Potential Ability of Probiotics in the Prevention and Treatment of Colorectal Cancer

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ABSTRACT: Colorectal cancer (CRC) is the third most common cancer in the world, and its incidence rate and mortality are on the rise in many countries. In recent years, with the improvement of economic conditions, people's living habits have changed, including lack of physical activity, poor diet patterns and circadian rhythm disorder. These risk factors can change the colon environment and the composition of intestinal microbiota. This state is called intestinal imbalance, which increases the risk of cancer. Probiotics, a class of microorganisms that help maintain gut microbial homeostasis and alleviate dysbiosis, may help prevent inflammation and colorectal cancer. These probiotics inhibit or ameliorate the effects of dysbiosis through the production of short-chain fatty acids (SCFAs), modulation of immunity, maintenance of the intestinal epithelial barrier, pro-apoptotic mechanisms, and other mechanisms. This review aims to explain the interaction between probiotics, the gut microenvironment and the gut microbiota, and summarize reports on the possibility of probiotics in the prevention and treatment of colorectal cancer.

KEYWORDS: Colorectal cancer, microbiome, probiotics, gut barrier, immunotherapy

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Introduction

Colorectal cancer (CRC) is the third most common type of cancer in the world, and the second-leading cause of cancer-related deaths. In 2020, there were approximately 1 900 000 new cases of CRC and 935 000 deaths attributed to the disease.[1] In China, lung cancer is still the most common cancer, followed by CRC and gastric cancer. The incidence rate of CRC is increasing year by year, which has caused a huge burden in the incidence rate and mortality of cancer. In 2020, gastrointestinal cancer accounted for 45% of all cancer deaths in China.[2] Colon cancer incidence and mortality have increased and this increase may be related to changes in lifestyle and dietary habits, including reduced physical activity, Increased intake of animal-derived foods and smoking.[3] The etiology of CRC is complex, involving genetic and environmental factors. CRC is divided into sporadic and hereditary, of which sporadic CRC accounts for the vast majority. The increase in the incidence rate of sporadic CRC is associated with long-term inflammatory bowel disease (IBD) and multiple lifestyle.[4] Most sporadic CRCs develop following a specific mutational sequence, the so-called adenoma-carcinoma sequence, in which polypoid adenomas progress to high-grade dysplastic adenomas, eventually leading to the formation of a malignant form.[5]

The human gut microbiota is composed of approximately 10^{13} to 10^{14} microorganisms, including bacteria, fungi, viruses, etc. The bacterial microbiome of the human gut is dominated by *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucobacteria*, with *Firmicutes* and *Bacteroides* accounting for 90% of the gut microbiota.[6-8] The gut microbiota plays an important role in the health of the host, which normally maintains a symbiotic relationship with the microbes in the gut.[9] Compared with healthy people, patients with CRC have reduced bacterial diversity and richness. In recent years, increasing evidence has demonstrated a causal relationship between gut microbial dysbiosis and CRC pathogenesis.[10] A healthy gut environment is governed by a complex balance of gut microbiota, its metabolites, and the host immune system, and imbalances in gut microbiota can contribute to disease states such as IBD.[11] Alterations in the gut microbiota can disrupt the gut epithelial barrier and promote inflammation, for example the gut ecology of patients with IBD is significantly less diverse than that of healthy individuals.[12,13] Imbalances in the gut microbiota promote colorectal carcinogenesis through multiple mechanisms, including inflammation, immune modulation, carcinogen activation, genotoxicity, and damage to host DNA.[14] The occurrence of CRC is closely related to intestinal dysbiosis and changes in the composition of the microbiota on the surface of the tumor intestinal mucosa and in adjacent tissues of the tumor. Inflammatory state driven by the gut microbiota is considered to be critical for the development and

progression of CRC. *Fusobacterium nucleatum*, anaerobic digestive *Streptococcus*, and enterotoxigenic *Bacteroides* fragilis, has been identified by inducing tumor proliferation, promoting inflammation, causing DNA damage, and protecting tumors from immunity attack to promote the development of CRC.[10] In addition, the gut microbiota modulates the tumor microenvironment by producing metabolites and even further affects the efficacy of tumor immunotherapy.

CRC remains a common cancer worldwide, and various studies have highlighted the role of the gut microbiota in promoting or inhibiting the development of CRC through modulation of immune responses, genetic damage, and apoptosis, and probiotic therapy. As an emerging field, probiotic therapy has great potential in the prevention and treatment of CRC. As a beneficial intestinal microorganism, probiotics have received more and more attention in the prevention and inhibition of CRC in recent years, and their mechanism of action has been gradually explored. Probiotics and their metabolites also offer new opportunities for the treatment of CRC. This article will explore the mechanism by which probiotics play a preventive and inhibitory role in the pathogenesis of CRC, and discuss the possibility of clinical application of probiotics.

Mechanism of Probiotics in Preventing and Inhibiting CRC

Currently, the standard treatment for CRC has been surgery, chemotherapy, and radiation therapy, which can be used in combination to treat patients. Current chemotherapy drugs can negatively impact quality of life or lead to drug resistance. While they are effective at killing cancer cells, they can also harm healthy cells.[15] According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics are defined as "living microorganisms that, when administered in sufficient doses, confer a health benefit to the host.."[16] Many clinical studies have demonstrated the therapeutic effect of probiotics on diseases such as obesity, insulin resistance syndrome, type 2 diabetes, and non-alcoholic fatty liver disease.[17,18] The role of probiotics in preventing and treating gastrointestinal diseases has gained increasing attention due to the rapid and in-depth development of related research. The research on probiotics has made remarkable progress, and a large number of studies have confirmed the beneficial effects of probiotics on gastrointestinal diseases (such as irritable bowel syndrome [IBS], gastrointestinal diseases, eradication of *Helicobacter pylori*, IBD). A large amount of evidence shows that probiotics have anti-CRC effects in *in vivo* or *in vitro* studies.[19] As an emerging CRC treatment modality, in addition to directly modulating and improving the host gut microbiota, probiotics have potential beneficial effects in cancer prevention and treatment. It mainly uses probiotics to reverse a series of inflammatory changes and abnormal proliferation caused by intestinal flora imbalance, which may lead to CRC lesions or play an antitumor effect in the intestinal tract of CRC patients. Their mechanisms of action include improvement of gut barrier function, immunomodulation, and gut modulation of microbiota composition, inhibiting colonization of pathogenic bacteria, anti-inflammatory and anti-pathogenic activity, production of anticancer compounds, degradation of carcinogenic compounds in the gut milieu, and induction of pro-apoptosis in cancer cells death and antiproliferative effects (see Figure 1).[20-22] Most probiotics belong to the natural intestinal flora. Human probiotics mainly include *Lactic Acid Bacteria*, *Bifidobacteria*, *Lactococcus*, *Streptococcus*, and *Enterococcus*. In addition, Gram-positive bacterial strains belonging to the genus *Bacillus* and some strains of yeast belonging to the genus *Saccharomyces* are commonly used in probiotic products.[18,23]

Probiotics and their metabolites enhance the function of intestinal barrier

The important role of intestinal barrier in protecting intestinal ecology and its preventive and protective effect on CRC. In the intestines of healthy people, the barrier function protects the gut from toxins, pathogens, and other damage. Gastrointestinal mucosa is a multilayer surface that interacts with the outside world. It is the largest interface between the human body and the external environment. The first line of defense in the gut barrier is the external mucus barrier, where the commensal microbiota and defense proteins (AMP and sIgA) reside.[24] Antimicrobial peptides (AMPs) and proteins secreted by epithelial cells enhance the barrier function of epithelial cells through immune exclusion and killing of bacteria.[25] The middle layer consists of intestinal epithelial cells (IECs) connected together by tight junctions (TJs), adherent junctions (AJs), and desmosomes.[26] As an important part of this barrier, the epithelial cells block the intestinal lumen from the deep colon tissue, thereby avoiding the invasion or colonization of the colon tissue by some pathogenic bacteria, and protecting the human intestine from direct contact with microorganisms, and selectively allow the absorption of water, electrolytes, and nutrients. The epithelium is composed of several different cell types, such as enterocytes, Paneth cells and goblet cells. Some IECs with special functions can promote host immunity by secreting antibacterial products such as cytokines and defensins. Paneth cells exert antibacterial and microbiota regulatory roles by secreting lysozyme, defensins, and other immunomodulatory proteins.[24] Goblet cells secrete mucus proteins that lubricate and protect the epithelial intestinal surface and act as antigen-presenting cells to deliver luminal antigens to dendritic cells (DCs), promoting the development of regulatory T cells (Tregs).Underneath the epithelial cells, the lamina propria containing innate and adaptive immune cells acts as the last line of defense for the intestinal barrier to protect the human body.[27,28]

The gut microbiota of normal people can maintain the microbial homeostasis in the gut and play a certain role in preventing the occurrence of some diseases. However, in some diseases, dysbiosis of intestinal flora leads to damage of intestinal

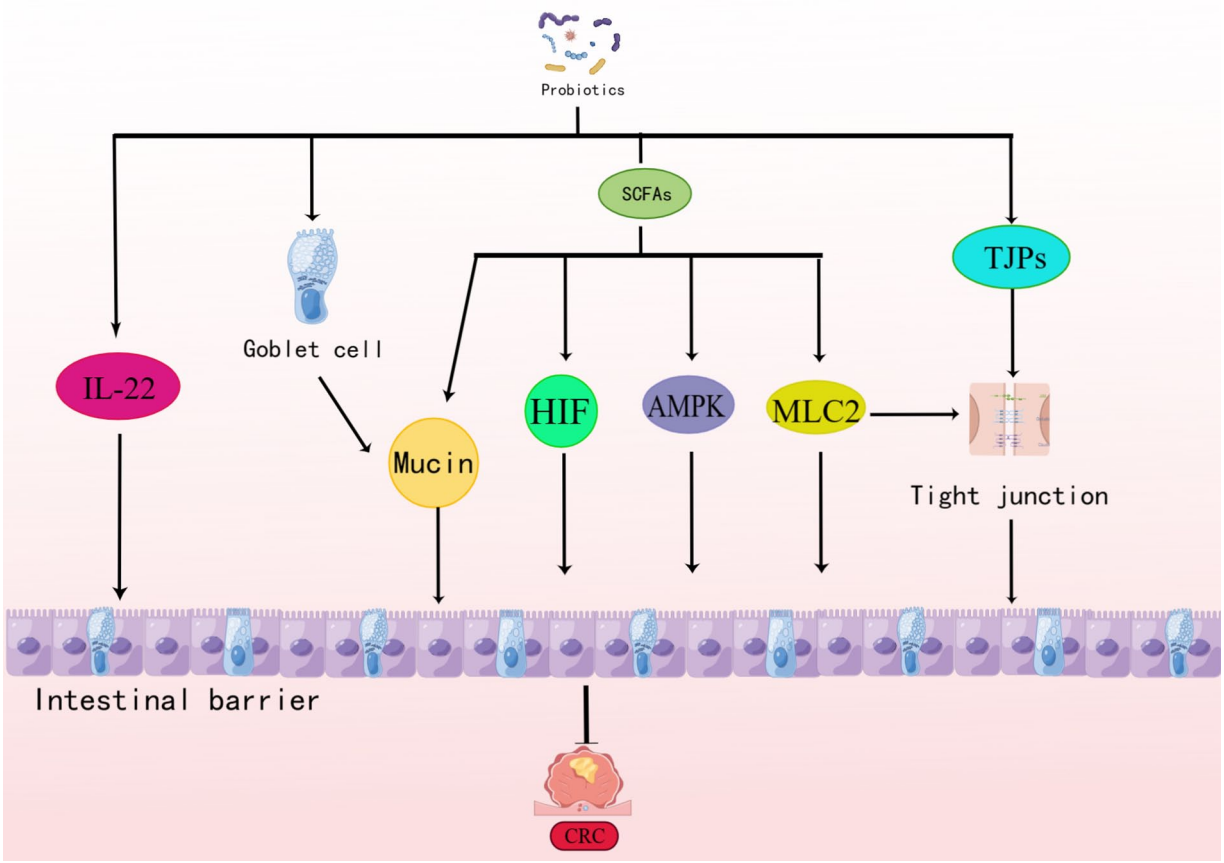

Figure 1. Mechanism of probiotics in preventing and inhibiting colorectal cancer. Probiotics and their metabolites enhance the function of intestinal barrier: Probiotics regulate mucin secretion by goblet cells to promote epithelial recovery and intestinal barrier enhancement. Some probiotics and their metabolites SCFAs strengthen the epithelial barrier and promote epithelial recovery by enhancing the expression of tight junction protein. Probiotics protect IL-1β-induced nuclear factor kappa B (NF-κB) activation in Caco-2 cells, thereby protecting intestinal permeability. In addition, short-chain fatty acids metabolized by probiotics can lead to local tissue oxygen consumption, maintain hypoxia inducible factors by reducing O2 concentration, and thus improve the function of epithelial barrier.
AMPK indicates activated protein kinase; CRC, colorectal cancer; HIF, hypoxia inducible factor; IL, interleukin; MLC2, myosin II-regulated light chain; SCFA, short-chain fatty acids; TJP, tight junction protein.

barrier resulting in intestinal diseases. Once the intestinal epithelial barrier is damaged, the intestinal wall mucosa is damaged and the permeability is increased, microorganisms and pathogens can invade the digestive system, causing direct contact between colon tissue and pathogens, thus causing chronic intestinal inflammation, which is the main cause of CRC.[29] Disruption of intestinal mucosal integrity and barrier dysfunction leads to increased allergen permeability leading to immune stress and inflammation.[30] The increase in gastrointestinal mucosal permeability and damage to gastrointestinal epithelial integrity are believed to play a role in the pathophysiology of various gastrointestinal diseases, including intestinal pathogen challenge, IBD, IBS, and gastrointestinal cancer. Research has shown that biological disorders and intestinal barrier damage are associated with the development of many chronic inflammation and systemic diseases, which may ultimately lead to cancer.[31] In mice lacking MUC2 mucin, the colon is invaded by bacteria in direct contact with the epithelium and penetrates down into the crypts and epithelium, eliciting a colonic immune system response characterized by inflammation and an increased risk of spontaneous colitis.[25,32] Dysbiosis of the microbiota is closely related to the disruption of the intestinal barrier and the occurrence of IBD. IBD patients had an average 25% reduction in microbial richness compared to healthy individuals. Disruption of the epithelial barrier and the combination of invasive pathological organisms lead to massive bacterial translocation, causing luminal bacteria that are not strain-specific, can freely flow through the paracellular space to the underlying lamina propria, and cause mucosal inflammation, resulting in morphological damage and clinical features of chronic inflammation. Other evidence also suggests that chronic inflammation may affect the gut microbiota and lead to loss of the epithelial death-dependent barrier, ultimately leading to a vicious cycle of uncontrolled colitis that induces colorectal carcinogenesis.[31] The enrichment of diverse bacteria in the gut contributes to colorectal carcinogenesis by inducing tumor proliferation, promoting inflammation, causing DNA damage, and protecting tumors from immune attack. In contrast, some bacteria, mainly probiotics, are depleted in CRC patients. Reduced microbiota diversity enables opportunistic

pathogenic organisms to infiltrate the gut via transcellular and paracellular pathways.[33] Thus, intestinal barrier dysfunction or increased TJ permeability is a common feature in the gut of patients with colon cancer.[10] The role of intestinal flora is crucial in maintaining physical barrier function and preventing disease progression, so maintaining the balance of intestinal flora is very important for preventing and improving gastrointestinal diseases. In recent years, studies on probiotics have found that they can regulate and maintain barrier integrity and balance of intestinal flora by reducing intestinal permeability and strengthening TJ, which has a potential effect on inhibiting the occurrence and development of CRC.

Protection and enhancement of probiotics on intestinal barrier. Probiotics prevent the occurrence and development of gastrointestinal tumors by enhancing intestinal barrier function. Probiotics promote the recovery of epithelial cells and enhance the barrier by regulating the secretion of mucin and the expression of TJ proteins (claudin-1, occludin, ZO-1, and ZO-2).[10] Alvarez et al demonstrated that probiotic *Escherichia coli Nissle 1917* (*EcN*) can enhance the expression of interleukin-22 (IL-22) to enhance the epithelial barrier, in addition to upregulating ZO-1 and claudin-14 and downregulating Leaky protein and claudin-2 through outer membrane vesicles.[34] *Bifidobacterium infantis* and *Lactobacillus acidophilus* regulate occludin and claudin-1 protein expression, protect IL-1β-induced nuclear factor kappa B (NF-κB) activation in Caco-2 cells, thereby protecting intestinal permeability. Besides, *Akkermansia muciniphila* enhances the integrity of IECs and the thickness of the mucus layer. If the content of *A. muciniphila* in the intestine is too low, the mucosa will become thinner, resulting in weakened intestinal barrier function.[35]

Short-chain fatty acids (SCFAs) are considered as the most important products in probiotic metabolism, with acetic acid, propionic acid, and butyric acid accounting for the vast majority of colon SCFAs.[34] SCFAs play an important role in intestinal homeostasis due to their anticancer, lipid metabolism, anti-inflammation and regulation of TJ proteins with protective effects on epithelial barrier integrity. Butyrate can suppress inflammatory responses and has growth-inhibitory and pro-apoptotic effects on cancer cells. Butyrate also induces apoptosis in colon cancer cell lines and prevents their growth by increasing p57 expression.[36]

Studies have shown that SCFAs enhance intestinal epithelial TJs and form a strong and healthy barrier by activating 5′-adenosine monophosphate activated protein kinase. The intestinal mucus layer contains a variety of mucins, of which epithelial mucin 2 (MUC2) plays a major role in both healthy and inflamed gut.[34] Loss of MUC2 leads to intestinal inflammation and increases intestinal barrier permeability.[33] SCFAs can increase the expression of not only MUC2 and other mucins such as MUC1, MUC3, and MUC4. Mucins form protective mucus gels through O-glycosylated tandem repeats that protect the human gut from microbes and inflammation. Butyrate can enhance MUC1 gene expression, increase mucus secretion and mucosal thickness, and repair mucosal damage by increasing cell migration to the site of injury. They modulate TJs by inducing increased expression of claudin-1 and ZO-1 and decreased expression of osmotic-promoting claudin-2, thereby affecting the gut Road barrier.[37] Sodium butyrate can enhance the intestinal epithelial barrier by regulating the activation of AMP-activated protein kinase (AMPK) and the phosphorylation of myosin II-regulated light chain (MLC2), thereby promoting TJ reorganization.[24]

Another important factor for SCFAs in maintaining the epithelial barrier is hypoxia-inducible factor. Disruption of hypoxia-inducible factor in intestinal epithelium leads to increased susceptibility to colitis. SCFAs can enhance barrier function through G protein-coupled receptor (GPCR)-mediated sensitization of IEC inflammasome and reduce oxygen concentration in IEC to induce hypoxia-inducible factor. Addition of butyrate and other SCFAs to cultured epithelial cells results in increased local tissue oxygen consumption, which helps maintain hypoxia-inducible factors by reducing O_2 concentrations, thereby improving the function of the epithelial barrier.[38,39]

By improving the connection and function of colorectal epithelial cells, restoring and maintaining the intestinal epithelial barrier, it can effectively prevent most pathogenic microorganisms from invading the deep intestinal tissue, and at the same time play an effective role in the occurrence of CRC by inhibiting intestinal inflammation.

Immunomodulatory effect of probiotics

Probiotics play a role in colorectal prevention and treatment through immunomodulation (see Figure 2). Probiotics can have pronounced immunomodulatory effects to reduce colonic inflammation or enhance antitumor immunity, depending on the selected species and strain. In terms of system regulation, probiotics can enhance the body's immune response and promote CRC cell apoptosis. In CRC patients, pro-inflammatory cytokine IL-1 β, IL-6, IL-8, IL-17, IL-12, and tumor necrosis factor-α (TNF- α) participated in the occurrence and development of cancer, while anti-inflammatory cytokines IL-10 and transforming growth factor- β (TGF-β) show inhibitory effects on cancer. Probiotics modulate innate and adaptive immunity, including promoting the development and maturation of the immune system, enhancing the viability of macrophages and natural killer (NK) cells, stimulating sIgA, and activating the production of TLR and related immune responses mediated by NLRs, regulate Th1/Th2 immune responses, and increase secretion of leukocytes IL-10 and TGF-β regulatory Treg numbers and enhance their function as well as reduce allergen-specific IgE levels. Probiotics affect the immune system in the gut mucosa, stimulating antibody production by activating toll-like receptors (TLRs) and T cofactor 1 differentiation. Dysregulation of the immune system reduces

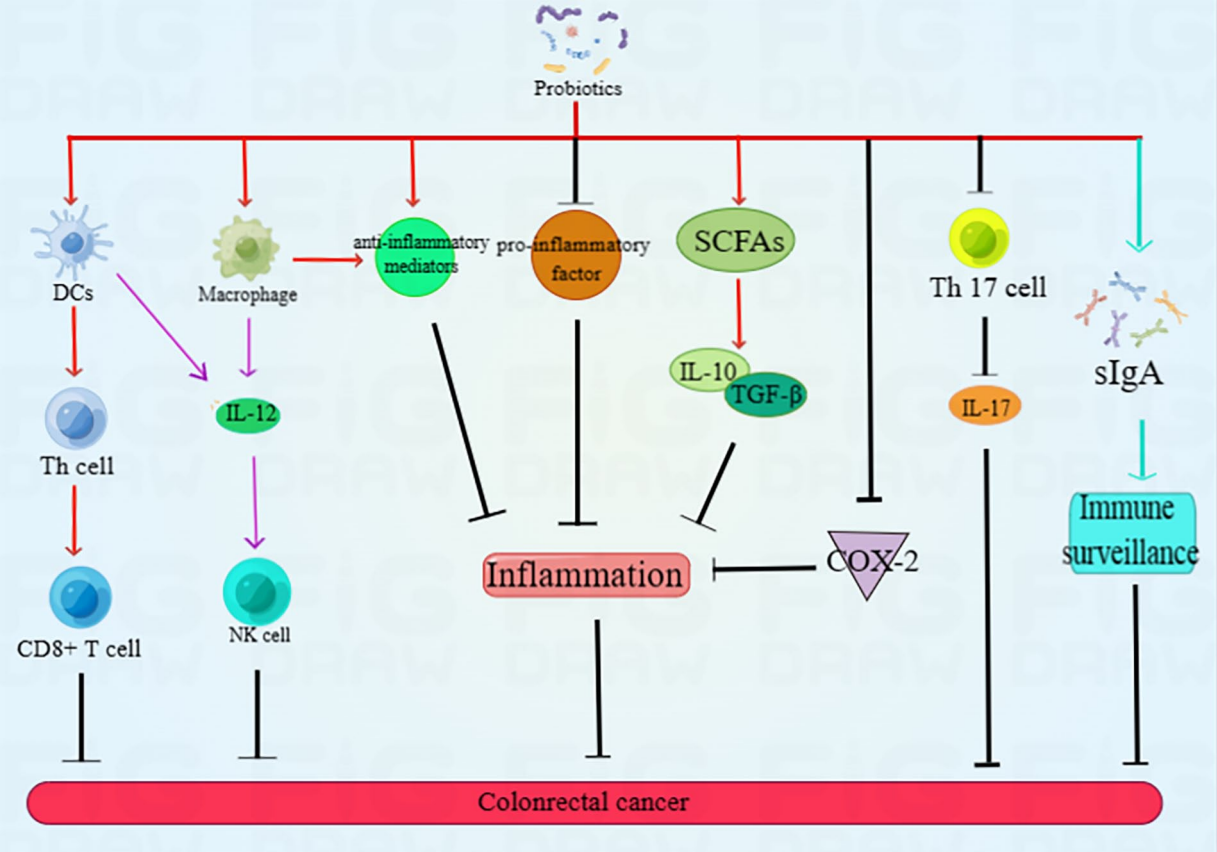

Figure 2. Immunomodulatory mechanism of probiotics in colorectal cancer. Probiotics regulate innate and adaptive immunity and promote the development and maturity of the immune system. Probiotics can enhance the activity of macrophages and NK cells and induce production of IL-12 to activate NK cells. Probiotics stimulate the production of IgA to strengthen and maintain the immune monitoring of isolated intestinal mucosa, and inhibit the production of IL-17 by reducing the number of Th17 cells to inhibit the activation of inflammation to prevent the occurrence of CRC in the early stage. Probiotics can promote the production of anti-inflammatory substances while inhibiting the production of pro-inflammatory substances. In addition, probiotics reduce the expression of COX-2, inhibit its stimulating cell proliferation and pro-inflammatory effects to reduce the risk of inflammation related CRC.
AMPK indicates activated protein kinase; COX-2, cyclooxygenase-2; DCs, dendritic cells; IgA, immunoglobulin A; IL, interleukin; NK, natural killer; SCFA, short-chain fatty acids; TGF β, transforming growth factor-β.

the number of Th17 and affects the balance of Th17/Treg. *Lactobacillus* and *L. acidophilus* can reduce the production of IL-8 and the expression of pro-inflammatory mediators, and increase the expression of TLR2. This anti-inflammatory effect is achieved through regulation of TLR2-mediated nuclear factor kappa-light chain enhancer (NF-κB) in activated B cells and mitogen-activated protein kinase (MAPK) signaling in inflammatory IECs.[40] Shi et al demonstrated that a specific commensal strain of *A. muciniphila* can enhance the efficacy of IL-2-based immunotherapy, and that combined IL-2 and *A. muciniphila* therapy can result in better tumor control while recruiting more tumor-specific CTLs in the tumor microenvironment and reduced immunosuppressive Tregs. The antitumor immune response induced by *A. muciniphila* may be attributed to its outer membrane protein Amuc through activation of TLR2 signaling, resulting in efficient tumor regression. Therefore, reinstatement of IL-2-based immunotherapy by precisely manipulating the gut microbiota of cancer patients becomes a potential therapeutic approach.[41] Lenoir et al found that *Lactobacillus casei BL23* can reduce the occurrence of CRC by reducing the number of Th17T cells. Th-17 is a type of accessory cell that secretes the cytokine IL-17 and plays an extremely important role in inflammatory activation, and it is a marker of early CRC. This suggests that *Lactobacillus* can prevent CRC by inhibiting Th17 production of IL-17.[42]

An important immunomodulatory pathway is increased production of immunoglobulin A (IgA). On one hand, this immunoglobulin acts on the intestinal barrier, limiting the contact of potentially carcinogenic compounds present in the intestinal lumen with colon cells due to its resistance to proteolysis. On the other hand, due to the inability to activate complementary systems and pro-inflammatory responses, IgA can create an anti-inflammatory environment. Probiotics can stimulate IgA production, induce IgA circulation, strengthen and maintain the immune surveillance of isolated intestinal mucosa, and promote the maturation of humoral immune mechanism.[43]

Probiotics promote phagocytosis, NK activity, and induce T cell apoptosis, thus stimulating the production of anti-inflammatory cytokines and reducing the production of pro-inflammatory

cytokines.[44] Probiotics can increase the number of macrophages and DCs in the lamina propria, enhance the ability to phagocytize cell debris and pathogens, activate immune cells such as lymphocytes to fight pathogens, and fix free cells. For example, probiotic *Lactobacillus* strains activate macrophages through the synthesis of pro-inflammatory mediators, including cytokines, reactive oxygen species (ROS), and participate in signal cascades such as nuclear factors κ *in vitro* inflammatory response of B (NF-κB) and TLR2 pathway cells.[45] NK cells are crucial for controlling immunity against cancer and infection, and the higher the activity of NK cells, the lower the risk of developing cancer. Probiotics enhance NK cell activity by inducing monocytes/macrophages to produce IL-12 to activate NK cells.[34] *Lactobacillus casei Shirota* can promote DCs to release IL-12 by enhancing the killing effect of NK cell, thereby inhibiting the occurrence of CRC. Some probiotics affect the immune response by activating phagocytes and help maintain a state of vigilance, which can eliminate cancer cells in their early stages of development.[46] Lactic acid bacteria have been proved to be able to induce not only the maturation of DC in the intestine, but also the subsequent Treg activation, and the cytotoxicity and cytokine secretion of NK cell in the cells of peripheral blood Monocyte. The expression of PD-L1 on the surface of tumor cells is abnormally upregulated, and excessive activation of PD-1/PD-L1 signaling induces T-cell exhaustion, causing immune escape of tumor cells, leading to tumor development and patient condition deterioration. In the context of immunotherapy, some bacterial species, such as Bifidobacterium, have been shown to promote antitumor immunity and correlate with the efficacy of programmed death ligand-1 (PD-L1) checkpoint blockade and reactivate exhausted T cells in the tumor immune microenvironment by inhibiting PD-1/PD-L1 signaling, restore their killing function against tumor cells, and improve antitumor immune activity.[47]

In addition, SCFAs act by binding to specific receptors on IECs, through which the NF-κB pathway, Treg cell suppression, and production of pro-inflammatory cytokines by neutrophils and macrophages are inhibited.[48] After metabolites are recognized by receptors such as TLRs and NLRs on immune cells and epithelial cells, immune cells and epithelial cells begin to secrete cytokines, helping to regulate innate and adaptive immune responses. Since probiotics can increase the production of anti-inflammatory cytokines and reduce the production of pro-inflammatory cytokines, the occurrence of colorectal inflammation is inhibited, and the incidence of CRC is thus reduced.

Effect of probiotics on resistance to pathogen colonization

There are many mechanisms by which probiotics can inhibit the colonization of pathogenic microorganisms in the intestine, such as competition for nutrients and ecological niches, changes in pH, and production of antibacterial substances.[49] Beneficial bacteria in the gut inhibit the penetration and growth of pathogenic bacteria by competing for space and resources.[43] Ingestion of specific probiotic strains can reduce pathogen colonization, John et al showed that non-virulent *Clostridium difficile* (*NTCD*) competes for a similar niche with toxigenic *C. ecological* niche and reduce its colonization, suggesting that probiotic treatment is a promising avenue.[10,50] Experiments by Fang et al showed that the probiotic *Escherichia coli nisle 1917* (*EcN*) can secrete DegP (a bifunctional periplasmic protein) to inhibit enterohaemorrhagic *Escherichia coli* (*EHEC*), allowing *EcN* to overcome pathogenic biofilms through extracellular DegP activity thereby inhibiting pathogenic organisms.[51] Probiotics can secrete antibacterial substances and directly exert antibacterial activity to inhibit the growth of pathogens. The study found that *Lactobacillus reuteri (L. reuteri)* not only exhibited the ability to inhibit the colonization of *H. pylori* on the human gastric mucosa, but also produced reutericin to effectively inhibit *H. pylori*.[52] Probiotics can promote goblet cells to produce mucus proteins and indirectly inhibit pathogen adhesion.[53] Probiotics indirectly inhibit the growth of pathogens by producing metabolites such as lactic acid and acetic acid to reduce intestinal pH.[54] SCFAs as organic acids have the potential to acidify the environment thereby providing a protective barrier against colonization by pathogenic microorganisms.[44] By excluding the invasion of pathogenic microorganisms, the intake of probiotics can help reduce the risk of intestinal infection and the resulting chronic inflammation, thereby potentially preventing the development of CRC and reducing complications in patients with CRC.

Lactobacillus and *L. acidophilus* can reduce the production of IL-8 and the expression of pro-inflammatory mediators, and increase the expression of TLR2. This anti-inflammatory effect is achieved through regulation of TLR2-mediated nuclear factor kappa-light chain enhancer (NF-κB) in activated B cells and MAPK signaling in inflammatory IECs.[40]

Anti-inflammatory properties of probiotics

A major cause of CRC is colitis-associated cancer (CAC). In IBD patients, CAC develops from long-term colitis, and over time, the increased risk of CAC is clearly related to the increased burden of inflammation. Compared with the general population, patients with IBD, including ulcerative colitis and Crohn's disease, have a higher risk of developing colitis-related CRC.[55] Probiotics can reduce gastrointestinal inflammation and reduce the incidence of inflammation-related CRC.

Bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* exhibit anti-inflammatory effects in the gut, and their reduction may lead to low-grade inflammation in which pro-inflammatory cytokines (IL-6, IL-8, TNF-α) are elevated in the patient's systemic circulation. *Lactobacillus* inhibits tumor-specific growth by producing antioxidants, anti-angiogenic factors, reducing DNA damage, reducing inflammation, and tumor size . The expression of sex proteins and polyamine components plays an important role in the

Table 1. The role and mechanism of some probiotics in the prevention of CRC.

PROBIOTICS	FUNCTION	MECHANISM
L. rhamnosus GG	Improve intestinal barrier function	Increase claudin-3 protein expression[59]
L. rhamnosus CNCM *I-3690*	Restore intestinal barrier integrity	Counteract inflammation-induced increased intestinal permeability and regulates levels of tight junction proteins occludin and E-cadherin22[60]
E. Coli Nissle 1917 (*EcN*)	Improve intestinal barrier function	Increased ZO-2 expression and robust ZO-2 relocalization to the TJs[59]; Stimulate mucin production and inhibit inflammation[61,62]; Induce expression of IL-22[61];
A. muciniphila MucT	Enhance intestinal barrier; Reduce the incidence rate of CRC caused by blunt colitis and CAC	Prevent CRC by inhibiting Th17 to produce IL-17[48]
L. plantarum BMCM12	Protects intestinal barrier	Secretion of extracellular proteins that weaken the adhesion of pathogens[63]
L. plantarum	Enhance intestinal barrier	Increase occludin protein expression and apical redistribution, increase ZO-1 apical redistribution[59]
B. polyfermenticus	Inhibition of cancer cell growth	Produce bacteriocin[34]
L. plantarum ZLP001	Enhance intestinal barrier	Reverse enterotoxic *E. coli*-induced decrease in claudin-1 and occludin protein levels[64]
L. reuteri	Inhibition of *Helicobacter pylori*; Reduction of enteropathogenic *E. coli* (EPEC) infection	Inhibits the colonization of *Helicobacter pylori* and produces reuterin[52]; Creation of a strong physical barrier against EPEC infection by binding to the mucus layer.[65]

Abbreviations: CAC, colitis-associated cancer; CRC, colorectal cancer.

prevention and treatment of cancer. In addition, probiotics reduce the expression of cyclooxygenase-2 (COX-2), leading to an increased risk of CRC due to its stimulation of cell proliferation and pro-inflammatory processes. *L. reuteri* has a significant anti-inflammatory effect. In *in vitro* studies, *L. reuteri CRL1098* was able to reduce the production of pro-inflammatory mediators, nitric oxide synthase (NOS) and pro-inflammatory cytokines.[56] *Lactobacillus* probiotic strains could activate the inflammatory response of macrophages through the synthesis of pro-inflammatory mediators, including cytokines, ROS, and involvement in signaling cascades.[43,45] Macrophages secrete anti-inflammatory mediators to prevent further tissue destruction after infection. Monocytes can also be transformed into intestinal macrophage-like cells under the action of epithelial cells, which have the same anti-inflammatory function.

The regulation of intestinal flora balance

Increasing evidence has demonstrated a causal relationship between gut microbial dysbiosis and CRC pathogenesis. Probiotics can prevent the development of CRC through intestinal flora balance when ingested in sufficient quantities. The numbers of *Bacteroides* and *Prevotella* were significantly higher in the CRC group.[57] *Fusobacterium nucleatum*, *anaerobic digestion Streptococcus*, and *enterotoxigenic Bacteroides fragilis*, have been identified as able to promote the occurrence of CRC by inducing tumor cell proliferation, promoting inflammation, causing DNA damage and protecting tumors from immune attacks, while some Probiotics with protective effect such as *Streptococcus thermophilus*, *Bifidobacterium* etc. are consumed in large quantities in the intestines of CRC patients.[10] Rebalancing of the gut ecosystem can be achieved through the administration of probiotics, prebiotics and synbiotics that can achieve homeostasis by neutralizing harmful pathogens, helping native beneficial bacteria grow, modulating immune responses, and repairing the intestinal mucosa.[58] By occupying the host tissue and preventing pathogen colonization, restore microbial microbiota imbalance and maintain intestinal microbiota balance. Probiotics can also produce metabolites such as lactic acid and acetic acid, or bacteriocins, which inhibit the growth of pathogens by lowering the pH value of the lumen and exerting direct antibacterial activity, respectively. It has been found that probiotic microorganisms can produce antibacterial substances such as bacteriocins, debonding bile acids, reuterins, hydrogen peroxide, and lactic acid.

Probiotics for Prevention and Treatment of CRC

With the continuous progress and deepening of research, the mechanism of action of probiotics has been gradually recognized and proved, and probiotics have gradually participated in clinical treatment. The intake of probiotics has been used to prevent subsequent possible CRC (see Table 1).

Clinical treatment of probiotics in patients with CRC

Probiotics gradually participate in clinical treatment as a drug for the treatment of CRC (see Table 2). *Lactobacillus* and *Bifidobacteria* are the main bacterial species currently used to treat gastrointestinal diseases. *Lactobacillus* and *Bifidobacterium*

Table 2. Inhibitory and therapeutic effects and mechanisms of some probiotics on colorectal cancer.

PROBIOTICS	FUNCTION	MECHANISM
L. gallinarum	Restrain the development of CRC	Secretion of protective metabolites including ILA to promote apoptosis of cancer cells.[67] Modulation of gut microbial composition by enriching probiotics and eliminating potential CRC pathogens inhibits CRC development.[67]
L. rhamnosus GG	Restrain the development of CRC; Inhibited cell growth and induced apoptosis	Increase the expression of Bax, casp3, p53, and other apoptosis promoting proteins[68] Inhibit the expression of inflammatory proteins[69] Increased claudin-3 protein expression[59]
Bifidobacterium	Anti-inflammatory effect. Inhibit proliferation of colorectal cancer cells	Increase the activity of the enzyme alkaline phosphatase[34] Enhance DC and CD8+ T-cell functions[70]
E. coli Nissle 1917 (*EcN*)	Anti-inflammatory effect	Inhibit inflammation[61,62]; Induce expression of IL-22[61];
A. muciniphila	Cancer immunotherapy treatments; Reduce the incidence rate of CRC Anti-inflammatory effect	Restore activity against cancer cells by blocking immune checkpoints such as PD-1;[71] Modulate CTLs;[72] Prevent CRC by inhibiting Th17 to produce IL-17;[48] Reduces the expression of TLR4, regulates the NF-κB pathway, reduces the secretion of IL-6 and IL-8.[73]
C. butyricum	Inhibit intestinal tumor development	Modulate Wnt-signaling and gut microbiota.[74]
B. polyfermenticus	Inhibition of cancer cell growth	Produce bacteriocin[34]
L. plantarum ZLP001	Anti-inflammatory effect	Decrease IL-6, IL8, and TNF-α levels[64]
L. reuteri	Inhibition of *Helicobacter pylori* Anti-inflammatory effect	Inhibits the colonization of *Helicobacter pylori* and produces reuterin[52] Reduced production of pro-inflammatory mediators, NOS and pro-inflammatory cytokines[56]
L. casei BL23	Immunomodulatory treatment Inhibit inflammation-associated CRC	Downregulate IL-22 cytokine, upregulate antiproliferative properties through Bik, caspase-7, and caspase-9;[75] Suppresses inflammation-associated colon cancer by producing histamine[76]

Abbreviations: CRC, colorectal cancer, DC, dendritic cell; IL, interleukin; NOS, nitric oxide synthase, TLR, toll-like receptor; TNF, tumor necrosis factor.

can inhibit the growth of CRC by inhibiting inflammation and angiogenesis and enhancing the function of the intestinal barrier through the secretion of SCFAs.[66]

Bifidobacterium:*Bifidobacterium* groups play an important role in intestinal homeostasis. On one hand, Bifidobacterial fingerprints can serve as a potential biomarker to understand gut state and thus indicate putative dysbiosis. On the other hand, increased levels of bifidobacteria in the gastrointestinal tract can be considered as a target for preventing and/or alleviating microbiota-related diseases. *Bifidobacterium* is one of the major genera of commensal bacteria present in the human gastrointestinal tract, and the presence of bifidobacteria has been associated with health benefits. Each type of bifidobacteria appears to exert different immune effects on the host, and it is noteworthy that bifidobacteria have the ability to amplify T-regulatory responses, which may be related to its use in chronic inflammatory diseases. The positive effects of bifidobacteria on human health have been widely evaluated in the past few years. Due to the potential impact of certain species of this genus on human health and GRAS, QPS status, some strains have been clinically studied and are currently being used as probiotics in human nutrition. The beneficial effects of consuming *Bifidobacteria* on patients with CRC are mainly related to the prevention and treatment of intestinal diseases and immune disorders.[77] The ability of bifidobacteria to induce altered fecal microbiota in CRC patients and to reduce some cancer risk factors by improving epithelial barrier function and reducing colorectal proliferation of commensal microorganisms. *Bifidobacteria* were able to induce changes in the fecal microbiota in CRC patients and reduce certain cancer risk factors by improving epithelial barrier function and reducing the proliferation of colorectal commensal microbes. Administration of bifidobacteria as part of perioperative probiotic therapy reduces the incidence of postoperative sepsis in patients undergoing colectomy and in patients undergoing CRC liver metastases. Preoperative administration of Bifidobacteria to CRC patients can reduce postoperative infectious complications by maintaining the gut microbiota, reducing the number of *E. coli*, and limiting the transfer of bacteria from the gut to the blood. Bifidobacteria aid in recovery after chemotherapy while reducing the likelihood of sepsis after surgery.[77] This potential probiotic has considerable protective anticancer properties,

comparable to the already used drugs cetuximab and trastuzumab, and is able to simultaneously downregulate cancer markers EGFR, HER-2, and PTGS-2 (COX-2); suppress tumor incidence; and prevent tumor progression to higher stages and grades.[78]

Lactobacillus:*Lactobacillus* can produce conjugated linoleic acid (CLA) from linoleic acid. This fatty acid is produced by bacteria in the distal ileum and can be absorbed by colon cells in the intestinal cavity and interact with them, thereby exerting its beneficial effects locally. The antiproliferative and pro-apoptotic activities of CLA stem from its ability to increase the expression of peroxisome proliferator-activated gamma receptor (PPARγ), which is involved in the regulation of lipid metabolism, apoptosis, and immune system function.[46] Small non-protein metabolites produced by *Lactobacillus gallinarum* inhibit the growth of CRC cells and organ-like organs from CRC patients by promoting apoptosis. The metabolites produced can inhibit the viability of CRC cells by inducing apoptosis. For example, *L. gallinarum* can produce L-tryptophan and convert L-tryptophan into ILA, which can inhibit the viability of CRC cells *in vitro* and the development of intestinal tumors *in vivo*. In addition, it can inhibit the development of CRC by regulating intestinal microbial composition.[67] Synergistic intervention of multiple live bacterial strains may be a promising approach for probiotic applications. Shang et al found that the probiotic mixture inhibited the invasion, migration, and proliferation of CT26 cells, indicating that the synergistic effect of multiple probiotics could enhance immune responses and inhibit tumor growth.[79]

The next generation of probiotics (NGPs): As a new concept microorganism, the difference from traditional probiotics is that traditional probiotics are isolated from the gut or fermented foods, while NGPs are modified bacteria. NGPs can enhance gastrointestinal immune function, maintain intestinal barrier integrity, produce beneficial metabolites, combat pathogens, improve immunotherapy efficacy, and reduce complications related to chemotherapy and radiation therapy.[80] NGPs can play a role in effectively preventing cancer development and treating cancer through various mechanisms. As the next generation of probiotics, *Faecalibacterium praussnitzii* (*F. prausnitzii*) can maintain an immune environment balance, inhibit the growth of cancer cells by inhibiting signaling pathways, produce SCFAs, maintain intestinal integrity, and other mechanisms to exert cancer suppressive effects, with enormous potential.[80] *Akkermansia muciniphila* exert anticancer effects through mechanisms such as maintaining intestinal immunity, enhancing intestinal barrier, immune regulation, and directly combating pathogenic bacteria.[35,81] Compared with healthy individuals, CRC patients have different gut microbiota composition and physiological activity, and SCFA-producing bacteria are depleted. This suggests that SCFAs-producing bacteria may have anti-inflammatory and anticancer properties, and may become NGP candidates in the prevention and treatment of CRC.

Probiotics cooperate with immune checkpoint inhibitors to play an anticancer role

Under normal physiological conditions, the combination of PD-1 and its ligand PD-L1 can inhibit the excessive activation of T cells to attack normal cells through downstream signals; but the expression of PD-L1 on the surface of tumor cells is abnormally upregulated, and through excessive activation of PD-1/PD- L1 signaling induces T-cell exhaustion, leading to immune escape of tumor cells, resulting in tumor development and patient deterioration. PD-1/PD-L1 blockade therapy reactivates tumor immune microbes by inhibiting PD-1/PD-L1 signaling. Exhausted T cells in the environment can restore their killing function against tumor cells and improve antitumor immune activity. Theoretically, probiotics and PD-1 inhibitors may have a synergistic effect to enhance their inhibitory effect on tumors, As reported by Chang et al, some strains of bifidobacteria may enhance the efficacy of immune checkpoint inhibitor (ICI) therapy in cancer; thus, the effect of anticancer therapy may be strain-dependent.[70] Probiotics can downregulate PD-1 expression in mice, activate DC function when combined with PD-1 inhibitor, the probiotic *L. reuteri*, and show antitumor responses. In addition, the colitis caused by immune checkpoint therapy is one of the complications caused by immune checkpoint therapy, and the probiotic *Lactobacillus* may alleviate immune checkpoint therapy-induced colitis.[20] Probiocis such as *Bifidobacterium* spp, *B. fragilis*, *A. muciniphila*, and *F. prausnitzii*, have opened up new therapeutic prospects for CRC. Different intestinal flora can make a huge difference in the effect of PD-1 immunotherapy in cancer patients. Gopalakrishnan et al found that melanoma patients who responded to PD-1 blockade therapy had higher levels of beneficial gut bacteria.[82] Matson et al reported that high numbers of *A. muciniphila* were observed in four patients with metastatic melanoma who responded clinically to PD-1-based immunotherapy.[83] In many experiments and reports, *A. muciniphila* has repeatedly shown its potential role in the treatment of cancer. Cancer immunotherapy combined with *A. muciniphila* as one of the important probiotics for selective flora transplantation is expected to be available to patients in the near future for better clinical outcomes.

Application of probiotics in perioperative period

The use of probiotics in patients undergoing abdominal surgery is a promising therapeutic approach in preventing postoperative infection at the surgical site with shallow incisions and improving the integrity of the intestinal mucosal barrier. In addition, patients' quality of life was improved, with shorter postoperative hospital stays and the time required for antibiotic administration.[84] Administration of bifidobacteria reduces the

incidence of postoperative sepsis in patients undergoing colectomy and in patients undergoing liver metastases from CRC. Preoperative administration of *Bifidobacteria* in CRC patients can reduce postoperative infectious complications by maintaining the gut microbiota, reducing the number of *Escherichia coli*, and limiting the transfer of bacteria from the gut to the blood. Postoperative application of probiotics is beneficial to the recovery and prognosis of CRC surgery. Salem Bajramagi et al demonstrated that probiotics are particularly important in reducing postoperative complications, and it can be argued that the introduction of routine probiotics in these tumor localizations is particularly important.[85] *Bifidobacteria* aid in recovery after chemotherapy while reducing the likelihood of sepsis after surgery.

Probiotics for the eradication of H. pylori

Probiotics can be used as adjunctive therapy to eradicate *H. pylori*, a Gram-negative bacterium that resides in the stomach and causes chronic ulcers, a pathology associated with the development of gastric cancer. In addition to being the most important risk factor for the development of gastric cancer, epidemiological data show that the risk of developing CRC in individuals infected with *H. pylori* has nearly tripled. One study found that *H. pylori* can cause the reduction of Tregs and pro-inflammatory T cells, and induce the loss of cancer promoting STAT3 signal transduction and goblet cell. The early use of antibiotics to eradicate *H. pylori* can restore the incidence rate of tumor in infected people to the level of uninfected people.[86] Probiotics stimulate the production of mucins that limit the adhesion of pathogens to intestinal surfaces, while producing SCFAs and other antibacterial substances that may reduce the density of *H. pylori. L. reuteri* exhibited the ability to inhibit the colonization of *H. pylori* on the human gastric mucosa and also produced a broad-spectrum antibiotic effective against *H. pylori*—reutericin.[52] This has been confirmed by different studies and meta-analyses, finding that in patients infected with *H. pylori*, supplementation of standard eradication therapy with probiotics may ultimately increase the eradication rate of the microbe by about 13% and reduce the overall rate of adverse effects by about 41%.[87] Therefore, probiotics can indirectly reduce the risk of CRC by eradicating *H. pylori* and reducing the incidence of chronic gastrointestinal inflammation.

Expectation

Accumulating evidence indicates that the integrity of the intestinal barrier is the prerequisite for the function of the intestinal barrier, and it is also the guarantee for maintaining the homeostasis of the intestinal flora. Therefore, the study of intestinal barrier homeostasis has become a challenge for the diagnosis and treatment of a large number of diseases. Probiotics can promote the normalization of intestinal flora, the improvement of gastrointestinal barrier and other mechanisms of action, so as to achieve the purpose of inhibiting the proliferation and growth of CRC. As one of the most important metabolites of probiotics, SCFAs not only serve as the energy source of the colonic mucosa, but also enhances the intestinal protective barrier and rebuilds the colonic epithelium, maintains the pH of the intestinal lumen, inhibits the growth of malignant cells, induces the apoptosis of cancer cells, and inhibits the growth of malignant cells. In addition, SCFAs also act as signaling molecules through GPCRs, reducing the production of pro-inflammatory cytokines and increasing the number of colonic Treg cells.

So far, the mechanism of action of probiotics in the prevention and treatment of CRC has not been fully elucidated. There are many kinds of probiotics with different characteristics and modes of action, and their effects are complex and diverse. Further clinical research is urgently needed to explore the regulatory mechanism of probiotics on CRC, clarify each mechanism and further use it as an adjuvant therapy for the prevention and treatment of CRC. Before CRC occurs, appropriate probiotic-related methods can be used to prevent CRC, such as direct oral administration of probiotics and their fermentation products, combined with probiotics or anticancer drugs. When aggressive tumors are formed, probiotics can also be used in surgery, chemotherapy, and immunotherapy to reduce the complications of surgery and chemotherapy, improve the efficacy of chemotherapy, and improve the quality of life of patients. Currently, traditional probiotics are used as adjuvant therapy in the treatment and management of CRC, mainly to reduce postoperative complications and alleviate side effects of chemotherapy. At present, probiotics need to solve the following problems to obtain a wide range of clinical applications: (1) Due to the diversity and complexity of probiotics, each strain may have a different mechanism of action, leading to varying clinical results. The combination of different probiotics may also have differential effects. Therefore, understanding the mode of action of each probiotic is crucial for personalized treatment, allowing the use of different strains to assist in the treatment of CRC with varying etiologies. (2) At present, there is a lack of sufficient follow-up results and safety issues in human clinical trials of probiotics for cancer biotherapy. Therefore, extensive clinical trials in humans are required to identify potential strains, dosages, and drug delivery regimens for specific types and stages of cancer as alternative therapies for cancer treatment. Probiotics provide more new strategies for the prevention and treatment of CRC. (3) The next generation of probiotics (NGPs) has emerged in recent years as a therapeutic agent that can change the intestinal microbiota and affect the development of cancer. Their presence in the digestive tract may affect the incidence of cancer. NGPs have demonstrated stronger therapeutic effects and fewer side effects and complications compared with traditional probiotics. However, further experimentation and testing are required to ensure their safety.

Author Contributions

JZ summarized the articles and wrote the article. YL, CW, YM, FW, YC, BZ, and HJ helped for article selection and gave valuable advice to the article. DT revised the article critically for important intellectual content and DW approved the version to be published. All authors have read and approved the final article.

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