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The current status and prospects of gut microbiota combined with PD-1/PD-L1 inhibitors in the treatment of colorectal cancer: a review

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Abstract

Background Colorectal cancer (CRC) is a common malignant tumor. Immune checkpoint inhibitors (ICIs), particularly those targeting programmed cell death protein 1(PD-1) and programmed cell death ligand 1(PD-L1), have shown promising potential in the treatment of CRC. Specific gut microbiota can modulate the efficacy of ICIs through immune or metabolic pathways. This review summarizes recent advances in the combined application of gut microbiota and PD-1/PD-L1 inhibitors in the treatment of CRC, aiming to provide insights for expanding clinical treatment options for CRC.

Materials and methods We employed a systematic search strategy to screen relevant literature from databases such as PubMed, EMBASE, Medline, Cochrane Library, and Clinical Trial registries, with the search period covering from the inception of each database to October 2024. This study includes animal models and human trial subjects. Data extraction and literature screening were strictly carried out by two independent researchers.

Results A total of 8 animal studies and 5 clinical trials were included to evaluate the effects of gut microbiota combined with PD-1/PD-L1 inhibitors in CRC. Tumor types included Microsatellite Stability(MSS), Microsatellite Instability-Low(MSI-L), and MSI-H CRC. Main outcomes were tumor volume, weight, and incidence; one study reported survival. Study durations ranged from 20 days to 26 weeks. Two studies used human fecal microbiota transplantation(FMT), and six applied experimental microbial interventions. The 5 clinical trials used ORR as the primary endpoint.Some also reported DCR, PFS, and OS. Two studies targeted Microsatellite Instability-High(MSI-H)/

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Deficient Mismatch Repair(dMMR), two MSS/Proficient Mismatch Repair(pMMR), and one lacked molecular subtype specification. All trials used full microbiota transplantation; one has released preliminary data.

Conclusion The treatment regimen combining gut microbiota with PD-1/PD-L1 inhibitors has shown promising therapeutic prospects in both animal studies and clinical research, although most clinical trials are data remain limited. Future studies should focus on: (1) gene-edited probiotic strains with targeted modifications; (2) the synergistic effects of multiple probiotics; and (3) conducting high-quality, multicenter clinical trials.

Keywords Gut microbiota, Colorectal cancer, PD-1, PD-L1, immunotherapy, Systematic review

Introduction

Colorectal cancer (CRC) is a malignancy with high incidence and mortality rates worldwide [1–3]. Although surgical resection remains the primary treatment for CRC [4–6], immunotherapy has emerged as a hot research area in CRC treatment, driven by advances in tumor immunology. Existing studies suggest that the development and progression of CRC are closely linked to immune evasion mediated by the programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) signaling pathway, making PD-1 and PD-L1 key molecular targets for immunotherapy in CRC [7]. Although PD-1/PD-L1 inhibitors have shown good clinical efficacy in the treatment of CRC [8], their effectiveness is limited to Microsatellite Instability-High(MSI-H) CRC patients, with no response observed in a larger proportion of Microsatellite Stability(MSS) patients. Their efficacy is also constrained by factors such as the tumor microenvironment, spatial heterogeneity, and T cell exhaustion.

In recent years, the key role of the gut microbiota in diseases affecting the nervous, endocrine, and urinary systems has become increasingly apparent, earning it the title of the "second genome" of humans. Its significance in regulating health and disease is now widely recognized [9–11]. Studies have shown that the gut microbiota not only influences the development and progression of various cancers but also significantly modulates the effectiveness of tumor immunotherapy [12]. Compared to healthy individuals, the diversity and abundance of the gut microbiota in CRC patients are significantly reduced [13], with a specific microbial imbalance that affects the biological progression of CRC. For example, *Fusobacterium nucleatum* (Fn) can activate the β-catenin signaling pathway through Toll-like receptor 4 (TLR4), promoting the onset of CRC [14], while *enterotoxigenic Bacteroides fragilis* (ETBF) secretes *Bacteroides fragilis toxin* (BFT) toxins [15] that lead to inflammation and CRC progression. In contrast, certain gut microbes, such as *Bifidobacterium spp.*, play a protective role in the prevention and treatment of CRC. In a study by Asadollahi et al. [16], *Bifidobacterium* significantly inhibited the growth of colon cancer cells by downregulating the expression of oncogenes such as EGFR, HER-2, and PTGS-2 (COX-2), and reduced tumor incidence in a mouse model. Furthermore, Lin et al.'s research also demonstrated that combining sprouted brown rice with *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* could suppress CRC development in rats, further supporting the protective effects of these probiotics [17].In summary, the gut microbiota in CRC primarily promotes the occurrence and progression of CRC through chronic inflammation (such as the release of pro-inflammatory factors induced by ETBF toxins and Fn bacteria) and metabolic regulation [18] (such as bile acid metabolism dysregulation and reduced short-chain fatty acids). Notably, Fn may induce myeloid-derived suppressor cells (MDSCs) to suppress anti-tumor immunity, leading to resistance to PD-1 inhibitor treatment in CRC [19]. Some probiotics, such as *Lactobacillus spp.* [20], can regulate the intestinal immune microenvironment, enhance T cell anti-tumor activity, and improve the efficacy of PD-1/PD-L1 immunotherapy. Additionally, fecal microbiota transplantation(FMT) combined with ICIs may improve $CD8^+$ T cell function [21] and antigen presentation by reshaping the gut microbiota, thereby enhancing the response to PD-1 blockade treatment and becoming a potential strategy for improving immunotherapy efficacy [22]. Despite the increasing research on combining gut microbiota with PD-1/PD-L1 immune checkpoint inhibitors (ICIs) in CRC treatment in recent years, clinical evidence remains limited, and the specific efficacy and mechanisms are yet to be fully elucidated. Therefore, this review discusses the mechanisms of CRC-related microbiota and their regulation of the efficacy of PD-1/PD-L1 inhibitors, aiming to provide new insights for personalized treatment of CRC.

Methods

Search strategy

This protocol has been developed and registered in PROSPERO (CRD42025630604). This study follows the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). All methods and reporting were conducted in accordance with the guidelines to ensure the transparency and reproducibility of the research [23]. To ensure methodological rigor, we followed the AMSTAR (A Measurement Tool to Assess

Systematic Reviews) guidelines for evaluating the methodological quality of systematic reviews.

The references for this review were obtained by searching databases including PubMed, Embase, MEDLINE, Cochrane Library, and clinical trial registries. the search phrases "Colorectal Carcinoma and Immunotherapy and Gut Microbiota", "Colorectal Carcinoma and Immunotherapy and Intestinal Microbiology", "Colorectal Carcinoma and PD-1/PD-L1 and Gut Microbiota", "Colorectal Carcinoma and PD-1/PD-L1 and Intestinal Microbiology", "Colorectal cancer and Immunotherapy and Gut Microbiota", "Colorectal cancer and Immunotherapy and Intestinal Microbiology", "Colorectal cancer and PD-1/PD-L1 and Gut Microbiota", "Colorectal cancer and PD-1/PD-L1 and Intestinal Microbiology"were used to retrieve relevant English-language literature. Search terms were combined and arranged as needed to ensure comprehensive retrieval of all relevant references.

Inclusion criteria and study selection

This review established strict inclusion criteria: For animal studies:1).The study subjects must be animals.2).The intervention must include both PD-1/PD-L1 inhibitors and gut microbiota modulation in CRC models.3).The study must provide a detailed description of the methodology, and the results must be scientifically reliable.4). Articles categorized as editorials, letters, case reports, or case series were excluded.For clinical studies:1).The study subjects must be human participants.2).The study design must be a randomized controlled trial (RCT).3).The intervention must include both PD-1/PD-L1 inhibitors and gut microbiota modulation in patients with CRC.4).The study must provide a detailed description of the methodology, and the results must be scientifically reliable.5). Articles categorized as editorials, letters, case reports, or case series were excluded.Two independent authors (M D and XY L) carefully screened the initial search results based on the inclusion criteria, removing duplicate and irrelevant studies. They then conducted a further review of the preliminary eligible studies, examining aspects such as study design and outcomes. Discrepancies were resolved through discussion and consensus to finalize the studies that met the inclusion criteria.

Results

Literature search

A total of 487 studies were identified in the initial search. After removing duplicates, 240 studies remained. Screening titles and abstracts yielded 50 potentially eligible studies. Of these, 13 studies met the final inclusion criteria after full-text evaluation. The study selection progress is presented in the PRISMA flow diagram (Fig. 1).

Characteristics of included studies

This study included eight animal experiments and five clinical studies. The eight animal studies (Table 1)investigated the therapeutic effects of gut microbiota combined with PD-1/PD-L1 inhibitors in mouse models of CRC. All included studies utilized subcutaneous tumor models, with three studies additionally employing orthotopic tumor models.Regarding tumor classification, one study focused on MSS CRC, five on microsatellite instability-low (MSI-L) CRC, and five on MSI-H CRC. Each study included more than 20 animals, with a minimum of 5 animals per experimental group.The outcomes recorded in all studies included key metrics such as tumor volume, tumor mass, and incidence rates. Additionally, one study provided data on animal survival. The experimental duration ranged from 20 days to as long as 26 weeks.In terms of gut microbiota transplantation, two studies utilized human gut microbiota, while six employed specific experimental bacterial consortia.

The five clinical trials (Table 2) investigated the efficacy of gut microbiota combined with PD-1/PD-L1 inhibitors in patients with CRC. As of now, these trials are ongoing, but preliminary results from one study have been reported in the literature.Among these trials, two specifically focused on patients with MSI-H/Deficient Mismatch Repair(dMMR) CRC, while another two targeted MSS/Proficient Mismatch Repair(pMMR) CRC patients. The remaining trial aimed to stratify patients based on their disease classification.In terms of efficacy endpoints, all five clinical studies evaluated the objective response rate (ORR). Additionally, one study also assessed the disease control rate (DCR), and another study included key metrics such as progression-free survival (PFS) and overall survival (OS).Regarding gut microbiota interventions, all five clinical studies employed full gut microbiota transplantation methods.

In preclinical models, three independent studies demonstrated that Lactobacillus rhamnosus (LGG) synergistically enhanced immunotherapy efficacy in both MSI-H and MSI-L CRC mice. Specific microbial interventions—Lactobacillus gallinarum, Roseburia intestinalis, and Fusobacterium nucleatum—were further validated in one study each for these subtypes. Notably, FMT showed efficacy in MSI-L models, while Lacticaseibacillus paracasei SH2020 targeted MSI-H tumors. Clinically, FMT also improved outcomes in MSS CRC patients, bridging preclinical mechanisms to human applications.

Literature analysis and review

In the preclinical and clinical studies included in the analysis, the impact of gut microbiota on the efficacy of PD-1/PD-L1 immune checkpoint inhibitors in CRC treatment was investigated through various gut microbiota interventions.

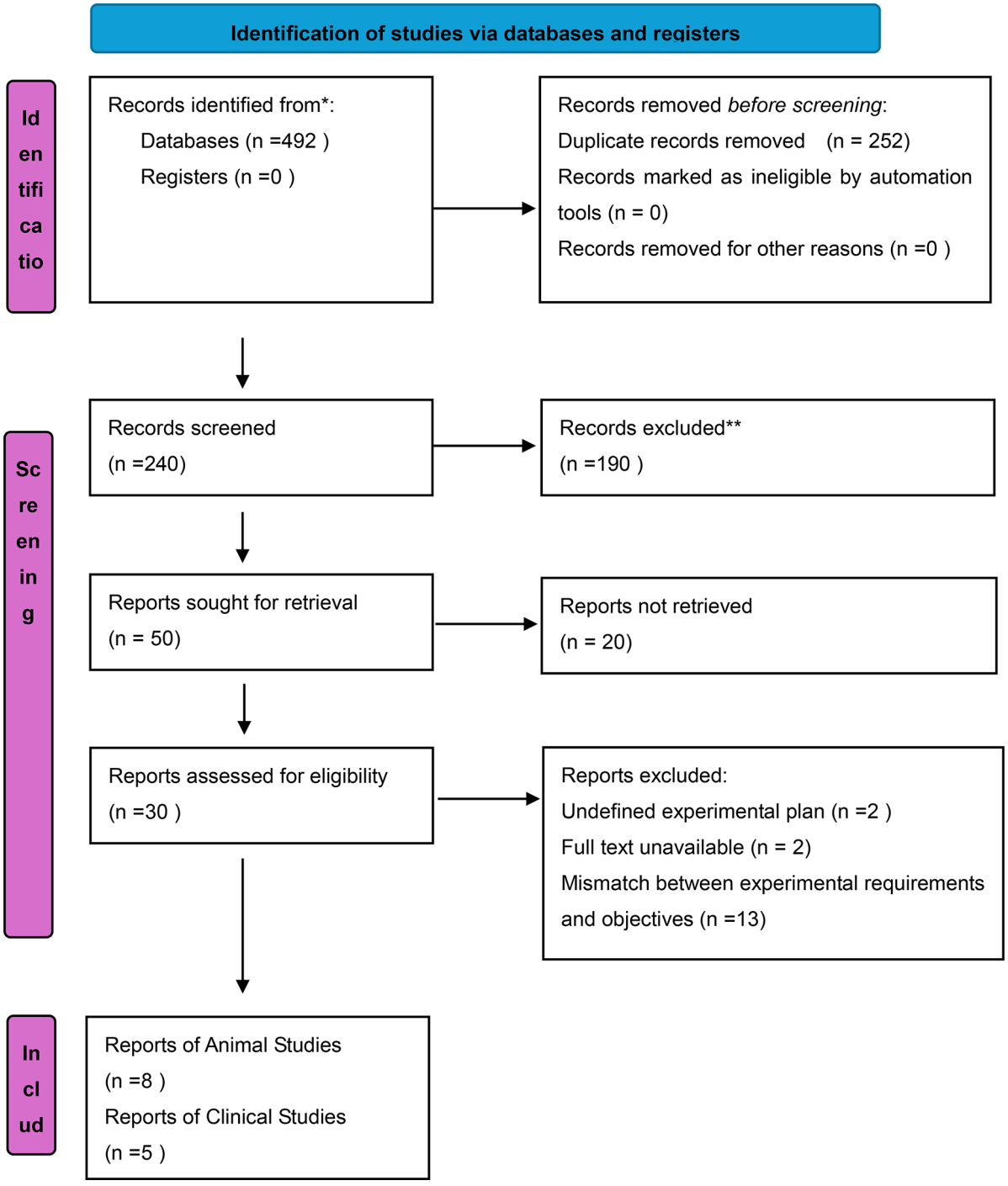

Fig. 1 PRISMA fow chart of study selection

Lacticaseibacillus rhamnosus

Lacticaseibacillus rhamnosus(LGG) is a common Gram-positive bacterium, a facultative anaerobic, non-spore-forming short rod, primarily residing in the gastrointestinal and reproductive tracts in humans. It is one of the most widely used probiotics in fermented foods. Numerous studies, both domestic and international, have confirmed that LGG not only exhibits resistance to gastric acid and multiple antibiotics but also possesses various functions, including the regulation of gut ecology, gut function, and the immune system [33, 34].

Live LGG

In the study by Wei Si et al. [29], it was demonstrated that oral administration of live LGG enhances the therapeutic efficacy of PD-1/PD-L1 immune checkpoint inhibitors.

Table 1 Characteristics of the included animal studies

Author	Country	Year	Animal	*n*	Groups	MSI Model	Tumor Model	Intervention Strategy	Duration(day)	Outcome Measures
Lu et al. [24]	China	2023	mice	20	4	MSI-H	Subcutaneous	LGG-EV	28	Tumor Weight、Tumor Volume
Wang et al. [25]	China	2024	mice	NA	4	MSI-L + MSS	Subcutaneous + Orthotopic	Fusobacterium nucleatum	182	Tumor Weight、Tumor Volume、Survival Time
Zhang et al. [26]	China	2022	mice	NA	2	MSI-H	Subcutaneous	FMT + Lacticaseibacillus paracasei sh2020	30	Tumor Weight、Tumor Volume、 Survival Rate
Gao et al. [27]	China	2023	mice	48	2	MSI-L	Subcutaneous	Lacticaseibacillus rhamnosus Probio-M9	33	Tumor Weight、Tumor Volume
Fong et al. [28]	China	2023	mice	NA	4	MSI-L + MSI-H	Subcutaneous + Orthotopic	Lactobacillus gallinarum	22	Tumor Weight、Tumor Volume、Tumor Number
Wei et al. [29]	USA	2022	mice	20	3	MSI-H	Subcutaneous	Lactobacillus rhamnosus GG	28	Tumor Weight、Tumor Volume 、Tumor Incidence
Huang et al. [30]	China	2022	mice	40	2	MSI-L	Subcutaneous	Fecal Microbiota Transplantation(FMT)	24	Tumor Weight、Tumor Volume、Survival Rate
Kang et al. [31]	China	2023	mice	38	4	MSI-H + MSI-L	Subcutaneous + Orthotopic	Roseburia intestinalis	30	Tumor Incidence、Tumor Weight、Tumor Volume、Tumor Number

Table 2 Characteristics of the included clinical studies

Study	Country	Year	Enrollment	MSI / MMR	Intervention Strategy	Primary outcome measures	Research status	Results
ChiCTR2100046768	China	2021	20	MSS/pMMR	FMTplus tislelizumab and fruquintinib	ORR DCR	Recruiting	YES[32]
NCT05279677	China	2022	30	MSS/pMMR	FMT + PD-1 ICIs + Sintilimab Injection + Fruquintinib	ORR	Recruiting	NO
NCT04729322	China	2021	15	dMMR	Pembrolizumab/Nivolumab + FMT	ORR	Recruiting	NO
ChiCTR2100052431	China	2021	—	MSI-H/dMMR	FMT + PD-1 ICIs	ORR PFS OS	Active, not recruiting	NO
NCT04130763	China	2019	10	—	FMT + PD-1 ICIs	ORR	Recruiting	NO

In vitro experiments confirmed that LGG did not affect the colony-forming ability of colon cancer cells, and oral administration of inactivated LGG similarly did not modulate the therapeutic effects of PD-1/PD-L1 inhibitors in mice. Additionally, the LGG microbiota in tumor tissues did not regulate the treatment efficacy of PD-1/PD-L1 inhibitors. Further mechanistic investigations revealed that both the combination therapy and LGG monotherapy resulted in increased levels of IFN and CD8 + T cells, confirming that the cGAS/STING pathway is essential for LGG's anti-tumor effects and induction of type I IFN. The cGAS-STING-TBK1-IRF7-IFN-β signaling cascade mediated a strong adaptive immune response to LGG in dendritic cells (DCs). Moreover, the combination therapy of LGG and anti-PD-1 shifted the gut microbiota towards an enrichment of *Lacticaseibacillus* and *Bacteroides uniformis*, increasing DC activation and CD8 + T cell recruitment.

LGG-EV

Building on the findings from the aforementioned study that confirmed the role of LGG in CRC immunotherapy,

Lu S et al. [24] conducted a study in which they isolated the active components from the LGG strain to derive extracellular vesicles (LGG-EV). These vesicles were cultivated in vitro to protect against damage caused by gastric acid and bile, thus enhancing their stability in the gastrointestinal tract. In preliminary in vitro experiments, the addition of LGG-EV to colon cancer cells showed anti-proliferative effects. In vivo experiments demonstrated that tumors formed by LGG-EV-treated colon cancer cells had significantly smaller volumes and weights.In the experiment by Wei Si et al. [29], it was already established that oral administration of LGG enhances the anti-tumor activity of PD-1 inhibitors by increasing tumor-infiltrating T cells and dendritic cells (DCs). In this study, the effects of purified LGG-EV on CRC treatment with PD-1 immune checkpoint inhibitors were further investigated. Four groups were established based on the presence or absence of LGG-EV gavage and anti-PD-1 treatment. The combination therapy group showed significantly reduced tumor volume and weight compared to the other groups. Cellular composition analysis demonstrated that oral LGG-EV enhanced lymphocyte infiltration into tumor tissues, including antigen-presenting dendritic cells and CD4 + and CD8 + T cells. Additionally, the CD8+/CD4 + ratio in the intestinal lymph nodes was elevated.This study also analyzed the gut microbiota of the experimental groups and confirmed that oral LGG-EV significantly altered the composition of the mouse microbiota. A correlation was observed between serum metabolites and changes in the gut microbiota, particularly an increase in serum uridine levels, suggesting that uridine may be a potential metabolite contributing to the enhanced efficacy of anti-PD-1 therapy.

LGG Probio-M9

In the study by Gao et al. [27], a series of experimental groups were set up, including a blank control group, preventive LGG Probio-M9 and anti-PD-1 combination therapy group, therapeutic LGG Probio-M9 and anti-PD-1 combination therapy group, and a control group with only anti-PD-1 treatment. The study found that both the preventive and therapeutic combination treatment groups significantly synergized with PD-1 immune checkpoint inhibitors to enhance anti-tumor effects. Further investigation revealed that supplementation with LGG Probio-M9 synergistically promoted the growth of beneficial microbes, such as lactobacilli and Bifidobacterium breve, while producing beneficial metabolites like butyrate in the gut. Additionally, it led to the accumulation of α-ketoglutarate, N-acetyl-L-glutamine, and pyridoxal in the blood. This process facilitated the infiltration and activation of cytotoxic T lymphocytes (CTLs) while inhibiting the function of regulatory T cells (Tregs) in the tumor microenvironment (TME), thereby enhancing the anti-tumor effects of anti-PD-1 treatment. Moreover, the study showed that transplantation of gut microbiota or gut metabolites from probiotic-treated mice into tumor-bearing mice could transfer this enhanced immune response, suggesting that microbiome-based immunotherapy strategies have potential clinical applications.

LGG is also involved in the immune modulation processes of various tumors. In a mouse model of breast cancer, the experimental group treated with oral LGG Probio-M9 showed significant increases in cytokines such as IL-9 and IL-27, as well as a notable elevation in serum metabolites, including niacin, pyridoxamine, glutamine, and β-hydroxybutyrate. This treatment enhanced the anti-tumor capabilities of the mouse model and inhibited tumor growth [35]. In a mouse model of lung adenocarcinoma [36], LGG aerosol treatment promoted the activation of the local immune microenvironment, suppressing tumor growth. The study verified the inhibitory effects on malignancies by examining the distinct roles and mechanisms of action in both the gut and lung microbiomes.

Fusobacterium nucleatum

Fusobacterium nucleatum (Fn) is a Gram-negative anaerobic bacterium belonging to the family Fusobacteriaceae and the order Fusobacteriales, which can inhabit the human gastrointestinal tract. Existing studies have confirmed that Fn is involved in the development of several system-related diseases in the human body. In the cardiovascular system, Fn promotes endothelial injury by increasing the permeability of human microvascular endothelial cells and decreasing the expression of cell adhesion molecules, thereby facilitating the progression of atherosclerosis [37]. In the digestive system, research suggests that Fn may promote tumor growth through the ERK/STAT3 signaling pathway and participate in tumor immunology processes [38].

Wang X et al. [25] focused on MSS CRC. Through microbiome analysis of 25 CRC patients undergoing PD-1 inhibitor treatment, they found a positive correlation between the abundance of Fn and CRC progression, and a negative correlation with PD-1 expression. Furthermore, patients with high tumor and intestinal Fn levels had favorable prognoses following anti-PD-1 treatment. In further mechanistic investigations, the primary focus was on tumor-associated microbiota. It was confirmed that Fn within the tumor produced abundant butyrate, which inhibited HDAC 3/8 to enrich H3K27 acetylation at the TBX 21 promoter in CD8 + tumor-infiltrating lymphocytes (TILs). This enhanced the expression of TBX21, which subsequently downregulated PD-1 expression. This process reactivated cytotoxic CD8 + TILs within the tumor, alleviating CD8 + TIL exhaustion in MSS CRC,

and ultimately enhancing the anti-tumor effects of PD-1 inhibitor therapy.

To date, the impact of *Fusobacterium nucleatum* on the efficacy of PD-1 inhibitors in CRC remains somewhat controversial. Various species of *Fusobacterium* can activate ALPK1 in CRC cells through the release of ADP-heptose or phosphorylated heptose, thereby regulating the downregulation of DNA mismatch repair genes and the upregulation of the checkpoint inhibitor protein PD-L1 [39]. Based on these findings, researchers have proposed another hypothesis that tumors activated by *F. nucleatum* through ADP-heptose release and ALPK1 activation may be more sensitive to immune checkpoint inhibitor (ICI) treatment. Although this requires further validation, it shares similarities with the results from Wang X et al. [25]. Another study also supports this hypothesis [40], where the injection of Fn into a CRC mouse model activated the STING signaling pathway in CRC cells, leading to the upregulation of PD-L1 expression via NF-κB transcription. Anti-PD-L1 antibody treatment blocked the interaction between PD-1 on TILs and PD-L1 on tumor cells, further increasing the number of IFN-γ + CD8 + TILs to kill tumor cells.Another viewpoint suggests that high levels of Fn in the gut are associated with reduced efficacy of immune therapy [41]. In a mouse model treated with *F. nucleatum* via oral gavage, its metabolites, such as succinate, impaired the cGAS-mediated IFN-β response and activated the SUCNR1-HIF-1α-EZH2 axis, which weakened the response to immune therapy. Other studies [42] have also confirmed the promotive effect of Fn on colorectal tumor growth and metastasis. Given the differing responses to combined therapies for the same bacterial species, we believe this discrepancy may be due to the differing roles played by the microbiome in the tumor microenvironment versus the gut microbiome.

Lacticaseibacillus paracasei

Lacticaseibacillus paracasei is a Gram-positive, facultative anaerobic bacterium, characterized by a rod shape, lack of flagella, and inability to form spores. It commonly appears as single cells, pairs, or short chains [43]. This bacterium is widely found in fermented foods (such as pickles, yogurt, cheese) and in the human gastrointestinal tract, and is primarily used in the production of fermented dairy products like yogurt and cheese. The role of L. lactis in humans is mainly to regulate gut function, participate in intestinal immunity, and play an important role in metabolism.

In the study by Zhang et al. [26], the transplantation of the gut microbiota from healthy human donors demonstrated a clear improvement in the effectiveness of anti-PD-1 therapy for CRC. Further 16 S rRNA sequencing revealed that the ratio of Firmicutes/Bacteroidetes in the FMT-HD group was significantly higher than that in the FMT-CRC group, while the relative abundances of Lactobacillaceae, Bifidobacteriaceae, and Ruminococcaceae gradually increased. In subsequent strain isolation and screening, a novel strain of Lacticaseibacillus paracasei was identified and named Lacticaseibacillus paracasei sh2020. Further mechanistic studies confirmed that Lacticaseibacillus paracasei sh2020 might induce the expression of chemokines such as CXCL10 in the tumor, which regulates CD8 + T cell infiltration into the tumor. Additionally, Lacticaseibacillus paracasei sh2020 increased the relative abundance of lactobacilli in anti-PD-1 treated mice, thereby exerting a synergistic anti-tumor effect with PD-1 immune checkpoint inhibitors.

Other Lacticaseibacillus paracasei strains have also shown inhibitory effects on the development and progression of CRC. Lacticaseibacillus paracasei K5, a strain isolated from sheep cheese, demonstrates effective adhesion to Caco-2 human colon cancer cells and exerts anti-proliferative regulatory effects [44]. Lacticaseibacillus paracasei PC-H1 [45] inhibits glycolysis mediated by HIF-1α to exert its anti-proliferative activity against colon cancer cells. Additionally, the Lacticaseibacillus paracasei strain CMU-Pb-L5 inhibits tumor proliferation and promotes apoptosis by suppressing polyamine synthesis [46, 47].

Roseburia intestinalis

Roseburia intestinalis is a Gram-positive, strictly anaerobic bacterium belonging to the phylum Firmicutes, class Clostridia, and family Lachnospiraceae. It is an important member of the human gut microbiota [48]. *Roseburia intestinalis* plays a crucial role in maintaining intestinal barrier integrity, suppressing inflammatory responses (such as regulating regulatory T cells), and preventing diseases such as CRC.

In the study by Kang et al. [31], *Roseburia intestinalis* was found to have a low abundance in the fecal microbiota of CRC patients. Further research using cell, mouse, and organoid models confirmed the inhibitory effect of *Roseburia intestinalis* on CRC. It was discovered that the intestinal metabolite butyrate produced by *Roseburia intestinalis* can activate cytotoxic CD8 + T cells, thereby enhancing the efficacy of anti-PD-1 treatment. These findings suggest that *Roseburia intestinalis* may serve as a potential adjunct to PD-1 inhibitor therapy, improving its effectiveness in CRC.

In addition, a study by Jiali Dong et al. [49] also found that *Roseburia intestinalis* plays a promoting role in the radiotherapy of CRC patients. The increased abundance of *Roseburia intestinalis* in the gut produces and releases butyrate, which activates the OR51E1/RALB signaling pathway, accelerating radiation-induced autophagy in

CRC cells. This, in turn, enhances the radiosensitivity of both primary and metastatic CRC.

Lactobacillus gallinarum

A study by Sugimura N et al. [50] pointed out that *Lactobacillus gallinarum* exhibits tumor-suppressive properties both in vitro and in vivo. In further research [28], the bacterium was found to secrete indole-3-carboxylic acid (ICA) by participating in the tryptophan metabolism pathway, thereby inhibiting the production of kynurenine (Kyn) in tumors. Additionally, *Lactobacillus gallinarum* can influence T cell activity through specific signaling pathways, enhancing the anti-tumor effects of PD-1 inhibitors. In CRC patients, supplementation with *Lactobacillus gallinarum* significantly improved the efficacy of anti-PD-1 treatment. The study found that this bacterium reduces the infiltration of Foxp3 + CD25 + regulatory T cells in tumors and enhances the effector function of CD8 + T cells, thereby synergizing with anti-PD-1 therapy.

FMT

In the study by Huang et al. [30], mice were divided into four groups based on whether they received FMT and PD-1 immune checkpoint inhibitor treatment. The results confirmed that the combination treatment group exhibited better survival rates and tumor control compared to the monotherapy groups. Further analysis of the gut microbiota in the combination treatment group revealed a significant increase in several species of *Bacteroides* and *Parabacteroides*. Additionally, the synthesis metabolic pathways of certain amino acids, such as ornithine and histidine, were notably enriched under FMT treatment. Nucleotides, including deoxyribonucleotides, guanosine nucleotides, and adenosine nucleotides, were significantly upregulated in the de novo biosynthesis pathway in both the EMT and combination treatment groups.

Excluding eight animal studies, clinical research with established results also employs FMT as an intervention measure [32]. In the study by Zhao W et al., the experimental group received a combination of FMT, PD-1 immune checkpoint inhibitors, and apatinib (a VEGFR inhibitor) for treatment. All patients were of the MSS type. Among the 20 patients involved in the study, the median progression-free survival (PFS) was 9.6 months (95% CI: 4.1–15.1), and the median overall survival (OS) was 13.7 months (95% CI: 9.3–17.7). The study included a responder group (PFS ≥ 6 months; $n = 12$) and a non-responder group (PFS < 6 months; $n = 8$). In the responder group, an increase in Firmicutes at the phylum level was observed, while at the genus level, significant increases were seen in *Lachnospira*, *Roseburia*, *Clostridium*, and *Escherichia* genera. Biological processes such as glycolysis, TCA cycle, amino acid metabolism, flagellum assembly, and bacterial chemotaxis were enriched, alongside the amplification of the TCR CDR3β cluster in peripheral blood.

MSS and MSI

In PD-1 inhibitor treatment for CRC, there are significant differences between dMMR/MSI-H and pMMR/MSS CRC. According to the KEYNOTE-016 study [51], the objective response rate (ORR) in dMMR mCRC patients can reach 40%, while pMMR mCRC patients do not benefit from monotherapy with immune checkpoint inhibitors, with an ORR of 0%. dMMR/MSI-H CRC patients, who account for 15% of all CRC cases, show a good response to PD-1 inhibitors and other immune therapies [52]. The significant efficacy is primarily due to the increased production of neoantigens mediated by high tumor mutation burden and substantial immune cell infiltration, which activates the immune function of the tumor microenvironment [53–55]. On the other hand, MSS and MSI-L CRC, which account for 85% of cases, have immune-suppressive cells [56] (such as myeloid-derived suppressor cells, MDSCs) infiltrating the tumor microenvironment, a lower tumor mutation burden [57], and limited activation of T cell-mediated immune responses, making immune therapy less effective. To improve the efficacy of immune checkpoint inhibitors in MSS and MSI-L CRC, extensive research has been conducted in recent years on combination immunotherapy [58–60], including VEGF inhibitors, anti-EGFR monoclonal antibodies, BRAF inhibitors, and radiation therapy, which have shown promising clinical outcomes.

Now, let's focus on the research related to gut microbiota combined with PD-1 inhibitors in the treatment of CRC. Among the 8 animal studies listed in this research, 5 studies have confirmed that the combination of gut microbiota enhances the therapeutic effect in MSI-H CRC. Additionally, 5 studies have demonstrated that the gut microbiota can improve the efficacy of immunotherapy in MSS and MSI-L CRC. Furthermore, one clinical study, which is supported by existing literature, has also confirmed that the involvement of gut microbiota improves the prognosis of MSS CRC patients in a clinical setting.

There are significant differences in the gut microbiota between MSS and MSI-H CRC [61]. Regarding the composition of the gut microbiota, MSI-H CRC patients have a more diverse microbiota [62], predominantly composed of Firmicutes, Bacteroidetes, Actinobacteria, and Clostridia [63, 64], while MSS CRC patients predominantly harbor Proteobacteria. In terms of gut microbiota metabolites, MSI-H CRC patients show higher levels of hydrogen sulfide and butyrate [65], which are mainly involved in nucleotide metabolism and peptide degradation. In

contrast, MSS CRC patients exhibit higher levels of propionate and lactate [66], with notably lower levels of short-chain fatty acids. These differences in gut microbiota and metabolites significantly impact the tumor microenvironment of different CRC subtypes, thereby influencing the therapeutic efficacy of CRC treatments.

The intratumoral microbiome and gut microbiota

In CRC immunotherapy, the intratumoral microbiome and gut microbiota exhibit significant differences in their mechanisms of action and clinical impacts. CRC patients have a reduced diversity and abundance of gut microbiota compared to healthy individuals, with a marked decrease in the abundance of Bacteroidetes and Firmicutes phyla, and a notable increase in levels of Escherichia coli, Enterococcus, and Clostridium perfringens, among others [67–70]. The gut microbiota regulates host immunity systemically through its metabolites, such as short-chain fatty acids (SCFAs) [71] and tryptophan derivatives [72]. In CRC patients, the abundance of SCFA-producing bacteria, such as Lactobacillus and Bifidobacterium, is significantly reduced [73]. These gut microbiota metabolites exert antitumor effects by downregulating the classic Wnt signaling pathway associated with CRC development and modulating various tumor cell characteristics, including cell proliferation, thereby inhibiting tumorigenesis.The tryptophan-derived metabolic pathways in the gut microbiota primarily include the indole pathway [74], serotonin system in enterochromaffin cells [75], and kynurenine pathways in immune cells and the intestinal epithelium [76], which regulate the inflammatory microenvironment [77] and reduce tumor-infiltrating CD8 + T cells to exert anticancer effects. On the other hand, the intratumoral microbiome influences tumorigenesis through various mechanisms, such as promoting DNA damage and mutations, cell proliferation, metastasis, and modulating the tumor immune microenvironment. For instance, the protein encoded by the pks loci of Escherichia coli can induce DNA double-strand breaks [78], leading to DNA damage and CRC development. Fusobacterium nucleatum activates the TLR4/MyD88/NF-κB signaling pathway, further triggering the RAS signaling pathway to promote tumor proliferation [79]. The genus Clostridium [80] affects the interferon (IFN)-γ signaling pathway, regulating the activity of cytotoxic T cells and regulatory T cells, as well as the sensitivity of tumors to immune checkpoint inhibitors (such as PD-1/PD-L1 inhibitors), thereby influencing tumorigenesis and progression.

In the studies listed in this research, *Lactobacillus gallinarum* [28] primarily participates in the indole and kynurenine pathways within the gut microbiota metabolites, demonstrating significant antitumor properties. By supplementing the gut with *Lactobacillus gallinarum*, tumor progression is delayed, and the therapeutic effect of anti-PD-1 inhibitors is enhanced. *Roseburia intestinalis* and *Lacticaseibacillus rhamnosus* both exhibit antitumor effects and enhance PD-1 treatment efficacy through gut microbiota-mediated pathways. In contrast, *Fusobacterium nucleatum* enhances the effect of anti-PD-1 treatment in CRC through both intratumoral and gut microbiota pathways.

Conclusion

Immunotherapy for CRC has made significant progress, with new PD-1/PD-L1 inhibitors continuously emerging. The combination of gut microbiota and PD-1/PD-L1 inhibitors has demonstrated promising therapeutic effects in both animal models and clinical studies, showing potential benefits in both MSS and MSI-H CRC. The synergistic mechanism of gut microbiota with anti-PD-1 immunotherapy is not merely the presence of microbiota but relies on the precise regulation of metabolites and immune signaling pathways. For instance, short-chain fatty acids such as butyrate [81, 82] influence immune cells like ILC3, T cells, and B cells [83] and the extracellular matrix, thereby impacting gut immunity and immune tolerance, allowing for personalized treatment based on the patient's microbiota profile.

Despite the promising results, several challenges remain: the composition of the microbiota is closely related to factors like host diet, antibiotics, and genetic background. While strict inclusion criteria are used in clinical studies, ensuring baseline consistency among enrolled patients remains difficult. Current microbiota studies mainly focus on single probiotics, yet microbiota function is ecologically network-dependent, and negative results from single-strain transplantation warrant further investigation. Future research should integrate multidisciplinary innovations: gene-editing technologies could be used to engineer probiotics as immunotherapy agents; further exploration of microbiota functions should investigate interactions between multiple strains to identify potential roles for "immune-activating bacteria," "metabolic-supporting bacteria," and "barrier-repairing bacteria" [84]; high-quality clinical trials should be conducted with standardized experimental protocols and intervention measures.

In conclusion, the combination of gut microbiota with anti-PD-1 immunotherapy has achieved significant research outcomes, offering valuable insights into clinical treatment strategies for CRC patients.

Abbreviations

ALPK1	Alpha Kinase 1
CRC	Colorectal cancer
CTL_s	Cytotoxic T lymphocytes
DCR	Disease control rate
DCs	Dendritic cells
dMMR	Deficient Mismatch Repair

EGFR	Epidermal Growth Factor Receptor
ETBF	Enterotoxigenic Bacteroides fragilis
FMT	Fecal microbiota transplantation
Fn	Fusobacterium nucleatum
ICA	Indole-3-carboxylic acid
ICI_s	Group 3 Innate Lymphoid Cells
Kyn	Kynurenine
LGG	Lactobacillus rhamnosus
$MDSC_s$	Myeloid-derived suppressor cells
MSI-H	Microsatellite Instability-High
MSI-L	Microsatellite Instability-Low
MSS	Microsatellite Stability
ORR	Objective response rate
OS	Overall survival
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
pMMR	Proficient Mismatch Repair
PFS	Progression-free survival
PTGS-2	Prostaglandin-Endoperoxide Synthase 2
SCFAs	Short-chain fatty acids
TIL_s	Tumor-infiltrating lymphocytes
TLR4	Toll-like receptor 4
TME	Microenvironment
Tregs	Regulatory T cells, Tregs
VEGFR	Vascular Endothelial Growth Factor Receptor
CXCL10	Interferon --inducible Protein 10
HER-2	Human Epidermal growth factor Receptor 2

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study is a review of previously published clinical research and does not involve any new studies with human participants or animals. Therefore, ethical approval, informed consent, clinical trial registration, and clinical trial identification number are not required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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