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Review

REVIEW

Dysbiosis of gut microbiota in promoting the development of colorectal cancer

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Abstract

Gastrointestinal microbiome, containing at least 100 trillion bacteria, resides in the mucosal surface of human intestine. Recent studies show that perturbations in the microbiota may influence physiology and link to a number of diseases, including colon tumorigenesis. Colorectal cancer (CRC), the third most common cancer, is the disease resulting from multi-genes and multi-factors, but the mechanistic details between gut microenvironment and CRC remain poorly characterized. Thanks to new technologies such as metagenome sequencing, progress in large-scale analysis of the genetic and metabolic profile of gut microbial has been possible, which has facilitated studies about microbiota composition, taxonomic alterations and host interactions. Different bacterial species and their metabolites play critical roles in the development of CRC. Also, microbiota is important in the inflammatory response and immune processes deregulation during the development and progression of CRC. This review summarizes current studies regarding the association between gastrointestinal microbiota and the development of CRC, which provides insights into the therapeutic strategy of CRC.

Key words: gut microbiota, microbiome dysbiosis, colorectal cancer, tumorigenesis

Introduction

Colorectal cancer (CRC), including carcinogenesis of the colon and rectum, is a major cause of incidence and mortality in the world [1]. CRC has been ranked third in terms of cancer death, causing near 500 000 deaths per year, and its incidence has been a health care challenge worldwide [2,3]. Despite the progress that has been made, CRC is still one of the deadliest cancer types, with different molecular phenotypes and strong resistance to therapies [4] and a very high mortality rate [5]. Thus, there is an urgent need to identify risk factors/biomarkers for CRC [6]. Recently, metagenome-wide association studies on fecal samples have characterized microbial markers of CRC [7,8]. Furthermore, the causal impact of bacteria on cancer has been recognized [9,10]. In this review, we will discuss the link between gut microbiota and CRC, as well as the potential cancer therapeutic strategy by employing the regulations involved.

Human gut microbiota and CRC

Due to new technologies that have allowed large-scale analysis of the genetic and metabolic profile of the gut microbial community, we now have a better understanding of the composition and functions of the human gut microbiota [7,8,11–14]. There are at least 100 trillion bacteria that live in our gut

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system, i.e. gut microbiome. The human gut microbiota is a complex ecosystem, with a biomass of about 1.5 kg. Moreover, the compositions of microbes are various in different parts of the gut, including ascending colon, distal colon, proximal ileum and jejunum, and they are critical for normal functioning of our homeostasis and health, including the digestion of food, vitamin biosynthesis, behavioral responses and protection from pathogens [15]. The majority of the endogenous bacteria in healthy adults are representatively two phyla, *Firmicutes* and *Bacteroidetes*, which account for approximately 90% of the microbial system [16]. The microbiome can work with the host to promote health but can sometimes initiate or promote disease [11,17,9].

Emerging evidence has shown that the dysbiosis of gut microbiota can lead to alteration of the host physiology, resulting in the pathogenic processes of different diseases [18, 19]. Gut microbiota can promote the development and progression of CRC by different processes, including the induction of a chronic inflammatory state or immune response, altering stem cell dynamics, the biosynthesis of toxic and genotoxic metabolites, and affecting the host metabolism [20,21]. Here we will list some of those roles in CRC tumorigenesis, focusing on inflammation, immune response and metabolites.

Microbial species and cancer

As a part of the tumor microenvironment, gastrointestinal microbiome participates in the development of a substantial number of gastrointestinal tract malignancies [22,23]. Several recent studies have implicated a link between dysbiosis of gut microbiota and CRC [24,25].

Emerging evidence has indicated that these microbes may induce inflammation, facilitate cell proliferation and provide a microenvironment for host cells to alter stem cell dynamics and produce metabolites that affect glycolysis or immune response [20]. However, details about the contribution or the molecular mechanism of the microbiome remain to be characterized [26].

Fortunately, the analysis of microbial composition and diversity has been facilitated by the advancement of next-generation sequencing technologies. By performing metagenomic sequencing on fecal samples, Yu *et al.* show that specific species, such as *Fusobacterium nucleatum* (*F. nucleatum*), *Peptostreptococcus stomatis* and *Parvimonas micra*, are enriched in colorectal carcinoma. The study confirms not only the associations between gut microbiota and CRC, but also the involvement of specific members of microorganisms in contributing to the development of CRC [7]. With conserved regions interspersed by specific variable regions, the 16S ribosomal RNA (rRNA) gene has been the most widely applied molecular signature for the studies of the microbial community. The 16S rRNA contains approximately 1500 base pairs and comprises nine variable regions, which can be used for taxonomic classification [27]. Studies have identified that several bacteria, such as *F. nucleatum*, *Escherichia coli* (*E. coli*), *Bacteroides fragiles* (*B. fragilis*) and *Enterococcus faecalis*, were increased in CRC patients [25], whereas the *Clostridiales*, *Faecalibacterium*, *Blautia* and *Bifidobacterium* were absent [24,28] (**Table 1**).

Fusobacterium nucleatum (F. nucleatum)

Using quantitative PCR, 16S rDNA sequencing or FISH analysis, increased abundance of symbiotic *Fusobacterium spp.* has been observed in colorectal adenomas and cancer [29–33]. Castellarin and colleagues uncovered the role that *F. nucleatum*, a Gram-negative oral anaerobe, played in CRC by showing that F. nucleatum DNA is enriched in tumor tissue and correlates with lymph node metastasis [29]. To evaluate the prognostic significance of F. nucleatum DNA in CRC, Mima *et al.* detected the F. nucleatum DNA in tissue of 1069 CRC cases and confirmed that F. nucleatum DNA is associated with shorter survival in CRC patients [30]. Through a series of animal experiments and human studies, Kostic *et al.* suggested that *F. nucleatum* can induce carcinogenesis through the inflammatory nuclear factor-kappab (NF-kb) signaling pathway and by down-regulating anti-tumor T cell-mediated adaptive immunity [32,34,35]. Using the APC (Min/+) mouse model treated with human isolates of *F. nucleatum*, they also found that *F. nucleatum* induced tumorigenesis by recruiting tumor-infiltrating myeloid cells, which facilitate cancer progression [32]. In other words, it creates a proinflammatory environment to promote cancer growth. The Fap2 protein of *F. nucleatum* can associate with TIGIT, an inhibitory receptor present on natural killer (NK) cells and T cells, to inhibit NK cell cytotoxicity [36]. Also, the adhesion and invasion of *F. nucleatum* into epithelial cells were mediated by FadA adhesin, which binds to E-cadherin and stimulates the beta-catenin pathway, leading to the activation of proinflammatory and oncogenic signals [37]. Another study shows that Fad causes vascular endothelial (VE)-cadherin to be away from cell–cell junctions, thereby increasing the permeability of endothelial cells to allow

Table 1. Special bacterium related to colorectal cancer (CRC)

Microorganism	Expression/role in affecting CRC	Function
Enterococcus faecalis	Driver	Producing extracellular superoxide causing DNA breaks [160]
Shigella	Driver	Inflammation induction
Escherichia coli NC101	Driver	Genotoxin production, synthesizing toxins cyclomodulins [44,45]
Bacteroides fragilis	Driver	*B. fragilis* toxin production; stimulating E-cadherin cleavage; inducing the Th17/IL-17 inflammatory response [52]
Streptococcus bovis	↑	Chronic inflammatory response [161]
Helicobacter pylori	↑	Producing multi-functional toxin VacA [162]
Fusobacterium nucleatum	↑	Enriched in CRC; instigating inflammatory nuclear factor-kappa b (NF-kb) signaling pathway [32,34]; triggering the Wnt signaling pathway [30,37]
Bifidobacterium	Protective	Reduced the β-glucuronidase activity [62]
Eubacterium rectale	↓	Butyrate producer [163]
Clostridium septicum	↓	Producing secondary bile acids [125]
Faecalibacterium prausnitzii	↓	Generating butyrate [164]
Lactobacillus	Protective	Reducing production of lactic acid; activation of Toll-like receptors [165]

bacteria to cross the junctions [38]. Furthermore, evidence from Mima *et al.* indicated that *F. nucleatum* promotes tumor growth by triggering the Wnt signaling pathway in colorectal carcinoma cells or down-regulating CD3+ T cell-mediated adaptive immunity [30]. These observations suggest that *F. nucleatum* facilitates the tumorigenesis via several critical signaling pathways. Further investigation is needed to unravel the interactive roles and mechanisms of *F. nucleatum* on host immunity. However, as a normal resident of the oral microflora, whether *F. nucleatum* is a cause or consequence of CRC is still not clearly understood.

Escherichia coli (E. coli)

E. coli, a member of intestinal microbiota, is a Gram-negative and facultatively anaerobic bacterium, which can be divided into five phylogenic groups (A, B1, B2, D and E). Various studies confirmed that pathogenic strains of *E. coli* played critical role in colorectal tumorigenesis [39–42]. *E. coli* induced CRC in interleukin 10 (IL-10)-deficient mice, suggesting that inflammation is essential for the tumorigenesis [41].

Based on the ability of *E. coli* to induce inflammation, Martin *et al.* showed that the levels of *E. coli* increased in Crohn's disease and CRC patients [43]. Buc *et al.* found that the *E. coli* strains of the B2 phylogroup favored the colonization of colon cancer [44]. Moreover, recent studies show that pathogenic *E. coli* could synthesize toxins designated cyclomodulins, such as cytolethal distending toxins (CDT), cytotoxic necrotizing factor (CNF), cycle inhibiting factor and colibactin, which were genotoxic or interfering with the cell cycle [44–46]. CDT could induce DNA damage through DNase activity [47]. In addition, two studies provide evidence that CDT has carcinogenic potential *in vivo* [48,49]. Ge and colleagues demonstrated that CDT could promote a NF-κB proinflammatory response, resulting in hepatocarcinogenesis [50]. The prevalence of cyclomodulin- and genotoxin-encoding genes in *E. coli* strains from CRC patients confirms the link between *E. coli* and CRC.

Bacteroides fragilis (B. fragilis)

The anaerobe *B. fragilis* is a commensal bacterium in gut, which can be classified into two subtypes: nontoxigenic *B. fragilis* (NTBF) and enterotoxigenic *B. fragilis* (ETBF) [51–53]. Studies indicate the inflammatory potential of ETBF and the contributions of ETBF in CRC [53]. Infection of ETBF will increase Th17 and T regulatory cells (Treg), resulting in early tumor development [54]. *B. fragilis* toxin (BFT), a zinc-dependent metalloprotease toxin, is involved in many colonic epithelial cell signal transductions, such as NF-κB, Wnt and mitogen-activated protein kinase (MAPK) signaling pathways, thereby inducing the production of inflammatory mediators that facilitate CRC development [55]. In addition, murine experiments have demonstrated the crucial roles of BFT and the Th17/IL-17 axis in ETBF carcinogenesis [52], which could promote the differentiation of myeloid cells into myeloid-derived suppressor cells, triggering colon tumorigenesis by the pathogenic inflammation pathway [56].

Bifidobacterium

Probiotics, some special bacteria existing in the gut, could exert numerous beneficial effects on the host. The most common types of probiotics are lactic acid bacteria (LAB), mainly the *Lactobacillus* and *Bifidobacterium* genera, including other genera such as *Enterococcus, Streptococcus* and *Leuconostoc* [57]. Gut microbiome studies have confirmed the preventive and treatment effects of theses probiotics in patients with inflammatory bowel disease or CRC [58]. Furthermore, the ratio of *Bifidobacterium* to *Escherichia* (B/E) is always used to indicate the intestinal flora. The number of *Bifidobacterium* will decrease significantly in CRC, while *Escherichia* increases [59]. Sivan *et al.* showed that oral administration of *Bifidobacterium* alone can influence immune response against tumors in several different mice models [60]. β-glucuronidase activity of gut bacteria reactivates chemotherapeutic CPT-11 in the gut, causing diarrhea during chemotherapy [61]. Drugs have been designed to inhibit this undesirable β-glucuronidase activity in gut bacteria to enhance chemotherapeutic efficacy [61]. *Bifidobacterium* influences the growth of CRC cells by reducing the glucuronidase activity, although the detailed mechanisms remain to be further characterized [62]. Together, it is possible that *Bifidobacterium* may also enhance chemotherapeutic efficacy.

Lactobacillus

Lactobacillus is a genus of Gram-positive, facultative anaerobic bacteria. Among many available probiotic strains, *Lactobacillus rhamnosus*, widely used clinically, is well characterized in terms of its anti-inflammatory role in modulating cytokine-producing human dendritic cells [63]. *Lactobacillus rhamnosus* reduces the expression of beta-catenin and the inflammatory proteins NFkappaB-p65 and induces the expression of p53 and BAX [64]. Thus, treatment of *L. rhamnosus* as a prophylactic measure could reduce the incidence and multiplicity of colon tumors, through inducing cell apoptosis and inhibiting the inflammation [64]. Moreover, it has been demonstrated that *Lactobacillus* administration to mice regulates the expression of Toll-like receptor 2 (TLR2), TLR4 and TLR9, especially TLR2, while it decreases the tumor incidence [65]. *Lactobacillus rhamnosus* GG could enhance the intestinal epithelial barrier function in a TLR2/cyclo-oxygenase-2-dependent manner [66,67]. The detailed mechanistic regulations remain to be investigated.

Taken together, evidence suggests a contributory role for microbiota in CRC development. Numerous pathogenic mechanisms have been demonstrated, although not fully illustrated. A burning question remains of whether microbial association with cancer is the cause or the consequence. It is known that several bacterial species have been identified and linked to colorectal carcinogenesis, such as *E. coli*, *Streptococcus bovis*, *B. fragilis*, *Enterococcus faecalis*, *Fusobacterium spp.* and *Clostridium septicum* [25]. And it seems that Gram-positive Clostridiales, including multiple members of Clostridium Group XIVa, are negatively correlated with tumors [28]. Thus, there is accumulating evidence that characteristic changes in the gut microbiome are associated with colorectal cancer development [23,68,69]. It is expected that an altered community of gut microbes is associated with CRC development. Because the gut bacteria have a modifiable nature, further studies may have potential implications for managing gut bacteria for CRC treatment.

Inflammation and immune responses

The interaction between microorganisms and the host immune system frequently happens in the gastrointestinal area [70]. Inflammation, an adaptive response triggered by internal and external stimuli, has become a hallmark of neoplastic transformation of epithelial cells and microbiome has roles in intestinal inflammation [71], thereby furthering CRC development [72]. During the process of inflammation, the leukocytes in the human body changed accordingly. Macrophages, dendritic cells, NK cells and neutrophils would produce reactive oxygen species

(ROS) to cause DNA damage of intestinal epithelial cells, and also increase levels of enzymes such as cyclooxygenase-2 (COX-2). All these events are critical for the induction of mucosal tumorigenesis [73].

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a group of inflammatory disorders affecting the colon and small intestine. It is well established that patients with IBD have an increased risk of 10–15% of developing CRC [74]. Interestingly, Taurog and colleagues found that the genetically modified animal model of IBD (B27 transgenic rat) did not develop colitis when it was raised in a germ-free (GF) environment [75]. Also, it has been reported that $CD45RB^{low}$ $CD4^{+}$ T lymphocytes have IBD-inducing potential [76]. But its ability to induce colitis in immune-deficient recipients was significantly compromised when the $CD45RB^{low}$ $CD4^{+}$ T cells were derived from GF mice [77]. Taken together, gut microbiota acts importantly in affecting the host immunity, thereby regulating the inflammatory process of IBD. Indeed, certain gut flora are involved in pathogenic processes of IBD. Many studies have demonstrated the role of *E. coli* in Crohn's disease [78]. It has been reported that ETBF, a member of the human commensal, triggers the activation of epithelial signal transducers and activator of transcription 3 (STAT3) [79], which is a protein family member that regulates the immune response. Significantly, the disruption of the intestinal barrier will be followed by IBD and CRC [80]. *Citrobacter rodentium*, one of the Gram-negative bacteria mainly colonizing the surfaces of the cecum and colon, can induce inflammation by the Th1/Th17 immune response [81]. Moreover, *Clostridium* clusters IV and XIVa have a critical role in maintaining mucosal homeostasis and preventing IBD [82]. Other microorganisms, such as *fusobactrium* and *mycobacterium*, were found to be increased in IBD [83,84]. Collectively, existing evidence suggests microbiome dysbiosis may lead to CRC by inducing inflammatory or immune response.

Pattern recognition receptors (PRRs) are a primitive part of the immune system, which can sense microbiota through molecular structures, including the Toll-like receptors (TLRs), the nucleotide-binding oligomerization (NOD)-like receptors (NLRs), the RIG-I-like receptors, the C-type lectin receptors, the absent in melanoma 2 (AIM2)-like receptors and the OAS-like receptor [85]. TLRs, a class of proteins, are commonly expressed in sentinel cells such as macrophages and dendritic cells. Once the intestinal barrier is disrupted by microorganisms, TLRs will recognize these microbes and induce the expression of some cytokines, finally activating immune responses.

TLR signaling has been characterized in recent years. There are two important TLR pathways: one dependent on myeloid differentiation factor 88 (MyD88) adaptor proteins and the other a TRIF-dependent pathway [86,87]. Most TLRs use MyD88 as the downstream adapter, except TLR3. In the MyD88-dependent pathway, once TLRs are activated, it will subsequently activate the downstream factors, including NF-κB, MAPK and interferon regulatory factors [87]. Previous studies revealed the role of microbiota in TLR-dependent recognition in CRC [87]. Calcineurin (Cn), a phosphatase responsible for the activation of the nuclear factor of the activated T cells (NFAT) family, is highly expressed in CRC and has been implicated in tumor development and metastasis [88]. In human CRC cells, after stimulating TLR2 and TLR4 by gut bacteria, both intracellular Ca^{++} and the DNA binding activity of NFAT are increased, which in turn contributes to the tumorigenesis of CRC [88] (**Figure 1**). In the $Apc^{min/+}$/$Myd88^{-/-}$ mice model, the levels of pERK and c-Myc are significantly decreased, which confirms the importance of microbiota-MyD88-ERK signaling during the carcinogenesis of intestinal epithelial cells (IECs) [89].

The ligand of TLR2 is bacterial peptidoglycans. The multiplicity and number of tumors in TLR2-deficient mice are significantly increased when compared with the wild-type control

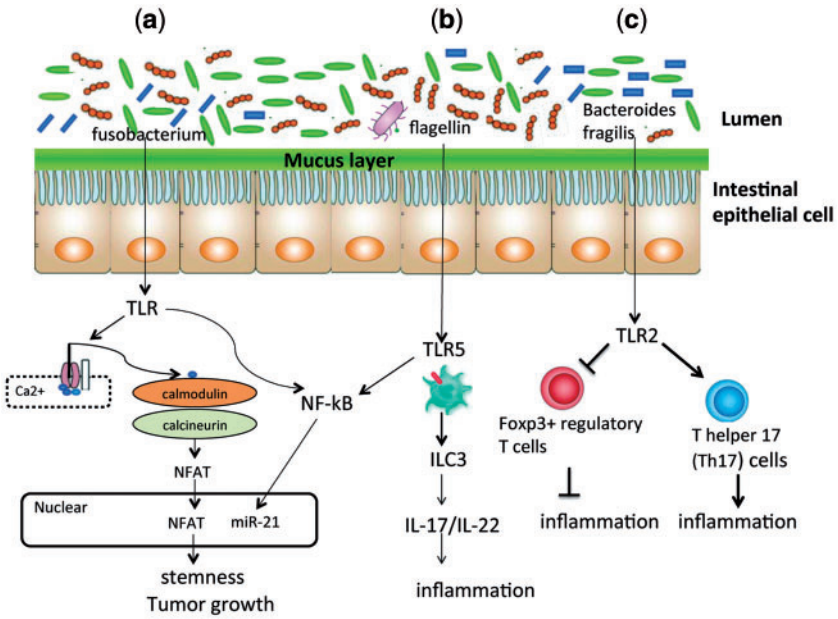

Figure 1. Influence of TLR signaling on carcinogenesis by microbiota. (**A**) Dysbiosis of the luminal microbiota, such as the increase of *Fusobacterium*, induces the expression of TLR4, which activates the calcium-dependent calcineurin and NFAT. In addition, once the TLR4 is activated, it leads to the activation of NF-κb, resulting in the change of several miRNA expressions, including miR21. Finally, tumor growth is promoted. (**B**) TLR5 could recognize bacterium flagellin and induce the activation of NF-κb, regulating the expression of some inflammation-associated cytokines. (**C**) *Bacteroides fragilis* produces polysaccharide A and suppresses anti-microbial immune responses via TLR2 signaling, which promotes the inflammatory T-helper 17 responses whilst inhibiting the Foxp3+ regulatory T cells. TLR, Toll-like receptor; NF-κb, nuclear factor κb; NFAT, nuclear factor of activated T cells; ILC3, Innate lymphoid cells 3.

mice [90]. Previous studies have demonstrated that the interaction between TLRs and commensal bacteria is essential for intestinal epithelial homeostasis [91], which is modulated by cell proliferation and apoptosis in the crypts. However, the loss of TLR2 has been shown to promote cell proliferation, inhibit cell apoptosis, even induce the formation of abnormal crypt foci, and activate IL-6 and STAT3s in early intestinal tumorigenesis, suggesting that TLR2 acts as an important protective factor in intestinal epithelial homeostasis [90]. Moreover, intestinal homeostasis is also maintained by regulatory T cells, including $Foxp3^+$ Treg cells and IL-10-producing type 1 regulatory T (Tr1) cells. Round and colleagues demonstrated that *Bacteroides fragilis* could exploit the TLR2-dependent signaling pathway for regulating the $Foxp3^+$ Treg cells [92] (**Figure 1**). These data suggest a pivotal TLR2-dependent impact on microbiota homeostasis.

TLR4 is a transmembrane protein, which is well known for its ability to bind with bacterial lipopolysaccharide, leading to the activation of inflammatory genes expression through the NF-KB signaling pathway (**Figure 1**). Based on mice models, Van Helden *et al.* proved that Gram-negative bacteria could induce DC migration by selectively activating TLR4, resulting in chronic infections [93]. It has been shown that the binding of TLR4 and MyD88 plays an important function in carcinogenesis by inducing tumor cell proliferation, invasion and migration, escaping from immunosurveillence and developing chemoresistance [92]. In previous studies, Wang *et al.* found that, in patients with CRC, high levels of TLR4 and MyD88 correlate with an increased risk of liver metastasis and worse survival [94,95]. It has been shown that overexpression of TLR4 will promote the activation of NF-κb, which in turn induces COX-2 expression, a biomarker of colorectal carcinogenesis [96] (**Figure 1**).

TLR9, another member of the TLR family expressed by numerous immune cells, such as B lymphocytes, monocytes and NK cells, could recognize unmethylated CpG sequences in DNA molecules, especially bacterial DNA [87]. Recent studies show that, under the condition of genotoxic stress, the level of TLR9 will increase in cancer cells [97]. However, the mechanisms of TLR9 signaling in colonic tumorigenesis remain unclear.

Other receptors also integrate microbial signals to adjust mucosal homeostasis in the human body. For example, NLRP6 is a member of the NLR family of receptors, expressed in IECs, especially in goblet cells. Studies have shown that NLRP6, implicated in inflammasome signaling, is essential for mucosal self-renewal and proliferation. Indeed, compared to the wild-type mice, the NLRP6-deficient mice are more susceptible to intestinal inflammation and chemically induced colitis as well as tumorigenesis in the colon [98].

Immunotherapy, such as using checkpoint inhibitors, has demonstrated clinical benefit in several types of cancer [99]. But the detailed mechanism behind the treatment efficacy is not well characterized. It is shown that optimal cancer immunotherapy responses depend on an intact commensal microbiota that can modulate myeloid-derived cell functions in the tumor microenvironment [100]. Also, the influence of microbiota on host immunity has been reported to affect cancer immunotherapy efficacy [101] (**Figure 2**). It was shown that increased gut bacteria *Bacteroidetes phylum* is related to the host's response to the treatment of immunologic checkpoint blockade with monoclonal antibody ipilimumab [102], which blocks cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4, a major negative regulator of T-cell activation) signaling [103]. In addition, it is shown that Bifidobacterium administration plus programmed cell death protein ligand 1 (PD-L1)-specific antibody therapy can block melanoma tumor growth in mouse models [60]. It remains to be verified in human clinical trials for future clinical application. It is therefore clear that understanding the inflammation and immune mechanisms of microbial-dependent CRC will open new avenues for cancer prevention and improving cancer therapeutic strategies.

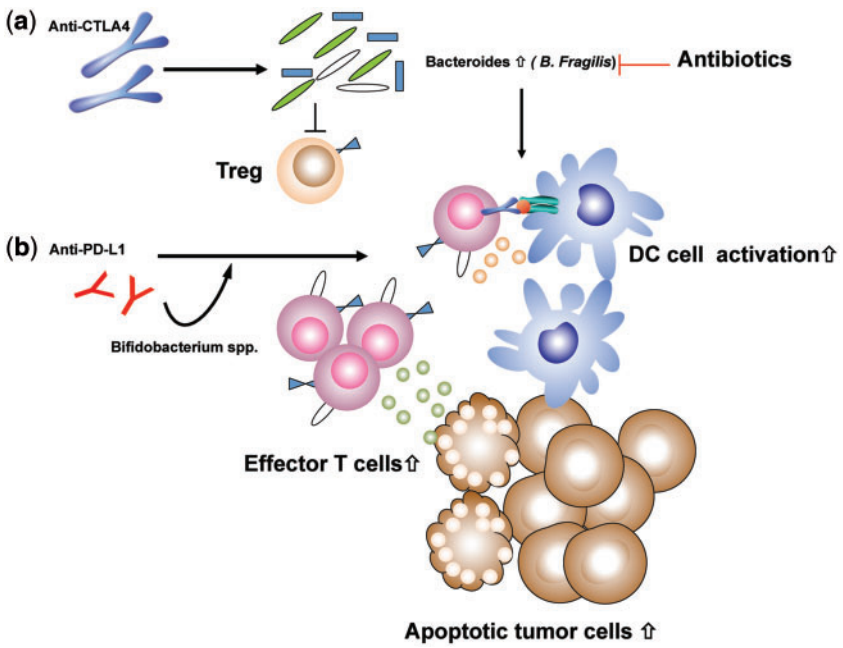

Figure 2. Microbiome affects immunotherapy efficacy. (**A**) Anti-CTLA4 antibodies impair function of Treg and increase *Bacteroides*, thereby improving the anti-tumor response mediated by immune checkpoint blockade (Anti-CTLA4). Antibiotics treatment can dampen the T-cell-mediated anti-tumor immune responses. (**B**) *Bifidobacterium* promotes DC activation and subsequent anti-tumor T-cell responses of anti-PD-L1 therapy. CTLA4, cytotoxic T-lymphocyte-associated antigen-4; Treg, regulatory T cells; DC, dentric cells; PD-L1, programmed cell death protein ligand 1.

Metabolic role of gut microbiota

Gut microbiota can create genotoxic stress or metabolite in the intestinal environment to facilitate genetic and epigenetic changes that lead to cancer [104]. Glycolysis generates adenosine triphosphate and nicotinamide adenine dinucleotide (NAD^+) for poly (ADP-ribose) polymerase (PARP) to repair DNA damage and provide energy for multidrug resistance efflux pumps to discard toxic chemotherapy agents. Thus enhanced glycolysis can impact on drug resistance. It is known that dietary fiber by increasing the abundance of Prevotella can improve glucose metabolism [105]. Barley kernel supplement or high fiber leads to increased Prevotella in gut microbiota, which subsequently protects against Bacteroides-induced glucose intolerance [105]. Glucose intolerance can affect tumor growth and may facilitate drug resistance [106,107]. CRC with KRAS or BRAF mutations often up-regulates GLUT1, a gene encoding glucose transporter-1 involved in glycolysis, to reprogram cancer energy metabolism [108]. In addition, increased glycolysis also enables the diversion of glycolytic metabolites into many other important biosynthetic pathways that are important for cell proliferation [109,110]. Thus, controlling gut microbiota or their metabolites may be a useful strategy in reducing drug resistance. It is therefore critical to illustrate interactions between gut microbiota, metabolism and the host in terms of CRC development. Several metabolites from microbiota are known to be involved in CRC carcinogenesis (**Table 2**).

Fatty acid

It is well known that diet plays an important role in the carcinogenesis of CRC. While high intake of processed meat induces an increased risk of CRC, it has been shown that increased intake of total dietary fiber can decrease the risk [111]. What is the role of dietary fiber in decreasing cancer risk? The discovery of short-chain fatty acids (SCFAs) (**Figure 3**), a kind of fatty acid synthesized by gut microbiota, could bridge the knowledge gap. SCFAs, such as acetate, propionate and butyrate, are products of the fermentation of dietary fiber and are synthesized by various microorganisms (*Bacteroides*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Prevotella*, *Propionibacterium* and others). Clinical studies have revealed that different compositions of gut microorganisms and SCFA levels may be linked to colorectal cancer mortality differentials [112–114]. Recently, SCFAs can change the global chromatin states, suggesting that gene expression change and transcriptional effects due to SCFAs play an important role in CRC development [115]. Butyrate attracts most attention, as it can act as a biomarker of cancer risk during cancer progression. It has been recognized that butyrate can modulate the apoptosis, proliferation and invasion of several cancer cell lines [116]. Butyrate could attenuate human colon cancer cell proliferation and promote apoptosis by reducing c-Myc and regulating p57 levels [117]. It also can induce apoptosis in colorectal cancer [118]. Various evidences demonstrate the inhibitory action of butyrate on inflammation and carcinogenesis. Major actions include inhibiting the production of proinflammatory mediators [119], influencing on NF-kB activation and histone deacetylation [120], and down-regulating Wnt signaling, a pathway that is well known, as it will be constitutively activated in CRC [121]. Butyrate could reinforce different components of the colonic defense barrier and decrease oxidative

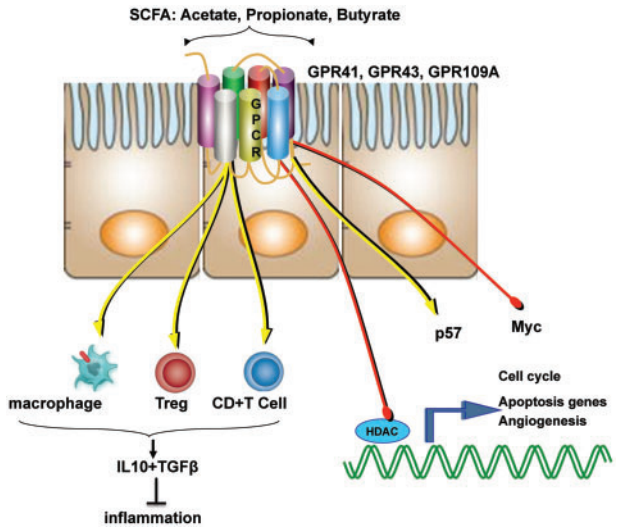

Figure 3. Bacterial metabolites affect inflammation and gene expression. Bacterial metabolites, including SCFAs, interact with GPR41, GPR43 and GPR109A on host cells. Butyrate interacts with GPR109A, promoting the differentiation of Treg and activating macrophages and CD^+ T cells to induce IL10 and TGFβ, thereby blocking inflammation. Butyrate and propionate, after being transported into host cells, cause HDAC inhibition, resulting in hyperacetylation of histones. The HDAC inhibition leads to cell cycle arrest, apoptosis induction and angiogenesis suppression. SCFAs, short-chain fatty acids; GPR, G protein-coupled receptor; Treg, regulatory T cells; IL10, interleukin 10; TGFβ, tumor growth factor β; HDAC, histone deacetylase.

Table 2. Metabolites involved in developing colorectal cancer (CRC)

Metabolites	Mechanism action	Microorganism	Signaling in CRC
Short-chain fatty acids (Butyrate)	Cell differentiation promotion, causing apoptosis, inhibiting tumor growth [116]. Histone deacetylase (HDAC) inhibitor. Binds GPR109A regulates gene expression, inflammation and autophagy	*Bifidobacterium*	Decreased in levels; having the anti-tumor activities
Deoxycholic acid	Activating β-catenin and epidermal growth factor receptor (EGFR) signaling [132]	*Clostridium*	Increased in levels; acting through FXR, PXR, VDR
Lithocholic acid	Promoting cancer invasion and MAPK signaling [136]	*Bacteroides fragilis*	Increased in levels
Ursodeoxycholic acid	Inhibiting the activation of COX-2 [146], blocking Ras activation [147]		Decreased in levels
Bacterial toxin (Fragilysin)	Activating the β-catenin nuclear signaling [157]	*Bacteroides fragilis*	Increased in levels
Trimethylamine-N-oxide (TMAO)	Use L-carnitine or choline to produce TMAO [18]	*Clostridium*	Increased in levels

stress [122]. Strategies, such as the consumption of probiotics and prebiotics, have been designed to stimulate the *Bifidobacterium* species and butyrate-producing bacterial species for maintaining intestinal homeostasis, since the beneficial effects of SCFAs have been reported. It is likely that the identification of mechanisms underlying butyrate may also be useful as a therapeutic strategy in the future.

Secondary bile acids

A wealth of evidence has demonstrated that high-fat diets are associated with increased risk of CRC [123]. Indeed, bile acid metabolism from high-fat diets is critical for this risk. Bile acids, kinds of steroid acids found in the bile, facilitate cholesterol elimination in the liver and absorption of lipids in the intestine. Primary bile acids such as cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized by the liver, mainly via the classic pathway starting with cholesterol 7α hydroxylase (CYP7A1) [124]. Most primary bile acids are involved in enterohepatic circulation, while there are still about 5% that escape and enter the gut cavity, from which the secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid (LCA) are produced by anaerobic microorganisms in the colon [125]. This step is catalysed by an important bacterial enzyme, 7α–dehydroxylase [125]. Human gut microbiota carrying out 7α-dehydroxylation has been shown to belong to the genus *Clostridium*, which are members of the Firmicutes. Insulin resistance has been shown to promote the increase of CA and DCA [126]. Famesoid X Receptor (FXR), an important receptor of bile acids, is not only involved in the regulation of lipid metabolism by influencing the gut microbiota, but also promotes glycogen synthesis and inhibits gluconeogenesis [127–129]. The FXR has been implicated as a tumor suppressor. APC mutation leads to the silencing of FXR expression as a consequence of increasing CpG hypermethylation of the FXR gene, which in turn causes increased expression of Myc and COX-2 [130]. The detailed mechanistic regulation remains to be further studied. However, there is considerable evidence supporting the role for FXR in modulating CRC tumorigenesis [130,131]. These studies show that bile acids have an important role in human body metabolism. Taken together, it is evident that the gut microbiota induces the carcinogenesis of CRC by affecting bile acids.

DCA, one metabolite of the gut microbiota, has been known as a significant contributor to the development of CRC. There is evidence indicating that DCA could modulate intracellular signaling and gene expression [132]. DCA can induce cancer stemness by regulating the muscarinic 3 receptor/Wnt signaling pathway [133]. It can also induce the expression of the orphan nuclear receptor Nur77 [134]. Elevated Nur77 is observed in a majority of human colon tumors to promote tumor growth and survival by serving as a mediator of the Wnt and AP1 signaling pathway [134]. Kong and colleagues showed that DCA would down-regulate the expression of miR-199a-5p in CRC [135]. miR-199a-5p can target CAC1, which contributes to carcinogenesis in patients with CRC, for degradation and functions as a tumor suppressor in colorectal cancer. Thus, DCA contributes to CRC tumorigenesis by decreasing miR-199a-5p expression and/or increasing the expression of CAC1.

Another secondary bile acid produced by gut flora is LCA, which is also proved to be an endogenous CRC promoter. Farhana *et al.* found that both DCA and LCA could promote the generation of cancer stem cells [133]. What is more, LCA can also induce the expression of urokinase-type plasminogen activator receptor (uPAR), which can affect cancer invasion, block inflammatory signals and promote the activation of MAPK signaling pathways in human colon cancer cells [136]. In addition, secondary bile acids have been found to modulate colon carcinogenesis, including the induction of cell proliferation by activating epidermal growth factor receptor (EGFR) pathway signaling [133,137–139], inducing DNA damage, causing oxidative or nitrosative stress [140,141], apoptosis, mutation, activation of protein kinase C pathway in epithelial cells [142], regulating of membrane permeability and gene transcription [140].

Interestingly, there is another bile acid, ursodeoxycholic acid (UDCA), whose chemical structure is similar to DCA. Although structurally similar, UDCA and DCA play different roles in the pathological process of CRC [143]. While DCA acts to promote the development of CRC, previous studies identified that UDCA would suppress the tumorigenesis of CRC [144]. Thus, UDCA may be used as a chemoprevention agent in the future. Khare *et al.* demonstrate that UDCA inhibits the activation and expression of COX-2 in the azoxymethane (AOM) model [145–148]. COX-2 induces carcinogenesis in colon cells. In addition, UDCA can prevent the effects that DCA exerted in the human colon cancer cells, including DCA-induced extracellular signal-regulated kinase (ERK) and Raf-1 kinase activity and the activation of EGFR [149]. Taken together, elucidating the mechanisms involved in bile acids, especially UDCA, may help in identifying a strategy for the prevention of colorectal cancer.

Trimethylamine-N-oxide (TMAO)

Trimethylamine (TMA), an intestinal microbial-dependent metabolite of red meat and fat, reacts with flavin monooxygenase (FMO), leading to the production of Trimethylamine-N-oxide (TMAO), an intestinal microbial metabolite involved in CRC development (Table 2). Omnivorous human subjects produce more TMAO when compared with vegans or vegetarians, since L-carnitine, a TMA in red meat, is processed by gut microbiota to produce TMAO [150]. This explains, at least in part, why vegetarian diets lead to an overall lower incidence of CRC [151].

Research data have revealed that TMAO links to the risk of cardiovascular diseases (CVDs) [150,152]. Wang *et al.* confirmed the link in both mice and humans [153]. They performed metabolomics studies to identify specific metabolic profiles in plasma, and showed that TMAO can act as a biomarker for increased CVD risk. Dietary supplementation of mice with TMAO promotes atherosclerosis [153]. Since CRC is similar to CVDs in risk association with red meat or fat intakes, the link between CRC and TMAO is possible. Indeed, study to detect the relationship between plasma factors of choline metabolism and CRC risk demonstrates that TMAO is the potential indicator of CRC [154]. Xu *et al.* performed a genome-wide analysis and concluded that TMAO correlates with CRC development [155], although the genetic pathway that links TMAO to CRC remains to be characterized.

In addition, there are many CRC-related metabolites produced by gut microbiota, including the bacterial toxin. For example, the fragilysin synthesized by *Bacteroides fragilis* could hydrolyse the extracellular domain of E-cadherin and activate the β-catenin nuclear signaling, which is closely related to Myc induction and CRC development [156,157]. The role of other bacterial toxins warrants further investigation.

Also, butyrate and niacin are bacterial products as a result of the fermentation of dietary fiber in the colon. They bind to GPR109A (Niacr1), a receptor for butyrate and niacin, to suppress intestinal inflammation and carcinogenesis [158], thereby

mediating the beneficial effects of gut microbiota. Basically, Niacr1 signaling promotes anti-inflammatory activities in macrophages and dendritic cells, and instigates induced differentiation of Treg cells and IL-10-producing T cells [158]. Taken together, investigating the metabolic role of microbiota in the development of colon cancer can provide insight into how to take care of the diet for preventing CRC.

Conclusions

Collectively, it has been shown that gut microbiota play a key role in the tumorigenesis of CRC in different ways, especially under the dysbiosis condition. The presence of certain bacteria species has an impact on the risk and development of CRC. It is then critical to investigate how the gut microbiome changes during the development of CRC and whether these changes can contribute to drug resistance or affect treatment efficacy. Investigating gut microbiome changes during tumorigenesis will provide promising insights into diagnostic tools, biomarkers and therapeutic intervention strategies for CRC. It is obvious that there are potentials for developing diagnostic tests based on the analysis of gut microbiota, which will offer improved accuracy, safety and non-intrusiveness and patient compliance. Study of microbiome dysbiosis will facilitate clinical application in CRC patient care.

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