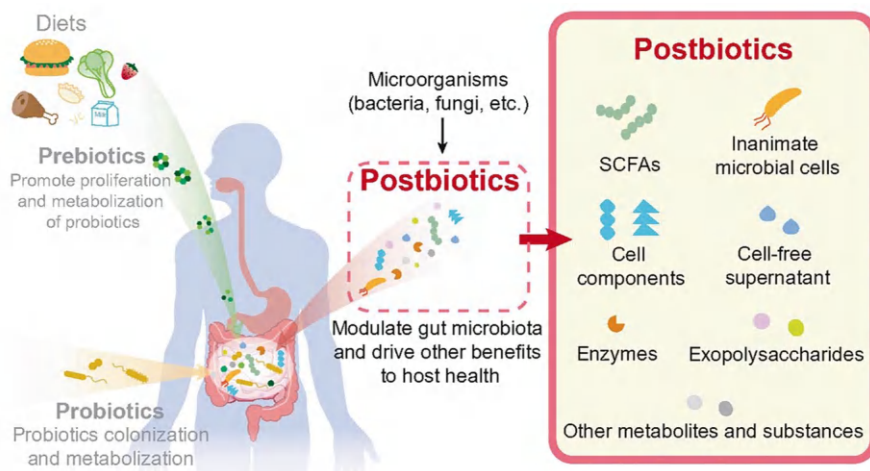


Fig. 7.1 Schematic representation of prebiotics, probiotics, and postbiotics in the human gut [39]

the intestinal barrier by facilitating tight junction formation, thus decreasing the likelihood of a “leaky gut” and ensuing systemic inflammation. Furthermore, short-chain fatty acids (SCFAs) exhibit anti-inflammatory characteristics by obstructing the nuclear factor kappa B (NF- κ B) pathway, which governs the synthesis of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) [35].

Short-chain fatty acids (SCFAs) have systemic metabolic effects, enhancing insulin sensitivity and lipid metabolism. Propionate, for example, is involved in the control of gluconeogenesis and cholesterol production. Acetate affects appetite management by interacting with the gut–brain axis, thereby influencing satiety signals [19]. These characteristics render SCFAs vital bioactive chemicals in postbiotics for the prevention of metabolic diseases, including obesity and type 2 diabetes [15].

7.4.2 *Bacteriocins and Peptides*

Bacteriocins are antimicrobial peptides synthesized by probiotic bacteria, including *Lactobacillus* and *Bifidobacterium* species, that impede the proliferation of pathogenic germs by compromising their membranes [31]. These peptides operate by creating breaches in bacterial cell membranes, resulting in the release of intracellular contents and subsequent cell death. Their targeted antibacterial properties preserve good gut microorganisms, thus maintaining microbial equilibrium and reducing infections.

Bacteriocins, including nisin and pediocin, are extensively used as food preservatives owing to their efficacy in suppressing spoilage microorganisms [33]. Moreover, several bioactive peptides included in postbiotics have immunomodulatory properties, augmenting immune responses by stimulating the activity of natural killer (NK) cells and macrophages. Certain peptides inhibit the angiotensin-converting enzyme (ACE), thus promoting cardiovascular health by lowering blood pressure [31, 34].

7.4.3 *Exopolysaccharides (EPSs)*

Exopolysaccharides (EPSs) are extracellular polysaccharides produced by bacteria during fermentation. These polysaccharides enhance the structural integrity of bacterial biofilms, promoting the colonization and persistence of good gut bacteria [36]. EPSs have prebiotic characteristics, acting as substrates for advantageous microorganisms, facilitating their proliferation, and augmenting gut microbial diversity [28, 30, 33]. In addition to their function in microbial colonization, EPSs exhibit immunomodulatory and antioxidant characteristics. They regulate immunological responses by engaging with receptors on immune cells, including toll-like receptors (TLRs), thus promoting the generation of anti-inflammatory cytokines

[31]. Furthermore, EPSs may mitigate reactive oxygen species (ROS), thereby alleviating oxidative stress and safeguarding tissues from injury. These attributes render EPSs advantageous in functional foods and nutraceuticals aimed at enhancing immune function and mitigating chronic illnesses [21].

7.4.4 Vitamins and Enzymes

Postbiotics include vital vitamins, notably B-group vitamins (such as B2, B6, B9, and B12) and vitamin K, synthesized by probiotic bacteria during fermentation. These vitamins are essential for energy metabolism, erythropoiesis, and brain activities [36]. Vitamin K, produced by gut bacteria, is crucial for blood coagulation and skeletal health. B-group vitamins, including folate (B9) and cobalamin (B12), are essential for DNA synthesis and neurological development, underscoring the significance of postbiotic-derived vitamins in health maintenance [28, 30].

Enzymes constitute a significant bioactive element of postbiotics. Bacterial enzymes, including amylase, protease, and lipase, enhance the digestion of carbohydrates, proteins, and lipids, thereby enhancing nutritional absorption and digestion [33]. Antioxidant enzymes, such as superoxide dismutase (SOD), are essential for neutralizing free radicals, mitigating oxidative stress, and safeguarding tissues from harm. These enzymes help bolster gut barrier integrity by maintaining a balanced redox state, thereby promoting general health [4].

Overall, the principal bioactive constituents of postbiotics, short-chain fatty acids, bacteriocins, exopolysaccharides, vitamins, and enzymes, demonstrate various health-enhancing effects. Postbiotics are crucial in the formulation of functional meals, supplements, and medicines for people and animals due to their functions in regulating gut health, immunological function, and metabolic processes [15].

7.5 Health Benefits of Postbiotics

7.5.1 Role in Gastrointestinal Health

Postbiotics are essential for sustaining and improving gastrointestinal (GI) health by boosting gut barrier integrity, fostering microbial equilibrium, and mitigating inflammation. Short-chain fatty acids (SCFAs), such as butyrate, acetate, and propionate, are essential bioactive chemicals in postbiotics that strengthen intestinal barrier integrity by facilitating the formation of tight junctions among epithelial cells [35]. Butyrate is the principal energy substrate for colonocytes, promoting mucosal repair and averting disorders like “leaky gut syndrome”, characterized by the translocation of toxins and pathogens into the bloodstream due to impaired intestinal barriers [19, 37].

Postbiotics not only enhance gut integrity but also modulate gut microbiota composition by suppressing pathogenic germs and increasing the proliferation of

beneficial bacteria, including *Bifidobacterium* and *Lactobacillus* species. Bacteriocins, antimicrobial peptides synthesized by probiotics, significantly inhibit pathogenic bacteria, thereby averting gastrointestinal illnesses and fostering microbial equilibrium [31]. These activities render postbiotics advantageous in the prevention and management of inflammatory bowel disorder (IBD) and irritable bowel syndrome (IBS).

7.5.2 Metabolic Regulation and Weight Management

Postbiotics have shown considerable promise in modulating metabolic processes, essential for the prevention of obesity, type 2 diabetes, and metabolic syndrome. Short-chain fatty acids, including acetate and propionate, affect energy metabolism by increasing insulin sensitivity and facilitating glucose absorption in the muscle and liver tissues [19]. Propionate affects lipid metabolism by inhibiting cholesterol production in the liver, thereby decreasing the risk of cardiovascular illnesses [32, 36].

Furthermore, postbiotics affect hunger control via the gut–brain axis. Acetate, for instance, promotes the secretion of appetite-inhibiting hormones including peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), which aid in diminishing food consumption and regulating weight [35]. The metabolic advantages render postbiotics significant elements in dietary strategies designed to address obesity and associated conditions. Postbiotics enhance mitochondrial activity and diminish systemic inflammation, thus improving overall metabolic health and lowering the risk of metabolic disorders [19].

7.5.3 Enhancement of Immune Function

Postbiotics markedly improve immune function by regulating both innate and adaptive immune responses. Short-chain fatty acids (SCFAs), including butyrate, facilitate the development of regulatory T-cells (Tregs), essential for sustaining immunological tolerance and averting autoimmune responses [36]. Butyrate diminishes the secretion of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), by inhibiting NF- κ B pathways, thus alleviating chronic inflammation and fostering immune homeostasis [35, 40].

Furthermore, bacteriocins and other peptides found in postbiotics augment the functionality of immune cells, including macrophages, dendritic cells, and natural killer (NK) cells, thus enhancing the body's capacity to combat infections [31]. Postbiotics enhance the synthesis of immunoglobulin A (IgA), thereby fortifying mucosal immunity by inhibiting the attachment of pathogens to the intestinal epithelium [33]. The immunomodulatory effects highlight the potential of postbiotics in infection prevention and the treatment of immunity-related illnesses.

7.5.4 *Neuroprotective and Cognitive Impacts*

Postbiotics have emerged as possible regulators of brain function via their interaction with the gut–brain axis, a bidirectional communication mechanism linking the stomach and the brain. Short-chain fatty acids (SCFAs), particularly butyrate, are essential for neuroprotection since they traverse the blood-brain barrier and mitigate neuroinflammation [19]. Butyrate operates as a histone deacetylase (HDAC) inhibitor, facilitating neuroplasticity and strengthening memory and cognitive abilities by increasing the production of brain-derived neurotrophic factor (BDNF) [13, 36].

Moreover, postbiotics affect neurotransmitter synthesis. Gut-derived metabolites influence serotonin production, which is crucial for mood control [31]. This underscores the potential function of postbiotics in the management of mental health disorders, such as anxiety, depression, and neurodegenerative illnesses. Postbiotics have remarkable therapeutic potential for improving cognitive performance and mental well-being by regulating inflammatory and neurotransmitter pathways [35].

7.5.5 *Antimicrobial and Antineoplastic Properties*

Postbiotics demonstrate significant antibacterial efficacy, mostly via the synthesis of bacteriocins and other bioactive peptides that suppress the proliferation of harmful microbes. Bacteriocins compromise the membrane integrity of pathogenic bacteria, inhibiting infections and fostering microbial equilibrium in the gut [33]. The antibacterial capabilities of postbiotics render them advantageous for food preservation and therapeutic uses in the prevention of gastrointestinal illnesses [17, 31].

Besides their antibacterial capabilities, postbiotics have potential anticancer effects. Butyrate, a principal short-chain fatty acid (SCFA), promotes apoptosis in cancer cells by altering gene expression via histone deacetylase (HDAC) inhibition [19]. Butyrate promotes the expression of tumor suppressor genes and slows cell proliferation, thus decreasing the risk of colorectal cancer [36]. Moreover, postbiotics enhance immune surveillance by stimulating the function of dendritic cells and NK cells, which are essential in recognizing and eliminating malignant cells [35]. These data indicate that postbiotics may be included in cancer preventive measures and treatment approaches.

In summary, postbiotics provide several health advantages, including the enhancement of gastrointestinal health, metabolic management, immune response modulation, and cognitive function improvement. Their antibacterial and anticancer activities underscore their potential for medicinal uses. These bioactive chemicals signify a potential domain for study and development, with applications in functional foods, medicines, and personalized nutrition.

7.6 Postbiotics in Food and Pharmaceuticals

7.6.1 *Applications in Fermented Foods and Beverages*

Postbiotics have major uses in fermented foods and drinks, improving their nutritional content and shelf life. Probiotic bacteria, including *Lactobacillus* and *Bifidobacterium* species, produce bioactive substances such as short-chain fatty acids (SCFAs), bacteriocins, and exopolysaccharides (EPSs) during fermentation, which retain their activity even after the bacteria cease to be alive [31]. These chemicals enhance sensory attributes, including flavor and texture, while augmenting product shelf durability by suppressing the proliferation of spoiling microorganisms [14].

Postbiotic-enriched fermented foods include yogurt, kefir, kombucha, and sauerkraut. Yogurt and kefir contain postbiotics such as short-chain fatty acids (SCFAs) and peptides that improve gut health and regulate the immune system [35]. Kombucha, a fermented tea, has organic acids and bacteriocins that have antibacterial properties, thus prolonging shelf life and inhibiting contamination. Furthermore, the postbiotics included in these meals possess antioxidant characteristics that might mitigate oxidative stress and enhance health [33]. The use of postbiotics in fermented goods is a novel strategy to address the increasing consumer demand for functional meals.

7.6.2 *Postbiotic Supplements: Advancement and Obstacles*

The advancement of postbiotic supplements is accelerating, emphasizing the formulation of items that provide specific health advantages. These supplements include bioactive substances like butyrate, bacteriocins, and antioxidant enzymes, intended to enhance gut health, immunological function, and metabolic control [19]. Postbiotic supplements often exhibit more stability than probiotic-based treatments since they do not need living bacterial cultures to maintain efficacy. The stability of postbiotics renders them suitable for incorporation into capsules, pills, and powders.

Nonetheless, several problems persist in the advancement of postbiotic supplements. A significant challenge is guaranteeing the bioavailability of active substances. Certain postbiotics, including short-chain fatty acids (SCFAs), may exhibit restricted absorption in the gastrointestinal system, thereby diminishing their efficiency [36]. Researchers are investigating improved delivery technologies, such as microencapsulation and nano-formulations, to improve the stability and tailored release of postbiotics [33]. Moreover, standardization is a considerable difficulty since discrepancies in manufacturing techniques and raw materials might influence the content and efficacy of postbiotic products.

A further problem pertains to customer acceptability and education. Although the advantages of probiotics are well acknowledged, the notion of postbiotics is rather novel, necessitating initiatives to enhance awareness of their benefits. Furthermore, the formulation of postbiotic supplements must include possible safety issues, especially for leftover bacterial fragments that may provoke immunological reactions in susceptible persons [31, 41].

7.6.3 Regulatory Guidelines and Quality Control

The regulatory environment for postbiotics is currently developing since these products occupy a distinct position between probiotics and functional food components. Regulatory bodies, including the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) are collaborating to create standards for the categorization, labeling, and safety of postbiotic products [36]. A primary problem lies in establishing precise criteria for classifying a postbiotic product since the lack of living microorganisms distinguishes them from conventional probiotics.

Quality control is crucial for guaranteeing the safety and effectiveness of postbiotic products. Manufacturers must comply with good manufacturing procedures (GMPs) and establish quality control techniques to confirm the composition and potency of their goods [35]. Analytical methods like high-performance liquid chromatography (HPLC) and mass spectrometry are often used to measure bioactive substances, including short-chain fatty acids (SCFAs) and bacteriocins, thus assuring product uniformity [19].

A vital component of regulatory control is the formulation of safety profiles for postbiotic products. Although postbiotics often possess a positive safety profile owing to the lack of live bacteria, it is crucial to assess possible concerns linked to leftover bacterial fragments or metabolites. Clinical studies are essential to substantiate the health claims of postbiotic products and confirm their compliance with regulatory standards for functional foods and dietary supplements [33, 43].

Alongside legislative restrictions, explicit labeling is essential for customer transparency. Labels must delineate the content of postbiotic goods, including the kind and concentration of active substances, to facilitate informed customer decisions. Moreover, postbiotic producers must address intellectual property issues since the manufacturing methods for postbiotics are often unique, necessitating meticulous attention to patents and trademarks. The incorporation of postbiotics into food and pharmaceutical products has promising opportunities for enhancing human health. Postbiotics in fermented foods and drinks improve sensory attributes, prolong shelf life, and provide health advantages via bioactive molecules such as SCFAs, bacteriocins, and EPSs. In the pharmaceutical industry, postbiotic supplements provide a reliable and efficacious alternative to probiotics, enhancing gut health, immune response, and metabolic balance.

Notwithstanding their promise, the advancement and commercialization of postbiotics encounter obstacles, including concerns over bioavailability,

standardization, and consumer awareness. Regulatory authorities are essential in formulating standards for the categorization, safety, and labeling of postbiotic goods, guaranteeing that customers get high-quality and safe items. As research on postbiotics progresses, these bioactive chemicals are set to become a crucial element of functional foods, nutritional supplements, and medicinal applications.

7.7 Postbiotics in Veterinary and Animal Health

7.7.1 *Role in Animal Nutrition and Disease Prevention*

Postbiotics provide considerable potential to promote animal nutrition and disease prevention by supplying bioactive substances that improve digestive health, immunity, and growth performance. Conventional antibiotics, once used as growth enhancers in animal feed, have elicited apprehensions over antibiotic resistance. Consequently, postbiotics have arisen as sustainable solutions for enhancing animal health [31].

In animal nutrition, postbiotics enhance feed efficiency by facilitating nutrient absorption and digestive processes. Short-chain fatty acids (SCFAs), including butyrate, function as energy substrates for intestinal epithelial cells, enhancing gut integrity and mitigating gastrointestinal diseases [35]. Furthermore, bioactive peptides and exopolysaccharides (EPSs) promote advantageous gut flora, inhibiting the growth of detrimental pathogens. By promoting a beneficial microbial ecosystem, postbiotics diminish the need for antibiotics, thus decreasing the likelihood of antibiotic resistance in livestock production [36].

Postbiotics demonstrate immunomodulatory characteristics, augmenting the immunological response in animals. They enhance the synthesis of immunoglobulin A (IgA) and augment the functionality of macrophages and natural killer (NK) cells, thereby improving resistance to infections [33]. Furthermore, postbiotic elements such as bacteriocins impede the proliferation of dangerous bacteria, thus diminishing the occurrence of disorders associated with *Salmonella* and *Escherichia coli* infections. The advantages of postbiotics render them a significant enhancement to animal feed, promoting improved animal health and production [48].

7.7.2 *Applications in Poultry, Aquaculture, and Livestock*

7.7.2.1 Poultry

Postbiotics have shown significant efficacy in chicken farming by enhancing gut health, growth performance, and disease resistance. Short-chain fatty acids (SCFAs), including butyrate, are included in chicken feeds to enhance the intestinal barrier, thus decreasing the risk of enteric disorders including necrotic enteritis [19].

Postbiotics augment the proliferation of advantageous gut bacteria, such as *Lactobacillus* species, which suppress the development of pathogens like *Clostridium perfringens* via competitive exclusion and bacteriocin synthesis [31].

Furthermore, postbiotic supplements enhance feed conversion efficiency, resulting in increased weight growth and decreased feed expenses. This is accomplished by enhancing nutrition absorption and minimizing the incidence of gastrointestinal problems, which may adversely affect growth performance. The immunomodulatory effects of postbiotics enhance chicken protection against infections, resulting in reduced mortality rates and improved flock health [33].

7.7.2.2 Aquaculture

In aquaculture, postbiotics are essential for enhancing growth and mitigating illnesses in fish and prawns since bacterial infections present considerable obstacles. The use of antibiotics in aquaculture is stringently controlled owing to apprehensions over antibiotic residues in aquatic products. Postbiotics have antibacterial properties against fish infections, including *Vibrio* and *Aeromonas* species [36].

Postbiotics enhance gut health in aquatic organisms by augmenting the microbial diversity of the gut microbiota. Bacteriocins generated by helpful bacteria suppress harmful species, while short-chain fatty acids enhance intestinal integrity, thus enhancing nutrient absorption and general health [35]. Furthermore, postbiotics augment immune function by promoting the synthesis of immune cells and cytokines, thereby enhancing the resistance of aquatic species to infections [31]. Postbiotics provide a sustainable and environmentally friendly approach to health maintenance in aquaculture.

7.7.2.3 Livestock (Cattle, Swine, and Others)

Postbiotics are increasingly favored in livestock husbandry, including cattle and swine, as a substitute for antibiotics to enhance growth and avoid infections. In cattle and swine, short-chain fatty acids such as propionate and butyrate are crucial for sustaining rumen health and modulating metabolism, thus enhancing feed efficiency and promoting weight growth [19]. Furthermore, bacteriocins in postbiotics inhibit the proliferation of harmful bacteria, including *Escherichia coli* and *Salmonella*, thereby enhancing gut health and diminishing the likelihood of infections [33].

Postbiotics provide advantages for reproductive health in cattle by improving immune function and decreasing the likelihood of infections. Enhanced gut health guarantees efficient food absorption, thus facilitating reproductive performance and fetal development in breeding animals [36]. Moreover, the immunomodulatory effects of postbiotics augment IgA synthesis and bolster mucosal immunity, thereby affording animals improved protection against respiratory and gastrointestinal diseases.

The use of postbiotics in veterinary and animal health has several benefits, such as increased nutrition, augmented immune function, and illness prevention. In poultry, aquaculture, and animal husbandry, postbiotics act as a sustainable substitute for antibiotics, enhancing growth performance while reducing the danger of antibiotic resistance. Postbiotics enhance overall animal health and production by altering gut microbiota, improving nutrition absorption, and strengthening immunity. As research on postbiotics progresses, their use in animal nutrition is anticipated to expand, offering a sustainable method for animal husbandry.

7.8 Recent Advances in Postbiotic Research

7.8.1 Postbiotics and Gut–Brain Axis Interaction

A recent study underscores the pivotal function of postbiotics in modulating the gut–brain axis, the bidirectional communication network between the gastrointestinal tract and the brain. Postbiotics, especially short-chain fatty acids (SCFAs) such as butyrate, influence brain function by diminishing neuroinflammation, enhancing neurogenesis, and augmenting cognitive performance [8]. Butyrate functions as a histone deacetylase (HDAC) inhibitor, enhancing the production of brain-derived neurotrophic factor (BDNF), thus promoting neuronal health and plasticity. Furthermore, metabolites generated from the gut affect the production of neurotransmitters, including serotonin, thereby influencing mood and mental health [19]. The results suggest that postbiotics may serve as possible treatments for neurodegenerative illnesses and mental health issues, such as depression and anxiety [7, 29].

7.8.2 Synergistic Interactions Between Probiotics and Prebiotics

Research demonstrates that the integration of postbiotics with probiotics and prebiotics has synergistic effects, amplifying their health advantages. Postbiotics provide bioactive compounds that enhance the efficacy of probiotics by stabilizing the gut microbiota and fortifying gut barrier integrity [31]. Prebiotics, including dietary fibers, act as substrates for beneficial microorganisms, which subsequently generate postbiotics such as short-chain fatty acids (SCFAs). The amalgamation of these components, referred to as synbiotic, provides extensive assistance for digestive health, immunological modulation, and metabolic management [33]. Research is investigating synbiotic formulations for the prevention of gastrointestinal illnesses, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [27].

7.8.3 Technological Advancements in Postbiotic Production

Recent technical advancements seek to enhance postbiotic production, guaranteeing increased yield and enhanced stability. Improvements in fermentation technology have facilitated the large-scale manufacture of postbiotics from probiotic bacteria, including optimized methods for extracting SCFAs, bacteriocins, and exopolysaccharides (EPSs) [36]. Microencapsulation methods are being devised to safeguard postbiotic substances against deterioration during storage and gastrointestinal passage, thus assuring their precise release and bioavailability. Moreover, bioengineering techniques are used to alter bacterial strains for the targeted synthesis of certain bioactive chemicals, enhancing the effectiveness of postbiotic products [33]. Technological advancements enable the integration of postbiotics into functional meals, supplements, and medications [23].

7.9 Challenges and Future Directions

Notwithstanding the promise of postbiotics, several obstacles persist regarding their manufacture, stability, and delivery. The synthesis of postbiotics depends on certain bacterial strains and fermentation parameters, which might influence the uniformity and quality of the end product [31]. Differences in fermentation substrates and microbial metabolism further add to batch variability, complicating standardization efforts. Stability is a critical issue since several postbiotic chemicals, such as SCFAs, are volatile and susceptible to deterioration during storage. Researchers are investigating sophisticated delivery systems, such as microencapsulation and nanoformulations, to safeguard postbiotics against degradation and guarantee their precise release [19]. These approaches improve the shelf life and bioavailability of postbiotics, making them appropriate for various uses. Further study is required to enhance manufacturing processes and establish standardized distribution systems that guarantee consistent product quality [9, 46].

The regulatory framework for postbiotics is still developing since they occupy a distinct niche between probiotics and functional food components. Regulatory bodies, including the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), are collaborating to create standards for the categorization, labeling, and safety of postbiotic products [36]. A primary problem lies in establishing precise criteria for designating a postbiotic since its distinction from probiotics is characterized by the lack of living bacteria.

Safety issues are essential in postbiotic development. Despite postbiotics often exhibiting a positive safety profile, the possible dangers linked to leftover bacterial fragments or hazardous metabolites need thorough assessment [33]. Clinical studies are crucial for substantiating health claims and verifying the safety and effectiveness of postbiotic products. Furthermore, producers are required to comply with good manufacturing practices (GMPs) and establish stringent quality control protocols to fulfill regulatory standards [6, 42].

The increasing interest in personalized nutrition and medicine offers new prospects for postbiotic applications. Progress in microbiome research has shown the significance of unique microbial assemblages in influencing health outcomes. Customized postbiotic formulations designed according to an individual's gut microbiota composition may improve treatment effectiveness and provide specific health advantages [31]. Individuals with metabolic problems may gain advantages from postbiotics that enhance insulin sensitivity and lipid metabolism, while those with inflammatory illnesses might benefit from postbiotics exhibiting strong anti-inflammatory characteristics.

Furthermore, postbiotics are under investigation as adjuncts in precision medicine, enhancing traditional treatments for chronic illnesses. Postbiotics have the potential to cure ailments from autoimmune disorders to neurodegenerative diseases by modifying immune responses and affecting the gut–brain axis [8]. The advancement of personalized postbiotic goods necessitates more investigation to comprehend the intricate connections between the microbiome, host physiology, and postbiotic chemicals [6, 42].

7.10 Conclusion

Postbiotics signify a potential advancement in health and wellness, providing several advantages, including the enhancement of gastrointestinal health, modulation of immunological function, and support for cognitive performance and metabolic control. Recent advancements in postbiotic research have highlighted their potential to impact the gut–brain axis, augmenting probiotics and prebiotics and fostering technical developments in manufacturing. Notwithstanding their potential, issues of manufacturing, stability, and regulatory adherence must be resolved to fully realize the capabilities of postbiotics in food, supplements, and medicine. The future of postbiotics is in their incorporation into personalized nutrition and medicine, where customized formulations may provide specific health advantages based on unique microbiome profiles. As research advances, postbiotics are anticipated to become fundamental to functional meals and treatments, enhancing health and well-being among many groups. The establishment of standardized manufacturing processes, efficient delivery systems, and explicit regulatory frameworks will be crucial for guaranteeing the safety, effectiveness, and accessibility of postbiotic products.

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Chapter 8

Impact of Diet, Microbiota, and Postbiotics in Colorectal Cancer (CRC) Management



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Abstract The occurrence of colorectal cancer (CRC), which is considered the most prevalent type of cancer globally, is majorly affected by the gut microbiome. The initiation, progression, and reaction of CRC to various systemic treatments are all influenced by the microbiome. Valuable information for CRC screening, early detection, and therapeutic treatment outcomes prediction is obtained by the composition of bacterial species and the contribution of diet to the microbiome. Food components, including proteins, fats, and fibers, stimulate the metabolism of bacteria, which helps with digestion and produces by-products that are extremely important to the host's function. On the other side, when this equilibrium is upset, the gut microorganisms produce hazardous compounds that have cytotoxic and genotoxic effects. Furthermore, by promoting microbial variety and nourishing the preexisting

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microbiota, meals high in probiotics and prebiotics can improve the diversity of the microbiome. One of the most promising novel approaches in medicine to enhance personal well-being is microbiome regulation. To ascertain the effectiveness of systemic therapy for CRC, reduce side effects, and raise survival rates, more investigation into the gut microbiome in oncology and in the care of CRC patients is therefore necessary.

Keywords Colorectal cancer · Microbiome · Dietary ingredients · Genotoxic · Prebiotics · Postbiotics

8.1 Introduction

Colorectal cancer (CRC), which comprises tumors of the rectum and colon, is the third major form of cancer in oncological pathology, behind lung and breast cancers. In the year 2020, almost 2 million instances were identified. While successful detection approaches are there that may lower the total amount of mortality, CRC remains the second leading reason for death due to cancer. The International Agency for Research on Cancer predicts that the worldwide incidence of CRC will rise by 56% between the time period of 2020 and 2040, with over 3 million instances projected each year [1].

At present, tumor resection chemotherapy, radiation, and surgery are the most often suggested cure for CRC [2]. In recent years, targeted treatment has become known as a viable anticancer treatment approach [3]. Yet, the treatment effectiveness of existing techniques is typically reduced in late-stage or metastatic CRCs, while they are generally effective in early-stage CRCs that have low relapse rates. It was observed that early CRC detection was followed by successful treatments that significantly increased the lifespan of patients and general well-being [4]. CRC treatment requires precise diagnosis and action at an early stage.

The connection between host and microorganisms is critical in both wellness and sickness. Nutrition, living conditions, age, and other external variables all have a significant influence on the variety of the gut microbiome. According to Simões et al., food is a significant moderator of the gut flora. The human microbiome has the potential to influence appetite, increase nutrient harvesting, and generate energy

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from different diet elements. Microorganisms also play a critical function in xenobiotic digestion. Numerous gut bacteria affect the molecular structures of numerous diet constituents, medications, contaminants, and pesticides in xenobiotic metabolism [5].

Various investigations have turned to postbiotics, described by the International Scientific Association of Probiotics and Prebiotics (ISSAP) as a “Mixture of inert microbes and/or their constituents that imparts well-being advantages.” It determined that the word “paraprobiotics” was employed in earlier research to characterize non-living microbe fractions or cells that provide health benefits to the host, as opposed to the prior description of postbiotics as “soluble substances or compounds with therapeutic purpose” [6]. In accordance with the current postbiotics guidelines, while also providing a comprehensive picture of recent achievements in adjacent domains, the present chapter aims to employ the ISSAP’s wider description of diet, microbiota, and postbiotics.

8.2 Overview of Gut Microbiota

The microbiota, known as the second “organ” of the body of a person, plays a growing part in the digestive system of humans, digestion, defenses, and a variety of other activities. It is thought that approximately 95% of bacteria live in the gut, particularly in the large intestine. Given its high bile contents and short passage time, the small intestine presents a more difficult atmosphere for bacterial colonization [7]. The colon, on the other hand, has the most diverse community of microbes due to its moderate rate of flow and neutral somewhat acidic pH. The International Probiotic Association describes microbiome in the following manner: A microbiota is a colony of organisms that live in particular conditions and on or in tissues in humans like the epidermis, pulmonary salivary glands, urogenital and intestinal tracts. Bacteria, archaeabiota, virobiota, and micobiota make up the human microbiota, and they communicate in a very complicated system with the host. The gastrointestinal system of humans includes over 100 trillion bacteria. The colon’s microbial cell density is expected to be 10¹¹–10¹² per milliliter [1].

The MetaHit and Human Microbiome Project information has been merged to create the greatest depiction of the man-related microbial repertoire yet. The combined information from these investigations discovered 2172 species derived from humans, categorized into 12 distinct phyla, with 93.5% belonging to Bacteroidetes, Actinobacteria, Firmicutes, and Proteobacteria. Three of the 12 discovered phyla had only one human-isolated species, comprising *Akkermansia muciniphila*, the Verrucomicrobia phyla’s sole representative. In human beings, 386 of the reported species are completely anaerobic, hence they usually occur in mucosal areas such as the cavities in the mouth and gastrointestinal system [8].

8.3 Gut Microbiota and Colorectal Cancer

In the last few decades, great emphasis has been placed on the function of microorganisms in the development of cancer. It has been discovered that bacteria are implicated in 20% of malignancies, particularly CRC. The initial investigation to demonstrate the function of the intestinal microbiome in regulating the cancer-causing properties of cycasin in mice without germs was reported in 1967. A number of studies have linked dysbiosis of the gut microbiome to the onset of CRC. There is increasing proof that modifying the gut microbiome influences CRC development [9]. Research on animals has indicated that the gut microbiome of individuals with CRC differs from that of individuals in good health, with less beneficial bacteria and more procarcinogenic microorganisms [10]. Researches have also discovered changes in the feces and mucosal microbiome of individuals with CRC. Touchefeu et al. discovered that *Eubacterium siraeum*, *Bacteroides eggerthii*, *Alistipes finegoldii*, *Barnesiella intestinihominis*, and *F. prausnitzii* were considerably lower in individuals with CRC than in controls. Many researches have attempted to delve further into the microbiome makeup linked with CRC in feces and mucous specimens [11]. These investigations discovered a worldwide composition alteration in the CRC microbiome. Certain kinds of bacteria have been linked to CRC through research including multiple populations. Numerous investigations have indicated that CRC has less diversity in bacteria and more pro-tumorigenic microorganisms [12]. *Peptostreptococcus anaerobius*, *Enterococcus faecalis*, *Clostridium septicum*, *Streptococcus bovis/gallolyticus*, *Bacteroides fragilis*, *E. coli*, and *Fusobacterium nucleatum* are among the kinds of bacteria most typically related with colorectal cancer. A systematic review of metagenomes revealed a core collection of bacterial species high in CRC. These included *Thermanaerovibrio acidaminovorans*, *Alistipes finegoldii*, *Prevotella intermedia*, *Porphyromonas asaccharolytica*, *Parvimonas micra*, *Bacteroides fragilis*, and *Fusobacterium nucleatum* [13].

A 2019 study found 29 key species abundant in CRC throughout eight geographic zones. According to Wang et al., *Peptostreptococcus*, *Streptococcus*, *Klebsiella* *Escherichia/Shigella*, *Enterococcus*, and *Bacteroides fragilis* were considerably more prevalent in the gut microbes of individuals with CRC than *Roseburia* and other bacteria that produce butyrate from the *Lachnospiraceae* family. Some bacteria, for instance, *Peptostreptococcus anaerobius*, *Bacteroides fragilis*, and *E. coli*, have been linked to CRC via the initiation of the Th17 cell response and the cause of DNA damage [14].

8.4 Gut Dysbiosis

Gut dysbiosis is defined as the composition and functional changes produced by a lack of symbiotic and opportunist bacteria. Dysbiosis is classified into three categories: (i) loss of helpful microorganisms, (ii) increase in harmful microbes, and (iii)

decrease of diversity in microbial life. Dysbiosis is linked to a variety of pathogenic illnesses, including diabetes, obesity, neurodegenerative diseases, and cancer [15].

At present, there are three identified genotoxins generated by organisms that attack DNA and cause strand breaks: cytolethal distending toxin (CDT) generated by Gram-negative bacterial species, typhoid toxin generated by *S. Typhi*, and colibactin generated by group B2 *E. coli*. *B. fragilis* generates and produces endotoxins which can induce damage to DNA, resulting in alterations and CRC development [16].

Genetic instability occurs when such strands rupture or genetic alterations are not fixed, which can lead to cancer development and recurrence. Furthermore, some pathogenic organisms, for instance, *Shigella flexneri*, block pathways for DNA repair through the enzymatic breakdown of p53, raising the likelihood of alteration escape repair. P53 is a tumor regulator gene that causes apoptosis and is responsible for inducing cell cycle arrest in damaged cells; an alteration or inhibition of this results in the unregulated proliferation of cells [7]. CRC has an elevated frequency of p53 mutations, similar to most malignancies. Patulin and Aflatoxin B1 which are generated by *Aspergillus*, result in the creation of DNA strand break and adducts respectively, as well as reactive oxygen species (ROS), are examples of fungal allergens that can also relate to the cause of cancer [17].

ROS induces CRC through apoptosis, angiogenesis, inflammation, DNA damage, and the transition of epithelial cells to mesenchymal transitions. Additionally, ROS triggers the development of mutations in different proteins that regulate the cell cycle (proto-oncogenes), such as c-Myc, Ras, and p53 which results in onco-gene stimulation. Enterotoxigenic *E. coli* that produces colivacin changes the progression of the cell cycle and causes DNA damage. The pathogenic bacterial species may additionally bind to epithelial cells in the gut and stimulate the proliferation and growth of cells, resulting in hyperplasia [16].

8.5 Dysbiosis Factors

Research has indicated a connection between an imbalance in the gut microbiota and external factors and CRC development and prognosis. A pro-inflammatory change in the microbiota of the gut may result from a number of reasons, including aging, obesity, antibiotic use, pro-inflammatory diets, and insufficient exercise. Gut microbiota and colorectal cancer are linked by the prolonged use of antibiotics. Growing older results in a decrease in CD4 T cells and a change to a pro-inflammatory microbiome. As a result, immune cells are less able to control colon inflammation [9]. Along with inflammation and dysbiosis, a decrease in bacteria that produce butyrate raises the intracolonic pH and causes colorectal cancer (CRC). In an animal model, smoking also causes CRC and modifies the composition of the intestinal microbiome [18]. Investigations comparing the gut microbial assemblages of breastfed and formula-fed infants have shown that diet has the greatest impact on the homeostasis of the gut microbiome, and this influence is present from birth [19].

Research has been done to find out what kinds of effects certain foods and nutrients from them have on the entire body. Numerous investigators worldwide have long discovered a link between nutrition and dysbiosis-related illnesses like obesity, diabetes, and allergy disorders; nevertheless, more recent studies have revealed that diet may also have an effect on conditions like IBD and some types of cancer, such as colorectal cancer (CRC) [20]. The makeup of the gut microbiota differed dramatically between an agrarian diet that mostly includes fewer animal products and high carbs and simple carbohydrates, and a “Western” eating habit that is high in animal products, sugar, and saturated fat. According to De Filippo et al., those who consumed a Western diet showed higher levels of Firmicutes, Proteobacteria, and some Bacteroides in their microbiota, whereas those who consumed an agrarian diet showed higher numbers of Actinobacteria, primarily Prevotella. A high sugar and fat diet has been shown to cause dysbiosis in mice with compromised host barrier activity. There was a decrease in SCFAs in those eating a Western diet and a rise in SCFAs in those eating an agrarian diet. SCFAs are produced in the colon as part of the fermenting procedure for dietary fiber. The three most prevalent SCFAs are butyric, propionic, and acetic acids. Since SCFAs, especially butyric acid, are believed to be preventive towards the growth of possibly harmful bacteria in the gut, it is possible that SCFAs play a regulatory function in the development of dysbiosis [20]. Genetic factors are also closely associated with the onset of dysbiosis in relation to external influences like food. This is especially common in dysbiosis linked to CRC. The research focuses solely on variations of IL-10R/IL-10, which have been linked to symptoms that appear IBD, in addition to variations of ATG16L1, and NOD2 the two most investigated IBD vulnerability genes and corresponding proteins, which have been shown to raise the possibility of CRC via changed relationship with the gut microbiome [20].

8.6 Impact of Diet for Maintaining Gut Microbiota

The human gut microbiota is a complex assembly of bacteria, viruses, fungi, and other microbes that have colonized the gastrointestinal tract over millions to billions of years. These microorganisms play important roles in several bodily functions, including digestion and metabolism, immune function, and mental health [8]. Gut microbiota, being an indispensable element of the intestinal environment functions to support a host as barriers and defenders (barrier function; protection of gut barrier), modulators (e.g., immune system); regulators (i.e., metabolic adjustment, nutrient/drug uptake) [21]. At birth, the human gut is nearly sterile, but it quickly becomes colonized by the mother’s microbiome during delivery [22]. The human gut microbiota comprises five dominant phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia. Firmicutes and Bacteroidetes are the most abundant phyla, representing 90% of gut microbiota [23]. The gut microbiota is a dynamic and ever-evolving ecosystem, influenced by various factors, with diet being one of the most significant modulators [24]. The makeup,

diversity, and function of the gut microbiota are greatly influenced by diet. The trillions of bacteria living in our digestive tract receive nutrition from the foods we eat in addition to our bodies. The human diet typically includes various food types such as proteins, fats, and carbohydrates, and maintaining a proper balance among these nutrients is essential. The type and amount of these dietary components are significantly influenced by the composition and diversity of the gut microbiome [25]. So, a balanced daily diet is crucial for maintaining a healthy gut microbiome, ensuring a normal distribution and balance among various groups of gut microorganisms. Dysbiosis refers to a state in which an unstable diet or depletion of nutrients triggers the pathogenic groups in gut microbiome growth and overgrowth, while other beneficial ones are drastically reduced. This can lead to the overproduction of toxic metabolites and an increase in pathogenic strains, which could ultimately damage intestinal integrity leading toxins into systemic circulation. These toxins would influence human organs and lead to different clinical presentations from mild illnesses, continuing to end-stage diseases after years of intake [26].

8.6.1 Different Components of Diets and Their Impact on Gut Microbiome

(i) Carbohydrates

It is important to note that the gut microbiome is influenced by carbohydrates, which determine various aspects of human health. It has been observed that the composition and function of gut microbiota are affected by the type and quality of carbohydrates consumed. Consuming simple sugars can lead to the proliferation of pathogenic bacteria that may cause harm. Conversely, dietary fiber, resistant starch, and oligosaccharides help in maintaining a balanced ecosystem of microflora [27]. This is important for overall wellness and prevention of various disorders such as chronic diseases. Simple sugars like glucose, fructose, and sucrose are rapidly absorbed in the small intestine, leaving little for the gut bacteria in the colon. However, excessive intake of simple sugars can lead to an overgrowth of certain bacteria, such as those in the Firmicutes phylum, which are associated with obesity and metabolic disorders.

(ii) Proteins

Through diet, the gut microbiome is molded by proteins. When proteins from red and processed meat are broken down, they produce gases such as hydrogen sulfide and ammonia that can cause inflammation in the intestines thereby increasing the risk of colorectal cancer. On the other hand, it has been found that plant-based proteins and dietary fiber can actually help to nurture useful bacteria such as Bifidobacteria and Lactobacilli. These bacteria play a role in keeping the gut lining healthy and promoting the production of anti-inflammatory short-chain fatty acids (SCFAs). In summary, maintaining a healthy and diverse gut microbiome which is crucial for overall health and disease prevention requires considering the type and balance of protein sources [28].

(iii) *Fats*

The variety of fats we consume greatly influences the gut microbiome. Fatty acids are categorized into two types; saturated and unsaturated fats. Saturated fatty acids found in animal products and processed foods might reduce the diversity of microbes, promoting pro-inflammatory bacteria. This results in inflammation within the intestines leading to conditions such as obesity, insulin resistance, and other metabolic disorders [29]. However, monounsaturated, and polyunsaturated fats from sources like olive oil, nuts, and fish can be helpful for gut flora. They also cause a reduction in inflammation and improve the functions of the gut barrier, thus supporting optimum intestinal health [30]. On top of everything else, omega-3 fatty acids boost both microbial diversity as well as short-chain fatty acid (SCFA) production. By doing so, they help maintain gut health, preventing inflammatory processes that can result in colorectal cancer. Nevertheless, all kinds of fats are important dietary components because focusing on monounsaturated fats or omega-3 fats could positively affect the composition of gut microbiota, reducing the chances of developing inflammation that can lead to colorectal cancer.

(iv) *Fiber*

Dietary fibers are essential for a healthy gut. These indigestible fibers make their way to the colon, where they become food for gut bacteria. When these bacteria break down the fibers, they produce short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate. These SCFAs are vital for maintaining a healthy gut, as they help reduce inflammation, strengthen the gut lining, and provide energy to colon cells. Fibers also create a favorable environment for beneficial bacteria like Bifidobacteria and Lactobacilli, promoting their growth while keeping harmful bacteria in check [31]. Consuming a fiber-rich diet not only supports gut health but also lowers the risk of chronic diseases such as colorectal cancer, type 2 diabetes, and heart disease. In short, dietary fibers play a crucial role in maintaining a balanced gut microbiome, which is key to overall well-being.

8.7 Impact of Diet on Colorectal Cancer

Colorectal cancer is influenced by diverse ways in which the dietary pattern affects it. There are specific eating habits that correspond to a typical Western type of nutrition and increase the risk of colorectal cancer (CRC), for instance; high intake of red meat, processed meats, and saturated fats with low intake of dietary fiber. Conversely, diets characterized by high intakes of fruits, vegetables, whole grains, and low-fat dairy have been associated with reduced risks for colorectal cancer [32]. Table 8.1, depicted below, shows various mechanisms such as nutrient composition, carcinogenic compounds, and antioxidants through which diet causes colorectal cancer risk.

Table 8.1 Mechanisms of diet influencing colorectal cancer risk

Mechanism	Description	Dietary influence	References
Nutrient composition and metabolism	Dietary components affect cellular processes and metabolism. High fiber promotes colonic health and reduces exposure to carcinogens.	High fiber (from fruits, vegetables, whole grains) Low fiber diets	[33]
Carcinogenic compounds	Some dietary components contain carcinogens that can damage DNA and promote tumor development.	Red and processed meats (nitrates, nitrites, heterocyclic amines) High-fat diets (saturated fats)	[34]
Antioxidant and anti-inflammatory effects	Antioxidants and phytochemicals from fruits and vegetables counteract oxidative stress and inflammation, reducing cancer risk.	Fruits and vegetables (vitamins, flavonoids, carotenoids) Low antioxidant diets	[35]
Microbiome influence	The gut microbiome's composition is influenced by diet, affecting inflammation and cancer risk.	High fiber diets (promote healthy microbiome) Low fiber, high-fat diets (dysbiosis, inflammation)	[36]

Certain nutrients and food compounds can either increase or decrease the likelihood of developing colorectal cancer by affecting processes within cells and the entire body. For example, fruits, vegetables, and whole grains enhance colon health because of their dietary fiber content, which increases fecal bulk, shortens transit time, and reduces the exposure of the colonic mucosa to toxic substances. Additionally, fiber influences changes in the gut microbiota and the subsequent production of short-chain fatty acids, particularly butyrate, which exhibits anti-inflammatory and anticancer properties [33].

Research suggests that consuming red and processed meats is associated with an increased risk of colorectal cancer (CRC). This link is attributed to the presence of carcinogenic substances like nitrates, nitrites, and heterocyclic amines in these meats. These substances have the potential to damage DNA, leading to mutations and fostering tumor development. Furthermore, high-fat diets, particularly those rich in saturated fats, may elevate the risk of colorectal cancer by affecting bile acid metabolism and promoting the production of inflammatory mediators that can alter the gut microbiome [37].

In conclusion, recognizing the link between diet and cancer risk is vital for addressing colorectal cancer (CRC), a significant global health concern. Implementing public health initiatives that encourage a healthy diet and conducting ongoing research into the root causes can help reduce the burden of CRC. Making dietary modifications can notably decrease the risk of developing this disease, leading to improved overall health and longevity.

8.8 Impact of Postbiotics on Colorectal Cancer

The gut microbiota, also known as the intestinal microbiota, produces a variety of metabolites that play a crucial role in the nutritional, metabolic, immunological, and other physiological functions of the host. Imbalances in the microbiota, characterized by a decrease in beneficial bacteria and an increase in harmful bacteria, commonly indicate dysbiosis. This imbalance can disrupt microbial metabolite production, leading to the accumulation of toxic metabolites [38]. Accumulation of toxic metabolites can compromise the integrity of the intestinal mucosal barrier, resulting in chronic inflammation and DNA damage, ultimately increasing the risk of colorectal cancer through cellular carcinogenesis.

Postbiotics are the products obtained from non-living microorganisms or their components, which provide health benefits to the host. These products primarily comprise bacterial components and metabolites. Postbiotics, or the end products produced by probiotic bacteria, have attracted considerable interest for their potential therapeutic roles in a range of diseases, including colorectal cancer (CRC). The primary constituents of postbiotics encompass nonviable bacteria, various bacterial components (such as cytosolic polypeptides, phosphoglycolic acids, intracellular and extracellular polysaccharides (EPSs), and surface proteins), and their by-products (including short-chain fatty acids (SCFAs), organic acids, bacteriocins, and enzymes) [39]. Postbiotics are bioactive molecules produced by the gut microbiota during the fermentation of dietary fibers. These molecules can have a significant impact on the growth of colorectal cancer (CRC) through various pathways as shown in Fig. 8.1. They can trigger the death of cancer cells, regulate immune responses, improve the integrity of the intestinal barrier, and modify gene expression [40].

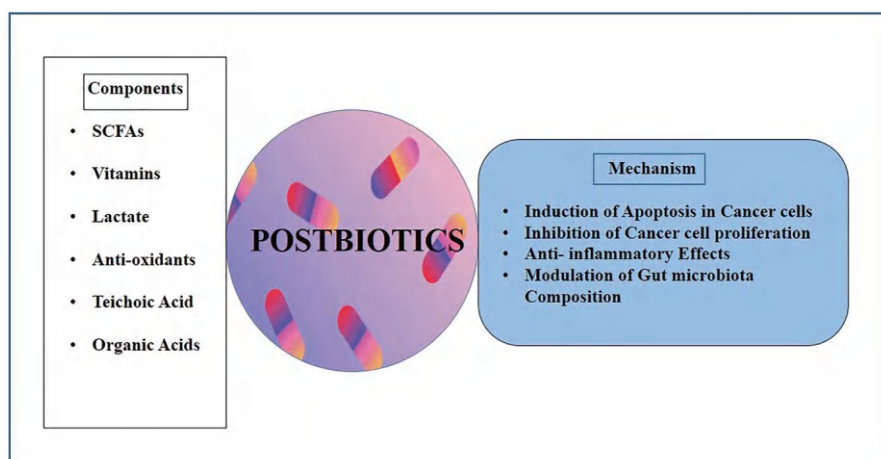


Fig. 8.1 Different components and mechanism of action of postbiotics in CRC

Postbiotics have been found to induce programmed cell death (apoptosis) in colorectal cancer cells, offering a crucial mechanism for impeding the proliferation and survival of cancer cells. One well-researched postbiotic, butyrate, functions by inhibiting histone deacetylases (HDACs), leading to the accumulation of acetylated histones. This alteration in chromatin structure fosters the expression of pro-apoptotic genes such as Bax and P21 while suppressing anti-apoptotic genes like Bcl-2. Ultimately, the activation of apoptotic pathways, such as caspase activation, leads to cell death [41]. Several postbiotics, such as butyrate and propionate, are known to stop the cell cycle at the G1 phase. This occurs by increasing the expression of cyclin-dependent kinase inhibitors (e.g., P21, P27), which leads to cell cycle arrest. This arrest can hinder the growth of CRC cells. Postbiotics, such as SCFAs, influence the gut environment in a way that supports beneficial bacteria like *Lactobacillus* and *Bifidobacterium*. These bacteria produce additional beneficial metabolites that help protect against colorectal cancer (CRC). This balanced composition of gut microbiota can decrease the presence of harmful bacteria, such as *Fusobacterium nucleatum*, which are associated with CRC [42].

Postbiotics play a multifaceted role, including inducing cancer cell apoptosis, inhibiting cell proliferation, reducing inflammation, enhancing gut barrier integrity, and modulating gut microbiota. By influencing key pathways involved in colorectal cancer (CRC) progression, postbiotics not only help prevent the onset of the disease but also offer promising strategies for slowing its progression and improving patient outcomes. The growing body of evidence supports the integration of postbiotics into CRC management. However, further research is necessary to fully understand their mechanisms of action and optimize their therapeutic application.

8.9 Synergistic Effect of Diet and Postbiotics in Cancer Prevention

Dietary practices are considered to be a contributing factor to the development of cancer, supported by various evidence indicating the impact of fruits and vegetables, whole grain products, and specific fats. These foods are rich in vitamins, minerals, fibers, and phytonutrients that possess antioxidants, anti-inflammatory, and anticarcinogenic properties. For example, the Mediterranean diet, known for its emphasis on plant-based foods and healthy fats, has been associated with a reduced risk of certain cancers such as colon, breast, and prostate cancer [43]. Historically, medical interventions such as early detection, vaccinations, and dietary and lifestyle adjustments have been the cornerstone of efforts to reduce cancer rates. However, with advancements in the understanding of the human microbiome and microbial metabolism, new avenues for cancer prevention have emerged. One such promising area of research is postbiotics, bioactive substances derived from the gut microbiota, which have the potential to impact cancer risk. These compounds not only benefit gut health but also have systemic effects, affecting immune function,

inflammation, and gene expression [44]. Recent research suggests that postbiotics can directly or indirectly influence cancer development by modulating various pathways involved in cell proliferation, apoptosis, and oxidative stress. Certain dietary components can impact the composition and function of the gut microbiota, leading to increased production of beneficial postbiotics. For example, gut bacteria ferment dietary fiber to produce SCFAs such as butyrate, which has been demonstrated to inhibit the growth of cancer cells and trigger apoptosis in colorectal cancer models [45]. Similarly, polyphenols present in fruits and vegetables can be transformed by the gut microbiota into bioactive compounds with potential anticancer properties. Furthermore, specific nutrients in the diet can collaborate with postbiotics to create a more potent anticancer effect. For instance, the combination of dietary fiber and SCFAs may boost the expression of tumor suppressor genes while reducing the expression of oncogenes. This synergistic effect can lead to a more effective prevention approach compared to diet or postbiotics alone. By understanding how the gut microbiota interacts with dietary components, it is possible to increase the production of beneficial postbiotics and enhance their antitumor effects. As our understanding of the gut microbiota and its metabolites continues to grow, future studies may provide more targeted dietary recommendations and postbiotic treatments for cancer prevention. This comprehensive approach, combining postbiotics with food, could be a powerful tool in the ongoing fight against cancer and help reduce the disease's devastating global impact.

8.10 Regulatory Aspect of Dietary Supplements

The consumption of dietary supplements is on the rise, yet they are often subject to minimal regulation compared to pharmaceuticals, especially in many regions. Regulating these products is challenging due to their deep cultural roots and the influence of industry marketing, which often operates independently of public health objectives. A significant driver behind supplement use is the belief that they can help prevent chronic diseases, including cancer. However, numerous expert committees and organizations have determined that there is little to no scientific evidence supporting the idea that supplements reduce cancer risk. In fact, some studies have shown that high doses of certain supplements may increase cancer risk. Despite this, the supplement industry frequently makes marketing claims suggesting anticancer benefits. The lack of stringent regulation around the marketing of dietary supplements may continue to lead to misleading information being provided to consumers. Therefore, both the scientific community and government regulators must work together to offer clear, evidence-based guidance to the public regarding the use of dietary supplements for cancer prevention.

8.10.1 History of Regulation of Dietary Supplements

Health claims for foods were not regulated by the FDA until 1990, when Congress responded to the increasing number of unverified health claims made by food manufacturers, including those suggesting anticancer properties, by passing the Nutrition Labeling and Education Act (NLEA). This act led the FDA to begin assessing and setting scientific standards for health claims related to nutritional supplements. However, this initiative was relatively short-lived. In 1994, the Dietary Supplement Health and Education Act (DSHEA) was passed, which reclassified dietary supplements as food products and significantly restricted the FDA's authority over the regulation of supplement manufacturing and marketing [46]. By 2010, recognizing the ongoing need for improved regulation of dietary supplements—highlighted by issues such as the undisclosed inclusion of steroids in some sports supplements—US Senators John McCain and Byron Dorgan introduced the Dietary Supplement Safety Act (DSSA). This proposed legislation aimed to empower the FDA with the authority to monitor the safety of dietary supplements and remove those found to pose potential health risks from the market [47].

8.11 Regulation Model for Dietary Supplements

Typically, two distinct regulatory approaches are used to align with domestic practices, national priorities, and public health requirements. The first approach, adopted by many jurisdictions, involves regulating dietary supplements under existing food and medicine laws. In this framework, various products—including herbal medicines, traditional remedies, and dietary or nutritional supplements—are grouped under broad categories. For instance, these are known as dietary supplements in the United States, complementary medicines in Australia, and natural health products (NHP) in Canada [48]. The second regulatory approach involves creating specific laws tailored to these products, resulting in distinct categories and detailed regulatory frameworks for various types of traditional and complementary medicines (T&CMs). This approach is often seen in countries with well-established traditional healthcare systems, such as China with its regulation of Chinese proprietary medicines (TCM), India with Ayurvedic medicines, and Japan with Kampo medicines [49]. Regardless of the regulatory approach, it is challenging for a single set of laws to encompass all products generally regarded as dietary supplements. For instance, advertising rules and regulations often apply to these products, regardless of whether they are specifically classified as dietary supplements.

8.12 Clinical Significance of Dietary Supplements and Postbiotics

Nutritional supplements are frequently recommended for patients with colorectal cancer (CRC). Clinical studies have shown that supplements like omega-3 fatty acids, arginine, vitamin D, glutamine, and probiotics can be effective in reducing inflammation, enhancing nutritional status, and improving overall clinical outcomes in CRC patients [50]. For instance, glutamine supplementation has been found to influence the immunometabolic response in CRC patients, which can help reduce their susceptibility to infections [51]. Additionally, probiotic supplementation has been shown to alter gut microbiota in CRC patients, decrease bacterial translocation and the release of inflammatory cytokines, and improve patient outcomes [52]. According to research in nutritional oncology, dietary vitamins and antioxidants are important in colorectal cancer (CRC) prevention. However, the relationship between these nutrients and CRC prevention is complex and depends on factors such as dosage, timing, and individual health status.

8.12.1 *Dietary Supplements in CRC Management*

No single dietary supplement is universally effective for all aspects of colorectal cancer (CRC) management. Clinical studies have demonstrated that omega-3 polyunsaturated fatty acids (PUFAs) possess anti-inflammatory properties and may offer preventive benefits against various cancers and age-related conditions. Omega-3s can be converted into anti-inflammatory lipid mediators that interact with specific receptors on immune cells to inhibit the production of proinflammatory cytokines. They also reduce the expression of NF- κ B and PPARs, leading to decreased inflammatory cytokine production. However, a meta-analysis showed that perioperative omega-3 supplementation did not significantly impact weight, BMI, or albumin (Alb) levels in patients with gastrointestinal cancer. Conversely, Yue et al. found that perioperative omega-3 supplementation significantly improved Alb levels in CRC patients. While omega-3 fatty acids are available as dietary supplements, evidence linking them to a reduced risk of colorectal cancer is limited. A systematic review of 20 prospective cohort studies concluded that dietary omega-3 supplementation is unlikely to prevent cancer [53]. Additionally, Brown et al. found that vitamin D supplementation during chemotherapy did not significantly affect weight or BMI in CRC patients, a result that aligns with our analysis. This discrepancy may be due to variations in research populations, including baseline weight loss, types of anticancer treatments, and medication tolerance. It is crucial to maintain the nutritional status of CRC patients during treatment, as this can help reduce treatment-related toxicity and complications, thereby improving patient prognosis and quality of life [54]. Additionally, our network meta-analysis (NMA) indicated that the combination of omega-3 and vitamin D was the most effective in maintaining albumin (Alb),

weight, and BMI levels in CRC patients during treatment. Malnutrition in CRC patients is often associated with cancer-induced systemic inflammation, leading to anabolic resistance and muscle loss [54]. Omega-3 and vitamin D can enhance immune cell function by improving response capabilities, maintaining a balanced immune response, regulating cytokine production and release, and mitigating excessive or harmful inflammatory reactions. These effects help preserve the nutritional status of CRC patients [55]. Based on the findings, the combination of omega-3 and vitamin D appears to be the most effective in maintaining the nutritional status of CRC patients during treatment. Glutamine was most effective in reducing TNF- α levels, shortening the length of hospital stay (LOH), and decreasing weight loss (WI) in CRC patients. Omega-3 combined with arginine was best for lowering IL-6 levels, while probiotics were most effective in reducing the incidence of pneumonia. Given some limitations in current clinical studies and data, future research should involve larger sample sizes, longer follow-ups, and more rigorous study designs to validate these results.

8.12.2 Postbiotics in CRC Management

Postbiotics are chemical substances derived from microorganisms, including short-chain fatty acids, enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, vitamins, plasmalogens, and organic acids [56]. They mainly consist of inactivated bacteria, bacterial fractions (such as cytosolic polypeptides, phosphoglycolic acids, intracellular and extracellular polysaccharides, and surface proteins), and their metabolites (including short-chain fatty acids, organic acids, bacteriocins, and enzymes) [57]. Both postbiotics and prebiotics share similar mechanisms in improving colorectal cancer (CRC) progression by managing gut microbiota, enhancing intestinal mucosal barrier function, regulating immune responses, and influencing systemic metabolism. Studies have demonstrated the chemopreventive effects of a mixture of acetate, butyrate, and propionate in AOM/DSS-treated mice, showing significant reductions in tumor incidence and size. Short-chain fatty acids (SCFAs) were found to lower the expression of pro-inflammatory cytokines like IL-6, TNF- α , IL-17, COX-2, and NF- κ B [58]. Research involving non-viable microbial cells, such as heat-killed *Enterococcus faecalis*, showed that pre-treatment of THP-1-derived macrophages with these cells reduced NLRP3 inflammasome activation in response to fecal content or commensal bacteria, leading to decreased caspase-1 activation and IL-1 β maturation. In vivo studies also indicated that *E. faecalis* alleviated intestinal inflammation and protected mice from colon cancer development. Despite these benefits, there are challenges in applying postbiotics for CRC prevention and treatment. For example, Kim et al. observed that heat-killed strains of *Bifidobacterium bifidum*, *Lactobacillus reuteri*, and *Lactobacillus casei* significantly delayed tumor growth in colorectal carcinoma models, providing direct evidence of the effectiveness of heat-killed bacterial cells against CRC [59]. However, given the vast number and diversity of

bacterial metabolites, the field of postbiotics remains relatively unexplored, and their safety profiles are still under evaluation in both preclinical and clinical settings.

8.13 Future Prospects

The future of colorectal cancer (CRC) management is individualized nutrition strategies based on individual microbiota profiles, which aim to promote gut health, prevent CRC, and improve treatment results. There is an increasing interest in creating targeted postbiotic medicines that can influence the gut microbiota or directly affect CRC cells. The integration of multiomics technologies provides more insight into the relationships between nutrition, microbiota, and host metabolism, potentially leading to new biomarkers and therapeutic targets. Robust clinical trials are essential to translate these findings into effective strategies. Standardized methodologies and regulatory guidelines are also needed to ensure the safety and efficacy of emerging microbiota-based treatments. Another important research area is the effect of dietary interventions on cancer survivors who have recently completed or are undergoing colorectal cancer treatments. While some studies have explored the benefits of prebiotics and probiotics in different patient groups, others suggest that nutritional care before and after colorectal cancer surgery, as well as during treatment, can improve treatment outcomes. On the other hand, little research has investigated this area, necessitating multi-institutional studies with a diverse spectrum of patients. More efforts are needed to create screening programs and control risk factors for CRC to lessen its burden. Molecular characterization and tumor-sidedness evaluation have emerged as critical methods for guiding CRC therapeutic decisions. The complex interplay of genetic and epigenetic changes inside colorectal cancers emphasizes the significance of a tailored treatment strategy. As precision medicine advances, the capacity to personalize therapy methods based on the specific genetic profile of each patient's tumor marks a paradigm shift in CRC management. As research progresses and our understanding of the molecular complexities of CRC grows, the prospect of more effective and customized treatments for CRC becomes more realistic.

8.14 Conclusion

Researchers are in search of ways to increase gut barrier function and host health utilizing various therapeutic options. Most nutritional therapies, including prebiotics, probiotics, or their combination, are believed to improve the mucus layer by modifying the gut microbiota. However, further research is required to establish their effective dosages and safety profiles. Understanding the formation and progression of CRC can help develop targeted prevention strategies and treatments to reduce the global burden of colorectal cancer. Diet, microbiota, and postbiotics have

a significant effect on colorectal cancer (CRC) management, emphasizing their importance in both prevention and treatment. A well-balanced diet that promotes a healthy microbiome can reduce the risk of CRC, whereas dysbiosis, or alterations in the gut microbiota, has been associated with an increased risk of cancer progression. Postbiotics, which are bioactive compounds produced by beneficial gut bacteria, have potential as therapeutic agents for controlling inflammation and boosting immune responses to cancer. For the development of effective CRC management strategies, a multidisciplinary strategy involving nutrition, microbiology, oncology, and emerging technologies is necessary, which will ultimately enhance patient outcomes and quality of life. Similarly, collaborating with dietitians, clinicians, and researchers to create evidence-based guidelines for the optimal intake of vitamins and antioxidants for CRC prevention and management can help individuals make more informed dietary choices and improve health outcomes. By establishing comprehensive, evidence-based recommendations, healthcare providers can offer clear guidance on the role of these nutrients in reducing colorectal cancer risk and enhancing treatment outcomes.

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Chapter 9

The Interplay Between Modulation Strategies of Gut Microbiota and Colorectal Cancer Development



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Abstract The scientific domain that studies the relationship between the human gut microbiome and cancers has changed throughout the last few decades. The bacteria that live in the digestive tract and work with the host to control behavioral and metabolic activities in the gut make up the gut microbiota. A growing number of evidence depicts that colorectal cancer (CRC) progression is correlated with the composition of the gut microbiome. The gut microbiota regulates inflammation by regulating the human immune system and shields the intestines from infections and other pathogens when they are in a healthy state. These gut bacteria affect the immune system's reaction to tumors in addition to being linked to the inflammation of the intestinal tract related to carcinogenesis. As such, they are associated with the growth of tumors and the efficacy of treatments aimed at preventing cancer. Various research depict gut microbiota could be utilized as a biomarker to predict the effects of immunotherapy and increase the effectiveness of this treatment for colorectal cancer by modifying. Here, we talk about the significance of the gut microbiota and how, as evidenced by current studies on the onset and course of colorectal cancer, it cooperates with anti-neoplastic treatment modalities.

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9.1 Introduction

With its incidence continuously rising in many nations, a major contributor to cancer-related death worldwide is colorectal cancer (CRC). Nearly 2.2 million additional cases and 1.1 million fatalities from CRC will occur each year by 2030, resulting in a 60% increase in the global burden [1, 2]. The CRC incidence is rapidly increasing in those under 50, particularly in high-income countries [3, 4]. This, together with the fact that most CRC episodes occur infrequently [5], further indicates the effect of lifestyle variables on the development of CRC.

The microbiota is made up of several bacterial communities that coexist in the host's organs' epithelial barriers and have a mutually advantageous connection. The microbiota is an ecosystem with a metabolic activity that interacts with stromal and epithelial cells and is vital to the physical state of humans [6]. The creation of several essential metabolites, preventing pathogen invasion, and controlling the overgrowth of certain bacterial groups to avoid hazardous bacteria from altering the local environment are just a few of the many roles that the microbiota performs [7]. Research has recently shown how important the gut microbiota is in regulating many aspects of human health, including the development of cancer. Trillions of bacteria make up the human gut microbiota, which is essential for preserving homeostasis and impacting the etiology of disease through processes like immunological regulation, metabolism, and inflammation. A wide range of metabolites that the gut microbiota creates can either be harmful or advantageous to the host. Variations in the quantities of gut microbial metabolites, such as polyphenols, vitamins, short-chain fatty acids, tryptophan catabolites, and polyamines, may be caused by shifts in the balance of intestinal bacteria [8]. These variations may have an impact on the pathophysiology of the aforementioned human disorders. More

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specifically, abnormal levels of short-chain fatty acids and substances related to the metabolism of amino acids, such as polyamines, have been connected to the initiation and spread of cancer in a number of tumor forms [9]. Managing gut microbiota has emerged as a promising treatment and prophylactic approach for colorectal cancer. Probiotics, dietary modifications, prebiotics, antibiotics, and fecal microbiota transplantation (FMT) are some of these tactics. Each of these strategies seeks to modify the gut microbiota's makeup and activity in order to reduce the environment that supports the development of CRC [10]. This study aims to provide a comprehensive overview of existing knowledge about the relationship between gut microbiota regulation and colorectal cancer development. We will examine the ways in which the gut microbiota affects colorectal cancer (CRC), the various techniques employed to alter the microbiota, and the possible effects of these approaches on CRC treatment and prevention. This study aims to shed light on innovative therapeutic options and future prospects in the area by exploring the complex interactions between gut microbiota and colorectal cancer.

9.2 Composition and Function of Gut Microbiota

The human gut microbiota is made up of a diverse range of microorganisms, including viruses, bacteria, archaea, and fungi. Bacteria are the most common and have been studied the most out of all of these [11]. There are several primary phyla that predominate in the gut bacterial ecosystem. Several activities vital to human health are carried out by the gut bacteria, such as [12].

Digestive purpose: Complex proteins, fibers, and carbohydrates that are indigestible to human enzymes are broken down by the intestinal flora. Short-chain fatty acids with anti-inflammatory properties, such as butyrate, acetate, and propionate, are produced as a consequence of this breakdown process and serve as sources of energy for colon cells.

Immune modulation: The immune system of the host interacts with gut microorganisms to help shape and control immunological responses. They enhance the formation of regulatory T cells and immunoglobulin A (IgA) secretion, both of which are essential for immunological tolerance and pathogen protection.

Metabolic aspect: Through its involvement in bile acid metabolism, vitamin synthesis (including that of vitamins K and B), and fat storage management, the microbiota affects host metabolism. Additionally, they create metabolites that may have an impact on lipid metabolism and insulin sensitivity.

Barrier function: The gut microbiota supports the function of the gut epithelium barrier. Some bacteria secrete mucins and other substances that fortify the mucus layer and stop poisons and infections from moving through it.

Neurological purpose: The two-way communication that occurs between the gut bacteria and the central nervous system is explained by the gut-brain axis. Neurotransmitters and other signaling chemicals that can affect behavior and brain function are produced by gut bacteria.

Dysbiosis is the term used to describe an imbalance in the composition and activity of the gut microbiota and is frequently linked to illness conditions. Numerous things, including the use of antibiotics, a poor diet, stress, and infections, can lead to dysbiosis. Dysbiosis has numerous effects and may be a factor in the emergence of various illnesses [6].

Inflammatory bowel disease (IBD): Dysbiosis is a hallmark of IBD, which encompasses Crohn's disease and ulcerative colitis. Variations in the microbiota's composition can lead to increased intestinal permeability, aberrant immune responses, and chronic inflammation.

Metabolic disorders: Changes in gut microbiota have been connected to conditions like metabolic syndrome, obesity, and type 2 diabetes. These metabolic problems are exacerbated by dysbiosis, which can impact insulin sensitivity, fat storage, and energy balance.

Colorectal cancer (CRC): The role of dysbiosis in the emergence of CRC is becoming more widely acknowledged. Some bacterial species—like *Fusobacterium nucleatum*—have been conjugated to the promotion of cancer by immune evasion, DNA damage, and inflammation.

Autoimmune diseases: Dysbiosis affects the gut-immune axis and promotes systemic inflammation, which can have an impact on the development of autoimmune illnesses including multiple sclerosis and rheumatoid arthritis.

Neurological disorders: Recent research indicates that dysbiosis may be related to neurological disorders, most likely via the gut-brain axis.

In a nutshell, the gut microbiota is an intricately dynamic ecology that is essential to human health. Dysbiosis, or disturbances to this system, can have serious consequences for a variety of illnesses, emphasizing the significance of preserving a healthy gut microbiota through nutrition, lifestyle, and possible therapeutic interventions [12].

9.3 Interplay Between Gut Microbiome and Colorectal Cancer

The gut microbiome is a burgeoning area of research. Trillions of tiny organisms residing in the gut maintain commensal relationships and have been found to play a decisive role in the development, progression, and treatment response of colorectal cancer. Altered microbial colony like an abundance of *Klebsiella* and *Streptococcus* is found in colon cancer patients, and their metabolites influence the host immune system and create a microenvironment that promotes cancer development [13]. Additionally, dietary intervention directly influences microbial dynamics. Certain diets can promote the count of beneficial microbes and suppress harmful ones that impact cancer pathways. Moreover, the link between gut microbes and colorectal cancer is dynamic, where microbial composition, metabolites, inflammation, genotoxicity, and diet lead to the growth of colorectal cancer.

9.4 Mechanisms Relating Colorectal Cancer and Gut Microbiota

9.4.1 Inflammation and Immune Modulation

The gut microbiota is closely related to inflammation and promotes colon cancer. Twenty percent of carcinogenic tumors in the colon are due to chronic inflammation. The abundance of *Fusobacterium nucleatum* and *Bacteroides fragilis* promotes chronic inflammation, which is a precursor to colorectal cancer [14]. Chronic inflammation due to microbial dysbiosis appoints myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), which promote metastasis. Enterotoxigenic *Bacteroides fragilis* (ETBF) produces toxins that promote inflammatory cytokines i.e., IL-17 and IL-23 and facilitate tumor growth. Additionally, the presence of *Fusobacterium nucleatum* in colonial tumors promotes tumor progression and inhibits T cell-mediated immunity. Sulfate-reducing bacteria produce hydrogen sulfide, stimulate butyrate oxidation, and disrupt membrane barriers. *Bacteroidales* and *E. coli* produce IL-6 and inhibit apoptosis via the JAK/STAT3 pathway [15]. Microbes can produce chemokines; for instance, LPS (lipopolysaccharides) aggregate immune cells and develop tumors.

9.4.2 Metabolites and Metabolic Pathways

Production of SCFAs by microbes occurs by multiple pathways. *Lactobacillaceae*, *Ruminococcaceae*, *Lachnospiraceae*, and *Bifidobacteriaceae* are the main producers. Bacteria break down complex polysaccharides by hydrolysis and form butyrate. Indigestible polysaccharides undergo anaerobic fermentation and produce butyrate, propionate, and acetate. Bacteria can also transform acetate into butyrate with the help of the enzyme butyryl-CoA:acetyl-CoA transferase. These SCFAs inhibit tumor growth by inhibiting the histone deacetylase enzyme. Therefore, apoptosis is triggered, and cell proliferation is inhibited. The cell cycle can be arrested by the activation of GPR43 and GPR41 in colon cancer cells by SCFAs. GPR43 interacts with propionate and acetate, whereas butyrate shows affinity towards the GPR41 and GPR109A receptors. Butyrate also interacts with GPR109A, promotes the differentiation of epithelial cells of the small intestine, and maintains the lining [16]. It helps the development of T cells and inhibits pro-inflammatory cytokines. Therefore, low SCFAs create a favorable environment for colorectal cancer. Moreover, the anti-inflammatory activity of SCFAs maintains homeostasis of the gut microbiota by suppressing potential microorganisms and restoring good bacteria.

Microbes can transform the bile acids by deconjugation, dehydroxylation, dehydrogenation, oxidation, and epimerization. Deoxycholic acid (DCA) and lithocholic

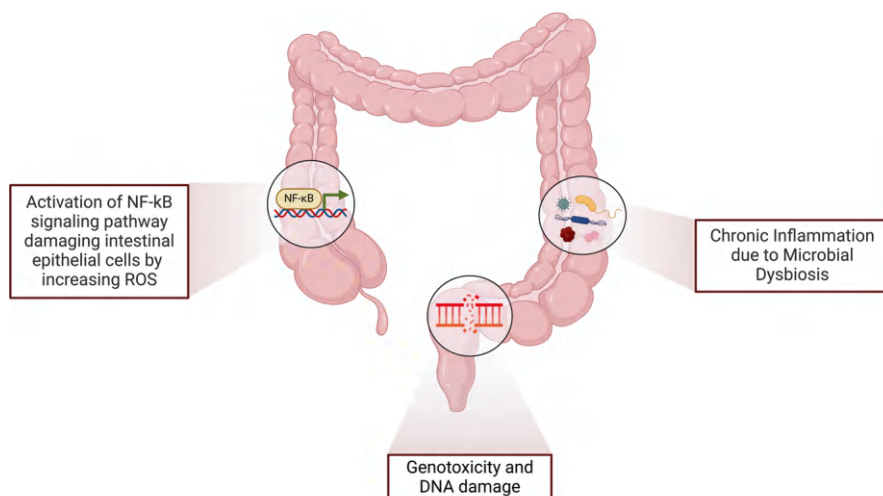
acid (LCA) are secondary bile acids that can be produced by modifying the hydroxyl group of the bile acid, which holds tumor-promoting properties [17]. The hydrophobic nature of secondary bile acid activates the NF- κ B signaling pathway and damages epithelial cells of the small intestine by increasing ROS. In addition, it produces matrix metalloproteinases (MMPs), which promote tumor invasion and metastasis. The β -catenin-cyclin D1 pathway is also upregulated, which facilitates cancer progression.

Gut microbes trigger the decarboxylase enzyme to produce biogenic amines from amino acids. *Clostridium* bacteria produce organic acids and other metabolites from amino acids via the Strickland reaction. Production of formate (oncometabolite) can increase glutamine metabolism and promote tumor growth and cancer cell proliferation. An increased count of *Fusobacterium* is observed in cancer patients, which utilize amino acids for their growth and increase amino acid metabolites [18]. Arginine decarboxylation, ornithine, and lysine decarboxylation are the major pathways through which polyamines are produced by gut bacteria. This polyamine upregulates oncogenes and downregulates tumor suppressor genes, and modulates mTOR and the WNT pathway that leads to cancer cell proliferation.

Gut microbes produce a variety of indole and phenolic metabolites, which have both a protective as well as a potentially harmful role in cancer development. *Enterococcus faecalis*, *Escherichia coli*, and *Proteus vulgaris* produce indole from tryptophan. Indole-propionic acid (IPA) is produced by *Clostridium sporogens* and *Clostridium bolulinum*. IPA activates AhR (aryl hydrocarbon receptor) and promotes IL-22, which maintains intestinal homeostasis, prevents tumorigenesis, and inhibits inflammation. Indole metabolites help the T cell differentiation process and produce Tregs, which prevent inflammation followed by cancer development [19].

9.4.3 Genotoxicity and DNA Damage

Gut bacteria and their metabolites are positively involved in genotoxicity and DNA damage, which can lead to cancer development. Colibactin produced by *Escherichia coli* breaks DNA double strands and may develop colorectal cancer [20]. Researchers identified the involvement of indolimines in the facilitation of tumor-supporting microenvironments. Additionally, metabolites of *Clostridium perfringens* and *Clostridium ramosum* have been identified, which show a diverse range of genotoxic effects. Genotoxins of *M. morganii* damage DNA and increase membrane permeability [21]. *Salmonella* damages DNA by producing typhoid toxin in colonic cells via the PI3K pathway. Microbial metabolites can activate the proliferation of intestinal epithelial cells and stem cells, which increase mutation and develop cancer cells. Figure 9.1 illustrates the possible mechanisms that play key roles in colorectal cancer progression.



Mechanisms Linking Gut Microbiota to Colorectal Cancer

Fig. 9.1 Mechanisms that link gut microbiota to progression of colorectal cancer

9.5 Evidence from Clinical and Preclinical Studies

Recent preclinical and clinical studies demonstrated the conjugation between gut microbiome and colorectal cancer. A metagenomic study revealed the existence of *Actinomyces odontolyticus*, *Fusobacterium nucleatum*, and *Atopobium parvulum* in samples of fecal from individuals with early-stage colorectal carcinoma [22]. A preclinical study shows that distinct gut bacteria, *Bacteroides fragilis*, and *Fusobacterium nucleatum*, in Apc mutant mice developed intestinal tumors. This bacterium produces colibactin, which modulates inflammation. In vivo studies on the pks + strain of *E. coli* produce genotoxin that damages DNA and promotes tumorigenesis in a mouse model [23]. *Porphyromonas*-treated mouse models induced senescence and accelerated tumor development. Studies have demonstrated that plenty of *Fusobacterium* and *Bacteroides* in colorectal cancer patients, low levels of SCFAs, and activation of inflammation. Long et al. found the growth of colorectal cancer in an APC +/- rodent model due to the expression of PCWBR2 of *P. anaerobius* via the PI3K-Akt signaling pathway [24].

9.6 Modulation Strategies of Gut Microbiota

Aiming that gut microbiota could be utilized as a remedy in the fight against colorectal cancer [10]. As an example, Bhalla et al. demonstrated how microbial metabolites, including folate, might inhibit the survival of colorectal cancer cells; thus,

altering intestinal-producing anticancer metabolites from microbiota could be a colorectal cancer treatment [25]. This section will discuss the techniques that have been used to alter the use of gut microbiota in medicine [10].

9.6.1 Dietary Interventions

Probiotics include microorganisms, such as molds, yeasts, and microbes, which, when provided in adequate amounts, can enhance the health of the host [26]. Three basic processes underline how probiotics work in the gut for the prevention and treatment of colorectal cancer: Enhancement of the functions of the gut barrier, prevention of the colonization of harmful microorganisms, and immune system modulation. Recent evidence indicates that probiotic supplementation restores the equilibrium of the microbiota of the intestinal tract by boosting the release of inflammation-inhibiting cytokines, including TGF- β 2 and IL-10, via Treg cells [10, 27]. Probiotics have the ability to boost phagocytosis and eradicate precancerous cells [28]. Microecological agents, particularly probiotics, have been found to have anticancer effects by inducing immunological responses, augmenting macrophage phagocytic activity, and boosting the activity of various cytokines [29]. Probiotics modulate immunological function, suppress the spread of potentially hazardous bacteria, decrease allergies, and regulate serum cholesterol levels, all of which prevent the growth of colorectal cancer [30]. Probiotics have also been shown in vitro research to impact colorectal cancer cells' malignant biological processes, including adhesion, apoptosis, and proliferation [31, 32]. *Bacillus polyfermenticus* with probiotic qualities, according to findings by Lee et al., decreased pro-inflammatory mediators such NO, COX-2, and TNF- α which in turn had anti-proliferative action on colorectal cancer cell lines (LoVo and HT-29) [10, 33].

Prebiotics have increasingly gained popularity as an additional treatment strategy for individuals with colorectal carcinoma in recent years. During a special prebiotics conference, the United Nations Agricultural and Food Organization broadened the term prebiotics in 2008 to include: Prebiotics are inert food ingredients that suppress disease-causing bacteria and encourage the production of good gut microorganisms [32, 34]. Polyphenols, polyunsaturated fatty acids, and carbohydrates like fructans, galacto oligosaccharides, Xylo oligosaccharides, and inulin have prebiotic characteristics. Prebiotics function in the intestine by stimulating the growth of good bacteria, promoting fermentation through the intestinal microbiota, directly absorbed into the intestine, causing anti-inflammatory effects, and interacting with pathogens to prevent colonization [35]. Prebiotics also prevent infectious bacteria from sticking to intestinal epithelial cells. For example, research by Ribeiro et al. demonstrated that prebiotic olive pomace powders support the microbiota's synthesis of SCFAs while simultaneously demonstrating antioxidant and anti-adhesive capabilities against infections. They showed that the initial form of insoluble dietary fiber, pulp-enriched powder, reduced the adherence of *L. monocytogenes* and *B. cereus* by up to 20% and 22%, respectively [10, 36].

Postbiotics are chemical substances derived from microorganisms. These include enzymes, endo- and exo-polysaccharides, teichoic acids, and peptides, vitamins, cell surface proteins, organic acids and plasmalogens [37]. The postbiotics are less dangerous than living microorganisms since they are found in the supernatant media of bacterial agriculture. The two methods by which postbiotics protect the intestinal epithelium against tumors are by specifically suppressing cancerous cells, preventing epithelial cell death, and raising IgA production [35]. Lee et al. used *L. fermentum* for colorectal cancer cells in a three-dimensional culture system to evaluate the tumoricidal effect of probiotics cell-free supernatant therapy. It was discovered that bacterial culture supernatant might cause colorectal cancer cell lines to undergo apoptosis by upregulating caspase-3, Bak, Bid, and Noxa [38].

Synbiotics have been utilized as a supplemental treatment for a number of illnesses, including colorectal cancer. Synbiotics are a blend of growth-promoting prebiotic substances and probiotic bacteria that produce “synergism” [39]. According to Kuugbee et al., the presence of MUC2, TRL2, occludin, and ZO-1 was upregulated in rats given a cocktail containing *Bifidobacteria bifidum*, oligofructose-maltodextrin-enriched *Lactobacillus acidophilus*, along with *Bifidobacteria infantum*. These medicines have the potential to contribute to numerous advantageous processes, including but not limited to slowed tumor growth, enhanced mucin secretion, tight junction preservation, and inflammation inhibition. Additionally, there was a drop in the degree of expression of COX-2, TRL4, caspase 3, as well as β -catenin, which are proteins that are known to promote apoptosis, the proinflammatory response, and tumor growth. This combination of probiotics and prebiotics altered gut flora and lowered the risk of colon cancer [39, 40].

9.6.2 Antibiotics and Their Impact

Antibiotic-induced modification on the gut microbiota was only partially studied in CRC, and there is not much research in the literature. In human CRC cells, erythromycin can inhibit the transcriptional activity of NF- κ B, activator protein-1, and its subsequent targets, IL-6 and cox-2 [39]. Additionally, antibiotics shield the intestinal mucosal barrier from erosion. Ijssennagger et al. explored the impact of heme and stand-in for red meat on gut flora and the growth of cancer of the colon because a greater consumption of red meat raises the chance of this disease. They showed that mice given heme-containing diets showed intestinal epithelial damage and hyperproliferation, which resulted in colon cancer, but mice given heme-plus antibiotic regimens did not exhibit these effects. The complex interconnection between the gut microbiota, antibiotics, and colorectal carcinoma requires more investigation in order to fully understand the method involved and how they may be used in colorectal cancer therapy [10, 41].

9.6.3 Fecal Microbiota Transplantation (FMT)

The dissemination of a donor's robust microbiological ecosystem into a patient, known as fecal microbiota transplantation, is presently being investigated as a treatment approach to restore normobiosis, the typical condition of the human gut microbiota, in various clinical circumstances [42]. FMT is regarded as a possible clinical setting in patients, as colorectal cancer is characterized by a dysbiosis condition. Through a decrease in the growth of the tumor, FMT restored the gut microbiota to normal in a CRC mice model [43]. By lowering IL-6, TNF- α , and IL-1 levels and raising non-inflammatory cytokines like TGF- β and IL-10 via the regulation of Cyclic NF- κ B signaling and cellular growth, FMT helped to lower the levels of inflammation. Furthermore, Tregs built up following FMT therapy, yet there were no Th1, Th2, or Th17 cells [37, 44]. FMT aims to replenish the variety of the gut microbiota. It has been demonstrated that FMT can help alleviate a number of GI diseases and restore microbial equilibrium by introducing a healthy and disease-free population of bacteria to an unbalanced community [45]. FMT is being used in a clinical trial (NCT04729322) to treat metastatic colorectal cancer in patients who do not react to anti-PD-1 therapy [10].

9.6.4 Phytochemicals and Natural Compounds

Targeting all phases of colorectal cancer from inception to progression, phytochemicals have been shown to affect crucial cellular signaling pathways. As a result, research efforts have focused on the functions of phytochemicals in signaling cascades that are thought to generate chemo-preventive properties. Natural product consumption affects the gut microbiota in a variety of ways, which has a significant biological impact. The gut microbiota's anticarcinogenic characteristics are mostly ascribed to two mechanisms: (a) enhancing the host's immune system or (b) producing compounds that can obstruct the pathways involved in the genesis of colorectal cancer. Table 9.1 presents various phytochemical effects on gut flora [46–48].

9.6.5 Pharmaceutical Targeting Gut Microbiota

The initiation of cancer is significantly influenced by the gut microbiome and certain types of bacteria. The balance of the microbiota may potentially be important in the therapy of cancer. By producing reactive oxygen species, nanomaterials with strong antibacterial activity have been employed to destroy microbes that cause cancer. Increased beneficial microbial species eliminated by broad-spectrum antibiotics may result from tailored delivery of antimicrobial nanoparticles to particular microbial species implicated in gut cancer, like *H. pylori*. Additionally,

Table 9.1 Effect of various phytochemical on gut flora

Name of phytoconstituent	Model studied	Effect on gut microbiota
Epigallocatechin-3-gallate	C57BL/6 J mice	Maintained dysbiosis and maintained the microbial ecological balance
Ginsenoside Rh4	C57BL/6 J and BALB/C mice	Rh4 reduced the imbalance in the gut microbiota brought on by colorectal cancer, enhanced the beneficial bacteria <i>Akkermansia muciniphila</i> , and broadened the variety of the gut microbiota. Reduced intestinal inflammation, restored the destruction to the intestinal barrier, and prevented the growth of cancer cells
Catechins	C57BL/6 J mice	Enhanced <i>Helicobacter</i> , gram-negative bacteria, and the <i>Bacteroidetes</i> to <i>Firmicutes</i> ratio. Maintained balance of gut microbiota
Phenolic acid	Mice	Despite low bioavailability, it reduced intestinal barrier dysfunction to modify chronic inflammatory disorders
Stilbenes	Wistar rats	Trans-resveratrol administration, either by itself or in conjunction with quercetin, significantly changed the intestinal microbiota profile by modifying the mRNA regulation of tight-junction proteins as well as genes linked to inflammation
Stilbenes	C57BL or 6 J mice	Changed the composition of intestinal microbiota by reducing <i>Bacteroidetes</i> and elevating <i>Firmicutes</i> and <i>Lactobacillus</i>
Organosulfur compounds	C57BL or 6 J mice	The gut microbiota's composition changed as a result of treatment

antimicrobial drugs can be delivered by nanomaterials through systemic injection to destroy tumor-associated bacteria at distant tumor locations [49].

A multimodal oral dextran-aspirin nanomedicine (P3C-Asp) has been employed to treat primary CRC orally since it possessed significant potential for modifying gut microbiota using prebiotic dextran and could produce salicylic acid while eliminating reactive oxygen species. Compared to free aspirin, oral P3C-Asp considerably enhanced salicylic acid buildup in cancer of colorectal tissues and was maintained in colorectal cancer tissues for more than 12 h. P3C-Asp enhanced the homeostasis of the microbiota and significantly raised the proportionate frequency of bacteria linked with a stronger systemic immune response to cancer, particularly *Lactobacillus* and *Akkermansia*, which rose by 6.66- and 103-fold, respectively, compared to the control group. This study reveals the complementary role that inflammation in the gut and microbiota modification play in the primary therapy for colorectal cancer and opens a novel pathway for immune system regulation in the tumor microenvironment through the use of oral nanomedicine [50].

Chemotherapeutic agents such as cyclophosphamide (CTX) have the ability to alter the gut microbiome and make it easier for gram-positive bacteria to enter supplementary lymphatic organs, resulting in the generation of Th17 cells. Treatment with CTX may be more effective if the microbiota is restored. Research using mice as models has demonstrated that bacteria including *Lactobacillus johnsonii*,

Enterococcus hirae, and *Barnesiella intestinihominis* increase the anticancer impact of CTX [51].

Yuan et al. studied the tumor development and gut microbiota of mice treated with 5-FU, ABX, an antibiotic combination or probiotics, utilizing the colorectal cancer animal model with high-throughput sequencing. According to the current findings, administering mice with ABX has disturbed their gut flora, which has lessened the antitumor efficaciousness of 5-FU [52].

9.6.6 Radio Immunotherapy

In addition to surgery, radiation has been shown to be the primary therapeutic choice among people with advanced colorectal cancer. Radiotherapy and gut microbiota interact in both directions. Radiation enteropathy is caused by alterations in the makeup and functioning of intestinal microbiota brought on by ionizing radiation. Intestinal microbiota influences the anticancer therapeutic efficacy of radiation therapy on the one hand. The potential use of intestinal flora in radiotherapy as a shielding component and indicator of exposure to radiation is highly intriguing. There are two primary mechanisms of intestinal flora dysbiosis caused by ionizing radiation. First, radiation can harm the intestinal epithelial cells, impairing their ability to serve as a barrier in the intestine. This allows bacteria to enter the body more deeply and encourages the entry of their by-products into the bloodstream, which in turn causes inflammation. Second, exposure to radiation can result in the generation of oxygen species that are reactive, which are active because of their unpaired electrons. ROS can harm the intestinal microbiota's DNA and other cellular components, changing the bacterial flora in the process.

In a preclinical model, the glycopeptide antibiotic vancomycin, which is effective towards Gram-positive bacteria, changes the makeup of the microbiota of the intestine in conjunction with radiation therapy. Additionally, it increases the presentation of antigens to CD11c dendritic cells in radiation-treated mice's tumor-draining lymph nodes, enhancing the anticancer impact [53]. By using high-throughput sequencing of the bacterial 16S rRNA gene to analyze the fecal microbial population of five radiation-exposed mice, a recent study discovered that radiation caused notable changes in the microbial diversity of the gut at the genus level. In the large intestine, radiation elevated the abundance of the genus *Alistipes* and lowered that of *Prevotella*.

Researchers have recently observed a strong correlation between the gut flora and the vulnerability to harmful side effects and radiation response. The main drawback of radiation therapy is the variation in radiation toxicity and radiosensitivity, which is correlated with tumor recurrence, patient mortality, and quality of life. Individuals who have undergone pelvic radiation therapy frequently experience fecal incontinence, tenesmus, diarrhea, and rectal hemorrhage. The cytotoxic action of radiotherapy to the rapidly reproducing epithelium mediates acute alterations in the digestive tract, which are further enhanced by inflammation. Because the

immune system finds it difficult to control bacterial translocation, ionizing radiation stimulates the coagulation system, causing the development of ulcers, which opens up the inner tissues to enteric bacteria and enhances the inflammatory response.

Future research is essential to comprehend the connection between gut flora and radiosensitivity despite radiation damage. The regulation of the host reaction toward radiation therapy by the gut bacteria is a relatively unknown phenomenon. Immune response mediates antitumor responses induced by radiotherapy. It is possible that the gut microbiota contributes to the immunological effect of radiation therapy as it has been depicted to affect the response of the immune system. The specific processes by which the gut flora influences the body’s response to radiation still need to be further investigated [54].

9.7 Evidence-Based Study Overview

9.7.1 Evidence from Animal Studies

The following studies depict the importance of modulation of gut microbiota in colorectal carcinoma. These studies include the results from animal studies conducted on different models for evaluating their efficacy.

Name of compound	Animal model	Inference	References
Echinacoside	Pseudo-sterile CRLM mice	This study showed that ECH has oral anti-metastatic activity through promoting gut bacteria that produce butyrate, which inhibits PI3K or AKT signaling and the transition of epithelial to mesenchymal growth. The antimetastatic impact of ECH was further demonstrated by alteration of gut microbes in the colorectal cancer mouse model, demonstrating the critical role that short chain fatty acids-producing gut bacteria played in mediating this effect	[55]
Hedysari radix praeparatacum Melle	Male C57BL/6 mice	The research conducted demonstrated a possible mechanism by which Hedysari radix reduces inflammation and symptoms associated with colitis associated colorectal cancer, modifying the function of the intestinal barrier and maintaining microbial equilibrium and fecal metabolism profile	[56]
B-glucan	Colon cancer mouse model	By repairing the intestinal mucosal barrier, increasing the amount of short chain fatty acids, regulating the metabolic processes of the gut microbiota, and rebuilding the gut microbiota framework with more Bacteroides and fewer Proteobacteria at the phylum level and more Muribaculum at the genus level, the lower-molecular mass β-glucan fraction demonstrated enhanced anti-colon cancer actions	[57]

(continued)

Name of compound	Animal model	Inference	References
Pien Tze Huang	Colorectal cancer mouse model	Pien Tze Huang inhibited pro-inflammatory and carcinogenic pathways, enhanced gut barrier activity, and altered the microbes in the gut and metabolites towards a more advantageous profile, all of which reduced the risk of colorectal cancer	[58]
Ginsenoside Rh4	Male C57BL/6J and BALB/C mice	The findings demonstrate that Rh4 reduces colorectal cancer in a way reliant on the gut microbiota by boosting the synthesis of ursodeoxycholic acid, which subsequently stimulates FXR receptor and controls TLR4-NF-κB signaling pathway, and by modifying intestinal microbiota-mediated bile acid metabolism	[34]

9.7.2 Evidence from Human Studies

The significance of diet and changes in the microbial diversity of the microbiota in the gut in the etiology of colorectal cancer is receiving more and more attention. Probiotics and synbiotics may be utilized as an intervention to lower the risk of colorectal cancer because it has been proposed that they alter enteric microbiota. Wierzbicka et al. determine how probiotic and synbiotic supplementation affected the gut microbiome of colorectal cancer patients. Adults with colorectal cancer who consumed probiotics or synbiotics for a minimum of 6 days participated in randomized controlled studies. A number of biochemical and inflammatory markers, as well as alterations in the gut flora, were identified. By decreasing the percentage of potentially harmful microbes like *Fusobacterium*, *Pseudomonas*, and *Porphyromonas*, along with *Enterococcus*, in addition to boosting the number of beneficial bacteria such as *Lactobacillus*, *Peptostreptococcus*, *Bacillus*, *Eubacterium*, and *Bifidobacterium*, the administration of probiotics and synbiotics improved the enteric microbiota. Furthermore, probiotic/synbiotic therapy enhanced the permeability of the gut, tight junction function, and antimicrobial release in colorectal cancer patients [59].

A traditional medicine called PRM1201 is effective in preventing the spread of colorectal cancer (CRC). Clinical experiments were analyzed in order to explore this possibility, and the relationship between PRM1201’s anti-metastatic effectiveness and the recovery of bacteria that produce short-chain fatty acids was examined. Clinical studies demonstrated that in the feces of CRC patients, PRM1201 enhanced the number of bacteria that form short-chain fatty acids. There is a positive conjugation between the regaining of short-chain fatty acids and PRM1201’s anti-metastatic activity. The administration of PRM1201 treatment changed the makeup of the gut microbiota by promoting the growth of bacteria like *Lachnospiraceae_NK4A136_* group, *Akkermansia*, as well as *Blautia* that produce short-chain fatty acids and reducing the number of dangerous bacteria like *Escherichia coli* [60].

9.7.3 Case Studies and Clinical Trials

A double-blind, randomized trial was conducted on 140 patients with colorectal cancer by Golkhalkhali et al. in an effort to reduce the negative effects of chemotherapy. Omega-3 fatty acids and microbial cell preparation were administered as part of the study. Two groups of subjects were created: a placebo group and a group that received therapy. In colorectal cancer patients undergoing chemotherapy, the combination supplementation of omega-3 fatty acid and microbial cell preparation alleviated some side effects, lowered specific inflammatory biomarkers, and enhanced quality of life [61].

In a different research, Dun et al. conducted a comprehensive analysis of randomized controlled studies to ascertain the effect of probiotics on the intestine-gut barrier in patients who acquired a colon cancer diagnosis after surgery. A total of 17 research involving 1242 patients were chosen for meta-analysis, comprising 12 Chinese and five English studies. After surgery, probiotics can successfully preserve the intestinal mucosa’s biological and physical barrier in patients having colorectal cancer [62].

Researchers should examine these different biotics and work to turn them into widely used, authorized anticancer treatments, considering the growing popularity of probiotics, prebiotics, postbiotics and synbiotics (Table 9.2).

9.8 Challenges and Limitations

The collaboration between gut microbiota modulation strategies and colorectal cancer (CRC) development presents several challenges and limitations, which are critical to understanding before applying these strategies clinically.

Table 9.2 Clinical trials on colorectal cancer by the modulation of gut microbiota [63]

Clinical trial no.	Title	Study type	Status of study
NCT05655780	UGT1A1 Genotype and Gut Microbiota Enzyme Activity-Based Irinotecan Dosing for Metastatic Colorectal Cancer Patients: Achieving Maximum Survival and Quality of Life with Dietary Intervention	Observational	Recruiting
NCT02947607	Evaluating the Microbiome’s Involvement in the Pathophysiology of Colorectal Cancer and Adenoma	Observational	Completed

9.8.1 Challenges of Modulation Strategies of Gut Microbiota and Colorectal Cancer (CRC) Development

9.8.1.1 Variability in Gut Microbiota Composition

Interindividual differences in the makeup of the gut microbiota can be seen even in healthy populations. The composition of an individual's gut microbiota might vary based on their race and country of origin. Genetics, nutrition, lifestyle, and environmental exposures all contribute to considerable differences in gut microbiota composition throughout individuals. This variation makes it difficult to develop one-size-fits-all modulation strategies. For instance, various bacterial strains may play distinct roles in CRC development in different populations, hindering the development of universal treatments [64].

9.8.1.2 Individual Responses to Modulation Strategies

Making changes to the gut microbiome may be a novel approach to cancer prevention and therapy if the processes by which the gut microbiota influences host health are more understood. Nowadays, it is believed that altering the gut microbiota, mostly with the addition of probiotics, offers CRC patients and high-risk persons three distinct advantages: preventing the development of CRC, lowering adverse effects associated with treatment, and boosting the effectiveness of anticancer therapy. Individuals respond differently to microbiota modulation, such as prebiotics, probiotics, or fecal microbiota transplants. These responses are influenced by the existing microbiota composition, the host's immune system, and genetic predispositions. This unpredictability can lead to inconsistent therapeutic outcomes, limiting the widespread application of these strategies.

9.8.1.3 Long-Term Effects and Safety Concerns

The long-term impact of microbiota modulation is not fully understood, raising concerns about safety. For example, while introducing specific bacterial strains may have immediate beneficial effects, the potential for long-term dysbiosis or adverse interactions with other microbiota components is a significant concern. Furthermore, the risk of inadvertently promoting pathogenic bacteria that could exacerbate CRC or other diseases adds another layer of complexity. In particular, the prevalence of opportunistic pathogens or virulence factors, as well as the propagation of resistance-causing genes in gut microbial communities, are safety issues for probiotic strategies. FMT's main obstacles are side effects such as abdominal pain, slight fever, diarrhea, flatulence, tiredness, and lethargy. In order to get reliable and thorough data, studies should evaluate each method's risk-benefit potential through lengthy trials, including a large sample size.

9.8.2 *Limitations of the Modulation Strategies of Gut Microbiota and Colorectal Cancer (CRC) Development*

The modulation of gut microbiota as a strategy for preventing or treating colorectal cancer (CRC) presents several significant limitations:

9.8.2.1 Heterogeneity of Gut Microbiota Composition

The gut microbiota is extremely customized and impacted by a variety of factors, including genetics, diet, lifestyle, and environment. This variation complicates the development of universally effective standardized modulation techniques. Variability in microbiota makeup might lead to different individuals having various microbial profiles that respond differently to the same intervention. For example, bacterial strains like *Fusobacterium nucleatum* and *Bacteroides fragilis* are conjugated to CRC, but their incidence and impact differ widely across people, making it difficult to develop a therapeutic strategy that works for everyone [65].

9.8.2.2 Unpredictable Individual Responses

Responses to microbiota modification techniques, such as probiotics, prebiotics, and dietary changes, are frequently unpredictable. This is because the gut's natural microbial community interacts in diverse ways with added bacteria or dietary changes, resulting in varying consequences. Some people may benefit from the treatment, while others may not see any improvement or may even suffer negative side effects, such as increasing dysbiosis or inflammation, which can potentially accelerate CRC progression.

9.8.2.3 Lack of Long-Term Safety Data

The long-term safety of gut microbiota modification techniques is a major concern. While short-term trials have yielded some favorable results, the long-term ramifications are unclear. There is a possibility that sustained change in the gut microbiota will result in unexpected outcomes such as pathogenic bacteria overgrowth, immune system dysregulation, or even the development of further gastrointestinal illnesses. Furthermore, some therapies may alter the delicate balance of the gut ecology, resulting in unexpected health consequences over time.

9.8.2.4 Complex Interaction with the Host Immune System

The immune response of the host is regulated by the gut microbiota and is intimately associated with the development of malignancy. However, this connection is extremely intricate and not completely understood. Modulating the gut microbiota may unintentionally influence immune responses, potentially resulting in poor tumor surveillance or excessive inflammation, both of which are harmful in the setting of CRC. For example, certain bacterial strains may trigger pro-inflammatory pathways, which, if not balanced, can induce cancer.

9.8.2.5 Challenges in Translating Research to Clinical Practice

However, preclinical investigations and limited clinical trials have yielded useful information, and turning these results into meaningful, large-scale clinical applications is difficult. The complexity of the gut microbiota, combined with individual response variability, makes it difficult to develop treatment interventions that are both safe and effective across varied populations. Furthermore, regulatory barriers and the requirement for thorough, long-term clinical trials hamper the development and approval of microbiota-based CRC therapy.

9.9 Future Directions and Research Opportunities

In the future, the gut microbiome is expected to show a crucial role in the prevention of colorectal cancer, the planning of systemic treatments, and the mitigation of treatment-related side effects. Dietary therapies, probiotic or prebiotic supplements, fecal microbiota transplantation, and antibiotic therapy are some approaches to regulating the gut microbiome. There is an urgent need for clinical research to account for various factors that influence the microbiome, such as geographical location, ethnicity, gender, and diet, as well as the impact of cancer treatment like chemotherapy and immunotherapy on the intestine microbiome. The linking between gut microbiota and cancer stem cells in the development of CRC represents one of the most promising yet challenging areas of research. Additionally, integrating this knowledge into new therapeutic strategies and applying them in clinical research and routine medical practice will pose significant challenges. Given that each individual possesses a unique gut microbiome from birth, personalized medicine tailored to the patient, supported via artificial intelligence and machine learning, is the ideal approach for achieving better outcomes. Furthermore, fostering a healthy gut microbiome not only enhances individual well-being but also positively impacts public health at large.

Many concerns remain unsolved about the optimal administration, kinetic efficiency, and long-term efficacy of modulating the intestinal microbiota with FMT, probiotics, and prebiotics. The strategies for comparing and combining different therapy approaches have yet to be investigated. The essential step in today's precision medicine will be to move away from the empirical approach of "one form of probiotic is suitable for everyone" and toward a personalized strategy for everyone. The significance of basic preventive measures, such as a healthy environment, sufficient nutrition, exercise, weight control, and the avoidance or reduction of stress through relaxation techniques, cannot be overstated in the fight against the development of colorectal cancer (CRC) during infancy and childhood (EOCRC). For the early identification of LOCRC, secondary preventive activities must be implemented in addition to primary preventative measures.

9.10 Conclusion

The chapter on the relationship between gut microbiota modification techniques and colorectal cancer development emphasizes the promising role of microbiome-based therapies in CRC management and prevention. However, it highlights the complexity of this strategy, given the great heterogeneity in individual microbiota compositions and the unexpected nature of responses to modulation tactics. Despite promising outcomes, there are still concerns about the tactics used to modulate gut microbiota. Probiotic techniques may pose safety issues due to variables such as the spread of resistant genes in gut microbial populations and the frequency of virulence factors or opportunistic infections.

Furthermore, side symptoms such as abdominal pain, diarrhea, moderate fever, flatulence, weariness, and lethargy are the primary problems of FMT. To generate trustworthy and comprehensive results, research should involve an assessment of each strategy's risk-benefit potential in long-term trials with a high sample size.

While personalized medicine and enhanced microbiome profiling provide routes to more targeted and effective treatments, their long-term safety and clinical application are questionable. Future research must address these issues by better understanding the complex interactions between the gut microbiome and the host, as well as designing solutions that may be tailored to individual needs. The ultimate goal is to use the gut microbiota in a safe and effective way to lower the burden of colorectal cancer in varied populations.

Taking all of this information into account, the gut microbiota should be regarded as having a crucial role in the beginning and progression of CRC. Additionally, dietary manipulation of the microbiome linked to cancer, such as consuming foods that can prevent an imbalance in bacteria and intestinal inflammation or modify the body's reaction to cancer treatment, maybe a useful tactic for delaying the onset and progression of colorectal cancer and enhancing the effectiveness of treatment.

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Chapter 10

The Interplay Between Postbiotics and Colorectal Cancer



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Abstract Recent advancements in microbiome research have underscored the critical role of gut microbiota in human health and disease. Among the emerging concepts in this field, postbiotics have gained significant attention for their potential therapeutic benefits, particularly in colorectal cancer (CRC). Postbiotics, which encompass various bioactive compounds produced by beneficial gut microbes, exert multifaceted effects on host physiology, immune response, and gut barrier integrity. This chapter explores the intricate interplay between postbiotics and CRC, encompassing their mechanisms of action, preclinical and clinical evidence, and therapeutic implications. Additionally, it discusses the potential of postbiotics as novel preventive and therapeutic strategies against CRC, highlighting their promise in modulating the gut microenvironment and influencing tumor development. By elucidating the complex interactions between postbiotics and CRC, this chapter aims to provide insights into future research directions and clinical applications in harnessing the therapeutic potential of postbiotics for colorectal cancer management.

Keywords Postbiotics · Colorectal cancer · Gut microbiota · Therapeutic benefits · Mechanisms of action · Clinical evidence · Therapeutic implications

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10.1 Introduction to Postbiotics and Colorectal Cancer

The beneficial bioactive compounds produced in the live microbial fermentation of the gastrointestinal tract (GIT) by beneficial bacteria are termed beneficial metabolites or postbiotics [46]. Postbiotics can effectively improve the health of the host, such as by reducing the risk of inflammation and cancer and even improving the ability of the immune system. By adjusting the host's response to metabolic disorders, some postbiotics can also increase energy intake and prevent weight loss [20]. The development of omics technologies revealed that in the presence of postbiotics, the host metabolism will undergo beneficial changes. Since postbiotics do not have any problems with storage and application, it can be said that postbiotics can be industrially developed and applied as a better alternative in comparison with live microbes. However, the lack of a clear regulatory definition of postbiotics is still an obstacle to its commercial utilization [38].

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths among all global cancer types [50]. Several studies have shown that the increase of atypical microbial fermentation products in the intestinal tract will promote the formation of CRC. The beneficial metabolites produced under the fermentation of beneficial gut bacteria in the host body are generally known as postbiotics. How do these postbiotics affect colorectal cancer? A comprehensive understanding of the relationship between them is helpful for the precise treatment of colorectal cancer [52].

10.2 Overview of Colorectal Cancer

In recent years, the beneficial effect of SCFAs has proposed an interesting alternative to managing cancer. Unfortunately, the application of SCFAs is not possible due to the histidine deacetylase inhibitory property for direct anti-colon cancer activity and involved other feedback signals as an indirect effect, respectively. Therefore, it is necessary to clarify the underlying molecular mechanisms and develop a general approach to translate these interesting findings for the design of novel therapeutic strategies for the treatment of CRC [47].

Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the second leading cause of cancer death globally. It is relatively known as the “black wolf” of the tumor due to few or no symptoms at the initial stage of the disease. A diagnosis is usually clinched only at an advanced stage when a cure is rare. Colorectal carcinogenesis can be generally categorized into three steps: initiation, promotion, and progression. Genetic as well as epigenetic changes contribute to the transformation of normal mucosa to adenoma, which accumulates additional mutations in oncogenes and tumor suppressor genes, followed by the promotion of the adenoma to CRC. Progression of the adenoma to CRC is associated with chromosomal instability (CIN) and microsatellite instability (MSI) in different pathways

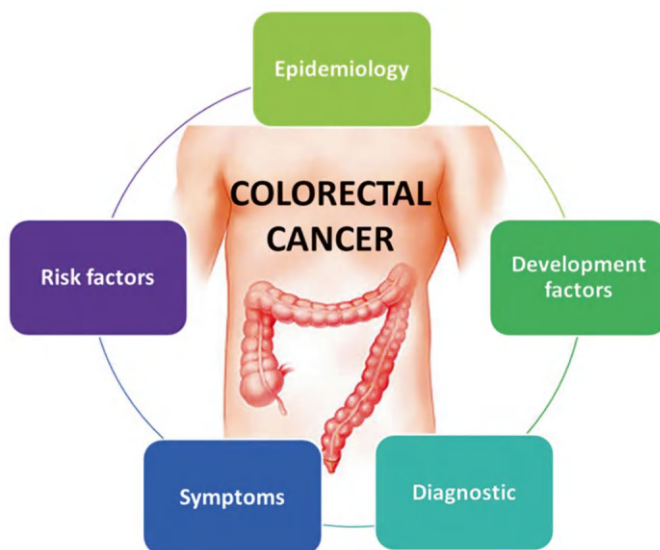


Fig. 10.1 Overview of colorectal cancer

[4]. Therefore, it is urgent to find alternative strategies to block CRC, as shown in Fig. 10.1. On the other hand, as a product of the fermentation of fibers and other nutrients contained in a healthy diet by the gut microbiota, butyrate can relieve oxidative stress and endoplasmic reticulum stress in severe colitis and colon cancer cells [44].

10.2.1 Epidemiology and Risk Factors

Colorectal cancer (CRC) is the third most common cause of cancer-related deaths, with a high mortality rate. Unfortunately, this proportion is rapidly increasing. Despite advances in the treatment and management of CRC, the survival rate remains poor. The development of CRC is preceded by the adenoma-carcinoma sequence, with dysplastic lesions being detected in more than 30% of people over the age of 50. Several risk factors have been identified, including sudden changes in diet, the use of antibiotics and other medications, and smoking [45]. However, age and genetic susceptibility appear to be the most important risk factors in younger individuals. This condition should also be suspected in frail, comorbid individuals. Given these negative influences and the need to find more accessible and effective therapeutic strategies, as well as early diagnosis through functional foods, postbiotics, prebiotics, and their combination may improve the health status and have a positive effect [15].

The acclaim that postbiotics are receiving these days is huge, thanks to the rising demand for alternatives to probiotic treatment. These trends suggest that the trend is driven by safety concerns and the current understanding of the subject. It is widely known that postbiotics have the same beneficial effects on health as traditional probiotics [17]. Both play a role in modulating the gut microbial community, improving immunity, degrading cholesterol, and reducing the risk of colorectal cancer. However, the use of probiotics in colorectal cancer has been controversial in some patients [23].

10.2.2 Pathophysiology and Molecular Mechanisms

After transcription, several particular molecular mechanisms determine the expression of a certain gene, as explained in Table 10.1. These mechanisms include the structure of the DNA and its compacting, processing of transcribed RNA into the template for the ribosomes, and specific mRNA stabilization. Moreover, the translational processes, equipment, and procedures determine the amount and allocation of the protein because the level of proteins present defines the available metabolic activity of the given cell [37]. Then, the functional and structural presence of metabolic markers like glycolytically associated enzymes, matrix metalloproteinases, cyclin-dependent kinases, and others are specific signs of the process that underwent significant changes in the malignant tissues of different origins and stages. The understanding of the specific connection underlying all these biological activities provides the opportunity for targeted intervention [6].

Microbes can contribute to neoplastic development and progression through various mechanisms, including the ability to promote chronic inflammation, formation of genotoxic molecules, interference with epithelial proliferation and differentiation processes, and modulation of the immune system [11]. Those findings explain the positive effects of probiotics, prebiotics, and synbiotics on health [9]. Nevertheless, there is established relevant evidence that one of the essential roles in the health-beneficial effects attributed to functional foods like fermented products

Table 10.1 Examples of postbiotics and their sources

Postbiotic	Source	Function
Short-chain fatty acids (SCFAs)	Fermentation of dietary fiber by gut microbiota	Maintenance of gut barrier integrity, modulation of immune response
Lactic acid	Lactic acid bacteria (e.g., lactobacillus spp.)	Acidification of gut environment, potential anti-inflammatory effects
Bacteriocins	Probiotic bacteria	Antibacterial activity against pathogenic bacteria
Exopolysaccharides	Bifidobacteria, lactobacilli	Modulation of gut microbiota composition, potential prebiotic effect
Peptides	Fermentation by gut bacteria	Bioactive compounds with antimicrobial and immunomodulatory properties

containing live beneficial bacteria, can be performed by its non-viable form, postbiotics [28]. The main novel insights about postbiotics and specific potential molecular mechanisms of their activity, contributing to antitumor effects, are provided in this chapter.

10.3 Understanding Postbiotics

Postbiotic: In 2017, a definition of postbiotics was drafted, which is a concept more recent compared to prebiotics and probiotics, created previously by the Food and Agriculture Organization of the United Nations. The definition proposed was “A postbiotic is a preparation of inanimate microorganisms and/or their components that confers a health benefit on the consumer.” The definition has three main points: microorganisms or components of these, inanimate, and justice-conferred health benefits [41]. Both prebiotics and postbiotics were not recognized as functional food ingredients by either the Food and Drug Administration or the European Food Safety Authority. Prebiotics have not been accepted by these agencies and still lack a widely accepted definition [5].

Postbiotics hold numerous beneficial effects, which are beneficial for improving health. In 2004, the Food and Agriculture Organization (FAO) defined prebiotics and probiotics; then, in 2017, the definition of postbiotics was given. Postbiotics is a recent concept, as compared to prebiotics and probiotics; as a consequence, there are no exact definitions. Draft both prebiotics and postbiotics, aiming for international consensus. Although the concept of postbiotics is recent, there was growing interest in the use and study of postbiotics [39]. In vitro and in vivo effects of postbiotics, which have been published by many authors, also contributed to the area's perception and development. As postbiotics have numerous beneficial effects, there is the perspective that postbiotics may have therapeutic use for several diseases, such as gastrointestinal disorders, neurological disorders, immune system defects, and some types of cancer, such as colorectal [35].

10.3.1 Definition and Classification

Prebiotics are fermented favorably by the microbiota, transformed prior to the development of PHGRs and beneficial antitumor markers by scarce species, and are also transformed into short-chain fatty acids. The resulting products are cell-proliferative agents and a simply absorbed energy source which contributes to the health of the normal colon [21]. Other possible prebiotics include dietary fruits, corn, and genetically modified vegetables, which have to date been investigated in less detail than, for instance, chicory roots and soybeans. Dietary fruits, corn, and genetically modified vegetables are also potential rich promoters of prebiotics and have been investigated less to date than, for example, chicory roots and soybeans

[18]. Dietary fruits, corn, and genetically modified vegetables are also likely rich prebiotic promoters, and their cancer prevention investigation is possibly less than the already extensively investigated bifidobacteria and lactobacilli and their derivatives [27]. To expound, in a concept that is neither prebiotics, nor probiotics, nor derivatives of these concepts, one must note that the clinical or health benefits of metabolites, i.e., mainly acetate, propionate, and butyrate, are already well established for the human host [36].

Since the terms “prebiotic” and “probiotic” were first introduced by Gibson, Roberfroid, and Parker in 1995, the benefits of these derivatives have been demonstrated across various aspects of life. Nevertheless, the majority of clinical and pre-clinical research thus far has been focused on probiotics and less so on prebiotics [8]. In comparison with probiotics, prebiotics are more bio-secure and thermal-stable, but since they are fermented in a less organized form, it is assumed that they possess reduced sensitivity and capability in the gut. For instance, bifidobacteria and lactobacilli are active and abundant in the gut of fetuses and infants, while prebiotics, which are also abundant by lactose diffusion, are present in breast milk. The main types of prebiotics, namely oligosaccharides and fermentation products, offer numerous benefits to the human body and are obvious constituents of different supplement micronutrients of probiotics [42].

10.3.2 Biological Activities and Mechanisms of Action

Postbiotics can originate from *Lactobacillus*, *Bifidobacterium*, *Akkermansia*, and *Lactococcus* spp. through fermentation and continue to exert gastrointestinal effects without the probiotic itself. However, these “newborn” postbiotics have been proven to carry biological properties, including anti-inflammatory activity, regulating the homeostasis of the intestinal mucosal barrier, increased integrity, and anticancer functions [30]. The beneficial effects of postbiotics are related to the surface components, solutes, and proteins released into the medium, especially the polysaccharides and non-digestible peptides. Through the presence of specific receptors or certain binding partners, even though postbiotics do not colonize in the gut as an exogenously applied bacteria do, they can still exhibit their role by binding to these receptors or partners as shown in Fig. 10.2 and trigger further signal pathways and trigger host responses. Moreover, postbiotics can also suppress inflammation by regulating three transcription factors: nuclear factor κ B (NF- κ B), cyclic adenosine monophosphate (cAMP), and peroxisome proliferation activated receptor gamma (PPAR γ). In addition, they are a key factor in the balance between pro- and anti-inflammatory compounds, enhancing the levels of interleukin (IL)-10 and immunoglobulin A (IgA) [32].

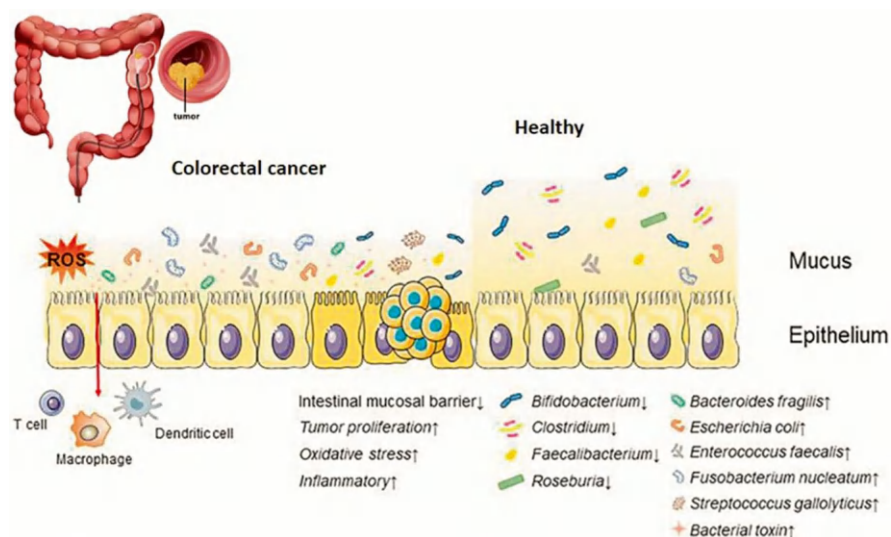


Fig. 10.2 Mechanisms of action of postbiotics in colorectal cancer

10.4 Postbiotics and Gut Microbiota

Prebiotics, probiotics, and postbiotics interact with host cells in different manners and to different extents. They mainly favor beneficial bacteria growth and down-regulate gene expression during inflammation. Some prebiotics are widely fermented by gut microbiota. Probiotics are complex functional foods prepared from live microorganisms with the purpose of affecting the host's health in a beneficial manner. The concept of the postbiotic is more recent, referring to bioactive compounds that are synthesized, broken down, or modulated by heat processing or enzymatic action, exerting a preventive effect on the host's health [34].

Human gut microbiota is a wide and complex assemblage of microorganisms that is able to exert important effects not only on the host's gut but also on the host's body. This includes chemical conditions and modulating immune system activity, affecting the nutritional status and energy balance, preventing bowel diseases, and even influencing the risk of developing distal bowel cancers, like colorectal cancer.

The term "postbiotics" encompasses a large assortment of bioactive compounds produced by different microorganisms during fermentation processes of dietary substrates within the human gastrointestinal tract. These include short-chain fatty acids, vitamins, enzymes, cell envelope components, and antimicrobial peptides, among others. Postbiotics are currently being sought after for the design of novel nutraceuticals, and promising results are being obtained.

10.4.1 Role of Gut Microbiota in Colorectal Cancer

The unique composition of the gut activities resembles a live organ due to the combined genetic material of 3.3 million genes and ranges from trillions of microorganisms, outnumbering the human genome. This significant combination greatly influences the host physiology and disease. A high number of scientific studies have concluded and proven that quantitative and qualitative changes in the composition of gut microbiota are associated with imbalances in populations of these microorganisms, which leads to various diseases, including infectious, neoplastic, chronic inflammatory, and neurodegenerative diseases [13]. With regard to colorectal cancer (CRC), abundant epidemiological studies have linked several members of gut microbiota with an increasing risk of developing CRC. To complete the picture, numerous in vitro and in vivo studies illustrate the potential mechanisms by which the gut microbiota affects CRC tumorigenesis. Moreover, the “two-hit” hypothesis, which was initially proposed in studies by Vogelstein and Kinzler, assembles a ground common for both genetic and environmental factors such as microbiota, supporting this is the logical consistency between several established risk factors associating microbiota and CRC, including genetic disorders and host immunity and adverse environmental conditions such as diet, smoking, and infections. Taken together, a promising way to prevent or treat CRC could be to moderate gut microbiota dysbiosis. The modulation of microbiota has garnered considerable attention, a fact that is evidenced by the burgeoning plethora of reviews in recent years with diverse therapeutic strategies for modulating microbiota, including appropriate diet, prebiotics, probiotics, synbiotics, fecal microbiota transplantation, and postbiotics. However, most strategies are still on a feasibility scale, emphasizing that there exist potential dangers and limitations.

Gut microbiota has rapidly gained attention over the last few years due to its significant role in the regulation of human health. The human gut microbiota comprises a complex array of microorganisms, including bacteria, fungi, archaea, and viruses, consisting of several hundred species. Most of these microorganisms live in the caecum and proximal colon, although variable composition is found in the gastrointestinal tract, with an increased diversity of bacterial microbiota existing in the large intestine in comparison to other parts of the gut. Often referred to as the “forgotten organ,” the gut microbiota plays a vital role in metabolism, synthesis of vitamins, fat storage, and xenobiotic metabolism, amongst others. A complex dialogue has been discovered between the gut microbiota and colonic mucosa, resulting in subsequent modulation of the host immune system. This continuous interaction can not only stimulate the host immune system but also protect the host from pathogenic organisms [12].

10.4.2 Impact of Postbiotics on Gut Microbiota

The gut microbiota not only plays an essential role in energy homeostasis and nutrient metabolism, forming a functional barrier to the host but also serves as a chemical barrier to the host and a direct cell product. A class of metabolites present in both fecal suspensions and organ-farming culture supernatants produced during the growth of gut microbiota, also known as postbiotics, have an established positive therapeutic effect. Various postbiotics produced by the complex gut microbiota are used for the treatment of chronic inflammation, obesity, type 2 diabetes, and gut barrier-related pathologies by modulating inflammation and functional recovery, rebalancing the gut microbiota, imparting a growth and angiogenic effect on intestinal epithelial cells, and maintaining gut barrier integrity [7]. The contribution of the gut microbiota metabolites, herein referred to as postbiotics, to gastrointestinal physiology and metabolism, is key, offering commercial potential alongside a real need for cellular integrity maintenance. In this review, known and potential postbiotics and relevant results from both *in vitro* and *in vivo* studies are presented and discussed. The effect of each postbiotic is thoroughly described and discussed [1].

Postbiotics, a term introduced to indicate the metabolic by-products of flora bacteria, act on the intestinal flora to provide beneficial effects on the health of the host. Postbiotics play a vital role in shaping the gut microbiota, maintaining gastrointestinal balance, and contributing to immunomodulatory effects. Studies are focusing on the relationship between postbiotics and disease progression, and extensive evidence is continuously enriching the treatment spectrum. In this review, we have briefly summarized the diseases associated with the imbalance of the gut microbiota and the relationship between postbiotics and diseases through existing literature. We hope that readers can increase their awareness and understanding of postbiotics and related diseases [26]. Future research directions and developments in related fields are also highlighted.

10.5 Postbiotics in Colorectal Cancer Prevention

In addition, much more knowledge is needed to establish safe and efficient dose-response relationships in mucosal disease contexts, but also with application to obtaining the potential reduction in the risk of systemic inflammation-related pathologies as well cardiovascular diseases. However, a slight increase is noticeable in the trend of postbiotic utilization in proctology, especially in the treatment of chronic inflammatory conditions of the anal canal [24]. However, to our knowledge, no current RCT studies have been developed regarding the systemic preoperative administration of PBP and the reduction in postoperative complications. In order to encourage more patients and surgical teams to use PBP in the near future, it is important to design preclinical and clinical intervention models to ensure, if justified and advantageous, the wide-spectrum application of the PBP.

The previously isolated beneficial effects of PBP in colorectal cancer may suggest their use for maintenance or improvement of gut homeostasis in subjects at risk for CRC or during cancer therapy. However, scientific evidence related to the direct effects of PBP on CRC is scarce, and much more remains unknown. Indeed, most PBP evaluation projects have an attractive focus for establishing an efficient use of postbiotic products on chronic diseases characterized by a high level of inflammation and a high degree of intestinal barrier dysfunction, such as inflammatory bowel diseases (IBD) and CRC. More preclinical models and clinical studies should be invited to be conducted in the future, combined with more research on the impact of isolation and manufacturing processes, but still expected to provide an important contribution to the use of isolation and prevention of colorectal diseases [40].

10.5.1 Preclinical Studies

Growth was checked, colorectal cancer was induced, and animals took pitolisant from early adulthood. Researchers verified that colony-forming units (CFU) + *Escherichia* genera and prominent crypt fission (output of the crypt fission number was used as a study endpoint) were increased at the colonic level during colorectal neoplasia development 37 t, but pitolisant modulated these biomarkers preventing polyp formation. There is room for postbiotics in the dialogue between colonic lumen and colorectal cancer, making them a therapeutic tool to act as a cancer chemopreventive or protective strategy. However, results have been considered minor, being far from clinical practice in the matter. Minimal progress has been made in developing postbiotic strategies that can specifically modulate primary tumor growth and kill quiescent colon CSCs [54].

On the topic of malignancies, Lee and colleagues, in 2018, reported that mice genetically predisposed to develop colorectal cancer received postbiotics from 2 days old until 2 months old. After colon analysis, researchers reported that postbiotics significantly decreased the number of large tumors and tumor volume [19]. It is also important to refer to pregnant mice, pregnant women, and neonatal life. Here, postbiotics supplemented during pregnancy and after breastfeeding were able to sustain glycosylation modifications of the fetal colon glycoproteins, suppressing proinflammatory signaling pathways. Moreover, postbiotic-supplemented offspring showed stimulation of the secretory immunoglobulin (SIgA) immune response and beneficial colonic inflammatory status regulation, as shown in Fig. 10.3. Other researchers have shown that a postbiotic-containing liquid can contribute to the inhibition of colorectal cancer in preclinical models. As for actual colorectal neoplasia, pitolisant can control the disease and body weight in mice fed with liquid [31].

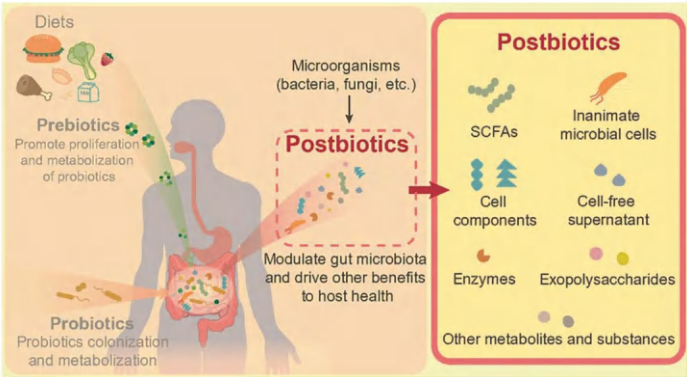


Fig. 10.3 preclinical evidence of postbiotics in colorectal cancer models

Table 10.2 Clinical studies on postbiotics in colorectal cancer

Study design	Function	Findings
Randomized controlled trial	Assessing therapeutic efficacy	Reduced inflammatory markers and improved gut barrier function in CRC patients receiving postbiotic supplementation
Prospective cohort study	Epidemiological observation	Lower incidence of CRC in individuals with high dietary intake of postbiotic-rich foods
Meta-analysis	Integrative data analysis	Meta-analysis suggests postbiotic use is associated with reduced risk of CRC recurrence
Case-control study	Observational assessment	Positive association between higher intake of yogurt (source of postbiotics) and reduced risk of CRC development
Systematic review and meta-analysis	Evidence synthesis	Systematic review supports the role of postbiotics in improving clinical outcomes in CRC patients

10.5.2 Clinical Evidence and Trials

Third, there are several reports focusing on extracellular vesicles, with and without known microRNA cargos, that include peptidoglycan, lipoteichoic acids, and lipopolysaccharide remnants from the cell walls of bacteria and their induced immune therapeutic effects. The reported results suggested that the formulation of postbiotic derivatives with better CRC preventive effects can be expected [16].

Second, postbiotic SCFAs and their derivatives have been reported to either inhibit or limit induced chronic inflammation in settings where SCFAs, particularly butyrate and acetate, play a pivotal role as anti-inflammatory mediators, as explained in Table 10.2. Since chronic inflammation is a well-recognized promoter of cancer, this preventive approach is reasonable for the improvement of CRC relapse in future clinical trials. Additionally, SCFA derivatives, such as 45 T-butyrate, have shown better immune-modulating properties compared to the parent SCFA molecule [48].

First, the administration of the postbiotic lactate suppressed the growth of CRC in both a mouse model of chemically induced CRC and a xenograft model, via meiosis and the suppression of the EPO receptor. The combined application of a low dose of the dietary lactate and a well-established CRC therapeutic, nab-paclitaxel, reinforced CRC-specific and site-specific tumor cell lysis *in vivo* via an immune mechanism. Compared to the prebiotic substrate (i.e., lactose), the oral bioavailability of lactate was greater and was well-tolerated by consumers in an evaluation using murine and porcine model systems.

Studies with postbiotics, many involving lactate, butyrate, or vitamin derivatives, have also shown promising preventive effects on the development and progression of CRC. We summarize the major findings here.

10.6 Therapeutic Potential of Postbiotics in Colorectal Cancer

To avoid potentially serious problems, the effectiveness of compounds that might be produced by infiltrated microorganisms in the postbiotic products should be demonstrated, especially in patients undergoing chemotherapy and clinical dysbiosis. Furthermore, to allow the multiplication of the positive effects, the containment of host responses that prevent postbiotic therapy-induced changes, such as diet or the host's pharmacotherapy changes, should be considered [49]. The utilization of postbiotics for CRC treatment depends on the priorities, intractable problems, such as anti-inflammatory effects, immune response influences, physical state, and the feasibility of rats or mice, and the safety and stability of bacterial components, that they may manifest [22].

Additionally, new postbiotics are being discovered, mainly by integrins that are linked to pollution sources, such as anthropogenic pollution, and the unique value of postbiotics for precise personalized therapy in cancer cannot be overemphasized. Furthermore, the establishment of clear risk factors needs to be prioritized. The safety of the postbiotics should be verified *in vivo*. The dose and host specificity of the selected postbiotics should be determined, and special attention should be given to their application in the host intestine whenever they are intended to be chronically consumed. Suitable formulations are required to guarantee the bioactivity of the postbiotics [3].

The potential of cancer prevention by bacterial metabolite trimethylamine-N-oxide (TMAO) or its precursors has also been explored in CRC. However, results from current studies are contradictory and inconclusive. Despite the extremely limited studies on postbiotics in preventing CRC, the current investigations revealed that postbiotics, microbial or microbially derived metabolites, could be used in cancer prevention and/or therapy along with different microbial aberrant toxins or some microbial-targeted chemicals that facilitate the regulation of the postbiotics. Future directions of the applications include further understanding the potential of the

agents, the mechanisms of the function of selected antimicrobial agents, and the combination of the postbiotics [51].

Another postbiotic that is used to prevent colon cancer is G418, which was demonstrated to inhibit glycoprotein biosynthesis, impact G protein, and block angiogenesis for cancer prevention in APC gene-mutated mice. CLA, another short-chain fatty acid, exhibits anti-inflammatory activity and apoptotic effects in cancerous cells and promotes the growth of normal colonic cells in xenografts. IL-12, *Dokdonia* sp., and linoleic acid produced by lactic acid bacteria, *Lactobacillus plantarum*, and *Bifidobacterium adolescentis*, respectively, could be used in immunotherapy for cancer therapy. Furthermore, the nonproteinaceous and nonantigenic character of immunomodulatory postbiotics was discussed, facilitating their therapeutic exploitation in situations with high variability in the host immune landscape, such as CRC [51].

Postbiotic manipulation represents a promising strategy for the prophylaxis and/or therapy of CRC. However, current data are mainly based on animal studies. Butyrate is a well-studied postbiotic extensively evaluated for its potential in cancer therapy. SCFAs, especially butyrate, exert their antineoplastic effects through multiple mechanisms, including histone acetylation and chromatin remodeling, macrophage function modulation, anti-inflammation, anticancer immunity stimulation, intestinal barrier function maintenance, oxidative stress and apoptosis induction, and glucose metabolism inhibition [43].

10.6.1 Mechanistic Insights

Research on colon cancer is faced with significant obstacles posed by the complexity and heterogeneity of the disease, including its etiology, susceptibility, biology, progression, prognosis, and therapy response. The constant progress in single-cell and spatial multi-omics technology will help address these obstacles. However, most large-scale studies conducted thus far have focused on DNA and RNA, missing the opportunity to comprehensively consider metabolites [10]. Samples studies are likely to yield metabolites as well, and with these novel opportunities, we may advance a more all-around understanding of postbiotic signaling in colorectal cancers. Ignorance in the understanding of colorectal cancers and postbiotics is intertwined with humans' unhealthy lifestyles. The increasing incidence of colon cancer poses a major threat to modern society due to unbalanced nutrition and environmental pollution. On the one hand, we should adopt a healthier diet and lifestyle by consuming more prebiotics, probiotics, and postbiotics. On the other hand, we should improve our technological capabilities and methods to better study the mechanisms underlying the interplay between postbiotics and colon cancer and thereby contribute to healthier living [2].

Postbiotics are important regulators and mediators of the host-bacteria cross talk. However, the mechanisms of postbiotic-mediated signaling or the players involved remain largely unexplored. SCFA, and butyrate in particular, have been extensively

studied in this respect as one class of postbiotics, and the main subjects studied thus far have been histone deacetylases, glycolysis, and anti-inflammatory effects. Our understanding of the direct colonic cancer signaling of butyrate, however, remains somewhat fragmented, and more in-depth analyses are expected. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is frequently discussed in terms of tumor cell growth arrest, but whether colonic epithelial cells can signal TRAIL remains unknown, and the direct postbiotic-mediated signaling events in other cell types present in the colon are less studied [27]. Although it is implied that there are multiple other postbiotic signaling paths yet to be discovered in the colon, current knowledge is limited to individual paths and has failed to provide a comprehensive understanding of their overall responses [33].

10.6.2 Combination Therapies with Postbiotics

A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health. Postbiotics are bioactive compounds produced by either the host or microorganisms that are metabolized by microorganisms and have an effect on the host. As studies in critical patients have shown, the combination of a prebiotic, probiotic, and antibiotic overcomes all of the drawbacks of each part of the mixture. It seems that this strategy could help patients at an advanced stage of the disease in metastasis prevention, and the achieved quality of life could be the basis for the therapy in other solid cancers [53]. To boost the antitumor effect encoded by the gut microbiota, pre- and postbiotics should be combined with the immune checkpoint inhibitors. They restore tumor-specific immunity by promoting the tumor infiltration of gut microbiota-reprogrammed T cells. With the help of programmed drug delivery systems, a novel drug-delivering system could be developed, and tumor-therapy optimization is possible with lower doses and minimal systemic activity and immune-adverse effects. Synergistic enzyme-activatable self-delivery drug nanovector could reduce the side effects, improve the killing effect, and keep the natural immunity system. The most interesting possibilities are related to drug-release mechanisms in colon cancer cells.

Colorectal cancer (CRC) is the second leading cause of cancer-related death globally, with poor prognosis in patients with advanced-stage disease. The cure is possible only in the early stages. However, tumor resection itself increases the risk of metastasis. CRC is not a systemic disease in most cases, but the manipulation of tumor and surrounding immune response seems to provide great changes in serum C-reactive protein, apolipoprotein, apo C-III, haptoglobin, hemopexin, beta-2-microglobulin, and others. Some of these are strong prognostic markers and could be used in clinical diagnosis without sampling in dangerous collections [29]. The microbiota seems to be involved in primary CRS-induction development and disturbances in long-term tumor prognosis. Preoperative probiotics and postbiotics may reduce the metastatic potential [54]. It is supposed that preoperative administration

would lower colonic local immune suppression, reduce surgical damage to both colon and mesenteric tissue, and avoid early relapse.

10.7 Future Directions and Research Gaps

The contact between bacteria, other microorganisms, and human beings is established long before their delivery and shapes the immune system, one of the earliest and most important protection barriers. Such an early relationship is key for the establishment of a stable and balanced relationship between the microbiota and our host. The human body is colonized by trillions of microorganisms, including bacteria, viruses, fungi, and archaea, which are mainly flourishing in the gut. It is now well established that a balanced gut ecosystem is involved in the well-being of the host, contributing to various complex metabolic and physiological systems, including digestion, vitamin synthesis, xenobiotic metabolism, and the immune system. However, the microbial functions go far beyond the gut, influencing the systemic health of the host. Indeed, disruptions in the microbial community composition, such as those attributed to antibiotic use, infections, stress, dietary changes, or aging, may lead to dysregulation in gut functions and gut linkages with non-digestive organs and/or systems [14].

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second most prevalent cause of morbidity and mortality in the Western world. However, CRC is preventable and curable when early diagnosed with appropriate screening strategies and lifestyle improvements. The contribution of the gut microbiota to the balance between health and disease has been widely discussed. Increasing evidence has pointed to the exploitation of microbial-derived metabolites as preventive and/or therapeutic strategies in CRC. Such compounds are known as postbiotics. This review provides state-of-the-art knowledge on the interplay between the gut microbiota, including postbiotics, and colorectal cancer. Furthermore, the potential of postbiotics in the prevention and therapy of colorectal cancer and the implications of this rising field are presented. Special attention is paid to the chemopreventive activities of postbiotics in murine models of chemically induced CRC and to the use of probiotics as adjuvant therapy for CRC patients [25].

10.8 Conclusion and Implications for Clinical Practice

Postbiotic agents can easily be formulated into existing dosage forms for direct oral consumption or made into rectally administered enemas or pessaries for local purpose treatment on the colonic mucosa in a more precise and potent way. If postbiotics demonstrate safety and efficacy through clinical studies, they can simplify existing complicated and demanding treatment regimens such as poorly compliant

high-dose antibiotic therapy. It should also be noteworthy that a left colon targeted delivery allows for decreasing systemic exposure of patients to potentially toxic substances. Postbiotic-based CRC management represents a cost-effective and affordable option for both developing and developed countries, avoiding problems with antibiotic-associated infections in oncology units and additional burdens on hospital healthcare resources. Despite showing promising therapeutic potential, the scope of postbiotics extends beyond this simple concept. More innovative applications expect scientific and clinical researchers to discover, develop, and exploit postbiotics in other fields for human beings, such as enriched probiotic products, human-based liquid foods, environmental use, etc. The potential of postbiotics is currently leading to its translation in the clinics and market. However, future research, expanding on the present limitations, is essential to assess the full potential and ensure the sustainable and beneficial use of these promising products.

An improved understanding of the complex interplay between postbiotics and CRC has provided investigators with new tools to manage and potentially eliminate the causes of CRC. The focus of the research should be the clinical applications of postbiotics to assess their safety and efficacy in the management of CRC. It is worth noting that whether postbiotic-based CRC management could be successfully commercialized should take into consideration that the universality of these biological properties is more useful than the difference among different antibacterial and anti-inflammatory agents, as well as their optimal production process. In the meantime, since the mammalian gut is endowed with an autopoietic structure, which is instrumental for creating abundant niches for supplying the microbial ecosystem, naturally, how to protect and prevent colorectal diseases using secreted biomacromolecules from populations of intestinal bacteria in 30 years' time is another challenge.

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Chapter 11

Postbiotics and Colorectal Cancer: Current Research and Clinical Insights



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Abstract The chapter details the emerging field of postbiotics and their potential impact on colorectal cancer (CRC). The fundamental concepts of postbiotics and their diverse health benefits have been explored to bridge the gap between microbiome science and oncology. It provides a comprehensive overview of colorectal cancer, its causes, symptoms, and risk factors. The crucial link between gut microbiota and CRC is explained, emphasizing the complex interplay between microbial communities and cancer progression. The literature underscores an in-depth analysis of postbiotics, their definitions, mechanisms, and therapeutic potential. Furthermore, the latest clinical research and trials are discussed to offer better insights into postbiotic interventions for CRC. The chapter concludes with future directions for postbiotic research and practical guidelines to integrate them into clinical practice, urging the necessity for continued exploration and innovation in this promising field. The chapter contents are essential for researchers, clinicians, and students investigating the association between microbiome science and cancer therapy.

Keywords Postbiotics · Clinical research · safety · ethical consideration guidelines

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11.1 Introduction to Postbiotics and Their Role in Health

The human gut microbiota refers to a diverse and ever-evolving population of microorganisms that resides in the gastrointestinal tract and serves as a critical system that affects multiple aspects of human physiology [1, 2]. Studying postbiotics, a relatively new field focus, has become relevant in recent years. On the other hand, the postbiotics or postbiotic substances are derived from the activities of the favorable microorganisms in the gut and are different from live probiotics. These compounds consist of short-chain fatty acids, enzymes, peptides, and cell wall components, and all share diverse health benefits [3]. Overcoming these drawbacks and considering the stability of postbiotics in comparison with live probiotics can be beneficial in their application for therapeutic purposes, as postbiotics are more resistant and easily standardized for application in various forms. Health promotion and management through postbiotics occurs in different ways, including regulating immune responses and engaging with the immune cells within the gut microbiota to enhance host protection and reduce inflammation, which is implicated in many chronic diseases. In the same regard, postbiotics, especially SCFAs such as butyrate, strengthen the intestinal barrier, a primary line of defense against deleterious compounds, by promoting the synthesis of mucus and optimizing the integrity of epithelial cells. Moreover, through the catabolism of nutrients, postbiotics directly impact the gut microbiota by promoting the growth of beneficial bacterial species while restraining the growth of pathogens, thus, the balanced composition of the microbial community [4]. Further understanding of the huge untapped potential of postbiotics in terms of CRC prevention and treatment is an interesting area of research. CRC is a common and normally fatal cancer and is associated with alterations in gut microbiota composition [5]. Metabolites like postbiotics, for example, butyrate, have an anti-inflammatory and anticarcinogenic effect that is beneficial to the gut and thereby possibly inducing cancer cell death [6]. Furthermore, postbiotics can also affect the expression of genes and pathways that are associated with the onset of cancer. With further advancements in research, postbiotics continue to present great prospects for altering the landscape of clinical practice and providing therapeutic possibilities as well as nutraceutical components to improve intestinal and overall human health.

11.2 Understanding Colorectal Cancer (CRC): Causes, Symptoms, and Risk Factors

Colorectal cancer, commonly known as CRC, is the world's leading health problem caused by the uncontrolled growth of cells in the colon or rectum as a result of gene mutations that may be inherited and may also be induced by certain extrinsic stimuli [7]. Some of these changes will result in the development of polyps, which are nearly always innocent growths, but if they are not checked, they can turn

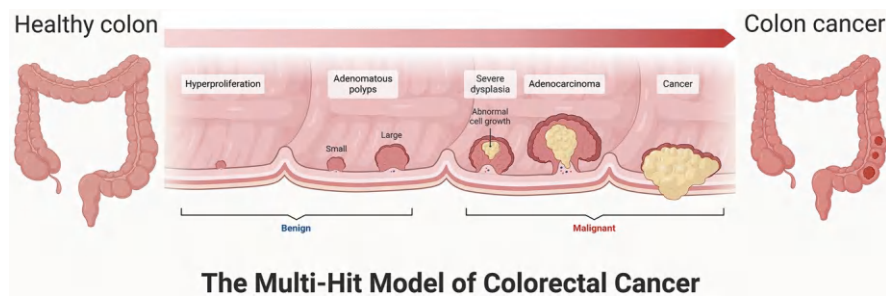


Fig. 11.1 The Multi-hit model of colorectal cancer

malignant. Causes of CRC are several and could be either hereditary or may include any extrinsic factors [8]. This is particularly so if the individual has some hereditary condition, for instance, FAP and Lynch syndrome, which are known to increase risk significantly. Lifestyle and environmental factors also contribute to the occurrence of the diseases [9]. Specific food habits like eating foods with high contents of red and processed meats, low fiber, and high unhealthy fats, habits such as smoking and drinking increase one's risk. In addition, conditions that cause chronic inflammation, such as Crohn's disease and ulcerative colitis, make the patient vulnerable due to inflammation and possible DNA damage (Fig. 11.1).

Another factor that has been postulated is age, CRC risk increases with age, which implies that older persons should possibly enhance their level of awareness. Difficulties in diagnosing early-stage CRC are caused by the fact that, in most cases, patients are asymptomatic [10]. However, the above disease presents in different ways, and as it progresses, the following symptoms are displayed: frequent changes in bowel movement, rectal bleeding or blood-stained stools, abdominal pain and discomfort, frequent loss of weight, and fatigue. This is why such symptoms should be identified clearly so that proper diagnosis can be made together with early treatment. The risk factors of CRC are also classified into two, that is, the reversible and the non-reversible risk factors [11]. These are factors that cannot be altered; these are age, family history, individual history of polyps or cancer, and hereditary syndromes. Lifestyle risk factors are those that can be changed; they include food choices, lack of exercise, being overweight or obese, smoking, and alcohol consumption. By recognizing these risk factors, one is in a position to change their lifestyle and hence avoid or prevent such evident risks [12]. Annual screening, especially for high-risk individuals, is therefore necessary to identify early signs that warrant adequate intervention [13]. Here are some preventive measures: one must take a diet that is high in fiber, fruits, and vegetables, engage in physical activity, and avoid smoking and excessive use of alcohol to lower the chances of getting CRC. Since we understand that there are possible genetic and environmental factors that can cause it, a person can reduce his or her risk and may seek medical help early enough in case symptoms are noticed [14].

11.3 The Relationship Between Gut Microbiota and Colorectal Cancer

The human gut microbiota is an enormous and complex population of microorganisms living inside a person's digestive system and plays an important role in their health [15]. It has been identified that the gut microbiota contributes to many physiological activities in the body, such as immune response, digestion and exclusion, and pathogenic defense. Current scientific studies have revealed that the gut microbiota is associated with the emergence of different diseases, including CRC [16]. Therefore, it is crucial to comprehend this complex link to design new effective preventive as well as therapeutic approaches to CRCs. Changed over the life span, the member of the gut microbiota depends on diet, genetics, age, antibiotic use, and other environmental factors [17]. The impression is that favorable gut microbiota is diverse and balanced with the beneficial microbial population dominating [18]. Disruptions of this balance are said to be dysbiosis, which they point out has been associated with various diseases, among them being CRC. Alteration of the gut microbiota, called dysbiosis, has been identified to play a crucial role in the initiation and progression of CRC [19]. They destroy useful species in the digestive system and increase the proliferation of pathogenic bacteria and the generation of pro-inflammatory molecules together with changed immunological response, carcinogen formation, and chronic inflammation that cause cancer. Chronically, inflammation, which is one of the well-established risk factors of CRC, may result from dysbiosis, which leads to the destruction of the mucosal barrier, which results in an invasion of harmful bacteria into the lining of the intestine. The immune system gets activated by this invasion and forms longer inflammation that can cause DNA damage and, consequently, promote the occurrence of cellular mutation that leads to CRC initiation and progression [20]. Some of the metabolites implicated in tumor development include carcinogens that act directly on colonic cells and cause mutation and cancer. Some components in the diet are also metabolized by the microbes, which in turn affect CRC risk. Inulin, a form of dietary fiber, is prebiotic and is fermented in the colon by beneficial bacteria that are known to produce SCFAs, which have negative effects on inflammation and cancer cell growth [21]. In contrast, the metabolism of protein and fat-rich diets produces metabolites which had been linked with increased CRC risk. Gut microbiota also has direct contact with the immune system, which plays an essential role in modulating the gut barrier [22] (Table 11.1).

This immune regulation can be disrupted by dysbiosis, which results in either inadequate or overstimulated immune response, which fosters cancer cell escape and multiplication. Some of the bacterial species that have been said to play a role in increasing the risk of CRC include *Fusobacterium nucleate*, enterotoxigenic *Bacteroides fragilis* (ETBF), and some strains of *Escherichia coli* through mechanisms such as inflammation, carcinogenic compounds, and immune system alterations. This improved understanding of CRC linked with the gut microbiota has pointed to dysbiosis as a possible therapeutic opportunity [23]. These are probiotics

Table 11.1 Gut microbiota and its implication in colorectal cancer

Aspect	Details
Gut microbiota	A complex community of microorganisms residing in the digestive system, significantly influencing human health
Role in CRC	Dysbiosis (imbalance in gut microbiota) plays a pivotal role in the initiation and progression of CRC
Mechanisms	Dysbiosis leads to: 1. Chronic inflammation 2. Production of carcinogenic metabolites 3. Impaired immune response
Key bacterial species	<i>Fusobacterium nucleate</i> , enterotoxigenic <i>Bacteroides fragilis</i> (ETBF), and some strains of <i>Escherichia coli</i> .
Therapeutic opportunities	Probiotics, prebiotics, postbiotics, fecal microbiota transplantation (FMT), and dietary modifications

and prebiotics to restore the balance, postbiotics with anticancer effects, fecal microbiota transplantation (FMT) to reinstate a healthy microbiota; and dietary modifications that favor the production of beneficial bacteria and SCFAs. Such an approach, which addresses the gut microbiota, has the potential to decrease the global disease incidence of CRC and enhance the patient’s prognosis [24].

11.4 Postbiotics: Definition, Mechanisms, and Therapeutic Potential

The focus on postbiotics as one of the newly discovered paradigms in the enhancement of health is slowly moving to the specificity of postbiotics [25]. Thus, postbiotics are the bioactive compounds derived from the fermentation of prebiotics, probiotics, and other active living microbes that take their distinct identity as postbiotics, encouraging the development of these probiotics. It includes a wide range of chemical substances like short-chain fatty acids (Scfa), enzymes, peptides, exopolysaccharides, and cell walls that have been known to possess quite several health benefits [26]. Compared to live probiotics that can spread cancer and cause further harm to their consumers, postbiotics are stable and, therefore, suitable for treatment [27] (Table 11.2).

The ones extracted from plants are generally more stable and possess the ability to be formed into standard weights present in supplements, functional foods, and pharmaceuticals; they are easy to store, often having a uniformly long shelf life and nearly predictable therapeutic consequences. Certain needs that are mostly met by postbiotics are highlighted below, especially regarding the gut and immune system [28]. They regulate the lens and the functional equilibrium of the mentioned microbiota, thus assisting in growing different, better bacteria strains and preventing pathogenic bacteria in addition. Persistent with this point, the SCFAs, especially butyrate, play an important role in maintaining the “Fortifying gut barrier to prevent

Table 11.2 Postbiotics—a potential therapeutic tool for CRC and beyond

Aspect	Details
Definition	Bioactive compounds resulting from the fermentation of prebiotics, probiotics, or other active microbes
Examples	Short-chain fatty acids (SCFAs), enzymes, peptides, exopolysaccharides, cell walls
Advantages over probiotics	Stable, standardized, predictable therapeutic effects, longer shelf life, safer
Benefits for gut and immune system	Modulate gut microbiota, strengthen gut barrier, anti-inflammatory, enhance immune response
Role in CRC prevention	Induce cancer cell apoptosis (especially butyrate), improve gut health, reduce inflammation
Potential applications	Treatment and prevention of various diseases, functional foods, dietary supplements
Future directions	More research needed on standardization, specific compounds, effects

corporal products from penetrating; they also contain strong anti-inflammatory properties that mitigate chronic inflammation, which is characteristic of many diseases, including CRC.” Furthermore, it indicates that postbiotics interact with the immune system and enhance the body’s response to pathogens, hence reducing the probability of autoimmune and inflammation diseases [29]. Among the described mechanisms regarding how postbiotics can rely on preventing CRC, one is the influence of butyrate, which can prompt cancer cell apoptosis. The potentials for postbiotics as a therapeutic tool look extensive, as these compounds might be helpful for the treatment of different diseases [30]. They can thereby offer new strategies for prevention and treatment, from modulating the functions and balance of gut microbiota and inflammation to serving as adjuvants in CRC treatment and improving gut health alongside cancer. This is because postbiotics are stable and have proven to be effective in improving the functioning of the consumers’ body; hence, they can be added to functional foods and diet supplements [31]. Despite these numerous opportunities, certain concerns should be addressed, such as various compounds and effects and how postbiotic products should be better standardized for greater effectiveness and safety in the future. As postbiotics are promising as a new area for gut health and disease prevention, including CRC, therefore more research has to be done regarding this phenomenon [32]. For this reason, given their prospective influencing characteristics on managing gut microbiota, affording improved intestinal integrity, reducing inflammation, and inducing apoptosis in cancer cells, they are viewed as a potential weapon against CRC and other chronic diseases. More research would provide postbiotic-based therapeutic products and functional foods that will revolutionize the concept and approach to diseases and health [33].

11.5 Clinical Research on Postbiotics and Colorectal Cancer

The link between postbiotics and CRC is a relatively new area of study with significant potential for identifying novel prevention and treatment strategies [34]. Despite the growing popularity of probiotics and prebiotics, postbiotics, which are the downstream metabolites of active probiotics, present another superior strategy to regulate GI health and associated disease processes. At present, the clinical research on postbiotics regarding CRC is less developed than the probiotics and prebiotics studies [35]. Despite this, data from the limited *in vitro* and *in vivo* studies give promising results for the use of postbiotics in the prevention and treatment of CRC. These studies have concentrated on the bioaction of postbiotics, the recognition of certain postbiotic compounds as having anticancer effects, and the expansion of postbiotics as healing agents [36]. Among all postbiotics, butyrate, a short-chain fatty acid derived from the bacterial fermentation of dietary fibers, has received the most attention regarding its effects on CRC (Fig. 11.2).

The anticarcinogenic capacities of butyrate comprise pro-apoptotic impact on cancer cells, the inhibition of HDAC, and changes in gene expression to counter tumor growth and inflammation, which is one of CRC's root factors [37]. In clinical trials that incorporated dietary interventions with the use of butyrate, the results demonstrated the effectiveness of butyrate in decreasing markers of CRC risk.

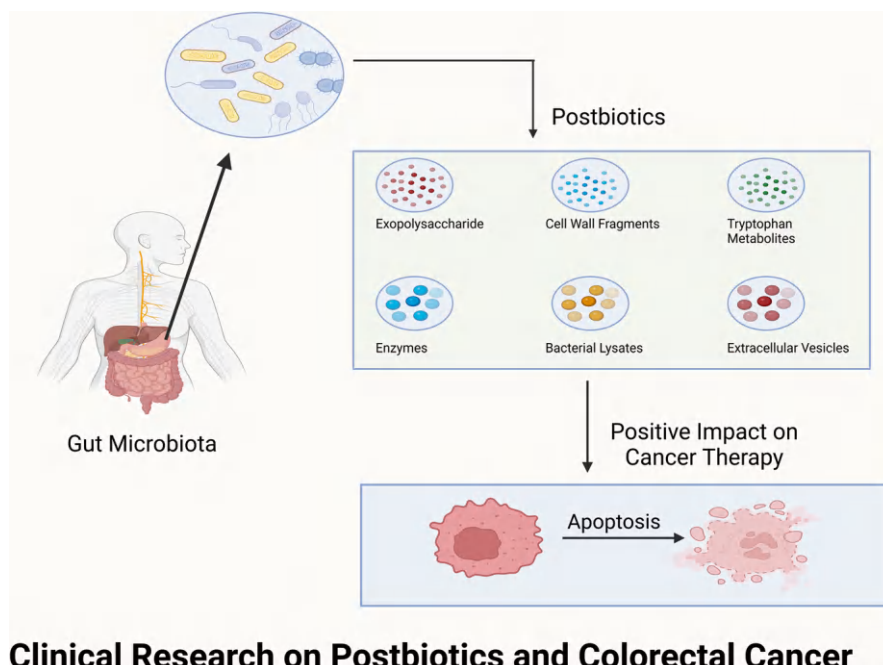


Fig. 11.2 Clinical research on postbiotics and colorectal cancer

Specific strains of *Lactobacillus* bacteria secrete both acid and specific metabolites that have been researched for their immunomodulatory potential that may help in the prevention or cure of CRC [38]. For instance, postbiotics from *Lactobacillus rhamnosus* GG (LGG) strengthen the immunosuppressive T-regulatory cells (Tregs), which are involved in immune tolerance and combating chronic inflammation. Postbiotics include peptidoglycan and lipoteichoic acid found in the bacterial cell wall, which can enhance the immune response and help the immune system identify and destroy cancer cells [39]. Small clinical trials that employed *Lactobacillus*-derived postbiotics showed its benefits to intestinal function and inhibition of inflammation biomarkers which might contrive to decreased risks of CRC [40]. Another type of postbiotics is exopolysaccharides (EPS), glycosylated polymers secreted by bacteria, including *Bifidobacterium* species, which have demonstrated potential for cancer applications [41]. EPS can stimulate the function of macrophages and dendritic cells that play a role in the activation of antitumor immunity and can suppress the growth of colorectal tumors in animal models. However, based on these results, further work indicates the potential for the future development of EPS as a therapeutic agent for CRC [42]. There are several ongoing clinical trials to establish the role of postbiotics in the prevention and management of CRC effectively. These investigations contribute to the existing knowledge base on postbiotics by assessing the safety and effectiveness of certain postbiotics in diverse and bigger groups of people [43]. Another trial concerns the use of butyrate in high-risk CRC populations: the effect of oral butyrate in decreasing biomarkers of CRC risk. Another cross-sectional study focuses on the effects of OR derived from *Lactobacillus* postbiotics as a complementary therapy in patients diagnosed with CRC receiving chemotherapy to establish if postbiotics can help alleviate the side effects of chemotherapy and enhance the prognosis of the disease [44]. Also, a trial is looking into the impact of postbiotic supplementation in subjects with colonic precancerous lesions to evaluate whether this diet helps in halting the development of CRC from the precancerous stage. Despite the encouraging evidence that postbiotics can be used in CRC, several issues must be discussed [45]. Among them, there is the challenge of standardizing and identifying active compounds, recognizing the action mechanisms, and determining the safety and long-term consequences of postbiotics. Potential research areas for the development of microbiota-based cancer treatment strategies are targeted therapy with a focus on the patient's microbiome, the use of microbiota-based therapies in addition to conventional cancer therapies, and further investigation of new postbiotic interventions. In conclusion, current literature on postbiotics and CRC clinical trials is somewhat scarce; however, the studies available show promising results [46]. Newer derivatives of probiotics known as postbiotics have been found to have features that could help in reducing the occurrence of CRC, possibly interact with immunity, and improve the outcomes of chemotherapy for cancer [47]. More extensive clinical trials are currently also being conducted to determine the efficiency of these compounds; therefore, it is expected that postbiotics will be an essential tool in combating CRC in the future, providing unique approaches to prevention, treatment, and better patient outcomes.

11.6 Postbiotic Interventions in Colorectal Cancer: Case Studies and Trials

Due to the increasing number of published studies detailing the use of postbiotics in CRC, the use of these bioactive compounds in clinical practices has been escalating [48]. The chapter specifically introduces sample postbiotics in CRC and the possible clinical application together with discussing the case studies in terms of challenges and success as well as strategies for the implementation. First, patients with CRC are the ones concerned with the given postbiotic, so this means that it is useful to approach this concept from a range of perspectives that case reports provide. A current example was the use of butyrate, a SCFA, short-chain fatty acid postbiotic in CRC [49]. A patient with severe gastrointestinal toxicity resulting from chemotherapy also benefited from butyrate enemas due to her improved toxicity rate, butyrate acts as an anti-inflammatory agent and promotes mucosal healing [50]. The final example described the use of postbiotics, such as *Lactobacillus rhamnosus* GG (LGG), in the postoperative care process [51]. A patient who took these postbiotics saw that their bowel movement was faster and they rarely got infected these highlighted the role played by postbiotics in helping the patient recover faster, and incidences of getting an infection were minimal. To date, several clinical trials have been in the process of being planned or have been performed to systematically assess the efficacy and the safety of postbiotics for the therapy and the prevention of CRC [52]. The BUTYRUS trial focused on whether butyrate could enhance the risk of adenoma recurrence in individuals at high risk. As stated in the trial, subjects who have taken butyrate have a lower percentage of adenoma recurrence compared to placebo subjects, meaning that butyrate is preventive. The trial named Lacto-Pro aimed to evaluate the possibility of increasing the chemotherapy efficacy and tolerability in CRC patients using postbiotics of *Lactobacillus*. The study seemingly provided hope that these postbiotics assist patients in coping with the ravages of chemotherapy by the latter not pulling out due to side effects.

Aspect	Details
Clinical applications	Butyrate enemas: improve chemotherapy-induced gastrointestinal toxicity, promote mucosal healing <i>Lactobacillus rhamnosus</i> GG (LGG): enhance postoperative recovery, reduce infection risk
Clinical trials	BUTYRUS: Assess butyrate’s efficacy in reducing adenoma recurrence in high-risk individuals Lacto-Pro: evaluate <i>Lactobacillus</i> postbiotics’ potential in improving chemotherapy efficacy and tolerability in CRC patients EPS-Colon: investigate exopolysaccharides’ effectiveness in reducing CRC risk in patients with IBD
Challenges	Standardization of postbiotic products Long-term safety and efficacy assessment Consideration of inter-individual variability Organizational and legal concerns
Future directions	Further research needed on postbiotic mechanisms and clinical applications in CRC Potential for postbiotics to become part of standard CRC treatment and prevention strategies

In the EPS-Colon trial, the effectiveness of exopolysaccharides from *Bifidobacterium* species in the reduction of CRC risk in patients with IBD, a risk factor, was assessed. Concerning the trends observed in the trial outcomes, EPS showed potential in cervical cancer prevention by reducing the formation of new pre-cancerous lesions and having comparably lower inflammatory markers, indicating successful prevention of CRC in high risk. However, there are well-defined limitations when it comes to the assessment of postbiotics; these challenges include; there are various challenges in postbiotic evaluations, including the standardization of postbiotic products, determining the safety and efficacy of postbiotics over a long period, and they do not consider inter-individual variability. The use of postbiotics in therapy influencing cancer patients also has several organizational and legal concerns [53]. However, it must be mentioned that the use of postbiotics in the frame of CRC treatment is still not investigated enough and is present as part of this process only in the development stage with a great number of possible achievements. Now, some drugs are derived from probiotics, so going further to investigate the efficiency of postbiotic management and its usage regarding the treatment of colorectal cancer is needed, which can open new perspectives for patients and practitioners [54].

11.7 Future Directions in Postbiotic Research for Colorectal Cancer

The future of postbiotic research in CRC is promising as researchers continue to try to demarcate the complex interaction between the gut microbiome, postbiotics, and cancer [55]. The way forward is depicted in several areas of research interest that may help in enhancing the understanding and use of postbiotics to tackle CRC. One is more focused on the specific effects and through which channels postbiotics affect cancer cells and tumor microenvironment outcomes. Nonetheless, most of the mechanisms have not been characterized, making it crucial to know the molecular impacts of postbiotics. Moreover, if the identification of postbiotics is not limited to these SCFAs, there might be other drugs that could be identified [1, 2]. Other opportunities include individualized treatments, also known as precision medicine, and “organism,” which are microbiome descriptors. This is because the gut microbiome is unique to the individual and, therefore, requires a gentler approach in its analysis. The directions of further research include the possibility of determining the microbiome features responsible for the definition of individual therapies included in the postbiotic approach, as well as the biomarkers that reflect the results of specific interventions. It is also important to use postbiotics in conjunction with existing cancer treatment products [56]. Further studies should explore whether it enhances the potency of chemotherapeutic agents, complement immunotherapy to raise the immune response against cancer cells, and promote gut welfare and CRC relapse prevention in postsurgical patients. Product safety and efficacy trials are needed in

the long term to identify the externalities of the constant use of postbiotics for the establishment of gut microbiome health and in the long-term success of the use of postbiotics. It is also important to identify new supply systems that would increase the delivery and effectiveness of combinations of prebiotics and probiotics or other technological encapsulations. It is also important that more effort should be made to try to cover a wider clinical trial. A cross-sectional study in multicenter with a different ethnic group would be useful to establish the research findings found in the present study. Another area that may be relevant for future trials is the question of preventive measures in the high-risk population. Finally, understanding the postbiotic regulation and development of clear strategies for postbiotic production, formulation, and testing will prove useful in launching postbiotics to the market. It will also be necessary to pay attention to the issue of ownership of the created intellectual property, as well as economic considerations in the formation of postbiotic. In conclusion, the discussion on postbiotics in radical CRC research has several potential opportunities. Taking into consideration the current approaches that have been used in the study of postbiotics, other directions of the future development of the field can be outlined as follows: Personalized medicine should be combined with the current approaches, large-term trials should be conducted, and various means of delivery of the postbiotics should be researched, which can further promote the development of postbiotics as one of the fundamental tools in prevention and treatment of colorectal cancer. Despite the uncertain future for postbiotics, the approach appears to be effective in addressing one of the most common and lethal forms of cancer at scale [57].

11.8 Integrating Postbiotics into Clinical Practice: Guidelines and Recommendations

Due to the application of postbiotics for clinical modalities of CRC prevention and management, there is a need to put strong policies in place. The present application of postbiotics in CRC is versatile with preventive care in susceptible groups and tertiary prevention in the active phase of disease control. From these observations, postbiotics might be proposed to CRC-suspected patients, such as a family history, a history of adenomas, or chronic inflammation [35]. They can also be administered together with other chemical substances or irradiation for cancer treatment and adjuvant therapy. In this respect, some of the sublists as follows should be prioritized. Among them, dose selection must be prioritized where universal postbiotic patterns should be adopted, starting with a low dose and increasing only in the presence or nature of response and tolerance by the patients. The maintenance duration of glutamine should depend on the case: whereas some patients might need long-term supplementation, others would need therapy right in connection with primary treatments. These risk factors are necessary to consider in the patient option based on the risk, active CRC, and post-surgical patient [58]. Some of the conditions that

are likely to give way to the treatment include severe gastrointestinal disorders or allergies. The possible disadvantages usually include a temporary alteration of the bacterial flora of the intestine and also rare but severe reactions, hence, constant monitoring and control is recommended. However, postbiotic intervention can and should be used as an adjunct to conventional management once a rigorous assessment has been made. They may assist in reducing side effects associated with chemotherapy, reduce toxicity resulting from radiotherapy as well as enhance immunotherapy [59]. When it comes to the application of postbiotics, one must educate the healthcare providers and the consumers about the science, hope, and expectations of postbiotic treatment. Future advancement and research are important because they play a major role in improving postbiotic treatments. This involves coordinating major clinical trials, gaining requisite approvals for these trials as well as promoting and defining the formulation and delivery technology [60]. This demonstrates that postbiotics have the potential to be widely implemented for the treatment of CRC. Professionals serving as clinicians are urged to take into consideration guidelines as well as recommendations developed to help them use postbiotics and obtain the intended therapeutic effect of improving the patient's quality of life. Future research and collaborations will enhance aspects of these guidelines and determine the postbiotics role in CRC prevention and treatment.

11.9 Conclusion

The topic of postbiotics as a concept in CRC is still relatively novel and could prove to be instrumental in redesigning approaches to both the prevention and cure of this deadly disease. The gut microbiota, which is composed of trillions of microorganisms residing in various body sites, has been closely associated with CRC occurrence and progression. Postbiotics, secondary metabolites from probiotics, have been identified as regulators of gut health and disease progression. Given the stability, multiple functions, and biological activities of postbiotics, such compounds have the potential as therapies in conventional cancer care, as standalone dietaries, or as complementary therapies to other conventional treatments. Scientific research gives promising prognoses for postbiotics as a tool for the prevention and treatment of CRC. Butyrate, an SCFA, has been known to possess anticarcinogenic properties, while postbiotics derived from *Lactobacillus* and exopolysaccharides have been shown to affect immune cells and inhibit the growth of tumors. More clinical trials are currently ongoing to determine the effectiveness and toxicity of these compounds in different populations ranging from high-risk to those on chemotherapy. There is a lot of potential for postbiotic research in CRC in the future. Further knowledge of the molecular targets and pathways, PH-based microbiomics medicine, combination with other systems, and the outcomes of long-term usage and effects are also important directions for future research. Regarding the clinical utility of postbiotics, the creation of new delivery systems for active ingredients and the continued growth of clinical trials will help further elucidate its upcoming utility in

clinical practice. Despite these limitations, the future for postbiotics in combating CRC is promising, and present difficulties such as standardization, safety evaluation, and interindividual differences should be further investigated. With time, as more information emerges, postbiotics can be embraced as an essential element of CRC management as a prevention and treatment option for additional support to the patients and health professionals. Translating research from the bench to the clinic is a slow and continuous process; nonetheless, postbiotics have the potential to transform this widely affecting and frequently fatal disease through future advancements and cooperation.

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Chapter 12

Gut-Brain Axis and Colorectal Cancer: Neuroimmune Interactions



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Abstract Colorectal cancer (CRC) is a concerning health issue worldwide, influenced by genetic, lifestyle, and environmental factors. Recent research highlights the importance of the gut-brain axis (GBA) in CRC development. The GBA connects the brain, gut, immune system, and gut bacteria, and imbalanced gut bacteria leads to dysbiosis, which can lead to inflammation and promote tumor growth. The gut-brain axis does not just impact cancer growth, it also affects mental health and brain function through inflammation, leading to cognitive issues and mood changes in CRC patients. Chemicals produced by the gut's nervous system, like neurotransmitters and cytokines, play a big role in how cancer cells behave, helping them grow and spread while impacting the immune system. Recently developed treatment options are being studied that focus on restoring gut health with probiotics, prebiotics, and psychobiotics, which may help to slow down tumor growth. Immunotherapy advancements, including CAR T cell therapy and new ways to deliver drugs through nanotechnology, are opening doors for more targeted cancer treatments. By combining advanced treatment strategies with traditional methods, doctors can create more personalized strategies for managing CRC. The connection between the gut, brain, and immune system is key to understanding how we can improve both cancer treatment and the quality of life for those with CRC.

Keywords Gut-brain axis · Colorectal cancer · Gut microbiota · Neurotransmitters · Immunotherapy · Personalized medicine · Treatment strategies

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12.1 Introduction

Colorectal cancer (CRC) is a rising health concern worldwide, affecting millions annually. It is the third most diagnosed cancer and the second pre-eminent cause of cancer-related deaths globally [1]. CRC manifests as the uncontrolled growth of cells in the large intestine, specifically the colon or rectum. The development of CRC is influenced by a variety of genetic, environmental, and lifestyle factors that can singularly or in combination affect its prognosis [2]. Current and ongoing research shows the proven and prospective role of the gut-brain axis in CRC, offering new insights into disease mechanisms and potential treatments. Colorectal cancer’s global impact is significant, with prevalence seen in both men and women and incidence rates varying significantly according to geographical locations. The countries most affected by CRC, with increasingly high incidence rates, are the United States, Australia, and parts of Europe, where lifestyle factors like diet and physical inactivity are prevalent [3]. Along similar lines, CRC incidence is seen to be rising in developing regions due to increasing urbanization and the adoption of Western lifestyles, which include higher consumption of processed foods and sedentary behaviors, as stated by GLOBOCAN 2024. As shown in Fig. 12.1, India is burdened with a rising incidence rate of colorectum cancer. At 9.6% globally, it is the third most common cancer to be diagnosed (Fig. 12.1), but it is also the second biggest cause of cancer-related deaths worldwide [4].

Developed countries often have robust screening programs that contribute to early detection and improved outcomes. For example, countries like the United States and the United Kingdom offer regular colonoscopy screenings for individuals over 50, significantly reducing CRC mortality rates [5]. In contrast, many developing countries lack widespread access to screening, leading to later-stage diagnoses and poorer outcomes.

Although genetic and lifestyle factors are recognized contributors to CRC development, the intricate interplay between the gut microbiome and the central nervous

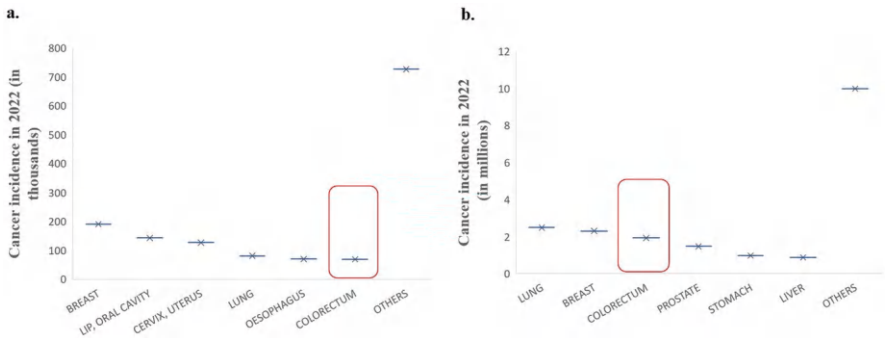


Fig. 12.1 Colorectal cancer incidence in India and worldwide. The histograms represent the status of different cancers in terms of individuals diagnosed with these cancers in India (a) and Worldwide (b) in the year 2022. (Data source: <https://gco.iarc.fr/en>)

system, termed the gut-brain axis, has emerged as a crucial area of investigation. This bidirectional communication network encompasses complex interactions between the enteric nervous system (ENS), the hypothalamic-pituitary-adrenal axis (HPA axis), the autonomic nervous system (ANS), and the gut microbiota. These components collectively influence the host's immune response and susceptibility to CRC.

Emerging evidence suggests that disruptions in the gut-brain axis, particularly alterations in the gut microbial composition known as dysbiosis, are closely associated with chronic inflammation, a hallmark of CRC development. Let us clearly understand about gut-brain axis and its components before moving toward neuroimmune interactions related to CRC.

12.2 Gut-Brain Axis

The gut-brain axis is broadly defined by the compact interaction of the autonomic nervous system (ANS), central nervous system (CNS), and gastric microbiota (Fig. 12.2). The autonomic nervous system, through sympathetic and parasympathetic branches, provides for the connection between the CNS and the

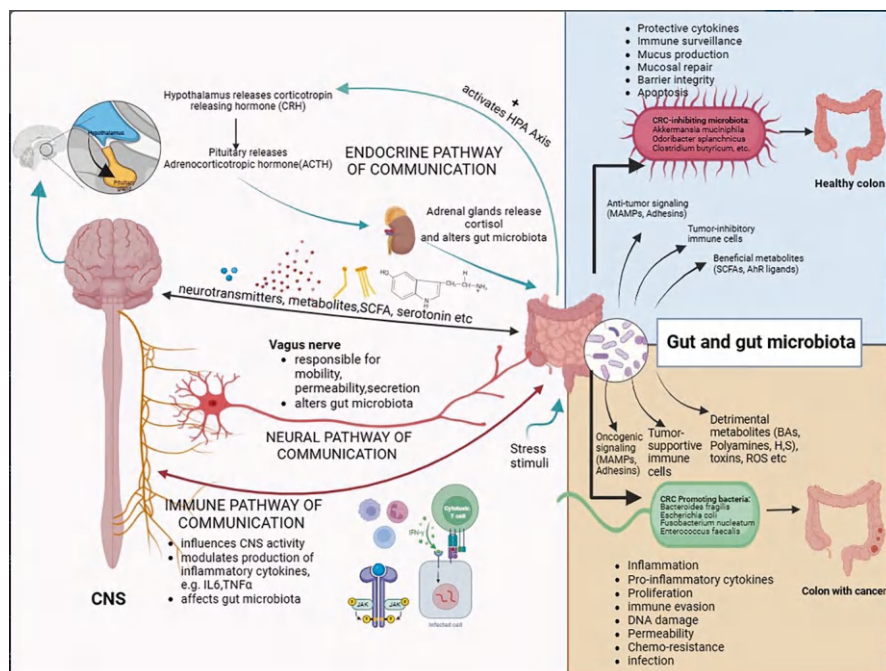


Fig. 12.2 Gut-brain axis: interaction of gut microbiota, autonomic nervous system, and central nervous system

gastrointestinal tract. As the body's main control center, the CNS, which is made up of the brain and spinal cord, regulates a wide range of physiological processes. It maintains general health, organizes voluntary and involuntary responses, and processes sensory information. The management of appetite and stress reactions by the brain can impact gut health and digestion, hence influencing gastrointestinal function [6]. The bidirectional communication in the gut-brain axis is primarily mediated through the vagus nerve of the enteric nervous system, while the HPA axis regulates stress response using vagus nerves and sensory neurons. The ENS, sometimes called the "second brain," is a massive network of neurons found in the gastrointestinal tract's walls. Much like the CNS, it regulates blood flow, secretion, and motility in the digestive system [7]. The ENS has autonomous control over gut function as evidenced by its ability to start peristalsis, the wave-like contractions that transport food through the digestive tract [8]. The vagus nerve, a part of the HPA axis, is a major connection for neural communication between the gut and brain. It transmits sensory information about gut status to the brain, influencing hunger, mood, and stress responses. The ENS also communicates with the CNS via spinal afferent neurons, providing feedback on gut function [9]. Activation of the vagus nerve has been shown to reduce inflammation and improve mood, highlighting its role in gut-brain communication [10].

The gut and brain are also mediated by hormones like cortisol, ghrelin, and leptin. Ghrelin is produced in the stomach and acts on the hypothalamus to stimulate appetite, while leptin is secreted by adipose tissue to suppress hunger and promote energy expenditure [11]. The release of cortisol in response to stress can alter gut motility and permeability, demonstrating the influence of hormonal signals on gut function [12]. Immune signaling is another aspect of the gut-brain axis; cytokines like tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) signal inflammation between the stomach and brain. According to [13], dysregulation of these immunological signals may be a factor in neuroinflammation and gastrointestinal problems. Persistent inflammation in the gastrointestinal tract can change the blood-brain barrier and may be a factor in neurological conditions [14].

Now the following questions arise: how does the gut interact with ENS, CNS, and HPA, how does the stimulation of the vagus nerve happen, and from where do neurotransmitters arrive in the gut? The gut is populated with a wide population of microbiota, which play a significant role in maintaining the epithelial barrier and synthesizing vitamins. However, gut microbiota also interacts with the brain by producing neuro-transmitters, e.g., serotonin, GABA neurotrophic factors, bacterial metabolites, or the modification of the intestinal barrier or directly by the modification of neuron survival. A signal sent by the gut using the vagus nerve is responded to by the ENS system, controlling the activity of the blood vessels and intestinal muscles. Diet regulates the composition of the gut microbes, which break down the components, producing short fatty acid chains (SCFA) that are required for the normal function of the CNS. A pathological change in the composition of the microbes of the gut can impact neurological health probably by altered production of neurotransmitters such as SCFA, dopamine, GABA, or serotonin and the overproduction of pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6.

Dysbiosis is reported to result in a “leaky gut” where the integrity of the intestinal epithelial layer is compromised. A leaky gut is characterized by the heightened release of neurotransmitters and toxins, which travel to the brain via the bloodstream and cause mood-related disorders such as anxiety and depression. Elevated levels of immunological regulators such as cytokines can result in neuroinflammation, hence contributing to diseases like Alzheimer’s, multiple sclerosis, and immune-related disorders such as inflammatory bowel disease.

Under stress conditions, the brain stimulates the vagus nerve to alter the gut function. The neurons in the GBA axis activate the HPA axis and also involve the interaction of the limbic system. The stimulated hypothalamus releases a hormone from the pituitary called adrenocorticotropin-releasing hormone (CRH), which further signals the synthesis and release of adrenocorticotrophic hormone (ACTH) [15]. As downstream signaling, the adrenal gland is stimulated to synthesize and release stress hormones such as cortisol, which cause the tight junctions in the intestine to leak and modify the permeability of the intestinal epithelia. As an immune response to the increased stress hormone, there can be an increased release of cytokines, which can cross the blood-brain barriers and, in turn, affect the overall balance of the gut microbiota.

12.3 Neuroimmune Interactions in the Gut-Brain Axis and Its Influence on CRC

The intricate relationship between the brain, gut, and immune system, often termed the gut-brain axis, has emerged as a focal point in understanding the pathogenesis of various diseases, including colorectal cancer (CRC). This complex network involves bidirectional communication, with the enteric nervous system (ENS) playing a pivotal role in modulating immune responses and gut microbiota composition.

We will delve into the complex interplay between these components, their influence on CRC, and the underlying mechanisms, specifically neurotransmitters and cytokines.

12.3.1 The Enteric Nervous System (ENS) and CRC

The ENS, also recognized as the “second brain,” is critical in governing gut motility and blood flow [16]. Below are the factors that suggest the ENS’ involvement and extent in CRC development.

12.3.1.1 Tumor microenvironment (TME) and its neural control

Cancer progression is directly dependent on TME, which includes various cell types, signaling molecules, and extracellular matrix components. ENS employs enteric neurons to release neurotransmitters and neuropeptides to modulate the TME by influencing cancer cells' growth, invasion, survival, and blood vessel development, either directly or indirectly

Neurotransmitters The microorganisms in the gut play a pivotal role in the release of a range of neurotransmitters. For example, some *Bacillus* species primarily secrete acetylcholine, dopamine, and norepinephrine, whereas some are known to secrete serotonin (Philip [17]). Additionally, there is a release of serotonin, GABA, and norepinephrine under the stimulus of gut microbiota and their secondary metabolites. These neurotransmitters further can directly reach the brain through the vascular system and impact the CNS in two possible ways, either through the blood-brain barrier (BBB) or through the intestinal epithelium.

Moreover, it has been shown that there are higher proportions of various neurotransmitters in the gut compared to the brain; it has also been found that substances like norepinephrine, dopamine, and serotonin can impact the proliferation and survival of cancer cells. For example, some studies have demonstrated that norepinephrine can stimulate cancer cell proliferation and confer resistance to apoptosis.

Epinephrine is said to promote carcinogenesis by upregulating the expression of matrix metalloproteinase (MMP-2, MMP-9) and vascular endothelial growth factor (VEGF) [18]. Acetylcholine mediates its role using the muscarinic acetylcholine receptor (mAChR) and nicotinic receptor (n-AChR) and promotes the proliferation of cancerous cells [19]. A high level of serotonin is observed in the case of colon carcinoma synthesized by serotonergic neurons of the gut mucosa and has implications for regulating the proliferation angiogenesis of cancerous cells [20].

Neuropeptides Molecules such as substance P, vasoactive intestinal peptide (VIP), and neuropeptide Y can affect various aspects of cancer progression. VIP, for instance, has been implicated in promoting cancer cell migration and invasion. Angiotensin II specifically indicated an antiapoptotic effect, promoting growth and invasion of colorectal cancer cell lines after treatment with angiotensin [21]. Endothelins promoted tumor growth through endothelin receptors (ETARs), as proved in vitro in SW480 and SW 620 cells [22].

Short-Chain Fatty Acids (SCFAs) Short-chain fatty acids (SCFAs) are a group of organic fatty acids characterized by their relatively small size, containing between two to six carbon atoms. These important compounds are produced as byproducts of the fermentation of indigestible polysaccharides, such as dietary fiber and resistant starch, that occur in the human gut, carried out by the diverse community of microorganisms known as the intestinal microbiota. The key SCFAs include acetate, propionate, and butyrate. Following their production, SCFAs require transporter proteins for absorption and can exert their effects by regulating genes associated

with multiple biological processes, including metabolism, differentiation, and proliferation. Furthermore, SCFAs are integral in maintaining the integrity of the intestinal epithelial barrier and are essential for supporting the innate tolerance of the intestinal immune system [23].

Notably, SCFAs possess the ability to cross the blood-brain barrier, where they can influence various functions of the central nervous system. Beyond this, research has also demonstrated that SCFAs have diverse impacts on host metabolism and the immune system. Their potential benefits extend to promoting gastrointestinal motility, modulating intestinal barrier function, and even ameliorating symptoms commonly associated with cognitive decline and depressive-like behavior. Given their multifaceted effects, SCFAs are increasingly recognized for their potential therapeutic applications in the prevention and treatment of various diseases [24].

Endotoxins The complex community of microorganisms residing in the gut, known as the gut microbiota, produces various by products, including endotoxins like lipopolysaccharides (LPS), derived from their cell walls. These endotoxins can exert their effects locally within the gut as well as in other areas, such as the gingiva and skin, leading to the dysregulation of gut-brain axis (GBA) signaling. Moreover, they can breach the intestinal barrier and enter the circulatory system, a phenomenon referred to as metabolic endotoxemia.

LPS is considered the innate immune sensing “alarm molecule” of the organism, which triggers an immune response in the host. Alhasson’s observations [25] in a mouse model of Gulf War disease revealed concomitant intestinal inflammation and neuroinflammation, suggesting that perturbations in the microbiota may contribute to increased intestinal permeability (leaky gut) and the occurrence of endotoxemia. These processes exacerbate neuroinflammation and activate Toll-like receptors, particularly TLR4, in both the small intestine and the brain.

TLR4, a crucial innate mammalian receptor integral to the immune system, plays a pivotal role in mediating LPS-induced inflammatory responses. Through its recognition of LPS and subsequent activation of the TLR4 signaling pathway, TLR4 facilitates the release of pro-inflammatory factors, thereby inducing inflammatory responses [26].

12.3.1.2 Neurogenic Inflammation

Neurogenic inflammation is a process by which the nervous system directly initiates an inflammatory response. The ENS can trigger neurogenic inflammation through the release of substances P and calcitonin gene-related peptide (CGRP), potentially promoting tumor growth and angiogenesis. Substance P interacts with receptors on cancer cells to enhance their growth and invasion while inducing pro-inflammatory cytokines. CGRP promotes angiogenesis and modulates immune responses within the tumor microenvironment (TME). Together, these peptides increase vascular permeability and recruit immune cells like mast cells, which release further inflammatory mediators. This pro-tumorigenic environment not only fuels tumor growth but also facilitates metastasis through enhanced angiogenesis and cell proliferation.

12.3.1.3 Neural Invasion

CRC is characterized by cells invading the nerves in the gut. The factors responsible are chemotactic factors, nerve growth factors, and other structural changes. Chemotactic factors such as glial cell-derived neurotrophic factor (GDNF) can attract CRC cells to nerves. These cells require growth factors for normal survival; hence, nerve growth factors produced by nerves support the growth of cancer cells e.g., nerve growth factors (NGF) and brain-derived neurotrophic factor (BDNF). Along with that, the growth of cancer cells in the nerves can also induce structural changes in the nerve, which provides for the spread of cancer locally as well as overall in the body.

12.4 Neuroimmune Cross Talk and CRC

In the current scenario, research is stressing on solving the complexities of CRC development by understanding the multiplex interaction of gut microbes and the host immune system. The gut houses a diversified plethora of microorganisms that control the host's health. The immune system is the primary player against all the foreign bodies entering the human body, and in turn, it is tightly interlinked with the gut microbiota. The strong interplay between the immune system and gut microbe controls tumor cell removal as well as the composition of the microbiota [27].

12.4.1 Innate Immune System and Signaling

Toll-like receptors (TLRs) on immune cells recognize microbial components and orchestrate innate immune responses. The microbiota generates a variety of molecules that can be recognized by TLRs, triggering downstream signaling pathways. One key pathway involves the activation of NF- κ B, a transcription factor that produces multiple cytokines crucial for immune responses. Several microorganisms, especially pathogenic bacteria, have been identified as capable of activating Toll-like receptors (TLRs) in the human body. *Bacteroides fragilis*, which produces polysaccharide A, segmented filamentous bacteria (SFB) that produce flagellin, *Yersinia enterocolitica*, *Salmonella enterica*, *Helicobacter hepaticus*, *Citrobacter rodentium*, and lipopolysaccharide (LPS)-producing *Serratia marcescens* and *Escherichia coli* are an example of such microorganisms. In a balanced state, TLR activation reinforces intestinal barrier integrity and promotes immune homeostasis by constant recognition of microbiota by TLR4 and TLR1/2, which could lead to cytokines (IL-6, IL-10, and TGF- β) production. Cytokine production is critical for the integrity of the intestinal epithelial cell barrier by promoting the expression of tight junction proteins (ZO-1, claudin-1, occludin) and maintaining their proliferation. However, dysregulation of TLR signaling can tip the scales toward chronic

inflammation, a hallmark of colorectal cancer (CRC) development. The regulation of TLR-induced NF- κ B signaling pathways involves specific negative regulators such as NLRX1, NLRC5, NLRP11, and LRRC25, which operate at different levels. These regulators play a critical role in maintaining the delicate equilibrium between bacterial composition, the mucosal immune system, and the integrity of the epithelial barrier. This delicate balance is essential for the body's overall health and the prevention of various diseases.

Pattern recognition receptors (PRRs) significantly help in detecting pathogens as they can identify and bind to conserved motifs, ubiquitously found on pathogens called pathogen-associated molecular patterns (PAMPs). PRRs can be categorized into three groups [28]:

- (a) Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are important in recognizing pathogen-derived cytosolic RNA.
- (b) Toll-like receptors (TLRs) are found in endosomes and can detect the genetic material in endosomes.
- (c) Cyclic GMP-AMP (cGAMP) synthase (cGAS) absent in melanoma 2 (AIM2) is one such another category.

Immune system counteracts invading pathogenic attacks by type -I interferon (IFN-1) signaling. Recently, some molecules were identified as the negative regulators controlling the IFN-I signaling, such as NLRC5, USP3, USP38, TRIM14, and LRRC25. These negative regulators offer potential benefits in maintaining gut mucosal system homeostasis during infections and regulating colitis and CRC development [29].

The microbiota regulates the innate immune system in various ways, such as releasing MAMPs like flagellin, EF-Tu, and LPS, which PRRs on innate immune cells can detect. Additionally, they can indirectly influence the innate immune system by producing metabolites or triggering production from colon epithelial cells. This has implications for colorectal cancer (CRC). *Prevotella intermedia* is associated with an increased risk of developing CRC (colorectal cancer) by evading innate immune control through disabling and killing tissue-infiltrating neutrophils in endodontic infection. *Lactobacillus rhamnosus* triggers anti-inflammatory effects in macrophages and suppresses TNF production through granulocyte-colony stimulating factor (G-CSF)-induced inhibition of c-Jun-N-terminal kinases (JNKs) [30]. *Bifidobacterium lactis* attenuates macrophage senescence and induces M2 macrophage polarization. Certain *Lactobacilli* and *Bifidobacteria* species can produce butyrate, which in turn controls the programming of macrophages for an anti-inflammatory phenotype. *Clostridium butyricum* triggers IL-10 production from intestinal macrophages via the TLR2/MyD88 signaling pathway and prevents mice from DSS-induced colitis. *Fusobacterium nucleatum* administration in mice triggers an increase in tumor-infiltrated immunosuppressive myeloid cells, including MDSCs, TAMs, TANs, and dendritic cells [30]. It can also act in a cytokine-independent manner and directly inhibit the cytotoxicity of NK cells against tumors. ETBF and *Peptostreptococcus anaerobius* are also reported to trigger the secretion of chemokines that recruit immunosuppressive MDSCs, TAMs, and TANs.

Additionally, *Clostridia* bacteria are reported to modulate the balance of retinoic acid and retinyl esters in intestinal epithelial cells, which further regulates the development of IL-22-producing ILC3s [30].

12.4.2 Adaptive Immune System

CD8 T cells unequivocally represent the most potent cytolytic population. Upon stimulation by CRC-derived modulators, such as IL-18, CD8 T cells generate pro-inflammatory cytokines, including IFN- γ , and cytotoxic molecules to eliminate cancer cells [31]. There exists a favorable correlation between tumor-infiltrating CD8 T cells and patients' prognosis and survival. Notably, the ratio of CD8 T cells to Treg cells is a critical determinant of prognostic outcomes. The microbial stimuli can also result in the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway. This pathway promotes the production of pro-inflammatory cytokines such as IL-6. Various CD4 T helper (Th) cells play unique roles in regulating CRC development. Cytotoxic Th1 cells are like cytolytic CD8 T cells in terms of functions and molecular production and are positively associated with prolonged survival of CRC patients. Regulatory T cells, also known as Treg cells, are responsible for regulating and suppressing the unwanted activation of the immune system. They play a crucial role in preventing autoimmune diseases. During the progression of colorectal cancer (CRC), Tregs significantly impact immune modulation by accumulating in the tumor microenvironment (TME) and suppressing immunity against tumor-specific factors, thereby promoting tumor progression. In colons of healthy individuals, the infiltration of Treg is low, which might be possible because, in a healthy state, Treg activity is not required, but at the early stages of CRC, there is a drastic hike in Treg infiltration. As colorectal cancer progresses to a metastatic stage, Treg infiltration decreases. Possible reasons can be the changes in the tumor microenvironment leading to a shift in immune dynamics; less Treg infiltration results in more aggressive immune responses against tumors. Further studies also suggest that the Tregs are five times more concentrated in the stromal (connective tissue) component of the tumor rather than in the epithelial (tumor cell) regions. The stroma plays a crucial role in supporting tumor growth, and a higher concentration of Tregs in this area might be contributing to the tumor's ability to suppress immune responses and promote cancer progression [30].

Th17 cells, a subtype of CD4+ T helper cells, are an integral part of maintaining gut homeostasis, present in intestinal lamina propria, and are activated by the gut's microbial flora, such as segmented filamentous bacteria (SFB). Within the immune system, Th17 cells are recognized for their pro-inflammatory role in autoimmune diseases and their defense against bacterial and fungal infections. However, it is worth noting that within the intestines, they also display immune-suppressive functions, showcasing their multifaceted and somewhat paradoxical role in different contexts. In CRC patients, an increase in Th17 cells is observed in tumor and peripheral blood than in healthy individuals. Th17 cells may exert their effects

through modified signaling pathways, such as STAT3, displaying distinct functions in the transition from healthy epithelial cells to cancerous cells. In the presence of IL-23 or serum amyloid A proteins, Th17 cells could develop a pathogenic pro-inflammatory behavior, in contrast to the non-pathogenic characteristics induced by IL-6 and TGF- β .

The microbial community that resides inside our gut microbiota controls adaptive immunity in various ways, notably the capability to differentiate T cells, Treg, and Th17 cells. *Bacteroides fragilis*, a bacterium inside the gut, is known for its immunomodulatory molecule, polysaccharide A (PSA), which converts CD4⁺ T cells into Foxp3⁺ Treg cells that produce IL-10, an anti-inflammatory cytokine. This process helps to maintain intestinal immune homeostasis and prevent inflammation. Species like *Akkermansia muciniphila*, *Helicobacter hepaticus*, *Odoribacter splanchnicus*, and others also play an active role in the activation of Treg cells. The microbiota can influence the ability of these cytotoxic T cells to recognize antigens and kill tumor cells as well. The gut microbiota has access to influence the inflammatory state of the colon, thus increasing the risk of CRC. The stimuli from bacteria can activate immune signaling pathways, leading to the loss of homeostasis and the creation of an inflammatory environment, leading to the activation of inflammasomes, multi-protein complexes having the potential to stimulate inflammation as a natural reaction to cellular distress or invading pathogens. The diversity in gut microbiota contains pro- and antitumor immune cells and various microbes, well acquainted with the environment created by multiple molecules secreted in the interplay of bidirectional pathways. This elucidates the reason behind the decrease in bacterial genera such as *Clostridium* and *Bacteroides*. These bacteria are unable to compete with the rapidly multiplying tumor cells and immune cells, which release inflammatory compounds that are toxic to the bacteria.

12.4.3 CRC-Related Neuroinflammation

In the context of CRC, inflammation is deeply involved in colorectal cancer (CRC) progression. The systemic inflammatory state associated with CRC affects many organs, including the cerebral part of the brain, and this condition is referred to as neuroinflammation. The nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signal pathway is one of the most important pathways mediating inflammation-induced CRC progression. After activation, NF- κ B translocates to the nucleus, stimulating the transcription and thus the secretion of pro-inflammatory cytokines, chemokines, and adhesion molecules, such as TNF- α , IL-6, IL-1 β , and COX-2. The increased expression of various pro-inflammatory mediators creates a pro-tumorigenic environment by initiating cell proliferation and angiogenesis and preventing apoptosis. Moreover, NF- κ B dependent inflammation eventually supports CRC progression by strengthening the viability of malignant cells' lifespan, promoting the epithelial-to-mesenchymal transition (EMT) process, hence metastasis. The Janus kinase/signal transducer and activator of transcription (JAK/STAT)

pathway is another pathway that also serves in CRC-related inflammation. The JAK/STAT signaling cascade gets activated by the involvement of cytokines (like IL-6, IL-10, and interferons) and subsequently promotes the transcription of genes engaged in processes like cell survival, proliferation, and immune evasion [32].

The expression of cyclooxygenase-2 (COX-2) induced by these inflammatory mediators further results in prostaglandin production, particularly PGE2. This enhances CRC progression by stimulating angiogenesis, cell proliferation, and invasion while inhibiting apoptosis. PGE2 shows some immunosuppressive effects as it regulates immune responses by suppressing natural killer (NK) cells and dendritic cells, thus assisting in immune evasion by tumor cells. The interaction between inflammation and CRC is not restricted to the primary site [33]. Pro-inflammatory cytokines released by the inflamed colon can affect the cerebral part either by crossing the blood-brain barrier or signaling via the vagus nerve. These cytokines activate the resident immune cells of the central nervous system, the microglia, thus releasing more pro-inflammatory cytokines, reactive oxygen species (ROS), and nitric oxide (NO), creating a neuroinflammatory environment. This neuroinflammation can result in synaptic dysfunction, neuronal damage, and cognitive impairment.

Furthermore, inflammation upregulates the expression of indoleamine 2,3-dioxygenase (IDO) in both peripheral and central tissues, causing the tryptophan breakdown into products kynurenine and other metabolites. Some metabolites of the kynurenine pathway, such as quinolinic acid, possess neurotoxicity and can contribute to neurodegeneration and cognitive dysfunction by affecting the mechanism of excitotoxicity and impacting cellular redox potential.

Furthermore, chronic inflammation in CRC can also disrupt the hypothalamic-pituitary-adrenal (HPA) axis functions with the increase in the production of corticotropin-releasing hormone (CRH) in the hypothalamus, resulting in elevated cortisol levels. High cortisol levels are toxic to the hippocampus, causing memory loss and mood changes as far as depression is concerned. Chronic cortisol exposure can exacerbate neuroinflammation, creating a feedback loop that further impairs cognitive function. The inflammation in CRC results in the generation of reactive oxygen species (ROS) and nitric oxide (NO), which can cause oxidative damage to DNA, proteins, and lipids as these molecules can pass through the central nervous system, contributing to neuroinflammation and neuronal damage. Furthermore, ROS can activate several signaling pathways, including NF- κ B, MAPKs (mitogen-activated protein kinases), and p53, enhancing inflammation and neuroinflammation and acting as a catalyst to cognitive decline as well as tumorigenesis [34].

The role of gut microbiota in this intricate relationship between inflammation and CRC should have a presidential role, as always. The reason behind this could be the gut microbiota produces products such as short-chain fatty acids (SCFAs) like butyrate, which show some anti-inflammatory effects in the gut. However, dysbiosis alters the production of SCFAs, causing a loss in their beneficial effects, such as the protective effects and contributing to inflammation. In addition to this, a number of gut bacteria synthesize neurotransmitters, like serotonin and gamma-aminobutyric acid (GABA), that affect brain operations. Dysbiosis in CRC can disrupt this production, attributing to neuropsychiatric symptoms [35]. To summarize, the complex

interactions between inflammation and CRC that operate beyond the colon to affect the brain and the rest of the nervous system can lead to neuroinflammation and a range of cognitive and psychological symptoms suffered by many CRC patients [36].

12.5 Cognitive and Psychological Effects of CRC

The intersection between inflammation, neuroinflammation, and colorectal cancer (CRC) is an area of emerging interest in exploring the way the body's immune response aids both the progression of cancer and the cognitive and psychological effects patients' experience. The cognitive and psychological effects of CRC comprise cognitive decline, psychological distress, and challenges faced in everyday life. Various other challenges contribute to this domain of CRC, including stress and trauma related to treatment processes such as chemotherapy and radiotherapy.

From the observations obtained from CRC patients in research, the key findings indicate that in CRC patients, cognitive impairments can be mild to severe and include: a. Mild to severe memory dysfunction: most patients experience short-term memory loss, b. Attention deficits: patient's ability to focus or concentrate was impaired; c. Executive function deficits: patients had problems with planning and organizing tasks and activities, and communication deficits: patients' verbal and written expression was impaired. Similarly, recent research indicates that CRC is responsible for affecting the psychological well-being and mental health state of patients, which may result in anxiety, psychological distress, and depression. The psychological impact of CRC often manifests as a decrease in the overall quality of life (QoL) [37]. Earlier, it was supposed that illness leads to psychological problems through a unidirectional effect on patients' well-being and quality of life. Still, surprisingly, the bidirectional communication network interplay between the gut-brain axis directly impacts the cognitive and psychological effects of CRC patients, including systemic and local inflammatory activities. The cognitive and psychological states can be influenced by different neuro-endocrine pathways with mechanisms including the release of neurotransmitters, inflammation, and microbiome composition [38].

The intricate web of inflammation in colorectal cancer (CRC) patients involves the upregulation of inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β), along with the presence of mediators like PGE2. These molecular signals are well known to be implicated in neuroinflammation, and prolonged neuroinflammation is further linked with implications like neurodegenerative processes, cognitive decline, and mood swings [39].

Furthermore, CRC comes with significant changes in the diverse composition of gut microbiota, which in the case of CRC brings changes in bioactive compounds secretion as short-chain fatty acids (SCFAs) and neurotransmitters like serotonin, so disturbances arise in gut-brain talk that will result in cognitive and psychological symptoms. On top of that, this chronic inflammatory elevated state disturbs the normal functioning of the HPA axis and causes our body's stress response to be

dysregulated. The dysregulation of these proteins significantly increases the severity of anxiety, depression, and cognitive deficits, thereby complicating the clinical perspective in CRC patients. The cognitive and psychopathological changes related to CRC can comprise cognition dysfunction as well as raise anxiety and depressive disorders depending on the degree of chronic neuroinflammation.

12.6 Therapeutics

After understanding the processes involved in the initiation, progression, and stimulation of CRC, let us take a look at therapies and treatment options available for the same. As previously stated, the inflammatory conditions in the colon are greatly influenced by the components of the gut microbiota. Dysbiosis, a negative imbalance in the gut, can cause increased intestinal permeability, which allows the bacterial products to enter into circulation and cause systemic inflammation. This inflammatory environment accelerates the development of tumors and promotes colorectal cancer. By altering the gut microbiota, probiotics and prebiotics have demonstrated the capability to be used in the management of colorectal cancer (CRC), also being crucial for preserving gut health. In addition to reducing pathogen proliferation and enhancing intestinal barrier integrity, these advantageous microorganisms and the dietary fibers that support their growth possess the essential factors needed to support the immune system, prevent metastasis, and control inflammation in patients with colorectal cancer [40]. Psychobiotics, a specific type of probiotics, have recently been recognized to be able to influence outcomes related to the central nervous system (CNS) via their action on the GBA. In addition to their direct impacts on mood and cognition, they can also create neurotransmitters like serotonin and GABA, which may have an impact on the systemic and gut-specific immune responses that are crucial for the advancement of cancer [41].

Taking a look at **probiotics**, which are live bacteria that, when given in sufficient quantities, offer various health advantages to the individual. As a result, they have gained attention as possible colorectal cancer (CRC) treatment agents. These good bacteria live in the gastrointestinal tract and provide a variety of health-promoting benefits. They mainly belong to the genera *Bifidobacterium* and *Lactobacillus*. Probiotics can influence and stimulate both adaptive and innate immune responses. *Lactobacilli* and *Bifidobacteria* enhance natural killer cell activity, increase cytokine production (e.g., IL-12, IFN- γ), and promote dendritic cell maturation, leading to more effective antigen presentation and T cell activation. They also produce short-chain fatty acids (SCFAs) and inhibit pro-inflammatory cytokines, which can significantly reduce chronic inflammation, a key driver of CRC [42, 43]. For instance, *Bifidobacterium longum* and *Lactobacillus acidophilus* have demonstrated anti-inflammatory properties in preclinical models [44]. Along with this, some probiotic strains, such as *Lactobacillus casei* and *Bifidobacterium animalis subsp. lactis*, have demonstrated positive abilities to reduce apoptotic behavior of the cells, inhibit cell proliferation, and reduce angiogenesis in CRC cell lines.

Prebiotic fibers enhanced the number of good gut bacteria like *Faecalibacterium prausnitzii*, which are linked to decreased inflammation and tumor growth in CRC patients, according to a randomized controlled trial by Dikeocha et al. [45]. In a study conducted on the probiotics on symptoms and surgical outcome after anterior resection of colon cancer (POSTCARE) patients, the group receiving the treatment in the form of probiotics experienced a decrease in bacteria linked to colorectal cancer (CRC), including *Prevotella*, *Alloprevotella*, *Fusobacterium*, and *Porphyromonas*, and an increase in the abundance of *Akkermansia*, *Bifidobacterium*, *Parabacteroides*, *Erysipelatoclostridium* and *Lactobacillus* [46]. In a similar study conducted earlier by [47], the diversity of the tumor microbiota was observed to be higher, and *Fusobacterium*, *Selenomonas*, and *Peptostreptococcus* were more abundant. Probiotic-treated colon cancer patients had a decrease in *Peptostreptococcus* and *Fusobacterium* and an increase in butyrate-producing bacteria such as *Clostridiales* and *Faecalibacterium* in their fecal microbiota. According to a different study, patients supplemented with probiotics, including six strains of live *Lactobacillus* and *Bifidobacteria* 4 weeks after surgery for colorectal cancer, exhibited lower levels of pro-inflammatory cytokines (IFN-gamma excepted). This gives encouraging results for the use of probiotics to alter the ecology of the gut, which would eventually reduce the harmful levels of pro-inflammatory cytokines [48].

Along similar lines, **Prebiotics** are those fiber-based food elements that aid the development and activity of useful bacteria in the colon. Food compounds classified as prebiotics come in a wide variety. The prebiotic properties of several dietary fibers and carbohydrates are the most reiterated among them. Dietary fibers can be fermented by colonic microbiota bacteria, but they essentially cannot be hydrolyzed in the small intestine by human digestive enzymes. Dietary fibers include a wide variety of carbohydrates. These include resistant starch, inulin, oligosaccharides including galactooligosaccharides, fructooligosaccharides, and xylooligosaccharides, and non-starch polysaccharides such as celluloses, hemicelluloses, gums, pectins, and mucilages. They may help to prevent and treat colorectal cancer (CRC) while also indirectly supporting gut health. Prebiotics help probiotic bacteria colonize and function more metabolically by giving them a substrate [49]. High dietary fiber consumption was also seen to lower the risk of colorectal cancer during a study. This protective effect, according to the researchers in the study conducted by [50], is caused by the fermentation of fiber into butyrate and other similar short-chain fatty acids (SCFAs), which has been demonstrated to have anti-carcinogenic qualities in the colon. Easy-to-ferment dietary fibers, such as inulin, have been shown in another study conducted on mice to enhance the generation of butyrate in the colon [51]. These elevated levels of butyrate have been linked to the prevention of cancer cell proliferation and the induction of apoptosis, indicating that certain dietary fiber types may offer significant protective value against colorectal cancer [52]. Similar studies show that higher consumption of fiber has been linked to a more stable and diversified gut flora [53]. Additionally, people with high-fiber diets had a decreased incidence of precancerous polyps, indicating that consuming dietary fiber over time may help to prevent the progression of colorectal cancer [54]. These results lend credibility to the theory that prebiotics in food not only promote a more favorable gut

flora but may also directly lower the risk of colorectal cancer. With further investigation, including particular prebiotic fibers in regular meals may prove to be an essential part of CRC prevention methods. In relation to psychobiotics, a study conducted by [55], showed that consumption of *L. casei* inhibited the growth of colorectal cancers, specifically inhibiting the development of tumors with varying levels of atypia, at least statistically significant, which was probably due to the supplementation's ability to modulate the gut-brain axis via inflammatory pathways.

The function of the gut microbiota in immunotherapy is becoming more widely acknowledged. According to a review by Griffin and Hang [56], changing the immunological conditions within the tumor microenvironment by adding particular bacterial strains to the gut microbiota may have a positive impact on checkpoint inhibitors. Furthermore, dietary interventions aimed at modulating the neuroimmune system have been investigated. Fitzgerald et al.'s clinical trial from 2022 showed that a ketogenic diet changed the composition of the gut microbiota, which decreased neuroinflammation and improved outcomes for CRC patients receiving immunotherapy [57]. The molecular features of the tumor, including mutations in HER2, BRAF, NRAS, KRAS, and other genes, are critical to the success rate of targeted therapy. These mutations can be found via molecular profiling, which opens up the possibility for further individualized and successful treatment plans [58]. Targeted therapy aims to focus on the vascular endothelial growth factor pathway (VEGF) and the epidermal growth factor receptor (EGFR) pathway [59]. EGFR emerged as a crucial target as about 60–80% showed overexpression of this. EGFR-inhibiting medications such as panitumumab and cetuximab, both being monoclonal antibodies, have been shown to be effective, especially in individuals with malignancies that are KRAS wild-type [60]. Cetuximab binds competitively to EGFR and its ligands, including epidermal growth factor (EGF). When combined with chemotherapy, cetuximab decreased the chance of progression, as demonstrated by the CRYSTAL study [61]. Similarly, panitumumab, being a fully humanized medication, reduced the risk of allergic reactions, especially when combined with chemotherapy [62].

The position of the tumor (left-versus right-sided colon cancers) affects how effective these medications are, whereas left-sided cancers have responded better to EGFR inhibitors. Left-sided tumors mostly exhibit APC, PIK3CA, KRAS, and p53 mutations, but right-sided tumors are more likely to have defects in DNA mismatch repair. Early-stage tumors on the right side are linked to a better overall prognosis, but late-stage cancers on the left side are linked to a better overall prognosis [63]. VEGF aids the process of blood vessel development, which aids the growth and metastasis of the tumor. Because VEGF inhibitors like bevacizumab prevent angiogenesis by altering receptor-binding, which is essential for tumor growth, they have been regarded as important in the treatment of metastatic colorectal cancer, as demonstrated by the PRODIGE 9 trial [64]. Afibercept and ramucirumab, two more recently developed medicines in combination with second-line FOLFIRI (fluorouracil, leucovorin, and irinotecan) that target the VEGF pathway, have shown variable degrees of competency in clinical trials. These more recent sets of medications are potentially reserved for patients showing failure in different sets of treatment [65, 66]. Various therapies are summarized in the table below (Table 12.1). However,

Table 12.1 Summary of immunotherapies currently in use for CRC

Targeted therapy	Pathway	Mechanism of action	References
Cetuximab	EGFR inhibitor	Binds to the extracellular sites of EGFR, blocking ligand-induced dimerization, and prevents signaling	Van Custem et al. [61]
Panitumumab	EGFR inhibitor	Similar to cetuximab, it alters EGFR activation	Amado et al. [62]
Bevacizumab	VEGF inhibitor	Binds to VEGF and inhibits its binding with VEGF receptors on the surface of endothelial cells, blocking angiogenesis	Aparicio et al. [64]
Ramucirumab	VEGF inhibitor	Targets VEGF2 receptor, directly inhibiting angiogenesis	Ottaiano et al. [65]
Aflibercept	VEGF inhibitor	Acts as a VEGF trap by binding VEGF ligands, preventing them from binding to endogenous receptors and inhibiting angiogenesis	Ottaiano et al. [65]
Fruquintinib	VEGF inhibitor	A kinase inhibitor, targeting VEGF receptors to prevent angiogenesis	Dasari et al. [67]
Encorafenib	BRAF inhibitor	Directly inhibits the BRAF protein, a part of the signaling pathway involved in cell growth and division	Kopetz et al. [68]
Trastuzumab	HER2 inhibitor	Targets the HER2/neu receptor, interfering with HER2 signal pathways involved in cell growth and differentiation	Sartore-Bianchi et al. [69]
Pertuzumab	HER2 inhibitor	Binds to a different epitope of the HER2 receptor than trastuzumab, preventing dimerization of HER2 with other HER receptors	Meric-Bernstam et al. [70]
Tucatinib	HER2 inhibitor	A tyrosine kinase inhibitor that selectively targets HER2	Kulukian et al. [71]
Larotrectinib	NTRK inhibitor	Targets NTRK fusions, inhibiting the TRK proteins that are activated in some cancers	Hong et al. [72]
Entrectinib	NTRK inhibitor	Inhibits TRK, ROS1, and ALK, targeting proteins involved in the survival and growth of tumor cells	Doebele et al. [73]
Regorafenib	Multi-kinase inhibitor	Inhibits multiple membrane-bound and intracellular kinases involved in normal cellular functions and in pathologic processes such as oncogenesis and tumor angiogenesis	van Custem et al. [61]
Nivolumab	PD-1 inhibitor	Blocks the PD-1 protein on immune cells, helping them to attack cancer cells more efficiently	Lenz et al. [74]
Pembrolizumab	PD-1 inhibitor	Similar to nivolumab, it targets PD-1 to prevent cancer cells from evading immune system detection	Diaz et al. [75]
Ipilimumab	CTLA-4 inhibitor	Blocks CTLA-4 on T cells to exhibit improved immune response against cancer cells	Lenz et al. [74]

each of the above-mentioned treatments includes common side effects such as hypertension, allergic reactions, thromboembolic events, etc.

Now, about 15% of colorectal tumors have also been observed to be caused by Microsatellite instability (MSI). Oncogenesis results from poor DNA mismatch repair in these cancers. Although they react differently to conventional chemotherapeutic regimens, tumors with MSI typically have a better prognosis. As immune checkpoint inhibitors (ICI), a number of immunotherapies have been used to enhance the antineoplastic immune response. Immune checkpoint inhibitors are mAbs that prevent the binding of IC proteins expressed by T cells and tumor cells, thus allowing the immune system to get rid of the cancer cells [76]. The core of ICI treatment is T cell reactivation, and the way in which T cells react to ICI blocking greatly influences the course of treatment. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), Programmed cell death–ligand 1 (PD-L1), and programmed cell death protein 1 (PD-1) are the main targets of these inhibitors [77]. Based on the KEYNOTE-177 study, pembrolizumab has been approved as first-line therapy for MSI-high mCRC, demonstrating significant survival improvements over chemotherapy [75]. For the same, nivolumab combined with low-dose ipilimumab as a dual checkpoint inhibitor showed long-lasting clinical benefits and was well tolerated as a first-line treatment [74]. These encouraging results call for randomized investigations. However, the majority of metastatic CRC cases are microsatellite stable (MSS) cancers, for which immunotherapy is less effective. The goal of increasing these tumors' immunogenicity is still being researched. Contrastingly, a few research studies suggest that ICIs also boost the tumoricidal activity of the host's innate immune system. Therefore, more in-depth study is required in this domain to better understand the pathways and molecules involved.

12.7 Current Research and Future Directions

Even with these developments in recent years, a lot of patients still run out of effective treatment choices. Since many CRC patients do not currently have detectable mutations or do not show effective results on current therapeutics due to toxicity and non-selectivity of the regime, they are not candidates for the targeted gut and immunotherapies. Emerging technologies like nanotechnology, drug delivery systems utilizing microorganisms, and cell modification for immunotherapy offer potential treatment options for patients who are not responding well to established treatment approaches. Large-scale randomized clinical trials may soon be conducted using the following strategies.

Currently, the phenomenon of fecal microbiota transplantation (FMT) is under great research. Various pre-clinical and clinical trials have shown promising results for the same. FMT is the process by which a donor's feces are administered into a recipient's digestive tract, aiming to directly alter the recipient's gut microbial environment and thus provide significant health benefits. According to [78], upon conducting a metagenomic investigation of the fecal matter of the mice under trial,

FMT notably altered the composition of the gut microbiota in tumor-bearing mice following anti-PD-1 therapy. In particular, the *Bacteroides* genus—which includes *B. fragilis*, FMT-decreased *B. ovatus*, and FMT-increased *B. thetaiotaomicron*—might be a reason for the improved effectiveness of anti-PD-1 therapy. Additionally, a metabolomic study performed on mouse plasma identified a number of probable metabolites that were elevated following FMT, such as aspirin and punicic acid, which may enhance the immune response to anti-PD-1 therapy. The current ongoing phase II trial investigates the efficacy of FMT and resuming anti-PD-1 therapy (nivolumab or pembrolizumab) in the treatment of metastatic colorectal cancer in patients who are non-responders to anti-PD-1 therapy. Monoclonal antibodies used in immunotherapy, such as pembrolizumab and nivolumab, may boost the body's defense against cancer and impede the growth and metastasis of tumor cells. Combining fecal microbiota transplants with pembrolizumab or nivolumab may aid in disease management, as stated by the study (NCT04729322).

Another promising avenue is that of adoptive cell therapy (ACT). Cancer patients undergoing ACT therapy receive cytotoxic cells specific to their tumors as a passive immunotherapy technique. CTLs and NK cells are the main effector cells utilized in the ACT method [79]. The process of chimeric antigen receptor (CAR) T cell therapy is a type of ACT. In this, the patient's T cells are extracted and subjected to genetic modification to produce CARs, anticancer receptors. Then, through the procedure of ACT, the patient receives the modified T cells again. The bloodstream is circulated by the modified T cells, which efficiently target the cancer cells that express antigens [80]. CAR T has shown significant success in pre-clinical and phase II clinical trials, especially in the ones involving hematological malignancies [81, 82]. However, while treating solid tumors, various challenges were seen due to a lack of suitable antigens, poor accessibility of these antigens due to the complex tumor microenvironment, and secretion of immunosuppressive factors. The effectiveness of CAR T cell therapy depends on the identification of certain tumor antigens. In preclinical research for colorectal cancer, a number of tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs) are being targeted, such as epithelial cell adhesion molecule (EpCAM). This has shown overexpression in about 90% of CRC cells, linking it to a poor prognosis [83]. Carcinoembryonic antigen (CEA) is a major target for CAR T cell therapy, which is again widely overexpressed in colorectal cancer (CRC) and other malignancies (NCT02349724). Another important target is the epidermal growth factor receptor (EGFR) (NCT03152435) and heat shock protein 70 (Hsp70), whose overexpression in colorectal cancer and absence in normal tissues led to its identification as a prospective target [84]. Other newly discovered targets for CAR T cell therapy in colorectal cancer (CRC) include guanylyl cyclase C (GUCY2C), a receptor found in most CRC metastases that has shown encouraging preclinical outcomes without endangering healthy tissues [85]. A receptor implicated in tumor immunosurveillance, natural killer Group 2 member D (NKG2D), has the potential to be a target for CAR T cell treatment [86]. DCLK1, or double cortin-like kinase 1, is a hallmark of cancer stem cells in colorectal cancer (CRC). In preclinical models, CAR T cells that target DCLK1 have demonstrated success. Subsequent studies ought to concentrate on

navigating the obstacles linked to CAR T cell therapy for solid malignancies [87]. Enhancing CAR T cell infiltration and persistence in the tumor microenvironment, identifying tumor-specific antigens more accurately, and formulating plans to reduce on-target, off-tumor.

As we mentioned, the receptor GUCY2C, which is expressed in the majority of metastatic CRC tumors, the successful production and characterization of a nanocarrier that can identify metastatic colorectal cancer cells in tertiary organs was reported in a study carried out in 2021. The field of cancer therapy has seen significant change owing to nanotechnology. In addition to improving tumor-targeting accumulation and reducing systemic exposure, nanoscale drug delivery systems also avoid poor solubility and susceptibility to adverse chemical as well as biological conditions, leading to a significantly improved therapeutic index. Also, nanocarriers can break through several biological barriers, including poor mucus and tumor penetration, as well as quick blood clearance, thus considerably improving the pharmacological and pharmacokinetic profiles. Lastly, bioactive and multifunctional nanocarriers are now developing to enable imaging, tumor-microenvironment manipulation, and intrinsic anticancer activity, further improving therapeutic outcomes. These nanocarriers differ from conventional biocompatible carriers [88]. CRC cells express the membrane protein GUCY2C, which is produced by the intestine's enterocytes. In a mouse xenograft model, injection of a nanocarrier that encapsulates the topoisomerase II inhibitor (chemotherapeutic medication etoposide) into the tumor site. This injection suppressed tumor development with high specificity due to the lack of expression of the receptors in other tissues, such as the liver and lung. Similarly, by employing hollow mesoporous copper sulfide with titanium dioxide coating, a novel self-thermophoretic nanoparticle system (CTPB) improves drug delivery efficiency in colorectal cancer (CRC). These nanoparticles that are covered with *Staphylococcus aureus* membranes target intestine segments afflicted by colorectal cancer (CRC) and, increase mucus penetration by a factor of 2.7 and decrease bacterial interception by a factor of 3.5 [89]. This study exhibited a 99.4% decrease in tumor rates, thus offering a new avenue for CRC therapy. In another study conducted by [90], oxaliplatin was administered using apoferritin (AF), a naturally occurring iron carrier, which was modified on the surface to bind panitumumab and form AFPO nanocages. These nanocages exhibit notable accumulation in EGFR-rich tumors in vivo, efficiently release oxaliplatin, and selectively target EGFR-expressing cells to inhibit tumor growth. This proved to be a promising method for clinical cancer therapy.

Another set of cells, enteric glial cells (EGCs), priorly seen to be supportive in the ENS, are now believed to be an active participant in various diseases and cancers. A study conducted by Van Baarle et al. [91] in in vitro and mouse models showed the tumorigenic role of these cells. The findings show that EGCs have an impact on how the CRC tumor's microenvironment is shaped. In particular, they encourage tumor-infiltrating monocytes to differentiate into pro-tumorigenic SPP1+ TAMs, which are linked to a poor prognosis in colorectal cancer. Osteopontin, or

SPP1, is expressed by SPP1+ TAMs, a subset of macrophages that help support tumor growth by modifying the extracellular matrix and encouraging angiogenesis. According to the findings, IL-1R signaling is a critical route that allows EGCs to change phenotypically to an immunomodulatory state inside the TME. IL-1 cytokines (IL-1 α and IL-1 β) released by monocytes and monocyte-derived macrophages within the microenvironment are responsible for inducing this transition. In response, activated EGCs release IL-6, which encourages monocyte differentiation into SPP1 + TAMs and so strengthens the pro-tumorigenic surroundings [91]. This shows the potential role of ILs as potential biomarkers as well as therapeutic agents.

Advances in cancer immunotherapy have taken a leap in recent years, focusing on anticancer vaccinations. Adjuvants are administered directly to the tumor and tumor-draining lymph nodes (TDLNs) during vaccination in order to elicit local immune responses. This directly activates resident dendritic cells (DCs) and enhances their migration from the tumor to TDLNs, thus strengthening the antitumor immune response [92]. In a study, antigenic peptides and photosensitive melanin were delivered via a nanovaccine consisting of DC hybrid zinc phosphate nanoparticles to a mouse model with MC38 tumors. This led to efficient DC maturation, the growth of CD8+ and CD4+ T cells, and substantial antitumor effects. Furthermore, the combination of tumor vaccines and immune checkpoint inhibitors (ICIs)—such as T cell-targeted exosomes (EXO-OVA-mAb), due to increased binding to T cells in TDLNs, modified with costimulatory molecules and anti-CTLA-4 antibodies raise the ratio of effector T cells to regulatory T cells and in turn inhibit tumor growth [93]. Along with TDLNs recently reported to have potential as biomarkers for diagnostic testing, they can also be used as therapeutic agents. In spite of the dMMR/MSI-H status, patients with colorectal cancer (CRC), having increased levels of CD8+ tumor-infiltrating lymphocytes and CD169+ macrophages in their regional lymph nodes (LNs) have better prognoses [94]. This demonstrates highly useful prognostic markers that the immune cell infiltration status of LNs can be used to differentiate the antitumor group from dMMR CRC. After local delivery of ICIs, TDLNs can be detected at high concentrations and show equally elevated drug bioavailability, which activates effector and memory T cells and provides protection against the chances of recurrence of CRC alongside lowering toxicity [95]. To investigate the usefulness of TDLNs for immunotherapy, the efficiency needs to be predicted based on molecular pathological diagnosis, additional clinical research is necessary.

12.8 Conclusion

After taking a closer look at how the gut-brain axis (GBA) relates to colorectal cancer (CRC), it becomes clear that there is a complicated relationship at play. On one hand, this connection involves how the immune system, the nervous system, and the

gut microbiome all influence each other, impacting how cancer develops and a person's overall health. On the other hand, this relationship is not just one-way; it is a two-way street where signals between the gut and the brain—via things like neurotransmitters and cytokines—can affect not only how a tumor behaves but also how inflammation spreads in the body, ultimately affecting the disease development and the patient's well-being. In terms of treatment, one promising approach is to focus on balancing the gut microbiome. Supplements involving probiotics, prebiotics, and psychobiotics are showing the potential to restore balance in the gut, which could even help to slow down tumor growth. At the same time, newer therapies are also in the spotlight. For instance, CAR T cell therapy and nanotechnology are emerging as targeted treatments that could offer more precision in tackling CRC. As we look towards future research, there is still a lot to figure out—especially when it comes to understanding the exact ways the GBA affects CRC, particularly how the immune system and microbial by products come into play. But by incorporating these innovative treatments with the conventional ones we already use, there is hope that healthcare providers could create more tailored and effective plans for CRC patients, potentially leading to better results and a higher quality of life.

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Chapter 13

Gut Microbiota, Postbiotics, and Personalized Medicine in CRC



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Abstract The interplay between gut microbiota, postbiotics, and personalized medicine presents a transformative approach to colorectal cancer (CRC) management. Research demonstrates that the gut microbiota, a diverse community of microorganisms, plays a significant role in regulating immune responses, maintaining intestinal integrity, and influencing CRC progression. Dysbiosis, an imbalance in microbial populations, promotes chronic inflammation, oxidative stress, and tumour development. Postbiotics, the bioactive metabolites or components derived from probiotics, offer promising therapeutic benefits. Their anti-inflammatory, anti-oxidant, and immunomodulatory properties reduce CRC risks while ensuring safety and stability compared to live probiotics. Personalized medicine, which tailors treatments based on genetic and microbial profiles, is enhancing CRC treatment strategies. Microbiome-targeted therapies, such as ‘fecal microbiota transplantation (FMT)’ and synthetic probiotics, have shown potential to improve patient outcomes. However, the clinical application of microbiome-based therapies and postbiotics faces challenges, including treatment variability, complex regulatory frameworks, and the need for long-term studies. Ethical concerns related to data privacy, informed consent, and equitable access to these novel therapies further complicate their integration into healthcare systems. Looking forward, emerging trends in artificial intelligence and machine learning are advancing predictive models to optimize CRC treatment responses. Advances in personalized microbiome therapeutics and the integration of genetic, microbial, and environmental data are expected to refine treatment strategies, improving precision and outcomes for CRC patients. The seamless combination of these elements marks a paradigm shift in CRC care, focusing on prevention, individualized therapies, and enhanced quality of life for patients.

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13.1 Overview

13.1.1 CRC Overview

Colorectal cancer is a common disease, ranking third in incidence and second in death worldwide [10]. The onset of colorectal cancer is affected by hereditary factors, dietary practices, and persistent inflammatory conditions. A recent study highlights the influence of gut microbiota on the start and progression of colorectal cancer by disturbing intestinal homeostasis and promoting chronic inflammation [44].

13.1.2 Significance of Gut Microbiota in Colorectal Cancer Development

The gut microbiota, a complex community of bacteria, is essential for preserving intestinal integrity and regulating the immune system [19]. Dysbiosis, defined as an imbalance between pathogenic and commensal bacteria, has been associated with the development of ‘colorectal cancer (CRC)’. This imbalance may result in the generation of deleterious metabolites, persistent inflammation, and DNA damage, all of which facilitate carcinogenesis [74]. Certain bacterial species, including ‘*Fusobacterium nucleatum*’, have shown the ability to enhance tumour proliferation and diminish the effectiveness of immunotherapy [88]. In contrast, advantageous metabolites generated by gut bacteria, such as ‘short-chain fatty acids (SCFA)’, have anti-inflammatory effects, hence decreasing the risk of colorectal cancer [44].

13.1.3 Emerging Role of Postbiotics

Postbiotics, defined as metabolites or components produced from probiotics, have garnered considerable interest in colorectal cancer research. In contrast to probiotics, which include live bacteria, postbiotics are non-living microbial preparations that provide comparable health advantages without the potential for uncontrolled bacterial proliferation [93]. Bioactive substances, including SCFAs, bacterial cell wall components, and enzymes, have anti-cancer, anti-inflammatory, and immunomodulatory properties [102]. Postbiotics are believed to enhance gut barrier integrity, diminish systemic inflammation, and provide a safer treatment strategy for controlling colorectal cancer [2, 88].

13.1.4 Relevance of Personalized Medicine in Oncology

Personalised medicine in colorectal cancer entails customising treatment approaches based on an individual's distinct genetic and microbiome characteristics [10]. This method is especially pertinent since alterations in the gut microbiota might affect the efficacy of cancer treatments, including immunotherapies. Recent studies indicate that some microbial profiles correlate with improved responses to immune checkpoint inhibitors, underscoring the promise of microbiome-based predictive biomarkers in colorectal cancer therapy [88]. The integration of personalised medicine and microbiome profiling may facilitate the creation of precision therapies, improving treatment results for colorectal cancer patients [93].

13.2 Gut Microbiota: Composition and Functionality

The human gut microbiota is a complex and dynamic ecosystem consisting of trillions of species, including bacteria, archaea, viruses, and fungi. This complex community is essential for preserving host health via its involvement in several physiological systems. Comprehending the makeup and functional dynamics of the gut microbiome is crucial for clarifying its influence on illnesses like colorectal cancer (CRC).

13.2.1 Diversity and Composition of the Gut Microbiome

The gut microbiome exhibits significant diversity, with estimations indicating the existence of more than 1000 distinct bacterial species in the human gastrointestinal tract [79]. The gut microbiota's composition differs across the gastrointestinal tract, with unique communities residing in the 'stomach, small intestine, and large intestine' [92]. The colon has the greatest density and variety of microorganisms, mostly consisting of the phylum Firmicutes and Bacteroidetes together comprising over 90% of the gut microbiota [62].

Firmicutes include species such as '*Clostridium*, *Lactobacillus*, and *Ruminococcus*', which mostly participate in the fermentation of dietary fibres into 'SCFA' [29]. Bacteroidetes, including *Bacteroides* and *Prevotella*, are essential for the breakdown of complex carbohydrates and proteins [4]. Additional notable 'phyla are Actinobacteria (e.g., '*Bifidobacterium*'), Proteobacteria (e.g., *Escherichia*), and *Verrucomicrobia* (e.g., Akkermansia)' [79].

Variability in gut microbiota composition across individuals is affected by genetics, nutrition, age, location, and lifestyle [34]. Notwithstanding this diversity, certain core microbial taxa are constantly found in healthy people, indicating a crucial function in sustaining intestinal homeostasis [108]. Dysbiosis, defined as an imbalance in microbial makeup, is linked to several clinical diseases, including colorectal cancer [33].

13.2.2 *Functional Roles of Gut Microbes in the Host*

The gut microbiota executes several roles vital for host health, including metabolic processes and immunological regulation. Essential functional responsibilities encompass:

13.2.2.1 Metabolism of Dietary Components

Intestinal microorganisms ferment non-digestible carbohydrates, yielding ‘short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate’, SCFAs function as energy substrates for colonocytes, modulate lipid metabolism and affect glucose homeostasis. Furthermore, microbiota-mediated metabolism promotes the production of vital vitamins, such as vitamin B and vitamin K [56].

13.2.2.2 Maintenance of Intestinal Barrier Integrity

The gut microbiota aids in preserving the intestinal epithelial barrier by enhancing the synthesis of mucus and tight junction proteins. *Akkermansia muciniphila* destroys mucin, hence promoting the renewal of the mucus layer and improving barrier function [25, 113].

13.2.2.3 Immune System Modulation

Microbial antigens and metabolites engage with the host immune system, facilitating immunological tolerance and defence against infections. Microbial signals regulate ‘regulatory T cells (Tregs)’ and the generation of anti-inflammatory cytokines, hence altering systemic immune responses [83].

13.2.2.4 Protection Against Pathogenic Microorganisms

Commensal bacteria vie with pathogens for resources and adhesion sites, synthesising antimicrobial substances that impede pathogen colonisation. Bacteriocins, synthesised by certain gut microorganisms, demonstrate antimicrobial efficacy against several pathogenic bacteria [20].

13.2.2.5 Influence on Host Gene Expression

Microbial metabolites may regulate host gene expression via epigenetic processes. Butyrate functions as a histone deacetylase inhibitor, affecting gene transcription and cellular differentiation [24].

Within the framework of CRC, these functional functions are very relevant. ‘Short-chain fatty acids (SCFAs)’, particularly butyrate, have anti-inflammatory and anti-proliferative characteristics, aiding in the inhibition of cancer [76]. Dysbiotic microbiota, in contrast, may generate genotoxic metabolites and pro-inflammatory agents that facilitate the development of colorectal cancer [84].

13.2.3 Factors Influencing Gut Microbiota Composition

Numerous internal and external variables affect the makeup and diversity of the gut microbiota:

13.2.3.1 Diet

The makeup of gut microbiota is mostly influenced by diet. High-fibre diets enhance the proliferation of SCFA-producing bacteria, whereas high-fat and high-sugar diets correlate with a heightened presence of pro-inflammatory taxa [22]. Dietary modifications may swiftly transform the microbiome, underscoring its sensitivity to dietary influences [104].

13.2.3.2 Host Genetics

Genetic Factors of the Host: Host genetic differences may affect microbial colonisation and composition. Genome-wide association studies (GWAS) have found host genetic loci linked to certain microbial taxa, indicating a genetic predisposition to particular microbiome profiles [34].

13.2.3.3 Antibiotic and Medication Use

Antibiotics disturb microbial communities by diminishing bacterial diversity and altering the equilibrium between commensal and pathogenic species [42]. Prolonged administration of alternative pharmaceuticals, like proton pump inhibitors and non-steroidal anti-inflammatory drugs, may also influence the gut flora [68].

13.2.3.4 Age

The makeup of gut microbiota changes over the course of a lifetime. Infants are colonised by facultative anaerobes, which subsequently give way to a preponderance of anaerobic bacteria in maturity. The ageing process correlates with diminished diversity and heightened occurrence of opportunistic infections [72].

13.2.3.5 Environmental Factors

The geographical location, sanitation, and environmental microbial exposure affect microbiota composition. Populations in varied habitats often demonstrate increased microbial diversity relative to those in urbanised areas [108].

13.2.3.6 Lifestyle Factors

Stress, physical activity, and sleep habits may influence the gut flora. Chronic stress has been associated with decreased microbial diversity and modified microbial activities [11].

13.2.3.7 Disease States

Specific microbiome profiles are connected with underlying health issues such as inflammatory bowel disease, obesity, and colorectal cancer. Disease-associated inflammation and modified metabolism may induce alterations in microbial populations [33].

Comprehending these characteristics is essential for modulating the gut microbiota to enhance wellness and alleviate sickness.

13.2.4 Methods for Analysing Gut Microbiota (e.g., 16S rRNA Sequencing, Metagenomics)

Advancements in molecular biology and bioinformatics have transformed the examination of gut microbiota, allowing thorough characterisation of microbial populations. Principal techniques comprise:

13.2.4.1 16S rRNA Gene Sequencing

16S ribosomal RNA (rRNA) gene sequencing is a prevalent method for characterising bacterial populations. The 16S rRNA gene comprises conserved and variable sections, facilitating the identification and categorisation of bacteria [13]. High-throughput sequencing systems, including Illumina MiSeq, enable the examination of microbial diversity and relative abundance [48]. This approach is restricted to bacterial identification and lacks species-level resolution [86].

13.2.4.2 Whole Genome Shotgun Metagenomics

Metagenomic sequencing entails the shotgun sequencing of all genetic material inside a sample, offering a comprehensive overview of the microbiome, including bacteria, archaea, viruses, and eukaryotes [79]. This method facilitates the identification of microbial species, functional genes, and metabolic pathways [59]. Metagenomics provides superior taxonomic resolution and insights into the functional capabilities of the microbiome relative to 16S rRNA sequencing [99].

13.2.4.3 Metatranscriptomics

Metatranscriptomic study entails sequencing the whole RNA from a microbial community, therefore elucidating gene expression levels and active metabolic pathways [55]. This approach elucidates the functional dynamics of the microbiome under varying settings [90].

13.2.4.4 Metaproteomics and Metabolomics

Metaproteomics and metabolomics concentrate on the examination of proteins and metabolites, respectively, providing an immediate evaluation of microbial activities and their interactions with the host [67]. These methodologies enhance genomic and transcriptome data, offering a comprehensive insight into microbiome functions [103].

13.2.4.5 Culturomics

Culturomics entails the use of high-throughput culture methodologies with mass spectrometry to detect and characterise microbial species [53]. Although culture-dependent approaches are limited by the cultivability of certain microorganisms, recent improvements have broadened the range of cultivable gut bacteria [94].

13.2.4.6 Bioinformatics Tools and Databases

Comprehensive bioinformatics pipelines are crucial for analysing and elucidating microbiome data. Instruments like QIIME, Mothur, and DADA2 provide data pre-processing, taxonomic classification, and diversity assessment [12, 13]. Platforms for metagenomic data analysis, such as MetaPhlAn and HUMAnN, provide functional profiling and route analysis [98]. Extensive databases, such as the Human Microbiome Project (HMP) and Integrated Microbial Genomes (IMG), facilitate microbiome research by providing substantial reference datasets [58].

13.2.4.7 Multi-omics Integration

‘The integration of multi-omics data (genomics, transcriptomics, proteomics, metabolomics) augments the comprehension of microbiome-host interactions’. Systems biology methodologies and machine learning techniques are used to examine large datasets, revealing complicated connections between microbial populations and host physiology [80].

Despite considerable progress, microbiome research encounters obstacles including the standardisation of methodology, data integration, and the interpretation of causation vs correlation [28]. Future advancements seek to improve the resolution and precision of microbiome analysis, enable the integration of multi-omics data, and convert discoveries into therapeutic applications [60]. The thorough examination of gut microbiota composition and activity is essential for elucidating its involvement in health and illness. Advanced sequencing technology and bioinformatics tools are enhancing our comprehension, and facilitating the development of microbiome-based diagnostics and therapies for colorectal cancer and other conditions.

13.3 Gut Microbiota and CRC

Colorectal cancer is a complex illness impacted by genetic, environmental, and lifestyle factors. Recent studies have emphasised the crucial role of gut bacteria in the development and progression of colorectal cancer (CRC). This section examines the epidemiological links between gut microbiota and colorectal cancer (CRC), investigates the mechanistic pathways by which gut microbes affect tumorigenesis, identifies significant microbial species associated with CRC, and evaluates the potential of dysbiosis as a biomarker for CRC risk and progression.

13.3.1 Epidemiological Association Relationship Between Gut Microbiota and Colorectal Cancer

Epidemiological studies have repeatedly shown a connection between changes in gut microbiota composition and the occurrence of colorectal cancer (CRC). Population-based research indicates that persons with colorectal cancer (CRC) have unique microbial profiles in contrast to healthy controls [110]. Meta-analyses reveal an increased presence of several bacterial taxa, including ‘*Fusobacterium nucleatum* and *Bacteroides fragilis*’, in colorectal cancer patients [47]. Longitudinal cohort studies indicate that alterations in microbiota diversity and abundance occur before CRC diagnosis, suggesting a possible causative association [107]. Geographical disparities further substantiate the microbiota-CRC association since areas with

elevated CRC incidence often exhibit distinct microbial profiles [112]. Dietary habits, which substantially affect gut microbiota composition, are also associated with colorectal cancer risk, highlighting the relationship between diet, microbiota, and cancer [22]. These epidemiological data highlight the potential of gut microbiota as both a risk factor and a diagnostic indicator for colorectal cancer (CRC).

13.3.2 Mechanistic Insights: How Gut Microbes Influence CRC Development

The gut microbiota has a role in colorectal cancer (CRC) development by several mechanisms, including inflammation and immunological regulation, metabolite generation and genotoxicity, and modification of host signalling pathways.

13.3.2.1 Inflammation and Immune Regulation

Chronic inflammation is a recognised risk factor for colorectal cancer, and gut microbiota significantly influence inflammatory responses. Specific bacteria, including '*Fusobacterium nucleatum* and *Enterotoxigenic Bacteroides fragilis* (ETBF)', may trigger inflammatory cascades in the colonic mucosa [38]. '*F. nucleatum*' stimulates the NF- κ B signalling pathway, resulting in the synthesis of pro-inflammatory cytokines such as IL-6 and TNF- α , which foster a tumorigenic milieu [84]. ETBF produces '*Bacteroides fragilis* toxin (BFT)', which undermines epithelial integrity and incites inflammatory responses, facilitating the spread of colorectal cancer [16].

Furthermore, gut microbiota affect the development and functionality of immune cells. '*F. nucleatum*' has been shown to attract 'myeloid-derived suppressor cells (MDSCs)' to the tumour microenvironment, inhibiting anti-tumour immune responses and promoting tumour proliferation [84]. The interactions between gut microbiota and the immune system underscore the significance of microbiota-induced inflammation in the aetiology of colorectal cancer (CRC).

13.3.2.2 Production of Metabolites and Genotoxicity

Gut microbiota metabolise food constituents into bioactive compounds that may affect colorectal cancer risk. 'Short-chain fatty acids (SCFA)', especially butyrate, are widely regarded as beneficial owing to their anti-inflammatory and anti-proliferative properties [61]. Dysbiotic microbiota may generate detrimental compounds, including secondary bile acids and hydrogen sulphide, which possess genotoxic characteristics [82]. Strains of '*Escherichia coli*' with the 'polyketide synthase (pks)' island synthesise colibactin, a genotoxin that causes DNA

double-strand breaks in host cells, hence facilitating mutagenesis and cancer [43]. High concentrations of deoxycholic acid (DCA), a secondary bile acid, have been associated with increased colorectal cancer (CRC) risk via pathways related to oxidative stress and DNA damage [77]. These microbial metabolites induce the chromosomal instability that facilitates the progression of colorectal cancer (CRC).

13.3.2.3 Modification of Host Signalling Pathways

Gut microbiota may influence essential host signalling pathways related to cell proliferation, death, and differentiation. *F. nucleatum* interacts with E-cadherin on colorectal epithelial cells by its FadA adhesin, therefore activating the β -catenin signalling pathway and enhancing oncogenic gene expression [84]. This connection promotes cellular proliferation and suppresses apoptosis, hence aiding tumour development.

Furthermore, 'Bacteroides fragilis toxin (BFT) cleaves E-cadherin, thereby disrupting adherens junctions and activating β -catenin signalling, which results in enhanced expression of genes associated with cell cycle progression and inflammation' [114]. The modifications in host signalling pathways highlight the direct impact of gut bacteria on the molecular processes underlying colorectal cancer (CRC).

13.3.3 Key Microbial Species Associated with CRC

Multiple microbial species have been reliably linked to colorectal cancer (CRC), each playing a role in carcinogenesis via unique methods.

***Fusobacterium nucleatum*:** Predominantly found in colorectal cancer tissues, *F. nucleatum* is associated with the promotion of inflammation, modulation of immunological responses, and alteration of cell signalling pathways [14]. Its presence correlates with a worse prognosis and resistance to treatment [84].

'Enterotoxigenic Bacteroides fragilis (ETBF)': ETBF synthesises Bacteroides fragilis toxin (BFT), resulting in epithelial cell injury, inflammation, and the activation of oncogenic pathways [105]. ETBF colonisation is associated with heightened CRC risk and carcinogenesis in animal models [57].

***Escherichia coli* (pks+ strains):** Strains of *E. coli* containing the pks genomic island synthesise colibactin, a genotoxin that induces DNA damage and facilitates the development of colorectal cancer [75].

***Akkermansia muciniphila*:** *A. muciniphila* is generally regarded as advantageous, although it plays a multifaceted function in colorectal cancer (CRC). Although it promotes mucosal health, certain settings and interactions may affect carcinogenesis [36].

***Bacteroides fragilis*:** Non-toxigenic strains of *B. fragilis* aid in gut homeostasis, whereas toxigenic strains (ETBF) have a direct role in colorectal cancer pathogenesis via toxin generation and immunological regulation [84].

Comprehending the functions of these pivotal bacteria species elucidates the microbial-mediated processes associated with CRC and underscores prospective targets for therapeutic intervention.

13.3.4 Dysbiosis as a Biomarker for CRC Risk and Progression

Dysbiosis, defined as an imbalance in the gut microbial ecology, is widely acknowledged as a possible biomarker for colorectal cancer risk and development. Distinct microbial profiles, characterised by elevated amounts of *F. nucleatum*, ETBF, and pks+ *E. coli*, along with decreased quantities of advantageous microorganisms such as *Faecalibacterium prausnitzii* and *Bifidobacterium*, have been linked to colorectal cancer [109].

Diagnostic models using microbiome profiles have shown efficacy in differentiating CRC patients from healthy persons with increased sensitivity and specificity [91]. A combination of microbial indicators might improve the precision of non-invasive colorectal cancer screening methods, supplementing conventional techniques such as colonoscopy and faecal occult blood tests [27].

Additionally, microbial profiles are associated with illness stage and prognosis. Elevated levels of “*F. nucleatum*” correlate with advanced tumour stages, metastasis, and reduced survival rates [73]. Assessing dysbiosis using faecal microbiota profiling provides a non-invasive method for early identification, prognostic evaluation, and tracking therapy responses in colorectal cancer patients [84].

13.4 Postbiotics in CRC Management

Postbiotics denote non-viable bacterial products or metabolites originating from probiotics that provide health advantages to the host (Fig. 13.1). In contrast to living probiotics, postbiotics include elements such as ‘short-chain fatty acids (SCFA)’, enzymes, peptides, cell wall fragments, and polysaccharides [3, 102]. They are generated by the fermentation of dietary fibres or as a result of microbial metabolic activities. Their stability and safety, in contrast to live probiotics, render them more appropriate for therapeutic applications [93].

The primary categories of postbiotics encompass ***bacterial metabolites***: they include short-chain fatty acids (SCFAs), lactic acid, and tryptophan metabolites. ***Cell-free supernatants (CFS)***: comprise bioactive substances such as antioxidants and antibacterial agents. ***Inactivated bacterial cells***: heat-killed bacterial

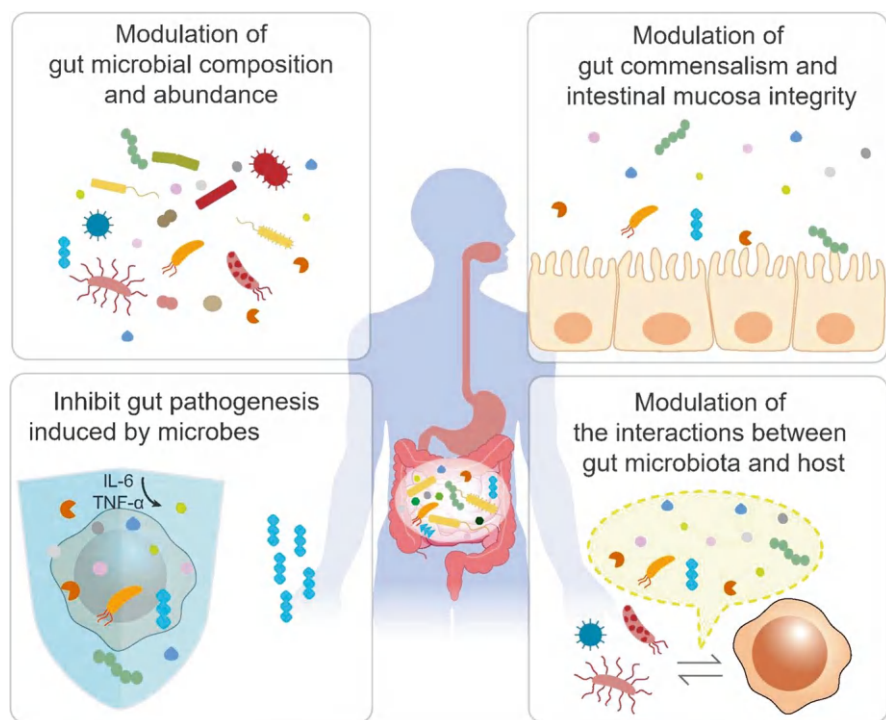


Fig. 13.1 Postbiotics and its biological activity [93]

preparations, including those derived from *Lactobacillus* and *Bifidobacterium*.

Microbial structural components: fragments of the cell wall, including exopolysaccharides (EPS), peptidoglycans, and surface proteins [88].

13.4.1 Mechanism of Action in CRC Prevention and Therapy

Postbiotics affect the development and progression of colorectal cancer (CRC) by many biological processes, including anti-inflammatory effects, regulation of immunological responses, induction of apoptosis in cancer cells, and augmentation of intestinal barrier function (Fig. 13.2).

13.4.1.1 Anti-inflammatory Effects

‘Chronic inflammation is a significant contributor to the pathogenesis of colorectal cancer (CRC)’. Postbiotics, especially short-chain fatty acids such as butyrate, have significant anti-inflammatory effects by suppressing the synthesis of

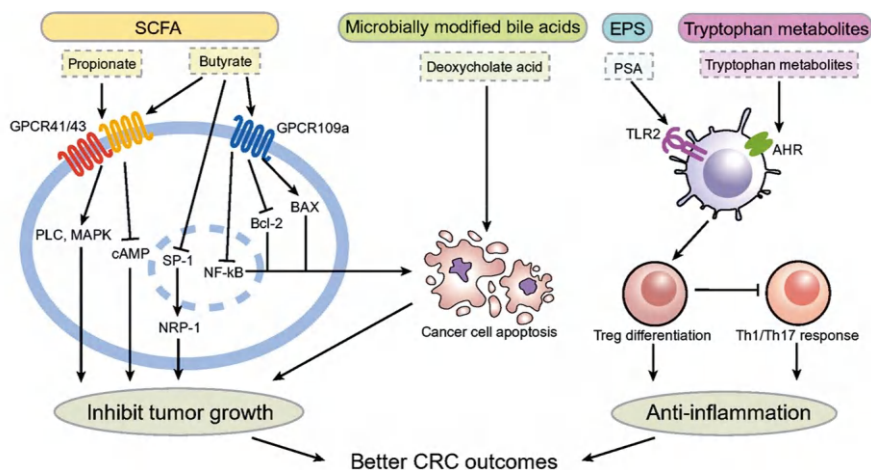


Fig. 13.2 Schematic illustration of postbiotics' molecular mechanisms for preventing and treating colorectal cancer [93]

pro-inflammatory cytokines (e.g., IL-6, TNF- α) and enhancing the expression of anti-inflammatory cytokines (e.g., IL-10) [15]. Butyrate does this by blocking histone deacetylases (HDACs), resulting in modified gene expression that promotes 'anti-inflammatory' pathways [8].

13.4.1.2 Modulation of Immune Responses

Postbiotics engage with the host immune system to augment anti-tumour immunity. Short-chain fatty acids (SCFAs) affect the development and functionality of regulatory T cells (Tregs), which are essential for sustaining immunological tolerance and averting excessive inflammatory responses [7]. Furthermore, 'extracellular vesicles (EVs)' originating from beneficial bacteria may activate dendritic cells and 'natural killer (NK) cells', hence augmenting their cytotoxic efficacy against tumour cells [92].

13.4.1.3 Induction of Apoptosis and Inhibition of Cell Proliferation:

Specific postbiotics directly trigger apoptosis in colorectal cancer cells and impede their growth. Butyrate promotes pro-apoptotic pathways by increasing BAX expression and decreasing BCL-2 expression, resulting in programmed cell death [47]. Furthermore, postbiotics may disrupt oncogenic signalling pathways, including the 'Wnt/ β -catenin pathway', thereby diminishing tumour cell growth [109].

13.4.1.4 Enhancement of Intestinal Barrier Function

Postbiotics fortify the intestinal barrier by stimulating the expression of tight junction proteins (e.g., occludin, claudins) and augmenting mucus production [100]. An effective intestinal barrier inhibits the transfer of harmful bacteria and their toxins, hence diminishing chronic inflammation and decreasing the risk of colorectal cancer [73].

13.4.1.5 Antioxidant Properties

Postbiotics with antioxidant properties counteract reactive oxygen species (ROS), alleviating oxidative stress-related DNA damage and mutagenesis in colonic epithelial cells [109]. This preventive impact is essential in averting the genetic modifications that initiate and advance CRC [77].

13.4.2 Clinical Studies and Evidence of Postbiotics in CRC

Preclinical investigations are essential for clarifying the processes by which postbiotics influence CRC and for evaluating their therapeutic potential before clinical trials. These investigations often include in vitro tests using CRC cell lines and in vivo studies employing animal models.

13.4.2.1 In Vitro Investigations

In vitro investigations have shown that postbiotics may suppress CRC cell growth and trigger apoptosis. For example, butyrate, a well-defined short-chain fatty acid (SCFA), has shown the ability to cause cell cycle arrest and death in colorectal cancer (CRC) cell lines, including HCT116 and HT29 [71]. The process entails the inhibition of ‘histone deacetylases (HDACs)’, resulting in the activation of pro-apoptotic genes and the downregulation of anti-apoptotic genes [97].

Moreover, extracellular vesicles (EVs) originating from *Bifidobacterium breve* have been shown to augment the cytotoxic efficacy of chemotherapeutic agents such as 5-fluorouracil (5-FU) on colorectal cancer (CRC) cells [106]. These EVs regulate signalling pathways related to cell survival and death, hence enhancing the susceptibility of cancer cells to chemotherapeutic drugs [49].

Functional components, including peptidoglycans and lipoteichoic acids derived from *Lactobacillus* species, have shown the capacity to diminish CRC cell viability and trigger apoptosis via the activation of caspase-3 and caspase-9 pathways [6]. These results underscore the potential of several postbiotic components to directly target CRC cells and augment the effectiveness of current therapies.

Animal Models Animal studies provide a more thorough comprehension of the in vivo impacts of postbiotics on the development and progression of colorectal cancer (CRC). In murine models of colorectal cancer, the administration of butyrate has been linked to a significant decrease in tumour incidence and size [31, 37]. This impact is facilitated by the inhibition of inflammatory cytokines and the improvement of intestinal barrier integrity, which together provide an inhospitable environment for tumour proliferation.

Moreover, research using xenograft models has shown that postbiotics from “*Bifidobacterium*” species may suppress tumour proliferation and alter the immune response by enhancing the infiltration of cytotoxic T cells into the tumour microenvironment [5]. The immunomodulatory impact is essential for improving the host’s capacity to identify and eradicate cancer cells.

A significant research used a genetically modified mouse model of colorectal cancer to examine the impact of postbiotic supplementation. The findings demonstrated that postbiotics decreased tumour burden and modified gut microbiota composition, enhancing the growth of beneficial bacteria while inhibiting pathogenic taxa linked to CRC [107]. These results highlight the complex function of postbiotics in influencing tumour biology and the gut microbiota.

Preclinical studies provide substantial evidence for the therapeutic efficacy of postbiotics in the therapy of colorectal cancer (CRC). They emphasise the capacity of postbiotics to directly target neoplastic cells, augment the effectiveness of standard treatments, and regulate the gut microbiota and immune responses to provide an inhospitable environment for tumour proliferation.

13.4.2.2 Clinical Evidence and Trials

Although preclinical research provides encouraging insights, clinical data is crucial to confirm the effectiveness and safety of postbiotics in colorectal cancer patients. Numerous clinical studies have started to investigate the function of postbiotics in colorectal cancer care, emphasising their potential to improve treatment results and augment patient quality of life.

Clinical Trials A seminal clinical study examined the impact of a butyrate-enriched enteral diet on colorectal cancer patients following surgical intervention. The research indicated that patients with a butyrate-enriched diet encountered fewer postoperative problems, including anastomotic leakage and inflammation, in contrast to those receiving normal nutrition [49]. The findings indicate that butyrate may facilitate intestinal repair and decrease the likelihood of postoperative complications in colorectal cancer patients.

A separate randomised controlled experiment assessed a postbiotic formulation of metabolites from *Lactobacillus* and *Bifidobacterium* species in colorectal cancer patients after chemotherapy. The research indicated that postbiotic supplementation markedly alleviated chemotherapy-induced gastrointestinal adverse effects, such as diarrhoea and mucositis, without compromising the effectiveness of chemotherapy

agents [66]. This suggests that postbiotics might enhance patient tolerance to chemotherapy, hence enhancing adherence and results.

A pilot research investigated the effects of a postbiotic combination on gut microbiota diversity and inflammatory markers in colorectal cancer survivors. The findings indicated that postbiotic supplementation enhanced microbial diversity and reduced pro-inflammatory cytokine levels, suggesting a favourable regulation of the gut microbiome and systemic inflammation [18]. Increased microbial diversity correlates with enhanced immune function and decreased risk of cancer recurrence, underscoring the significance of postbiotics in the long-term therapy of colorectal cancer.

A clinical experiment evaluated the use of extracellular vesicles (EVs) derived from '*Bifidobacterium*' in conjunction with immunotherapy in colorectal cancer (CRC) patients. The research indicated that EVs augmented the immune response to tumour cells by elevating the activation of 'cytotoxic T cells and natural killer (NK) cells'. This synergistic impact highlights the potential of postbiotics to enhance immunotherapeutic approaches in colorectal cancer therapy.

Safety and Tolerability Clinical investigations have consistently shown that postbiotics are well-tolerated, exhibiting little side effects. In contrast to probiotics, postbiotics do not pose the danger of infections or immunological responses linked to living microorganisms, making them a safer option, particularly for immunocompromised individuals. Comprehensive safety evaluations are essential to validate these results across various patient demographics and treatment protocols.

Limitations and Future Directions Notwithstanding the encouraging outcomes, clinical investigations on postbiotics in colorectal cancer remain nascent, with several trials being either limited in size or preliminary. Comprehensive, multi-centre randomised controlled studies are necessary to provide conclusive evidence of effectiveness and safety. Furthermore, the standardisation of postbiotic formulations and doses is crucial for ensuring uniformity and repeatability of outcomes [93].

Future clinical research should investigate the mechanisms via which postbiotics engage with the host immune system and gut microbiota to improve CRC therapy results. Comprehending these interactions would enable the creation of targeted postbiotic therapy customised to specific patient profiles, according to the tenets of personalised medicine [6].

13.4.3 Postbiotics as Adjunct Therapies in CRC Treatment

Postbiotics have considerable promise as supplementary medicines in the management of colorectal cancer, enhancing traditional treatments such as surgery, chemotherapy, and radiation therapy. Their incorporation within treatment protocols may augment therapeutic effectiveness, reduce side effects, and boost overall patient outcomes.

Enhancing Therapeutic Efficacy Postbiotics may enhance the efficacy of chemotherapy by influencing signalling pathways related to cancer cell survival and apoptosis. Butyrate has been shown to increase the susceptibility of colorectal cancer (CRC) cells to 5-fluorouracil (5-FU) by upregulating pro-apoptotic proteins and downregulating anti-apoptotic proteins, therefore facilitating cancer cell apoptosis [9]. Furthermore, postbiotics may disrupt drug resistance pathways, rendering cancer cells more vulnerable to chemotherapeutic treatments [107].

Mitigating Adverse Effects Chemotherapy and radiation treatment may induce considerable gastrointestinal damage, including mucositis, diarrhoea, and inflammation. Postbiotics may mitigate these negative effects by preserving gut barrier integrity and decreasing inflammation. For example, short-chain fatty acids such as butyrate augment the production of tight junction proteins, so inhibiting the translocation of pathogens and toxins that worsen chemotherapy-induced gut inflammation. Additionally, postbiotics have been shown to diminish oxidative stress and facilitate mucosal repair, thereby alleviating mucositis and improving patient comfort and adherence to treatment regimens [1, 52]. Postbiotics mitigate these detrimental effects, hence enhancing quality of life and promoting adherence to cancer treatment protocols.

Mitigating Cancer Recurrence Postbiotics may contribute to the prevention of colorectal cancer recurrence by preserving healthy gut flora and enhancing immune surveillance. Consistent consumption of postbiotics maintains microbial equilibrium, averting dysbiosis that may result in chronic inflammation and cancer [40]. Furthermore, postbiotics may augment the functionality of immune cells, facilitating more efficient identification and eradication of remaining cancer cells [107].

Research indicates that postbiotic supplementation may result in enduring alterations in the gut microbiota linked to diminished cancer recurrence rates [40]. Postbiotics cultivate an ‘anti-inflammatory’ and immunoactive milieu, establishing circumstances that are detrimental to cancer recurrence and metastasis.

Synergistic Effects with Immunotherapy Immunotherapy has emerged as a viable intervention for colorectal cancer (CRC), especially in tumours with high microsatellite instability (MSI-H). Postbiotics may improve the effectiveness of immunotherapies by regulating gut microbiota and immunological responses. Extracellular vesicles derived from ‘*Bifidobacterium*’ have shown the ability to augment the activation and proliferation of cytotoxic T cells, hence improving the anti-tumour immune response.

Additionally, postbiotics may diminish immunosuppressive elements in the tumour microenvironment, enhancing the sensitivity of colorectal cancer tumours to immune checkpoint inhibitors [51]. The synergistic relationship between postbiotics and immunotherapy presents opportunities for creating more effective and personalised treatment approaches for CRC patients.

Safety, Efficacy, and Regulatory Considerations The effective incorporation of postbiotics into colorectal cancer care depends on resolving safety, effectiveness,

and regulatory issues. It is crucial to verify that postbiotics are safe for consumption, effective in delivering therapeutic results, and adhere to regulatory criteria for their clinical use.

Postbiotics are often considered harmless because to their non-viable characteristics, which preclude the dangers linked with living microorganisms, including infections or immunological responses. The safety profile of postbiotics may differ based on their particular components and origins. Thorough toxicological assessments are essential to confirm that postbiotics do not cause harmful consequences, particularly in susceptible groups such as immunocompromised individuals or patients with advanced colorectal cancer [107].

Research has shown that postbiotics, including short-chain fatty acids and bacterial cell wall components, are well-tolerated in both healthy persons and patients with colorectal cancer. However, further safety studies are necessary to evaluate the risk of unexpected consequences, especially when postbiotics are used with other treatments [115].

Determining the usefulness of postbiotics in colorectal cancer therapy necessitates comprehensive clinical studies that validate their capacity to enhance treatment results, mitigate adverse effects, and avert cancer recurrence. Although preclinical investigations provide robust evidence of their therapeutic potential, clinical trials are necessary to confirm these results in human populations [93].

Standardisation of postbiotic formulations and doses is essential for guaranteeing uniform treatment outcomes. Inconsistencies in postbiotic formulations might result in variations in effectiveness, necessitating the establishment of standardised methods for their manufacturing and administration [96]. Moreover, pinpointing certain postbiotic constituents that provide the most substantial advantages might improve the focused implementation of postbiotic treatments [95].

The regulatory framework for postbiotics is still developing, with some countries categorising them differently as dietary supplements, functional foods, or biotherapeutics [95]. Explicit regulatory criteria are crucial to guarantee the quality, safety, and effectiveness of postbiotic products. This entails the use of standardised production protocols, quality assurance methods, and substantiated efficacy assertions [21].

In the United States, postbiotics intended for therapeutic applications may need clearance from the Food and Drug Administration (FDA) as biotherapeutic drugs, contingent upon stringent clinical testing and validation. The European Union's regulatory frameworks are governed by the European Medicines Agency (EMA), which requires extensive safety and effectiveness evidence prior to approval.

Cooperative endeavours among researchers, physicians, and regulatory authorities are essential for traversing the approval procedure and enabling the clinical incorporation of postbiotic medicines. Establishing worldwide agreement on the definition and categorisation of postbiotics may facilitate regulatory procedures and encourage global standardisation [40].

Standardisation and Quality Control Maintaining the stability and repeatability of postbiotic products is essential for their therapeutic use. This entails the standardisa-

tion of extraction, purification, and formulation procedures to preserve the integrity and concentration of active constituents. Advanced analytical methods, including mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy, are used to characterise postbiotic profiles and confirm their bioactive components [87].

Quality control protocols must also include possible contaminants and guarantee that postbiotic goods adhere to rigorous purity requirements. Implementing good manufacturing practices (GMP) and complying with regulatory criteria are crucial for ensuring the quality of postbiotic formulations [69].

Future research should concentrate on refining postbiotic formulations to improve effectiveness and safety, while also investigating new postbiotic substances with therapeutic promise in colorectal cancer (CRC). Personalised medicine strategies, which customise postbiotic therapy according to individual microbiota profiles and genetic predispositions, may enhance therapeutic efficacy [6].

Furthermore, 'Integrating multi-omics data (genomics, transcriptomics, proteomics, metabolomics)' might provide an enhanced understanding of the pathways via which postbiotics affect CRC development and treatment [40]. This thorough comprehension will enable the creation of tailored postbiotic therapies that tackle particular elements of CRC biology.

Postbiotics serve as a potential adjuvant in the therapy of colorectal cancer, providing several therapeutic advantages via their anti-inflammatory, immunomodulatory, and anti-tumorigenic capabilities. Preclinical research has shown the fundamental effectiveness of postbiotics while accumulating clinical data endorses their potential to improve treatment results and reduce side effects. Further study is essential to comprehensively understand their processes, refine their formulations, and develop standardised regulatory frameworks. Overcoming these hurdles would allow the effective incorporation of postbiotics into personalised medicine approaches for colorectal cancer therapy, hence enhancing patient results and quality of life [70].

13.5 Personalised Medicine in CRC: Integrating Gut Microbiota and Postbiotics

Personalised medicine signifies a transformative approach in the treatment of colorectal cancer, focusing on customised therapeutic options informed by unique patient attributes. Incorporating gut microbiota and postbiotics into personalised medicine frameworks has exciting opportunities to improve colorectal cancer prevention, diagnosis, and therapy. This section examines the fundamental concepts of personalised medicine in oncology, the categorisation of colorectal cancer patients according to microbiota profiles, the tailoring of postbiotic therapy, relevant case studies, and the problems related to the implementation of personalised strategies.

13.5.1 Principles of Personalised Medicine in Oncology

Personalised medicine, sometimes equated with precision medicine, seeks to individualise healthcare by tailoring medical choices and treatments to the specific genetic, environmental, and lifestyle characteristics of each patient [17]. This methodology has transformed cancer treatment in oncology by facilitating the creation of tailored treatments that directly target the genetic anomalies responsible for carcinogenesis [32].

Key Principles

Genomic Profiling: An exhaustive examination of tumour genomes to detect mutations, gene amplifications, and other genetic modifications amenable to targeted therapy [41].

Biomarker Identification: The use of biomarkers to forecast disease development, therapeutic efficacy, and prognosis, therefore informing therapy choices [39].

Multi-omics Integration: The amalgamation of data from genomes, transcriptomics, proteomics, and metabolomics to get a comprehensive knowledge of cancer biology and individualised disease causes [39].

Patient Stratification: Classifying patients into subgroups according to molecular and clinical traits to guarantee that medicines are suitably aligned with those most likely to benefit [101].

Adaptive Treatment Strategies: Ongoing assessment of patient reactions and real-time modification of treatment regimens to enhance therapeutic results [17].

The integration of gut microbiota and postbiotics into personalised medicine enriches these concepts by introducing an additional layer of complexity and potential. The gut microbiome closely interacts with the host's immune system, metabolism, and the effectiveness of cancer medicines, consequently affecting individualised treatment responses [35].

13.5.2 Microbiota-Driven Stratification of Colorectal Cancer Patients

Stratifying colorectal cancer patients according to their gut microbiota patterns facilitates more accurate therapeutic treatments and prognostic evaluations. This stratification entails classifying patients into several microbiome-associated subgroups, each characterised by diverse microbial compositions and functional capabilities that affect CRC development and therapy efficacy [47].

Methods of Stratification

Analysis of Microbial Composition: Employing high-throughput sequencing techniques, including 16S rRNA gene sequencing and metagenomics, to detect and quantify microbial species in colorectal cancer patients [13]. Particular bacterial

signatures, such as the prevalence of '*Fusobacterium nucleatum* or *Bacteroides fragilis*', have been linked to specific colorectal cancer subtypes and clinical outcomes [14].

Functional Profiling: Evaluating the metabolic and functional capacities of the gut microbiota by 'metatranscriptomics, metaproteomics, and metabolomics' [30]. This functional stratification may elucidate the correlation between microbial activities, such as short-chain fatty acid synthesis or bile acid metabolism, and colorectal cancer growth and therapy success [89].

Integrative Multi-omics Approaches: Integrating microbiome data with host genomic, transcriptomic, and immunological characteristics to develop complete stratification models [59]. Integrating microbial fingerprints with tumour mutation profiles might elucidate synergistic relationships that contribute to CRC development [55].

Clinical Implications

Prognostic Indicators: Microbiota-based stratification may function as prognostic markers, with certain microbial profiles associated with a worse prognosis or heightened risk of metastasis [47].

Therapeutic Targets: Identifying particular bacteria that either promote or inhibit colorectal cancer (CRC) facilitates targeted therapeutics, such as antimicrobial medicines or microbiota regulation via diet, probiotics, or postbiotics [33].

Predictive Biomarkers: Microbiota profiles may forecast patient responses to medications such as chemotherapy, immunotherapy, and targeted treatments, facilitating personalised therapeutic strategies [45].

13.5.3 Tailoring Postbiotic Therapies to Individual Microbiome Profiles

Personalising postbiotic therapy entails tailoring the selection and delivery of postbiotics according to the unique gut microbiota makeup and functional condition of each CRC patient. This customised strategy seeks to optimise therapy effectiveness, reduce unwanted effects, and improve overall patient outcomes [78].

Steps in Tailoring Postbiotic Therapies

Microbiome Assessment: Performing extensive microbiome profiling using sequencing and metabolomic analysis to detect dysbiotic patterns and advantageous bacteria activities [64].

Postbiotic Selection: Selecting certain postbiotics that might mitigate the detected dysbiosis or augment advantageous microbial functions. For example, butyrate supplements may be recommended for patients with diminished levels of 'SCFA-producing bacteria' [85].

Dosage Optimisation: Establishing the ideal dosage and administration technique of postbiotics to guarantee effective absorption and stability in the gastrointesti-

nal environment [63]. This may include encapsulation methods or the incorporation of dietary fibres to augment short-chain fatty acid (SCFA) synthesis [109].

Combination Therapies: Integrating postbiotics with traditional cancer treatments to synergistically improve therapeutic effectiveness. Combining postbiotics with immunotherapy may augment immune cell infiltration and activity in tumours [91].

Monitoring and Adjustment: Ongoing evaluation of patient responses by microbiome and clinical evaluations, allowing real-time modifications to postbiotic therapy as required [46].

Examples of Tailored Postbiotic Interventions

Butyrate Supplementation: Administering butyrate or butyrate-producing bacteria to patients with diminished levels of ‘butyrate-producing Firmicutes’ enhances intestinal barrier function and exerts ‘anti-inflammatory’ and anti-proliferative effects on colorectal cancer cells [37, 61].

Anti-inflammatory Postbiotics: Employing postbiotics with anti-inflammatory characteristics, such as specific short-chain fatty acids (SCFAs) or components of bacterial cell walls, to mitigate chronic inflammation linked to colorectal cancer (CRC) [26].

Genotoxin-Blocking Postbiotics: Choosing postbiotics capable of neutralising microbial genotoxins such as colibactin, therefore averting DNA damage and mutagenesis in colonic epithelial cells [84].

13.5.4 Case Studies: Success Stories and Lessons Learned

Empirical case studies demonstrate the practical uses and advantages of incorporating gut microbiota and postbiotics into personalised treatment for colorectal cancer. These instances highlight achievements, obstacles, and essential insights that guide forthcoming plans.

Case Study 1: Improved Chemotherapy Efficacy via Butyrate Supplementation

A group of CRC patients receiving chemotherapy was provided with a butyrate-enriched enteral diet. The research indicated that individuals administered butyrate had decreased chemotherapy-induced mucositis and diarrhoea relative to the control group. Moreover, tumour reduction was more significant in the butyrate-supplemented cohort, indicating that butyrate amplifies the cytotoxic efficacy of chemotherapeutic drugs [106].

Case Study 2: Enhancement of Immunotherapy via Postbiotic-Derived Extracellular Vesicles

A clinical experiment examined CRC patients undergoing immune checkpoint inhibitor treatment, focussing on the supplementation of extracellular vesicles (EVs) sourced from ‘*Bifidobacterium*’ species. The findings indicated enhanced

immune cell activation and greater infiltration of cytotoxic T lymphocytes into tumours, corresponding with superior patient responses to immunotherapy [71].

Case Study 3: Microbiota-Informed Customised Postbiotic Treatment

A tailored postbiotic regimen was created for a CRC patient with a distinct dysbiotic microbiota profile marked by elevated levels of *Fusobacterium nucleatum* and diminished short-chain fatty acid production. The intervention included the administration of short-chain fatty acids (SCFAs) and anti-*Fusobacterium* postbiotics, leading to a significant decrease in tumour markers and stabilisation of disease development [71].

Comprehensive Observations These case reports highlight the potential of postbiotics to augment traditional CRC therapies, promote patient tolerance, and aid in disease management. They emphasise the significance of individualised strategies and the need for thorough microbiome evaluations in clinical practice.

13.5.5 Challenges in Implementing Personalised Approaches

Although the integration of gut microbiota and postbiotics into personalised treatment for colorectal cancer shows significant promise, several difficulties must be overcome to enable broad clinical use.

13.5.5.1 Complexity of the Microbiome

The gut microbiome is complex and dynamic, shaped by several variables including nutrition, genetics, environment, and pharmacological interventions. The intricacy complicates the precise characterisation of individual microbiome profiles and the prediction of their interactions with postbiotic therapy [28].

13.5.5.2 Standardisation of Microbiome Profiling

The absence of standardised techniques for microbiome collection, sequencing, and data processing leads to heterogeneity in outcomes across research [13]. Implementing standardised procedures is essential for dependable patient classification and therapy targeting [30].

13.5.5.3 Inter-Individual Variability

Substantial inter-individual diversity in microbiome composition requires highly personalised methodologies, which may be resource-demanding and time-consuming (36). Formulating scalable and economical solutions for individualised microbiome evaluation is crucial [23].

13.5.5.4 Limited Clinical Data

Although preclinical and first clinical studies show promise, substantial data from extensive randomised controlled trials is required to confirm the effectiveness and safety of postbiotic treatments in colorectal cancer (CRC). The scarcity of high-quality clinical evidence restricts the incorporation of postbiotics into routine treatment [91].

13.5.5.5 Regulatory Obstacles

Postbiotics occupy a regulatory grey area, with classifications differing among dietary supplements, functional foods, and biotherapeutics according to their intended use and jurisdiction [95]. Successfully navigating these regulatory frameworks needs explicit norms and standards to guarantee product safety, quality, and effectiveness [85].

13.5.5.6 Manufacturing and Quality Control

The production of postbiotics with uniform quality and efficacy is difficult owing to the heterogeneous characteristics of bioactive chemicals and their susceptibility to environmental influences. Enforcing rigorous production protocols and quality assurance methods is crucial for preserving the integrity of postbiotic goods [40].

13.5.5.7 Integration into Clinical Practice

The incorporation of microbiome and postbiotic evaluations into standard clinical procedures requires training, infrastructure, and multidisciplinary cooperation among oncologists, microbiologists, and nutritionists. Facilitating smooth incorporation into clinical practice is essential for the optimal use of personalised postbiotic therapy [6].

13.5.5.8 Ethical and Privacy Concerns

Microbiome profiling entails the acquisition and examination of sensitive biological data, which raises ethical and privacy issues related to data ownership, permission, and security [110]. Implementing stringent ethical rules and data protection protocols is essential to mitigate these issues [50, 54, 65].

Incorporating gut microbiota and postbiotics into personalised medicine frameworks has substantial potential for improving colorectal cancer care. Personalised postbiotic therapy may enhance treatment success, minimise unwanted effects, and avert cancer recurrence by customising therapeutic tactics according to individual microbiome profiles. Nonetheless, surmounting the intrinsic obstacles needs coordinated efforts in research, standardisation, regulatory alignment, and clinical integration. Ongoing progress in microbiome science and postbiotic research, together with collaborative and multidisciplinary methodologies, will be crucial in achieving the full promise of personalised medicine in colorectal cancer therapy.

13.6 Interplay Between Gut Microbiota, Postbiotics, and Personalised Medicine

13.6.1 Synergistic Effects in CRC Prevention and Treatment

The amalgamation of gut microbiota, postbiotics, and personalised medicine presents novel opportunities in the prevention and treatment of colorectal cancer (CRC). The gut microbiota plays a crucial role in general health by sustaining immunological homeostasis and generating bioactive metabolites, including short-chain fatty acids (SCFAs). Postbiotics, as microbial byproducts, augment these advantages without the hazards linked to active probiotics. Personalised medicine utilises information from an individual's genetic and microbiological profiles to customise therapies for the best results [93].

The interplay among these components is apparent as postbiotics facilitate the modulation of immune responses and diminish chronic inflammation, hence fostering an environment conducive to cancer prevention. Tailored interventions, informed by an individual's microbiological and genetic profiles, improve the effectiveness of therapies such as immunotherapy. Furthermore, modifying the gut microbiota with postbiotic supplements or dietary changes enhances gut integrity, hence inhibiting the development and recurrence of colorectal cancer [88].

13.6.2 Case Studies or Recent Trials Illustrating Integrated Approaches

Recent clinical studies underscore the efficacy of integrating microbiome-based strategies with traditional colorectal cancer therapies. For instance, ‘faecal microbiota transplantation (FMT)’ has been used to re-establish microbial equilibrium, enhancing responses to immune checkpoint inhibitors [93]. Research on postbiotic elements, including short-chain fatty acids (SCFAs), indicates decreased tumour proliferation and improved chemotherapy tolerance, highlighting the promise of microbiome-targeted interventions [102].

Furthermore, studies are investigating personalised dietary treatments designed to enhance the proliferation of good bacteria linked to improved treatment outcomes. Patients with advantageous microbial compositions have shown enhanced results in immunotherapy, emphasising the predictive significance of microbiome data in personalised medicine [74]. The amalgamation of these tactics not only provides more effective treatment alternatives but also facilitates preventative interventions for high-risk patients.

The interaction of gut microbiota, postbiotics, and personalised medicine illustrates the future of colorectal cancer therapy, emphasising preventative care, enhanced treatment techniques, and superior patient outcomes. As research progresses in elucidating the intricate interactions among these parameters, it is anticipated that more targeted therapies will arise, facilitating improved disease management and higher quality of life for CRC patients.

13.7 Current Challenges and Future Directions

Despite the significant potential of incorporating gut microbiota and postbiotics into personalised treatment for colorectal cancer, several difficulties hinder their broad clinical implementation. Overcoming these challenges is crucial to fully use the advantages of microbiome-based therapies in colorectal cancer care. This section delineates the principal difficulties and examines prospective strategies to surmount these obstacles.

13.7.1 Technical and Methodological Constraints

A primary problem in microbiome research is the absence of standardised techniques for sample collection, processing, and data analysis. Divergences in methods may result in discrepancies in microbiome profiling, complicating the comparison of findings across research [30]. The intricate nature and elevated dimensionality of microbiome data need sophisticated bioinformatics tools and experience, which are

not consistently accessible in research and clinical environments [81]. The amalgamation of multi-omics data (genomics, transcriptomics, proteomics, metabolomics) complicates data interpretation, necessitating sophisticated computational frameworks and multidisciplinary cooperation [39].

13.7.2 Inter-Individual Variability

The gut microbiota has considerable inter-individual heterogeneity shaped by genetic, dietary, environmental, and lifestyle variables [34]. The variety presents a difficulty in creating universal postbiotic therapeutics, necessitating highly personalised interventions to accommodate individual microbiome patterns [45]. Furthermore, temporal variations in microbiome composition provide further complexity, requiring longitudinal investigations to document dynamic changes and their consequences for colorectal cancer [109].

13.7.3 Limited Clinical Evidence

Although preclinical research has shown the therapeutic potential of postbiotics in colorectal cancer, clinical data is still few. The majority of current clinical studies are either small-scale or preliminary, lacking the statistical power and comprehensive endpoints necessary to determine definite effectiveness and safety [66]. Extensive, randomised controlled studies are crucial to substantiate the advantages of postbiotic therapies and to determine appropriate doses, formulations, and methods of delivery.

13.7.4 Translational Gaps

Translating microbiome research results into clinical practice necessitates overcoming substantial translational barriers. This entails the development of dependable biomarkers for colorectal cancer risk and progression derived from microbiome profiles and the formulation of standardised postbiotic products for smooth integration into current treatment procedures. Furthermore, healthcare practitioners need training and resources to successfully integrate microbiome data into individualised treatment strategies.

13.7.5 *Regulatory Obstacles*

The regulatory framework for postbiotics is still developing, with differing classifications and approval procedures in various countries. Postbiotics may be classified as dietary supplements, functional foods, or biotherapeutic medicines, each governed by specific regulatory standards. It is essential to establish explicit recommendations and standards for the safety, effectiveness, and quality of postbiotic products to promote their clinical acceptance and safeguard patient safety [21, 95].

13.7.6 *Future Perspectives*

To address these issues, other prospective strategies are suggested:

Standardisation and Protocol Harmonisation: Establishing uniform methods for microbiome research, including sample collection, sequencing, and data analysis, would improve reproducibility and comparability across investigations.

Advancements in Bioinformatics and Artificial Intelligence: Utilising artificial intelligence and machine learning may enhance data integration and interpretation, facilitating the detection of intricate microbiome-disease connections and the creation of prediction models for colorectal cancer.

Customised Interventions: Customising postbiotic treatments according to individual microbiome profiles may improve therapeutic effectiveness and reduce side effects. Customised diet and certain postbiotic formulations provide potential pathways [111].

Comprehensive Clinical Trials: Executing extensive, multi-centre clinical studies will provide substantial data about the effectiveness and safety of postbiotic treatments in colorectal cancer, hence promoting their incorporation into therapeutic guidelines [66].

Development of Regulatory Framework: Engaging with regulatory authorities to formulate explicit rules for postbiotics would facilitate the approval process and guarantee the uniform quality and safety of microbiome-based therapeutics [95].

Interdisciplinary Collaboration: Promoting cooperation among microbiologists, oncologists, bioinformaticians, and regulatory specialists would enhance innovation and expedite the use of microbiome research in clinical settings [85].

Public and Professional Education: Informing healthcare professionals and the public about the microbiome's involvement in colorectal cancer and the advantages of postbiotics may improve the acceptability and use of personalised microbiome-based treatments [60].

Incorporating gut microbiota and postbiotics into personalised therapy for colorectal cancer offers substantial prospects for improving prevention, diagnosis, and treatment. Addressing technological, methodological, and regulatory constraints is crucial to fully realise their promise. Future progress in standardisation,

bioinformatics, personalised therapies, and multidisciplinary cooperation will be crucial in surmounting these obstacles, allowing creative and successful microbiome-based methods in colorectal cancer care.

13.8 Conclusion

Colorectal cancer (CRC) continues to pose a considerable global health challenge, requiring novel strategies for its prevention, detection, and management. The complex interplay between gut microbiota and colorectal cancer (CRC) has become a crucial focus of study, providing new perspectives on cancer biology and possible treatment approaches. This study has clarified the diverse functions of gut microbiota and postbiotics in colorectal cancer, highlighting their incorporation into personalised treatment as a possible strategy to improve patient outcomes. The gut microbiota, a complex and dynamic ecosystem, is essential for sustaining intestinal homeostasis, regulating immune responses, and affecting metabolic activities. Dysbiosis, defined as an imbalance in microbial composition, has been repeatedly linked to the development and progression of colorectal cancer (CRC). Particular microbial taxa, like '*Fusobacterium nucleatum* and *Bacteroides fragilis*', have been associated with cancer by processes that include inflammation, genotoxicity, and modification of host signalling pathways. These results underscore the potential of microbiota-derived indicators for colorectal cancer risk evaluation and early identification, in addition to serving as targets for therapeutic intervention. Postbiotics, the bioactive metabolites and constituents resulting from microbial fermentation, signify a new area in microbiome research. In contrast to probiotics, postbiotics provide improved stability and safety, alleviating apprehensions associated with the use of living microorganisms. Short-chain fatty acids (SCFAs), especially butyrate, have anti-inflammatory and anti-proliferative properties in colorectal cancer (CRC) models, although other postbiotic substances have shown potential in modifying immune responses and promoting apoptosis in cancer cells. Preclinical research and rising clinical data indicate the therapeutic promise of postbiotics as adjunct medicines in colorectal cancer therapy, indicating their ability to enhance the effectiveness of conventional therapies and reduce unwanted effects. Incorporating gut microbiota and postbiotics into personalised medicine frameworks has considerable potential for colorectal cancer care. Customised strategies that adjust therapies according to individual microbiome profiles and genetic predispositions may enhance therapeutic effectiveness and reduce adverse effects. The classification of CRC patients according to microbiota makeup facilitates the discovery of distinct microbial signatures linked to disease progression and therapy efficacy. Customised postbiotic treatments aimed at re-establishing microbial equilibrium and addressing pathogenic processes, signify a significant progression in personalised oncology. Nonetheless, many obstacles hinder the extensive practical implementation of microbiome and postbiotic-based therapies. Technical and methodological constraints, such as the absence of standardised techniques and the intricacy of

microbiome data, need progress in bioinformatics and multi-omics integration. The heterogeneity in microbiota makeup across individuals and the restricted scope of clinical studies hinder the effective translation of preclinical results into reliable clinical applications. Moreover, regulatory obstacles and the need for explicit standards on postbiotic classification and quality control persist as substantial impediments. Future outlooks in this domain highlight the need for extensive, multi-centre clinical studies to confirm the effectiveness and safety of postbiotic treatments across varied patient demographics. Improvements in sequencing technology and bioinformatics tools will augment our capacity to precisely characterise the microbiome and find relevant microbial targets. Cooperative endeavours among academics, doctors, and regulatory agencies are crucial for formulating standardised procedures and creating explicit regulatory frameworks. Furthermore, promoting multidisciplinary cooperation and incorporating educational activities would enable the smooth integration of microbiome-based methods into clinical practice. The incorporation of gut microbiota and postbiotics into personalised therapy signifies a revolutionary strategy in combating colorectal cancer (CRC). Utilising the symbiotic link between the host and its microbial residents, personalised therapies may provide more effective and customised strategies for CRC prevention and therapy. Confronting present hurdles via collaborative research and technological innovation will be crucial in unlocking the whole potential of microbiome-based therapeutics, therefore enhancing patient outcomes and progressing the field of cancer [2].

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Chapter 14

Therapeutic Strategies of Targeting Gut Microbiota in Colorectal Cancer Management



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Abstract The collective group of bacteria, fungi, viruses, Protista, and *Archaea* predominantly regulate the gastrointestinal tract and produce metabolites for a life-time. The gut microbiota affects several processes, such as colonization, pathogen resistance, intestinal epithelium maintenance, metabolism of food and medication, immune function regulation, and behavior regulation. Humans contain trillions of different microbiotas, but in the case of dysbiosis, a change in microbiota from

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healthy to unhealthy promotes disease condition. Dysbiosis can be altered by certain factors such as antibiotics, drugs, diet, genetics, physical and psychological stress, etc. Dysbiosis is the main responsible key to colorectal cancer. The gut microbiota influences epigenetic modifications in colorectal carcinogenesis. The Pathogenic bacteria can replace harmless commensal bacteria such as *Lactobacillus*, *Bacteroides*, *E. coli*, and *S. epidermidis*, causing inflammation in the host, promoting cell proliferation, and inducing carcinogenic signals and metabolites. Strategies of gut microbiota modulation are explained in this topic, such as postbiotics, probiotics, prebiotics, nutraceuticals, mucosal immunomodulation, enhancement of gut barrier function, gene therapy, dietary habits, microbiota and CRC RT, fecal microbiota transplantation, and drugs. Interaction between intestinal flora and colorectal cancer with a detailed explanation of this can stimulate inflammation and promote tumor formation through many inflammation-related signaling pathways such as NF- κ B, STAT3, Wnt/ β -catenin epigenetic changes, and carcinogenetic changes. It is found to be the impact of gut microbiota on treatment toxicity in CRC with inhibition of CRC proliferation but also induces its metastasis via succinate receptor signaling.

Keywords Radiotherapy · Fecal microbiota transplantation · Colorectal cancer · Gut barrier · Probiotics

Abbreviations

5-FU	5-Fluorouracil
APC	Adenomatous polyposis coli
BGs	β -glucuronidases
CD4 ⁺	Cluster of differentiation 4
CDT	Cytolethal distending toxins
COX-2	Cyclooxygenase-2
CT	Chemotherapy
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CVS	Cardio vascular diseases
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
FadA	Adhesion A - <i>Fusobacterium nucleatum</i>
FM	Fecal microbiota
FMT	Fecal microbiota transplantation
Foxp3	Forkhead box protein 3 gene
GALT	Gut-associated lymphoid tissue
GBM	Gut-brain axis
GIMs	Gastrointestinal microbes
GIT	Gastrointestinal tract
GM	Gut microbiota
GPCR	G-protein-coupled receptors
HDAC	Histone deacetylases

IBD	Inflammatory bowel diseases
IECs	Intestinal epithelial cells
IgA	Immunoglobulin A
IL	Interleukins
IT	Immunotherapy
LPS	Lipopolysaccharides
miRNAs	Micro ribonucleic acid
siRNAs	Small interfering RNAs
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
PD-1	Programmed cell death protein 1
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
PRRs	Pattern recognition receptors
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
Smad4	Suppressor of mothers against decapentaplegic homolog 4
STAT3	Signal transducer and activator of transcription 3
TGF- β	Transforming growth factor-beta
Th17	T helper 17
TLR	Toll-like receptor
TIMER	Translocation, Immunomodulation, Metabolism, Enzymatic degradation, and Reduced diversity
TME	Tumor microenvironment
TNF- α	Tumor necrosis factor alpha
UC	Ulcerative colitis
Wnt	Wingless-related integration site

14.1 Introduction to GM and CRC

The stomach is the primary origin of the human microbial community. The gut-brain axis is a complex network that regulates the immunological function and influences colonization, resistance to pathogens, medicine, and food utilization, intestinal epithelial cell restoration, and cognition. There is a wide variety of different microbes in the gut's microbiota, with 300–1000 species recorded in the colon, the section of the GIT with the highest microbe population. So far, bacteria have been the most studied and abundant portion of the gut's ecosystem, with an estimated 30–40 species accounting for 99% of the species. In terms of dry mass, bacteria account for up to 60% of excrement. Anaerobic organisms make up more than 99.9% of the microbes in the gut, whereas aerobic microbes may grow in significant concentrations in the cecum. Human gut bacteria may contain genes with a density 100 times greater than that of human genetic material as a whole [1]. Some gut microorganisms assist the human host by breaking down food fiber into SCFAs like butyric acid and acetic acid. In addition, intestinal bacteria are involved in the

metabolism; a few types of bacteria are found in the stomach and small intestine; the majority of gut bacteria, more than 99.9%, are anaerobes [2]. There is evidence that the gut microbiome influences host phenotypes through a number of signaling connections, including the gut and its organ axis (and brain, kidney, and liver). These interactions can occur directly or indirectly, either due to bacteria directly interacting with different intestinal cells or due to the microbial composition or their molecules [3]. GM affects the growth and operation of neutrophils, APCs, and several types of natural cells; it plays a fundamental role in the body's defenses, including peripherally and centrally [4]. The term "intestinal microbiota" describes the communities of bacteria that individuals have in their intestines. Protozoa, fungi, viruses, bacteriophages, and bacteria make up this ecosystem. The gut bacteria influence both metabolic regulation and host immune response maturation. Microbes serve as a protective barrier against harmful organisms. The microbes are essential for the breakdown and metabolization of breastfeeding milk, colostrum, and nursing meals in newborns, as well as a wide range of foods in individuals. They also aid in the decomposition of toxins and medication, enzyme synthesis, and the uptake of the homeostasis regulation of the autoimmune system, including resistance to food antigens, and the expansion and maturation of epithelium cells covering the lumen of the GIT are among the physiological activities [5]. CRC, also known as bowel, colon, or rectal cancer, starts in the colonic region or rectum. Some of the indications that may have occurred include changes in bowel habits, lack of appetite, abdominal discomfort, and extreme fatigue [6]. While inherited defects do play a role in a small percentage of CRC cancer cases, aging and lifestyle changes account for a large percentage of cases [7]. Consumption of alcoholic beverages, animal products, and processed food all contribute toward an elevated risk. Some of the inherited genetic disorders that can cause CRC include familial adenomatous polyposis and hereditary nonpolyposis colon cancer. However, these represent less than 5% of cases. It usually manifests as an adenoma or other benign growth at the outset, but it may develop into a malignant tumor later on [8]. Approximately 75–95% of CRC incidences happen in people who have no or little inherent threat. Potential risk variables include gender categories, aging, excessive consumption of sweets, animal products, processed food, beverages, and fats, being overweight, and inadequate fitness. Insufficient activity accounts for approximately 10% of cases. The dangers of liquor seem to increase with daily use. Moreover, consistently consuming five glasses of water daily reduces the incidence of CRC and adenomatous polyps [9]. People with inflammatory bowel illness have a higher risk of CRC. The length of the illness and the intensity of the inflammation accelerate its severity. In this high-risk group, regular colonoscopies are recommended. The detection of CRC risk factors through endoscopy surveillance may aid in preventing them [10]. The epidermal cells lining the GIT are the origin of the disease. Genetic alterations typically trigger the disease by boosting Wnt signaling activity, which affects these cells' capacity to function correctly, including maintaining the lining. Both hereditary and acquired mutations are possible, and they are most likely to start in the intestinal crypt cells. In all CRC cases, the APC gene is considered the most often altered gene [11]. So, maintaining homeostasis requires a healthy microbiome, and

certain species have demonstrated a clear correlation with cancer prevention. For instance, *Bifidobacterium* and *Lactobacillus* species are the most researched protective microorganisms that possess anticancer capabilities in a variety of ways. These processes include their impact on the divisions and death cells, control over the host defense system, and elimination of harmful substances besides antibiotics [12]. These processes include their impact on host immunity, cellular proliferation, apoptosis, and the neutralization of xenobiotic and carcinogenic chemicals. The anti-inflammatory and anticarcinogenic properties can also be shown by other colonic bacteria, such as *Rubrococcus*, *Clostridium*, *Eubacterium*, and *Faecalibacterium*, when they are consumed, resulting in the production of butyrate SCFA. Colonic epithelium and immune cells' histone deacetylase is inhibited by SCFA through its interaction with GPCR [12]. During the process of cancer development, helper T cells that cause inflammation or primitive myeloid stem cells that show signs of cancer attract T lymphocytes, inflammatory cytokines, and chemokines. What makes this environment favorable for tumor growth? It includes things that cause angiogenesis and its progression, tissue remodeling enzymes, and the blocking of antitumor T cell responses [13]. It is anticipated that the incidence of CRC will rise gradually due to changes in Western eating patterns around the world. By 2030, the number of new cases is expected to reach 2.2 million. Research has indicated that around 90% of CRC is incidental, with the remaining 4% being due to genetic predispositions or exposure to particular environmental variables. The external environment is a major cause of cancer. Specifically, lifestyle variables that include obesity, poor fiber intake, alcohol consumption, smoking history, physical activity, and western diet are key contributors to CRC [13, 14]. In addition to providing a highly resolved picture of the human microbial profile, metagenomics data gathered from fecal and tissue samples also shed light on individual host-gut microbial interactions. Many fundamental and clinical studies have demonstrated that a major feature of CRC is the imbalance of gut microbes. There is a big rise in types of bacteria that can cause cancer, like *E. coli*, *B. fragmentis*, *Peptostreptococcus anerobius*, *Micromonas parvum*, and *F. nucleatum* [15]. Genetic and epigenetic changes accumulate as a result of the progressive development of rectal cancer, which leads to genetic and epigenetic changes. Throughout this phase, hyperplasia, or the transition from normal to hyperproliferative epithelium, occurs. When hyperplasia occurs, the intestinal epithelium becomes dysplastic, losing its distinctive architecture and functions. These polyps have the potential to penetrate. Research has demonstrated the crucial role of microbiota in the transition from adenoma to carcinoma, as well as in its genesis and proliferation in the genesis and proliferation of it [16].

14.2 Concept of Gut and Its Microbiota Dysbiosis

Dysbiosis, also known as dysbacteriosis, occurs when the microbiota malfunctions. These changes can affect microbes' metabolism, physiology, and distribution. The human microbiota, including the flora in the gut or vagina, may disappear, resulting

in the underrepresentation of typically dominant species and the proliferation of typically outcompeted or confirmed species to replace them. People most frequently describe dysbiosis as a GI system disease [17]. The most varied bacteria are located in the gut, and they are essential to human health. Small intestine bacterial overgrowth, which is frequently caused by a reduction in food and waste flow through the GIT after surgery or other preexisting disease, is one scenario in which dysbiosis in the GI system is most noticeable. Small intestine bacterial overgrowths can cause symptoms such as diarrhea, discomfort after eating, malnourishment, and stomach pain, as well as an overabundance of fungus in the gut, which may be indicative of small intestine bacterial overgrowth [18]. A variety of factors can cause dysbiosis to develop at various stages of life. Antibiotics, for example, frequently play a major role in microbiome disturbance. Antibiotic use during early childhood development may cause dysbiosis or unfavorable GI problems later in life. The use of antibiotics alters the gut flora and increases the risk of developing disorders like UC, IBD, and obesity, among others. A clear correlation between the gut microbiome and the GI immune mechanism, which is why antibiotic damage can make it difficult to heal [17].

14.2.1 Causes of Gut Microbiota Imbalance

14.2.1.1 Physical and Psychological COX-2

The BBB and intestinal barrier must remain intact in order to shield the body from outside stimulants and internal environment disruption. Chronic stressful situations may alter the distribution and architecture of the intestinal microbiome. It can also cause changes in metabolites produced by microbes, including chemokines, cytokines, and transmitters, which influence performance by activating the central as well as peripheral neurological systems [19].

14.2.1.2 Antibiotics and Antimicrobial Agent

Dysbiosis can manifest at different stages of life for a variety of reasons. Antibiotics frequently play a major role in microbiome disturbances. This occurs because different types of microorganisms will respond differently to antibiotics, meaning that the antibiotic may alter both the overall population of microbes and their balance. The use of antibiotics during early childhood development might cause dysbiosis or unfavorable gastrointestinal problems in later life [20]. Additionally, it heightens susceptibility to colonization, modifies metabolism, and fosters the development of antibiotic resistance in bacteria [21].

14.2.1.3 Food

Colic bacteria ferment a wide range of dietary carbohydrates, including resistant starch, dietary fibers, polyols, and nondigestible oligosaccharides, to form colonic food. Junk food lacks diversity and can lead to detrimental effects such as increased proliferation of harmful germs and inflammation. A lot of research has shown that eating processed foods may increase the number of bad microbes in the GI tract. These microbes cause inflammation and changes in metabolism, and they also help pathogenic *Firmicutes* grow, which are linked to inflammation and obesity.

14.2.1.4 Genetics

Genetics influences the quantity of certain bacteria in the gut microbiota. Genetically linked family members have more microbiota similarities than unrelated family members. The microbiotic similarity of monozygotic twins is higher than that of dizygotic twins [22].

14.2.2 The Connection Between Dysbiosis and CRC

The changes in the gut environment eventually cause colorectal cancer, which is good for pathogens like *Fusobacterium*, *Roseburia*, and *Streptococcus bovis* that are looking for a chance to grow. Symbiotic bacteria cause tissue malignancy through cellular DNA damage. Researchers have discovered that resident bacteria, a specific subset of colonizing gut bacteria, play a crucial role in preserving gut homeostasis. Reduced population growth of *Blautia*, *Bifidobacterium*, and *Faecalibacterium*, as well as the occurrence of *Porphyromonas* and *Mogobacterium* species, result in mucosa degradation, which results in the formation of cancer cells [23]. Sometimes the microbiome consists of a signaling pathway; chemicals may raise the chances of CRC. Ectopic proliferation of oral pathogens and reduction of certain commensal species may contribute to the development of CRC. We found some bacteria to be crucial mutants, extensively promoting tumor-positive *E. coli*, *F. nucleatum*, and other related species. In addition, *S. typhimurium* attaches to the intestinal epithelium and steals from mucosal macrophages, causing acute gut inflammation. It is known that acute infections change the microbial ecology over time. During inflammation, the body produces endogenous luminal sulfur and reactive oxygen species. Persistent chronic infections have the potential to alter the composition and function of the intestinal microbiome, thereby increasing the risk of tumor formation.

14.3 Methods for Metabolic Regulation of CRC by the Gut Microbiome

Every person's digestive system is home to a unique collection of microbes called the intestinal microflora. It is very important for human health because it affects many bodily functions, metabolic processes, immunological reactions, and absorption. You can change the gut microbiota to help avoid and treat many diseases, including CRC. This part talks about different ways to change the gut microbiota, such as using nutraceuticals, postbiotics, probiotics, prebiotics, mucosal immunomodulation, improving the function of the gut barrier, gene therapy, and changing your eating habits. It also talks about how these changes can affect CRC RT, RIT, FMT, and drug development. Figure 14.1 depicts several gut-microbiome modulation strategies in CRC [24].

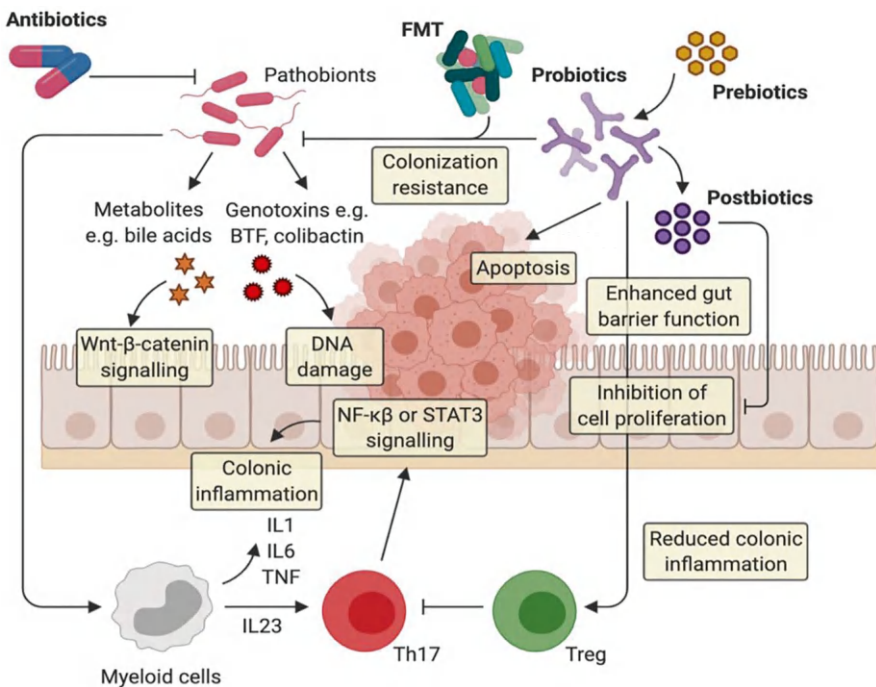


Fig. 14.1 Gut-microbiome modulation in CRC with the help of antibiotics, FMT, pre-, pro- and postbiotics. (Taken from Perillo et al. [24]; this article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license)

14.3.1 *Postbiotics*

When probiotics feed on food, they produce postbiotics, which are good things. Chemicals such as vitamins, peptides, SCFAs, and others can improve health without using live bacteria. Research demonstrates that postbiotics can aid in reducing swelling, safeguarding against free radicals, and regulating the immune system. In other words, they could help change the microbiome in the gut [25]. Postbiotics may help to maintain gut balance, improve the barrier between the gut and the outside world, and stop the spread of bad bacteria [26]. For instance, studies have demonstrated that butyrate suppresses CRC cellular function and slows down tumoral growth. This means that they might help people avoid getting CRC [27].

14.3.2 *Probiotics*

Humans can consume probiotics, which are beneficial microbes. They can change the components of the microbiome, accelerate the gut barrier function, and change how the immune system reacts [28, 29]. For example, researchers are considering research on *Lactobacillus* and *Bifidobacterium* species to understand their role in maintaining gut health and suppressing diseases. Probiotics have demonstrated the ability to decrease swelling, inhibit the growth of harmful microbes, and enhance the efficacy of CRC treatments [30]. A study revealed that a combination of prebiotics and probiotics could alter gut bacteria and reduce oxidative stress, thereby reducing the incidence of CRC in animal models [31].

14.3.3 *Prebiotics*

Prebiotic-containing foods do not undergo digestion, but they aid in the growth and function of beneficial gut bacteria. A few of them are inulin, *fructooligosaccharides*, and *galactooligosaccharides*, which are fibers. *Bifidobacteria* and *Lactobacilli* are two types of probiotics that help grow [32]. Different kinds of bacteria can live in the gut, more SCFAs can be made, and the gut barrier can work better [33]. Prebiotics reduce CRC by maintaining beneficial bacteria homeostasis while preventing harmful bacterial populations. One study found that prebiotics given to mice with CRC decreased tumor growth and raised their odds of survival [34].

14.3.4 Nutraceuticals

Nutraceuticals are bioactive compounds found in foods. These compounds have health effects beyond basic nutrition. These compounds, such as omega-3 fatty acids, flavonoids, carotenoids, and polyphenols, have demonstrated the ability to alter gut flora and combat cancer. In addition to helping good bacteria grow, nutraceuticals can also lower inflammation and stop cancer cells from spreading [35, 36]. Fruits and vegetables contain polyphenols that alter the gut microbiota and lower CRC by encouraging good bacteria growth and reducing inflammation [37].

14.3.5 Mucosal Immunomodulation

It refers to changing the immune mechanism at the surface of the gut mucosa to maintain homeostasis and downregulate inflammation. Interesting fact: GALT is an important component of the immune system's monitoring and response to bacteria [38]. One method for altering mucosal defense is to increase cytokines, suppress inflammation, and regulate T cell expression through probiotics, prebiotics, and postbiotics [39]. Evidence suggests that modifying the mucosa's immune system can lower inflammation and halt the spread of CRC by preserving gut homeostasis and averting dysbiosis [40].

14.3.6 Improvement of Gut Barrier Impairment

It acts as a physical and functional barrier known as a leaky gut, preventing harmful substances and germs from passing across the intestinal lumen and entering the bloodstream. It appears to be associated with many health issues, one of which is colon carcinoma [41]. Prebiotics, postbiotics, and probiotics can improve gut barrier function by strengthening tight junctions, reducing inflammation, and speeding up mucosal healing [42]. In animal models, probiotics altered gut flora and reduced inflammation, improving gut barrier function and reducing the growth of CRC [43].

14.3.7 Gene Therapy

Gene therapy is a novel approach to monitoring gut microbiota and preventing disease progression, although it is still in its early phases [44]. Gene therapy treats disease by transferring, removing, or changing genetic material in a patient's cell. Gene therapy can either increase the expression of good genes or downregulate the expression of bad genes in gut microbiota regulation [45]. This technique may

influence the make-up and role of the microbiome throughout the digestive tract, reducing inflammation and slowing the progression of CRC [46].

14.3.8 Dietary Habits

What you eat has a big effect on the gut bacteria and how it works. Researchers have found that eating a diet rich in plant-based foods and cereals lowers the risk of heart disease and helps good bugs grow in the gut [47]. Research has demonstrated a link between an imbalance and an increased risk of CRC [48]. Reduced red meat consumption and increased fiber intake have been found to alter gut bacteria and reduce the risk of CRC [49].

14.3.9 Microbiota and CRC RT

Ionizing radiation, which is used in RT, kills cancer cells. This is a typical approach to treating CRC. Radiation treatment can damage normal gut tissue and alter gut flora, thereby causing dysbiosis and inflammation [50]. Probiotics, prebiotics, and postbiotics can change the gut flora, lowering inflammation, enhancing gut barrier function, and accelerating mucosal regeneration after radiation [51]. Researchers have found that changing the gut bacteria can make radiation treatment for people with colorectal cancer work better and have fewer side effects [52].

14.3.10 Radioimmunotherapy (RIT)

It combines RT and IT to help the immune system fight cancer cells better. Changing the bacteria in the gut can help RIT work better by reducing inflammation and improving the immune system's ability to fight cancer [53]. Animal tests with colon cancer show that probiotics help RIT work better and the immune system responds better to them [54].

14.3.11 Fecal Microbiota Transplantation (FMT)

It entails transmitting microbes from a donor in good health to a recipient's GIT and drastically altering the recipient's gut microbiome. FMT may be beneficial in treating a variety of digestive issues, including infections with *Clostridium difficile* and IBD [55]. FMT can improve CRC treatment by regulating gut flora, reducing inflammation, and improving overall health [56]. By enhancing survival rates, FMT

changed gut flora and reduced inflammation to prevent tumor growth in CRC in vivo models [57].

14.3.12 Drugs

Researchers developed a wide range of medications specifically to alter the composition and activity of the gut microorganisms. Antibiotics, probiotics, prebiotics, and postbiotics modify the gut microbiome to enhance gut barrier performance and decrease inflammation [58]. Prebiotics, postbiotics, and probiotics help to produce beneficial bacteria and improve gut health, whereas therapeutic antibiotics target and kill pathogenic bacteria [59]. According to a recent study, modulating the gut microbiota with medication reduces CRC and improves the efficacy of conventional cancer therapies [60].

14.4 Interactions Between Intestinal Flora and CRC

The human gut microbiota has received a lot of attention recently, particularly with the advancement of metagenomic studies, which have allowed for the elucidation of its species variety and potential applications. The gut microbiome comprises trillions of microbes inhabiting the digestive system [61]. Host genetics, diet, and environmental factors initiate the process in a newborn and modulate it. Changes in gut microbiota composition have an impact on GB function, digestion, metabolism, and immune defense. Additionally, it might cause inflammation through an imbalance in gut microbiota and result in illnesses like gastrointestinal, metabolic, immunological, and neuropsychiatric diseases [61].

A growing body of evidence links genetic modifications to a variety of persistent nontransmissible illnesses, including overweight or obese individuals, CVS, and T2DM [62]. One of the key challenges in current research is to gain a better understanding of the molecular mechanisms that underlie such associations. For example, this complex microbial community acts as a metabolic interface, converting food and other ingested compounds into metabolites finally detected in host feces, urine, and blood [62]. Many of these metabolites are microbe-specific and influence human physiology. For example, the gut microbiota ferments dietary complex polysaccharides such as fiber into SCFA metabolites that play a positive role in nutritive and immune regulation [62].

In the 2019 study, Zhang et al. sought to discover the impact of GMs on CRC initiation and development by studying alterations of the microbiota in CRC patients. They performed Amplicon 16S rRNA gene sequencing and analyzed the mucosa-luminal interface microbiome [63]. We used PICRUST to predict the bacterial functional profiles at the microbial level. Comparisons of the genotypic compositions between the CRC patients and the healthy individuals showed differences in

the microbial composition. Actually, there wasn't much change in the types of butyrate-producing *Eubacterium* bacteria in people with CRC, but there was a big rise in *Devosia* bacteria. Such variations may indicate the potential abundance of *Eubacterium* and *Devosia* for early detection biomarkers in CRC [63]. Some types of microbes and the chemicals that make them dangerous work in different ways, including direct effects on epithelial cells that turn into cancerous cells and connections that help CRC grow by weakening the host's defenses [64]. The mechanisms involve DNA damage induction, Wnt/ β -catenin stimulation, NF- κ B activation, and impairment of nutrients or changes in cancer cell metabolism [64]. The accumulation of β -catenin was seen in laboratory samples of inflammatory or bowel cancer caused by oxidative stress. This suggests that it could be used as a diagnostic tool for CRC [65]. By affecting these and other ways, such as by changing how immune cells are recruited, stimulated, and worked, malignancy-associated fibroblasts and ECM parts in the GMs weaken the immune system and cause a TME that changes the course of the disease. Understanding the role of intestinal microbes in cancer may illuminate novel areas for therapeutic intervention in CRC prevention and treatment [64].

14.4.1 Intestinal Microbiome and CRC

The intestine hosts a complex community of microorganisms that coexist with the intestinal cells in a symbiotic relationship and maintain homeostasis; its perturbation may give rise to several intestinal disorders. Researchers have conducted extensive research on the relationship between GM and CRC since the 1960s. For instance, Laqueur et al. (1967) showed that intestinal microbes play a part in Cycas-mediated carcinogenesis. They found that Cycas caused cancer in normal rats but not in rats that did not have any germs [66]. More recently, Zhang et al. (2018) discovered 24 bacteria associated with CRC by examining inflammation and FM indicators across different stages of CRC. Its associated bacteria comprise *Campylobacter jejuni*, *Escherichia coli*, *Bacteroides fragilis*, and *Fusobacterium nucleatum* [66].

Moreover, *Fusobacterium nucleatum* promotes the growth of normal cells into cancerous cells via activation of β -catenin/Wnt pathways and through FadA binding to E-cadherin, promoting NF- κ B and TLR4, which primes to an increase in carcinogenic miR21 [66]. In this way, *F. nucleatum* can raise levels of proliferating cell nuclear antigen and inflammatory markers, which helps the Apc^{Min/+} mouse model develop tumors. It can also work with other bacteria to help cancer spread and become resistant to treatment by increasing the expression of miRNAs related to autophagy and starting up genes related to glycolysis [66]. Gram-negative anaerobic *Escherichia coli* colonizes the gut soon after birth. Fecal strains typically belong to taxonomic categories A and B1, while infectious strains carrying virulent agents are primarily classified into groups B2 and D. Some of the strains isolated from these groups have been associated with IBD, a known CRC risk factor [66]. The

pathogenic *E. coli* can induce CRC through DNA breaks and chromosomal aberrations. *E. coli* secretes colibactin, which speeds up the development of CRC in mouse models. CRC genomes have accumulated sequences that bind colibactin [66].

Bacteroides fragilis is an enteric bacterium that symbiotically exists in the gut but, under appropriate conditions, can cause CRC. Enterotoxigenic *B. fragilis* (ETBF) makes a poison called BFT that breaks down the epithelial barrier, cuts E-cadherin, and starts the Wnt signaling pathway. This causes inflammation and the growth of tumors [66]. ETBF turns on STAT3 pathways and boosts NF- κ B and Wnt even more by starting IL-17 production. This creates an inflammatory environment that is good for the growth of CRC. Furthermore, ETBF stimulates the expression of spermine oxidase, resulting in ROS production and cytokine release. This leads to DNA damage, which in turn promotes the initiation of CRC [66].

Campylobacter species, including *C. jejuni*, express **cryptolectal** distending toxin that generates broken DNA double-strands through the function of the enzyme DNase. The ability of enteric pathogen *C. jejuni* strain, isolated from humans, to starkly increase tumor incidence and size in sterile **Apc^{Min/+} mice** was determined, changing both macro transcriptome and gut microbiota. A mutant variant of *C. jejuni*, bearing a defective CDTb subunit that is unable to produce CDT, is virtually noncarcinogenic; therefore, the DNA damage responses in intestinal tissues seem to play a role [66].

14.4.2 Mechanism Linking Gut Microbiota to CRC

Changes in intestinal microbiota abundance and structure can disrupt the microbial balance, reducing probiotics and increasing pathogenic bacteria. The latter produces toxins that directly damage IECs, causing chronic inflammation [66]. During this inflammation process, cytokines and chemokines activate CRC signaling pathways. Furthermore, epigenetic modifications allow invader microbes to take over host genomes and modify gene regulation in recipient cells, allowing them to participate critically in CRC development [66]. Therefore, monitoring these changes in diseased situations may provide insight into how to control the host immunity [67].

The GIMs modulate naturally occurring and adaptive immunity communications, which in turn govern mucous and systemic immune responses. In this regard, microorganisms are able to activate IECs, PRRs, macrophages, and lymphocyte (B and T) cells [66]. Microorganisms stimulate immune responses through M cell activation of epithelial macrophages, thereby stimulating innate immunity and promoting CRC. In a similar way, adaptive immunity works by having B cells find tumor antigens and make antibodies against them with help from CD4⁺ T cells [66]. Adaptive immunity modulates the composition of GMs, particularly the secretion of IgA by B and T cells, by interacting with intestinal microorganisms to model the microbiota in various ways [66]. The release of TNF- α and ILs (6, 8, and 17) from the inflammatory cells is responsible for CRC development. In the end, this causes an infection with *Enterococcus faecalis*, the production of TGF- β , stimulation of

epithelial Smad4 in the intestine, and the production of ROS and H₂O₂. DNA damage is indirectly known to activate the NF- κ B inflammatory cascade, which promotes CRC progression [66]. Along with this, *E. faecalis* is involved in the COX-2 bystander effect. This means that macrophages release TNF- α , which causes chromosomal instability and cell transformation. *Helicobacter pylori* promotes CRC through epithelial damage via IL-8-mediated mechanisms. The STAT3 induction was required for Th17 cells to release and promote inflammation and cancer, as well as other known markers such as NF- κ B. ETBF also turned on STAT3, which helped Th17 cells release IL-17, Wnt, and NF- κ B, which created an inflammatory TME [66].

Further ahead, epigenetic modifications are heritable and reversible, and they regulate gene expression [67]. These modifications are influenced by the intestinal flora, which in turn influences CRC development. Notably, DNA methylation, histone modifications, and chromatin aberrations are among the most important epigenetic events. *Leuconostoc mesenteroide* treatment downregulates carcinogenic miRNAs, such as miRNA-21 and miRNA-200b [66]. Some metabolites from microbes, like butyric acid, which blocks HDAC, help Treg cells by modifying the promoter, histone H3, and Foxp3. The SCFAs, which are made up of acetic and propionic acids, cause the production of anti-inflammatory IL-10RA and antimicrobial peptides. These peptides lower the expression of NF- κ B and stop colitis from happening. In another way, GM may epigenetically alter gene expression related to tumors by enhancing DNA methylation at CpG sites in CRC cells [66].

14.4.3 Diagnosis

Causality studies have demonstrated the contribution of GMs to the onset and progression of CRC, leading to their consideration as another causative factor in CRC development. Each of the CRC stages in both fecal and mucosal samples exhibited alterations of specific gut microbes, indicating their potential as diagnostic tools in CRC [15]. Although the diversity of microbes varies greatly from person to person, this research hints at a possible association between feces microbial imbalance and CRC prognosis [15].

Researchers have linked CRC to bacteria such as *Parvimonas micra*, *Fusobacterium nucleatum*, and *Solobacterium moorei*. Studies have also demonstrated the potential of microbe gene markers to differentiate CRC from healthy subjects. These findings have been recently extended to the early stages of CRC with the identification of specific bacteria and metabolites that may potentially be used as early diagnostic markers [15]. Furthermore, the gut fungal and viral communities have some potential as CRC biomarkers, as evidenced by alterations in their fungal and viral profiles in CRC patients compared to healthy controls. This implies the potential integration of microbial, fungal, and viral markers into clinical practice for CRC detection [15].

14.5 The GM Effect on the Toxicity of CRC Treatment

In the treatment of CRC, GM interacts with chemotherapeutic agents such as irinotecan and 5-FU. The gut absorbs them, and they trigger effects that involve drug metabolism and enzymatic degradation, leading to varying therapeutic outcomes. Bacteria, among them *Fusobacterium nucleatum*, are particularly involved in the resistance of CRC treatment [68]. In cases of CRC that are going through CT, chemoresistance is linked to bacteria species like *Sutterella* and *Veillonella dispar*. Such microbial candidates modulate drug responses or reduce side effects, which may be useful in the optimization of CRC treatments [68].

The liver metabolically activates irinotecan, a major chemotherapeutic drug for metastatic colorectal cancer, through activation and inactivation processes, producing the active metabolite SN-38 and its glucuronidase form, SN-38G. Microbial BGs in the colon may reactivate SN-38G, leading to damage to IECs and subsequent profuse stools [69]. Severe diarrhea, that is grade 3–4, with irinotecan use as a single agent occurs in 30–40% of patients and 11–37% with combination therapy, once more underscoring the critical role of microbiome variation in drug toxicity [69].

Emerging evidence suggests that the host's microbiome influences CT's three primary therapeutic outcomes: improving medication effectiveness, decreasing tumor incidence, and mediating toxicity. This raises the notion that GMs are important considerations for the expansion of individual therapies and demands a better understanding of microbial co-metabolism of CT agents [70]. Reports indicate a direct relationship between GMs and the pharmacological activity of several CT drugs, including cyclophosphamide, methotrexate 5-FU, oxaliplatin, irinotecan, gemcitabine, and methotrexate, as well as new immunotherapies targeting CTLA-4 and PD-L1. We can summarize these mechanisms within the framework of TIMER. Targeting GM offers a way to improve CT effectiveness while reducing its toxicity [70].

We elucidated the influence of GMs on CRC risk and progression through inflammatory inflection, DNA damage, and metabolite production, which in turn affects tumor growth. In CRC patients, dysbiosis is characterized by the suppression of beneficial commensal and bacteria-secreting butyrate, as well as an increase in harmful pro-inflammatory microbes [71]. Changes in the production of microbial metabolites (including SCFA) and polyamines that are involved in cancer metabolism are linked to CRC [71]. High-fiber diets and supplements, including polyunsaturated fatty acids, polyphenols, and probiotics, apparently may reduce the risk of CRC and improve therapeutic responses. As a result, it is likely that personalized dietary prevention of CRC will rely on strategies that modulate the GM, slow the development of established disease, and improve responses to therapies directed at cancer [71].

CT stands out among the available therapeutic options; however, GI side effects can cause unbearable pain and potentially be life-threatening if not properly managed. Intestinal mucosal inflammation caused by CT and radiation alters the

composition of the GMs [72]. These, in turn, affect host autophagy and the metabolic response to treatment. As a result of the treatments, the makeup of the gut environment changes, which is an important modulator of the side effects of treatment. Researchers have shown the usefulness of probiotics and synbiotics in preventing mucosal inflammation and diarrhea, but their role in preventing diarrhea or gastrointestinal toxicity associated with CT remains unclear [72].

Targeting GMs appears to be a promising means for CRC prevention and treatment, now implemented with a new view to dysbiosis reversal. Probiotics, prebiotics, postbiotics, antibiotics, and some of the possible benefits of FMT include restoring ecological harmony, boosting immunity, promoting gut barrier functioning, avoiding colonization by pathogens, and targeting cancerous cells with specific cytotoxicity [73]. It is thought that prebiotics work by fermenting to help good bacteria grow, selectively interact with harmful compounds, and reduce inflammation. The magnitude of such effects might be in persons in whom the genetic variation is person-dependent. Postbiotics change only the abilities of tumor cells while protecting healthy epithelial cells from apoptosis and increasing immune functions like IgA release [73].

Studies have shown that some species of *Lactobacillus* may block penetration and promote cell death in colorectal cancer cell lines. Conversely, antibiotic-induced microbiota depletion can worsen treatment toxicity, frequently requiring dose reductions or treatment discontinuation [73]. Dietary prebiotic fiber has been shown to be beneficial in mitigating irinotecan toxicity, likely through increased butyrate production. Research also demonstrates that infusing FMT from healthy donors into CT-treated or irradiated subjects effectively restores GM balance, prevents gastrointestinal toxicity, and improves survival. Myelosuppression remains one of the most common dose-limiting toxicities for many chemotherapeutic regimens [73].

14.6 Gut Microbiota's Potential Use as a Treatment Strategy

Recent research links the intestinal microbiome to CRC initiation, expansion, and death. Knowing microbial alterations in CRC progression is critical to mapping GM with this disease. Specific bacteria might turn out to be appropriate indicators for assessing the likelihood of CRC and predicting the response to CT and IT. Intestinal microflora profiles, combined with other factors such as chronological age, dietary habits, familial background, and regional context, could provide a basis for estimating CRC risk [30]. To prevent CRC, we must avoid the indiscriminate use of antibiotics in light of the intestinal microecological balance. Dietary interventions that reshape GM may contribute to the prevention and treatment of CRC. GM-based diagnostics may permit accurate identification of CRC risk factors, hence making possible the development of personalized probiotic therapies for its treatment [30].

Recent research suggests that the nanoparticle-based LPS entrapment device enhances PD-1-based resistance in CRC and offers new strategies for future microbiome-targeted therapies. When identified in various clinical contexts, these

optimal microbial compositions could serve as biomarkers, diagnostic tools, or treatment targets, potentially leading to the development of microbial therapies that enhance anticancer activity while reducing systemic toxicity [74]. Precision and personalized therapies targeting specific microbial groups are likely to be a priority in the near future, enabling breakthroughs in IT and vaccine development, the delivery of drugs against intestinal diseases, and the prevention of inflammation. One potential extension of this approach in a clinical trial is further development of the application potential of microbes as anticancer medication delivery systems and as adjuvants in conventional therapies [74].

Cancer risk factors encompass chemicals and polypeptides that detect the presence of a tumor. Antibiotics such as prebiotics, probiotics, FMT, and synbiotics, which have varying results and potential side effects, are among the various methods employed to counteract dysbiosis. People increasingly recognize personalized treatment strategies, especially those that target each individual's GM profile, as the key to preventing and managing colon cancer [75].

14.7 Conclusion

The GM is considered critical in the CRC and in response to treatment. CRC is very closely associated with dysbiosis, driven by several factors. Researchers are investigating strategies to modulate GM using postbiotics, probiotics, prebiotics, nutraceuticals, and gene therapy to restore balance and enhance treatment outcomes. Interventions like mucosal immunomodulation, diet, and FMT present an open area of research with potential benefits in modulating the gut environment in terms of its role in CRC therapy. Additional roles that link it to personalized medicine include the GM's involvement in RT and RIT, as well as in drug metabolism, both of which have the potential to alter the toxicity of treatments. This opens the door to the next generation of therapeutic approaches, which will focus on using the bad effects of therapy to their advantage by changing the intestinal microbiome and making treatment more effective overall. This will make the GM a key part of managing CRC.

Competing Interest None

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Chapter 15

Integrating Probiotics, Postbiotics, and Nanotechnology for Enhanced Treatment Efficacy in Colorectal Cancer



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Abstract Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. While conventional cancer therapies like chemotherapy and radiation can be effective, they often have significant side effects and limited efficacy. Emerging evidence suggests that modulating the gut microbiome through probiotics and postbiotics may offer a promising complementary approach for CRC treatment and prevention. Probiotics are live microorganisms that, when administered in adequate amounts, can confer health benefits to the host. Postbiotics are the bioactive compounds secreted by probiotic bacteria, including metabolites, cell wall components, and extracellular vesicles. These probiotic-derived compounds have demonstrated potent anti-inflammatory, antiproliferative, and immunomodulatory properties that can target various hallmarks of cancer. Furthermore, nanotechnology has the potential to enhance the delivery and efficacy of probiotics and postbiotics for CRC treatment. Nanoparticle-based systems can improve the stability, bioavailability, and targeted delivery of these bioactive compounds, leading to more effective modulation of the gut microbiome and its interactions with the host. This book chapter will provide a comprehensive overview of the integration of probiotics, postbiotics, and nanotechnology for enhanced treatment efficacy in CRC. It will discuss the role of gut microbiome dysbiosis in CRC development, the mechanisms of action of probiotic-derived bioactive compounds, the application of nanotechnology to improve the delivery of probiotics and postbiotics, preclinical and clinical

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evidence on the synergistic effects of these integrated strategies, and challenges and future perspectives in the development of innovative CRC treatment approaches.

Keywords Colorectal cancer · Probiotic · Postbiotic · Nanotechnology

15.1 Introduction

Cancer is characterized by uncontrolled cell growth and abnormal spread of cells in the body. Nearly every organ or tissue, including the lungs, intestines, breast, skin, bones, and blood, is susceptible to cancer development [1]. More than 100 forms of cancer exist, and they are typically classified according to the tissue or organ from which they originate [2]. One of the most prevalent malignancies in the world, colorectal cancer (CRC), affects the colon and the rectum and presents a serious health risk. It is the second most prevalent cause of cancer-related deaths worldwide and the third most common type of cancer diagnosed worldwide [3]. CRC remains a significant financial burden on healthcare systems despite advancements in screening, prevention, and treatment, particularly in high- and low-income nations [4].

Annual incidence rates (AARs) of CRC in India is 5.36 cases of colon cancer per 100,000 men, men's rate of rectal cancer is 5.17 per 100,000, and women's colon cancer rate is 4.3 per 100,000. Women have a lifetime risk of 167–500 cases of colon cancer, while men have a lifetime risk of 100–353 cases of rectal cancer. In India's Northeast and South, the risk is greatest. With 10% of all cancer cases worldwide, CRC ranks third in terms of frequency [5]. According to ACS predictions, in 2024, there will be 152,810 new instances of CRC [6]. According to WHO, it was anticipated that there were over 1.9 million new instances of CRC and over 930,000 deaths from the disease in 2020 [7].

There were significant regional differences in the incidence and fatality rates. Europe, Australia, and New Zealand had the highest incidence rates, whereas Eastern Europe had the highest fatality rates. The annual burden of CRC is expected to rise to 3.2 million new cases (a 63% increase) and 1.6 million deaths (a 73% increase) by 2040 [8]. In high-income nations, colorectal cancer incidence rates have been declining, partly as a result of successful screening initiatives. Nonetheless, since the mid-1990s, incidence rates for Americans under the age of 55 have increased by 1–2% annually. These days, colorectal cancer ranks second in women in the same age group and first in men under 50 in the US for cancer-related deaths. In the United States, approximately 30% of people under 50 who have been diagnosed with colorectal cancer attribute their illness to either genetic or family history [6, 9].

The main risk factors for CRC are age, family history, consuming alcohol, smoking, and lifestyle factors. 10–20% of patients with CRC seem to have a positive family history [10]. Patients who are infected with conditions like ulcerative colitis or Crohn's disease are at increased risk for CRC due to chronic inflammation [11].

However, the treatment for irritable bowel disease does not eliminate cancer risk, and managing the condition itself can be complex and lifelong [12].

CRC can be treatable if detected early, which makes regular screening critical for reducing mortality rates. Tumor-related characteristics of CRC can be treated by surgery, radiation, targeted therapy, immunotherapy, and chemotherapy [13]. The most promising intervention to decrease the CRC mortality rate is palliative chemotherapy intervention [14]. Nevertheless, the effectiveness of existing approaches is typically jeopardized since medications are unable to distinguish between cancerous and healthy cells, intensifying toxicity and producing unwanted side effects in humans [15]. Furthermore, chemotherapy might encourage the emergence of drug resistance while rarely succeeding in the total elimination of malignant cells [16]. Cancer cells exhibit a wide range of amazing physiologic alterations. The impact of gut microbiota composition on the start and/or course of carcinogenesis has recently been added to the list [17].

More than three million genes are encoded by the approximately 100 trillion microbes that make up the human gut microbiota, three times greater than all human cells. This varied microbial community, which is confined to the intestinal lumen of the GIT, is a habitat for 500–1000 different bacterial species, the majority of which are members of the phyla Firmicutes and Bacteroidetes [18]. The gut microbiota is established in infancy and reaches its greatest complexity in maturity and reaches its stability [19]. The composition of gut microbiota varies between individuals and it depends on age, antibiotic use, ethnicity, and nutrition [20]. The generation of anti-bacterial and anti-inflammatory chemicals, immunomodulation, mucosal barrier integrity, host nutrition metabolism, and defense against invasive pathogens are all significantly impacted by healthy gut microbiota [21].

Specific bacterial species, such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Escherichia coli*, *Streptococcus bovis* and *Streptococcus gallolyticus*, *Helicobacter pylori*, *Salmonella typhimurium*, *Clostridium sp.*, and *Citrobacter rodentium* are generally causes of CRC [22]. Carcinogenesis is induced by the microorganisms via activation of signaling pathways, toxic metabolite production, and extensive DNA damage [23]. Different studies suggest that drugs can have a major impact on the gut microbiota, which plays a major role in the development of the disease and its treatment [24]. There has been evidence that the gut microbiota contributes to improved therapeutic efficacy and that the metabolic activity of gut bacteria plays a crucial role in the worsening of adverse effects [25]. To treat CRC, for example, irinotecan is primarily administered intravenously. However, when microbial β -glucuronidase enzymes convert it into the active metabolic form, it frequently results in severe diarrhea [26]. The use of β -glucuronidase inhibitors reduces those adverse effects. Furthermore, it was shown that 5-Fluorouracil (5-FU) suppresses the growth of *F. nucleatum*, a bacterium linked to CRC. However, certain members of the intratumoral microbiota can convert 5-FU into a nontoxic form, which promotes the proliferation of cancer epithelial cells [27].

This chapter explores the cutting-edge integration of probiotics, postbiotics, and nanotechnology for enhanced therapeutic strategies in CRC. It delves into the role

of gut microbiome dysbiosis in CRC progression, highlighting the bioactive compounds derived from probiotics and their mechanisms of action. Additionally, it examines how nanotechnology can optimize the delivery of probiotics and postbiotics, supported by preclinical and clinical evidence while addressing challenges and future directions in this emerging field.

15.2 Probiotics and Colorectal Cancer

According to the Food and Agriculture Organisation (FAO) of the United Nations and WHO, “probiotics are living microorganisms when consumed in adequate amounts confer a health benefit on the host” [28]. In the early twentieth century, Nobel laureate Elie Metchnikoff from Russia first observed the beneficial effect of some bacterial strains in humans and their importance for various physiological activities in the body. Most probiotics recognized to date are Gram-positive species like *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Propionibacterium*, but a few Gram-negative species are also used like *Escherichia coli* Nissle 1917 (EcN) [29, 30].

The probiotics generally used as foods for human consumption are considered safe, with most being certified by the US FDA as generally regarded as safe (GRAS) or by the EU EFSA as the qualified presumption of safety (QPS) [31]. The consumption of probiotics has been linked to various beneficial effects. The positive influence of probiotic bacteria is primarily related to the way gut microbiota is composed, the ability to survive in the intestine when consumed orally, to alter the immune system, and the reduction of the nonbeneficial bacteria in the gut by competing for nutrients, growth factors and host cell adhesion for survival [32].

Probiotics are essential for preventing and treating many human diseases, according to recent research using animal models as well as clinical interventions [33]. The human host's disease process is influenced by the interaction between probiotics and with gastrointestinal tract (GIT), which includes the mucus layer, epithelium layer, and gut-associated lymphoid tissue. The interaction between probiotics and the human GIT, which includes the layers of mucus, epithelial, and gut-associated lymphoid tissue, plays a role in influencing disease processes within the host [34, 35]. Probiotics adhere to intestinal epithelial cells (IECs), which can stimulate mucus production, strengthening the intestinal barrier [36]. This interaction allows probiotics to outcompete pathogenic bacteria for space and resources, inhibit their growth by competing for nutrients, and lower intestinal pH by fermenting dietary fiber into short-chain fatty acids (SCFAs) [34].

CRC originates as a malignant tumor on the inner lining of the colon or rectum and typically develops over a prolonged period. The progression of colorectal cancer follows a sequence from normal epithelial cells to the formation of adenomatoid polyps, eventually leading to adenocarcinoma [37]. Factors such as gut microbiota imbalance, immune system dysfunction, and chronic inflammation may play a role in the pathogenesis of CRC [38].

Recent research indicates that the gut microbiota, the community of bacteria in the intestines, can either contribute to or protect against CRC by mediating various modifiable risk factors. Several mechanisms have been proposed to explain how changes in microbial composition may drive CRC development. These include triggering inflammatory pathways, causing direct or indirect DNA damage through harmful metabolites, altering histone function, activating cancer-promoting genes, inducing treatment resistance in tumors, and increasing the generation of reactive oxygen species (ROS) [39].

Probiotics are recognized for their role in modulating the immune system and offering various health benefits. They can help prevent and treat CRC by restoring the balance of gut microflora and interacting with immune cells to combat the disease. Through multiple pathways, probiotics protect colon epithelial cells from cancer [40]. Probiotics can offer protective effects against CRC through various mechanisms [41, 42]. These mechanisms typically function constitutively, benefiting the intestinal microbiota and shielding the colon and rectum from environmental stressors that can induce cancerogenesis. Key mechanisms include promoting eubiosis, improving the intestinal barrier, modulating the immune system in the gut, producing anticarcinogenic substances, and breaking down carcinogenic compounds in the intestines. Additionally, probiotics can trigger pro-apoptotic and antiproliferative effects on cancer cells, indicating their potential in CRC treatment. They also influence the secretion of metabolic enzymes (which convert precursor compounds into carcinogens) by interacting with other intestinal microbes [40].

15.2.1 Mechanisms of Action of Probiotics in CRC

15.2.1.1 Limiting the Proliferation of Cancerous Cells

Probiotics can inhibit the growth of cancer cells by activating apoptotic pathways, both intrinsic and extrinsic. Through a multitude of in vitro studies, the validation of pro-apoptotic effects was confirmed, showing changes in the expression or suppression of apoptosis-related proteins such as death-ligand receptors, pro-caspase, caspase-3, -8, and -9, Bax/Bak, and Bcl-2/Bcl-x proteins [43]. Probiotics also regulate the cell cycle, limiting cell differentiation and proliferation marked by altered cyclin expression. Studies have shown that *Propionibacterium acidipropionici* as well as *Propionibacterium freudenreichii*, two probiotic species, generate SCFAs like propionate, butyrate, and acetate, that trigger apoptosis in HT-29 (human colon cancer cells) and Caco-2 (colorectal adenocarcinoma cells). This process occurs through the activation of caspase-3, leading to chromatin condensation, formation of apoptotic bodies, and ROS production [44]. Additionally, regular consumption of dietary fibers indirectly increases SCFA production by modulating gut flora.

15.2.1.2 Improvement of Intestinal Mucosal Barrier

The intestinal mucosal barrier comprises physical, chemical, biological, and immune defenses. In a healthy state, it protects the gut from toxins and harmful pathogens [45]. Probiotics promote mucus secretion by intestinal epithelial cells (IECs), creating a barrier that prevents bacterial and toxin translocation while also inhibiting the adhesion and invasion of harmful bacteria in IECs [46]. They enhance the intestinal barrier by modulating the expression of tight junction proteins, such as claudin-1 and occludin, and by promoting mucin secretion, which helps suppress inflammation and supports epithelial cell repair [34]. Occludin, a transmembrane tight junction protein, plays a key role in forming the mechanical barrier of epithelial cells, and its levels are considered a marker of the intestinal barrier's integrity [47]. Research has shown that *Bifidobacterium infantis* and *Lactobacillus acidophilus* help protect intestinal permeability by regulating occludin and claudin-1 expression and preventing IL-1 β -induced activation of nuclear factor kappa-B (NF- κ B) in Caco-2 cells [48].

15.2.1.3 Regulation of the Immune System

The interaction between gut microbiota and the intestinal immune system is crucial for maintaining homeostasis. The gut microbiome helps train immune cells to effectively eliminate harmful pathogens while tolerating beneficial commensal microbes. Toll-like receptors (TLRs) on epithelial cells detect microbial factors, acting as ligands or microbe-associated molecular patterns (MAMPs), and trigger a cascade of immune responses. When gut flora becomes imbalanced (dysbiosis), it can activate the MAPK pathway and cause the nuclear translocation of NF- κ B, leading to the release of pro-inflammatory cytokines such as IL-8 and nitric oxide. This inflammation can contribute to conditions like inflammatory bowel disease (IBD) and colorectal cancer. Probiotic administration helps restore balance (eubiosis) in the gut microbiota, thereby promoting the secretion of anti-inflammatory cytokines like IL-10 and TGF- β 2 through regulatory T (Treg) cells. Treg cells, specialized T cells, suppress the immune system's response to self-antigens and commensal microbes, fostering immune tolerance [49, 50]. A conditioned medium derived from *Bacillus subtilis* (ATCC 23857) and *Clostridium butyricum* (ATCC 19398) has demonstrated apoptotic and cell cycle arrest effects on HCT116 (human colon cancer) along with SW-1116 (human colorectal adenocarcinoma) cells. This effect is linked to a reduction in inflammation, indicated by lower TLR4 and NF- κ B expression [51].

15.2.1.4 Regulating the Generation of ROS

Oxidative stress plays a significant role in the progression of CRC [52]. ROS, which are by-products of normal cellular metabolism in the GIT, are central to this process. Maintaining redox balance between antioxidation and oxidative stress by the

intestinal epithelium is essential for several intestinal functions, including nutrient digestion and absorption, immune responses, stem cell proliferation, and the apoptosis of enterocytes [53, 54]. ROS and their oxidation products can impair the antioxidant defense system of the tissues of the intestine, disrupting normal intestinal function and potentially leading to mucosal hyperplasia [55]. Research shows that the host's ROS levels are related to gut microbiota composition; for example, increased oxidative stress is linked to a reduction in *Lactobacillus* and *Bifidobacterium* populations and an observed increase in *E. coli* [56]. Studies using mouse models have revealed that a high abundance of *Bacteroidetes* in the colon helps control pathogen levels by inducing proinflammatory and prooxidative responses, which are key to preventing intestinal infections [57]. Another study using a mouse model of azoxymethane-induced CRC found that administering *Clostridium butyricum* reduced the *Firmicutes/Bacteroidetes* ratio, increased the relative prevalence of probiotics, enhanced tumor cell apoptosis, inhibited the NF- κ B pathway and IL-6 levels, and ultimately reduced CRC incidence [58, 59].

15.2.1.5 Synthesis of Anticarcinogenic Compounds

Anticarcinogens exhibit bioactive properties capable of inhibiting cancer development or neutralizing carcinogenic compounds. Probiotics can help prevent carcinogenic processes by secreting such compounds. In addition to SCFAs, probiotics generate conjugated linoleic acids along with other bioactive compounds that have cytotoxic effects on cancer cells. These extracellular bioactive compounds can be extracted and tested on colon cancer cell lines, with some demonstrating stronger tumor-suppressive effects than certain approved chemotherapeutic agents for colorectal cancer. For example, the anticarcinogenic compound ferrichrome, produced by *Lactobacillus casei* ATCC334, has shown tumor-suppressive effects on human colorectal adenocarcinoma cell lines like Caco-2/bbe, SKCO-1, and SW620. This anticarcinogenic activity is thought to be mediated through the activation of c-jun N-terminal kinase (JNK) [60].

15.2.1.6 Reprogramming the Gut Microbiota Composition

The colon hosts a large population of microbes, most of which are harmless, though some pathogenic bacteria can contribute to acute or chronic conditions such as obesity, inflammatory bowel disease, and CRC [61]. *E. coli*, a symbiotic intestinal bacterium, includes certain strains that promote inflammation in the gut and lead to the production of colicin, a potential carcinogen [62]. Pathogenic *E. coli* is often present in CRC tissues and is used as a tumor marker for staging and prognosis [63]. Additionally, *E. coli* strains with pks islands, which code for colibactin, can cause single-stranded DNA breaks, affecting disease progression [64]. Studies have shown that mice injected with *Bacillus polyfermenticus* had reduced tumor size compared to those injected with *E. coli*, and *B. polyfermenticus* reduced the expression of

ErbB2 and ErbB3 proteins and mRNA in HT-29 cells [65]. Pathogenic gut microbes, like *Bacteroides* and *Clostridium*, are also linked to CRC development [66]. In a double-blind trial with 37 CRC patients and 43 colonic polypectomy patients, the use of synbiotics (LGG, *Bifidobacterium lactis* Bb12, and oligofructose) enhanced the presence of beneficial *Lactobacillus* and *Bifidobacterium* while reducing *Clostridium perfringens* in CRC patients. The synbiotic intervention also inhibited colorectal cell proliferation, reduced colon cell necrosis, and improved epithelial cell barrier function in polypectomy patients [67].

15.2.2 Preclinical and Clinical Evidence on Probiotics in CRC

Numerous clinical trials have produced compelling evidence supporting probiotics as a supplemental treatment approach. These trials have shown various positive outcomes, such as increased diversity of beneficial bacteria, reduction of *Fusobacterium* and *Peptostreptococcus* species, enhanced gut function, decreased transmucosal permeability, improved epithelial barrier function, reduced tumor cell proliferation, and relief from symptoms of irritable bowel disease [40, 68]. A randomized study involving 398 participants, both male and female, demonstrated a lower incidence of moderate to high-grade atypia tumors when treated with *Lactobacillus casei*. These findings point to the potential preventative benefits of probiotics in CRC [69].

Moreover, administering probiotics or synbiotics at the time of surgery led to fewer postoperative complications and improvements in gastrointestinal symptoms. Results included shorter hospital stays, reduced cases of septicemia and postoperative infections, lower diarrhea rates, faster recovery, and decreased need for postoperative antibiotics [70]. Additionally, combined therapy of strain-specific probiotics and omega-3 fatty acids was found to enhance the quality of life as well as reduce chemotherapy side effects [71].

Despite these promising outcomes, the number of relevant clinical trials in this area remains limited. Larger, well-randomized, and thoroughly analyzed trials are needed to provide stronger, more definitive evidence.

15.3 Postbiotics and Colorectal Cancer

The term postbiotic was defined by the International Scientific Association of Probiotics and Prebiotics (ISAPP) as “a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.”[72]. So, postbiotic refers to substances that are generated after microorganisms cease to be alive, meaning they are dead, inanimate, or inactivated. The microorganisms in a postbiotic may consist of whole inanimate cells or structural fragments like cell walls. Many postbiotic formulations also contain microbe-derived substances, usually metabolites, peptides, or proteins, which can enhance the health benefits, though

these components are not required for a substance to be considered a postbiotic. A true postbiotic must originate from a precisely identified microorganism or a specific combination of microorganisms with known genomic sequences and be created through a clear, reproducible method of biomass generation and inactivation [73, 74].

15.3.1 *Types of Postbiotics and Molecular Mechanisms*

15.3.1.1 Short-Chain Fatty Acids (SCFAs)

SCFAs are a type of saturated fatty acids that contain six or fewer carbon atoms, including acetate, propionate, butyrate, pentanoic (valeric) acid, and hexanoic (caproic) acid [75]. SCFAs are produced by bacteria such as *Firmicutes*, *Bacteroides*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, and they serve as an energy source for intestinal cells in the host. They are important for maintaining gut homeostasis, play protective roles in CRC development, and fluctuate in response to shifts in gut microbiota composition. As a result, SCFAs are strongly linked to CRC progression [76]. Whitehead et al. [77] discovered that butyrate significantly reduced the proliferation of human CRC cell line LIM1215, with similar results showing that both propionate and butyrate inhibited the growth of HT-29 human colorectal cancer cells [78]. Additionally, butyric acid regulates the neuropilin-1/vascular endothelial growth factor pathway to block CRC angiogenesis [79]. It additionally encourages apoptosis in CRC cells by suppressing the signaling pathway of Wnt/ β -catenin [80].

15.3.1.2 Exopolysaccharides (EPS)

Extracellular polysaccharides (EPSs) are large bacterial surface molecules found in structures like capsules or pericellular mucus, and they play several key roles, including immunomodulatory, antitumor, and antioxidant functions. Research has demonstrated that EPSs can deactivate the oncogenic compound Trp-P-1 and help slow down cancer progression [81]. A study investigating the effects of exopolysaccharides (EPSs) on the growth of HT-29 cells revealed that EPSs derived from *Lactobacillus lactis* facilitate apoptosis and suppress cell proliferation. This is achieved through the upregulation of Bax, Caspase3, and Caspase9, alongside a downregulation of Bcl-2 and Survivin gene expression [82].

15.3.1.3 Heat-Killed Bacterial Cells

Heat-killed microbial cells were not recognized as part of postbiotics until 2021 when the International Scientific Association of Probiotics and Prebiotics (ISAPP) expanded the definition of postbiotics to include deliberately inactivated microbial

cells, primarily heat-killed probiotic bacteria [83]. To explore the biological functions of these microbial cells, they were inactivated through methods such as heating, chemical treatments (e.g., formalin), gamma or ultraviolet radiation, and sonication [84, 85]. Ueno et al. [86] demonstrated that administering heat-killed *Lactobacillus brevis* SBC8803 successfully preserved intestinal balance and treated intestinal inflammation, showing potential for CRC therapy. In a study by Kim et al. [87], heat-killed *B. bifidum* MG731, *L. reuteri* MG5346, and *L. casei* MG4584 were found to significantly slow tumor growth in colorectal carcinoma, providing direct evidence of the efficacy of heat-killed bacterial cells in CRC treatment.

15.3.1.4 Cell-Free Supernatant (CFS)

Cell-free supernatant (CFS) refers to biologically active metabolites produced and secreted by microorganisms. These supernatants contain compounds such as organic acids, short-chain fatty acids, bacteriocins, and other bioactive substances, and research has demonstrated that probiotic CFS can promote human health [76]. Studies have shown that CFS possesses antimicrobial, antioxidant, and antitumor properties [88, 89]. Recently, *Lactobacillus* cell-free supernatant (LCFS) has gained increasing attention for its potential role in CRC prevention [88]. The CFS from *Lactobacillus plantarum* was found to not only reverse tumor cell resistance to 5-FU and reduce CRC stem cells but also specifically inhibit tumor cell proliferation and induce apoptosis, all while sparing normal cells [90].

15.3.1.5 Tryptophan Metabolite

Tryptophan is a vital amino acid found in foods like cheese, chocolate, chicken, and eggs. In the human gut, it is metabolized by bacteria, primarily *Clostridium sporogenes* and *Lactobacillus reuteri*, into various compounds such as 5-hydroxytryptamine (5-HT), indole, and kynurenine. These metabolites act as ligands for aromatic hydrocarbon receptors (AhR), playing key roles in maintaining intestinal immune balance, mucosal barrier integrity, inflammatory responses, and neural function [76]. A recent investigation into the chemopreventive effects of statins on CRC demonstrated that atorvastatin, in a mouse model of CRC, inhibited the utilization of tryptophan by decreasing the expression of indole derivatives in the intestinal epithelium. This resulted in elevated levels of tryptophan in the gut and an increased presence of *Lactobacillus reuteri*, which metabolizes tryptophan to produce indole-3-lactic acid (ILA). ILA interacts with the transcription factor ROR γ t, leading to the suppression of Th17 differentiation, a reduction in IL-17 expression, and a deceleration of CRC progression [91].

15.4 Nanotechnology for Enhanced Delivery of Probiotics and Postbiotics

CRC remains one of the most prevalent cancers worldwide, prompting the exploration of innovative therapeutic approaches. Probiotics and postbiotics have gained attention as potential adjuvants in cancer therapy due to their roles in modulating gut microbiota, enhancing the immune response, and exerting anti-inflammatory effects. However, challenges associated with the delivery of these bioactive compounds hinder their full potential. Nanotechnology presents a promising solution by enhancing the stability, bioavailability, and targeted delivery of probiotics and postbiotics to tumor sites. This section delves into the integration of nanotechnology to overcome conventional delivery challenges and improve therapeutic outcomes in CRC.

15.4.1 Limitations of Conventional Delivery of Probiotics and Postbiotics

Probiotics, live microorganisms that confer health benefits, and postbiotics, their nonviable metabolic by-products, face several limitations when administered through traditional delivery systems.

15.4.1.1 Viability and Stability Issues

The gastrointestinal (GI) tract poses numerous challenges, including the acidic environment of the stomach, the presence of digestive enzymes, and bile salts, which can reduce the viability of probiotics and degrade postbiotics before they reach the colon [92]. Research suggests that a minimum of 10⁸ viable cells are necessary to produce therapeutic effects. However, several formulations prove inadequate in delivering this amount effectively because they become inactivated during transportation [93, 94].

The stability of live microorganisms is often compromised by the absence of protective formulations in conventional probiotic products. Probiotics may not be able to survive the processing and storage conditions without sufficient encapsulation or protective coatings, which would further diminish their viability [94, 95]. Additionally, the stability of probiotics during storage and transportation remains a concern. The need for cold chain storage and limited shelf life further complicates their use in clinical settings [96].

15.4.1.2 Limited Colon Targeting

Both probiotics and postbiotics are often nonspecific in their effects, which can reduce their therapeutic efficacy. Probiotics do not always get to the colon, where they do their good work, thanks to conventional delivery methods. Probiotics cannot fully colonize the lower intestines because they are released in large quantities in the upper GI tract. This is especially problematic for CRC treatment because the colon's localized effects are so important [93, 94]. Conventional oral administration leads to nontargeted distribution, where only a fraction of the administered dose reaches the tumor site. This lack of targeted delivery is particularly problematic in the treatment of CRC, as the tumor microenvironment (TME) requires precise modulation for optimal therapeutic outcomes [97].

15.4.1.3 Strain-Specific Efficacy

Different strains of probiotics can differ greatly in their efficacy. In clinical applications, conventional delivery methods frequently produce inconsistent results because they fail to take this strain-specific variability into account. Certain strains may have a higher specificity than others when it comes to modifying the immune response or preventing the growth of cancer cells, but standard formulations may not always account for this [98]. For instance, while some *Lactobacillus* strains may show anticancer properties, others do not exhibit similar effects, highlighting the need for strain-specific evaluations in clinical applications [99]. Probiotic efficacy may also vary depending on the particular disease setting. The fact that strain specificity implies that a probiotic that is beneficial for one illness might not be for another complicates the quest for probiotic therapies that are effective for CRC in all cases. Research has indicated that while some strains may be useful in treating the symptoms of disorders such as irritable bowel syndrome (IBS), they are not effective in treating colorectal cancer [83, 99].

15.4.1.4 Safety Concerns

Using live probiotics increases the risk of infection in patients with compromised immune systems. Because of this worry, scientists are looking into postbiotics—nonliving components made from probiotics—as safer substitutes. More thorough research is necessary to verify the safety and effectiveness of postbiotics in the treatment of colorectal cancer, as their safety profiles are still being examined [98, 100]. In certain situations, using probiotics—especially if they come from strains of bacteria resistant to antibiotics—may contribute to the emergence of antibiotic resistance. In clinical settings, where patients may already be susceptible to infections, this presents a serious risk [100].

Above all, large-scale, randomized clinical trials that definitively demonstrate the safety and efficacy of pro and postbiotics in the treatment of CRC are hard to

come by despite encouraging preclinical data supporting their use. The acceptance and incorporation of these therapies into conventional treatment protocols are restricted by this disparity. Probiotics and postbiotics have the potential to improve the treatment of CRC, but traditional delivery methods have several drawbacks, such as nonspecific delivery, safety concerns, variability in strains, and a lack of clinical evidence.

15.4.2 Nanoparticle-Based Delivery Systems

Nanotechnology offers a groundbreaking approach to address these challenges through nanoparticle-based delivery systems. These systems can protect probiotics and postbiotics from the harsh conditions of the GI tract, ensuring their safe passage to the colon, where they exert their beneficial effects. Nanoparticles (NPs), typically ranging from 1 to 100 nanometers, are designed to encapsulate bioactive compounds, providing a protective barrier that enhances their stability and viability [101]. Nanoparticle formulations can improve the stability of probiotics during storage, protecting them from environmental factors such as light, temperature, and oxygen, which can degrade their viability [102].

The most common types of nanoparticles used for probiotic and postbiotic delivery include liposomes, polymeric nanoparticles, and solid lipid nanoparticles (SLNs). Liposomes are phospholipid bilayer vesicles that can encapsulate both hydrophilic and hydrophobic substances, making them ideal for the dual delivery of probiotics and postbiotics. Polymeric nanoparticles, made from biodegradable materials like chitosan and poly(lactic-co-glycolic acid) (PLGA), offer sustained release properties, allowing for prolonged therapeutic effects. SLNs, composed of solid lipids, provide excellent protection against degradation and have been shown to improve the bioavailability of encapsulated compounds [103] (Table 15.1).

15.4.2.1 Improved Stability and Bioavailability

One of the primary advantages of nanoparticle-based delivery systems is the significant improvement in the stability and bioavailability of probiotics and postbiotics. Probiotics are sensitive to environmental factors such as temperature, humidity, and pH, which can lead to reduced viability during storage and administration. Nanoparticles provide a protective shell, shielding probiotics from these adverse conditions, ensuring their survival until they reach the target site [102]. For postbiotics, encapsulation in nanoparticles prevents their premature degradation in the GI tract. This encapsulation enhances their bioavailability, allowing for a more controlled release at the site of action. For instance, studies have shown that encapsulating short-chain fatty acids (SCFAs), a class of postbiotics, in polymeric nanoparticles significantly increases their stability and prolongs their therapeutic effects in the colon [98].

Table 15.1 Types of nanoparticle-based delivery systems for probiotics and postbiotics

Nanoparticle type	Composition	Advantages	Challenges	References
Liposomes	Phospholipid bilayer	Encapsulates hydrophilic and hydrophobic agents	Limited stability in biological fluids	[104]
Polymeric nanoparticles	Natural or synthetic polymers (e.g., chitosan, alginate, PLGA)	Improved stability and viability of probiotics Controlled release mechanisms Biocompatibility	Variability in polymer properties Potential toxicity of synthetic polymers	[95]
Solid lipid nanoparticles	Lipids (e.g., phospholipids, triglycerides)	Enhanced bioavailability Ability to encapsulate both hydrophilic and hydrophobic compounds Improved absorption	Stability issues during storage Complexity in formulation	[105]
Inorganic nanoparticle	Metal oxides (e.g., silica, titanium dioxide)	High stability and durability Potential antimicrobial properties Controlled release capabilities	Biocompatibility concerns Potential toxicity in vivo	[102]
Nanocapsule	Polymer or lipid shells encapsulating probiotics	Targeted delivery to specific sites in the gut Protection against harsh GI conditions	Challenges in scaling production Regulatory hurdles for new materials	[106]
Nanostructured lipid carriers (NLCs)	Mixture of solid and liquid lipids	Enhanced loading capacity Improved stability compared to conventional lipid carriers	Complex preparation methods Limited research on long-term effects	[107]
Mesoporous silica nanoparticles	Silica with ordered pore structures	High surface area for drug loading Controlled release properties	Potential leaching of silica Regulatory concerns regarding silica use	[108]
Chitosan nanoparticles	Chitosan (a natural polymer)	Biodegradable and biocompatible Enhanced mucoadhesion and stability	Variability in chitosan quality Limited scalability for commercial production	[106]

Nanoparticles also enhance the bioavailability of encapsulated compounds by improving their absorption through biological membranes. The small size of nanoparticles allows them to cross the epithelial barrier more efficiently, facilitating the uptake of probiotics and postbiotics into the bloodstream and their subsequent delivery to the tumor site [108, 109]. Additionally, nanoparticles can be combined with other bioactive substances to produce synergistic effects that will boost the treatment's overall effectiveness. For instance, co-encapsulating probiotics and anti-inflammatory drugs can offer a more thorough method of treating inflammatory bowel disorders and colorectal cancer [105]. Certain nanoparticles, like those with antimicrobial or immunomodulatory effects, have inherent therapeutic qualities. These nanoparticles can improve the overall therapeutic result when used in conjunction with probiotics or postbiotics because they not only deliver the probiotics but also offer extra health benefits. Natural polymers, such as chitosan and alginate, as well as synthetic polymers, can be used to formulate nanoparticle-based systems, enabling customization based on unique therapeutic requirements and patient populations [95, 102].

15.4.2.2 Targeted Delivery to the Tumor Microenvironment (TME)

Targeted delivery of therapeutics to the TME is critical for improving the efficacy of CRC treatment. Nanoparticles can be engineered to achieve this through surface modifications that enable active targeting. By functionalizing the surface of nanoparticles with ligands such as antibodies, peptides, or small molecules, researchers can direct the delivery system specifically to tumor cells or components of the TME [110].

By stimulating the production of anti-inflammatory cytokines and augmenting the function of immune cells like T cells and natural killer (NK) cells, probiotics and postbiotics can have an impact on the immune system [111–113]. Within the TME, this modulation may result in a stronger antitumor response. Dysbiosis is frequently caused by cancer treatments, particularly chemotherapy, which alters the gut microbiota. In order to sustain gut health and boost systemic immunity, a balanced microbiota composition must be restored. Probiotics and postbiotics can assist in this process [83]. A few postbiotics have demonstrated the capacity to stop tumor growth and cause apoptosis in cancer cells. For instance, by inhibiting angiogenesis and encouraging apoptosis, postbiotics made from particular *Lactobacillus* strains have shown antiproliferative effects against colorectal cancer cell lines [114, 115]. Probiotics and postbiotics can be encapsulated using nanoparticle-based delivery systems, which guarantees their stability and targeted release in the TME. This strategy can improve these therapeutic agents' interactions with cancer cells and increase their bioavailability [116]. Probiotics can aid in reducing TME inflammation, which is frequently linked to the development of tumors. Through the modulation of inflammatory pathways and the reduction of pro-inflammatory cytokine levels, probiotics can enhance anti-tumor immunity and restrict tumor growth [113].

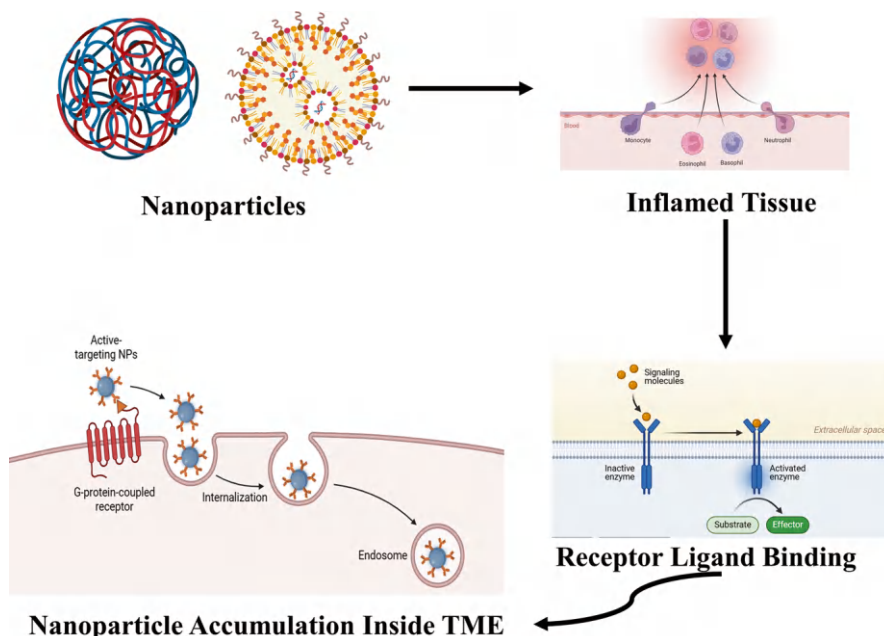


Fig. 15.1 Mechanism of targeted nanoparticle delivery to tumor microenvironment

In CRC, the TME is characterized by an abnormal vasculature, hypoxia, and an immunosuppressive milieu. Nanoparticles can exploit the enhanced permeability and retention (EPR) effect, which is a hallmark of tumor vasculature, to preferentially accumulate in tumor tissues. Additionally, ligands targeting specific receptors overexpressed on tumor cells, such as epidermal growth factor receptor (EGFR) or folate receptors, can further enhance the specificity of nanoparticle-based delivery systems [117]. Targeted delivery not only increases the concentration of probiotics and postbiotics at the tumor site but also reduces off-target effects, minimizing potential toxicity and enhancing overall treatment efficacy (Fig. 15.1).

15.4.3 Preclinical Studies on Nanoformulations of Probiotics and Postbiotics in CRC

Numerous preclinical studies have demonstrated the potential of nanoformulations of probiotics and postbiotics in CRC treatment. In vitro and in vivo models have shown that these formulations not only improve the delivery of bioactive compounds but also enhance their anticancer effects. For example, a study by Owens et al. [118] encapsulated *Lactobacillus rhamnosus* in chitosan nanoparticles and

demonstrated enhanced probiotic stability and anticancer activity in a mouse model of CRC. The nanoformulated probiotics induced apoptosis in cancer cells, reduced tumor growth, and modulated the immune response in favor of an antitumorigenic environment.

Similarly, postbiotics such as butyrate have shown enhanced anticancer activity when delivered using nanoparticle systems. Garavaglia et al. [112] encapsulated butyrate in PLGA nanoparticles and observed significant tumor suppression in CRC models. The encapsulated postbiotic not only exhibited increased stability but also showed a sustained release profile, leading to prolonged therapeutic effects. These studies highlight the potential of nanoformulations in enhancing the therapeutic efficacy of probiotics and postbiotics in CRC. However, further research is needed to translate these findings into clinical practice.

Nanotechnology has opened new avenues for improving the delivery and therapeutic efficacy of probiotics and postbiotics in the treatment of colorectal cancer. By addressing the limitations of conventional delivery systems, nanoparticle-based approaches offer enhanced stability, bioavailability, and targeted delivery to the tumor microenvironment. Preclinical studies have provided promising results, demonstrating the potential of these nanoformulations to improve cancer treatment outcomes. Future research should focus on optimizing these delivery systems for clinical application and further exploring their synergistic effects with conventional cancer therapies (Table 15.2).

Table 15.2 Preclinical studies on various nanoformulations with probiotics and postbiotics in CRC

Nanoparticle type	Probiotic/postbiotic	Findings	References
Polymeric nanoparticles	<i>Lactobacillus</i> spp.	Enhanced viability and colonization in a simulated gastrointestinal model, leading to improved anticancer effects in CRC cells	[16]
Lipid-based nanoparticles	Probiotic lysate	Demonstrated protective effects against oxidative stress and inflammation in a colitis model, suggesting potential for CRC treatment	[95]
Silica nanoparticles	<i>Bifidobacterium</i> spp.	Showed improved stability and targeted delivery to tumor cells, enhancing the anticancer activity of probiotics	[107]
Chitosan nanoparticles	Postbiotics derived from <i>Lactobacillus</i>	Induced apoptosis in CRC cells and modulated gut microbiota, showing promise in CRC prevention and treatment	[83]
Metal oxide nanoparticles	Probiotic extracts	Exhibited synergistic effects with chemotherapy, enhancing the efficacy of treatment in CRC models	[104]
Carbon nanotubes	<i>Lactobacillus rhamnosus</i>	Enhanced bioavailability and therapeutic effects against CRC, with improved immune modulation observed	[105]

15.5 Integrating Probiotics, Postbiotics, and Nanotechnology for CRC Treatment

15.5.1 Synergistic Effects of the Integrated Approach

The integration of probiotics, postbiotics, and nanotechnology in CRC treatment represents a synergistic approach aimed at enhancing therapeutic efficacy. Probiotics, which are live microorganisms, have shown the potential to modulate gut microbiota composition and improve immune responses. Postbiotics, derived from probiotic fermentation, include bioactive compounds such as short-chain fatty acids (SCFAs), enzymes, and peptides, which further enhance anticancer effects [119]. A number of probiotic strains have demonstrated promise in halting the growth and recurrence of colorectal tumors, including *Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* Bb12, and *Lactobacillus casei*. Probiotic-produced short-chain fatty acids (SCFAs) are one type of postbiotic that can stop the growth of colorectal cancer cells and trigger apoptosis [97, 116, 120]. Probiotics and postbiotics can be co-delivered by nanoparticles with chemotherapy to help overcome treatment resistance and stop metastasis. Nanotechnology, on the other hand, provides advanced delivery mechanisms that protect probiotics and postbiotics from degradation in the gastrointestinal (GI) tract, ensuring they reach the tumor site effectively [121].

The combination of these three strategies creates a synergistic effect where the probiotic microorganisms can modify the gut microenvironment to reduce inflammation and improve immune surveillance. Concurrently, postbiotics like butyrate have been shown to inhibit tumor cell proliferation and induce apoptosis in CRC cells. Nanotechnology, as a delivery tool, ensures precise targeting and prolonged release of probiotics and postbiotics, maximizing their therapeutic potential [122]. This integrated approach enhances not only the bioavailability of therapeutic agents but also their effectiveness, leading to better CRC outcomes.

15.5.2 Modulation of Gut Microbiome and Tumor-Immune Interactions

Gut microbiome dysbiosis has been recognized as a contributing factor to CRC development and progression. Probiotics and postbiotics play a critical role in modulating the gut microbiome, restoring its balance, and promoting beneficial microbial populations that support anticancer activity. Nanoparticle-encapsulated probiotic and postbiotic therapies can specifically target the colon to restore the composition of a healthy gut microbiome [83]. Several studies have demonstrated that probiotics, such as *Lactobacillus* and *Bifidobacterium* strains, can suppress inflammation by producing anti-inflammatory cytokines, thereby reducing the risk of CRC [123]. For example, *Lactobacillus fermentum* could affect tumor responses in mice using TLR9 ligands, reducing tumor DNA damage. To improve antitumor

immune responses, nanoparticles can be designed to specifically target tumor microenvironment or specific CRC cell surface markers [116].

The gut microbiota is closely linked to the host immune system, and its dysregulation can affect tumor-immune interactions. The use of probiotics and postbiotics can enhance antitumor immunity by promoting the production of SCFAs, which act as immunomodulators. For instance, butyrate produced by certain probiotic strains has been shown to promote the activation of T regulatory cells (Tregs), reduce inflammation, and limit tumor progression [124]. Nanotechnology plays a key role in this modulation by encapsulating probiotics and postbiotics in biocompatible nanoparticles, protecting them from harsh gastric conditions and allowing them to modulate the gut-tumor-immune axis more effectively [125].

15.5.3 Enhancing the Efficacy of Conventional Cancer Therapies

In addition to their standalone benefits, probiotics, postbiotics, and nanotechnology have shown promise in enhancing the efficacy of conventional cancer therapies like chemotherapy and radiotherapy. One of the major challenges in CRC treatment is the toxicity and adverse effects associated with these therapies. Probiotics have been studied for their ability to reduce the gastrointestinal side effects of chemotherapy, such as diarrhea and mucositis, by restoring gut microbiome balance [126]. Postbiotics, particularly SCFAs, have also demonstrated potential in sensitizing tumor cells to chemotherapy agents. For example, butyrate has been shown to increase the sensitivity of CRC cells to 5-fluorouracil, a commonly used chemotherapeutic drug, thereby improving treatment efficacy [127]. By modulating inflammation and enhancing immune function, postbiotics may help to reduce the systemic toxicity of conventional treatments [128].

Nanotechnology offers an innovative platform to improve drug delivery in CRC treatment. Nanoparticles can be engineered to deliver both chemotherapy agents and probiotics/postbiotics to the tumor site, enhancing the therapeutic effect while minimizing damage to healthy tissues. For instance, nanocarriers loaded with both chemotherapeutic drugs and probiotics have been shown to improve drug uptake by tumor cells, reduce drug resistance, and promote apoptosis [129]. This combination strategy enhances the overall efficacy of conventional cancer treatments and offers a promising approach for CRC management.

15.5.4 Clinical Evidence and Ongoing Trials

The potential benefits of integrating probiotics, postbiotics, and nanotechnology in CRC treatment have been supported by preclinical and clinical studies. Clinical trials investigating the use of probiotics in CRC have demonstrated improvements

Table 15.3 Synergistic mechanisms of probiotics, postbiotics, and nanotechnology in CRC treatment

Component	Primary mechanism of action	Therapeutic role in CRC	References
Probiotics	Restores gut microbiota balance, reduces inflammation	Modulates immune response, enhances gut barrier function, reduces side effects of chemotherapy	[123, 126]
Postbiotics	Produces bioactive metabolites (e.g., SCFAs, butyrate)	Promotes apoptosis, inhibits tumor growth, improves sensitivity to chemotherapy	[124, 127]
Nanotechnology	Encapsulation of probiotics/postbiotics, targeted delivery	Protects probiotics from degradation, ensures targeted delivery to tumor sites	[121, 129]

in gut health and reductions in treatment-related side effects. For example, a clinical trial by Wei et al. [130] found that the administration of probiotics to CRC patients undergoing chemotherapy reduced the incidence of chemotherapy-induced diarrhea, highlighting the supportive role of probiotics in enhancing patient outcomes.

Similarly, ongoing research into postbiotics has shown that SCFAs, especially butyrate, can reduce inflammation and tumor growth in CRC patients [83]. Clinical studies have also explored the potential of postbiotics in enhancing the effects of immunotherapy by improving gut microbiota composition and immune function [129].

Nanotechnology is at the forefront of clinical innovation for CRC treatment, with numerous clinical trials investigating the use of nanoparticles for targeted drug delivery. Nanoparticles designed to carry both chemotherapeutic agents and probiotics have shown promising results in preclinical models, enhancing drug efficacy while reducing toxicity [131]. Ongoing clinical trials are now evaluating the safety and efficacy of these nanotechnology-based interventions in CRC patients [132]. For instance, in CRC patients receiving chemotherapy, a randomized trial is assessing the benefits of a probiotic mixture containing *Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* Bb12, and *Lactobacillus casei* (NCT03024684).

Despite the promising results, several challenges remain in translating these integrated strategies into clinical practice. The stability and safety of probiotics and postbiotics during storage, the potential for immune reactions to nanoparticles, and the long-term effects of altering the gut microbiome are important considerations. Nonetheless, the future of CRC treatment is likely to benefit from continued advancements in this field, with ongoing trials paving the way for innovative, multimodal treatment strategies (Table 15.3).

15.6 Challenges and Future Perspectives

In recent years, there have been a lot of advancements to control the gut microbiota for cancer treatment. The inability of conventional CRC treatments to precisely target cancer cells is one of their main drawbacks. Chemoresistance also develops, and there are a lot of negative effects on the human being [133]. Probiotic supplementation has been suggested as a useful adjunctive treatment for CRC patients receiving chemotherapy to reduce gut dysbiosis and eliminate gastrointestinal pain [58, 59]. Nevertheless, a few studies have indicated barriers to the use of probiotics despite their proven positive health effects, which include epithelial colonization, the restoration of microbial diversity, and the detoxification of carcinogens. In the food sector, bacteriocins are employed as natural biopreservatives [134]. In addition to their use in food, bacteriocins are gaining interest as potential anticancer medicines because of their selective action against cancer cells and negligible to no effect on healthy cells. However, even with their beneficial qualities that are relevant to medicine, like their biocompatibility, biodegradability, and lack of immunogenicity, they exhibit certain basic drawbacks when taken orally, like reduced bioavailability and vulnerability to proteolytic enzymes when exposed to the GIT [135]. High-throughput sequencing may improve the discovery of new bacteriocins, while a variety of strategies, including encapsulation technologies, bioengineering, and semi-synthetic techniques, could be employed to overcome such limitations by improving their physicochemical characteristics and biological activity. Furthermore, the high complexity of bacteriocins resulting from prolonged posttranslational changes and the high cost of production associated with their effective purification continue to be significant obstacles to their large-scale synthesis [136].

Exopolysaccharides (EPSs) are multifunctional and complex carbohydrates that have garnered interest in the pharmaceutical and therapeutic domain. These properties promote health, which include immune system modulation, scavenging of free radicals, and inhibition of cancer cell growth [137]. Due to their nontoxic nature, thermal stability, biocompatibility, and biodegradability, EPSs are considered a prospective alternative to synthetic anticancer agents [138]. The various physicochemical properties of EPSs, such as their molecular weight, electric charge, branching degree, and monosaccharide composition, are closely related to their functioning. So, special methods are acquired to elucidate their chemical structure [139]. Furthermore, their commercialization is currently limited by their high production costs, low polysaccharide yields, and time-consuming purification procedures. However, there is some limited research done on gut microbiota modulation. So, there is currently insufficient clinical evidence to support the administration of the drug to humans [140].

Short-chain fatty acids (SCFAs) constitute the biggest class of bioactive compounds that are detected in the intestinal lumen. SCFAs help improve the host's health by acting as signaling molecules entering the bloodstream [141]. SCFAs exert anticancer activity by regulating the immune response, reducing HDAC activity, and repairing intestinal microecology [142]. Although SCFAs act as anticancer

agents still, some limitations should be taken care of. There are inconsistent results from in vitro and in vivo experiments. Therefore, the assessment of the local and systemic effects of SCFAs and the identification of the most advantageous route of distribution to the host remain hampered by the absence of pre-clinical and clinical evidence [143].

15.7 Conclusion

Probiotics, postbiotics, and nanotechnology together present a promising new avenue for colorectal cancer (CRC) treatment. Probiotics and postbiotics can alter the gut microbiota, lower inflammation, and strengthen the immune system—all of which are important aspects of the prevention and treatment of colorectal cancer. Their innate capacity to strengthen immune system function, lessen adverse effects, and possibly even stop tumor growth makes them an excellent addition to conventional therapies for gut health.

The therapeutic efficacy of both established and newly developed cancer treatments is improved by nanotechnology due to its precision in medication administration. Drugs, probiotics, or postbiotics can be directly delivered to malignant tissues using nanoparticles, increasing bioavailability and reducing toxicity to neighboring healthy tissues. This focused approach lessens adverse effects while also improving therapeutic success.

Combining these advancements can have a synergistic impact in which nanotechnology improves medication delivery and treatment precision while probiotics and postbiotics preserve gut health. With more individualized, minimally invasive, and effective treatment options available, this integrated approach may greatly increase the efficacy of colorectal cancer therapies. Nevertheless, more investigation and clinical testing are required to completely grasp the possibilities and maximize these state-of-the-art treatments for general clinical application.

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Chapter 16

Postbiotics, Gut Microbiota, and Colorectal Cancer: Implications for Immunotherapy



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Abstract Colorectal cancer (CRC), a leading cause of cancer-related mortality worldwide, is a multifactorial disease influenced by genetic, epigenetic, and environmental factors, including the gut microbiota. The gut microbiota, a complex ecosystem of microorganisms, plays a pivotal role in CRC pathogenesis, progression, and response to therapy. Dysbiosis—imbalances in the microbiome—has been linked to tumor initiation and immune evasion, highlighting its role as a potential therapeutic target.

This chapter explores the emerging significance of microbiome-targeting strategies in CRC management. These approaches, including probiotics, prebiotics, synbiotics, dietary modifications, and fecal microbiota transplantation (FMT), aim to restore microbial homeostasis and improve treatment outcomes. Additionally, the gut microbiome influences the efficacy and side effects of chemotherapy and immunotherapy, with specific microbial profiles correlating with treatment response.

Postbiotics, bioactive compounds produced by beneficial microbes, further enhance therapeutic potential by modulating the immune system and exerting anti-inflammatory effects. Personalized microbiome modulation, tailored to an individual's microbial profile, offers a promising avenue for optimizing CRC treatments. Dietary interventions, particularly those rich in fiber, polyphenols, and omega-3 fatty acids, also demonstrate significant potential in enhancing gut health and mitigating CRC progression.

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Future research should focus on integrating microbiome data into personalized treatment regimens, optimizing delivery mechanisms, and understanding geographical and demographic variations in microbiome composition. By advancing microbiome-targeting strategies, this work underscores the potential of leveraging gut microbiota and postbiotics to revolutionize CRC prevention, treatment, and prognosis, providing a foundation for personalized medicine in oncology.

Keywords Colorectal cancer (CRC) · Gut microbiota · Microbiome-targeting strategies · Postbiotics

16.1 Introduction

Colorectal cancer (CRC), encompassing both colon and rectal cancers, originates in the lining of the colon or rectum and has the potential to metastasize to other organ systems and lymph nodes. Approximately 95% of CRC cases are adenocarcinomas [33]. It is the third most common type of cancer worldwide, accounting for 10.2% of new cases annually, and the second leading cause of cancer-related deaths at 9.2% [48]. While only 10–15% of CRC cases are hereditary, environmental factors play a significant role in its development through genetic and epigenetic mechanisms [4].

The human gut microbiota, a complex community of viruses, bacteria, fungi, and protozoa, holds critical functions such as aiding digestion, producing vitamins (B and K), protecting against pathogens, and stimulating the immune system. Two primary models explain its influence on CRC [14]. The first model describes microbiota as pro-carcinogenic drivers, causing DNA damage in epithelial cells, which are later replaced by passenger bacteria that either promote or hinder carcinogenesis. The second model emphasizes the role of host genetics in dysbiosis, which leads to pro-inflammatory responses and epithelial damage, ultimately contributing to cancer formation [48].

In addition, postbiotics, a subset of the biotics family that also includes probiotics, prebiotics, and synbiotics, represent non-living microbial components or metabolites that exert health benefits. The microbes comprising postbiotics may be inanimate, intact cells or maybe a structure fragment from the organism such as cell walls [3]. These substances are deliberately prepared using reproducible methods for inactivation and must demonstrate health-promoting properties to be classified as postbiotics [53].

Recent advancements in CRC treatment include immunotherapy, particularly effective for tumors with deficient mismatch repair (dMMR) or microsatellite instability-high (MSI-H) features. There are different modes of action to affect cancer such as PD-1 inhibitors and CTLA-4 inhibitors. PD-1 inhibitor drugs target PD-1, a protein on T cells that prevents them from attacking other cells. By blocking PD-1, these drugs enhance the immune response against CRC cells, specifically in cases exhibiting dMMR or MSI-H ([15]) CTLA-4 inhibitors are another

immunotherapeutic agent that boosts the immune response by targeting CTLA-4, a regulatory protein on T cells. Blocking CTLA-4 promotes an enhanced immune attack on CRC cells [11].

This chapter examines the relationship between CRC and the influence of both microbiota and postbiotics on its progression and management. It also discusses immunotherapy as a treatment approach for CRC, highlighting the latest therapeutic families utilized in clinical practice.

16.2 Gut Microbiota and Colorectal Cancer

16.2.1 Composition and Function of Gut Microbiota

The gut microbiota comprises approximately 40 trillion microorganisms, which is roughly three times the number of human cells [51]. The majority of these microorganisms reside in the colon, accounting for about 30 trillion cells. This microbial community interacts closely with the intestinal epithelium, immune cells, and the mucosal barrier, playing a vital role in maintaining gut homeostasis and overall health. The initial exposure to gut microbiota occurs during birth and is influenced by the mode of delivery. In vaginal deliveries, newborns are exposed to vaginal microbes such as *Lactobacillus*, whereas those delivered via cesarean section encounter skin-associated microbes, including *Staphylococcus* and *Corynebacterium* [65]. These early microbial exposures significantly shape the composition of the gut microbiota and influence long-term health outcomes. The gut microbiota is integral to normal gut physiology, contributing to processes such as digestion, vitamin biosynthesis, heat generation, immune regulation, and the maintenance of gut homeostasis [31]. It plays a pivotal role in the development and functioning of both the innate and adaptive immune systems [27]. Beyond serving as a physical and biological barrier against infections and bacterial invasions, the gut microbiota also influences the effectiveness of hematopoietic cell transplantation and chemotherapy [48].

16.2.2 Dysbiosis and Colorectal Cancer: Mechanisms and Pathways

Dysbiosis refers to functional and compositional alterations in the gut microbiota caused by an imbalance between opportunistic and symbiotic microbes. It manifests in one of three ways: the loss of beneficial microbes, the expansion of pathogenic microbes, or a reduction in microbial diversity. Dysbiosis is influenced by pathological conditions such as diabetes, obesity, neurodegenerative diseases, and cancers [27].

Various factors, including antibiotic usage, age, diet, environment, and exercise, significantly impact gut microbiota composition. These factors often shift the microbiota toward a pro-inflammatory state. Advanced age, in particular, is associated with a decline in CD4 T cells and a transition of the gut microbiota to a more pro-inflammatory profile [50]. Additionally, aging is linked to a reduction in butyrate-producing bacteria, an increase in intracolonic pH, and chronic inflammation. These changes, in combination with dysbiosis, contribute to the development and progression of colorectal cancer (CRC) [27].

The gut microbiota influences the development of colorectal cancer (CRC) through multiple mechanisms, including the production of microbial-derived factors such as metabolites and genotoxins. Certain bacterial species exhibit direct carcinogenic properties or proliferate as opportunistic microorganisms within the tumor-associated microenvironment, further promoting cancer progression. Host-microorganism interactions play a crucial role in activating carcinogenic signaling pathways, which drive molecular alterations and contribute to the initiation and progression of CRC [55].

16.2.3 Key Bacterial Species Involved in CRC

Specific microorganisms play a significant role in colorectal cancer (CRC) development. Certain species within the *Escherichia*, *Enterococcus*, *Bacteroides*, and *Clostridium* genera are implicated in promoting colorectal carcinogenesis, partly by increasing aberrant crypt foci induced by 1,2-dimethylhydrazine [26]. In comparison to a healthy microbiota, the microbiota of CRC patients shows a lower abundance of protective taxa, such as *Roseburia*, and an enrichment of pathogenic organisms like *Bacteroides*, *Escherichia*, *Fusobacterium*, and *Porphyromonas* [56]. 16S ribosomal RNA sequencing has been widely used to characterize CRC-associated microbiota in fecal and mucosal samples. This approach has identified an increased abundance of *Fusobacterium nucleatum* and other bacteria from the *Parvimonas*, *Peptostreptococcus*, *Porphyromonas*, and *Prevotella* genera in CRC patients.

Further, meta-analyses, including one examining 526 fecal samples, have associated additional organisms with CRC. These include *Bacteroides fragilis*, several oral bacteria such as *Fusobacterium nucleatum*, *Parvimonas micra*, *Porphyromonas asaccharolytica*, and *Prevotella intermedia*, as well as other species like *Alistipes finegoldii* and *Thermanaerovibrio acidaminovorans* [40].

Beyond bacterial contributions, viral involvement in CRC has also been explored. Viruses such as cytomegalovirus, John Cunningham (JC) virus, and human papillomavirus (HPV) have been linked to CRC, further emphasizing the multifactorial microbial influence on cancer development and progression [55].

16.2.4 *Potential as Biomarkers for CRC Prognosis*

The diagnosis and prognosis of colorectal cancer (CRC) involve various approaches, including immunological assays, blood tests, and microbial profiling. Increasingly, microbial tests are gaining attention due to their potential to identify microbial signatures associated with CRC [16].

Studies have identified specific microbial genes that can predict CRC across different populations. Notably, 22 genes have been highlighted, including *butyryl-CoA dehydrogenase* from *Fusobacterium nucleatum*, two transposases from *Peptostreptococcus anaerobius*, and the RNA polymerase subunit (*rpoB*) from *Parvimonas micra* [56].

Additionally, CRC-associated microbial species have been identified, including *Clostridium symbiosum*, *Fusobacterium nucleatum*, *Roseburia torque*, *Gemella morbillorum*, *Solobacterium moorei*, *Parvimonas micra*, and *Clostridium citroniae* [58]. These findings underscore the potential of microbial biomarkers for improving CRC diagnosis and prognosis, providing a foundation for precision medicine approaches in CRC management.

16.3 Postbiotics: Definition, Mechanisms of Action, and Tumor Microenvironment Remodeling

16.3.1 *Definition and Classification of Postbiotics*

According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), a postbiotic is defined as “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.” The concept implies that effective postbiotic preparation must contain an inactivated whole, or a component of, a microbial cell with or without metabolites that benefit the body [38].

There are various postbiotic classifications based on their structure, physiological functions, elemental composition, and the metabolites generated by the microbiota. Table 16.1 shows different postbiotic classes and summarizes their functions based on studies that were mainly done on rodents as sufficient human data are still lacking [49].

16.3.2 *Biosynthesis of Postbiotics by Gut Microbiota*

Different classes of postbiotics mentioned in Table 16.1 are produced by various processes. The biosynthesis of postbiotics may be the result of probiotics action on prebiotics. For example, short-chain fatty acids (SCFAs) are produced from fermentation of plant polysaccharides by enteric bacteria. Enzymes are mostly derived

Table 16.1 Classification and functions of postbiotics

Class	Functions
Short-chain fatty acids (SCFAs) e.g., acetate, propionate, and butyrate	Intestinal epithelium regeneration Anti-inflammatory effects Substrates in different metabolic pathways as propionate in gluconeogenesis
Exopolysaccharides (EPS)	Antimicrobial and antioxidant effects Improving lipid metabolism Antioxidant activity Immunomodulatory effect
Enzymes	Glutathione peroxidase from <i>Lactobacillus fermentum</i> has in vitro antioxidant activity Catalase produced by <i>Lactobacillus lactis</i> inhibits chemically induced colon cancer in mice
Cell wall fragments e.g., teichoic acid and lipoteichoic acid	Anticancer, antioxidant, and immunomodulatory activities were demonstrated, however, further studies concerning their safety are needed as they may produce an excessive inflammatory immune response
Cell-free supernatants (CFS)	Anti-inflammatory, antioxidant, anticancer, and antimicrobial effects
Bacterial lysates	Immunomodulators act by stimulating immune response and antibodies against pathogens
Other metabolites	They include other metabolites produced by bacteria such as vitamin K and some of the B vitamins which are de novo synthesized by Gut microbiomes

from bacterial strains, *Bacillus subtilis* and *Bacillus licheniformis*. Cell wall fragments, for example, teichoic acid and lipoteichoic acid, are structural derivatives from bacterial cell walls as they represent 60% of cell wall mass in gram-positive bacteria. Cell-free supernatants (CFS) are liquids containing remaining metabolites from microbial growth and unabsorbed nutrients in the medium [49].

16.3.3 Mechanisms by Which Postbiotics Influence Gut Health

By definition, postbiotics must have a beneficial effect on host health. Recent studies demonstrated the benefits of probiotics are not always related to their viability, and a significant portion is related to their metabolites, structural components, enzymes, and inactivated organisms. Moreover, postbiotics are considered safer and more stable due to their non-viable state. So, they can be used in immunocompromised individuals and without the need for cold chains in industrial use [12]. However, further research on their safety is still required. Focusing on the gut health benefits provided by postbiotics through many mechanisms, studies, both in vivo and in vitro, show the following benefits.

16.3.3.1 Gut Protection Against Pathogens

Postbiotics inhibit the growth of harmful bacteria and improve the functionality of beneficial bacteria. Results of many studies suggest that lactic acid and bacteriocins from lactic acid bacteria (LAB) may be the alternatives to antibiotics due to their expressed antimicrobial activity. An example of antimicrobial activity is the bactericidal action of a bacteriocin purified from *L. helveticus* on *E. coli* and *E. faecalis*. Another mechanism is the adhesion of postbiotics and competition for binding sites in the gut epithelium which inhibits the growth of diarrheagenic bacteria such as enterotoxigenic and enteropathogenic *E. coli*. Additionally, postbiotics can possibly inhibit the growth of pathogens via quorum quenching, blocking quorum sensing of the bacteria that regulate their behavior collectively.

16.3.3.2 Improvement of Gut Barrier Function

Both in vitro and in vivo studies revealed that many postbiotics can contribute to the improvement of the gut barrier functions, such as integrity and permeability, through restoring different protein expression, inhibiting apoptosis mediators, and enhancing mucin expression. As a result, intestinal injuries and infections are prevented due to improved intestinal defense [63].

16.3.4 Postbiotic Effects on Colorectal Cancer (CRC) Tumor Microenvironment

16.3.4.1 Immune and Inflammation Modulation

Postbiotics can modulate inflammatory responses within the tumor microenvironment (TME). For instance, lipoteichoic acid from *Lactobacillus plantarum* has been shown to reduce pro-inflammatory cytokines such as TNF- α , contributing to a less immunosuppressive environment that supports antitumor immunity. Additionally, postbiotics can recruit immune cells, such as T cells and natural killer (NK) cells, to the TME. Butyrate, a short-chain fatty acid (SCFA), further enhances immune modulation by increasing the production of anti-inflammatory cytokines, such as IL-10, while reducing pro-inflammatory cytokines like IL-6 [35].

16.3.4.2 Antiangiogenic Effects

Certain postbiotics can impact CRC cell growth, invasion, and migration by inhibiting the expression and secretion of the VEGFA gene and protein, which is crucial for angiogenesis. By limiting the formation of new blood vessels, postbiotics restrict tumor growth and metastasis [60].

16.3.4.3 Apoptosis Induction

Several studies have demonstrated that postbiotics can induce apoptosis in CRC cells. For example, postbiotics derived from *Lactobacillus acidophilus* have been shown to inhibit proliferation in human colon cancer cell lines such as HT-29 [18]. Similarly, butyrate, produced by the gut microbiota, induces apoptosis in CRC cells by suppressing cyclin D1 expression and arresting the cell cycle at the G2/M phase. This reduces tumor cell viability and creates a local environment that is less conducive to cancer progression ([47]).

16.3.4.4 Inhibition of Cell Proliferation and Migration

Postbiotics can also inhibit the growth, invasion, and migration of CRC cells by modulating key signaling pathways involved in tumor progression. The Wnt signaling pathway, which regulates cell proliferation, differentiation, migration, and apoptosis, is a critical driver of CRC. Dysregulation of this pathway leads to uncontrolled cell proliferation and tumorigenesis. Targeting this pathway with postbiotics holds therapeutic promise for CRC treatment [7].

16.3.4.5 Restoration of Gut Microbiota Balance

An imbalance in the gut microbiota is associated with CRC development. Postbiotics help restore microbial balance, potentially reducing cancer development by promoting a healthier microbiota composition [45].

16.4 Postbiotics and Immunotherapy: A Synergistic Approach

16.4.1 Postbiotics in Enhancing Immunotherapy Outcomes

The gut microbiome plays a significant role in modulating the immune system's response to cancer treatments (Fig. 16.1). It influences both adaptive and innate immunity, as demonstrated in germ-free mice, which show impaired immune responses compared to normal mice. The microbiome aids in maintaining immune system functions, such as the production of immune cells and antigen presentation. Other examples of their importance include:

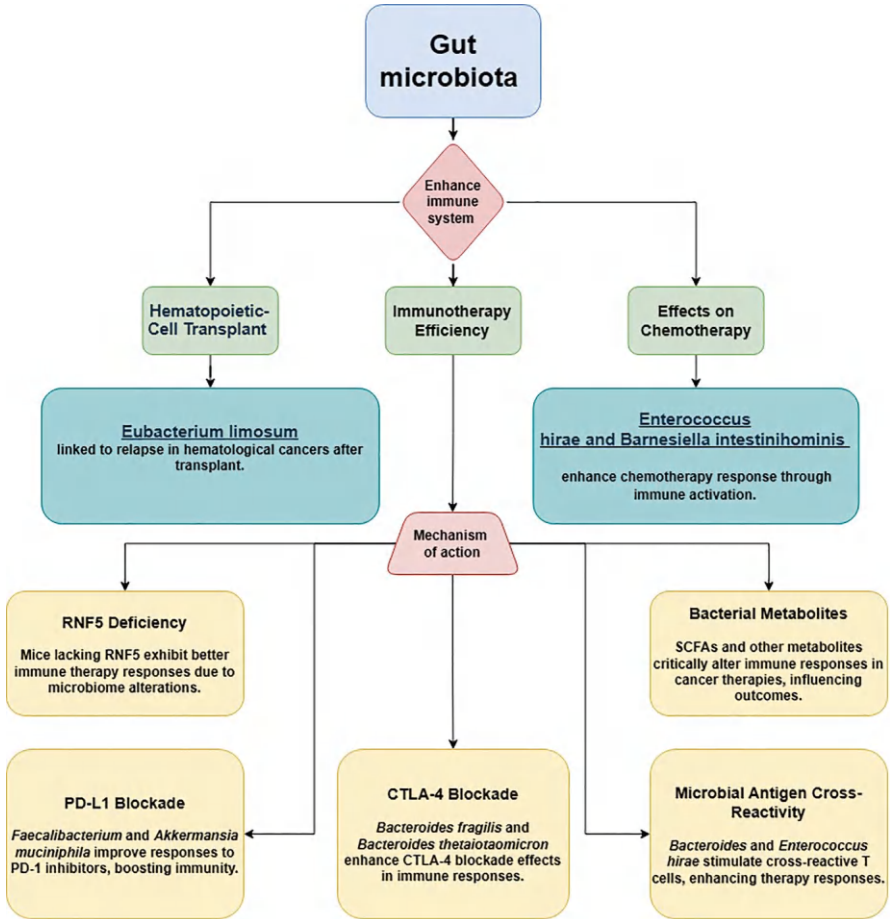


Fig. 16.1 Summary of the gut microbiome’s essential role in response to cancer treatment. (Modified from Ref. [48])

16.4.1.1 Hematopoietic Cell Transplant

Sally Temraz et al. have shown that specific bacterial populations, like *Eubacterium limosum*, are linked to relapse or progression of hematological cancers following hematopoietic cell transplantation [29, 48].

16.4.1.2 Chemotherapy

Chemotherapy medications like cyclophosphamide are more effective when certain gut microorganisms are present. These microorganisms trigger immunological responses, including the production of T helper 17 cells, in mouse models [48].

Certain strains, like *Enterococcus hirae* and *Barnesiella intestinihominis*, increase the intertumoral CD8/Treg ratio in secondary lymphoid organs and infiltrate $\gamma\delta$ T cells that produce IFN- γ into cancer lesions, respectively, to enhance immune responses in cancer treatment [5].

16.4.1.3 Immunotherapy

Immunotherapy's efficacy is largely dependent on the gut microbiota. The gut microbiota has a major impact on the effectiveness of cancer treatments by modifying immune responses, affecting the tumor microenvironment, and interacting with different cancer medications (Fig. 16.1) [48].

16.4.1.4 The Mechanisms of Enhancing Immunotherapy Outcomes

(A) PD-L1 Blockade

Certain bacteria, such as *Faecalibacterium*, *Ruminococcaceae*, and *Clostridiales*, have been linked to improved responses to PD-1 inhibitors by promoting an increase in CD8+ T and CD4+ cells in the bloodstream (Fig. 16.1). Additionally, *Akkermansia muciniphila* enhances PD-1 blockade efficacy by inducing IL-12 secretion and recruiting tumor-infiltrating lymphocytes, thereby strengthening antitumor immunity (Melo et al. 2023). Conversely, dysbiosis caused by antibiotics, as observed in non-small-cell lung carcinoma, renal cell carcinoma, and urothelial carcinoma, can reduce responses to PD-1 inhibitors and shorten survival. Patients receiving antibiotics showed diminished responses to PD-1 blockade compared to those who did not use antibiotics [36].

(B) CTLA-4 Blockade

Zhang et al. [61] identified several *Bacteroides* species, including *B. fragilis*, *B. thetaiotaomicron*, and *Burkholderiales*, as critical for the effectiveness of CTLA-4 blockade. A preclinical study demonstrated that immunization with *B. fragilis* polysaccharides enhanced the response to CTLA-4 blockade in germ-free mice, suggesting a potential microbiome-based strategy to improve immunotherapy outcomes [52, 61].

(C) Microbial Antigen Cross-Reactivity

The gut microbiome has the ability to mimic tumor antigens, which can enhance the effectiveness of immunotherapies. Specific bacteria, such as *Bacteroides* species and bacteriophages from *Enterococcus hirae*, can promote immune responses by stimulating cross-reactive T cells, potentially improving antitumor immunity [5].

(D) Bacterial Metabolites

Metabolites produced by the gut microbiota, such as short-chain fatty acids (SCFAs), have a significant impact on immune responses. SCFAs may suppress immune activity, potentially limiting the effectiveness of CTLA-4 inhibitors. In contrast, metabolites like inosine and anacardic acid have been shown to enhance T cell responses, which can improve the efficacy of immunotherapy ([9, 21, 61]).

(E) RNF5 Deficiency

A recent study demonstrated that mice lacking RNF5, a protein involved in stress responses, exhibited altered gut microbiomes that enhanced responses to immunotherapy. Reduced activity of the unfolded protein response (UPR) in these mice and patients correlates with improved outcomes during immune checkpoint therapy. Several strains, including *B. rodentium*, were identified as contributing to these improved therapeutic responses (Fig. 16.1) [19].

16.4.2 Gut Microbiome Role in CRC Response to Immunotherapy

Recent studies highlight the critical role of the gut microbiota in regulating immune responses within the tumor microenvironment (TME) and influencing immune-related cytokines. The composition of gut microorganisms is essential for maintaining symbiosis and producing metabolites, such as butyrate and tryptophan derivatives, that activate lymphoid cells to secrete IL-22. These metabolites enhance immune cell activity in the gut, contributing to both local and systemic immunity [10, 24]. Cytokines play a pivotal role in preserving gut homeostasis, supporting tissue repair during infections, and facilitating immune responses through the action of short-chain fatty acids (SCFAs). SCFAs are particularly linked to innate immunity and antibody production, underscoring their importance in intestinal immune regulation [10].

The adaptive immune response, particularly T cell sensitivity to luminal signals, is crucial in intestinal immune regulation. This sensitivity triggers both pro-inflammatory and anti-inflammatory processes, influencing the differentiation of CD4+ and CD8+ T cells [22]. However, tumors can disrupt these immune responses by inducing cytokine production that promotes regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), while inhibiting CD8+ cytotoxic T cells. Conversely, certain gut microorganisms can counteract these effects, enhancing the function of effector T cells and promoting antitumor immunity [20].

Changes in gut microbiota diversity are closely linked to disease progression. A reduction in common species, such as *Firmicutes* and *Bacteroidetes*, is associated with various diseases, including colorectal cancer (CRC). Recent cohort studies reveal significant shifts in gut microbiome composition in CRC patients, particularly at the phylum level [32]. The proliferation of *Fusobacterium nucleatum* in

Table 16.2 The effect of gut microbiota on CRC response to immunotherapy [48]

Bacteria	Effects
<i>Fusobacterium nucleatum</i>	The capability to attract immune cells and induce a pro-inflammatory environment
<i>Bacteroides</i>	Improves responsiveness to anti-CTLA4 therapy, correlated with enhanced antitumor immune reactions and increased presence of antitumor CD8+ T cells. Moreover, it hinders IL-17 production and boosts the functionality of Tregs within the gastrointestinal tract, penetration, and infiltration of effector CD8+ T cells into the tumor tissue
<i>Akkermansia muciniphila</i>	Enhances IL-12 production and is linked to the reinstatement of anti-PD1 efficacy during immunotherapy
<i>Bifidobacteria</i>	When combined with CD294 inhibitors, it exhibits nearly complete inhibition of cancer cell growth through the augmentation of trafficking
<i>Lactobacillus acidophilus</i>	When used in conjunction with anti-CTLA4, it has the potential to amplify antitumor immune responses by activating effector T cells that infiltrate the tumor microenvironment

CRC correlates with its ability to recruit immune cells and create a pro-inflammatory environment conducive to tumor progression [32].

The relationship between gut microbiota and immunotherapy is an emerging area of interest, as gut microorganisms exhibit both pro-tumor and antitumor effects. Certain species enhance CRC immunotherapy by boosting antitumor activity. For example, *Bacteroides fragilis* improves outcomes in anti-CTLA4 therapy by enhancing CD8+ T cell activity, similar to the effects of *Lactobacillus acidophilus* [52]. *Akkermansia muciniphila* increases IL-12 production, restoring the efficacy of anti-PD1 therapy (Table 16.2), while *Bifidobacteria*, in combination with PD-L1 inhibitors, promote CD8+ T cell infiltration and tumor eradication, addressing a critical challenge in cancer immunotherapy [36, 44].

IL-12 also plays a vital role in improving dendritic cell (DC) function, essential for gut immune tolerance. DCs promote the differentiation of CD4+ T cells into Tregs, initiating an anti-inflammatory IL-10 response that protects against inflammatory lesions. *Bacteroides fragilis* contributes to this regulatory process by producing polysaccharide A, which inhibits IL-17 production and enhances Treg activity [10]. However, not all microorganisms are beneficial. For instance, *Escherichia coli* and *Fusobacterium nucleatum* foster a pro-tumor environment by promoting M2 macrophage activity and reducing FOXP3+ Tregs (Table 16.2) [10].

Combining beneficial microorganisms with immunotherapies offers promising therapeutic potential. For instance, combining *Lactobacillus acidophilus* lysate with anti-CTLA4 therapy significantly enhances effector T cell activation in the TME [64]. These findings underscore the dual role of gut microbiota in modulating intestinal immune cell function, promoting antitumor defenses, and counteracting tumor-induced immunosuppression.

Collectively, these studies highlight the pivotal role of gut microbiota in cancer immunotherapy. While specific bacterial species show therapeutic potential, the complexity and diversity of the gut microbiome present significant challenges. Further research is needed to fully elucidate the distinct functions of individual

microorganisms and to determine the optimal microbiota composition for supporting antitumor immune responses. Table 16.2 shows the effects of gut microbiota bacteria on CRC immunotherapy.

16.5 Microbiome-Targeting Strategies in Colorectal Cancer

16.5.1 Overview of Microbiome-Targeting Strategies

The human microbiome, a complex collection of microorganisms that reside within the human body, forms a fluctuating ecosystem that greatly influences human health. The diversity and variation in the composition of this microbiome have been linked to several physiological processes and disease conditions and progression. The possibility of microbiome-targeting strategies to ameliorate human health conditions by altering the microbial composition of the gut and other body regions has attracted a lot of interest in the field of medical microbiology in recent years. Harnessing the microbiome for personalized medicine has become a new method to personalize therapies for each patient according to their highly distinctive microbial profile. Interestingly, treatment response is also thought to be influenced by the diversity profile of the microbiota living in a patient's body, which influences how people metabolize drugs and react to treatments [43]. This variability in response underscores the importance of integrating microbiome data into personalized treatment regimens. In fact, the composition of an individual's microbiome can influence the efficacy and safety of certain drugs. A physician can also predict potential side effects and increase dosages by taking into account the microbiota variability profile, resulting in more individualized and efficient therapies. This has led medical microbiology researchers to develop treatments for a number of diseases that involve changes in the human microbiome environment, for example, autoimmune diseases, mental health disorders, or even cancer [37, 39, 42, 62].

Important approaches to microbiome-targeted medicine include probiotics, prebiotics, synbiotics, dietary modification, and fecal microbiota transplantation (FMT) [43]. One important goal of using probiotics, which are healthy live microorganisms, is to restore or increase the numbers of bacteria that are beneficial to human health. For example, strains of *Lactobacillus* and *Bifidobacterium* are common probiotics that have shown encouraging results in treating conditions such as drug-associated diarrhea and irritable bowel syndrome (IBS) [54]. In contrast, prebiotics consist of undigestible substances that only provide nutrients to beneficial bacteria and can thus help adjust the microbial balance. Common prebiotics, such as inulin and fructooligosaccharides (FOS), have been studied for their effects on the growth of *Lactobacillus* and *Bifidobacterium*, which support human gut health [6]. Synbiotics combine probiotics and prebiotics, creating a synergistic ecosystem of support to enhance the survival and activity of beneficial microbes; a prominent example is the combination of *Lactobacillus* and inulin, which has been shown to

improve lipid profiles and immune responses. Another approach is dietary modification, where specific dietary components are tailored to directly influence the composition of the microbiome [46]. Diets rich in fiber, polyphenols, and omega-3 fatty acids have been associated with increased gut microbiota diversity and improved health outcomes, while Western-style diets high in fat and sugar may decrease microbiome diversity, potentially leading to metabolic and inflammatory disorders [41]. Finally, fecal microbial transplantation (FMT) is a powerful and exciting strategy that involves transferring the fecal microbiome from a healthy donor to a recipient patient with microbiome dysbiosis, an imbalance or disturbance in the balance of the microbiome [25, 30]. FMT has been shown to be highly effective in treating *Clostridium difficile* infections that often cause recurrent pseudomembranous colitis and is being investigated for other conditions such as ulcerative colitis and metabolic syndrome [2, 30]. Each of these strategies has its own advantages and limitations, often requiring tailoring efforts based on an individual's microbiome profile. With rapid advances in microbiome research, these approaches may soon offer personalized microbiome-based therapies for a variety of diseases.

16.5.2 Gut Microbiome–Targeting Strategies’ Role and Efficacy in CRC Treatment

Colorectal cancer (CRC) is the second leading cause of cancer death and the third most common type of cancer. In 2018, it caused 1.8 million new cases and 881,000 deaths worldwide [4]. While the prevalence and morbidity of CRC have decreased in developed countries due to population-based colonoscopy screening and treatment, some developing countries continue to experience increasing incidence and mortality [1]. Many reasons that require further investigation may be the cause. A multifactorial disease consisting of genetic, epigenetic, and environmental factors combines to form colorectal cancer (CRC) [28]. Environmental variables, such as smoking, consuming cancer-causing foods, lack of exercise, and the state of the gut microbiota, are believed to be the most significant contributors to the development and progression of colorectal cancer. Until now, research related to the relationship between gut microbiome variability conditions and colorectal cancer development is still interesting to do. This is very necessary to solve the mystery of the microbiome-targeting strategy for CRC [8, 13, 17]. The main probiotics currently receiving attention that are beneficial for treating CRC are *Lactobacillus*, *Clostridium butyricum*, *Akkermansia muciniphila*, and *Bifidobacterium* [13]. *Streptococcus thermophilus* secretes β -galactosidase, a key protein that triggers apoptosis in colorectal cancer cells by triggering the AMPK pathway and inhibiting the “Warburg effect,” also called aerobic glycolysis, a metabolic process in which cancer cells generate energy by converting glucose to lactate, even in the presence of sufficient oxygen. The production of short-chain fatty acids by *Clostridium casei* and the upregulation of short-chain fatty acid receptors (SLC5A8 and gpr43) by

Butyricicoccus pullicaecorum are two ways in which this bacterium exhibits anti-cancer effects. Amuc_1434* and Amuc_1100, two proteins found in *Akkermansia muciniphila*, exhibit unique anticancer qualities. Amuc_1100 stimulates cytotoxic T lymphocytes in mesenteric lymph nodes and enhances their cytotoxicity to prevent colorectal cancer due to colitis, while Amuc_1434* can break down mucin 2 of CRC cells to accelerate CRC cell death [13].

Gut microbiota has also been shown to influence how CRC patients respond to immunotherapy and chemotherapy. Strong evidence suggests a close relationship between gut microbiota and immunotherapy drugs such as programmed cell death receptor 1 (PD-1) inhibitors and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors, as well as chemotherapy drugs such as 5-fluorouracil, oxaliplatin, and cyclophosphamide. Particularly in the case of probiotics such as *Bifidobacterium* spp., *Lactobacillus* spp., and *Bacteroides* spp., which enhance efficacy, numerous studies have found significant differences in gut microbiota between patients who respond to treatment and those who do not, as well as between patients who have no side effects and those who have mild or severe side effects [13, 62]. The gut microbiome has also been shown to play a key role in regulating the side effects of treatments used to manage colorectal cancer. Intestinal mucosal inflammation, diarrhea, and weight loss are often major side effects of immunotherapy and chemotherapy treatments, and the gut microbiota is closely linked to modulating the immune system by controlling the inflammatory response [13].

16.5.3 General to Specific Approaches to Manipulating Gut Microbiota

There are several important approaches to modulate or manipulate the gut microbiota that aim to shift microbial communities toward a composition that supports human health, using various strategies collectively known as microbiome-targeted strategies. These strategies are the implementation of probiotics, prebiotics, synbiotics, dietary modification, and fecal microbiota transplantation (FMT). As we have already discussed the benefits of using probiotics, prebiotics, and synbiotics in modulating the gut microbiome, in this section, we will delve deeper into how FMT and dietary interventions can modulate the gut microbiome so they can alter and support the CRC treatment.

16.5.3.1 Fecal Microbiota Transplantation (FMT)

Most of the bacteria found in the gut have been known to be involved in regulating the gut microenvironment. The interaction and dynamic balance of the gut microbiota are essential for maintaining normal gut function. Through several studies, it has been found that CRC patients have a very different microbiome profile from

normal patients. For example, some bacteria such as *Porphyromonas*, *Enterococcus*, *Streptococcus*, or *Peptostreptococcus*; on the contrary, some bacteria show decreased abundance, such as *Roseburia* and other butyrate-producing bacteria in the *Lachnospiraceae* family [59]. Among these microbiota, oncogenic bacteria have been found in CRC patients. For example, *Streptococcus bovis* biotype I bacteria are known to be associated with colorectal adenoma and CRC [2]. This suggests that there is a very close relationship between the interaction and dynamic balance of the gut microbiota and normal gut function. Fecal microbiota transplantation is considered an effective method to transfer healthy gut microbiota. For this, the selection of the “ideal” gut microbiota is a key step for successful FMT, because the composition and quality of the gut microbiota for each individual can be very different and influenced by many factors, such as diet, environment, and heredity. Currently, washed microbiota transplantation and artificially cultivated formula flora are being developed as safer and more effective methods for fecal microbiota transplantation. In addition, the frequency of transplantation is another key point for successful FMT [30, 59]. In the process of FMT, some bacteria may be released due to their weak adhesion or colonization resistance, resulting in the failure of colonization of the introduced bacteria. Therefore, multiple transplantations may be beneficial for bacterial colonization [59].

16.5.3.2 Dietary Interventions

Dietary factors may be important in tumor development and response to treatment and are generally considered to impact the course and prevention of colorectal cancer disease. Because dietary factors can significantly alter the composition of the gut microbiota, and several nutritional interventions have been associated with improved disease prognosis. Because the gut microbiota can ferment food waste and food components to produce short-chain fatty acids (SCFAs), which are well-established bacterial metabolites that maintain epithelial barrier function, prevent infection, suppress ammonia absorption, and promote tumor cell apoptosis, this may be a promising strategy to prevent complications in patients undergoing CRC treatment, including surgery [23]. Dietary fiber has been shown to exert beneficial effects on metabolic activity in the gastrointestinal tract by modulating the gut microbiota. Most importantly, a diet rich in whole grains and dietary fiber has been associated with a lower risk of developing *F. nucleatum*-positive CRC, supporting a potential role for the gut microbiota in mediating the association between diet and colorectal neoplasm development. Several studies have also recently defined the impact of omega-3 polyunsaturated fatty acids (PUFAs) on the gut microbiota [23, 41].

16.6 Future Directions and Research Opportunities for Gut Microbiota and Postbiotics in Colorectal Cancer

The relationship between gut microbiota and colorectal cancer (CRC) has attracted significant attention in recent years, unveiling complex mechanisms through which gut microbes influence cancer development, progression, and treatment responses. This rapidly evolving field offers various opportunities for future research, particularly in exploring the role of postbiotics.

16.6.1 Personalized Microbiome Modulation in the Treatment of CRC

Traditional approaches have often employed a “one-size-fits-all” strategy for microbiome modulation. Future efforts should focus on developing and optimizing personalized microbiome treatments tailored to each patient’s unique microbial profile. Such individualized interventions could enhance the efficacy of chemotherapy and immunotherapy while reducing toxicity, ultimately leading to improved outcomes [34, 57].

16.6.2 Development of Interventional Approaches and Delivery Mechanisms

Several intervention methods, including probiotics, prebiotics, fecal microbiota transplantation (FMT), antibiotics, and lifestyle changes, have been evaluated in clinical trials. However, challenges remain in optimizing delivery routes, dosing, kinetics, effectiveness, and durability. Future research should prioritize defining the most suitable delivery methods and dosing regimens to maximize the stability and effectiveness of microbiota-modulating therapies [34].

16.6.3 Integration of Primary and Secondary Prevention Strategies

The rising incidence of early-onset CRC underscores the importance of early prevention. Primary prevention measures include adopting healthy environmental and dietary practices, regular exercise, stress management, and weight control from birth through childhood. Further research is needed to explore whether early-life modulation of the microbiome can lower CRC risk later in life. For late-onset CRC,

early detection and management can be enhanced through screening programs and microbiome-focused health interventions. Integrating primary and secondary preventive strategies, tailored to individual life stages and risk levels, could provide a comprehensive approach to CRC prevention [34].

16.6.4 Geographic and Demographic Variation Analysis of Microbiome Composition

Significant geographical, ethnic, and dietary variations in gut microbiome composition influence CRC risk and therapeutic responses. Future studies should focus on understanding these variations through large-scale, multicenter trials, and international collaborations. Insights from such research could inform the development of culturally and regionally specific guidelines for CRC prevention and treatment [34].

16.6.5 Public Health Implications

Improving gut microbiome health could yield extensive public health benefits beyond CRC. A healthy microbiome contributes to overall well-being, reducing the risk of various diseases and improving mental health. Public health initiatives promoting dietary and lifestyle habits that support a balanced microbiome could positively impact CRC incidence and enhance population health.

16.7 Conclusion

Due to the rising incidence of colorectal cancer (CRC), the roles of gut microbiota and postbiotics are gaining significant attention for their potential to treat, prevent, and influence the prognosis of CRC.

This chapter discusses the multifaceted roles of gut microbiota in CRC, which range from contributing to disease pathogenesis through dysbiosis to modulating responses to immunotherapy. The microbiome has emerged as a valuable agent, demonstrating multiple mechanisms that affect the tumor and its microenvironment. These mechanisms include immune modulation, antiangiogenic effects, and the induction of apoptosis in CRC cells.

Integrating microbiome-targeting strategies, such as postbiotics, with immunotherapy holds significant promise. By enhancing the efficacy of checkpoint inhibitors and other therapeutic agents, postbiotics, and related interventions have the potential to revolutionize the treatment of CRC, offering renewed hope for thousands of patients facing this debilitating and lethal disease.

However, the complexity of interactions between microbiota, cancer pathways, and therapies necessitates continued research. This research should focus on clinical validation, safety, and understanding the exact mechanisms involved. Doing so will generate robust experimental evidence and optimize these approaches for individual patients.

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Chapter 17

Microbiota-Targeted Therapies for Colorectal Cancer: Current Challenges and Future Directions



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Abstract Colorectal cancer (CRC) is among the most prevalent cancers world-wide, with the microbiome playing a significant role in cancer progression and treatment. Numerous studies have demonstrated a strong correlation between microbial metabolites and the development of CRC. Current CRC treatments, such as chemotherapy, radiotherapy, and immunotherapy, while effective, are constrained by issues of toxicity and resistance. The gut microbiota interacts with anticancer drugs through pharmacokinetic and pharmacodynamic mechanisms, significantly impacting treatment outcomes. Additionally, gut microbiota can serve as valuable biomarkers for CRC diagnosis and therapy. This chapter delves into targeted CRC therapies utilizing probiotics, prebiotics, fecal microbiota transplantation (FMT), and bacteriophages. These therapies offer a novel approach to CRC treatment but present several challenges, including individual variability, lack of standardized protocols, potential adverse effects, safety concerns, and regulatory and ethical issues. The future of CRC treatment will likely focus on the modulation of the gut microbiome, which can be readily altered to enhance treatment efficacy. This strategy is anticipated to be pivotal in next-generation personalized medicine, improving CRC treatment outcomes through precise microbiome modulation.

Keywords Colorectal cancer · Microbiota-targeted therapy · Probiotics · Fecal microbiota transplantation · Immune modulation

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17.1 Introduction

In epidemiological studies in the United States, cancer is the leading cause of death mostly in people aged above 85 years [1]. With about 2 million new cases annually, colorectal cancer (CRC) is the second most frequent kind of cancer globally and the second largest cause of cancer-related deaths. According to statistical analysis, there are 152,810 new cases in males and females, out of which affected males are 81,540 and females are 71,270. The estimated death in both sexes is 53,010, out of which males are 28,700 and females are 24,310 [1]. Hereditary and environmental risk factors contribute to the development of colorectal cancer (CRC). A systematic review by Nora B. et al. indicates that individuals with a familial history of CRC have an elevated risk of developing the disease, with the likelihood increasing based on the number of affected relatives and their age at diagnosis [2]. CRC is also progressed in patients with ulcerative colitis (UC), inflammatory bowel diseases, adenoma, and Crohn's disease [3]. Hereditary CRC can be subdivided into two types: nonpolyposis syndrome and polyposis syndrome [4]. "Polyp" is derived from the Greek word polypus, which means "morbid lump." A polyp can be described as any projecting mass into the lumen of a hollow vessel in the gastrointestinal, genitourinary, or respiratory tract [5]. The pathogenesis of CRC in many cases is initiated by the formation of a polyp which further forms a neoplastic lesion and eventually develops into CRC [4]. The human microbiome is the term for microbes, their genes, and gene products that are transferred vertically from mother to newborn and colonized from birth [6]. More than 10^4 microorganisms reside in the human gut, including fungi, viruses, bacteria, and protozoa. These microorganisms play a crucial role in maintaining gastrointestinal health by supporting physiological functions such as energy harvest and immune maturation [7, 8]. Due to environmental changes and alterations in diet, there is a transformation of protective microflora to harmful which causes susceptibility to various diseases; this condition is called dysbiosis [9, 10].

Available conventional treatment for CRC is based on chemotherapy and surgery. The chemotherapeutic agent most commonly works by altering rapidly dividing cells by interfering with the cell cycle. Chemotherapeutic agents commonly used are platinum derivatives like oxaplatin, antimetabolites like 5-fluorouracil, and topoisomerase including irinotecan. However, certain disadvantages cause the failure of treatment. These are mainly drug resistance and toxicity caused by nonspecific cytotoxicity. Chemotherapeutic agents act nonspecifically, leading to the destruction of nonmalignant cells and disrupting normal physiological functions. To overcome these problems associated with conventional therapy, microbiota-associated targeted therapy has emerged [11]. Microbiota-targeted therapies include the use of prebiotics, probiotics, symbiotic, adjuvant and neoadjuvant therapies, and fecal microbiota transplantation [12].

17.2 Microbiota and Colorectal Cancer

17.2.1 Composition and Diversity of Gut Microbiota

Bacteria, fungi, viruses, and protozoa are the main components of human gut microbiota. Healthy gut microbiota consists of almost 1000 different species and 7000 different strains of bacteria [13]. Large intestines have an abundance of anaerobic bacteria. The most common phyla present are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* (Fig. 17.1) [14]. Variations in the gastrointestinal tract's pH, transit time, oxygen exposure, and host secretions, like bile and digestive enzymes, all have an impact on the number of bacteria that live there [15]. Diet is a key determinant of gut microbial composition and diversity. Changes in diet or environmental conditions can cause a shift from healthy gut microflora to pathogenic microbes, a condition known as dysbiosis [16].

17.2.2 Mechanism Linking Microbiota and CRC

One of the worst malignancies in the world, colorectal cancer (CRC) ranks second in mortality rates and third in annual incidence [12]. The incidence of CRC has complex reasons. CRC occurs in multifactor and multistep processes. Age, environment, lifestyle, genetic factors, and dietary patterns can lead to CRC progenesis [17]. Among environmental factors, gut microbiota has emerged as a significant influence, according to research from the last five years. In the gastrointestinal tract

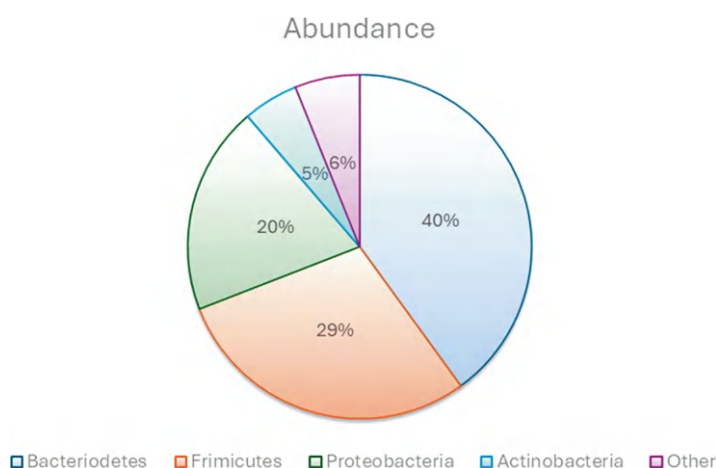


Fig. 17.1 Bacterial phylum abundance based on complete bacterial genomic studies. Others include *Spirochaetes*, *Fusobacteria*, *Deferribacteres*, *Cyanobacteria*, *Planctomycetes*, *Lentisphaerae*, *TM7*, and *Tenericutes*

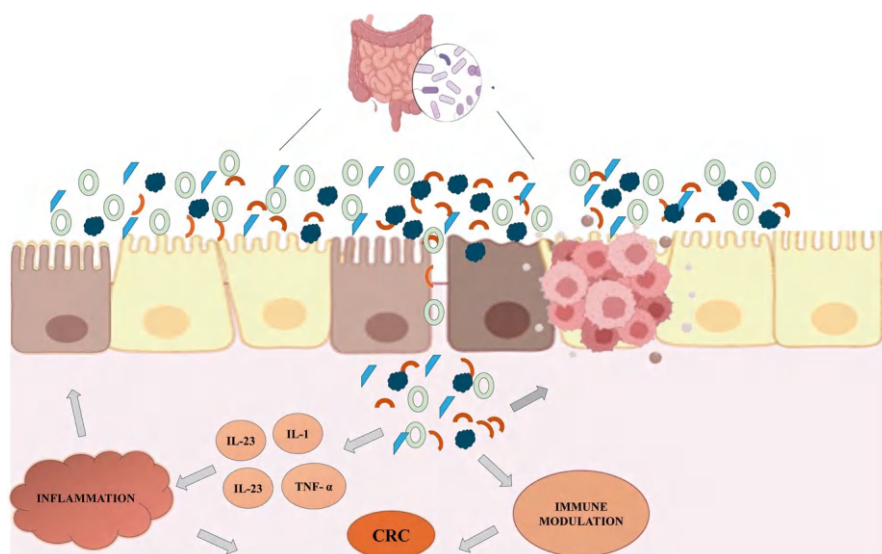


Fig. 17.2 Mechanism of CRC progression by cell proliferation, inflammation, and immune modulation due to dysbiosis

(GIT), the microbial population is comparatively high. In the gastrointestinal tract, the microbial population is comparatively high. Microbes in the gastrointestinal tract are involved in normal physiology such as energy harvesting and immune maturation. Any change in their abundance due to environmental factors leads to dysbiosis which results in the development of intestinal and extraintestinal diseases [8]. Reddy et al. reported for the first time a link between gut microbiota and the development of CRC in 1975 [18]. CRC carcinogenesis is a convoluted process, which is affected by environmental factors and genetic mutations with different mechanisms (Fig. 17.2). Mechanisms involved in CRC carcinogenesis are inflammation, immune regulation, metabolism of dietary components, and genotoxin production [19].

17.2.2.1 Inflammation and Immune Modulation

17.2.2.1.1 Inflammation

Microbiota has a major role in developing chronic inflammation and further development of CRC [20]. Chronic inflammation is the cause of malignancy [21]. Patients with inflammatory bowel disease are at higher risk of CRC [22]. Rudolf Virchow 1863 reported a link between inflammation and CRC due to the presence of leukocytes in cancer tissue which gave rise to the hypothesis of a connecting link between inflammation and CRC [22]. Based on a 16S rDNA sequence analysis conducted for 95 carcinoma/normal DNA pairs and quantitative polymerase chain reaction, it came to light that fusobacterium sequences were high in colorectal carcinomas.

Fusobacteria within colorectal cancers were also observable using fluorescent in situ hybridization. These findings suggest that the *Fusobacterium* species may be linked to inflammatory bowel disease (IBD), which covers Crohn's disease as well as ulcerative colitis (UC) [23]. Chronic inflammation inhibits apoptosis and mutations and promotes cell proliferation [24]. Gut microbiota gets exposed to enteric bacteria. Regardless of having different defense mechanisms against infection, pathogenic bacteria adapted alternate strategies to invade across the mucus layer and with the help of bacterial pili and surface adhesive molecules get attached to the epithelium [25, 26]. When epithelial cells get adhered to by pathogens, they recognize various classes of host molecules including transmembrane glycoproteins like integrin and cadherin, receptors on the intestinal epithelial cells (IECs) or extracellular matrix material [26]. Through direct interaction with pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), epithelial cells sense these pathogenic foreign molecules. These interactions overcome immunological tolerance, hyperactivate several signaling pathways, and ultimately cause the expression of genes that code for cytokines, resulting in increased inflammation [25, 27, 28]. When pathogens bind to the epithelial cell surface some pathogenic bacteria can be internalized which initiates three major consequences: (1) Inflammatory cytokine expression (2) Disruption of epithelial tight junction. and (3) Cell cycle interruption. Pathogens associated with these mechanisms are *Salmonella typhimurium* or *Helicobacter species* [29, 30]. The conventional, serrated, and microsatellite instability (MSI) pathways majorly affect the CRC-associated molecular pathway. The conventional pathway of CRC starts with *adenomatous polyposis coli* (APC) mutation, along with mutation of *KRAS*, *PIK3CA*, and *SMAD4*. Loss of heterozygosity of chromosomes 18 and *Tp53* mutation. Conventional pathway is observed in 70% of cases. The conventional pathway is characterized by high levels of CIN (CIN+++), microsatellite stability (MSS), and no or low levels of the CpG island methylation pathway (CIMP-). Second is serrated pathway occurs in 20–30% of cases. The serrated pathway is further divided into two sub-pathways namely CIMP^{low} MSS tumors and BRAF-mutant Camp^{aign} MSS tumors/BRAF-mutant CIMP^{high} microsatellite instability (MSI) tumors. The third pathway in CRC development is the microsatellite instability (MSI) pathway. It is initiated by damage to repair gene of DNA mismatch like gene encoding MLH proteins/MSH proteins [31]. There are different types of mechanisms by which bacteria contribute to the development of CRC some of them are explained here. First, bacteria and their metabolites can be genotoxic in nature which can transform healthy intestinal epithelial cells (IECs). For example, *Escherichia coli* that express the genomic island polyketide synthase, which further produces colibactin. Colibactin generates mutagenic DNA adducts [32]. Gallic acid produced by microbes has a local effect on tumor suppressor genes like TP53. Mutation of this gene induced by gallic acid can lead to oncogenicity. However, the microbiome can help regulate such mutations [33]. The third mechanism is associated with the induction of inflammation and tumorigenesis by an anaerobic Grampositive bacterium like *Fusobacterium nucleatum*. CRC tissue was found to be rich in *Fusobacterium nucleatum* [34]. *Fusobacterium nucleatum*

activates β -catenin signaling in colon epithelial cells, which further leads to upregulation of oncogenes like genes encoding cyclin D1 and MYC, and pro-inflammatory signals such as tumor necrosis factor (TNF) and IL-17 [32]. *Fusobacterium nucleatum* also leads to the induction of chemokines CXCL8 and CXCL1.

17.2.2.1.2 Immune Modulation

Oncogenic transformation of healthy cells is done by antigens, which are recognized by the immune system. Tumor antigens are of two types: (1) self-antigens and (2) neoantigens. Self-antigens are associated with normal and tumor cells but overexpressed in tumor cells than in normal cells [35]. Self-antigens that are shared are represented by characterized antigens like NY-ESO-1, MART-1, MUC1, CYCLIN B1, CEA, and Her 2/neu that are found to be overexpressed in tumor cells than normal cells. The second type of antigen is neoantigen. Neoantigens are produced by mutation, which include *KRAS*, *TP53*, *BRAF*, and *PIK3CA* [35]. Tumor antigens are represented by major histocompatibility factors (class I and II) and dendritic cells which act as antigen-presenting cells. Antigen-presenting cells process antigens and give it them to T cells [36]. The pathogenesis of CRC in many cases is initiated by the formation of a polyp which further forms a neoplastic lesion and eventually develops into CRC [4]. Then, tumor progression is promoted by oncogenic mutations in *KRAS*, *BRAF*, and *PIK3CA*. Adenomatous polyposis coli (APC), a key tumor suppressor, plays a crucial role in this process. Loss of the APC pathway leads to the activation of *c-Myc* and *cyclin D*, which are critical downstream effectors. The function of *c-Myc* is as a transcription factor that, upon activation due to the loss of the APC pathway, leads to cell proliferation and the survival of cancerous cells. *c-Myc* also regulates immune checkpoint proteins such as PDL1, which deactivate adaptive immunity, and similarly affect innate immunity. Another protein overexpressed in tumor cells is cyclin D1, also recognized as a tumor antigen. Cyclin D1 synthesizes peptide sequences that stimulate the activity of CD4+ OT ccd8+ cytotoxic T cells [37].

17.2.2.2 Metabolic Byproducts

Gut microbiota synthesizes metabolites that take part in the promotion of disease. Metabolites are produced from host compounds that are synthesized from endogenous substances by the metabolism process of hosts and microorganisms [38]. Microbial gut metabolites present in the gastrointestinal tract are volatile small molecules, lipids, proteins, peptides, sugars, and secondary bile products. By biotransformation of different dietary products available in human gut microbiota, it generates different metabolites that alter the normal physiology of the host [39]. Certain types of metabolites with increased amounts in gut promote CRC progression like deoxycholic acid (DCA); however, a decrease in the number of useful

bacteria also leads to the promotion of CRC [40]. During tumor development, various signaling pathways are affected due to alterations in the tumor microenvironment (TME). Metabolic byproducts can be classified as tumor-suppressing metabolites and tumor-promoting metabolites.

17.2.2.3 Tumor-Suppressing Metabolites

17.2.2.3.1 Short-Chain Fatty Acids

Short-chain fatty acids (SCFA) are the most commonly found metabolite in the body. By fermentation process in the gastrointestinal tract, starches and nonabsorbable dietary fibers get converted into short-chain fatty acids namely propionate, butyrate, and acetate [41]. By binding to the 7 transmembrane receptor43, SCFAs show anti-inflammatory effects [42]. SCFAs enter the cell via two main pathways: cell diffusion and carrier-mediated transportation [43]. Among all SCFAs butyrate has a big contribution in antagonism of colonic inflammation. Its major producing sources were found to be *Frimictus*, *Clostridia*, *Eubaterium*, and *Ruminococcaceae* [44]. Mechanisms by which butyrate shows an anti-inflammatory effect in diseased cells are suppression of cell proliferation, colony formation, and invasion that promotes CRC cell death [45]. Butyrate induces cell apoptosis by decreasing the transcription of microRNA-92a (miR-92a) and increasing the expression of phosphatase and tensin homolog (PTEN). Transcription of RNA is decreased by controlling the expression of c-Myc gene [46]. Butyrate also prevents the formation of tumors in CRC by altering the translation of tumor suppressor genes by specifically inhibiting the function of histone deacetylases (HDACs). Acetate and propionate are prominently produced by *Bacteroides bacteria* [47]. Acetate acts by reducing CRC proliferation and also induces apoptosis in CRC cells. Likewise, propionate leads to the induction of downregulation of PRMT1 resulting in apoptosis of cells.

17.2.2.3.2 Bacteriocin

Bacteriocins are peptide in nature which is produced by bacterial ribosomes with bacteriostatic and/or bacteriolytic action. Based on different studies, it can be believed that bacteriocin can inactivate tumor growth with various mechanisms. Bacteriocin can bind selectively to the tumor cells as tumor cells carry a negative charge with higher membrane fluidity on them, and there is overexpression of microvilli than healthy cells. The interaction of bacteriocin with tumor cells occurs through channels on the tumor cell membrane, leading to the accumulation of intracellular reactive oxygen species, which further promote apoptosis and necrosis. Bacteriocin also exhibits anti-inflammatory and immunomodulatory effects. Bacteriocins can also amplify phagocytosis of macrophages which enhances antitumor immunity (c) [47, 48].

17.2.2.3.3 Indole Derivatives

Indole is a metabolic product of tryptophan metabolism indole and its derivatives like indole lactic acid and indole acetic acid. Indole and its derivative were found to inhibit the progression of CRC [49, 50]. Activation of aryl hydrocarbon receptor (AhR) leads to the increased expression of mucin in epithelial cells that improves the intestinal barrier and protects against pathogenic infection. Ligand for aryl hydrocarbon receptors are indole derivatives, which eventually leads to the mitigation of inflammation [51]. In 2021, Sugimura reported that *Lactobacillus gallinarum* directly shows anti-CRC effect [50]. Indole-3-lactic acid induces apoptosis and suppresses proliferation of CRC cells.

17.2.2.3.4 Tumor-Promoting Metabolites

Secondary Bile Acids

Secondary bile acids (BAs) are oncogenic which causes CRC. Excessive intestinal BAs concentrations could change the gut microbiota's composition leading to microbial dysbiosis, eventually leading to the emergence of CRC by enriching opportunistic pathobionts and depleting helpful commensals. Significant signaling molecules, known as BAs, control both the innate and acquired immune responses. Several investigations have revealed that BAs can alter host immunity in order to stimulate carcinogenesis [52]. Two BAs that can encourage the growth of immunosuppressive regulatory T cells (Treg) are isoalloLCA and 3-hydroxydeoxycholic acid (isoDCA). IsomelloLCA and isoDCA, respectively, induce Treg differentiation with increased FOXP3 expression in naïve CD4+ T cells and farnesoid X receptor activity in dendritic cells [53].

Polyamine

Polyamines are metabolic products of the spermidine derivatives that are formed by the host tissue and intestinal microbiota. Microbes involved in the synthesis of polyamines are *Lactobacillus* spp., *Veronococcus* spp., *Bifidobacterium* spp., and *Clostridium* [54]. A study reported an increase in polyamine metabolites in CRC patients, along with elevated levels of N(2),N(12)-diacetylspermine, an end product of spermine metabolism [55]. Polyamine possesses tumor-promoting and tumor-suppressor action. Intestinal health is progressed by polyamine, as polyamine makes more improvements in intestinal epithelial barrier function. This intestinal epithelial barrier is made strong by mucin production, expression of Toll-like receptor TLR2, and induction of E-calcine mucin induction. However, polyamine shows a carcinogenic effect as it was found that the intestinal barrier gets weakened with histamine and spermine by regulating NLRP6 inflammatory vesicles that cause IL-18 secretion [56–58].

17.2.2.4 Interaction with Dietary Factor

Human gut microbial alterations brought on by food are thought to be the root cause of the rising CRC epidemics. The human gut is home to trillions of bacteria. The gut microbiota of individuals with and without colorectal cancer (CRC) and animal models have been used to compare the gut microbiota during the past 10 years to uncover experimental evidence of a possible connection between the two. The four bacterial phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*, together with a few more bacterial phyla, made up the gut microbiotas. Dietary patterns may have an impact on the changes in the composition and quantity of gut microbiota that occur before or during the development of colorectal cancer [59].

17.2.2.4.1 Red or Processed Meat

Incidence of CRC is observed to occur in developed countries, as in these countries there is a high consumption of red meat and low fiber intake. Research on the environment's impact on colorectal cancer (CRC) has found that second-generation Japanese and African immigrants to the United States have a higher prevalence of the disease than other ethnicities, despite their underlying low risk [60]. The intestinal mucosa comes into contact with several components of red meat, such as protein, animal fat, heme, N-nitroso compounds (NOC), polycyclic aromatic hydrocarbons (PAH), and heterocyclic amines (HCA) with associated carcinogenic properties [59, 61]. Increased quantities of secondary bile acids, which are mutagenic and have antiapoptotic effects on colonic epithelial cells (CECs), are produced in the gut due to a diet high in red meat and animal fat and greater counts of 7a dehydroxylating colonic bacteria. Red or processed meat contributes to CRC through metabolism of mutagens, such as polycyclic aromatic hydrocarbons, heterocyclic amines, and dietary N-nitroso compounds [62]. Secondary bile acids that accumulate and heme end products promote cytotoxicity and inflammation. This leads to the production of cytokines and reactive oxygen and nitrogen species, which in turn drives the hyperproliferation of epithelial cells. By adding nitrates and nitrites to processed meats, increasing the cytotoxicity of nitrosyl heme and reactive chlorine species, endogenous and exogenous N-nitroso compound synthesis increases [63]. Red meat-rich diets encourage the development of sulfate-reducing bacteria, which create the deadly gas hydrogen sulfide.

17.2.2.4.2 Alcohol

Although the exact mechanisms behind alcohol's carcinogenic effects are unresolved, some well-established or plausible theories include: (1) alcohol acting as a solvent to allow carcinogens—such as those found in tobacco—to penetrate mucosal cells; (2) alcohol's reactive and genotoxic metabolites, such as acetaldehyde; (3) alcohol's role in mediating the concentration of estrogen and prostaglandin; (4)

alcohol's production of reactive oxygen and reactive nitrogen species; (5) alcohol's interactions with DNA repair and folate metabolism; and (6) high alcohol consumers' diets being comparatively deficient in vital nutrients [63]. Research on animals led to the discovery that drinking alcohol has an impact on the quantity and make-up of gut bacteria. Mice given alcohol for 3 weeks were shown to exhibit intestinal bacterial overgrowth, a relative excess of *Bacteroidetes* and *Verrucomicrobia* bacteria, and a relative disadvantage of *Firmicutes* bacteria as compared to control mice [64]. By analyzing bacteria in aspirates from the jejunum, Bode et al. observed that chronic alcoholic patients displayed a larger quantity of aerobic and anaerobic microbes compared to healthy people [64]. According to Mutlu et al., colonic biopsy samples from dysbiotic alcoholics showed higher median abundances of *Proteobacteria* and decreased median abundances of *Bacteroidetes*. Similar fecal microbial communities were also found in the HBV-related cirrhosis group, which may lead one to believe that changes in the fecal microbiota composition were primarily caused by cirrhosis. Similarly, the alcohol-related cirrhosis group had a lower proportion of *Bacteroidetes* and highly enriched *Proteobacteria* and *Fusobacteria* [65]. Drinking alcohol seems to be a strong risk factor for colorectal cancer. It is advised to limit alcohol consumption, particularly for those who consume large amounts of it.

17.2.2.4.3 Dietary Fiber

Burkitt proposed the theory that dietary fiber may reduce the risk of colorectal cancer when he saw a geographic overlap between low colorectal cancer incidence and high-fiber diet patterns [66]. Numerous mechanisms associated to explain how fiber reduces the risk of colorectal cancer. These include general stool bulking, changes to gut flora and local metabolism, and shorter stool transit times, which result in more frequent stools. Numerous mechanisms associated to explain how fiber reduces the risk of colorectal cancer. These include general stool bulking, changes to gut flora and local metabolism, and shorter stool transit times, which result in more frequent stools [67, 68]. Diets high in fat or high in fiber had the opposite effects on microbiological taxa. In animal-based diets (no fiber and high amounts of fat and protein), the abundance of *Bacteroidetes* increased while numerous members of the butyrate-producing *Firmicutes* declined when compared to extreme plant-based diets (high levels of fiber) [69]. Given that most cancer and adenoma patients had *Bacteroides* instead of *Ruminococcus* in their represented enterotype, and that CRC cases it was observed to have a depletion of fiber fermenting *Clostridia*, these findings may indirectly support the link between a high-fiber diet and a lower risk of CRC [70, 71].

17.3 Cancer-Related Microorganisms and Effectors

Microbes itself behave as a pathogen and carcinogen. For instance, a group of poisons called cell death toxins (CDTs) are exuded by *E. coli*. They induce a highly proliferative epithelium in normal intestinal epithelial cells by acting directly on the intestinal epithelial cells. The intestinal epithelial cells that are multiplying create an adenoma and proceed to infiltrate the intestinal mucosa's submucosa, ultimately resulting in malignant alterations. *Enterococcus faecalis* uses free radicals like active nitrogen and oxygen to break down DNA [72]. Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) are examples of innate receptors that enable recognition of molecular patterns and are crucial to stimulate the innate immune response. Nod-like receptors (NLRs), C-lectin-like receptors (CLRs), RIG-I-like receptors (RLRs), and toll-like receptors (TLRs) are examples of these molecular patterns. NLRs are cytoplasmic receptors that have shown strong evolutionary conservation, indicating their critical function in host defense [73].

17.3.1 *Microorganism Associated with CRC with Their Effectors*

17.3.1.1 *Escherichia coli*

In the typical gut microbiota, *Escherichia coli* is the most common aerobic-anaerobic gram-negative bacterium. The intestines of humans can become infected with certain pathogenicity islands containing virulent strains of *E. coli* and thereafter become ill. *E. coli* is classified into four groups which are A, B1, B2 and D. Some strains of *E. coli* from phylo group B2 are at high risk of CRC by the development of IBD and CD. Polyketone acid synthetase (PKS) island which has genotoxins, is present in the group B2 in *E. coli*. Anaphase bridging, chromosome abnormalities, aneuploidy, and tetraploidy are all brought on by PKS⁺ *E. coli* infection [74]. Mucus degradation promotes pks⁺ *E. coli* adherence, which in turn causes colibactin to cause more DNA damage to colonic epithelial cells [75]. There is a need for contact between bacteria and host cells to produce a genotoxic effect of pks⁺ colibactin acts as an effector to have a genotoxic response. PKS island deletion reduced the number of tumors without changing inflammation, indicating that colibactin promotes tumors apart from inflammation [76].

17.3.1.2 *Bacteroides fragilis*

B. fragilis typically coexist symbiotically and is thought to support systemic and mucosal immunity as well as the nutritional state of the host. On the other hand, *B. fragilis* also causes illness in humans when it interferes with intestinal integrity. This allows it to enter the sterile peritoneum and function as an opportunistic pathogen there [77]. Only one strain of *B. fragilis*, called enterotoxigenic *B. fragilis* (ETBF), is linked to diarrheal illness. On the other hand, nonenterotoxigenic *B. fragilis* (NTBF) strains are thought to have the ability to cause colonic inflammation, while being thought of as a potential probiotic [78]. The production of the *B. fragilis* toxin (BFT) is the fundamental reason of ETBF's pathogenicity. So BFT acts as an effector. The 20-kDa zinc-dependent metalloproteinase toxin known as BFT attaches itself to colonic epithelial cells (CECs) and induces the tumor suppressor protein (E-cadherin) to be cleaved [79]. Murine models of colon carcinogenesis show that E-cadherin cleavage promotes procarcinogenic stimuli, including Wnt signaling, CEC proliferation, and epithelial barrier breakdown, which contribute to mucosal inflammation and the development of colon cancers [80].

17.3.1.3 *Streptococcus bovis*/*Streptococcus gallolyticus*

Hidden links exist between *S. bovis* and a wider range of cellular and molecular alterations that could be connected to the development of CRC COX-2 expression induced by the wall-extracted *S. bovis* antigen. COX-2 inhibits apoptosis and encourages cellangiogenesis with prostaglandins' assistance. *Streptococcus* contributes to CRC by inducing inflammation and inhibiting apoptosis; however, its effector remains unknown [81].

17.3.1.4 *Fusobacterium nucleatum*

The gram-negative, obligatory, commensal, opportunistic bacteria *F. nucleatum* is a native of the human oral cavity and contributes to periodontal disease. Recent findings offer experimental evidence in favor of *F. nucleatum*-induced tumor formation. In contrast to normal tissue, *F. nucleatum* is more prevalent in CRC tumors [81]. *F. nucleatum* and host cells communicate directly. Additionally, *F. nucleatum* and other intestinal bacteria can cling directly to enterocytes, changing the integrity of the endothelium and potentially having carcinogenic effects according to Rubinstein's research. FadA92, the external adhesin, is the related factor that has been most described. *F. nucleatum*'s special FadA adhesive can bind to E-cadherin, activating β -catenin signaling and causing carcinogenic reactions in CRC cells [82].

Table 17.1 Microbes associated with CRC and their effectors

Serial no.	Microbes associated with CRC	Effector	References
1.	<i>Escherichia coli</i>	Colibactin	[75]
2.	<i>Bacteroides fragilis</i>	<i>B. fragilis</i> toxin (BFT)	[79]
3.	<i>Streptococcus bovis</i> / <i>Streptococcus gallolyticus</i>	Cox-2	[81]
4.	<i>Fusobacterium nucleatum</i>	FadA	[83]
5.	<i>Helicobacter pylori</i>	CagA	[86]

17.3.1.5 *Helicobacter pylori*

The development of stomach disorders and gastric cancer (GC) is significantly influenced by *H. pylori*. While some reports have suggested a possible link between colorectal neoplasms and *H. pylori* infection, others have refuted this. It is still unknown how *H. pylori* causes colorectal neoplasia pathophysiologically. *H. pylori* causes CRC through three mechanisms, one of which is the inhibition of gastric mucosal proton secretion [83]. Secondly, the secretion of inflammatory mediators like IL-8 and the expression of *CagA* gene contribute to the progression of CRC [84]. Shmuely et al. examined the expression of CagA protein and serum IgG antibodies in 67 instances of colorectal adenocarcinomas to see if there was a connection between the protein and colorectal cancer. They discovered a link between increased risk of colonic and stomach cancer and CagA+ seropositivity. Additionally, it promotes gastrin secretion, leading to cell proliferation and, consequently, CRC [85] (Table 17.1).

17.4 Current Microbiota-Targeted Therapies

17.4.1 Prebiotics

Prebiotics are generally described as “nondigestible food components that pass into the colon in the unchanged state, are resistant to the action of hydrolytic enzymes at the top of the GIT, and beneficially affect the microflora of the host organism by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon and thereby improving the host’s health” [86]. One may use the following two criteria to distinguish that this substance is a prebiotic: substances that are carbohydrates that have a degree of polymerization (DP) of three or more, and the small intestine’s natural enzymes cannot hydrolyze them [87]. Due to fermentation, various metabolites are produced, such as organic acids (e.g., lactate, pyruvate, and succinate), gases (e.g., H₂, H₂S, CO₂, and CH₄), and SCFAs (e.g., acetate, propionate, and butyrate), which results in a decline in fecal enzymes, nitrogenous end products, and a moderate fall in colonic pH [88]. These metabolites are responsible for histone modification, and DNA methylation and are thus used in treating colorectal cancer [88]. Prebiotics boost the growth of advantageous

Table 17.2 Different prebiotics and their therapeutic outcome

Serial no.	Category	Composition	Outcome	References
1.	Fructo oligosaccharide	Inulin	Decrease the level of inflammatory mediators and inflammation in colon	[94]
2.	4-O- β -D-galactopyranosyl-D-fructofuranose	Lactulose	Rapid increase in the growth of probiotics	[95]
3.	β -galactosidase	<i>Bacillus circulans</i>	Inhibits inflammation	[96]
4.	Resistant starch	Green bananas	Activates apoptosis of cancer cells	[97]
5.	<i>L. plantarum</i>	Honey bee gut	Decreases inflammation	[98]

Lactobacilli and *Bifidobacteria* while reducing the viability of several enteropathogenic species (Table 17.2). This resistance to the colonization of the gut by pathogens can lower the incidence of acute and chronic inflammation, which may cause damage to DNA and oncogenesis [89]. Prebiotics can affect the synthesis of pro- and anti-inflammatory cytokines, which reduces inflammation. Prebiotics elevate IgA secretion in the ileum and boost IL-10 production in Peyer's patches. Prebiotics prevent colorectal cancer by inhibiting proinflammatory cytokines IFN- γ and IL-2, nuclear factor kappa B (NF- κ B) activation [90]. In a meta-analysis, Friedenreich et al. found that consuming more than 27 g of fiber daily reduced the risk of colorectal cancer by 50% when compared to consuming less than 11 g [91]. Role of probiotics, prebiotics, and symbiotic in chemoprevention for colorectal cancer. In a clinical trial study conducted in China, oral administration of a 30 g prebiotic supplement (Hangzhou Niuqu Biotech Co., Hainengbo, China) containing 25% fructooligosaccharide, 25% xylooligosaccharide, 25% polydextrose, and 25% resistant dextrin had a significant impact on immunologic indices in both the preoperative and postoperative periods of patients with colorectal cancer (CRC). The study involved 140 perioperative patients (90 men and 50 women, aged 40–75 years). Additionally, the patients' opportunistic pathogens and four commensal microbio-tas—*Bacteroides*, *Bifidobacterium*, *Escherichia coli*, and *Enterococcus*—had different abundances after prebiotics [92, 93].

17.4.2 Probiotics

Probiotics are live, nonpathogenic microorganisms that are essential to the host's health advantages when given in appropriate amounts. Probiotics are mainly classified as lactic acid-producing and non-lactic acid-producing bacteria. Probiotics' primary attributes include bile tolerance, acid resistance, bile salt hydrolase

Table 17.3 Different probiotics and their therapeutic usage

Category	Species	Therapeutic outcome	References
Lactic acid-producing	<i>Lactobacillus gallinarum</i>	Reduces intestinal tumor number and size	[50]
	<i>Lactobacillus acidophilus LA102</i>	Inhibits the proliferation of tumor cells	[101]
	<i>Lactobacillus casei</i>	Produces linoleic acid (LA) from linoleic acid and exert local beneficial action	[102]
	<i>Lactobacillus plantarum</i>	Inhibits colon cancer in mouse model	[103]
Bifidobacterium species	<i>Bifidobacterium adolescentis</i>	Generates CD143+ CAFs with highly expressed GAS1 and inhibited colonic carcinogenesis via the Wnt/ β -catenin pathway	[104]

potential, adhesion of mucosal or epithelial cells, antimicrobial resistance, immunostimulation, antagonistic action against pathogens, and antimutagenic and anticarcinogenic properties [99]. Probiotics often act as protective agents against different enteric infections and as supplements to the host microbiota. Probiotics also activate particular genes in localized host cells, which in turn stimulate, modify, and regulate the host's immune response [100] (Table 17.3).

An inflammatory immune response and immunological stress were triggered by disrupting the intestinal mucosa's integrity and barrier dysfunction, which increased allergen permeability. Disruption of the epithelial barrier caused by pathogenic microorganisms entering the intestinal epithelium raises the risk of colorectal cancer. Probiotics can preserve the integrity of the epithelium and avert damage caused by tumor necrosis factor (TNF). Prebiotics also enhance the structural integrity of the intestinal barrier and tight junction [105].

Probiotics have shown encouraging results in reducing the growth of tumors and triggering apoptosis by upregulating proapoptotic mediators and squelching antiapoptotic signals. To initiate extrinsic apoptotic pathways and signaling cascades involving caspase 8 and B-cell lymphoma 2 (BCL2), probiotics bind to death receptors 4 and 5. Probiotics use the NF- κ B and myeloid differentiation factor 88 (MYD88) pathways to limit cancer cell proliferation and reduce the risk of colorectal cancer. It has been proposed that MYD88, a regulatory factor in inflammation and the innate immune system, can predict the prognosis of colorectal cancer. When MYD88 is activated, its transcription factor NF- κ B and signaling cascades are activated downstream, promoting inflammation and suppressing apoptotic signals [106]. Probiotics are also used after chemotherapy to reduce gastrointestinal complications and improve gut microbiota dysbiosis. Huang et al. evaluated 100 eligible CRC patients who required chemotherapy after undergoing major surgery as part of their treatment. Randomly assigned to the Probio group, half of them were given a probiotic combination to consume from the time of the procedure until the end of the first round of chemotherapy. The placebo group included the other half of

patients who were given a placebo instead. During chemotherapy, reports of gastrointestinal side effects including nausea, acid reflux, stomach pain, abdominal distention, constipation, and diarrhea were recorded. Fecal samples were obtained for short-chain fatty acid (SCFA) analysis and 16S rRNA high-throughput sequencing both before surgery and following the first postoperative chemotherapy round. The introduction of probiotics was found to be an effective way to minimize gastrointestinal issues caused by chemotherapy, especially in cases of diarrhea ($p < 0.01$) [107].

Probiotics' capacity to act as antioxidants makes them a valuable tool in the prevention of colorectal cancer. Thus, Sah and colleagues showed that some probiotic strains found in yogurts can generate antioxidant peptides with the ability to scavenge free radicals, suggesting that they may lessen oxidative stress in the lumen and, as a result, prevent or postpone the start of CRC [108]. The terms "post- and paraprobiotics" have been used to characterize nonviable microorganisms or bacterial cell-free culture supernatants that may benefit hosts by supplying extra bioactivities beyond those of probiotics. Postbiotics are also known as cell extracts, biogenic, metabolites, cell-free culture supernatants, and metabiotics. Conversely, paraprobiotics are intact, inactivated (nonviable) microbial (probiotics or nonprobiotics) cells [109].

17.4.3 Synbiotics

Synbiotics are preparations that overlap prebiotics and synbiotics. This category of compounds has both prebiotics and synbiotics for better therapeutic effects and superior performance [110]. The International Scientific Association for Probiotics and Prebiotics (ISAPP) panel recently updated the definition of a synbiotic to read as follows: "a mixture consisting of live microorganisms and substrate(s) selectively utilized by host microorganisms that confer a health benefit on the host." Such a preparation can be made in a way that targets the host microorganisms in a complementary manner, or it can be made in a way that the coadministered probiotics selectively use the prebiotic to accomplish one or more health advantages [111] (Fig. 17.3). In a study, Polakowski investigated the impact of preoperative synbiotic use in colorectal cancer patients. The study was conducted in 73 patients who were given either synbiotics (Simbioflora®) or placebo (maltodextrin). The levels of IL-6 (163.2 ± 19.5 vs. 138.8 ± 12.5 , $p < 0.001$) and CRP (10.0 ± 5.2 vs. 7.17 ± 3.2 , $p < 0.001$) significantly decreased after 7 days of synbiotic intake, while the control group showed no significant changes (IL-6: 154.2 ± 18.3 vs. 160.9 ± 18.6 , NS and CRP: 10.6 ± 6.18 vs 10.4 ± 6.1 , NS). Transferrin and serum albumin did not exhibit any appreciable alterations. In the synbiotic group, the mean duration of hospital stay was 3.0 ± 1.0 days, while in the control group, it was 4.0 ± 18 days ($p < 0.001$). In the synbiotic group, there was no mortality and three fatalities in the control group ($p = 0.115$). Infection problems following surgery happened in 2.8% of patients in the synbiotic group and 18.9% of patients in the control group ($p = 0.02$). This indicates that synbiotics have the potential to treat colorectal cancer [112]. Oh

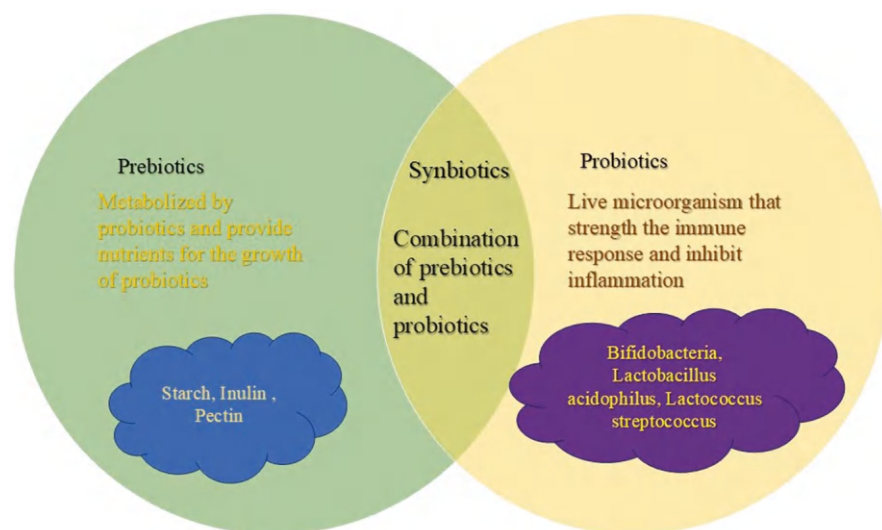


Fig. 17.3 Different microbiota-targeted therapies

et al. investigated the cancer-protective effect of a synbiotic combination between *Lactobacillus gasseri* 505 and a *Cudrania tricuspidata* leaf extract on colitis-associated colorectal cancer. After 10 weeks of therapy, the incidence of colonic tumors and damage to the colonic mucosa in the colitis-associated colorectal cancer (CAC) animal model produced by azoxymethane (AOM)/dextran sodium sulfate (DSS) was successfully decreased in LG, CT, or FCT, indicating a cancer-protective effect. The AOM/DSS group was observed to compare with the LG/CT/FCT group for biomarkers linked to inflammation, colon barrier, apoptosis, and cancer cell proliferation to get an understanding of these. Inflammation-associated enzymes (iNOS and COX-2) as well as pro-inflammatory cytokines (TNF- α and IL-6) were down-regulated while anti-inflammatory cytokines (IL-4 and IL-10) were up-regulated when fed in combination (FCT). Furthermore, the colon barrier experiment demonstrated the upregulation of tight junction (occludin and ZO-1) and mucus layer (MUC-2 and TFF3) biomarkers. Leu et al. examined the potential of the probiotic bacterium *Bifidobacterium lactis*, carbohydrate-resistant starch (RS), and their combination as a synbiotic. Semi-purified diets were given to six equal groups of Sprague-Dawley rats for 30 weeks. The two weekly injections of azoxymethane (15 mg/kg body weight) caused colonic neoplasms. The following were the experimental groups: control: dietary fiber and RS were not added; RS was added in two different forms (Hi-maize 260 or 958); *B. lactis* (lyophilized) was added to both the control and RS diets (a total of six treatment groups). Rats given RS together with *B. lactis* had a substantial ($P < 0.01$) reduction in the frequency and multiplicity of colonic neoplasms by more than 50% when compared to the control group [113]. The synbiotic intervention of *Bifidobacterium lactis* and resistant starch protects against colorectal cancer development in rats (Table 17.4).

Table 17.4 Different synbiotics and their therapeutic outcome

Serial no.	Synbiotics	Therapeutic outcome	References
1.	<i>Bifidobacterium lactis</i> and resistant starch	Apoptosis of cancer cells	[113]
2.	<i>Lactobacillus rhamnosus</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , and <i>Bifidobacterium lactis</i> + fructooligosaccharides	Reduction in IL-6 levels	[114]
3.	<i>Bifidobacterium lactis</i> Bb12, <i>Lactobacillus delbrueckii</i> , oligosaccharides enriched inulin	Decreased proliferation of colorectal cancer cells	[115]
4.	<i>Lactobacillus casei</i> , <i>Bifidobacterium breve</i> and β -galactosyl-sucrose	Decreased IL-6, STAT-3, COX-2, and TNF α gene transcripts	[116]
5.	<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium sp.</i> , <i>Streptococcus thermophilus</i> , <i>Lactobacillus casei</i> , <i>L. delbrueckii subsp Bulgaricus</i> , and <i>Bifidobacterium longum</i> and chicory fructo oligosaccharides (FOS)	Reduced cytokines and inflammation	[117]

17.4.4 Adjuvants and Neoadjuvant Therapies

17.4.4.1 Oxaliplatin

Adjuvants are compounds that alter vaccine effects and enhance the body's defenses against antigens. This galectin inhibitor induces a stronger secondary response and longer-lasting immunity. Immune checkpoint inhibitors and cytokines are examples of adjuvants [109]. QUASAR Collaborative Group* analyzed 3239 patients suffering from colorectal cancer. They impede DNA replication and cause cellular death. Two phase III clinical trials assessed oxaliplatin in patients with metastatic colorectal cancer. The results showed that oxaliplatin added to infusional fluorouracil and leucovorin improved overall survival, increased response rate, and disease-free survival [118]. According to a recent French study, the inclusion of oxaliplatin raised the 5-FU response rate from 26% to 57%. More research is being done to determine whether using oxaliplatin in conjunction with fluorinated pyrimidines improves survival in patients with advanced colorectal cancer and to validate the combination's efficacy. In the CO-7 trial, the National Surgical Adjuvant Breast and Bowel Project (NSABP's) planned large bowel adjuvant therapy research, one of the treatment arms is presently scheduled to be the 5-FU-plus-leucovorin-plus-oxaliplatin combination [119]. Twenty-four hundred previously untreated patients with detectable disease were randomized to receive either a 2-h infusion of LV (200 mg/m²/d), in combination with an 85 mg/m² oxaliplatin 2-h infusion on day one, or a 5-FU bolus (400 mg/m²/d) and 22-h infusion (600 mg/m²/d) for 2 days in a row every 2 weeks. When compared to the control arm, patients assigned to oxaliplatin + LV5FU2 had a substantially longer progression-free survival (median, 9.0 vs 6.2 months; $P = .0003$) and a superior response rate (50.7% vs 22.3%; $P = .0001$) [119].

17.4.4.2 5-Fluorouracil

5-FU is a commonly prescribed chemotherapeutic drug for the treatment of many malignant tumors, including those of the skin, stomach, esophagus, breast, pancreas, and head and neck areas. Since the 1990s, oral and intravenous 5-FU, or other fluoropyrimidines (FPs), have been the cornerstone of systemic treatment for colorectal cancer (CRC). 5-FU inhibits TS which depletes the initial precursor of DNA dTTP which causes misincorporation of Dntp into DNA [120]. 5-FU has lots of side effects and an unpredictable pharmacokinetics profile thus, it is used in conjugation with many drugs and has shown encouraging benefits. To enhance the efficiency and to limit the ill effects of drug 5-FU was loaded on gold nanoparticles. Safwat et al. synthesized 5-FU gold nanoparticles using glutathione (GSH) and thioglycolic acid (TGA). In colorectal cancer cells, 5-FU/GSH-GNPs triggered apoptosis and halted the cell cycle progression. In addition, their anticancer impact was two times more than that of free 5-FU [121].

Adjuvant chemotherapy aims to increase survival rates and eliminate micrometastatic illness. Many adjuvant chemotherapy experiments have been carried out during the past three decades to increase survival. The advantages of adjuvant chemotherapy were initially shown by Moertel et al. in a crucial study in 1990. Adjuvant chemotherapy with 5-FU and levamisole given for 12 months following surgery was shown to increase survival and minimize recurrence more than surgery alone.

17.4.4.3 Herbal Adjuncts

The main polyphenol pigment in *Curcuma longa*, a plant that is widely consumed as a wholesome meal with antioxidant properties, is known as curcumin. Curcumin works in concert with antineoplastics such as oxaliplatin and 5-fluorouracil as well as anti-inflammatory drugs by blocking cyclooxygenase-2 and nuclear factor kappa B. Moreover, curcumin has anticancer characteristics by blocking the signaling pathways linked to the development of colorectal cancer (CRC), including PI3K/Akt, Hedgehog, Notch, Wnt/ β -catenin, and mTOR [122]. One of the most well-known polyphenols is resveratrol, a naturally occurring plant antibiotic found in many different plants, nuts, and fruits, but especially in grapes and red wine. Resveratrol has been demonstrated in CRC to chemo-sensitize CRC cells to 5-FU in 3D alginate cultures and to inhibit TNF- β -induced tumor metastasis. Furthermore, resveratrol has been shown by Khaleel and colleagues to be able to increase the susceptibility of colorectal cancer cells to Doxorubicin (a chemotherapeutic agent) (DOX) by inducing apoptosis and improving DOX intracellular entrapment through inhibition of the P-gp pump. Interestingly, Didox, a synthetic polyphenolic molecule that has significant metabolic targets with resveratrol, was also able to exhibit the same capacity [123].

17.4.4.4 Antibodies

Antibodies directed against vascular endothelial growth factor, like bevacizumab, and epidermal growth factor receptors, like cetuximab, have both demonstrated effectiveness in the metastatic context [114]. CIKs are produced by cultivating autologous peripheral blood mononuclear cells with anti-CD3 monoclonal antibodies, interleukin-2, and interferon-gamma. When patients receive these cells, they can use their cytotoxic properties against cancer cells. Individuals who had CKI treatment also reported higher quality of life. Clinical studies are now being conducted to assess CKI therapy as a potential treatment for colorectal cancer. CAR-T cell therapy, a form of immunotherapy, involves extracting leukocytes from a patient's whole blood sample, genetically modifying them to express chimeric antigen receptors (CARs) on their surface, and reintroducing them to elicit an immune response against cancer cells expressing the targeted antigens [124]. Adjuvant chemotherapy aims to increase survival rates and eliminate micrometastatic illness (Fig. 17.4).

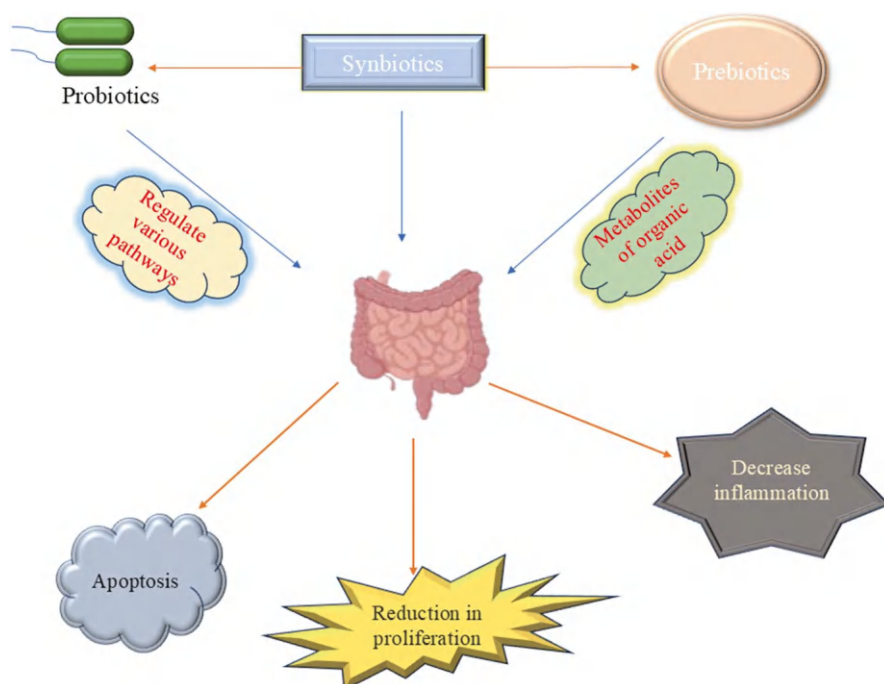


Fig. 17.4 Illustration of microbiota-targeted therapies

17.5 Challenges in Microbiota Targeting Therapies for CRC

The results may be influenced by factors such as the complexity of carcinogenesis, trial design, challenges in obtaining sufficient sample sizes, variations in probiotics strains, and differences in participants' tumor stages. Furthermore, it has been shown that the dosage of probiotics taken impacts the intended protective benefits. Rat colon cells exposed to carcinogens showed protection against *L. casei*-induced lesions when probiotics were administered at a dose of 1×10^{10} bacteria in 10 mL NaCl/kg body weight. In a similar vein, it was discovered that a single dosage of live *Streptococcus thermophilus*, *L. acidophilus*, *L. gasseri*, *L. confusus*, *B. breve*, and *B. longum* could stop MNNG-induced DNA damage in the colon. Nonetheless, the carcinogen protective effects were lost at a 50–90% lower bacterial dosage. This might be explained by the probiotics' dose-dependent activation of the immune system in the gut, which releases inflammatory and regulatory cytokines such as IL-14, IL-10, gamma interferon, and interleukin 12 (IL-12) [125]. Targeting colorectal cancer is challenging because intestinal microorganisms' ecological makeup and metabolic activity can be reconstructed by gut microbiota-derived metabolites such as trimethylamine-N-oxide, secondary bile acids, hydrogen sulfide, and N-nitroso compounds. These metabolites can also create an environment that increases susceptibility to carcinogenic stimuli. Through several methods, such as causing inflammation and DNA damage, activating tumorigenic signaling pathways, and modulating tumor immunity, they are implicated in the incidence, progression, and metastasis of colorectal cancer (CRC) [126]. More research has shown that not all patients respond to treatments that modify the gut microbiota similarly; rather, the response largely depends on the baseline traits, such as genetic background, gut barrier function, and microbiome diversity [12]. The efficiency of microbiota-targeted therapies also depends on the country where they originate. The same species of the same strain belonging to different countries showed different survival and stability of probiotics. In addition to having to withstand the harsh conditions of storage and transit, probiotics also need to be able to withstand the acidic environment of the stomach and the bile salts of the small intestine before they can take action in the colon. Overages are added to compensate for the dead bacteria during transportation or storage but it also creates a new challenge towards therapy because one can't predict the number of bacteria that will die and one also can't predict the interaction of these death bacteria metabolism.

17.5.1 Regulatory Considerations

Regulatory considerations for microbiota-targeted therapies are lacking due to the responsible authorities for food and drugs did not have any explicit guidelines or protocols for evaluating living microorganisms at the time, therefore there was no agreement on how to evaluate and prove their advantages. There is no single,

globally accepted framework governing the use of probiotics, so regulations vary between countries. Probiotics and dietary supplements are subject to EU regulations under the Food Products Directive and Regulation (Directive 2000/13/EU; Regulation 178/2002/EC). Probiotic health claims must all be approved by the EFSA. A list of microbial cultures that do not need safety studies is available thanks to the EFSA's Qualified Presumption of Safety²² designation. Additionally, the EFSA is in charge of evaluating health claims related to certain diseases [127]. In terms of quality and safety, sometimes synbiotics are the least sight-seen products in research and regulatory standards. Regulations related to synbiotic products vary from country to country. Before being marketed in the market, synbiotic products need to meet country-specific safety and labeling regulatory requirements [128]. Prebiotics continue to be studied by scientists, but there are still issues with product regulation and choice that affect the general consumer. Patients may inquire about prebiotics, probiotics, or synbiotics in the practice of medicine. It is crucial to determine the right dosage, duration, and product that would best serve the patient. It is imperative to carry out further study and create the best possible application of prebiotics as a weapon in the fight against colorectal cancer. There is now no "separate status" for live biotherapeutic products (LBPs) in the European legislative framework. Since the active ingredients in LBPs are living microbes, which are biological substances, they are by definition biological medical products. The legal and regulatory framework pertaining to biological medical products must thus be followed by LBPs. Developers should thus depend on pertinent and appropriate regulatory ideas that are available for the biological medical product category in the lack of a dedicated LBP regulation. The Food and Drug Administration (FDA) has clarified pharmaceutical expectations by defining a new category called "live biotherapeutic products (LBP)." Since 2019, the European Pharmacopoeia (Ph. Eur.) has also provided clarification on the quality criteria for this category of medicinal goods. LBPs must be registered as medicinal products to be sold in the USA and Europe, just like any other product used to cure or prevent a disease. The "International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use" (ICH) guidelines are frequently used in this field by regulatory bodies and the pharmaceutical industry [129]. According to the regulations the products containing microbiota must fulfill these given statements on their label.

1. Substances/allergens: All active and inert substances are enumerated by weight in descending order (allergens must be included in this list).
2. By current taxonomy, the probiotic's genus, species (subspecies), and strain.
3. A viable count is expressed as the overall number of colony-forming units (CFUs), while counts specific to each strain are favored. The CFUs stated on the label ought to be guaranteed until the end of the shelf life and should, at mini-

mum, match the quantity that has been demonstrated to be effective in human research.

4. Daily dosage: the required daily intake must equal or exceed the daily dosage examined in human trials [130]. There is no single, globally accepted framework governing the use of probiotics, hence regulations vary between countries. Probiotics and dietary supplements are subject to EU regulations under the Food Products Directive and Regulation (Directive 2000/13/EU; Regulation 178/2002/EC). Probiotic health claims must all be approved by the EFSA. A list of microbial cultures that do not need safety studies is available thanks to the EFSA's Qualified Presumption of Safety²² designation. Additionally, the EFSA is in charge of evaluating health claims related to certain diseases [127].

17.6 Future of Microbiota-Targeted Therapies

The subject of microbiota-targeted therapy research is fast emerging and has considerable potential for the development of pharmacological treatments for colorectal cancer. There is now more interest in researching novel probiotic strains for cancer treatment due to the rapid advancement of sophisticated genetic sequencing technologies, bioinformatics platforms, and potent methods for modifying bacterial genomes. The existing restrictions may be solved by next-generation probiotic formulations genetically designed to target certain species for different kinds of cancer. The development of genome editing has made probiotic immunotherapy a viable treatment for tumor resistance in colorectal cancer. Sec. Cancer Immunity and Immunotherapy Volume 14—2023 [131]. Recognizing that the human gut microbiota is very customized, one important avenue of research is the development of tailored probiotics.

Scientists are working to pinpoint certain bacterial strains that will assist each patient to the greatest extent possible using methods such as metagenomic sequencing. This customized method offers more precise and potent treatments by customizing probiotic treatments to each individual's specific needs [132]. To prevent the degradation of synbiotics in acid conditions and to efficiently work on specific colorectal cancer cells one must integrate the synbiotics with nanocarriers to deliver synbiotics in targeted areas. Zhu et al. encapsulate *Lactobacillus reuteri* lysate in Dimethyl phosphate (DMP) to create a novel nonviral siRNA vector (DMPLAC). DMPLAC can decrease tumor growth, enhance immune cell maturation and activation, and boost the tumor microenvironment's APC and cytotoxic T cell infiltration. In some animals, loading siRNA directed against Stat3, DMPLAC/siStat3, further suppresses tumor growth [133]. Mousavi et al. formulated silver nanoparticles containing a probiotic strain of *Lactobacillus rhamnosus*. During analysis, it was found that live cells of colorectal cancer (HT-29 cell line) decreased significantly, leading to apoptosis [134].

17.7 Conclusion

Microbiota-targeted therapies can be the future of colorectal treatment. Microbiota-targeted therapies are the epitome of therapies for the prevention and treatment of colorectal cancer. Microbiota-targeted therapies are also used to reduce the post-surgery complications. Microbiota-targeted therapies are also used as supplements with adjuvant therapies to decrease their side effects and lead to better outcomes [135]. The working mechanisms of these therapies are very diverse but majorly it can be concluded that they reduce the factors that cause progression in colorectal cancer and also increment of beneficial factors. These therapies work on the base levels to prevent cancer. They release metabolites which provide relief in complications. These therapies will advance significantly if various clinical trials are conducted on human subjects. So far, only animal studies have been performed, leading to uncertainty due to physiological differences between animals and humans. One another pivotal route to have beneficial results from microbiota therapies is the opportunity of fecal microbiota transplantation. It is the best way to transfer the beneficial microbes in the cancer area and subside the cancer-causing microbes. This therapy needs lots of attention to reduce the risk of infection. To use these targeted therapies, one must be aware of the interactions among prebiotics, probiotics, and drug adjuvants. Neither every prebiotic is beneficial for probiotics nor every probiotic is favorable to drug adjuvants. One should consider strain, specific colony-forming units, and the process of cultivation of probiotics before accruing them with adjuvant therapies. The regulations of these microbiota-targeted products are lacking which leads to more complications but efforts are being made to have stringent regulations for these products. Summing up one can conclude that microbiota-targeted therapies can themselves be adjuvant in colorectal cancer and they can be added up with another drug.

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Chapter 18

Diagnostic Approaches for Colorectal Cancer: Gut Microbiota–Based Biomarkers for Colorectal Cancer Detection and Monitoring



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Abstract Colorectal cancer (CRC) is the third most common disease in the world and one of the leading causes of cancer-related death worldwide. Even with its severity, good results are frequently achieved with early discovery and treatment. As a result, CRC screening initiatives have been launched internationally. A growing body of research reveals the gut microbiome's role in CRC development and its potential as an early detection biomarker. Studies show that the gut microbiome can affect the growth of adenomas and the risk of colorectal cancer (CRC) through various processes such as co-metabolic dysregulation, inflammation, epigenetic modifications, and DNA damage. Researchers have identified specific microbiomes such as *Fusobacterium nucleatum*, *Escherichia coli*, and *Bacteroides fragilis* within precursor lesions, highlighting microbiome alterations as distinguishing features in CRC progression. Early methods of screening for colorectal cancer often use fecal biomarkers such as the guaiac fecal occult blood test (gFOBT) and fecal immunochemical tests (FITs). These tests are usually followed by an endoscopy because the gFOBT and FITs don't show everything. Although the oral and fecal gut microbiota exhibit potential as a supplement to the current CRC screening instruments, the

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available data indicates that these microbiomes are not yet ready for widespread clinical use. Metaproteomics, metabolomics, and microbial cell-free DNA (microbial cfDNA) are noninvasive ways to look at the microbiome for CRC. Deep sequencing, novel laboratory approaches, and computational methods are increasingly evaluating the potential of microbial cfDNA for cancer detection, despite technical and biological challenges in CRC. Liquid biopsies could target the microbiome and improve early cancer detection. Overall, gut microbiota analysis represents a rapidly advancing field with significant promise for noninvasive CRC diagnostics.

Keywords Colorectal cancer · Microbiome · Cancer · cfDNA

18.1 Introduction

Globally, colorectal cancer (CRC) is the third most frequent cancer when compared to all types of cancers. In recent years, the incidence and mortality of CRC cancer have sharply risen in many lower-income countries. By 2030, CRC incidence is predicted to have increased by more than 60%. Over half of the CRC cases and mortality rates are attributed to modifiable risk factors including smoking, a poor diet (Western diet), too much alcohol consumption, physical inactivity, and obesity [1]. A continuous accumulation of genetic and epigenetic modifications resulting from CRC generates significant genomic instability. CRC has been associated with several mutations including mutational inactivation of tumor-suppressor genes like (p53, adenomatous polyposis coli (APC), and transforming growth factor- β (TGF- β)) and activation of oncogene pathways like (Ras, BRAF, and phosphoinositide 3-kinase (PI3K)) [2]. The APC gene proved to be the first gene mutation found in CRC [3]. In CRC, primarily it forms polyps in response to mutations in tumor-suppressor genes, such as APC a component of the Wnt/ β -catenin pathway that regulates the cell cycle or cell growth. Furthermore, CRCs are mutations occurring in genes related to deoxyribonucleic acid (DNA) repair, including human DNA mismatch repair protein MSH2 [4]. Among these genetic changes are some hereditary CRC cases that are generally infrequent. Genetically, three forms of CRC exist, sporadic (60%), which describes patients without a family history, with familial history (30%), which includes patients with at least one blood relative with CRC or an adenoma, and hereditary (10%), which is brought on by mutations traveled down to the germline [5]. Nowadays most noninvasive screening methods of stool-based tests are applied in the stool to identify CRC or precancerous polyps. The most commonly utilized type of stool-based testing is fecal occult blood tests (FOBTs). Hidden blood in the stool found by FOBT could indicate CRC or polyps. Fecal immunochemical testing (FIT) has a high sensitivity for detecting CRC (79%), but it has a low sensitivity for identifying colorectal adenomas (CRAs) (25–27%) [6]. Primary stages of CRC or precancerous polyps can be found with convenient, non-invasive, stool-based testing. Current clinical diagnostic tools, including the FIT and FOBT, have poor CRC diagnosis sensitivity. Research studies show that early

diagnosis and treatment of precancerous growths can reduce the incidence and death rate of CRC. Moreover, with regular screening, thorough monitoring, and first-line treatment, an increase in CRC incidence and mortality can be prevented [7].

To overcome the above limitation in the detection of CRC, it is ideal to identify creative biomarkers as reliable and noninvasive. The identification of gut microbial biomarkers helps noninvasive instrument development for CRC diagnosis to advance. The recent research on gut microbiome–based screening of CRC was elucidated in bibliography mapping, as shown in Fig. 18.1. Abundant CRC bacteria could be helpful as bacterial biomarkers for the CRC diagnosis [8]. Extensive studies on fecal metagenomes have revealed microbial signals that might predict CRC in diverse populations. Extensive studies on fecal metagenomes have revealed microbial signals that might predict CRC in many different cultures. CRC is associated with gut microbes including *Solobacterium moorei*, *Fusobacterium nucleatum*, *Clostridium citroniae*, *Gemella morbillorum*, and *Parvimonas micra* among other species [9]. Six significant genera such as *Alloprevotella*, *Parvimonas*, *Gemella*,

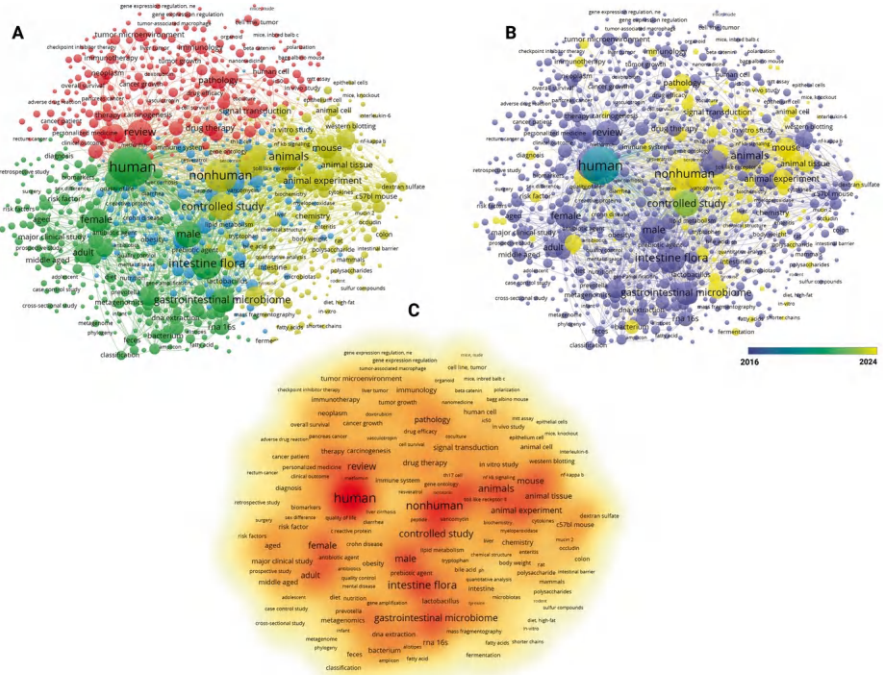


Fig. 18.1 (a) Bibliography map of scientific research on gut microbiome–based diagnostic approaches for colorectal cancer (CRC). The network visualization based on four colored clusters indicates the relatedness of the scientific journals and an overlay visualization. (b) It specifies a period of the occurrence of the keyword from 2016 (blue) to 2024 (yellow). This figure is utilized from the Scopus and PubMed database by keywords “Gut” and “Microbiome” and “Diagnostic” and “Biomarkers” and “Dysbiosis” and a time range of 2014–2020. (c) Density visualization of scientific research on gut microbiome based diagnostic approaches for screening of colorectal cancer

Peptostreptococcus, *Campylobacter*, and *Fusobacterium* were repeatedly overexpressed in the tumor mucosa of CRC [10].

Bile acids and short-chain fatty acids (SCFAs) are the microbial metabolites associated with the development of CRC and could be the biomarkers for early CRC identification. Many studies have found in the fecal samples of patients with CRC varied concentrations of microbiological metabolites, including lowered butyrate levels, ursodeoxycholic acid, and raised acetate levels [11, 12]. Preliminary CRC diagnosis can be diagnosed using the enrichment of genes linked to protein and mucin catabolism, as well as the depletion of genes linked to carbohydrate breakdown, based on the study of CRC metagenomes [9].

Microbial metabolites, such as SCFAs and bile acids, that have been associated with CRC progression may also serve as biomarkers for the early screening of CRC. Many studies have reported differentiating levels of microbial metabolites in fecal samples of patients with CRC, including ursodeoxycholic acid, lower levels of butyrate, and higher levels of acetate [13]. Analysis of CRC metagenomes suggests an enrichment of protein and mucin catabolism genes and a depletion of carbohydrate degradation genes, which may be used as a marker for preliminary CRC diagnosis [14]. A study by Zhang et al. found that *Parvimonas* and *Peptostreptococcus* species were used for the screening of stage I CRC. For stage II species like *Oxalobacteraceae*, *Parvimonas*, *Burkholderiales*, *Fusobacterium*, *Caulobacteraceae*, *Delftia*, and *Streptococcus*, and for Stage-III species such as *Oxalobacteraceae*, *Burkholderiales*, *Fusobacterium*, *Caulobacteraceae*, and *Faecalibacterium Sutterella* are analyzed [15]. Chang et al., found that the CRC was enriched with 37 bacterial species such as *Coriobacteriaceae*, *Citrobacter portucalensis*, *Shigella sonnei*, *Parvimonas micra*, *Gemella morbillorum*, *Fusobacterium nucleatum*, and *Alloprevotella sp.* [16]. Shen et al. conducted research and identified the three phage bacteria biomarkers were identified in CRC such as *Parvimonas micra* phage, *Fusobacterium nucleatum*, and *Peptacetobacter hiranonis* [17]. Osman et al. found that four bacterial markers such as *Akkermansia muciniphila*, *Parvimonas micra*, *Peptostreptococcus stomatis*, and *Fusobacterium nucleatum* differentiate CRC patients from normal persons [18]. The presence of specific gut microbiome species may serve as a biomarker to screen the risk of developing CRC. Reliable diagnostics by gut microbiome to screen those with average risk should help to lower the incidence and death of CRC. In this chapter, we discussed the various novel gut microbiome-based diagnostic features for CRC detection and monitoring.

18.2 Role of Gut Microbiota in Colorectal Cancer Development

18.2.1 The Ecology of Gut Microbiota in CRC

CRC is the third greatest prevalent cancer globally, yet its specific origin remains unclear [19–21]. The proportion of CRC occurrences are intermittent (almost 90%), with exogenous and genetic risk variables have been recognized. In developing

nations, lifestyle variables such as sedentary behavior, tobacco smoking, poor nutritional choices (e.g., high trans-fat meals, refined carbohydrates, and processed meats), consumption of alcohol, and obesity substantially elevate the possibility of CRC [22, 23]. These exogenous stimuli can alter the intestinal environment of pathogenic microbes [24]. In preclinical models, gut microbiome may play an essential role in CRC formation by producing microbial oncometabolites (lactate, polyamines, short-chain fatty acids, acetate, formate, acetaldehyde, cytotoxic necrotizing factors, glucuronidase, colibactin, and deoxycholic acid) which compete with the host gene response and produce genotoxic pathogenicity effectors [24, 25]. According to a recent investigation, colonic mucosa and fecal specimens from CRC individuals show less variety and richness of gut-based pathogenic microbes in comparison with healthy people [26, 27]. Furthermore, in comparison to healthy subjects, CRC individuals exhibit notable changes in certain microbial populations that could influence the mucosal immune response [27]. For example, individuals with CRC have higher levels of *Streptococcus gallolyticus*, *Shigella*, *Escherichia coli*, *Enterococcus faecalis*, *Peptostreptococcus*, *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *Campylobacter* and lower levels of *Roseburia*, *Bifidobacterium*, *Clostridium*, *Blautia*, and *Faecalibacterium* [28]. Such alterations could raise pro-inflammatory adaptive microbes while reducing butyrate-generating microbes, thereby causing dysbacteriosis (disturbances in intestinal microbiome homeostasis) and tumor growth [29]. Ahn et al. reported a reduction in bacterial richness in fecal specimens of individuals with CRC, including an elevation in *Porphyromonas* and *Fusobacterium nucleatum* and a decline in chemoorganotrophic *Clostridia* [30]. Individuals with an initial phase of colorectal tumors exhibit a distinctive microbiome profile compared to those having late-phase tumors, implying that the gut microbiome may have contributed in tumor development [31]. Tjalsma et al. developed a bacterial driver-passenger approach for microbiological participation in CRC formation. The kinds of bacteria in the intestinal mucosa have different sequential relationships with tumor development [32]. The initial imbalance of the microbiome in adenomatous polyps and the increasing amount of *Fusobacterium nucleatum* have been attributed to an elevated generation of pro-inflammatory mediators in intestinal mucosa from individuals with CRC [33].

The gut microbes can cause pro-tumorigenic functions in mice with genetically susceptible colorectal cancer models. For example, Li et al. demonstrated that gut microbiome promotes the formation of tumors in adenomatous polyposis coli (APC)^{Min/+} mice via activating the STAT-3 and JNK/c-Jun signaling systems when combined with anemia [34]. In IL-10 deficient mouse, an elevated microbiota-mediated Th1 function aggravated chronic enterocolitis and accelerated adenocarcinoma development [35]. However, in addition to bacteria, the gut microbiota also comprises multiple fungal and virus strains. Numerous investigations have documented an elevated viral DNA expression in cancerous cells compared with healthy noncancerous cells. Several research has explored how viral transmission, including herpesviruses, polyomaviruses, and human papillomaviruses, may contribute to the possibility of CRC [36]. The community-driven viral shotgun next-generation sequencing (NGS) approaches have identified changes in colon virome heterogeneity in individuals with CRC. Specifically, CRC populations showed an increase in

viral variety, with abundance of the genera viz *Tunalikevirus*, *Inovirus*, and *Orthobunyavirus*. Surprisingly, the first two genera of the virus have been shown to infiltrate gram-negative bacterium hosts, such as *Escherichia coli*, *Fusobacterium nucleatum*, and *Bacteroides fragilis* which are linked to the occurrence of CRC. The findings demonstrate that fecal virome profiling can distinguish patients at initial and advanced phases of CRC and anticipate the patient's CRC condition [37]. However, other than the virome, the fungal microbiome metagenome was additionally investigated in CRC. The upregulated levels of *Candida* and *Phoma* (genus of fungi) in colonic adenoma samples indicated a correlation between modified host-associated fungus communities and the development of CRC [38]. In a study conducted by Coker et al. reported that the CRC individuals are significantly affected by fungal imbalance, with an abundance of Basidiomycota/Ascomycota proportion and the genus of *Malassezia* in comparison with healthy subjects. In patients with CRC, there has been an impairment in the quantity of *Saccharomyces cerevisiae*, a yeast recognized for its immune system modulation and anti-inflammatory activity. This suggests that it might be a possible treatment option [39]. Gao et al. reported a fungal imbalance in CRC and colon polyps, including an elevation within the Ascomycota/Basidiomycota proportion and aggressive fungus *Malassezia* and *Trichosporon*. This might contribute to the advancement of CRC [40]. The initial stages of cancers had less variety and substantially altered microbiome, as demonstrated by further research. Research implies that CRC is caused by a disturbance in gut virome and microbiome homeostasis and a disbalance in gut-microorganisms makeup.

18.2.2 The Interconnection Between Inflammation and Gut Microbiota Dysbacteriosis Leading to CRC

Prolonged inflammation has been associated with the progression of cancer. Therefore, it is predicted that long-term inflammation mechanism promotes up to 20% of all tumor growth [41]. Under carcinogenesis condition, cancerous cells discharge chemokines and inflammatory mediators, which bind to the pro-inflammatory T helper cells or naive myeloid cells. The pro-tumorigenic milieu produces tissue remodeling enzymes, angiogenic and growth factors, and suppresses antitumor T cell functioning, thereby facilitating tumor development [42]. The dysbacteriosis of the gut microbiome and higher intestinal penetration are strongly linked to colonic inflammatory conditions, which may be a major contributing factor to the development and/or advancement of CRC [43]. Therefore, as intestinal penetration increases, certain bacterial lipopolysaccharides from their outermost cell membranes permeate the host body. This causes the immune system to release cytokines and initiates a series of events that culminate in inflammation. Through the synthesis of chemokines and pro-tumorigenic cytokines, which function as growth factors and stimulate angiogenesis, localized inflammation facilitates the formation of

tumors [42]. Recent investigation has shown that polyps' formation in preclinical mice models is connected with microbial invasion, impairments in the functioning of the intestinal wall, and upregulated the production of several inflammatory mediators, including IL-1, TNF- α , CXCL2, and IL-17. Furthermore, changes in the intestinal wall permitted microorganisms to cause inflammation, leading to polyp production and cancer growth in mice [44].

18.2.3 Pathogenic Microorganism Leading to CRC

Apart from alterations in the diversity of the microbiome, the emergence of CRC has also been linked to pathogenic microorganism species. The pathogenic microorganism implicated in CRC such as *Peptostreptococcus*, *Fusobacterium prausnitzii*, *Streptococcus hansenii*, *Ruminococcus* (*Ruminococcus albus* and *Ruminococcus torques*), *Bifidobacterium* (*Bifidobacterium angulatum* and *Bifidobacterium longum*), and *Bacteroides* (*Bacteroides stercoris* and *Bacteroides vulgatus*) [45, 46]. These microorganisms may contribute to CRC carcinogenesis by promoting epithelial cell migration, causing membrane degradation, and triggering inflammation. However, genotoxins can cause mutations in DNA, leading to tumor formation. For example, colibactin (genotoxic metabolite derived from *Escherichia coli*) toxin induces DNA cross-linking and double-strand DNA damage and *Bacteroides fragilis* toxin (BFT) stimulates NF- κ B and Wnt signaling cascades, leading to increased pro-inflammatory mediator's production from epithelial cells [47, 48]. AvrA, a multifunctional protein from *Salmonella*, regulates the STAT3 system in a mice model of CRC, leading to increased β -catenin signaling and carcinogenesis [49]. *Fusobacterium nucleatum*, which adheres to E-cadherin on colon cells by FadA adhesion protein, stimulating the Wnt/ β -catenin signaling, resulting in a significant inflammatory and carcinogenic response, which makes it a plausible candidate for CRC susceptibility [50]. *Fusobacterium nucleatum* has been linked to becoming resistant to antineoplastic drugs (oxaliplatin), primarily triggering autophagy through Toll-like receptors (TLRs) [51].

18.3 Gut Microbiota–Based Biomarkers for CRC Detection

Clinical studies have revealed that the gut microbiota of CRC patients is different from that of healthy individuals, having a greater concentration of carcinogenic bacteria and a lower quantity of commensal bacteria. Research has also revealed variations in CRC patient's mucosal and fecal microbiota. Many investigations have attempted to delve deeply into the makeup of the microbiota in fecal and mucosal samples linked to CRC [52]. These investigations have discovered a change in the CRC microbiota's worldwide makeup. Research data on many populations have

demonstrated the connection between specific bacterial species and CRC. Numerous investigations have revealed that CRC has less bacterial diversity and more of some pro-tumorigenic microorganisms. *Fusobacterium nucleatum*, *Escherichia coli*, *Bacteroides fragilis*, *Streptococcus bovis/gallolyticus*, *Clostridium septicum*, *Enterococcus faecalis*, and *Peptostreptococcus anaerobius* are among the bacterial species that are frequently linked to CRC. Seven bacterial species were enriched in CRC in a core sample of 536 fecal shotgun metagenomes that underwent meta-analysis. The pathogenic microbes like *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Parvimonas micra*, *Prevotella intermedia*, *Porphyromonas asaccharolytica*, *Alistipes finegoldii*, and *Thermanaerovibrio acidaminovorans* are increased in CRC individuals [14]. Individuals with CRC had a substantial rise in *Candida albicans*, *Aspergillus rambellii*, *Cordyceps sp.*, *Erysiphe pulchra*, *Moniliophthora perniciosa*, *Sphaerulina musiva*, and *Phytophthora capsici* were discovered to be more abundant in CRC individual's microbiome. Significant enrichment of *Lachancea waltii*, *Aspergillus rambellii*, and *Phanerochaete chrysosporium* was observed in CRC by another recent investigation. According to Coker et al., individuals with CRC have altered *archaeomes*, showing a decrease in methanogens and enrichment of halophiles [53].

18.3.1 *Fusobacterium nucleatum*

For adhesion and invasion into epithelial cells, *Fusobacterium nucleatum* employs the FadA adhesion protein. FadA binds to E-cadherin, stimulates the Wnt/ β -catenin pathway, and increases the permeability of endothelial cells, thereby enabling *Fusobacterium nucleatum* to traverse cell-cell junctions. The development of CRC is suppressed by the synthetic peptides that obstruct the binding of *Fusobacterium nucleatum* to E-cadherin. Fap2, an additional protein, can inhibit natural killer (NK) cells by forming an association with TIGIT, an inhibitory receptor on NK cells. Through the inflammatory NF- κ B signaling pathway, *Fusobacterium nucleatum* can induce carcinogenesis and suppress CD3+ T-cell-mediated adaptive immunity [54]. The potential of *Fusobacterium nucleatum* as a prognostic biomarker for CRC is indicated by a substantial correlation between patient outcome and *Fusobacterium nucleatum*. In mice, *Fusobacterium nucleatum* can also activate TLR4 signaling to facilitate the development of tumors. According to a recent study, *Fusobacterium nucleatum* activates yes-associated protein (YAP) signaling, inhibits FOXD3 expression, and reduces METTL3 transcription, thereby reducing m6A modifications in CRC cells. Formate, a metabolite produced by *Fusobacterium nucleatum*, promotes the development of CRC by increasing the incidence of tumors and the expansion of Th-17 cells [55].

18.3.2 *Enterococcus faecalis*

Enterococcus faecalis is a facultative anaerobic, gram-positive bacterium that is commensal. Studies have verified an elevated prevalence of *Enterococcus faecalis* in the feces of patients with CRC. Nevertheless, the significance of *Enterococcus faecalis* in the development of CRCs continues to be a topic of debate. Numerous studies have indicated that it can produce reactive oxygen species (ROS) and extra-cellular superoxide, which can cause DNA damage to colonic epithelial cells, resulting in mutations and CRC. *Enterococcus faecalis* also generates metalloprotease, which has the potential to induce inflammation and affect the intestinal epithelial barrier. In contrast, other research suggests that *Enterococcus faecalis* is a significant probiotic microorganism and may play a role in the prevention of CRC [56].

18.3.3 *Streptococcus bovis/gallolyticus*

Streptococcus bovis/gallolyticus is a gram-positive bacterium that is linked to endocarditis. The prevalence of *Streptococcus bovis/gallolyticus* in CRC tissues and the association between *Streptococcus bovis/gallolyticus* infection and CRC have been confirmed by research. The precise mechanism by which *Streptococcus bovis/gallolyticus* induces carcinogenesis remains to be elucidated. However, research has suggested that it is involved in the early phases of CRC. Consequently, it may be used as an early marker for CRC screening. Pil3 pilus is employed by *Streptococcus bovis/gallolyticus* for adhesion to and translocation across colonic epithelial cells. Furthermore, the interactions between *Streptococcus bovis/gallolyticus* and host cells may be mediated by bacterial type VII secretion systems (T7SS), which are crucial for the bacterium's virulence. By upregulating β -catenin levels and inducing inflammation through IL-1, IL-8, and COX-2, *Streptococcus bovis/gallolyticus* likely contributed to the development of CRC. In azoxymethane (AOM) and dextran sulfate sodium (DSS) mouse models of malignancy, oral gavage with *S. bovis/gallolyticus* has been demonstrated to elevate the tumor burden [57].

18.3.4 *Peptostreptococcus anaerobius*

Peptostreptococcus anaerobius is a gram-negative anaerobic bacterium that is frequently observed in the buccal cavity and the gastrointestinal tract. It induces the formation of reactive ROS by modulating immune cells and interacting with Toll-like receptors (TLR-2 and TLR-4) on colon cells, thereby promoting carcinogenesis. *Peptostreptococcus anaerobius* binds to colon cancer cells through its surface

protein, putative cell walls binding repeat 2 (PCWBR2), which interacts with $\alpha 2/\beta 1$ integrins on colon cells. The PI3K-Akt pathway was activated by the binding of *Peptostreptococcus anaerobius*, which subsequently stimulated cell proliferation and inflammation [58].

18.3.5 *Parvimonas micra*

Parvimonas micra is frequently linked to a variety of oral diseases, such as endodontic abscesses, odontogenic infections, periodontitis lesions, and other infections. A recent study conducted on an isolated strain from a CRC patient demonstrated that it could promote colony proliferation by enhancing Th17 cell infiltration and the oncogenic Wnt signaling pathway [59].

18.3.6 *Escherichia coli*

Escherichia coli is a rod-shaped gram-negative bacillus with four phylogenetic groupings (A, B1, B2, and D) that are ubiquitous in various populations of humans [60]. Several research investigations have associated *Escherichia coli*, specifically from the B2 phylogenetic group, with CRC [61]. The majority of virulent *Escherichia coli* variants are linked to inflammatory bowel conditions, including Crohn's disease, which doubles the possibility of CRC [62]. Furthermore, people who have CRC have been identified to have a higher rate of colonic mucosal infection caused by *Escherichia coli* [63]. The genotoxins generated by infectious *Escherichia coli* variants include cytotoxic necrotizing factors, cycle-inhibiting factors, cytolethal distending toxins (CDTs), and colibactin. Colibactin is co-expressed by the pks island in *Escherichia coli* [64]. It causes the alkylation of adenine sequences in DNA, which further triggers double-stranded breakage in DNA, and disrupts the cell cycle mechanism [64]. Research demonstrates that colibactin-generating *Escherichia coli* promotes carcinogenesis by activating inflammatory pathways [65]. The colibactin-generating *Escherichia coli* is commonly detected in individuals having advanced stages of CRC [64].

In summary, the development of CRC is associated with changes in the GIT microbiota, with specific microorganisms such as *Fusobacterium nucleatum* and *Escherichia coli* promoting carcinogenesis. *Streptococcus gallolyticus*, *Enterococcus faecalis*, and *Peptostreptococcus anaerobius* are key markers. The potential for non-invasive CRC screening is indicated by the quantitative analysis of these markers in fecal samples.

18.4 Technological Advances in Microbiome Profiling to Detect the CRC

The development of models that integrate both metabolomics and metagenomics data of sampled individuals is strongly advocated for as such models may improve the sensitivity and specificity of detecting CRC at treatable stages which will in turn, prolong the life span of such individuals. Figure 18.2 shows the microbial profiling of CRC by fecal metagenomics, metabolomics, and mucosal microbiota analysis.

18.4.1 Fecal Metagenomics

Using fecal samples, differences in the gut microbiota diversity and composition between patients with adenoma or CRC and healthy persons highlight the potential use of fecal microbiome in early, noninvasive CRC diagnosis [66]. The gut microbiome signatures in CRC were studied using a variety of methodologies by different researchers. Techniques include shot-gun metagenome sequencing of the fecal samples and amplification and sequencing of the several variable regions (V1, V2, and

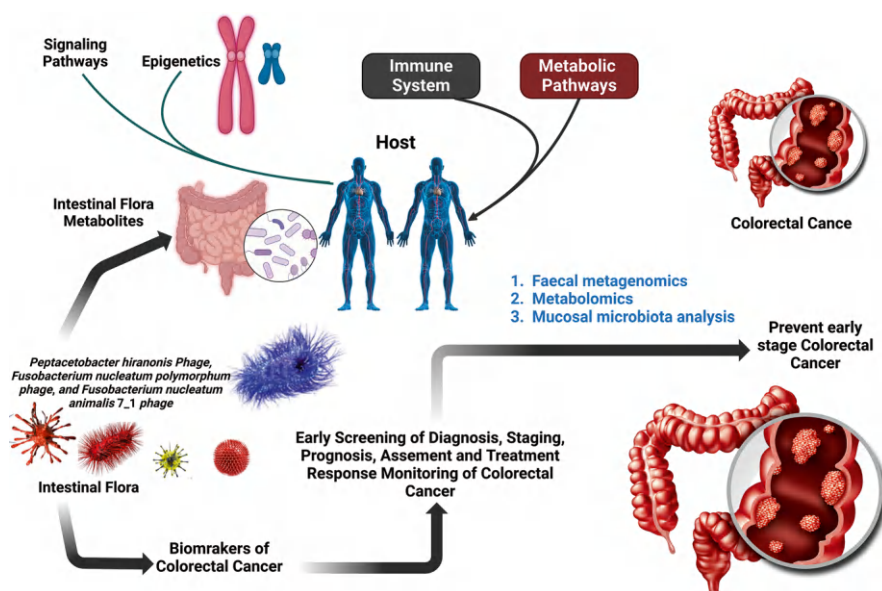


Fig. 18.2 The comprehensive overview of gut microbiome profiling by fecal metagenomics for taxonomic profiling, metabolomics for functional profiling, and mucosal microbiota analysis for assessing the microbiome at the intestinal interface; it provides a powerful approach to screen for and detecting CRC at an early stage. (Created with Biorender.Com)

V4) of the 16S rRNA of the extracted stool DNA. In several investigations, the target microbial gene marker's abundances in the samples of interest were also measured using qPCR in comparison to the chosen controls [67]. *Peptostreptococcus stomatis*, *Porphyromonas asaccharolytica*, and *Fusobacterium species* (*Fusobacterium nucleatum* subspp. *Vicentii* and *Fusobacterium nucleatum* subspp. *animalis*) were the most prevalent and discriminatively enriched species in the fecal samples of CRC patients compared to the controls. The development of CRC from early to late and metastasizing stages of the disease was likewise connected with these microbial indicators. *Peptostreptococcus stomatis* and the two species of *Fusobacterium* showed a strong enrichment in the early stage of CRC patients when compared to the controls [68]. Recent research discovered 20 microbial gene markers that differentiated CRC from control microbiomes. Additionally, the report confirmed the validity of four markers in Danish, French, and Austrian cohorts, two transposases from *Peptostreptococcus anaerobius*, butyryl-CoA dehydrogenase from *Fusobacterium nucleatum*, and rpoB gene encoding RNA polymerase subunit β from *Parvimonas micra*. Two of these genes' qPCR results accurately identified CRC patients in a separate Chinese population. These genes have the potential to be used in the early diagnosis of CRC because it has been demonstrated that they are enriched in the microbiome of patients in the early stages (I and II) of the disease [69]. A dysbiotic gut linked to CRC nearly invariably contains certain bacterial genera, such as *Fusobacterium* and *Peptostreptococcus*. A further indication of their potential use (although in conjunction with other particular microbial indicators) in the early diagnosis of the disease is the accumulation of *Fusobacterium* in certain patients even in the early stages of CRC. The ratio of *Fusobacterium nucleatum* (Fn) to a few key probiotics in the gut may be a helpful biomarker for the early detection of CRC [70]. The combination of Fn/Bb (*Bifidobacterium*) and Fn/Fp (*Fecalibacterium prausnitzii*) gave a sensitivity of 90.0% and a reduced specificity of 60.0% in the detection of stage I (early) CRC. The microbial ratio of Fn to Bb shows high sensitivity and specificity of 84.6% and 92.3%, respectively, in the detection of CRC. In addition, there was a strong inverse correlation between Fn and Fp in the CRC group as compared to the control groups, suggesting a dysbiosis linked to CRC. In patients with colorectal adenoma (CRA), early/advanced CRC patients, and healthy controls, the relative abundance of *Clostridium symbiosum* was assessed by qPCR. The prediction accuracy of the results was compared with that of *Fusobacterium nucleatum*, FIT, and carcinoembryonic antigen (CEA) (a blood-based biomarker). When compared to the healthy controls, CRA, early CRC, and advanced CRC patients showed a substantial stepwise increase in the organism's abundance level, indicating that *Cenarchaeum symbiosum* performed better than all the other biomarkers used. According to this study, *Cenarchaeum symbiosum* is a viable biomarker for the early, noninvasive detection of CRC. A five bacteria panel consisting of *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Streptococcus bovis*, *Enterotoxigenic Bacteroides fragilis* (ETBF), and *Porphyromonas* spp. showed improved diagnostic performance for differentiating

adenomatous polyps (AP) and CRC patients from the normal control group [71]. Using the simple linear combination model, the sensitivity was found to be 91.4%, the specificity to be 93.5%, and the AUROC climbed to 0.97 with this panel of bacteria. When it came to CRC, biomarkers for *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Porphyromonas* spp., and *Porphyromonas gingivalis* showed a statistically significant rising range that allowed patients to be discriminated from both AP patients and healthy controls [72].

When comparing the fecal samples of CRC patients to the normal controls, a notably elevated concentration of *Parvimonas micra* was discovered. This finding was utilized exclusively to differentiate early-stage CRC patients from the controls. The bacterium was combined with other microbiological indicators (*Fusobacterium nucleatum* and colibactin toxin-producing clb A + bacteria) to increase the sensitivity and specificity, which were 60.5% and 87.3%, respectively. *Parvimonas micra* may be used in conjunction with these microbial fecal biomarkers to identify people who have “high-risk” microbial patterns, which are indicative of an elevated risk of CRC [73].

Two gut microbes, *Holdemanella biformis*, which is the human equivalent of *Faecalibaculum rodentium*, were not only antitumorigenic but also showed a significant decrease in composition during the development of CRC. Interestingly, differences in behavior were seen between the ApcMin/+ animals and their control littermates, the C57BL/6 wild-type WT mice, as early as 8 weeks (when the tumor growth had begun). The quantitative decrease of *F. rodentium* was shown by the paired-end reads, and at 12 weeks, this reduction was even more pronounced. When comparing the Apc^{Min/+} mice to the WT mice, it was discovered that only the readings attributed to *Faecalibaculum rodentium* one of the ten taxonomic units most abundantly represented in the WT mice were significantly under-represented. Through a series of in vitro and in vivo experiments using CRC cell lines, tumor tissues from CRC patients, and mice, as well as a data set of shot-gun microbiome analyses conducted in patients with advanced CRA they were able to determine that human *Holdemanella biformis* is closely related to mouse *Faecalibaculum rodentium* and may be considered a human homolog of the bacterium. Essentially, there is a chance to use these research findings in the early identification of CRC at the adenoma stage. Recent research data shows that combined information from metagenomics and metabolomics found that patients with CRC had higher concentrations of the genera *Fusobacterium*, *Parvimonas*, and *Staphylococcus*, which grew more prevalent as the disease advanced. Conversely, there was a decrease in the *Lachnospiraceae*. The discovery that the genus *Adlercreutzia* was more prevalent in the feces of patients with adenoma than in the control and carcinoma groups is noteworthy and raises the prospect of using it as a biomarker for the early diagnosis of CRC [74].

18.4.2 *Metabolomics*

Various studies have suggested that both the concerted activities of the gut microbiota and the influence of its metabolome contribute to the etiology of CRC. Thus, the possible application of these metabolites from a CRC-influenced dysbiotic gut relative to that of healthy individuals as biomarkers for early CRC diagnosis emerged. The major approach used in the gut metabolome study in CRC patients is the ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS) technique and proton nuclear magnetic resonance spectroscopy (^1H NMR)-based metabolomics approach. SCFAs, particularly butyrate, have been reported to be anticarcinogenic due to their anti-inflammatory properties. On the other hand, some gut bacteria can metabolize primary bile acids into secondary bile acids which could promote CRC pathogenesis via the generation of ROS and reactive nitrogen species (RNS), known to be genotoxic [75]. Recent studies have shown that an altered state of the gut microbiota results in the reduction of the concentration of SCFAs. These SCFAs usually obtained from carbohydrate fermentation in the colon are known to be essential components needed for the maintenance of gut homeostasis. However, in gut microbiota dysbiosis, fermentation of these carbohydrates yields a much lower concentration of the SCFAs than they would in healthy states. Their quantification in stools has been said to possibly function as biomarkers for noninvasive diagnosis for various gut ailments. The presence of AP and CRC disease could exhibit a specific fecal SCFA characteristic, and the concentration of SCFAs in fecal samples of AP and CRC patients found that the total amount of SCFAs was significantly lower in CRC patients in comparison with the healthy controls. The percentage composition of the fecal SCFAs was different in the healthy controls, compared to CRC and AP patients, and the healthy control groups could be separated from the AP and CRC groups. This would suggest the potential application of fecal SCFA determination as a biomarker in the early detection of AP and CRC [76]. Microbial metabolites may contribute to CRC development as they observed a reduction in butyrate-producing bacteria in the feces of CRC patients and some studies also implied that there is an association between bacterial dysbiosis, metabolites, and CRA More recent studies found that this association especially, between gut metabolites and CRC is insignificant. SCFA concentrations and tumor burden were still simply weak and could not serve as a predictive marker in the detection of AP and CRC [77].

Kinross et al. sampled the gut mucosal microbiota using tumor tissues rather than feces of patients and reported that 16S rRNA gene sequencing revealed that the ecology of the gut microbiota appears to be cancer-stage specific and is strongly connected with features of poor prognosis. While fusobacteria and ϵ -proteobacteria were more abundant in tumor compared to adjacent normal mucosal tissue, fusobacteria and β -proteobacteria levels increased as the cancer stage advanced. However, network analysis revealed that the bacteria associated with these poor

prognostic features were not responsible for the alteration of the cancer mucosal metabonome. They further proposed that the mucosal microbiota in CRC only evolves with the cancer stage to meet the requirements of cancer metabolism. Thus, even though the ^1H magic angle spinning nuclear magnetic resonance (MAS-NMR) spectroscopy which was employed for metabonomic analysis exhibited an increased abundance of taurine, isoglutamine, choline, lactate, phenylalanine, and tyrosine and decreased levels of lipids and triglycerides in tumor relative to adjacent healthy tissue, they cannot be used to determine the stage of the CRC and may have limited application as biomarkers for early-stage detection of the CRC [78]. Proton nuclear magnetic resonance spectroscopy (^1H NMR)-based metabolomic approach in combination with pattern recognition through principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) was employed to profile fecal metabolites of CRC patients at different stages against healthy controls (HCs). OPLS-DA revealed that each stage of CRC could be clearly distinguished from HC based on their metabolomics profiles. Relative to HCs, the CRC group depleted levels of acetate, butyrate, propionate, glucose, glutamine and increased quantities of succinate, proline, alanine, dimethylglycine, valine, glutamate, leucine, isoleucine and lactate were found in the CRC group. Particular interest is that the fecal metabolic profiles of HCs can be distinguished from CRC patients, even in the early stage (stage I/II), emphasizing the potential utility of NMR-based fecal metabolomics fingerprinting as predictors of earlier diagnosis in CRC patients [79]. Additionally, glucose, lactate, SCFAs, glutamate, and succinate at stages I/II significantly differed from those at stages III and IV. A later study by the same group investigated colonic tumor tissues and their normal adjacent tissues together with patient-matched fecal samples obtained pre- and post-operatively revealing the relationship between fecal metabolic phenotypes and changes in CRC metabolites. This analysis which also employed ^1H NMR spectroscopy combined with pattern recognition technique showed that acetate compared to other identified metabolites was most profound in distinguishing the CRC group from healthy controls. Interestingly, fecal acetate correlated positively with changes in glucose and myo-inositol in the tumor tissues indicating enhanced energy generation needed for rapid cell proliferation [80]. Furthermore, significantly higher levels of lactate, glutamate, alanine, succinate, and reduced concentrations of butyrate, relative to the controls were found to be the overlapping discriminatory metabolites detected across sampled CRC tissues and feces. They inferred that these metabolites may reflect tumor cell shedding and could also show metabolic pathway anomalies possibly revealing associations to enhanced glycolysis, tricarboxylic acid cycle, glutaminolysis, and SCFA metabolism. This study, however, did not include adenoma patients, and this is essential and should be investigated and explored for future studies [81].

In the study conducted by Clos-Garcia et al. however, observable differences existed in fecal levels of sphingolipids and cholesteryl esters in CRC patients. Partial least squares discriminant analysis (PLS-DA) was reported to differentiate

the CRC groups from the adenoma/control groups but was not distinct for the control and adenoma groups. The metabolites which mostly contributed to the distinction were the cholesteryl esters and sphingomyelins. Glycerophosphatidylcholine (GPC) species were also partly involved and PLS-DA analysis in a pairwise manner clearly distinguished the CRC group from both the healthy control and adenoma groups. These researchers also showed that integrating metabolomics data with that of metagenomics was distinct in the separation of the healthy control group from the CRC group as well as the adenoma group from the CRC group [82]. More research into these metabolites (sphingomyelins and cholesteryl esters) in healthy, adenoma, and CRC patients is recommended for the gut microbiota and fecal biomarker study. It is intriguing to note that the bacteria genera found to be differential in the three groups of individuals studied also correlated with the very same metabolite classes majorly observed to be discriminating and differential between sample groups.

18.4.3 Mucosal Microbiota Analysis

Mucosal microbiota analysis has emerged as a useful tool for the detection and monitoring of CRC. Research indicates a high correlation between dysbiosis, or alterations in the gut microbiota and the development of CRC. Some microbial profiles, particularly those including *Escherichia coli*, have been identified as potential biomarkers for CRC. Patients with CRC frequently have these bacteria in their colonic mucosa, which is known to promote carcinogenesis. Recent research has used state-of-the-art techniques such as 16S rRNA sequencing to identify the gut microbiome from stool samples and mucosal tissues. There have been notable differences in microbial diversity between patients with adenomatous polyps, CRC, and healthy controls. For instance, a rise in the *Bacteroides* and *Parabacteroides* species is connected with the transition from adenomas to carcinomas, suggesting a potential role for these pathogens in the pathogenesis of CRC [83]. Furthermore, the identification of opportunistic infections in patients with colorectal cancer CRC, including *Erysipelatoclostridium ramosum*, suggests that certain mucosal microbiota may serve as noninvasive markers for early diagnosis. Quantitative real-time PCR has demonstrated that the pathogen can predict CRC with a respectable degree of specificity and sensitivity, underscoring the diagnostic utility of studying the mucosal microbiota. Incorporating microbiota profiling into CRC screening protocols could enhance patient outcomes and facilitate early identification [84]. While initial results are promising, larger cohort studies are required for further validation before the adoption of these microbial indicators as reliable diagnostic tools in clinical settings. All things considered, the analysis of mucosal microbiota holds a great deal of potential for the development of noninvasive biomarkers for the identification and monitoring of CRC, potentially leading to the development of new cancer treatment approaches [85].

18.5 Conclusion and Future Directions

Diagnostic methods undergo a radical change when gut bacteria are investigated as biomarkers for CRC diagnosis and surveillance. It is clear from our growing knowledge of the complex interaction between the microbiome and CRC that changes in gut bacteria are active players in carcinogenesis rather than only effects of illness. The discovery of certain microbial fingerprints, like *Fusobacterium nucleatum* and *Escherichia coli*, etc., emphasizes the possibility of these species being consistent markers of CRC risk and advancement. Future initiatives should concentrate on the inclusion of microbiome analysis into normal clinical practice. Standardized techniques for microbiota profiling must thus be developed to guarantee consistency and dependability in diagnosis results. Refining our knowledge of microbial communities and their functional roles in CRC will depend much on the use of bioinformatics tools and improved sequencing technology. Moreover, validation of the therapeutic relevance of gut microbiota–based biomarkers throughout several demographics and geographical areas depends on large-scale, multicentric investigations. Using personalized therapy and harnessing individual microbiome profiles presents a potential path for colonization control of CRC. Healthcare professionals might improve therapy efficacy and reduce side effects by customizing treatments including dietary changes, probiotics, or fecal microbiota transplantation. Furthermore, the investigation of liquid biopsies aiming at microbial cell-free DNA (cfDNA) might transform early detection methods and enable noninvasive tracking of CRC development and treatment response. Still, limitations are the complicated relationships between gut bacteria and host variables including genes, food, and environmental impacts. Developing thorough models that precisely estimate CRC risk and guide preventative plans will depend on addressing these elements. Finally, the inclusion of gut microbiota profiling into CRC diagnosis has great potential to enhance early identification and patient outcomes. Overcoming current obstacles and completely realizing the possibilities of microbiome-based indicators in clinical environments will depend on ongoing research and multidisciplinary cooperation. Maintaining a patient-centered approach is essential as this area develops to guarantee that treatments are safe, efficient, and catered to specific demands.

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Chapter 19

Beyond Treatment: Integrating Gut Microbiota and Postbiotic Survivorship and Long-Term Management of Colorectal Cancer



Suparna Garai, Ashish Garg, and Amalesh Samanta

Abstract Colorectal cancer (CRC) is among the most common and life-threatening cancer types with minimal therapeutic options worldwide. Gut microbiota is recognized as the crucial determinant in sustaining gastrointestinal tract homeostasis. Alterations within the gut microbiome impact the growth and advancement of cancer. The beneficial role of postbiotics, in describing microorganism-sourced substances, in colorectal cancer has been brought to light by various studies. The association between food, gut microbiota, and colorectal cancer is usually characterized as a correlation rather than a causal link. Given the significant dysbiosis in the microbiota associated with colorectal cancer, postbiotics seem to be a viable therapeutic agent for the therapy and long-term management of this condition. Gut microbiota biomarkers possess the potential for translational applications in colorectal cancer screening and early diagnosis. Gut microbiota-associated interventions improve antitumor therapy's effectiveness and serious intestinal toxic effects. Evidence shows that along with gut dysbiosis, specific pathogenic microbes, virulence factors, metabolites, and microbial carcinogenic mechanisms facilitate to the inception and progression of colorectal cancer.

Keywords Colorectal cancer · Gut microbiota · Postbiotics · Dysbiosis · Cancer biomarkers

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19.1 Introduction

Colorectal cancer is one of the dominant causes of cancer-related health complications and fatality worldwide, with an approximate 1.9 million new cases and 935,000 deaths reported in 2020 [1]. Regardless of the advances in intervention and treatment modalities, which include chemotherapy, surgery and targeted drug therapies, the long-term supervision and survivorship of colorectal cancer patients possess significant challenges. These hurdles obligate a paradigm shift to focused approaches for a more holistic view from the traditional treatment. This approach includes survivorship strategies intended at improving the quality of life and overall health outcomes for survivors.

Recent studies highlight the essential role of the “gut microbiota” in impacting various aspects of health which include immune function, disease pathogenesis, and metabolism [2]. The “gut microbiota” includes a complex population of microorganisms that reside inside the gastrointestinal tract and play an important role in sustaining homeostasis. Dysbiosis, or a discrepancy in the “gut microbiota,” has been associated with the development and growth of colorectal cancer, with indication suggesting that distinct microbial communities may either promote tumorigenesis or exercise effects which are protective against cancer [3].

Postbiotics, which can be defined as bioactive compounds generated while the process of fermentation of prebiotics by probiotics occurs, have gained focus as a promising adjunctive approach in cancer management [4]. Contrary to probiotics, which consist of live microorganisms typically bacteria or yeast, postbiotics are heat stable and can provide therapeutic benefits devoid of the risks associated with the live strains of bacteria. The ability of postbiotics to regulate gut health, increase immune responses, and improve comprehensive well-being makes them a valuable contemplation in the long-term management of the survivors. By examining the interconnection between gut health and cancer outcomes, studies can provide insights into how these elements can improve the quality of life and longevity of the individuals recovering from colorectal cancer.

19.2 Understanding Colorectal Cancer (CRC)

Epidemiological Studies of Colorectal Cancer CRC is a substantial public health concern worldwide, marked by the uncontrolled proliferation of cells in the rectum or colon. It is one of the major causes of cancer advance health outcomes and mortality, with varying occurrence and survival rates affected by factors such as lifestyle, genetics, geography, and screening practices. Interpreting the epidemiology of the colorectal cancer is essential for developing efficient prevention and control strategies.

Disease Incidence and Disease Prevalence Colorectal cancer is the third most frequently diagnosed cancer globally, followed by lung cancer and breast cancer. In accordance with the Global Cancer Observatory, nearly 1.93 million recent cases of colorectal cancer were detected worldwide in 2020, and an estimation of 935,000 deaths, making it the second largest cause of deaths related to cancer globally [5].

Geographical Variation The occurrence of colorectal cancer varies significantly over different regions. Affluent countries, particularly North America and Western Europe, report the maximum number of colorectal cancer incidents. Comparatively lower rates of colorectal cancer are observed in Africa and Asia [6]. This geographical disparity is believed to arise from differences in dietary habits, lifestyle, and healthcare access.

Age Colorectal cancer incidence rises with age, with majority of incidents occurring in individuals above the age of 50. The average age of diagnosis is estimated 66 years, and it is uncommon in individuals under 40 years [7].

Gender Colorectal cancer affects all the genders almost equally, although men have a slightly higher rate of occurrence of the disease. The male-to-female ratio varies depending on region, where it is found men generally having 1.5 times increased risk compared to women [1].

Risk Factors

Various risk factors associated with an enhanced likelihood of progressing colorectal cancer:

Diet: A diet with high amount of red and processed meats but low in vegetables and fruits and also low in fiber is linked to a higher risk of colorectal cancer [8]. Dietary factors, includes physical inactivity and obesity, which also significantly contribute to the risk profile.

Genetic Factors: Hereditary syndromes, including “Lynch syndrome (hereditary nonpolyposis colorectal cancer)” and familial adenomatous polyposis, significantly enhance the risk of colorectal cancer. Individuals having a family history of colorectal cancer can also possess a higher risk [9].

Lifestyle Factors: Excessive alcohol consumption, tobacco use, and sedentary lifestyles are thoroughly documented risk factors. Conversely, routine physical engagement has been correlated with diminished risk of colorectal cancer [10].

Comorbid Conditions: Chronic inflammatory conditions like inflammatory bowel disease (IBD), which include ulcerative colitis and Crohn’s disease, are associated with an elevated risk of colorectal cancer [11].

Screening and Early Detection

Preliminary detection of the disease through screening has shown to substantially decrease the colorectal cancer morbidity and mortality. Several screening methods are employed which includes:

Colonoscopy: It is the gold standard for screening colorectal cancer that is recommended every ten years for people aged 50 and older and earlier for those who have increased risk factors [12].

Fecal Immunochemical Test (FIT): It is a noninvasive test that usually detects blood in stool that is recommended annually [13].

Flexible Sigmoidoscopy: This procedure is recommended every five years and it allows the direct depiction or visualization of the lower colon and rectum.

Screening programs have contributed to a rise in rates of early diagnoses of colorectal cancer, thereby improving treatment and survival rates.

Survival Rates

Survival rates of colorectal cancer are mostly influenced by the stage at which it is diagnose:

Localized Disease: The relative survival rate is five years for localized colorectal cancer, which is confined to the rectum or colon, and the survival rate is approximately 90% [14].

Regional Spread: As the cancer spreads to the nearby lymph nodes, there is a reduction in survival rate which comes to about 71%.

Distant Metastasis: For patients with metastatic colorectal cancer, the five-year survival rate comes down to around 14% [15].

The epidemiology of colorectal cancer highlights the importance for community health initiatives which are aimed at prevention, detection (early), and efficient management of the disease. By addressing modifiable risk factors and encouraging screening, it is certainly possible to decrease the incidence and mortality, which are associated with colorectal cancer.

19.3 Current Treatment Modalities (Surgery, Chemotherapy, and Radiotherapy)

The strategy for the treatment of colorectal cancer is dictated by the stage at which it is diagnosed, and this also determines the probability of metastasis of the disease and the survival outcomes. The stages of the disease range from localized, then early-stage disease (stage 0) and to advanced, metastatic disease (stage IV), and so the treatment evolves accordingly.

19.3.1 Stage 0: Carcinoma In Situ: Fundamental Treatment Modalities

Polypectomy and endoscopic mucosal resection (EMR) are primary interventions, given their success in completely removing localized lesions [16].

Endoscopic mucosal resection (EMR) and polypectomy are pivotal interventions in the monitoring of colorectal lesions, specifically for inhibiting the intensification to colorectal cancer (CRC). These ostensibly invasive procedures have been suggested to successfully eliminate the localized lesions and substantially impact the patient end results and strategies for the prevention of cancer.

19.3.1.1 Polypectomy

Definition and Technique: This is the surgical extraction of polyps from the epithelial layer of the colon or rectum, usually performed while conducting a colonoscopy. It involves different techniques based on the size, morphology, and anatomical characteristics of the polyp. The most prevalent methods include:

Cold Snare Polypectomy This method incorporates a snare that excludes the utilization of heat. It is exceptionally effective for small polyps, since it reduces thermal injury to neighboring tissues. Studies suggest that cold snare polypectomy can accomplish complete resection rates in compare to hot snare methods, and also with a lower incidence of difficulties, particularly regarding post-procedural bleeding [17].

Hot Snare Polypectomy Using this technique, a heated wire snare is implemented to remove the polyp while undergoing cauterization of the base to attenuate bleeding. This technique is appropriate for larger polyps. A reflective analysis showed that the hot snare polypectomy received complete surgical resection rate of estimating 93% for polyps ≤ 2 cm, at reduced rates of immediate complications [18].

Indications Polypectomy is recommended for adenomatous polyps (with the potential for cancerous progression); sessile serrated lesions; selected or specific hyperplastic polyps.

19.3.1.2 Endoscopic Mucosal Resection (EMR)

EMR is a more sophisticated technique used to eliminate larger or flat lesions which cannot be easily extracted through standard polypectomy procedure. The intervention involves various steps:

Submucosal Injection It is a solution which is usually saline and is injected under the lesion to raise it from the subjacent tissue and allow for an easy removal.

Resection The elevated growth (lesion) is excised using a snare or a specialized cutting device.

Cauterization The root of the resected lesion is then cauterized to reduce bleeding and ensure the complete removal of the lesion.

The ability of EMR to accomplish complete resection of the precancerous lesions is very well documented. A structured review studied that the complete resection occurrence rate of the lesions is removed via EMR and was approximately 90%, along with long-term monitoring and indicating low recurrence rates in the case of early-stage cancers [19].

Indications: EMR is specifically suitable for large sessile polyps, lesions not receptive to standard polypectomy because of the size or morphology, and flat lesions which are suspected to be early cancers.

Safety and Complications Both polypectomy and EMR are usually safe as they are not lacking risks. Prospective complications are:

- **Bleeding:** It is the most frequent complication, which is usually supervised endoscopically or conservatively. The meta-analysis stated that significant bleeding appeared in approximately 1.4% of cases which is followed by polypectomy.
- **Perforation:** An uncommon but serious complication connected with EMR, especially for the larger lesions, with a projected incidence rate of 0.5–1% [20].
- **Stricture Formation:** This can take place after the elimination of large lesions, compelling further endoscopic intervention.

Significance in Reducing Colorectal Cancer Risk The efficiency of polypectomy and EMR in avoiding colorectal cancer is very well established. After the removal of precancerous polyps, all these interventions play a very crucial role in the early detection, treatment, and further saving lives potentially. Consistent surveillance colonoscopy is always recommended for the individuals having a history of polyps which enables the timely intervention and further monitoring for the recurrence [21].

Polypectomy and EMR are necessary interventions in the supervision of localized colorectal lesions. The success of the procedures in completely extracting the localized lesions is essential for the prevention of the colorectal cancer. The endoscopic techniques persist to advance the safety and effectiveness of these procedures, which will further increase their role in the management of colorectal disease. In situations where lesions are difficult to remove, localized surgical resection may be recommended.

Follow-up and Prognosis Regular surveillance colonoscopies are essential to monitor for recurrence.

19.3.2 Stage I: Localized Cancer

The surgical intervention is colectomy, which includes regional lymph node dissection and that is the primary treatment for stage I CRC. Laparoscopic and robotic techniques may be used which depends on the location of the tumor.

Prognosis and Survival: For stage 1 the survival rates of 5 years are high alone with the surgical procedure. As per the studies showing 90% of survival rates in the early-stage interventions [22].

19.3.3 Stage II: The Locally Advanced Disease

Surgical Resection: Here partial colectomy remains the established standard for extracting the localized tumors. Here high-risk stage II patients can get benefit from an adjuvant chemotherapy.

Adjuvant Therapy: In this stage, chemotherapy with capecitabine or 5-fluorouracil (5-FU) is considered in the high-risk cases.

Emerging Approaches: The strategy of genomic profiling to determine the microsatellite instability (MSI) status helps in personalizing treatment [23, 24].

19.3.4 Stage III: Regional Lymph Node Involvement

Combination Therapy Approach: In this stage, surgery is followed by adjuvant chemotherapy which is a standard. Here regimens like FOLFOX (oxaliplatin, leucovorin, and 5-FU) are commonly followed.

Chemotherapy Regimens: In this procedure alternatives such as capecitabine and oxaliplatin (CAPOX) are found effective for stage III cases.

Radiation therapy for Rectal Cancer: In this stage, neoadjuvant chemoradiation is advised for rectal cancer to lower local recurrence [25].

Genetic Considerations: The testing for KRAS, NRAS, and MSI status helps the usage of targeted therapies in various cases [26].

19.3.5 Stage IV: Metastatic Disease

Systemic Chemotherapy: In this stage, the treatment includes chemotherapy regimens which include FOLFOX, CAPOX, and FOLFIRI, often paired with targeted agents (e.g., bevacizumab and cetuximab).

Targeted Therapy: For patients of RAS wild-type, agents like cetuximab or panitumumab are used, which have proved to be beneficial [27].

Surgical Resection of Metastases: The procedure of resection of liver or lung metastases might help in the extended survival in selected patients.

Immunotherapy in MSI-High Tumors: The immunotherapy options, like pembrolizumab have demonstrated effectiveness for MSI-high stage IV CRC [28].

Emerging and Experimental Therapies

Immunotherapy Advances: Latest studies in “PD-1/PD-L1 inhibitors” show promising results for certain genetic subtypes of CRC treatment.

CAR-T Therapy and Precision Oncology: Gene based is an emerging and highly effective approach, with clinical trials currently assessing the potential of CAR-T cells in the treatment of CRC.

Patient-Specific Treatment Which Is Based on Molecular Profiling: Tumor molecular profiling, e.g., FoundationOne, is one of the advancing personalized treatment options [29].

Side Effects Management and Assuring Quality of Life: Management of Side Effects of **Chemotherapy:** Gastrointestinal toxicity, fatigue, and neuropathy are the common side effects and require a comprehensive support.

Radiation Toxicity: The monitoring and managing of the radiation-induced side effects are crucial, specifically in rectal cancer.

Supportive Care: In this care plan, pain management, psychosocial support, and nutrition are important parts [30].

Future Directions in Colorectal Cancer Treatment

Early Detection and Screening: The advancements in imaging and liquid biopsy could increase early detection rates of colorectal cancer.

Development of New Therapies: Various treatments like combination therapies include novel immunotherapy approaches, are under study.

Clinical Trials: The ongoing clinical trials are adding value in refining the treatment approaches for better patient outcomes [31].

19.4 Understanding the Gut Microbiota in the Colorectal Cancer (CRC)

The “gut microbiota” is a complex community comprising trillions of microorganisms, which serves as a crucial function in several physiological processes that includes metabolism, maintenance, and immune function of the intestinal barrier. Several researches have highly linked dysbiosis (imbalance in the microbial composition) along with the development and advancement of CRC. A pro-inflammatory environment, created by dysbiosis may increase the mutagenic potential, and also compromise gut integrity, that can participate in the tumorigenesis inside the colon and rectum [32, 33].

19.4.1 Composition and Functions of Gut Microbiota

The “gut microbiota” of human comprises a diverse compilation of bacteria, viruses, archaea, and fungi, where there is a dominance of bacterial phyla, *Firmicutes* and *Bacteroidetes*, which is followed by smaller populations of *Actinobacteria* and

Proteobacteria [34]. All these microbes perform an essential function like fermenting dietary fibers which can produce the “short-chain fatty acids (SCFAs) such as butyrate,” which provide the energy for colonocytes and then exerts anti-inflammatory effects [35]. Moreover, a balanced “gut microbiota” can support immune modulation performed by training the immune system which facilitates to recognize the harmful pathogens, meanwhile tolerates the beneficial microbes [36].

19.4.2 Microbiota Alterations in Patients with Colorectal Cancer

Studies suggest that CRC patients possess a unique gut microbial profile in comparison to healthy individuals. For example, pathogenic bacteria like *Escherichia coli* and *Fusobacterium nucleatum* are often enhanced in CRC patients, while the beneficial microbes like *Faecalibacterium prausnitzii*, which is known for its anti-inflammatory characteristics, are reduced [37, 38]. The state of dysbiosis has been often associated with elevated levels of pro-inflammatory cytokines, genotoxic compounds, and altered metabolic byproducts, that leads to epithelial DNA damage, promoting malignant transformation of cells [39]. For example, the presence of *F. nucleatum*, has been connected with advanced tumor stages and also poor survival prognosis in CRC patients. *F. nucleatum* is also known to promote carcinogenesis via mechanisms like activation of pro-inflammatory pathways and immune evasion [40]. Strains of *E. coli* harboring the polyketide synthase (pks) pathogenicity island are capable of producing colibactin, a genotoxin associated with DNA double-strand breaks in host cells, potentially causing mutations that promote tumor development [41].

19.4.3 Impact of Treatment on Health of Gut Microbiota

CRC treatments, which includes chemotherapy, surgery, and radiation, can significantly impact the “gut microbiota.” For instance, chemotherapy has shown to decrease the microbial diversity and modify microbial composition, which often result in an excessive growth of opportunistic pathogens which can exacerbate side effects gastrointestinal system [42]. Antibiotic use is common in perioperative and treatment settings of cancer, which further disrupts the microbial balance and leads to dysbiosis, which may persist beyond treatment [43].

A study examining the impact of chemotherapy on “gut microbiota” in the CRC patients found that chemotherapeutic agents like oxaliplatin and 5-fluorouracil led to significant decline in beneficial bacteria which includes *Bifidobacterium* and *Lactobacillus* species, eventually promoting the growth of possibly potential harmful bacteria [44]. All these alterations can damage intestinal barrier function and allow the translocation of bacterial endotoxins inside systemic circulation, which potentially worsens inflammation and impact patients’ all over health and recovery [45].

19.5 CRC and the Role of Microbial Metabolites

Metabolites derived from “gut microbiota,” like SCFAs (butyrate, acetate, and propionate), polyamines, and bile acids, play distinct roles in maintaining the intestinal homeostasis and influencing risk of cancer. Butyrate, an SCFA which is primarily produced by the fermentation process of dietary fibers, evidenced to suppress cell proliferation in CRC by modulating the activity of histone deacetylase (HDAC) and inducing apoptosis [46]. Moreover, dysbiosis may reduce bacteria producing butyrate by decreasing this protective effect in patients with CRC [47].

Another group of microbial metabolites is secondary bile acids which is produced from the primary bile acids inside the colon through enzymatic activity of bacteria. While primary bile acids facilitate digestion of fats, the secondary bile acids like deoxycholic acid are linked to DNA damage, colorectal tumor formation, and oxidative stress [32]. This dichotomy highlights the bidirectional and complex relationship between the microbial metabolites and risk related to CRC. Table 19.1 represents the gut microbes which have reported to have a mechanistic role in CRC treatment [48]:

19.6 Postbiotics and Their Potential in Survivorship in CRC

Postbiotics are metabolites which are produced by gut bacteria while fermentation of dietary fibers occurs. It includes short-chain fatty acids (SCFAs) like butyrate, propionate, and acetate. These metabolites exert numerous health benefits, specifically in the context of CRC. For example, butyrate is known for its anti-inflammatory, gut-barrier-enhancing, and anticancer properties [49]. SCFAs has shown to inhibit the proliferation of CRC cells by inducing regulating immune responses and apoptosis [50].

Including postbiotics into the CRC-long-term management could increase recovery, improve the gut health, and simultaneously reduce inflammation [51].

19.6.1 *Combining Gut Microbiota and Postbiotics in the Survivorship Care of CRC*

Dietary Modifications and Probiotic or Prebiotic Supplementation: A crucial strategy in the prolonged management of survivors of CRC involves dietary modifications which promises at supporting the healthy “gut microbiota.” Fiber-rich diets, specifically prebiotics such as fructo-oligosaccharides and inulin, promote the growth of gut bacteria which are beneficial and produces SCFAs. Current studies suggest that diets with high-fiber can reduce CRC recurrence with the help of modulation of the microbiome to favor protective species [52]. Moreover, supplementing with probiotics, like *Lactobacillus* and *Bifidobacterium*, helps in restoring microbial balance even after chemotherapy and surgery [53].

Table 19.1 Gut microbes which have reported to have a mechanistic role in CRC treatment [48]

Serial no.	Microbe	Mechanism	Virulence factor or effector
1.	Enterotoxigenic <i>Bacteroides fragilis</i>	Activates β -catenin and STAT3 pathway, which increases the expression of COX-2 and NF- κ B	<i>B. Fragilis</i> toxin (BFT)
2.	<i>Fusobacterium nucleatum</i>	It modulates E-cadherins/ β -catenin pathway which blocks the antitumor immune response	FadA, Fap2
3.	<i>E. Coli</i>	It causes break in DNA double strands	Colibactin, cytolethal distending toxins (CDTs)
4.	<i>Enterococcus faecalis</i>	It damages DNA by producing the reactive oxygen species (ROS) and the extracellular superoxide	Metalloprotease
5.	<i>Streptococcus bovis/gallolyticus</i>	It upregulates β -catenin and promotes cell proliferation and inflammation	Pil3 pilus
6.	<i>Helicobacter pylori</i>	It activates the β -catenin/ MAPK signaling pathway	CagA and VacA
7.	<i>Peptostreptococcus anaerobius</i>	It interacts with TLR-2 and TLR-4 on colon cells so that it can induce ROS formation	Putative cell wall-binding repeat 2 (PCWBR2) protein

19.6.2 Microbiota Transplantation

Another promising approach in the management of colorectal cancer (CRC) is fecal microbiota transplantation (FMT), particularly for patients with dysbiosis who have not shown improvement or responded to conventional therapies. FMT includes the transfer of the fecal microbiota from a healthy donor to restore the microbiota composition of the recipient. Current studies show that FMT can improve treatment outcomes in patients with CRC by strengthening immune function and supporting the post-therapy recovery [54].

19.6.3 Use of Postbiotic Supplementation

Supplementation with postbiotics, specifically SCFAs, holds capability of improving the outcomes in CRC survivors. For example, butyrate has shown to enhance the gut epithelial integrity, reduce the growth of malignant cells, and suppress inflammation. Clinical trials have also explored the effectiveness of butyrate and other SCFAs in the prevention of CRC and treating CRC with promising results, which indicate that postbiotic therapy can increase the antitumor effects of the chemotherapy [55].

19.6.4 Anti-inflammatory Effects and Immune Modulation

As chronic inflammation is observed in CRC patients and through microbiota modulation its recurrence, inflammation will be beneficial. Postbiotics, specifically butyrate, play an important role in the modulation of immune responses and reduction of chronic inflammation. SCFAs can also enhance the population of regulatory T cell, which are crucial in prevention of tumor recurrence as well as its metastasis. Moreover, butyrate has shown to impact the expression of the immune checkpoint molecules, resulting in potentially enhancing the efficacy of immunotherapies in CRC treatment [56].

19.6.4.1 Personalized Microbiome Interventions and Long-Term Monitoring

As part of the survivorship care, patients with CRC should regularly undergo microbiome monitoring so that early signs of dysbiosis or microbial imbalance can be detected, which may further lead to recurrence and other complications. Interventions with personalized microbiome, which include tailored probiotic and postbiotic regimens that is based on the microbiota profiles of the individuals, represent an evolving frontier in long-term management of CRC. These interventions can optimize recovery, maintain overall health, and prevent recurrence or aggravation of the disease.

19.7 Clinical Implications and Future Research Directions

Integrating postbiotics and “gut microbiota” into the survivorship and the continuing management of the CRC is a potentially promising approach which can significantly enhance outcomes for the CRC patients. With the help of dietary interventions, microbiota transplantation, probiotic supplementation, and postbiotic therapies, it is feasible to support a healthy microbiota, reduce the risk of recurrence, and enhance immune responses. Continuous research in this field is necessary to properly understand the complex relationship between the microbiota and CRC, and to develop the effective strategies for the incorporation of microbiome-based therapies into the clinical practice. Understanding and studying the role of “gut microbiota” in CRC uncover new approaches for therapeutic interventions. Modulation of the gut microbiome through the dietary interventions, prebiotics, probiotics, and also fecal microbiota transplants (FMT) is in the process of evaluation and is being researched as a potential adjuvant treatment to the standard CRC therapies. For example, FMT has shown assurance in restoring the microbiota balance after chemotherapy, which potentially reduces gastrointestinal toxicity and improves patient outcomes [57].

Future studies should aim on identifying distinct microbial signatures that could function as biomarkers for early detection or prognosis of CRC. Therefore, more

clinical trials are needed so that the efficacy of microbiota-modulating therapies can be established in CRC treatment and survivorship care, which aim for personalized treatments based on microbes tailored to individual profiles of CRC patients [58].

19.8 Conclusion

The integration of “gut microbiota” and postbiotics into the survivorship and long-term management of colorectal cancer represent a promising frontier in oncology. Colorectal cancer remains a major global health challenge, with conventional treatments often failing to address long-term health and quality of life for survivors. Emerging evidence underscores the critical role of “gut microbiota” in influencing cancer progression, treatment response, and overall patient outcomes. Dysbiosis in colorectal cancer patients highlights the need for interventions targeting microbial imbalances. Postbiotics, with their anti-inflammatory and anticancer properties, offer a safe and effective strategy to enhance gut health, modulate immune responses, and support recovery. Dietary modifications, probiotic and postbiotic supplementation, fecal microbiota transplantation, and personalized microbiome interventions are all promising approaches to improve survivorship care. These strategies not only mitigate the side effects of conventional therapies but also enhance their effectiveness, reduce inflammation, and prevent disease recurrence. Future research should focus on personalized microbiome interventions, the development of biomarkers for early detection and monitoring, and the exploration of postbiotics’ synergistic effects with existing therapies. By adopting a holistic approach that integrates “gut microbiota” and postbiotic strategies, it is possible to transform colorectal cancer management and improve long-term outcomes for patients.

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Chapter 20

Future Perspectives: Advancements in Postbiotics and Gut Microbiota Research and Potential Therapeutic Breakthroughs



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Abstract CRC is impacted by the gut microbiota's anti-inflammatory, antioxidant, and anticarcinogenic postbiotics. They control immune, inflammatory, and tumor suppression pathways. Postbiotics help prevent and treat CRC by restoring gut balance and lowering gut dysbiosis. Innovative delivery systems like nanotechnology boost efficacy and focus. This chapter examines the complex association among gut microbiota as well as colorectal cancer (CRC), focusing on postbiotics as a treatment. Beginning with a summary of gut microbiota composition and gut dysbiosis causes, this can lead to colorectal cancer. This study studies how gut microbiota affects inflammation, immunological response, and carcinogenesis in cancer progression. A postbiotic investigation of gut microbiota therapy follows. Postbiotics, bioactive molecules produced by gut microbes, are promising colorectal cancer (CRC) treatments due to their anti-inflammatory, antioxidant, and anticarcinogenic

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properties. This study examines how postbiotics affect colorectal cancer (CRC), including tumor suppression signaling pathways. New technologies like nanotechnology-based postbiotic administration highlight the bioactive chemicals' increased efficacy and focused effects. The chapter covers therapeutic and nonclinical uses of postbiotics, toxicity issues, and regulatory status, emphasizing the need for comprehensive assessment and advice. After reviewing postbiotics' potential as precise therapeutic instruments in human illness management, the chapter suggests that further research could enable their application in oncology and other chronic diseases. This comprehensive analysis makes postbiotics a priority in developing innovative colorectal cancer (CRC) treatments.

Keywords Colorectal cancer · Gastrointestinal tract · Microbiota · Postbiotics · Tumor suppression · Apoptosis · Cell proliferation

20.1 Introduction

The gastrointestinal tract (GI) of humans contains a vast number of bacteria, archaea, fungi, and viruses that form a complex network of interconnected microorganisms. This microbial ecosystem is unique and consists of trillions of these organisms. The establishment of the community of microbes commences from birth, with every phase of life being characterized by a distinct dominant population of microbes that significantly influences the host's physiology [1]. The host, microbiome, released metabolites, and activated metabolic pathways are gaining increasing recognition due to the tremendous advances made in understanding their critical significance. The phenomenon of cell-cell communication called quorum sensing (QS) has yet to be fully understood about the microbiota of the gut as well as its impact on human physiology as well as nutrition [2]. To completely comprehend the true

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part of bacteria in the gut environment, comprising their interaction with host cells and the production of various bioactive materials, further research is required despite the progress made in the field of biological sciences. The functional mechanism of intestinal microbiota is influenced by factors like microbial diversity, the connection between different microbes, nutritional intake, and the complete health status of the host. Additionally, research indicates that deactivated or lifeless microbial cells, together with their structural-functional components and by-products, can stimulate certain signaling pathways within host cells and also produce specific biological or physiological effects [3]. Regarding this matter, we can mention bioactive metabolites (postbiotics) generated via intestinal microbiota. These metabolites can inhibit the development of dangerous bacteria, preserve the integrity and proper functioning of the intestinal lining, and also alter the gut microbial community composition [4]. This approach shows promise in delivering therapeutic advantages that are crucial for establishing and sustaining a state of healthy gut microbial balance, known as eubiosis. Because of postbiotics' distinctive composition, they engage through host cells and exert their cellular and molecular functions by disrupting immunological as well as nervous system regulatory systems. Instances of this phenomenon consist of enhancing the efficacy of the innate immune system, mitigating inflammatory reactions produced by infections, and fortifying intestinal barrier function [5].

Research findings indicate that each individual possesses a distinct microbiome, and there is mounting evidence that a discrepancy in the composition, as well as gut microbiome diversity, known as intestinal dysbiosis, can disrupt the normal communication between the brain and intestines [6]. This disruption can result in various neurological, behavioral, and physiological disorders. Some instances of diseases include autism spectrum disorder (ASD), inflammatory bowel disease (IBD), neurodegenerative disorders like Parkinson's and Alzheimer's diseases, multiple sclerosis (MS), and chronic non-communicable diseases like type 2 diabetes, obesity, and cancer [7]. Currently, researchers are gathering further proof to support the beneficial impact of gut microbiota on human physiology. They are also exploring different aspects of this relationship [8]. Therefore, any biological method focused on adjusting the population of microorganisms in the intestines may likewise impact illnesses of the central nervous system. Consequently, it is regarded as a strategy with several suitable results.

The concept of postbiotics is suggested for indigestible food components to be produced through anaerobic fermentation or digestible materials like complex carbohydrates, lipids, and proteins, as well as through bacterial metabolism in the gastrointestinal tract (Fig. 20.1). This review seeks to elucidate the essential function of postbiotics as the primary agents mediating interactions between the microbiota and the host [9]. Furthermore, it provides the existing evidence on how postbiotics interact with host cells by potentially binding to cell receptors, stimulating and improving immune system function, and enhancing the composition and purpose of the human microbiome.

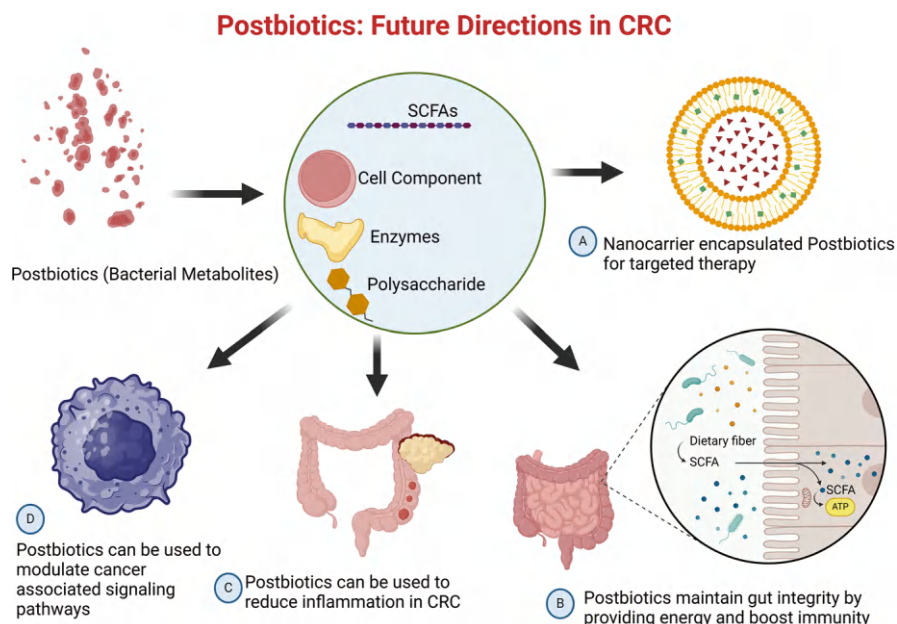


Fig. 20.1 Future role of postbiotics in CRC

20.2 Gut Microbiota Role in Colorectal Cancer

The digestive tract has a multitude of bacteria that establish a symbiotic relationship through cells of the intestine, therefore ensuring a lively equilibrium as well as preserving the intestinal milieu. Disruption of equilibrium will result in an imbalance of the intestinal flora, causing a cascade of intestinal disorders [10]. Association among intestinal flora, as well as colorectal cancer (CRC), was investigated as early as the 1960s. The present work demonstrates that *Cycas* has a cancer-causing influence on conventional rats; however, it does not induce tumors in disinfected rats. This finding suggests that microorganisms of the intestine are significant contributors to the carcinogenesis facilitated by *Cycas*, as previously reported by Laqueur et al. in 1967 [11]. A recent case-control study undertaken by the investigation crew examined features of fecal flora and factors of blood inflammation in different phases of colorectal tumor genesis (from benign polyps to progressive adenoma). The study recognized 24 bacteria associated with colorectal cancer (CRC) and demonstrated flora plays a part in facilitating the progress of the CRC microenvironment during the gradual malignant transformation process [12]. Currently, the established microbiota linked to colorectal cancer (CRC) mostly consists of *Escherichia coli* (*E. coli*), *Fusobacterium nucleatum* (*F. nucleatum*), *Campylobacter jejuni* (*C. jejuni*), *Bacteroides fragilis* (*B. fragilis*), and others [13].

20.2.1 *Fusobacterium nucleatum*

A frequent oral symbiotic Gram-negative bacterium is *Fusobacterium nucleatum*. It is oral cavity-specific and crucial in periodontal disease. A variety of adhesins connect biofilm bacteria. *F. nucleatum* impacts several CRC stages, according to growing evidence. *Fusobacterium nucleatum* increases cancer cell proliferation in two ways: (1) FadA and E-cadherin binding activates β -catenin and Wnt pathways [14]. (2) TLR4 and NF- κ B activation increases carcinogenic miR21 expression [15]. *F. nucleatum* caused Apcmin/+ mice to have elevated levels of multiplying cell nuclear antigen, supporting the proliferation of cells, also inflammatory mediators in mouse serum will be increased, and myeloid cells infiltrate into the tumor, stimulating the growth of the tumor [16]. In addition, *F. nucleatum* may impact metastatic spread, from liver and lymph node metastases it can be isolated and works with Bacteroides, Selenomonas, and Prevotella to encourage CRC metastasis [17]. Subsequently treatment of CRC, *F. nucleatum* blocks autophagy-related mir-18a* and mir-4802, enhancing CRC recurrence and chemotherapy resistance [18]. *F. nucleatum* also promotes CRC glycolysis and carcinogenesis by upregulating the elongated non-coding RNA enolase 1 (ENO1)-intron transcript (ENO1-IT1), which histone modifies ENO1 and other genes [19].

20.2.2 *Escherichia coli*

Escherichia coli, an anaerobic Gram-negative symbiotic bacterium, colonizes the gut soon next to the birth. Certain highly contagious *E. coli* by a pathogenicity island can enter the digestive tract and cause illness. Four *E. coli* strain evolutionary groupings exist: A, B1, B2, and D. The most harmful virulent strains are in groups B2 as well as D, but maximum feces strains are in A as well as B1 [20]. IBD is linked to some pathogenic types in groups B2 and D, and IBD makes you more likely to get colorectal cancer. Colibactin is a gene poison found in bacteria. Nougayrede et al. were the first to write about it in 2006. *E. coli* is the main organism that makes it. Colibactin is thoroughly linked to CRC, which can break double-strand DNA, stop the cell cycle in eukaryotic cells, change the shape of chromosomes, as well as cause CRC. Colibactin-producing *E. coli* can make colon cancer more likely to happen in the CRC mouse model [21]. Colibactin destroys DNA that is high in AT-enriched hexamer sequence patterns more efficiently in human colorectal cancer cells. This is because DNA has different shape properties and an electrostatic potential. The colibactin-binding series was found to be gathered in colorectal cancer [22]. As soon as somatic mutation of colibactin targets were looked at in thousands of tumor genomes.

20.2.3 *Bacteroides fragilis* Strain

Bacteroides fragilis is a bacteria that resides in the intestines and works well with the host. Most bacteria are good for you because they help your body digest food and keep your gut healthy. In certain circumstances, though, these bacteria make a poison that damages the cells on the gut's exterior, which makes CRC more likely. The two kinds of *B. fragilis*, which are not poisonous, also enterotoxigenic *B. fragilis* (ETBF), make a *B. fragilis* toxin (BFT). The epithelial barrier can get damaged by BFT and cause E-cadherin to break apart. Simultaneously, cutting E-cadherin starts the Wnt signaling pathway, which can lead to inflammation of mucosa and the growth of colon tumors. It is also important for the growth of Th17 cells as well as the STAT3 pathway is what colon change is seeking. We can see that ETBF helps Th17 cells make IL-17 by quickly activating the STAT3 pathway. This then helps the NF- κ B and Wnt pathways become active, producing an inflammatory tumor microenvironment in the gut. IL-17 is needed for this process to happen, and giving mice an IL-17-blocking antibody can stop tumors from growing [23]. ETBF increases the activity of spermine oxidase (SMO) in colonic epithelial cells. This raises the level of SMO-dependent reactive oxygen species (ROS), which leads to the discharge of inflammatory cytokines and damage of DNA, eventually helping to make colorectal cancer happen.

20.2.4 *Weak Campylobacter jejuni*

According to Zheng He et al. from a human body, *C. jejuni* 81–176 can colonize sterile Apcmin/+ mice and dramatically raise tumor quantity and size. The mice's gut flora and macro transcriptome changed significantly [24].

20.3 Gut Microbiota as Therapeutic Target to Treat Colorectal Cancer (CRC) Using Postbiotics

Table 20.1 represents the mechanism of postbiotics against colorectal cancer specifically mentioning the cell lines and mechanism of action.

Table 20.1 Postbiotics as therapeutic targets to treat colorectal cancer

Probiotic strain	Originated postbiotic	Cancer cell line	Mechanism of action	References
<i>Bifidobacterium adolescentis</i>	Cell-free supernatant	Caco-2, HT-29 and SW480	Decreases cell proliferation	[25]
<i>Lactobacillus rhamnosus</i>	Decellularized supernatant	Caco-2 and HT-29	Decreases cell proliferation	[26]
<i>Lactobacillus casei</i>	Sonicated-cell suspension	HT-29	Antiproliferative	[27]
<i>Pediococcus pentosaceus</i>	Indeterminate	Caco-2	Decreases cell proliferation Activation of apoptosis	[28]
<i>Clostridium butyricum</i>	Indeterminate	HCT116, SW1116 and Caco-2	Decreases cell proliferation	[29]
<i>Bacillus polyfermenticus</i>	Indeterminate	LoVo, HT-29 and AGS	Decreases cell proliferation	[30]
<i>Lactobacillus rhamnosus</i>	Indeterminate	Caco-2	Increases apoptosis	[31]
<i>Faecalibacterium prausnitzii</i>	Cytosolic fraction	Caco-2 cells	Immunomodulation	[32]
<i>Lactobacillus plantarum sp</i>	Cell-free supernatant	MCF-7	To induce apoptosis, increase DECAY, FADD, and RAS64B gene expression while lowering BCL-2 and BUFFY gene expression.	[33]
<i>Clostridium butyricum</i>	Short-chain fatty acid	HCT-116, Caco-2, and HCT-8	Reduces Wnt/b-catenin signaling and alters gut microbiota composition	[34]

20.4 Mechanism of Postbiotics in CRC Management

20.4.1 Reduced Bacterial Translocation and Preserved Intestinal Barrier

The intestinal cell barrier protects the gut in normal conditions. The gastrointestinal mucosal layer shields the gut epithelium from hazardous substances [35]. Pathogenic bacteria, toxins, exogenous antigens, and other dangerous chemicals can permeate mucous [36]. This is bacterial translocation. Colonocyte junction injury causes inflammation and tumor progression [16]. Mucin glycoproteins in the intestinal barrier’s mucous layer regulate homeostasis and inflammation. The gut has a balance of bacteria that live with the host. The gut microbiota-postbiotic interaction maintains intestinal integrity [37]. In vitro studies suggest that SCFA postbiotics protect the gut by inhibiting HDAC, modulating gene expression, and raising MUC2, MUC1, MUC3, and MUC4 expression. SCFA postbiotics stimulate 50-adenosine

monophosphate-activated protein kinase to strengthen epithelial cell tight junctions, promoting a healthy intestinal barrier [38]. Yogurt postbiotics from *Streptococcus thermophilus*, *L. acidophilus*, and *L. bulgaricus* protect intestinal cell walls. Pro-inflammatory cytokines stimulate NO production in intestinal cancer Caco-2 cell lines [39]. Additionally, intestinal cell epithelial barrier function requires a hypoxia-inducible factor. Disrupting these factors in epithelial cells raises inflammation and colitis risk. SCFAs (butyrate) postbiotics improve barrier function in cultured intestinal epithelial cells with enhanced local oxygen intake by sustaining hypoxia induction [39].

20.4.2 Immunomodulation

Probiotics and postbiotics have a crucial role in regulating the gut immune system and providing therapeutic and preventative effects against chronic inflammatory illnesses. Various studies indicate that postbiotics, like probiotics, can change immune system response and have immunomodulating properties. Miyazawa et al. (2011) found that both the probiotic and postbiotic of *L. gasseri* TMC0356 have immunomodulatory effects in vitro, with the postbiotic leading to a greater increase in IL-12 production in macrophages than the live probiotic cells [40]. The study found that optimal heat treatment does not harm the potential of postbiotics to activate IL-12 production in macrophages. Immunomodulation activity in LAB bacteria may be linked to differences in microbial cell wall components, such as peptidoglycan and lipoteichoic acid. LAB bacteria's immunomodulation action involves increasing Th1 cytokines and decreasing Th2 cytokines [41]. Lin et al. (2007) found that LAB-derived postbiotics (*L. acidophilus* LAP5, LAF1, and LAH7) can suppress *S. Typhimurium* invasion in intestinal cell cultures and mouse models via immunomodulating macrophages [42]. Various factors, such as probiotic strains, postbiotic components, and human immune cells, can impact immunomodulation activity and response [37]. Inflammation is an immune system reaction that involves leukocytes and plasma proteins aggregating and activating at infection, toxin exposure, or cell damage. Inflammation can prevent infection and promote tissue regeneration when balanced [43]. Inflammation is linked to cancer by the presence of immune cells in the tumor. Chronic inflammation, such as inflammatory bowel disease, gastritis caused by *H. pylori* infection, and hepatitis, can lead to colon, gastric, and hepatocellular cancer [44]. Chronic illnesses, uncontrolled inflammation, and genomic instability can increase incursion proliferation and apoptotic responses, promoting cancer growth. SCFA postbiotics protect the mucosal layer by lowering COX-2-produced prostaglandins, which cause inflammation and tumor growth in colon cancer [38]. Laboratory studies suggest that postbiotic components of SCFAs, such as butyrate, propionate, and acetate, can reduce COX-2 expression in malignant cells and mitigate the negative effects of prostaglandins on the mucosal layer [42]. There is evidence that SCFA postbiotics influence OS by regulating HDAC. In contrast, in vivo studies indicate that high ROS levels in serum enhance the risk of

CRC. Postbiotics such as butyrate, acetate, and propionate from probiotics protect colon epithelial cells against DNA damage from ROSs. In vivo studies show that SCFA postbiotics inhibit the TLR-4 signaling pathway, reduce inflammatory cytokines (TNF- α , IL-6, and IL12), stimulate anti-inflammatory cytokines (IL-10), and suppress immune system function. Attaching SCFA postbiotics to GPRs like GPR41, GPR43, and GPR109a activates signaling pathways, suppresses NF- κ B expression, and promotes colon Treg cell differentiation [38]. The postbiotic *L. casei* Zhang (LcZ) enhances macrophage innate immune system response by increasing inflammatory cytokines and Toll-like receptor transcription (TLR2, TLR3, TLR4, and TLR9) [45]. Lahtinen (2012) states that postbiotics, including exopolysaccharides (EPSs), protein monolayers, peptidoglycan, conjugated linoleic acid, lipoteichoic acid, and peptides, regulate the immune system. Fujiki et al. (2012) found that heat-inactivated postbiotics from *L. plantarum* L-137 had an immunomodulatory effect and increased IL-12 p40 production in vitro. The primary role of activated inflammatory cells is to promote INF- γ production, which links the innate and adaptive immune systems. The study found that inactivating probiotic strains with TT may not negatively impact postbiotics' ability to regulate IL-12 p40 production. Postbiotics have similar immunomodulation properties to live probiotics, and optimum inactivation procedures (e.g., thermal treatment) may not negatively impact their anti-inflammatory properties [46].

20.4.3 Induction of Antiproliferative and Apoptotic Mechanisms in CRC Cells

Cancerous cells exhibit uncontrolled growth, proliferation, and resistance to apoptosis. The majority of apoptotic responses and survival mechanisms in afflicted cells suffer negative changes. The apoptosis mechanism, which is the host's natural response without artificial inflammation, is a key factor in the demise of aberrant cells in CRC patients. Colorectal and stomach cancers can be prevented through screening programs and nutritional/pharmacological treatment (chemoprevention) [47]. Research suggests that probiotics and postbiotics can reduce cancer risk as effective chemopreventive agents [48]. Postbiotics from *Lactobacillus* spp., including inactivated cells, cell-wall components, peptidoglycan, cytoplasm extracts, and cell-free supernatants, can inhibit CRC cell proliferation and induce apoptosis [48]. Song et al. (2018) found that *Lactobacillus* spp. postbiotics inhibit LT-97 and HT-29 cancer cell lines by promoting apoptosis and slowing cell cycle progression in the S phase. CRC cells, using glycolysis, produce energy and lactate in the gut [49]. Eslami et al. (2019) found that *Propionibacterium* sp. creates excessive SCFAs, leading to CRC cell obstruction in the sub-G1 stage [38]. Bacteriocin, an important postbiotic, helps prevent cancer cell growth and colonization in HT-29 and Caco-2 cell lines by negatively regulating ErbB2 and ErbB3 expression at the mRNA and protein levels [50]. SCFAs from *Propionibacterium freudenreichii* postbiotics reduce tumor growth by activating the apoptotic pathway in gut cancer cell lines

[49]. SCFAs, particularly butyrate, acetate, and propionate, have antiproliferative properties and promote apoptosis by affecting mitochondrial permeability potential, producing ROS, activating caspase-3, and causing chromatin condensation in colon cancer cells [50]. In apoptosis, FRSs, caspase 3, and mitochondrial membrane permeability were found. Orlando et al. (2012) found that postbiotics from *L. paracasei* IMPC2.1 and *L. rhamnosus* GG suppress colorectal (DLD-1) and gastric (HGC-27) cancer cell proliferation by activating proapoptotic pathways [51]. Research suggests that ferrichrome from *L. casei* postbiotics can induce apoptosis in tumor cells by activating the JNK pathway (Konishi et al. 2016). Evidence suggests that postbiotics generated from particular probiotics have antiproliferative characteristics and increase proapoptotic pathways in cancer cells. Postbiotics' antiproliferative actions are influenced by factors such as parent probiotic cell strains, production methods, bioactive compounds, functionality, and molecular processes [52].

20.5 Possible Signaling Mechanism of Postbiotics

The NF- κ B and MAPK pathways play life-threatening roles in regulating inflammatory responses, cell proliferation, and survival [53, 54]. Dysregulation of these pathways is a common character in various inflammatory diseases, including inflammatory bowel disease (IBD), arthritis, gastritis, asthma, and atherosclerosis [55–57]. Both pathways are activated in response to stress signals, such as infection or tissue injury, prominent to the creation of pro-inflammatory cytokines. Postbiotic metabolites produced by probiotics have gained attention to control these pathways for their potential [58–61]. They can interact with the NF- κ B and MAPK pathways, influencing inflammation and cell cycle regulation. However, the effects of postbiotics can differ significantly depending on the particular bacterial strains involved [62]. This strain specificity highlights the complexity of the immune response and the need for tailored therapeutic strategies. Research indicates that certain probiotics may effectively alleviate indications of IBD and further inflammatory gastrointestinal diseases by targeting these pathways [63–65]. Their ability to modulate NF- κ B and MAPK signaling suggests a promising therapeutic avenue for managing prolonged inflammation. Ongoing research is essential to fully elucidate the mechanisms as well as optimize the use of postbiotics in clinical settings [66].

20.5.1 Molecular Mechanism of NF- κ B Signaling

The NF- κ B pathway shows a vital role in modifiable inflammatory responses through a well-characterized network of signaling events. At its core are the NF- κ B transcription factors, including RelA (p65), RelB, c-Rel, NF- κ B1 (p50/p105), and NF- κ B2 (p52/p100) [67]. These proteins can be various homo- and heterodimers,

with the p50/RelA combination being the most prevalent. In inactive cells, NF- κ B is sequestered in the cytoplasm through inhibitors known as I κ Bs. Upon inflammatory stimuli, the I κ B kinase (IKK) complex phosphorylates I κ Bs, marking them for proteasomal degradation. This process releases NF- κ B, allowing it to transfer to the nucleus, which initiates the transcription of numerous target genes involved in immune responses [68]. These genes include chemokines, interleukins, adhesion molecules (ICAM and VCAM), and antiapoptotic proteins like Bcl-2 and Bcl-xL. The effects of NF- κ B activation can differ based on the cell type and the signaling context, influencing T cell differentiation, dendritic cell maturation, and macrophage polarization [69]. Additionally, various postbiotic elements can interact with different receptor families, triggering signaling cascades that promote the phosphorylation and degradation of I κ Bs, thereby further modulating NF- κ B activity. This multifaceted regulation underscores the pathway's importance in orchestrating immune responses and maintaining homeostasis.

20.5.2 MAPK Signaling Pathway

Mitogen-activated protein kinases (MAPKs) exist as a crucial family of serine/threonine kinases that play significant roles in various cellular processes. They are categorized into three primary subfamilies: those are c-Jun N-terminal kinases (JNKs), extracellular signal-regulated kinases (ERKs), as well as p38 MAP kinases [70].

20.5.3 ERK Pathway

This pathway primarily responds to mitogens as well as growth factors, regulating cell cycle progression and differentiation.

20.5.4 JNK and p38 Pathways

These pathways have broader roles, influencing apoptosis, production of cytokine, proliferation, and inflammatory responses.

20.5.5 Signal Transduction

The MAPK signaling cascade is initiated by the phosphorylation of MAP kinase kinases (MKKs), which are activated by various second messengers, including RAS/RAC proteins, receptor tyrosine kinases (RTKs), G-protein-coupled receptors

(GPCRs), integrins, small GTPases (Ras and Rap), tumor necrosis factor receptors (TNFRs), and pattern recognition receptors (PRRs). Once activated, MAPKKs phosphorylate MAP kinase kinases (MAPKKs) such as MKK4 and MKK7, which then activate the MAPKs (ERK, JNK, and p38) [70]. The phosphorylated MAPKs implement cellular responses through various protein effectors, including other kinases and transcription factors, mediating diverse biological outcomes. This intricate signaling network allows cells to respond effectively to a wide range of stimuli [71].

20.5.6 Postbiotics' Influence on NF- κ B and MAPK Pathways

The properties of postbiotics on pathways of inflammation are complex and depend on various factors, including their origin and the specific bacterial strains used [72]. Research indicates that postbiotics can use both proinflammatory and anti-inflammatory effects, highlighting a diverse and heterogeneous nature among different postbiotic preparations. Key findings show that postbiotics from cell-free supernatants (CFS), as well as heat-killed bacteria (HKB), mainly reduce inflammation by inhibiting NF- κ B activity [73]. This is achieved through mechanisms such as blocking the phosphorylation of I κ B, inhibiting its deprivation, and enhancing the expression of NFKBIA, which sequesters NF- κ B in the cytoplasm. Both NF- κ B and MAPK pathways can be activated through pattern recognition receptors (PRRs), and their simultaneous modulation leads to a reduced inflammatory response [74].

Specific examples include CFS from *L. rhamnosus* and *L. plantarum*, which showed decreased NF- κ B activity and p38 MAPK phosphorylation. HKB from various *Lactobacillus* strains can stimulate NF- κ B translocation, promoting an inflammatory response, while others induce apoptosis via MAPK activation. Dual signaling modes were observed, where some postbiotics activate together NF- κ B and MAPK pathways reliant on the type of cell and situations, demonstrating their context-dependent effects. Despite numerous studies focusing on NF- κ B and MAPK signaling, there is a need for more in-depth investigations into downstream signaling pathways and structure-activity relationships to clarify how different postbiotics exert their effects. This knowledge could lead to more targeted applications of postbiotics in managing inflammation and enhancing gut health.

20.5.7 Other Mechanisms and Molecular Pathways

The intestinal obstruction faces various harmful factors that can compromise cell survival and disrupt its integrity. Recent discussions have highlighted how probiotic components and their metabolites can positively influence the regulation of intestinal epithelial barriers. These substances may help enhance barrier function, promote cell survival, and mitigate damage from harmful agents. Understanding these interactions can overlay the manner for developing beneficial approaches to support

gut health [75]. From a molecular perspective, the PI3K/AKT pathway is often highlighted as a crucial goal for postbiotic factors that regulate intestinal barrier functionality. This pathway plays an important part in the survival of cells, apoptosis, and maintaining intestinal integrity, including mucin production. Additionally, PI3K/AKT may interact with NF- κ B and MAPK cascades, influencing immune responses to probiotic and postbiotic compounds. Furthermore, it is interrelated through the AMP-activated protein kinase (AMPK) pathway and serves as an additional important cellular effector of probiotics and postbiotics, linking metabolic regulation to intestinal health [75]. The stimulation of protein kinase B (PKB/Akt) through postbiotics is well-documented and associated with various health benefits. PKB plays a dangerous part in modulating inflammatory responses, like reducing inflammation triggered by sequestered LGG peptides p40 and p75. It also enhances the production of mucin (including muc2 gene expression) in reaction to butyrate besides purified protein HM0539 from LGG, as well as increases the production of tight junction proteins (claudins-3 and 4) following butyrate treatment as well as exposure to conditioned supernatant fluid (CSF) of *Lactobacillus plantarum* RG14. Notably, the variation of the PI3K/AKT pathway by postbiotics has been witnessed in various types of cells beyond the intestinal environment, suggesting their potential as drug candidates. For instance, an extract from *Lactobacillus reuteri* has been shown to promote wound healing in mice, relying on the initiation of the PI3K/AKT/ β -catenin/TGF- β pathway. Furthermore, the CSF of *Lactobacillus fermentum* mitigated H₂O₂-induced senescence in murine adipocytes through inhibition of the PI3K/Akt/mTOR pathway. These discoveries focus on the versatile therapeutic prospective of postbiotics in various biological contexts [75].

20.6 Nanotechnology-Based Postbiotics Delivery

Nanotechnology is revolutionizing the field of nutraceuticals by enhancing the bioavailability of active compounds, such as antioxidants and vitamins, thereby improving their therapeutic efficacy. The development of nano nutraceuticals enables more effective delivery and absorption of these beneficial substances. Recent advances focus on harnessing bioactive micro and macromolecules—known as postbiotics-produced through beneficial gut microorganisms [76]. These tiny biomolecules offer various physiological health benefits and represent a promising area of research. Active targeting systems utilizing nanoparticles present a novel approach for the controlled in vivo transport of postbiotics. While taking postbiotics is quite emerging, extensive research, including randomized, double-blind clinical trials, is essential to validate their therapeutic potential [77]. Despite the growing interest in postbiotics, understanding their mechanistic actions remains a challenge, which is crucial for substantiating their health claims. Future investigations could integrate disciplines such as food science, microbiology, and personalized medicine, potentially transforming microorganisms into functional ingredients for health and wellness. This interdisciplinary research could unlock new pathways for developing effective treatments tailored to individual needs [77].

20.7 Non-clinical Application of Postbiotics

Biopreservation	<i>Lactobacillus rhamnosus</i> , <i>Lactobacillus paracasei</i>	Prolongs the shelf life and inhibits microbial spoilage of food [78]
Biopreservation of fish and meat products	<i>Lactobacillus curvatus</i> BCS35, <i>Bifidobacterium lactis</i> Bb-12	Which improves shelf life up to 3 months [79]
Biopreservation of dairy products	<i>Lactobacillus. acidophilus</i> , <i>Bifidobacterium bifidum</i> , <i>Lactobacillus plantarum</i>	Fungal spoilage growth prevented in sour cream and semi-hard cheese [80]
Biopreservation of vegetables	<i>Lactobacillus brevis</i> , <i>Leuconostoc mesenteroides</i>	Reduces the sums of native microbiota (aerobic mesophilic bacteria, coliforms, as well as yeasts/molds) of ready-to-eat baby leaf vegetables [81]
Biopreservation of breads	<i>Lactobacillus reuteri</i>	Antifungal ability inhibits the mold contagion for 15 days and increases the consistency of the bread [82]
Food packaging	<i>Lactobacillus sakei</i>	Inhibits contamination, antimicrobial action [83]
Biofilm control	<i>Lactobacillus salivarius</i> , <i>Yersinia enterocolitica</i> , <i>Campylobacter jejuni</i>	Distorts biofilms formation, disrupts and remove them [84]
Cosmetics	<i>Enterococcus faecalis</i> SL-5	Decreases the inflammation in acne lesions [85]
Biological doping	<i>Veillonella</i> species	Increases physical performance in mice [85]

20.8 Clinical Significance of Postbiotics

The word “postbiotics,” coined lately, refers to an extensive category of bioactive substances comprising inactive or nonliving microbial cells, components of cells, as well as any soluble materials or metabolic products made by microorganisms (probiotics), when consumed by humans have positive biological effects [78, 86]. For industrial uses, postbiotics are more dependable and protected than probiotics. Several factors, like pH, protein concentration, fat and carbohydrate content, activity of water, the occurrence of natural antibiotics, handling out and storing conditions, and activity of water, may all have an influence on probiotic cell existence. These constituents also impact food’s nutritional value [87]. Additionally, it concerns the bioactive soluble substances (products or metabolic by-products) formed through microorganisms, for example, enzymes, peptides, teichoic acids, muropeptides originated from peptidoglycan, endo- as well as exopolysaccharides, cell surface proteins, organic acids, vitamins, and plasmalogens [88]. Postbiotics are divided into groups according to two criteria: either their composition can include bacterial cell components, microbial metabolites, and products of enzymatic activity on the food matrix, or their physiological benefits, such as antioxidant,

anti-inflammatory, antiproliferative, hypocholesterolemic, antihypertensive, antimicrobial, and immunomodulatory qualities [88, 89]. Although *Lactobacillus* and *Bifidobacterium* strains are the primary source of postbiotics, reports have also linked postbiotics to *Streptococcus* and *Faecali* bacteria species. The favorable action of postbiotics in element absorption, like calcium, zinc, magnesium, and iron, has been confirmed in animal and human models [90]. Postbiotic supplements have been revealed in studies to reduce blood pressure, which accounts for their antihypertensive qualities. Variations in the gut microbiota as well as its metabolic by-products, improvements in the function of the gut barrier, effects on inflammation, endotoxemia, as well as renal sympathetic nerve activity are the causes of this [91]. Studies reveal gut microbiota gives to the immune system development, guard against pathogens and inflammation, and among other digestive system processes [92]. Short-chain fatty acids (SCFAs) created in the gut by bacteria help control lipid metabolism, glucose levels, and insulin sensitivity by activating receptors like G protein-coupled receptors (GPRs), therefore aiding in maintaining energy steadiness and metabolic stability [93, 94]. It has also been demonstrated that a few short-chain fatty acids, comprising butyrate, acetate, and propionate, are present in keeping the body's plasma cholesterol levels in check [95]. Additionally, it has been shown that postbiotics can inhibit pathogenic bacteria such as *Salmonella enterica* S-1000, *Escherichia coli* E-30, *Listeria monocytogenes* L-MS, and vancomycin-resistant Enterococci. Cell-free supernatant culture from strains of *L. plantarum*, including RG11, RG14, RI11, UL4, TL1, and RS5, is used to achieve this inhibition [96]. A distinct antiproliferative effect of postbiotics on colon cancer cells has also been shown; this effect may be attributed to their ability to modulate immunological responses, which in turn triggers proapoptotic cell death pathways [97].

20.9 Future Aspects of Postbiotics

Probiotics thrive and multiply in the gut, while postbiotics cannot do so, leading to a lack of persistent consequences as soon as treatment is stopped. Further investigation is required to recognize the key postbiotic markers that need to be present in specific concentrations to positively impact health. Measuring postbiotic markers can also assist in shaping if the stages in an exact host's microbiome remain inadequate and/or signal illness, as well as whether the host would gain after postbiotic supplements. Certain postbiotics may undergo metabolism through oral microorganisms before reaching the large intestine, thus reducing their positive impact on health [98]. Postbiotics are essential for the gut environment, barrier integrity, and, eventually, the gut microbiota's composition, which helps the immune system mature. Postbiotics can be used to treat or inhibit an extensive variety of diseases, comprising ones for which there are certainly no reliable treatments. Postbiotics establish an ideal environment for the growth of advantageous bacteria critical for maintaining general health and well-being. Currently, scientists have discovered different types of postbiotic molecules produced by probiotic cells both inside and

outside the cell. These elements consist of muropeptides originating from peptidoglycans, EPS, teichoic acids, and surface protruding structures, for example, fimbriae, pili, or flagella representing cell wall parts, as well as secreted proteins/peptides, bacteriocins like acidophilin, reuterin, and bifidin, cell-free supernatant, lactic acid and acetic acid organic acids, vitamins, butyric acid and propionate short-chain fatty acids, neurotransmitters, biosurfactants, and more [99–101]. Aiming to mimic the restorative effects of probiotics, postbiotics also offer increased stability and extended shelf life while avoiding risks of exposing live bacteria to immune systems that may be impaired or intestinal walls that may be affected [101, 102]. Postbiotics are revealed to have an extensive variety of potential well-being benefits, comprising the capability to modify the immune system, reduce blood pressure, lower cholesterol, inhibit cell growth, enhance gut barrier function, and have anti-inflammatory and antibacterial effects [89, 101]. It has been shown that muramyl dipeptide in peptidoglycan is an insulin-sensitizing postbiotic via activating NOD2, which reduces inflammation as well as glucose intolerance in overweight mice without fluctuating body weight or microbiota composition [103]. Lactic acid bacteria containing exopolysaccharides (EPS) have been shown in pre-clinical investigations to exhibit a variety of bifunctional features, including immune system modulation, gut microbiota regulation, antioxidative capabilities, and the ability to bind to free cholesterol and reduce cholesterol levels [99, 104–106]. Cell-free extracts obtained after lactic acid bacteria show a notable ability to counteract oxidation, in contrast to whole cell cultures, due to the presence of either enzymatic or nonenzymatic antioxidants within the cells [107, 108]. In addition to helping with the metabolism of fats and carbohydrates, SCFAs have an influence on the immune system, adipose tissue, and also other tissues. They also preserve the integrity of the gut barrier. These molecules may help lower the risk of inflammatory illnesses, obesity, diabetes, and other metabolic disorders because of their involvement in energy and lipid metabolism [109]. Bacteriocins are known to contribute significantly to the variety of the gut microbiota and then provide evidence of probiotics' anti-inflammatory qualities [110].

20.10 Conclusion

Postbiotics are substances that have positive effects on the host. They can be metabolites or parts of the cell wall secreted by living bacteria or released by the following bacterial lysis. Postbiotics have the ability to promote several health benefits, such as immunomodulatory, antiobesogenic, anti-inflammatory, antihypertensive, antioxidant, hypocholesterolemic, and antiproliferative. These characteristics imply that postbiotics may, even though precise processes are still being investigated, help to support host healthiness by producing particular physiological effects. More work is required to assist in the identification and description of novel postbiotics, which possibly will advance our knowledge of the modulation of signaling pathways. Innovative research will make it possible to produce comprehensive data that

will guarantee postbiotic product efficacy and stability throughout the production process. Though uncontrolled environmental influences can easily modify metabolism and also experience unanticipated temporary inconsistency, different considerations ought to be devoted to the creation of identical and well-defined cultural practices to avoid probable inconsistency in the postbiotics group. Furthermore, substantiate health claims associated with the supplementation of postbiotics, carefully planned randomized placebo-controlled human/clinical intervention trials, and metabolomics investigations must be carried out.

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