

Progress in Research on Colorectal Cancer-Related Microorganisms and Metabolites

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Abstract: Intestinal flora is an important component in the human body, which have been reported to be involved in the occurrence and development of colorectal cancer (CRC). Indeed, changes in the intestinal flora in CRC patients compared to those in control subjects have been reported. Several bacterial species have been shown to exhibit the pro-inflammatory and pro-carcinogenic properties, which could consequently have an impact on colorectal carcinogenesis. In this review, we summarize the current knowledge on the potential links between the intestinal microbiota and CRC. We illustrated the mechanisms by which intestinal flora imbalance affects CRC, mainly focusing on inflammation, microbial metabolites, and specific bacteria species. In addition, we discuss how a diet exhibits a strong impact on microbial composition and provides risks for developing CRC. Finally, we describe the potential future directions that are based on intestinal microbiota manipulation for CRC diagnosis and treatment.

Keywords: intestinal flora, colorectal cancer, inflammation, microbial metabolites, diet

Introduction

Colorectal cancer (CRC) is a common gastrointestinal cancer. According to the Global Cancer Statistics 2018, in both sexes combined, CRC is the fourth commonly diagnosed cancer based on incidence (6.1%), and the second diagnosed cancer based on mortality (9.2%).¹ According to the reports, the incidence of CRC is positively correlated with social economic development.² It is expected that the number of new cases will increase to 2.2 million and that of death cases will increase to 1.1 million by 2030, especially in developed countries.³ Thus far, the cause of CRC is not very clear. It may be related to genetic factors, inflammatory response, virus infection, and even dietary practices (Figure 1). Some papers have reported that people who were eating red meat had a higher incidence of CRC.⁴ Determining the pathogenesis of CRC will bring benefits to the treatment of this disease.

With advanced developments in high-throughput sequencing technology, intestinal microecology has received increasing attention in recent years.⁵ The steady state of intestinal microecology is important for the body to maintain a healthy balance.⁶ Recent studies have shown that changes in the gut microbiota could be a major factor to trigger CRC.^{7,8} There are many types and numbers of microorganisms in the intestinal tract. They can participate in the substitution of sugar, protein, starch, vitamins, and other nutrients to determine the body's immune function and maintain the health of the host. Initial microflora is always in a state of dynamic equilibrium with the intestinal micro-environment. Once the dynamic equilibrium of the group is broken, it may lead to various

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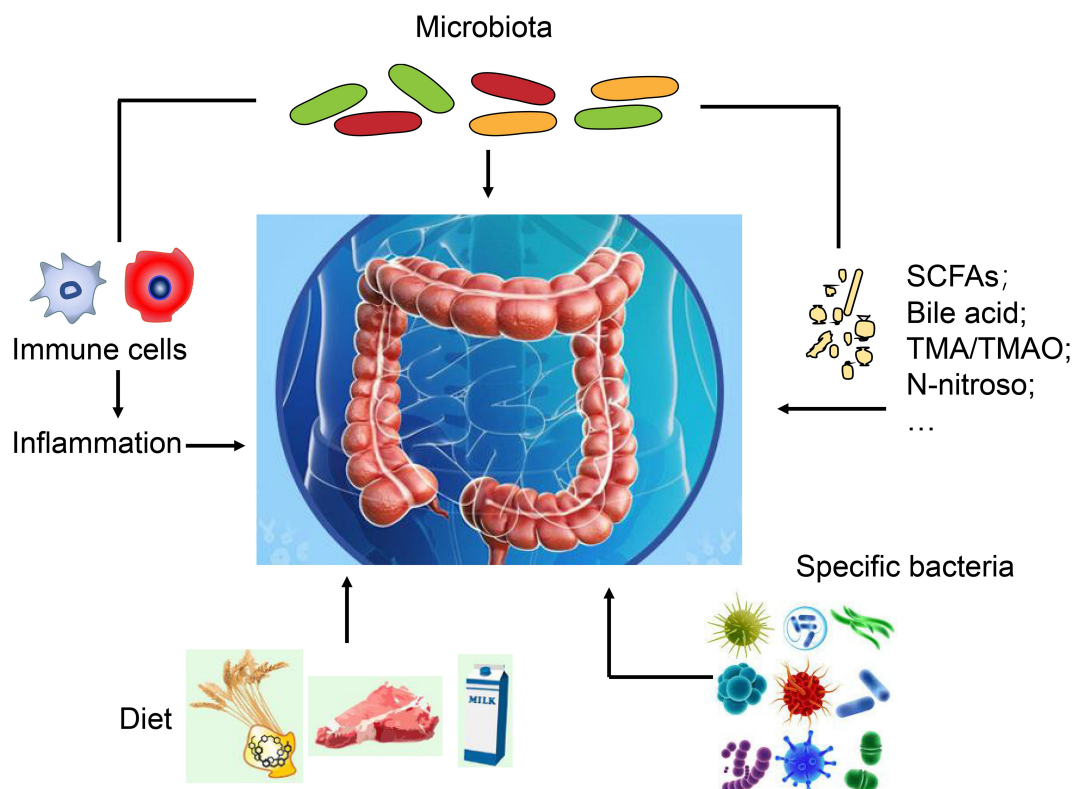


Figure 1 Multidimensional Framework of CRC Evolution. The development of colorectal cancer (CRC) involves a complex interplay between various layers of extrinsic and intrinsic factors. (1) Inflammation represents an important intrinsic factor that promotes carcinogenesis by inducing DNA damage, and reactive oxygen and nitrogen species. (2) Metabolites of intestinal flora can influence intestinal tumorigenesis. (3) Specific bacterial species can influence CRC evolution. (4) Diet can modulate the composition and metabolic activity of the gut microbiota and influence CRC.

diseases. Research results suggest that there is a difference in the composition of intestinal microorganisms between CRC patients and healthy individuals.⁵ The occurrence and development of CRC are in relationship with risk factors such as heredity, immunity, environment, dietary practices, and lifestyle, but the exact pathological mechanism underlying CRC is currently unclear. Animal experiment results^{9,10} indicate that the incidence of intestinal inflammation and CRC in sterile animals is extremely low. However, once the dysregulated intestinal flora is transplanted into the intestinal tract of sterile animals, the incidence of intestinal inflammation and CRC increases significantly. These results imply that the steady state of intestinal flora is the foundation for maintaining intestinal health. Further, there is also a close connection between the microenvironment of the intestinal flora and CRC. However, the above-mentioned conclusions still lack direct evidence from human subjects, and there is a need for further exploration and in-depth research in the future.

In recent years, studies have shown that the metabolites of intestinal flora are closely related to the occurrence of CRC.^{11,12} Primary metabolites of intestinal flora, including amino acids, nucleotides, polysaccharides, lipids, and

vitamins, are necessary to maintain the growth and reproduction of the intestinal flora.¹³ The synthesis of primary metabolites is a constant process, and any obstacles in this synthesis will affect the normal activity of the microorganisms. The secondary metabolites of intestinal flora, including alkaloids, phenols, and antibiotics, can determine the function and specificity of the intestinal flora.¹⁴ All these findings form studies indicate that the intestinal flora is very valuable in maintaining the intestinal microecological balance. Thus, controlling the metabolites of intestinal flora may be a useful strategy in the treatment of CRC. It is critical to illustrate the interactions between intestinal flora, metabolites and CRC development. In this review, we explore the mechanisms underlying intestinal flora imbalance that affects CRC, mainly including inflammation, microbial metabolites, specific bacteria, and diet. We also elaborate the potential clinical practice implications in this microbiota era.

Overview of the Intestinal Flora

Intestinal flora is a research hotspot in microbiology and medicine in recent years. Intestinal flora refers to the various microorganisms that reside in the gastrointestinal

tract of the body, which are combined in a certain ratio. Species are mutually restricted and interdependent to maintain the ecological balance. The human gut has a huge number of microorganisms including bacteria, viruses, and archaea.^{15–17} Among them, bacteria are present in the largest number, reaching to 10.¹⁴ Intestinal flora is mainly divided into three categories: (1) Probiotics such as *Bifidobacterium*, *Lactobacillus*, etc., which are beneficial to health; (2) Conditionally pathogenic bacteria such as *Escherichia coli*, which are not pathogenic under normal conditions but can cause disease when the intestinal microenvironment changes or the balance of the flora is broken; (3) Pathogenic bacteria such as *Staphylococcus*, *PneumoniaCocci*, *Neisseria*, etc., which can cause disease even under normal conditions.

The initial microflora has an important role in maintaining the survival and health of the host organism. Previous research results suggested that the intestinal flora has the following physiological functions at minimum: (1) Preventing the invasion of pathogenic bacteria, adjusting the balance between the human body and the microorganisms state, and maintaining the body's initial health or physiological state; (2) Immunity function during which the bacterial flora can produce an immune response by stimulating the host to inhibit the propagation of pathogenic bacteria in the intestine; (3) Detoxification function in which the bacterial flora can adjust the peristaltic movement of intermediates and the absorption of water to promote stool and excretion of harmful substances; (4) Nutritional effect, in which the initial bacterial flora can synthesize or promote the absorption of nutrients; (5) The bacterial flora can activate antitumor cytokines by degrading and removing carcinogens to exert its antitumor role; (6) The initial flora can reduce the production of oxygen free radicals and control the arthritis response to delay senescence.

In a healthy gut, the dominant bacteria mainly include *Firmucutes*, *Actinobacteria**Proteobacteria*, and *Bacteroidetes*. However, the intestinal flora has a diverse structure at the genus and species levels. Intestinal flora communicates with the host, enhances the epithelial defense against pathogens, and accelerates the maturity of the immune system.^{18,19} Previous research reported that the intestinal flora has the capacity to defend the body against pathogens by recognizing the conserved antigen of bacteria.^{20,21} The intestinal flora was reported to protect the local homeostasis. For example, Wang et al reported that gut microbiota could decrease alcohol-associated steatohepatitis.²² Except that, *Salmonella typhi* was reported as a well-known pathogen that can cause great damage to human health. A variety of factors such as age,

diet, drugs, sports, and genotype also have been reported to impact the gut microbial community.^{23–27}

In addition, the relationship between gut microbiota and CRC has become a research hotspot in recent years. Studies have shown that people who begin to take antibiotics from a young age are more likely to develop CRC after 60 years of age. This prompted us to suggest that the disruption of the intestinal microbiota balance may be a main factor of CRC.²⁸ Compared with healthy people, CRC patients have significantly decreased intestinal microbiota diversity and obviously changed microbial abundance.²⁹ Many studies showed that the bacteria in the initial microflora species such as *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Escherichia coli*, and so on, were obviously increased in CRC patients.^{30,31}

Pathogenic Mechanism of Intestinal Flora

Inflammatory Microenvironment and CRC

Inflammation is the key factor that promotes CRC progression. Among patients with inflammatory disorders, 10%–15% are more likely to develop CRC compared with that in normal people.³² The dysregulation of intestinal microbiome can make the intestinal tract environment worsen and stimulate intestinal epithelial cells to activate the NF- κ B pathway to drive the inflammation (Figure 2). A study on *ApcMin*⁺ mouse reported that colonitis was driven by the high density of microorganisms.³³ Microorganisms can accumulate in the polypus and trigger local inflammation. Antibiotics can relieve these symptoms. Previous studies reported that inflammation and gut microbiota are closely linked through a two-way relationship. On the one hand, IL-33 can activate B cell to produce IgA to maintain the homeostasis of the intestinal microbiome. On the other hand, the remodeling of the intestinal microbiota can activate the release of IL-1 α to induce tumor formation related to colitis and inflammation.³⁴ Long-term chronic mild inflammation that accompanies the aging process is the main cause of inflammatory-related tumors. Immune depletion may be the main reason. Studies have found that mild chronic inflammation was often accompanied by the reconstruction of intestinal microbiota. Microbial diversity was reduced. The change in gut microbiota can indeed promote the inflammation. According to Wong's study, they fed the sterile mouse with CRC patients' fecal samples and found that the treatment group had more polypus, intestinal dysplasia, increased inflammatory factors including CXCR1, CXCR2, IL-17A, IL-22, IL-23A, and

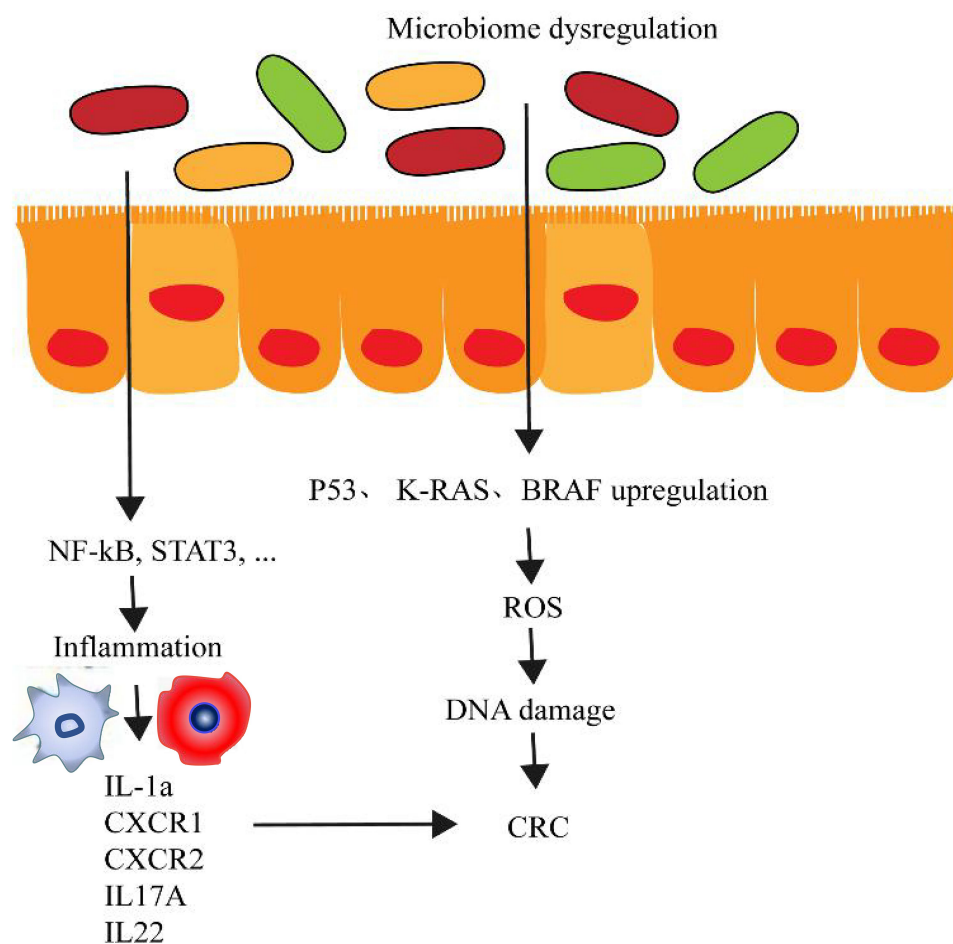


Figure 2 Inflammatory induction of the colorectal cancer (CRC) occurrence. Deterioration of the intestinal ecological environment leads to the propagation of pathogenic microorganisms and imbalance in the microbial flora. Intestinal epithelial cells activate the NF- κ B pathway to drive the inflammation. As the result, a large number of inflammation factors are released to induce the occurrence of CRC.

enhanced Th1 and Th17 cells than did the control group.³⁵ However, determining the reasons why the inflammation and microbiology reconstruction are the starting factors for CRC development further study is needed.³⁶

Immune cell infiltration has been reported to be closely related to the prognosis of CRC patients. However, the role of chemokines in promoting immune cells to enter colorectal cancer needs to be confirmed by more studies. Some studies found that tumor cells may be an important source of the expression of chemokine CCL5, CXCL9, and CXCL10. Tumor cells that are exposed to gut bacteria can recruit more T cells than the control cells, which suggests that intestinal microbes may play an indispensable role during the chemotaxis process of T cells.³⁷

Bacterial Metabolites and CRC

Intestinal microbes play an important role in human health and disease. The metabolic function of intestinal microbes

can be considered as a contributing factor for disease development, and their biologically active substances have an important impact on the host's physiological and pathological processes. After intestinal microflora disorders, intestinal anaerobic bacteria can produce a series of metabolic enzymes to change the metabolic capacity. These metabolic enzymes can act on different substrates (including bile acid, fatty acid, and so on) to produce carcinogens, which, in turn, cause colon cancer.⁵ According to previous reports, carcinogens produced as a result of bacterial metabolism mainly included hydrogen sulfide, reactive oxygen species (ROS), secondary bile acids, and so on. The type and number of intestinal flora can directly affect the occurrence and development of tumors, and intestinal flora can also indirectly affect CRC cells by regulating body metabolism. At the same time, tumor cells escape the killing effect of these substrates on tumors by downregulating the metabolites of beneficial flora in the process of tumor growth (Figure 3).

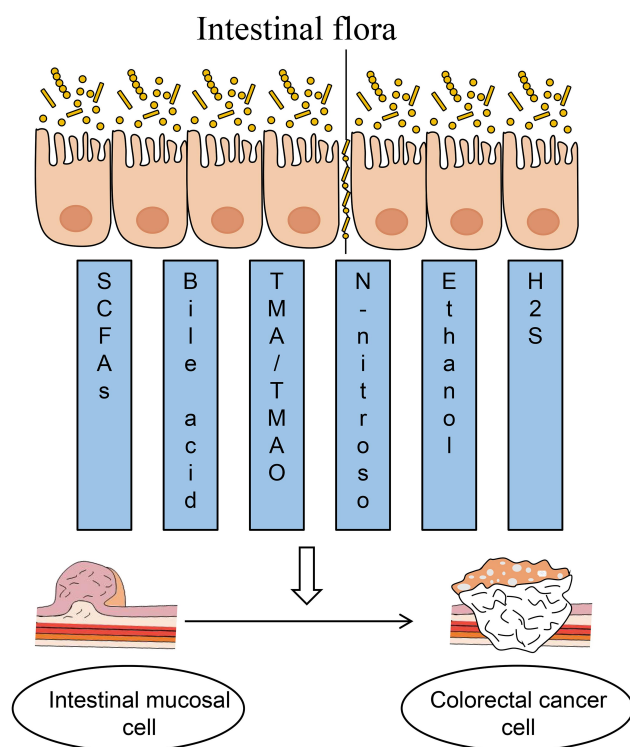


Figure 3 Bacterial metabolites of intestinal flora infect the development of colorectal cancer (CRC). The common metabolites of intestinal flora including short-chain fatty acids, bile acids, TMA/TMAO, N-nitroso, ethanol, sulfured hydrogen, etc., which can be secreted by the intestinal flora to promote CRC development.

Short-Chain Fatty Acids (SCFAs)

The beneficial flora can produce short-chain fatty acids (SCFAs) after fermentation. Common SCFAs include acetate, propionate, and butyrate. Among them, butyrate can enter into the nucleus of tumor cells and function as a histone deacetylase inhibitor to block tumor cell proliferation.³⁸

G-protein-coupled receptor 43 (GPR43) and GPR109A are the main receptors of SCFAs. They are important molecules that mediate the cancer suppressive effect of the bacterial fermentation product SCFA.³⁹ Studies have shown that the expression of GPR109A in CRC can upregulate the expression of apoptotic factors and downregulate the expression of tumor proliferation-related genes, which, in turn, promote tumor cell apoptosis and reduce tumor cell colonization and growth.⁴⁰ Another study had shown that GPR43 had a low expression level in the primary and metastatic foci of colon cancer.⁴¹

The ectopic expression of GPR43 in CRC can lead to cell cycle stagnation and eventually cause apoptosis of tumor cells. Some researchers reported that the ectopic expression of the sodium ion-coupled SCFA transport

protein can cause translocation of the apoptosis inhibitor protein from the nucleus to the cell membrane through protein interaction and repress survivin transcription to promote the apoptosis of tumor cells.⁴² Our previous study also demonstrated that the levels of SCFAs in CRC patients and individuals with a high risk of CRC were higher than those in healthy individuals.⁸

Bile Acids

High levels of bile acids have been confirmed to be closely related to human colon cancer (Figure 4). Intestinal bacteria can produce bile acid, especially under a high-fat diet. *Clostridium* can produce secondary bile acids. These secondary bile acids affect the mitotic processes, which induces DNA damage and the production of ROS, thereby leading to the increase in the incidence of colon cancer risk.⁴³

Bile acid is a product of cholesterol catabolism in the liver. It is a general term for a class of bile acids. It can be divided into primary bile acid and secondary bile acid from the source. Primary bile acid is synthesized in the liver cells using cholesterol as a raw material. After the entry of the primary bile acid into the large intestine, it is decomposed and dehydroxylated by bacteria to generate secondary bile acid, deoxycholic acid, and lithocholic acid.⁴⁴ Secondary bile acids have biological toxic effects such as mutagenesis, cell lysis, and DNA band breakage, which are known inducers of intestinal tumors.⁴⁵

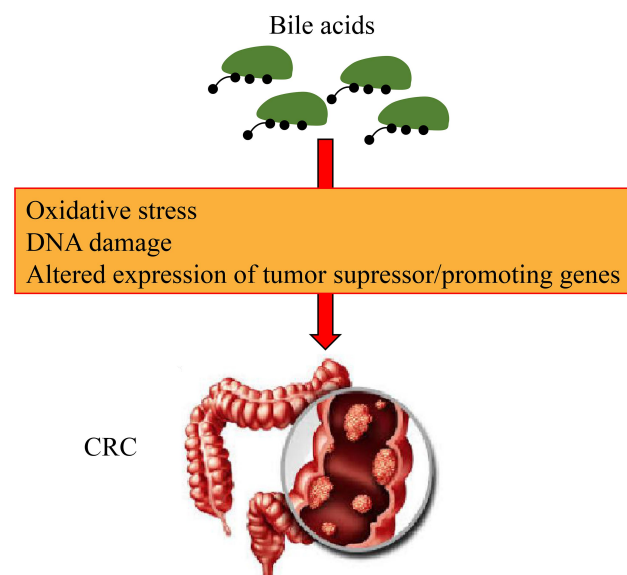


Figure 4 Mechanism of bile acids leading to the development of colorectal cancer (CRC). Bile acids can promote oxidative stress and induce DNA damage, and altered expression of tumor suppressor and lead to the development of CRC.

In vivo studies have shown that the content of secondary cholic acid in the serum and colon of patients with adenomatous polyps and colon cancer was significantly increased.⁴⁶ In vitro experiments also confirmed that deoxycholic acid and chenodeoxycholic acid in the colon can promote the proliferation of human colon adenoma AA/C1 cells and decrease the apoptosis of cancer cells.⁴⁶ More importantly, long-term high-fat, high-protein, and low-fiber diets will produce large amounts of secondary bile acids and bile acid fecal enzymes in the colon, which can, in turn, cause colon cancer.⁴⁷

Hydrogen Sulfide

Hydrogen sulfide is produced by intestinal flora by the reduction of diet-derived sulfate and the metabolism of other compounds including sulfur amino acids and taurine.⁴⁸ Food residues with a high protein content can stimulate the growth of sulfate-reducing bacteria. Hydrogen sulfide plays a role in promoting inflammation and genotoxic substances, indicating that it is associated with the development of CRC.⁴⁹ Hydrogen sulfide can cause cell proliferation, differentiation, apoptosis, and inflammation, eventually leading to the malignant transformation of intestinal epithelial cells.⁵⁰ Thus, hydrogen sulfide levels might be primarily driven by the changes in bacterial activity rather than by bacterial abundance.

Researchers have found that CRC patients had higher hydrogen sulfide levels than healthy people. Furthermore, the detoxification capacity of the colon tissue for hydrogen sulfide in CRC patients was weakened.⁵¹ Hydrogen sulfide induces the formation of colon cancer mainly through the induction of DNA damage, the release of free radicals, inflammation of the colonic mucosa, excessive colonic mucosa hyperplasia, and inhibiting cytochrome oxidase, butyrate utilization, mucus synthesis, and DNA methylation.⁵²

Methylamines (TMA/TMAO)

Trimethylamine oxide (TMAO) is one of the important metabolites of intestinal microorganisms. It is first decomposed into trimethylamine (TMA) by the nutrients that are rich in phosphatidylcholine (PC) and L-carnitine under the action of the intestinal microbe TMA lyase. Then it was formed after oxidation by flavin monooxygenase (FMO) 3 in the liver.⁵³ With increasing research on TMAO, it was found that TMAO was related to not only cardiovascular disease, kidney disease, and diabetes but also cancers. Studies have found⁴⁶ that plasma TMAO levels were lower in patients with stroke and transient ischemic attack (TIA),

and it was speculated that stroke or its treatment will reduce plasma TMAO levels. In addition, studies have shown⁵⁴ that TMAO and Alzheimer's disease have a strong positive correlation. Clinical data showed⁵⁵ that plasma TMAO levels were positively correlated with CRC. Experimental studies suggested⁵⁶ that urine TMAO can be used as a predictor of CRC. On the contrary, a few studies have shown that^{57,58} TMAO can correct the folding defects in mutant proteins and have a protective effect in the process of CRC cancer. Therefore, the effect of TMAO on cancer needs further study. In addition, some studies have shown that TMAO can cause oxidative damage to the liver⁵⁹ and can also be used as a factor of poor prognosis of community-acquired pneumonia.⁶⁰ At the same time, it is also related to inflammatory bowel disease, ulcerative colitis, chronic gastritis, and gastric ulcer.^{61,62} Some scholars have studied the correlation among diet, microbiome metabolism, and diseases. They found that TMAO-related genes and CRC-related genes share a common genetic pathway in the immune system, cell cycle, cancer pathway, and Wnt signaling pathway. Hence, TMAO may be connected to a high-protein and high-fat diet. Moreover, it may be an important intermediate marker of intestinal microbiome metabolism and CRC risk.⁶³

N-Nitroso Compounds

After dimethylnitrosamine was confirmed to have carcinogenic effects in 1956, more than 200 kinds of nitroso compounds were proved to be carcinogenic. There are two types of nitroso compounds: N-nitrosamine and N-nitrosamide.⁶⁴ N-nitrosamines are mostly volatile and have no direct mutagenic effect on organs and tissue cells. N-nitrosamide can directly damage DNA and is a direct carcinogen. N-nitroso compounds are ubiquitous in nature and can also be synthesized in the body by nitrates and nitrites in food, and the gastrointestinal tract is the main site for the endogenous synthesis of nitrous compounds.⁶⁴ Recently, Abu-Ghazaleh et al reported that N-nitroso compounds can modulate CRC progression.⁶⁵ The content of nitrite in pickled foods such as kimchi and sauerkraut is very high. Therefore, its intake is closely related to the incidence of human CRC.⁶⁶ N-nitroso compounds have a strong carcinogenic effect,⁶⁷ especially in inducing human digestive tract cancer such as CRC.⁶⁸

Ethanol

At present, it is widely recognized that excessive drinking is an important risk factor for carcinogenesis,^{69,70} while ethanol metabolism by the intestinal flora may further

increase its toxicity. Ethanol can be produced by many anaerobic bacteria in vitro when they are grown in pure culture. However, the level of endogenous ethanol production by the colonic microbiota in vivo is unknown. Although ethanol itself is not a substance with obvious carcinogenic effects, its oxidation product acetaldehyde is recognized as a strong carcinogen, which can have a series of effects on the body, including the degradation of vitamin folate and DNA damage.⁷¹ Interestingly, studies of the oral microbiota have shown that microorganisms contributed to the production of acetaldehyde from ethanol, which suggested that the gut microbiota might also contribute to this process.^{71,72}

Specific Bacterial Strains Associated with CRC

Current research believes that the composition of intestinal microbes is an important factor affecting tumorigenesis, and some intestinal flora such as *Desulfovibrio*, *Escherichia coli*, and those found in fecal intestines (Figure 5) promote the occurrence of CRC.

Fusobacterium nucleatum

Currently, the relationship between *Fusobacillus nucleatum* (*F. nucleatum*) and CRC occurrence has become the hotspot. Gur et al⁷³ found that *F. nucleum* can protect various tumors from the killing effect of natural killer (NK) cells, and this function was mediated by the ITIM domain (TIGIT). The inhibitory receptor TIGIT exists on human NK cells and T cells, and this inhibitory effect depends on the Fap2 protein in *F. Nucleatum*. This indicates that the derivative factors of *F. Nucleatum* can promote tumor immune escape. In addition, proliferation rate, invasion activity, and tumor growth rate in mouse xenograft models can be significantly increased when CRC cells are infected by *F. nucleatum*.⁷⁴ *F. nucleatum* can regulate the tumor immune microenvironment and activate the E-cadherin/ β -catenin signaling pathway. Rubinstein et al⁷⁵ proved that *F. nucleatum* can bind to E-cadherin by adhering to FadA, thereby activating the β -catenin signaling pathway to induce carcinogenesis and inflammation. Importantly, compared to the CRC tissue, the expression of FadA in healthy tissues is significantly reduced, suggesting that inhibition of this signaling pathway can protect the body from an oncogenic

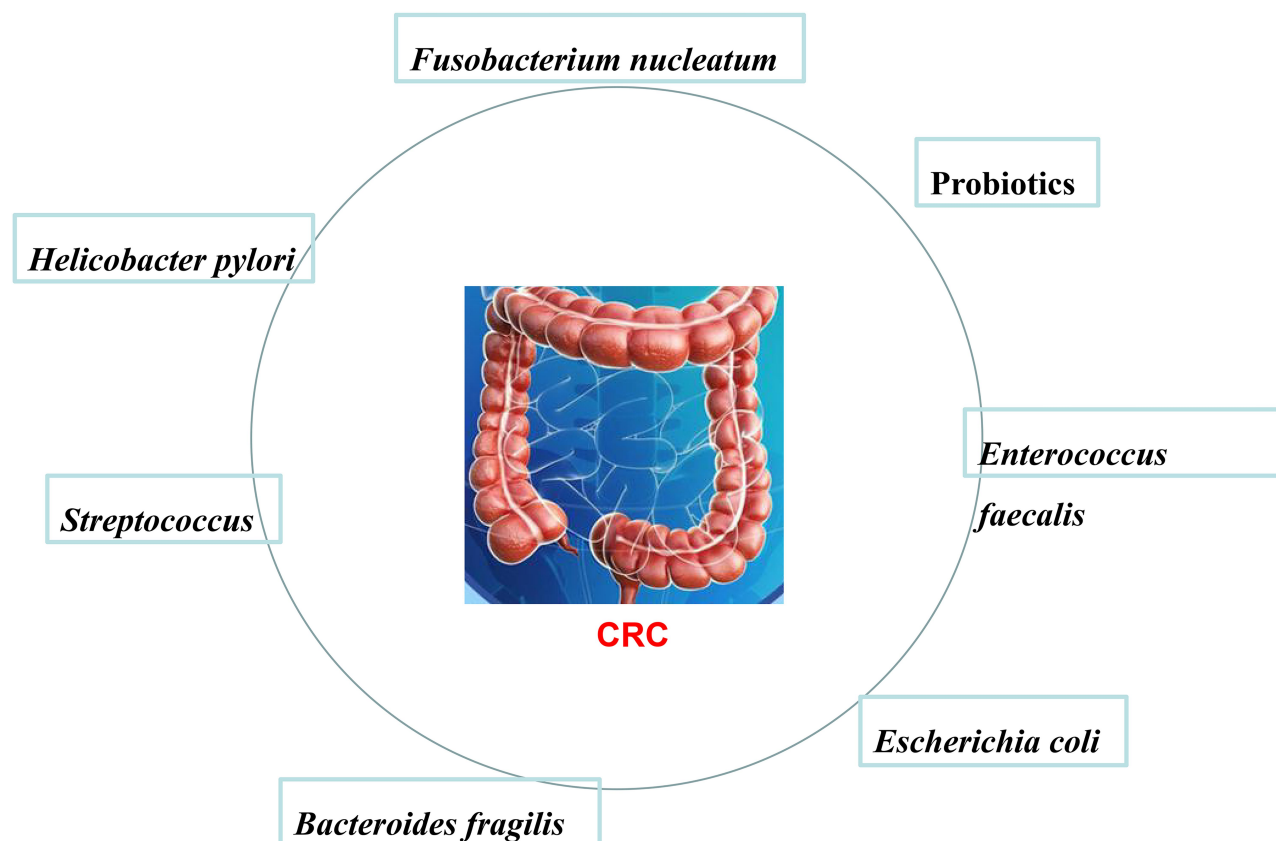


Figure 5 Specific bacteria promote the occurrence of colorectal cancer (CRC). Specific bacterial strains can be associated with CRC, such as *Fusobacterium nucleatum*, Probiotics, *Enterococcus faecalis*, *Escherichia coli*, *Bacteroides fragilis*, *Streptococcus*, *Helicobacter pylori*, and so on.

effect. Yu et al⁷⁶ showed that the relative abundance of *F. nucleatum* not only was related to the occurrence of CRC but also resulted in resistance to chemotherapy drugs and increased relapse rate by interfering with the signal transmission of TLR4 and MyD88 in CRC patients. The possible mechanism might be that *F. nucleatum* targets specific miRNAs, resulting in the activation of the autophagy pathway to change the patient's response to chemotherapy drugs. The enrichment of *F. nucleatum* in CRC tissues had a positive correlation with a shorter survival rate.⁷⁷ Therefore, it may serve as a potential prognostic marker for CRC.

Probiotics

In addition to the pathogenic microorganisms in the human intestine, there is also a category of intestinal microorganisms called "probiotic." They regulate host mucosa and system immune function. They also can produce a beneficial physiological effect on the host by improving the intestinal nutrition and the flora balance. Shadnouch et al⁷⁸ proved that a stable intestinal environment is the balance between intestine immune and anti-inflammatory reactions, which can be maintained by probiotics. Probiotics can not only relieve lactose intolerance and reduce constipation but also reduce the recurrence of inflammatory bowel disease and prolong the remission period.⁷⁹ A previous clinical study was conducted on 92 patients with inflammatory bowel disease. Subjective evaluation and clinical remission rate in patients in the treatment group with the addition of probiotics were higher than those in the control group. Sood et al⁸⁰ also confirmed that probiotics can significantly improve the remission rate in patients with mild-to-moderate active cancer.

Prebiotics are food components that cannot be broken down by enzymes in the intestine. They can promote the growth of probiotics to become the predominant flora. The combination of prebiotics and probiotics is called synbiotics. The application of synbiotics can make fecal flora change significantly. As the number of *Bifidobacterium* and *Lactobacillus* increases, the number of *Clostridium perfringens* decreases, and it can improve the function of the colonic epithelial barrier in patients with polypectomy.⁸¹ Probiotic bacteria participate in suppressing allergies, controlling serum cholesterol levels, and regulating immune function to inhibit the growth of potentially harmful bacteria to prevent the occurrence of CRC.⁸² Based on the anticancer properties of probiotics, it can be used in combination with traditional CRC treatment (including surgery, chemotherapy, etc.).^{83,84} It can improve

the integrity of the intestinal mucosal barrier and reduce the incidence of infectious complications in surgical CRC patients.⁸⁵ Some studies have analyzed the effects of probiotics and synbiotics and found that both can effectively combat the risk factors of CRC. *Yorkshire milk Bacillus* (*L. johnsonii*) can adhere to the surface of the colonic mucosa, reduce the invasion of pathogens into the intestinal tract of patients with CRC during the perioperative period, and adjust its local immune function.⁸⁶ To counterbalance the adverse complications related to tumor treatment, probiotics can also prevent CRC recurrence and improve the quality of life of patients.⁸⁷

Enterococcus faecalis

Enterococcus faecalis (*E. faecalis*) is a gram-positive facultative anaerobic commensal bacterium. Most of them are harmless to humans. However, it has emerged as a human pathogen.⁸⁸ Balamurugan et al⁸⁹ reported that significantly higher *E. faecalis* fecal populations were found in fecal samples collected from CRC patients than in healthy control individuals. *E. faecalis* has been reported to induce chronic inflammation and to produce extracellular superoxide and hydrogen peroxide.⁹⁰ Furthermore, production of extra cellular free radicals was shown to induce DNA damage in vitro.⁹¹ In addition, *E. faecalis* was also able to induce DNA damage in colonic cells.⁹¹ Reactive oxygen species (ROS) are able to induce chromosomal instability,⁹² which could be associated with CRC occurrence.⁹³ Moreover, *E. faecalis* can trigger colitis, dysplasia, and CRC.⁹⁴ Wang and colleagues also showed that *E. faecalis* was able to polarize colon macrophages to an M1 phenotype. All of these findings could explain the mechanisms by which *E. faecalis* exerts effects on colorectal carcinogenesis. Extracellular superoxide produced by *E. faecalis* is converted to hydrogen peroxide, thereby causing DNA damage to colonic epithelial cells.^{93,95}

Escherichia coli

The content of *E. faecalis* in CRC patients is higher than that in healthy people. In addition, some studies have found^{96–98} that some special types of *E. coli*, *Proteobacteria*, and enterotoxin-producing *Bacteroides fragilis* (*Enterotoxigenic Bacteroides fragilis*, ETBF), *Bacteroides/Prevotella* spp., *Campylobacter*, *Peptostreptococcus*, etc. were found in the colon of CRC patients in much higher quantities than those in normal individuals. Using adenocarcinomas and normal colonic mucosa from CRC patients, mucosa-associated *E. coli* was found in 50% of adenomas compared to that of 15% of normal mucosal samples.⁹⁹ Moreover, there is a

correlation between poor prognostic factors of CRC and colonization of mucosa by *E. coli*.¹⁰⁰ Some research had reported that *E. coli* might influence CRC progression by persisting in immune cells and controlling the secretion of pro-tumoral mediators.¹⁰¹

The polyketide synthase (pks) gene of some *E. coli* strains can encode the colibactin protein. This genotoxic product can induce single-stranded DNA damage.^{102,103} CRC *E. coli* causes continuous and low-level colonization in the intestine to induce an asymptomatic and continuous inflammatory response in the colonic mucosa. At the same time, it produces genotoxic substances that can cause DNA damage in colonic epithelial cells, thereby increasing the individual's vulnerability to CRC.^{104,105}

Bacteroides fragilis

Professor Cynthia recently published a study in *Science*. She found that enterotoxin-producing *Bacteroides fragilis* (ETBF) was able to “engulf” a part of the intestinal mucus layer, thus destroying the intestinal barrier and allowing itself and the “genotoxic island” sequence encoded by the pks gene of *E. coli* (pks+*E. coli*) invade the inner layer of intestinal mucus and form a biofilm.¹⁰⁶ These two common bacteria are important promoters of CRC.

ETBF is an anaerobic bacterium in the colon of healthy people and animals. It is a resident bacterium in the human intestine. ETBF is one of subtypes of the *B. fragilis*, that can be asymptotically colonized after the infection and can also cause diseases such as diarrhea. ETBF is also the cause of clinically independent endogenous suppurative infections and is associated with the onset of colorectal tumors. Wu et al¹⁰⁷ had focused on the function of ETBF in mice. They found that ETBF and non-toxicogenic ETBF can be colonized in mice, but only the ETBF can cause an inflammatory reaction in the colon and significantly increase the incidence of colon tumors in the multiple intestinal neoplasia (Min) mouse model. Goodwin et al¹⁰⁸ found that ETBF infection can upregulate spermine oxidase (SMO), which can promote reactive oxygen species, DNA damage etc., thereby promoting tumorigenesis. Treatment with SMO inhibitors can inhibit the inflammatory response produced by ETBF in the Min mouse model and significantly reduce the number of colon tumors. Rhee et al¹⁰⁹ found that C57BL/6 mice with intragastric administration of ETBF did not only produce an inflammatory reaction in the colonic mucosa but also caused the proliferation of intestinal mucosa epithelial cells. Toprak et al¹¹⁰ found that the detection rate of fecal ETBF in patients with CRC was 38% and that in normal people was 12%. It was confirmed for the first time

that ETBF was highly prevalent in patients with CRC. The pathogenicity of ETBF is related to its products containing sugar capsules, outer membrane proteins, and special enzymes, including enterotoxin fragilysin. Studies have shown that the target of fragilysin is the cell surface protein E-cadherin. ETBF enhances the transcription of proto-oncogenes c-myc and cyclin D1 by activating the Wnt/Wingless signal transduction pathway, leading to tumorigenesis. ETBF directly acts on colonic epithelial cells through *Bacteroides fragilis* toxins, and at the same time, it can promote the development of CRC by causing an immune inflammation reaction.^{108,110,111}

Streptococcus

Studies have found that *Streptococcus bovis* was highly enriched in CRC patients.^{112–117} The role of *S. bovis* in CRC is currently unclear. Klein et al¹¹⁸ found that most *S. bovis* can induce colon adenoma or asymptomatic tumors in patients with endocarditis, indicating that *S. bovis* was involved in the early stages of CRC. In addition, another study found that the level of *S. bovis*-induced antigen expression of RpL7/L12 was significantly increased in patients with colon polyps and stage I/II CRC but not in patients with advanced lymph nodes or distant metastases.¹¹⁹ These findings suggest that *S. bovis* may promote the development of CRC at an early stage. At the same time, *Streptococcus bovis* can also cause specific boarding crypts provided by colorectal tumor lesions, which can cause the release of inflammatory cytokines such as interleukin-8 (IL-8) through direct contact or antigen-stimulated cells, which, in turn, further promotes abnormal colonic recession overproliferation of litters.^{52,120} In addition, *S. bovis* also produces inflammatory cytokines such as IL-8 and prostaglandin E2 (PGE2), causing chronic inflammation of the colon. This long-term adverse stimulation will gradually promote normal colonic epithelial cells to become cancerous.¹²¹

Helicobacter pylori

Some scholars have found the presence of *Helicobacter pylori*-DNA in the intestine of patients with CRC.¹²² In recent years, many domestic and foreign scholars have reported the relationship between *Helicobacter pylori* infection and CRC.¹²³ At present, the mechanism of colorectal tumors caused by *Helicobacter pylori* infection is not clear, but hypergastrinemia and the expression of cyclooxygenase-2 (COX-2) are currently considered to be possible mechanisms of CRC caused by *Helicobacter pylori* infection.¹²⁴ In animal experiments in mice, it was found that overexpression of gastrin can cause intestinal metaplasia-atypical hyperplasia and eventually

gastric cancer in 20 months.¹²⁵ Therefore, there is a hypothesis that *H. pylori* infection may indirectly lead to CRC through changes in gastrin levels: *H. pylori* infection causes atrophic changes in the gastric mucosa and high gastrin levels through the negative feedback mechanism of gastric antrum G cells, thereby promoting colonic mucosa growth.^{126,127} Studies have shown that high gastrin levels can promote the growth of colon cancer cells cultured in vitro and increase the incidence of CRC in animal models.^{128–130} In addition, many studies have shown in animal experiments that non-amidated gastrin (including progastrin and glycine extended gastrin) acting as a growth factor for colonic epithelial cells and tumors, may be involved in the development of CRC.^{131,132} Glycine-extended gastrin promoted the proliferation of the colonic mucosa through Rho/ROCK-dependent pathways, which was mainly manifested by thickening of the intestinal mucosa and increased goblet cells of the gland duct.^{133,134} Studies have shown that *H. pylori* infection-induced hypergastrinemia was often accompanied by high expression of COX-2 in the large intestinal mucosa.^{135,136} Hartwich et al¹³⁷ further showed that the expression of gastrin, COX-2, and anti-apoptotic mRNA receptors increased in *H. pylori*-infected colorectal tumor tissues, suggesting that hypergastrinemia and COX-2 may interact and lead to the formation of large intestine tumors. In addition, *H. pylori* infection may also lead to CRC through immune tolerance mechanisms. Frumento et al¹³⁸ studies have shown that low tryptophan levels and increase in the concentration of its degradation product kynurenine may directly affect the immune response to antigen-stimulated T cells in tumor patients. However, Engin et al¹³⁹ found that the *H. pylori*-positive group of patients with CRC had a significantly higher kynurenine/tryptophan ratio than the *H. pylori*-negative group. It was speculated that *H. pylori* infection may lead to cancer development through immune tolerance. At present, hypergastrinemia, high expression of COX-2, and immune tolerance can be considered as one of the mechanisms of the development of colorectal tumors caused by *H. pylori* infection, but the specific physiological mechanism is still unclear and needs further investigation.

Diet and CRC

High-Fat, High-Protein Diet

In recent years, with continuous improvement in people's living standards and reduction of dietary fiber content, the proportion of meat and fried grilled food has increased significantly, resulting in an increased risk of CRC. Studies have found that the incidence of CRC was lower in countries with

a low-fat diet, while the incidence is higher in countries with a high-fat diet (Figure 6). Studies in Shanghai (China) have also found that the increase in the incidence of CRC was related to the intake of a large number of high-fat diet. The carcinogenic effect may be related to the production of oxides and fatty acids broken down by fat. A high-fat, high-protein diet leads to the onset of CRC.¹⁴⁰ It can cause the liver to synthesize and secrete too much of bile acid. Under the influence of anaerobic bacteria in the intestine, the bile acid remaining in the intestine becomes secondary cholic acids such as deoxycholic acid, lithocholic acid, and so on. Secondary cholic acid can directly interfere with DNA metabolism, change DNA synthesis, increase the activity of ornithine decarboxylase in colorectal mucosa cells, reduce immune function, and thereby promote the proliferation of cancer cells. After eight years' study, Tiemersma et al¹⁴¹ showed that the increase in lean meat intake by men was positively correlated with the incidence of CRC, while the intake of poultry and fish by women could reduce the CRC incidence. An animal experiment in Europe showed that a high-fat diet can induce mutations in the K-ras gene, dysregulate intestinal flora, and promote the development of gastrointestinal tumors.¹⁴² While some studies have shown that high-protein diets can produce some potential carcinogenic effects, there is insufficient evidence to prove the relationship between protein diet and CRC, and further proof is needed.

Dietary Fiber

The protective effect of dietary fiber is due to the fact that cellulose can selectively promote the growth of intestinal

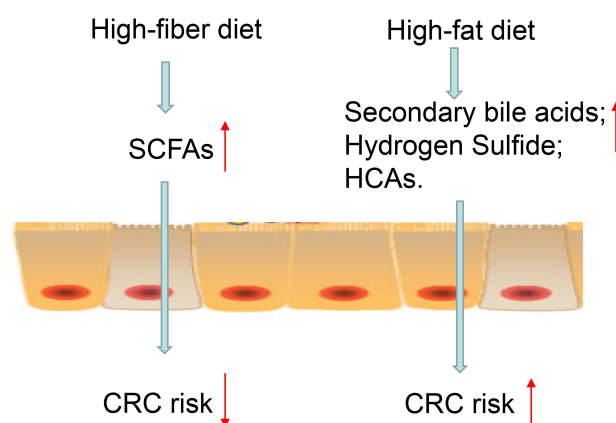


Figure 6 Relationship between diet, gut microbes, and colorectal cancer (CRC). The figure shows a series of interactions between diet and CRC. High fiber diet is fermented into short-chain fatty acids (SCFAs) by bacteria in the colon. SCFAs are the key metabolites linking gut microbes and a significantly reduced risk of CRC. High-fat diets and red meat are metabolized by the gut microbiota into metabolites such as secondary bile acids, heterocyclic amines (HCAs), and hydrogen sulfide, increasing the risk of CRC.

flora and increase the intestinal motility, increase stool volume, reduce the contact time between stool and carcinogens and intestinal mucosa and dilute the concentration of intestinal carcinogens.¹⁴³ For example, YEH et al¹⁴⁴ found that the intake of fresh vegetables and fruits was negatively correlated with the risk of CRC through a case-control study. Huang Xiuhai et al¹⁴⁵ also found that the intake of high fiber, fresh fruits, and vegetables and low saturated fatty acids can reduce the risk of gastrointestinal tumors. Moreover, Bingham et al¹⁴⁶ found that dietary fiber intake was negatively correlated with the incidence of CRC through 519,978 case-control studies in 10 European countries. It was not related to the source of dietary fiber and had the greatest protective effect on the left colon. Therefore, the American Cancer Institute of the World Cancer Research Foundation has shown that increasing daily intake of dietary fiber by 10 g can effectively reduce the incidence of CRC by about 10%.¹⁴⁷

Trace Elements and Vitamins

A large number of studies have shown that intake of appropriate amounts of vitamins and other nutrients can effectively reduce the incidence of CRC. Important factors for preventing CRC are dietary calcium, folic acid, selenium, and vitamins A, C, D, E, β -carotene, and other micronutrients. Among them, studies have found that long-term use of high-calcium preparations can reduce the risk of distant colon cancer.¹⁴⁸ Calcium ions and vitamin D can combine with cholic acid and fatty acids to form an insoluble calcium soap, which can inhibit the growth of intestinal epithelial cells, improve the final differentiation of cells, and play a chemical protective role in intestinal epithelium. In recent years, the observed protective effect of selenium on colon cancer has received increasing attention. Selenomethionine can inhibit tumor cell proliferation by activating the p53 tumor suppressor protein. Studies have also found that the reduction in the risk of colon cancer was related to long-term consumption of foods rich in folic acid.¹⁴⁹

Intestinal Flora and Clinical Application in CRC

Immunotherapy can suppress tumor growth by suppressing cell cycle checkpoints, which has a therapeutic effect. However, it is not effective in all populations. Increasing evidence shows that the efficacy of immunotherapy depends on the steady state of the intestinal microbiota, and changes

in the intestinal microbiota will have a certain effect on the efficacy of tumor chemotherapy.^{150,151} Certain intestinal microorganisms such as *Bifidobacteria* can enhance the activity of dendritic cells, thereby increasing the responsiveness of T cells to checkpoint blockade.¹⁵² Currently, anti-CTLA-4 is a commonly used immunotherapy. Sterile mice have no sensitivity to ipilimumab, indicating that the efficacy of ipilimumab, especially the activation of CD4 + T cells, is inseparable from the participation of intestinal microbiota. Further research found that anti-CTLA-4 therapy can change the composition of the gut microbiota in the tumor microenvironment. In mice and patients, the abundance of *Bacteroides fragilis* increases, which is conducive to killing cancer cells. Fecal transplantation in sterile mice can restore their drug sensitivity. This result gives us hope to control the efficacy of drugs by providing probiotics.¹⁵³ Understanding microbial-dependent inflammation and immune mechanisms will open up new ways for cancer prevention and improved cancer treatment strategies. Anti-PD-1 immunochemical drugs are currently effective means of treating malignant tumors. There is a significant difference in the sensitivity among different patients to this chemotherapy drug, which may be due to the difference in intestinal microbiota. The tumor growth in mice transplanted with the feces of drug-sensitive patients was slower than that in the control group, and after anti-PD-1 drug treatment, the tumor volume in sensitive mice shrank and the efficacy was better. Matson et al¹⁵⁴ found that the abundance of *Bifidobacterium longum*, *Corinella aerogenes* and *Enterococcus faecium* in the feces of patients who were sensitive to chemotherapy drugs was higher. Routy et al¹⁵⁵ identified *Akkermansia muciniphila* (AKK) as a beneficial bacterium with antitumor effects and found that it was highly present in drug-sensitive patients. Gopalakrishnan et al¹⁵⁶ identified *Faecalibacterium* as a beneficial bacterium that can play a positive role in anti-PD-1 immunotherapy. On the contrary, *Bacteroides* is a harmful bacterium that inhibits the efficacy of anti-PD-1 immunotherapy. The CD8 + T cell activity was higher in the tumor environment of drug-sensitive patients, which suggests that specific intestinal bacteria may enhance the ability of T cells to enter the tumor microenvironment and kill the cancer cells, thereby making the body durable and result in a strong immunotherapy response.

There is a very close relationship between the occurrence and development of malignant tumors and the imbalance in the intestinal flora.¹⁵⁷ Studies have found that the concentration of bacteria in tumor tissues was much higher than that in normal tissues. The hypoxic tumor

microenvironment provides good conditions for the growth of anaerobic bacteria. At the same time, abnormal blood vessels and high pressure in the tissue gap restrict immune components (granulocytes, antibodies, serum complement, etc.) to enter the bloodstream, protecting bacteria from the body's immunity. We can see that special microenvironment composition of the tumor tissue will lead to the growth of a large number of bacteria, and excessively proliferating bacteria can compete with tumor cells for nutrition to inhibit tumor growth, and some special metabolites of bacteria can directly inhibit tumors.¹⁵⁸ Cell proliferation and induction of tumor cell apoptosis may be achieved through regulation of the intestinal flora to reach the purpose of treating malignant tumors. Colley's research confirms this point of view. Colley¹⁵⁹ found that lumps of tumors in patients with sarcoma infected with acute streptococcus have shrunk; thus, starting the history of using bacteria or bacterial extracts to treat tumors. With improvement in living standards, obesity triggers intestinal flora imbalance, which, in turn, induces a high incidence of colon cancer. Currently, prevention and treatment methods are urgently needed. Therefore, based on the regulation of intestinal flora, exploration of colon cancer treatment methods and mechanisms of action are the main issues that our research group is concerned about. The hope is that through this exploration and research, a certain theoretical reference for the development of drugs based on the intestinal flora can be found, leading to the treatment of colon cancer.

In addition, one emerging translational application of the gut microbiota is its use as a biomarker to indicate the presence of a disease. Nowadays, several studies have reported associations between bacterial markers and treatment efficacies or clinical outcomes. Analysis of the gut microbiota serves as a rich source of potential biomarkers. A higher level of *F. nucleatum* was found in fecal samples in patients with colorectal adenomas than in healthy individuals.¹⁶⁰ Furthermore, the robust association of CRC with *S. gallolyticus* resulted in the development of a positive test which was used for CRC diagnosis for up to 10 years.¹⁶¹ Moreover, Wang et al found that serum antibodies against *F. nucleatum* might also serve as a potential biomarker for detecting CRC. Other studies also found the metabolites of intestinal flora, including SCFAs, bile acids, and butyrate,¹⁶¹ were higher in patients with CRC than in healthy controls.¹⁶³

Current Limitations and Future Directions

With the development of genomics technology, in recent years, intestinal microbiota research on CRC has made rapid progress. However, there are still a series of problems. There are individual differences in the intestinal microbiota. Furthermore, a small sample size in a single experiment makes it difficult to obtain a unified and convincing conclusion. Therefore, it is necessary to establish a multicenter, large sample dataset. The experimental process of similar research lacks standardization. At this moment, it is not clear whether the fecal sampling can completely represent gut microbiota status, and whether there are differences among the different detection platforms. Therefore, the unified collection process and detection methods should be built. Non-cultivated metagenomic big data analysis can only help us understand the state of microbes in the gut from the genetic level. Combining with culture-based culture omics related research will be able to provide a more realistic analysis of the gut microbial composition. We hope to learn more about the gut microbiota to help the clinical detection and prognosis. In addition to routine testing, we hope that microbial markers can be used to detect abnormal states in the early stages of cancer. In addition, we can also use adjuvant probiotic treatment during chemotherapy to achieve better results. As expected, research on the gut microbiota is still in development, and more experimental research is needed. This review summarizes the relationship between intestinal flora and its metabolites and colorectal cancer. It can provide potential microbial targets for screening and diagnosis of colorectal cancer. Moreover, it can provide further research direction for microbial agents as adjuvant therapy for colorectal cancer.

Conclusion

CRC is a disease characterized by complicated causes and pathogenesis. The intestinal microecology is closely related to the occurrence of CRC. Intestinal flora, especially some special bacteria, affect the development of CRC through metabolic and structural changes, which provides research data that can be used to establish prevention strategies of CRC and identify its pathogenic mechanism. We have discussed the important impact of the inflammation of intestinal flora on the development of CRC. The studies that are discussed in this review

highlight that progression to CRC is influenced not only by the presence of intestinal flora but also by the metabolic output of the entire microbiota. In the progress of CRC development, the function of metabolites still needs to be further studied. It is expected to provide new ideas and clues. Diet is the most important factor to determine the dynamic changes of intestinal flora composition and function. The intake of dietary fiber and supplementation with beneficial bacteria may maintain intestinal health, which can be very promising for preventing CRC. Moreover, these data have provided an unprecedented opportunity to move microbiota discoveries toward clinical applications. In the future, targeted removal of early-stage carcinogenic members of the gut microbial community, might be a desirable approach to reducing risk factors for CRC.

Methods

We mainly searched for literature paper in PubMed database by using both medical subject heading (MeSH) terminology and relevant keywords. We summarized the correlation between CRC and microorganisms and their metabolites. There are some limitations in the paper, it is inevitable that some literature will be omitted in the process of literature screening. In the process of summarizing relevant literature again, it is possible to misinterpret the author's original intention.

Author Contributions

All authors participated in the conception and design of the study; wrote the manuscript; designed and draw figures; reviewed and sorted out the literature; read and approved the paper; agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that no potential conflicts of interest exist.

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