Gastrointestinal Tract

REVIEW

The influence of gut microbiota on immunotherapy for colorectal cancer

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ABSTRACT

Colorectal cancer (CRC) stands as the third most prevalent cancer worldwide and ranks second in mortality for both men and women. Novel therapeutic approaches have emerged to address this alarming situation, with immunotherapy showing great promise. This cutting-edge technique harnesses patients' immune system to recognize and eliminate cancer cells. Despite its tremendous potential, the complex interplay between the intestinal microbiota and CRC development remains a critical area of investigation. The intestinal microbiota, comprised of diverse microorganisms such as bacteria, viruses, protozoa, and fungi, plays pivotal roles in digestion, vitamin production, defense against pathogens, and immune system modulation. Perturbations in this delicate balance have been implicated in carcinogenesis. Interestingly, the intestinal microbiota is now recognized to significantly impact immunotherapy's effectiveness by influencing immune checkpoint regulation, particularly in the activation of cytotoxic T cells. Consequently, a better understanding of the intricate mechanisms underlying this interplay between microbiota and CRC could revolutionize cancer treatment outcomes. In this review, we aim to elucidate the mechanisms through which the intestinal microbiota directly influences CRC development and, specifically, explore its potential in generating improved prognoses for cancer patients. By delving into the link between the microbiome and CRC, this review highlights the significance of incorporating microbiota-related considerations in the design of novel therapeutic strategies, fostering a new era of precision medicine in colorectal cancer treatment.

Key words: colorectal cancer, immunotherapy, gut microbiota, immune checkpoints, cytotoxic activity of T cells, tumor microenvironment

INTRODUCTION

Colorectal cancer (CRC) ranks as the third most prevalent cancer worldwide and represents a significant health concern due to its high mortality rate, placing it as the second deadliest cancer for both men and women.[1–3] The carcinogenesis of CRC is an intricate and multifactorial process, involving mucosal inflammation, interactions between the host and the microbiota, and genetic abnormalities. Accordingly, most CRC cases emerge from intestinal polyps that initiate inflammation, leading to genetic changes that culminate in tumor development.[4–6] This process is influenced by a myriad of risk factors, including genetic predisposition, lifestyle habits (such as smoking, sedentary behaviors, and an unhealthy diet), as well as pre-existing conditions like diabetes and chronic inflammation of the gut.[7–9]

The therapy of CRC typically relies on well-established methods such as surgery, radiotherapy, and chemotherapy.[10–12] Nevertheless, novel therapeutic approaches are being explored and implemented in the treatment of cancer patients. Notably, immunotherapy has emerged as a promising strategy aimed at

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augmenting the efficacy of the individual's immune system in combating tumors. This encompasses various techniques such as immune checkpoint inhibitors, adoptive cell transfer, and cancer vaccines, all of which are already employed strategies in CRC therapy.[13–15]

The human microbiota, consisting of approximately 10 to 100 trillion microbes, including bacteria, viruses, fungi, and protozoa, is primarily concentrated in the human intestine. The gut microbiota functions as a distinct organ with vital roles in food digestion, vitamin production, protection against harmful/pathogenic microorganisms, and modulation of the immune system.[16–19] Dysregulation of the microbiota has been linked to various health changes, including CRC, suggesting a potential role of microbiome dysregulation in the development or progression of CRC. Moreover, recent studies have demonstrated that the composition and diversity of the microbiome can impact the efficacy and toxicity of immunotherapeutic treatments.[20,21]

Thus, this review provides an analysis of the direct association between the microbiota and colorectal cancer, examining its role in carcinogenesis and its impact on CRC immunotherapy. Additionally, the article discusses the potential of manipulating the microbiota in medicine to improve patient outcomes, enhance immunotherapy effectiveness, and improve the quality of life for individuals with cancer.

RELATIONSHIP BETWEEN GUT MICROBIOTA AND THE ONSET OF COLORECTAL CANCER

The gut microbiota, an intricate ecosystem, comprises a diverse assemblage of microorganisms that colonize the entire gastrointestinal tract. Predominantly, the bacterial phyla *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Verrucomicrobia*, and *Euryarchaeota* constitute approximately 90% of its microbial composition.[22,23] This highly complex environment serves as a critical determinant of human homeostasis, playing a pivotal role in various essential functions, including metabolic processes, synthesis of vitamins, and neuromodulation. The gut microbiota's multifaceted regulatory functions further underscore its significance as both a metabolic and immunological regulator, impacting various aspects of human health and disease.[24–26]

Dysbiosis, a condition marked by an altered balance of the gut microbiota, has garnered considerable attention as a potential contributory factor in the pathogenesis of autoimmune diseases, exemplified by irritable bowel syndrome.[27–29] These investigations have unveiled previously undiscovered aspects of the complex gut microbiome. Additionally, a notable finding in the context of CRC is the discernible disparity in the gut microbiota composition between CRC patients and healthy individuals. Specifically, CRC patients frequently exhibit a diminished diversity of microorganisms constituting the gut microbiota, as evidenced by various studies.[22,23,30]

The role of gut microbiota in the development of colorectal cancer can be comprehensively understood through an integrated theoretical model that combines insights from two prominent perspectives. This unified model highlights how both the intestinal microorganisms and the host's genetic predisposition interact to shape the pathogenesis of CRC.[31,32] The first aspect of the model focuses on the role of the host's genetic predisposition in driving the establishment of a pro-inflammatory microenvironment in the gut. Individuals with specific genetic traits may exhibit recurrent and sustained inflammatory responses, leading to significant modifications in the normal cellular landscape of the gut microenvironment.[33–37] These genetic factors influence the immune response, cellular signaling pathways, and inflammatory mediators, collectively contributing to the development of CRC.

In turn, the second aspect of the model emphasizes the active contribution of intestinal microorganisms in remodeling the gut environment, fostering a pro-carcinogenic milieu. According to this perspective, damage to the intestinal mucosa creates an opportunity for transient bacteria to infiltrate, thereby increasing susceptibility to carcinogenesis.[38–40] Alterations in the composition and behavior of gut microorganisms play a critical role in initiating and promoting CRC. The interplay between these microorganisms and the host cells can lead to the generation of pro-inflammatory signals and other tumorigenic factors.[41]

Recently, Chattopadhyay *et al.*[42] comprehensively compiled a list of key bacteria associated with CRC, stemming from disturbances in the healthy gut microbiota. The bacteria implicated in CRC pathogenesis include *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Escherichia coli* (*E. coli*), *Helicobacter pylori* (*H. pylori*), and *Streptococcus gallolyticus/Streptococcus bovis*[42]. Accordingly, Kharrat *et al.*[43] conducted a metagenomic analysis, revealing a significant elevation of *F. nucleatum* in individuals with CRC compared to those with adenomas, implicating its potential role in adenoma-to-CRC progression.[43] The principal pro-carcinogenic mechanism of *F. nucleatum* seems to involve the adhesion molecule FadA, which interacts with E-cadherin on intestinal epithelial cells, leading to the activation of β-catenin and subsequent stimulation of transcription factors and oncogenes.[43,44] Furthermore, *F. nucleatum* appear to induce an increase in inflammatory

cytokines (IL-1β, IL-6, IL-8, and TNF-α), fostering a pro-oncogenic microenvironment in the colorectal region.[44]

Similarly, enterotoxigenic *B. fragilis* (ETBF) plays a significant role in CRC oncogenesis, particularly when prevailing in high proportions within a dysregulated gut microbiota.[45] The toxin produced by ETBF stimulates β -catenin expression, eliciting inflammatory responses akin to those triggered by *F. nucleatum*. This process involves the induction of cyclooxygenase (COX)-2 production, resulting in the release of PGE2 and instigating the inflammatory cascade, leading to elevated pro-inflammatory cytokines, such as IL-17.[46,47]

Enterococcus faecalis exhibits a dual role, serving as a protective agent under certain conditions, yet demonstrating pro-carcinogenic potential in cases of gut dysbiosis.[48,49] While it modulates the immune system by inducing the release of anti-inflammatory cytokines and exerting a protective effect,[48] *E. faecalis* can also activate β-catenin and transcription factors, potentially leading to cellular alterations.[49] On the other hand, Pleguezuelos-Manzano *et al.*[50] report the involvement of *E. coli* in CRC oncogenesis, particularly in strains harboring the pks genomic island, which synthesizes colibactin, causing direct DNA damage to cells.[50,51] Furthermore, Iyadorai *et al.*[52] further corroborate this association by observing a higher prevalence of pks+ *E. coli* in individuals with CRC compared to healthy individuals.

The involvement of *H. pylori* in CRC is partially elucidated, with underlying mechanisms still being investigated. Ralser *et al.*[53] recently demonstrated that *H. pylori* triggers a Th17 pro-inflammatory response in the intestine, concurrently leading to a decrease in regulatory T cells, ultimately resulting in tumorigenesis. Moreover, the pro-inflammatory activity of *H. pylori* seems to activate transcription factors NF-κB and STAT3, thereby further augmenting its potential carcinogenic effects.[53,54]

Finally, the relationship between *S. bovis/gallolyticus* and CRC also remains a subject of ongoing investigation, with mechanisms being actively explored. Abu-Ghazaleh *et al.*[55] suggest that this bacterium elicits the release of inflammatory cytokines, creating an environment rich in free radicals and nitric oxide, which directly influence oncogenes in intestinal epithelial cells. Furthermore, it activates the COX-2 pathway, contributing to the altered environment.[56] Table 1 presents an overview of the primary mechanisms associated with the carcinogenic impact of the aforementioned microorganisms.

In conclusion, the past few years have witnessed substantial progress in the field of research concerning the link between gut microbiota and CRC development. This advancement has primarily been centered around the exploration of potential bacterial profiles and the underlying mechanisms by which they might contribute to CRC initiation and progression. The emerging body of knowledge in this area offers promising insights into the complex interplay between the gut microbiome and colorectal carcinogenesis. However, it also highlights the need for further investigations to unravel the intricacies of this relationship fully.

IMMUNOTHERAPY FOR COLORECTAL CANCER

Immunotherapy has revolutionized the treatment outlook for several types of solid tumors, such as malignant melanoma, non-small-cell lung cancer, and renal cell carcinoma, and has emerged as the primary treatment approach for recurrent or metastatic solid tumors, surpassing traditional chemotherapy and targeted therapy.[57] Immunotherapy is a treatment approach that utilizes peptides, cells, viruses, small molecules, or antibodies to activate or modulate the immune system to attack cancer cells. Research conducted in preclinical and clinical settings to evaluate the effectiveness of immunotherapy in treating colorectal cancer has yielded positive results.[58] Here, we aim to synthesize the main concepts of immunotherapy in colorectal cancer and the main clinical results.

Due to their remarkable effectiveness, immune checkpoint inhibitors (ICIs) have quickly emerged as a prominent therapeutic approach for several solid tumors. ICI function by obstructing immunosuppressive tumor signaling pathways that target receptor or ligand checkpoint proteins, such as programmed cell death 1 (PD-1), PD-1 ligand 1 (PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4), thereby restoring the antitumor immune response.[59,60] The FDA has authorized the use of treatments, including pembrolizumab, nivolumab, and ipilimumab, which target the PD-1, PD-L1, and CTLA-4 immune checkpoints, for managing dMMR/MSI-H CRC.[57,60,62] Nevertheless, not all dMMR/MSI-H CRC cases respond to immunotherapy, and about 50% of patients with this subtype show primary resistance, which implies the existence of considerable molecular heterogeneity among dMMR/MSI-H CRCs. This heterogeneity could be attributed to high CIMP levels, oncogenic BRAF mutations, and CMS1 subtypes.[63] Furthermore, recent research into combined ICI treatment has suggested that it may enhance the effectiveness of immunotherapy in this patient population. Several studies on combination therapy with ICIs have investigated their potential when administered alongside chemotherapy, targeted therapy, other ICIs, or radiotherapy to investigate methods of transforming cold tumors into hot ones, thereby

Table 1: The impact of key gut microbiota bacteria on colorectal cancer carcinogenesis

Bacteria	Involvement in colorectal cancer carcinogenesis	References
Fusobacterium nucleatum	The bacterium promotes the activation of β-catenin, leading to the upregulation of transcription factors and oncogenes. Simultaneously, it induces the secretion of inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α.	[43,44]
Bacteroides fragilis (ETBF)	The toxin triggers the production of COX-2, leading to the release of prostaglandin E2 (PGE2). Consequently, this initiates the inflammatory cascade, characterized by the recruitment of T cells and the upregulation of pro-inflammatory cytokines, particularly interleukin-17 (IL-17).	[46,47]
Enterococcus faecalis	Stimulates the liberation of β-catenin, thereby activating transcription factors associated with oncogenesis.	[50,51]
Pks+ Escherichia coli	The *pks genomic island* facilitates the synthesis of colibactin, a compound that directly damages cellular DNA.	[50,51]
Helicobacter pylori	Elicits a Th17 pro-inflammatory response in the gut, leading to a decrease in regulatory T cells within gut tissues and the activation of transcription factors NF-κB and STAT3.	[53,54]
Streptococcus gallolyticus/ Streptococcus bovis	The bacterium induces the release of free radicals that directly modulate oncogenes. Additionally, it influences the release of COX-2, leading to the generation of inflammation.	[55,56]

enhancing their sensitivity to immunotherapy.[64,65]

Despite existing for over a century, cancer vaccines have had limited success in treating CRC patients. However, with the promising efficacy of immunotherapy, cancer vaccines have garnered renewed attention. Several trials are currently underway to identify appropriate antigenic stimulants for cancer vaccines.[66]

Adoptive cell therapy (ACT) is another eagerly anticipated novel treatment method aimed at stimulating tumor immunity. ACT involves selecting either host cells that demonstrate antitumor activity or host cells that have been genetically engineered with chimeric antigen receptors (CARs) or antitumor T cell receptors (TCRs) to enhance the host's antitumor immune response. While both CAR T therapy and TIL (tumor-infiltrating lymphocyte) therapy have shown promising preliminary results, their wider applicability still needs to be demonstrated.[67]

As further clinical trials produce valuable knowledge in the search for effective therapeutic approaches, the identification of novel combinations and biomarkers may assist clinicians in administering personalized treatments to CRC patients. These advancements in treatment are anticipated to transform the treatment landscape for CRC, with immunotherapy emerging as a potential game-changer.

INFLUENCE OF GUT MICROBIOTA COMPOSITION ON THE RESPONSE OF CRC TO IMMUNOTHERAPY

Recent research highlights the critical role of the gut microbiota in regulating immune responses within the tumor microenvironment and influencing immune-related cytokines.[68] The composition of these microorganisms is crucial for maintaining gut symbiosis and producing metabolites like butyrate and tryptophan degrading metabolites, which stimulate lymphoid cells to produce IL-22.[69,70] Moreover, they contribute to increased immune cell activity in the gut.[71,72] Cytokines are essential for preserving gut homeostasis and supporting repair mechanisms during infectious events.[72,73] Additionally, bacterial metabolites, such as short-chain fatty acids (SCFAs), play a role in immune responses and are closely associated with innate immunity and antibody production.[74]

In the field of intestinal immune regulation, the adaptive response is of utmost importance, especially considering T cells' high responsiveness to signals from the intestinal lumen. This responsiveness leads to the generation of both inflammatory and anti-inflammatory responses, as well as the facilitation of differentiation between CD8+ and CD4+ T cells.[75] This phenomenon is intricately linked to tumors' ability to induce cytokine production, bolstering Tregs and myeloid-derived suppressor cells (MDSCs), while inhibiting CD8+ cytotoxic T cell function.[76] These events underscore the potential of certain microorganisms to modulate the function of specific intestinal immune cells through activation pathways, potentially contributing to anti-tumor defense by enhancing the activity of CD4+ and CD8+ effector T cells, thereby countering the suppressive effects induced by tumors.[76]

Altered microbiota diversity significantly impacts disease development, with a decline in typical gut microbiome species, like *Firmicutes* and *Bacteroidetes*, playing a crucial role in triggering numerous diseases.[70,77] In the context of CRCs, recent cohort studies have identified significant changes in the gut microbiome's composition at the phylum level.[78] Notably, the proliferation of the *Fusobacterium nucleatum* group, commonly observed in CRC patients, has been linked to its ability to recruit immune cells and create a pro-inflammatory environment that favors CRC development.[78] Further

investigation is necessary to elucidate its precise role in CRC pathogenesis and its impact on immune responses.

The relationship between immunotherapy and the gut microbiota has become an increasingly interesting topic of research, as it appears to exert both anti-tumor and pro-tumor influences.[79] Some microorganisms have demonstrated beneficial effects in CRC immunotherapy and other cancers by exerting an anti-tumor role. For instance, Bacteroides fragilis has been associated with improved treatment outcomes, such as enhanced anti-CTLA4 therapy,[80] leading to improved anti-tumor immune responses and increased activity of anti-tumor CD8+ T cells, similar to the effects observed with *Lactobacillus acidophilus*.[81] Similarly, *Akkermansia muciniphila* has been found to increase IL-12 production, which restores anti-PD1 efficacy during immunotherapy.[82] *Bifidobacteria*, when combined with PD-L1 inhibitors, demonstrated almost complete inhibition of cancer cell growth by enhancing the trafficking, penetration, and infiltration of effector CD8+ T cells into tumor tissue, addressing a critical challenge in cancer immunotherapy[83] (Figure 1).

Pathways involving increased IL-12 production are associated with improved dendritic cell (DC) function, crucial for gut immune tolerance by promoting the differentiation of CD4+ T cells into regulatory T cells.[84,85] These regulatory T cells induce an anti-inflammatory IL-10 response to defend against inflammatory lesions.[85,86] *Bacteroides fragilis* in the gut microbiome also plays a regulatory role due to its production of polysaccharide A, which exerts anti-inflammatory effects by inhibiting IL-17 production and enhancing the activity of Tregs in the gut.[86] Understanding these intricate interactions between microorganisms and immune responses holds promise for advancing cancer immunotherapy and gut immune regulation.

Another significant finding indicates that combining *Lactobacillus acidophilus* lysate with anti-CTLA4 can enhance antitumor immune responses by activating TME-infiltrated effector T cells.[87] On the other hand, some microorganisms are associated with a pro-tumor environment, such as *E. coli* and *F. nucleatum*, which promote an increase in M2 macrophages and a decrease in FOXP3+.[69–72] The effects of gut microbiota bacteria on CRC immunotherapy are summarized in Table 2.

Collectively, these studies suggest that the gut microbiota may play a key role in cancer immunotherapy, and certain bacterial species could be highly effective in combination therapies. However, due to the complexity and high diversity of microbiomes, further research is necessary to understand the specific functions of each microorganism. Additionally, the optimal composition of the gut microbiome for supporting anti-tumor immune responses remains unclear.

ROLE OF THE GUT MICROBIOTA IN THE MODULATION OF SPECIFIC IMMUNOTHERAPEUTIC APPROACHES

Influence of microbiota on anti-CTLA-4 efficacy

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a protein receptor with inhibitory features that plays pivotal role in T-cell homeostasis and immune regulation(88). This immune checkpoint can compete with CD28, a costimulatory molecule, by binding to CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells, thus inhibiting T cell activation and proliferation[89] (Figure 2A). As a result, several studies have evaluated the effect of CTLA-4 blockade in enhancing the immune response against tumors, which led to the development of ipilimumab, a monoclonal antibody currently used in the treatment of certain types of cancer.[90–92]

Regarding CTLA-4-based immunotherapy in CRC, ipilimumab is the only approved for the treatment of colorectal cancer. Currently, its association with nivolumab for patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer have been assessed and is apparently a promising alternative.[65] On the other hand, tremelimumab has been the scope of several clinical trials, but is still pending approval.[93]

Increasing evidence suggests that the microbiota plays a role in the effectiveness of immunotherapy. Certain types of bacteria may either enhance the immune system's response to cancer cells, reduce toxic effects, or even hinder immunotherapy.[94,95] In this regard, Vétizou *et al.*[80] demonstrated that CTLA-4 blockade efficacy is influenced by the presence of specific bacterial species in the gut, especially Bacteroides in mice and patients. The mechanism underlying this finding is probably related to the effect of the microbiota on IL-12 production. This cytokine stimulates Th1 cells and consequently enhances anti-tumor effects.[80,96]

As for colorectal cancer, there are still few evidences assessing the role of microbiota in the efficacy of immunotherapy in humans. To date, Zhuo *et al.* [87] has pointed out a possible synergistic action between Lactobacillus acidophilus cell lysates and the anti-CTLA4 blocking antibody. The combined therapy was able to increase CD8+ T cells, IFN-γ and IL-2 production and reduce the percentage of regulatory T cells (Tregs) in mice, which suggests that combining L. acidophilus lysates with CTLA-4 blockade may enhance

Table 2: Influence of gut microbiota bacteria on colorectal cancer response to immunotherapy

Bacteria	Effects	Reference
Fusobacterium nucleatum	The capability to attract immune cells and induce a pro-inflammatory environment.	[30,78]
Bacteroides fragilis	Leads to improved responsiveness to anti-CTLA4 therapy, correlated with enhanced anti-tumor immune reactions and increased presence of anti-tumor CD8+ T cells. Moreover, it hinders IL-17 production and boosts the functionality of Tregs within the gastrointestinal tract.	[80,81,86]
Akkermansia muciniphila	Enhances IL-12 production and is linked to the reinstatement of anti-PD1 efficacy during immunotherapy.	[82]
Bifidobacteria	When combined with CD274 inhibitors, it exhibited nearly complete inhibition of cancer cell growth through the augmentation of trafficking, penetration, and infiltration of effector CD8+ T cells into the tumor tissue.	[83]
Lactobacillus acidophilus	When used in conjunction with anti-CTLA4, it has the potential to amplify anti-tumor immune responses by activating effector T cells that infiltrate the tumor microenvironment.	[87]

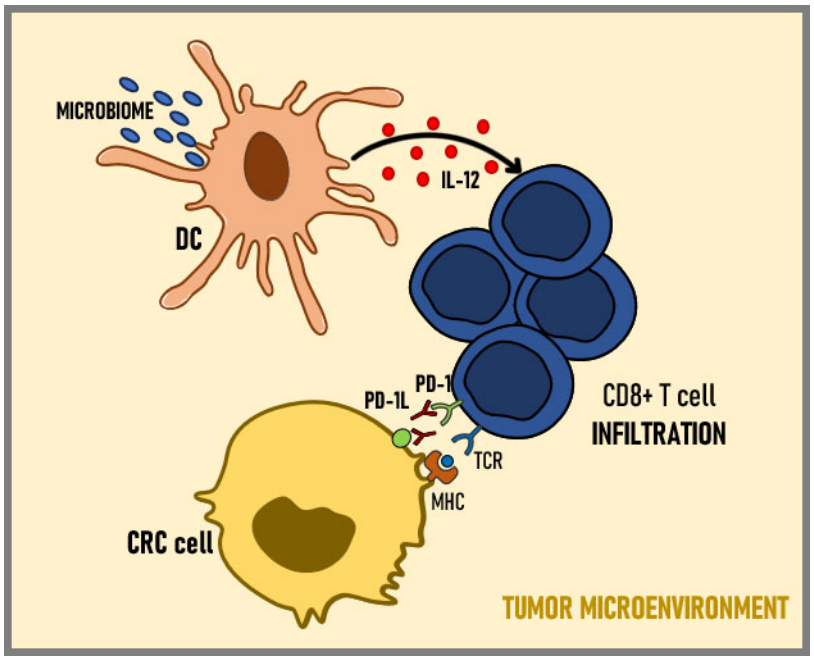

Figure 1. Microbiome-induced changes in the tumor microenvironment. DC: dendritic cell. CRC: colorectal cancer. PD-1: programmed cell death 1. PD-L1: programmed cell death ligand 1. IL-12: interleukin 12.

antitumor immunity and reduce immunosuppression.[87]

Overall, even though there is increasing evidence on the benefits of anti-CTLA4 based immunotherapy, its use in colorectal cancer is still not well-established, mainly due to the high incidence of adverse effects. However, the modulation of the microbiota and association with CTLA-4 blockade may hold promising results. For this purpose, further studies are strongly required, especially in humans.

Influence of microbiota on anti-PD-1/PD-L1 efficacy

PD-1 is an essential immune checkpoint receptor expressed in activated T cells that regulates immunosuppression.[97] On the other hand, membrane-bound PD-L1 engages PD-1, leading to T cell dysfunction and apoptosis. In turn, targeting PD-1 or its ligand PD-L1 with antibodies can rescue T cells from exhaustion and revive the immune response against cancer cells[98–100] (Figure 2B). Increasing evidence suggest that the commensal microbiome might play a mechanistic role in the antitumor immune response of human cancer patients.[82,101] Nevertheless, the influence of the microbiome in variable therapeutic outcomes of PD-1/PD-L1 therapy in CRC still needs to be addressed.[102]

To investigate the effects of the gut microbiome on PD-1 antibody immunotherapy, Xu *et al.*[68] compared its relative efficacy against established CT26 tumor-bearing mice treated with different antibiotics. These authors observed that after introducing the PD-1 antibody treatment, the administration of antibiotics resulted in an increase in tumor growth as compared to the group that consumed sterile drinking water without antibiotics. In

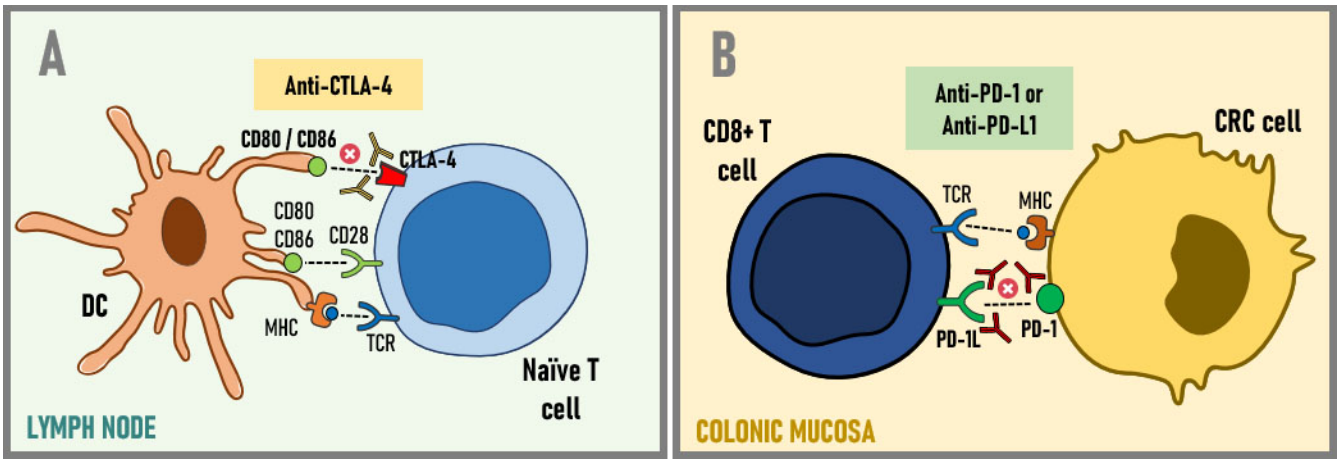

Figure 2. Immune checkpoint inhibition targets: PD-1/PDL-1, and CTLA-4/CD80 or CD86. **A:** CTLA-4/CD80 or CD86 inhibition. CTLA-4 is an additional immune checkpoint molecule predominantly found on the surface of regulatory T cells (Tregs) and certain cancer cells. When CTLA-4 interacts with its ligands CD80 or CD86 on antigen-presenting cells, it inhibits the activation of effector T cells, dampening the overall immune response. By blocking CTLA-4 with immune checkpoint inhibitors, this inhibition is relieved, enhancing the activation and proliferation of effector T cells, which can then more effectively target cancer cells or other threats. CTLA-4: cytotoxic T-lymphocyte-associated protein 4**. B.** PD-1/PDL-1 inhibition. PD-1, present on the surface of activated T cells, interacts with its ligand PDL-1, expressed on some tumor cells and other immune cells. This interaction acts as a 'brake' on the immune response, preventing T cells from attacking these cells, including tumor cells. Immune checkpoint inhibitors that target PD-1 or PDL-1 interrupt this interaction, releasing the brake and allowing T cells to launch a stronger attack against tumor cells. PD-1: programmed cell death 1. PD-L1: programmed cell death ligand 1. CRC: colorectal cancer.

addition, the combined use of broad-spectrum antibiotics (ampicillin, streptomycin, and colistin) hindered the antitumor effects of anti-PD-1.[68] This was evident from the lack of response in the group that received the ampicillin, streptomycin, and colistin antibiotics. Alterations in the gut microbiome also resulted in changes of glycerophospholipid metabolism, which could impact the expression of immune-related cytokines IFN-γ and IL-2 within the tumor microenvironment. The observed changes and their impact suggest a potential association with the variable therapeutic outcomes of PD-1 antibody treatment.[68] Overall, these findings indeed emphasize the critical role of the gut microbiota in the efficacy of PD-1 antibody-mediated anticancer effects.

In parallel, Goc *et al.*[103] demonstrated that the establishment of microbiota-induced anti-tumoral type-1 immunity in the intestine and tumor microenvironment requires a conversation between T cells and group 3 innate lymphoid cells (ILC3s) through major histocompatibility complex class II (MHCII). Indeed, the lack of ILC3-specific MHC-II results in invasive CRC and resistance to anti-PD-1 immunotherapy in mice. Additionally, these authors showed that humans with dysfunctional intestinal ILC3s carry microbiota that do not induce type-1 immunity or promote immunotherapy responsiveness when transferred to mice.[103] These results reinforce the importance of the ILC3s-microbiota binomial in modulating anti-tumor responses and demonstrate that disruption of their function in CRC leads to impaired adaptive immunity, tumor progression and resistance to immunotherapy.

Huang *et al.* [104], in turn, obtained exciting results by employing a multi-omics approach to evaluate the synergistic impact of fecal microbiota transplantation (FMT) and anti-PD-1 therapy in curing colorectal tumor-bearing mice. The combination therapy resulted in significantly higher tumor control and survival rate in mice compared to those treated with FMT or anti-PD-1 therapy alone. Metagenomic analysis showed that the gut microbiota composition of tumor-bearing mice treated with anti-PD-1 therapy was notably altered following FMT. Additionally, metabolomic analysis of mouse plasma identified several potential metabolites, such as punicic acid and aspirin, which increased after FMT and could promote the response to anti-PD-1 therapy *via* their immunomodulatory properties.[104]

Similarly, Zhang *et al.*[105] demonstrated that the gut microbiota of healthy individuals significantly increased the sensitivity of CRC tumor-bearing mice to anti-PD-1 therapy, whereas the gut microbiota of CRC patients did not have the same effect. Through 16S rRNA gene sequencing, these authors isolated a novel strain of Lacticaseibacillus, which was named L. paracasei sh2020. Mechanistically, the antitumor immune response induced by L. paracasei sh2020 was found to be CD8+ T cell-dependent. *In vitro* and *in vivo* experiments revealed that *L. paracasei* sh2020 also stimulated the upregulation of CXCL10 expression in tumors, which subsequently enhanced the recruitment of CD8+ T cells. Interestingly, the modulation of gut microbiota caused by L. paracasei sh2020 improved both its antitumor effect and gut barrier function.[105]

Therefore, the differential composition of the patient microbiome has been shown to affect antitumor immunity and the efficacy of anti-PD-1/PD-L1 immunotherapy in preclinical mouse models. Nevertheless, further studies are needed to investigate the extension of these effects in humans.

BIOMARKERS FOR CRC DIAGNOSIS AND PROGNOSIS BASED ON GUT MICROBIOTA

Studies investigating the influence of gut microbiota on the development and treatment of CRC have led to a new stage, focused on exploring potential biomarkers for non-invasive diagnosis and prognosis evaluation. These biomarkers are identified through the detection of dysbiosis in microbial taxa and metabolites within the affected individual's microbiota.[106] Genomic analysis of the microbiota has enabled the association of specific strains, such as *F. nucleatum* and *B. fragilis*, with CRC, making them promising biomarkers and prognostic targets.[106,107]

Among the techniques adopted for identifying CRC-linked strains, fecal 16S rRNA sequencing has gained popularity due to its specificity and sensitivity, serving as a potential biomarker for prognosis. However, further research is required to address the limitations of this approach, such as defining its sensitivity and specificity more precisely.[108] Additionally, exploring the relationship between intestinal metabolites and bacterial taxa associated with CRC holds promise for enhancing non-invasive diagnosis. In a specific study, the association of 11 metabolites and 6 bacterial species demonstrated substantial potential as colorectal cancer markers.[109] This association includes SCFAs and their producing bacteria, which play a protective role on the intestinal mucosa.[106] Investigating the interplay between microbial taxa and metabolites is crucial for identifying robust and reliable biomarkers for CRC diagnosis and prognosis. Future advancements in this area may lead to improved early detection and personalized treatment options for CRC patients.

MANIPULATION OF THE MICROBIOTA TO ENHANCE THERAPEUTIC RESPONSE

The therapeutic response of CRC patients is intricately linked to the composition of the microbiota, as previously mentioned above. To enhance treatment efficacy, researchers are investigating the effects of secondary interventions aimed at manipulating the microbiota.[110]

Antibiotics

Recent studies have shown conflicting findings regarding the impact of antibiotics on immunotherapy efficiency in oncologic treatment. A meta-analysis of 2,740 patients revealed that antibiotic use negatively affected the efficacy of ICIs due to alterations in the individual's microbiota composition and potential elimination of beneficial bacterial strains linked to improved immunotherapy outcomes.[111] However, some researchers advocate for the prophylactic use of antibiotics to prevent CRC development, particularly by eliminating aggressive pathogens like *Fusobacterium nucleatum*, which is associated with poor prognosis in CRC tissues.[112] Physicians treating oncologic patients undergoing immunotherapy should carefully consider the use of antibiotics to preserve the resident microbiota while also exploring their potential role in preventing carcinogenesis.[111–113]

Fecal microbiota transplant

Fecal microbiota transplant (FMT) involves the transfer of a healthy and complete microbiota ecosystem from a donor to a recipient to restore homeostasis in a dysbiotic region, potentially enhancing the response to immunotherapy.[114,116] While FMT has shown promise in treating intestinal infections, such as Clostridium difficile infections, its application in CRC is still underexplored. Studies suggest that FMT may positively impact the treatment of cancer patients and even serve as a preventive measure against tumor genesis in high-risk individuals.[117,118]

Nevertheless, research concerning the correlation between FMT and CRC remains scarce. While some knowledge exists regarding the advantageous effects on chronic inflammation, the association of this process with cancer remains unexplored. Consequently, further investigations in this domain are imperative, given the highly promising potential of FMT to enhance the efficacy of cancer treatment and even serve as a preventive measure against tumor development in high-risk individuals.[117]

Prebiotics

Prebiotics are substrates that positively influence the development of the microbiota, promoting probiotic growth and strengthening the individual's immune response.[117] Studies have demonstrated that prebiotics may have a potential role in preventing CRC development by modulating the microbiome, restoring homeostasis, and producing SCFAs, which protect the intestinal mucosal barrier.[118] Furthermore, SCFAs can reduce the release of pro-inflammatory cytokines, associated with cancer development, through their interaction with G protein-coupled receptors on colon cells and immune cells.[118]

Probiotics

Probiotics consist of live microorganisms that, upon ingestion, induce a novel composition of an individual's microbiota, resulting in positive health impacts and intestinal protection through modulation of the microbiome.[117,119] In the field of medicine, probiotics are already employed for the prevention and treatment of various diseases, particularly those with inflammatory aspects. This efficacy stems from their ability to fortify the intestinal mucosa, thereby creating a natural barrier against harmful pathogens.[119] As such, the potential of probiotics in the treatment and prevention of CRC is substantial.

Probiotics offer specific mechanisms that play a crucial role in both preventing and treating CRC. One such mechanism is the (1) reinforcement of the intestinal mucosal barrier. Within probiotics, there are selective bacteria that stimulate the production of mucus, leading to the strengthening of the intestinal barrier. Moreover, these beneficial bacteria outcompete aggressive pathogens, effectively preventing their colonization.[117] Another significant mechanism is the (2) reduction of intestinal inflammation. Specific probiotic strains, including *Bifidobacterium infantis* and *Lactobacillus acidophilus*, possess the ability to modulate the immune system. Through the regulation of pro-inflammatory cytokine expression, they promote immune system homeostasis and effectively mitigate intestinal inflammation.[117] Finally, probiotics also demonstrate the ability to (3) inhibit pathogenic toxic activity. In the gut, certain microorganisms release toxins that have been linked to carcinogenesis due to their aggressive nature. However, the introduction of probiotic bacteria creates a natural competition, regulating the production of these toxins and mitigating their harmful effects.[119]

Furthermore, a randomized double-blind study involving 52 CRC patients, who received probiotics four weeks after the surgical procedure, demonstrated the safety and positive impact of this intervention on the treatment. Notably, inflammation was reduced, as evidenced by a clear decrease in pro-inflammatory cytokine levels among the subjects.[120] This finding underscores the potential therapeutic value of probiotics in managing CRC.

Postbiotics

Postbiotics are by-products and metabolites excreted by microbiota microorganisms that play vital roles in host health. Specific postbiotics, such as SCFAs and the p40 protein produced by *Lactobacillus rhamnosus*, have been identified as crucial in preventing CRC development.[121,122] SCFAs regulate gene expression, induce apoptosis in cancer cell lines, and contribute to mucosal protection, while the p40 protein aids in reducing intestinal epithelial inflammation and increasing IgA production.[121,122] Although the use of postbiotics shows promise in CRC prevention, further research is also required to confirm their potential.

Diet

Research has indicated that diet, particularly non-digestible fermentable carbohydrates like resistant starches, plays a significant role in modulating the gut microbiota. Consuming whole grains, fruits, and vegetables has been associated with favorable changes in the microbiome and increased production of SCFAs, which possess anticancer effects.[123,124] Studies also suggest that a Mediterranean diet may reduce inflammation, protect the intestinal barrier, and decrease aggressive microorganisms such as *F. nucleatum*.[125] However, more extensive studies are needed to explore the impact of diet on microbiota modulation in CRC patient.

Manipulating the microbiota presents a promising avenue to enhance therapeutic responses in CRC patients. Secondary treatments such as antibiotics, prebiotics, probiotics, postbiotics, and FMT hold potential for optimizing treatment outcomes by influencing the composition of the microbiome. Additionally, diet plays a significant role in microbiota modulation and may have implications for CRC prognosis. However, further research is warranted to fully understand and leverage the therapeutic potential of microbiota manipulation in CRC treatment and prevention.

CONCLUSION

The relationship between the microbiota and colorectal cancer emerges as a complex process involving both positive and negative aspects. Dysregulation of the microbiota can impact carcinogenesis and lead to a poor prognosis, while a balanced microbiota can positively influence immunotherapy outcomes, thereby offering potential benefits for cancer patients. The microbiome's direct involvement in intestinal homeostasis and its active role in organ protection through the production of mucus and stimulation of the immune system *via* cytokines are well-established.

Furthermore, the discovery of the microbiota's influence on immune checkpoint inhibition has opened a new and promising avenue for immunotherapy. Nevertheless, while some advances have been made in manipulating the microbiota to enhance colorectal cancer treatment, many underlying processes remain incompletely understood, necessitating further and more targeted research.

Despite these uncertainties, the manipulation of the

microbiota holds great promise, potentially revolutionizing the treatment of CRC and offering a renewed outlook for thousands of patients who face the challenges of this debilitating and lethal disease daily. By unlocking the full potential of these novel techniques, we may pave the way for improved quality of life and better prognoses for those affected by colorectal cancer. Therefore, comprehensive, and rigorous investigations in this field are warranted to advance our understanding and translate these discoveries into tangible clinical benefits.

DECLARATIONS

Author contributions

All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version. All authors agree to be accountable for all aspects of the work in ensuring that questions that are related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of interest

All authors declare no potential conflicts of interest.

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