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The role of microbiota in the development of colorectal cancer

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Mini Review

Colorectal cancer is the third largest cancer in worldwide and has been proven to be closely related to the intestinal microbiota. Many reports and clinical studies have shown that intestinal microbial behavior may lead to pathological changes in the host intestines. The changes can be divided into epigenetic changes and carcinogenic changes at the gene level, which ultimately promote the production and development of colorectal cancer. This article reviews the pathways of microbial signaling in the intestinal epithelial barrier, the role of microbiota in inflammatory colorectal tumors, and typical microbial carcinogenesis. Finally, by gaining a deeper understanding of the intestinal microbiota, we hope to achieve the goal of treating colorectal cancer using current microbiota technologies, such as fecal microbiological transplantation.

Introduction

As the world's third-largest cancer, colorectal cancer (CRC) retains a high morbidity and mortality in our country, which imposes a severe burden on the health system and on patients.[1] There are hundreds of kinds of microorganisms in human intestines, forming a symbiotic system with intestinal cells to maintain the intestinal environment. The typical bacteria include *Escherichia coli*, *Enterococcus faecalis*, and *Bacteroides fragilis*.[2] Microbes have their own pathogenicity and carcinogenicity. For example, a class of toxins released from *E. coli* is known as cell death toxins (CDTs). They act directly on the intestinal epithelial cells and cause a highly proliferative epithelium in normal intestinal epithelial cells. The proliferating intestinal epithelial cells form an adenoma and continue to invade the submucosa of the intestinal mucosa, eventually leading to cancerous changes. *Enterococcus*

Key words: microbiota, inflammation, carcinogenesis, colorectal cancer

Abbreviations: AP-1: activating protein 1; AMPs: antimicrobial peptides; BFT: *B. fragilis* toxin; CDI: *C. difficile* infection; CDT: cell death toxin; CIN: chromosome instability; CLR: C-lectin-like receptor; CECs: colonic epithelial cells; CRC: colorectal cancer; CIMP: CpG island methylator phenotype; CD: Crohn's disease; COX-2: cyclooxygenase-2; CDT: cytolethal distending toxin; CNF: cytotoxic necrotizing factor; DAMPs: damage-associated molecular patterns; DCs: dendritic cells; ETBF: enterotoxigenic *Bacteroides fragilis*; FMT: fecal microorganism transplantation; GC: gastric cancer; IBD: inflammatory bowel disease; IRF: interferon regulatory factor; IRAK1: IL-1R-associated kinases 1; IFN: interferon; IL: interleukin; IEC: intestinal epithelial cell; LRRs: leucine-rich repeats; MAMPs: microbe-associated molecular patterns; MDP: muramyl dipeptide; MSI: microsatellite instability; MMR: mismatch repair; MAPK: mitogen-activated protein kinase; MyD88: myeloid differentiation factor 88; NF-κB: NF-kappaB; NLR: nod-like receptor; NTBF: nonenterotoxigenic *B. fragilis*; PAMPs: pathogen-associated molecular patterns; PRR: pattern recognition receptor; PKS: polyketone acid synthetase; RNS: reactive nitrogen species; ROS: reactive oxygen species; RELMβ: resistin-like moleculesβ; RLR: Rig-I-like receptor; sIgA: secretory immunoglobulin A; SMO: spermine oxidase; TAK: transforming growth factor kinase; Th17: T helper cell 17; TJs: tight junctions; TLR: toll-like receptor; TRAF: TNF receptor-associated factor; TFFs: trefoil factor peptides; TNF: tumor necrosis factor; IFN-β: type I IFN; UC: ulcerative colitis

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faecalis destroys DNA *via* free radicals, such as active oxygen and active nitrogen.[3] Some carcinogenic mechanisms may involve a variety of different signaling pathways. Their interactions, promotions, substitutions, and combinations result in intestinal microorganism induced CRC. The role of these mechanisms is inseparable from the subsequent inflammatory response, which eventually leads to CRC.[4] This review provides an overview of the intestinal epithelial barrier structure and microbial signal transduction pathways, the role of microbes in inflammation-induced colorectal neoplasms, and a detailed review of typical microbial carcinogenesis. Finally, we expect colorectal cancer may be treated using certain current technologies for the typical microbiota, such as fecal microbiological transplantation.

The Importance of the Intestinal Epithelial Barrier and Microbial Signals in the Regulation of Intestinal Homeostasis

Intestinal epithelial cells (IECs) are continuous physical barriers formed by single cells that separate the intestinal flora from the deeper intestinal tissue. Epithelial cells are networked together by tight junctions (TJs) and provide a paracellular seal (Fig. 1). This not only blocks the paracellular space, indicating ion flux between tissues, but also maintains cell polarity.[5] Intestinal mucus is the first barrier between the intestinal tract and mucous tissue. It is mainly comprises a large amount of modified glycoprotein mucus.[6] Jakobsson *et al.*[7] found that the filtration function of colonic mucus depends on the microbial community. The intestinal microbiota are also active participants in maintaining intestinal homeostasis. Lipopolysaccharide acts as an endotoxin, and microbiota containing lipopolysaccharide interfere with the function of the epithelial barrier, leading to chronic inflammation and CRC. However, the intestinal microbiota can regulate the renewal and reorganization of TJs of intestinal epithelial cells, thereby enhancing the barrier function.[8] The mucus layer of the intestinal epithelium is a sterile environment containing some biomolecules, such as secretory immunoglobulin A (sIgA), antimicrobial peptides (AMPs),[9] microbe-associated molecular patterns (MAMPs), trefoil factor peptides (TFFs), resistin-like molecules β (RELMβ), and Fc-γ binding proteins.[10] A study using aseptic mice showed that the thickness of the mucus layer was reduced compared to the rodents with an intact microbiota.[11] On the basis of IECs, there are also plasma cells, macrophages, and dendritic cells (DCs). These cells have a simple nature and limited inflammatory cytokine expression in the healthy state.[12] However, in inflammatory bowel disease (IBD), the number of these immune cells will increase.[13] Meanwhile, the expression of endothelial cell adhesion molecules in IBD also increases.[14]

Innate receptors, such as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), play an important role in the innate immune response and are able to identify molecular patterns. These molecular patterns include Nod-like receptors (NLRs), C-lectin-like receptors (CLRs), Rig-I-like receptors (RLRs), and toll-like receptors (TLRs).[15] Although mice with pattern recognition receptor (PRR) and signal transduction defects have been modeled, skin-specific PRR knockout mice are still needed to demonstrate the role of bacterially derived signals in intestinal homeostasis.[16] Over the last few years, we have witnessed a significant expansion in the number of reports associated with the contribution of the NLR family members to IBD pathobiology.[17]

NLRs are cytoplasmic receptors and are highly conserved throughout evolution, attesting to their important role in host defense.[18] MAMPs activate muramyl dipeptide (MDP) and recognize NLRs. Among them, nucleotide binding oligomerization domain containing (NOD)1 and NOD2 are active in intestinal cells and can recognize caspase recruitment domains (card-card).[19] Under the stimulation of leucine-rich repeats (LRRs), which are involved in bacterial-sensing during pathogenesis, NOD1 and NOD2 interact with the receptor-interacting protein 2 (RIP2), which stimulates tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and recognizes transforming growth factor kinase 1(TAK1), triggering mitogen-activated protein kinase (MAPK) and NF-kappa B (NF-κB) signaling.[20] By contrast, there has been little research on CLRs.

The RLR signaling pathway induces the phosphorylation and homodimerization of interferon regulatory factor 3 (IRF3) and upregulates the transcription of type I IFN (IFN-β).[21] Meanwhile, RLRs can be used for virus detection. RNA viral infection plays a key role in the early production and subsequent expression of IFN-β, which induces an inflammatory response, thereby inhibiting viral replication.[22] Therefore, IFN-β identifies the virus RNA and induces the congenital antiviral response.[23] Remarkably, like other innate immune pathways, upon stimulation, RLR signaling is markedly amplified by multiple feed-forward loops in self-regulatory or autocrine/paracrine ways.[24] TLRs are usually sensitive to microbial components, DNA, and RNA fragments.[25] TLRs can be located on the cell surface or in the intracellular compartment, with the specific ligand completing the feedback and associated with a specific adaptor that activates the cascade of downstream signals.[26]

TLRs include TLR1, TLR2, and TLR4, and bind to myeloid differentiation factor 88 (MyD88) and activate NF-κB by binding to IL-1R-associated kinases 1, 2, and 4 (IRAK1, 2, and 4).[27] TRAF6 mediates the activation of NF-κB induced by MyD88 and IRAK.[28] TLRs are strongly expressed in human rectal adenocarcinoma cells, especially TLR2 and TLR4.[29] In addition to TLR3, MyD88[30] signaling is usually triggered by adapters, which initiates a signal cascade, which eventually activates transcription factors, such as NF-κB, IRF,[31] and activating protein 1 (AP-1).[32]

Inflammatory Cytokine-Mediated Signaling Pathway Leading to Colorectal Cancer

The occurrence of malignant tumors is inseparable from chronic inflammation. Accumulating evidence confirms that

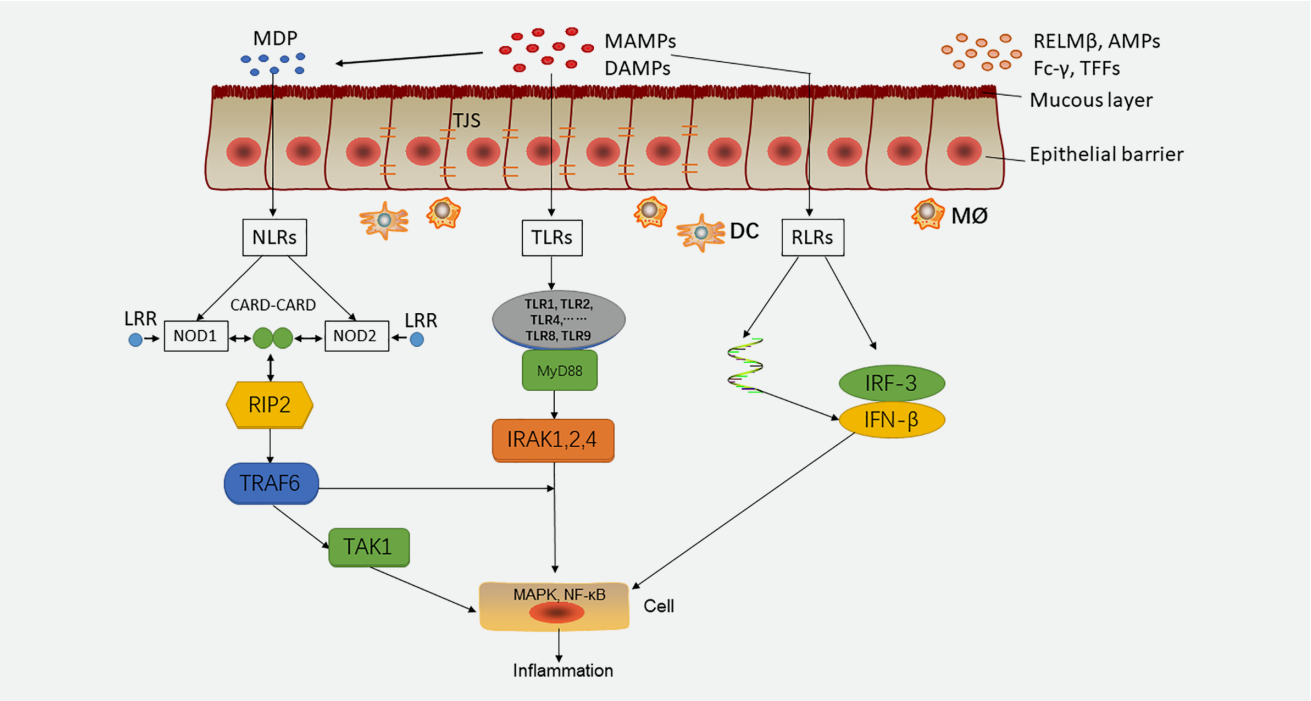

Figure 1. A schematic diagram of the signaling pathways of microorganisms in the intestinal epithelium. Among them, NLR, TLR and RLR family members provide significant microbial signaling pathways in the intestinal epithelium. DAMPs and MAMPs on the epithelial barrier activate signaling pathways through different receptors. MAMPs activate muramyl dipeptide (MDP) and recognize NLRs. NOD1 and NOD2 are active in intestinal cells and can recognize caspase recruitment domains (card-card). NOD1 and NOD2 interact with RIP2, stimulates TRAF6 and recognizes TAK1, triggers MAPK and NF-κB. TLRs include TLR1, TLR2, and TLR4, which bind to MyD88 and activate NF-κB by binding to IRAK1, 2, and 4. RLRs are capable of recognizing viral RNA, releasing IFN-β, and activating NF-κB. If the signal pathways are disrupted, inflammation may occur, leading to cancerous lesions.

the collapse of the symbiotic relationship is important in the pathogenesis of IBD.[33] It is generally believed that chronic colon inflammation from ulcerative colitis (UC) or Crohn's disease (CD) may increase the risk of colon cancer.[34] In the past few decades, the incidence of UC in western countries has been very high. However, recent studies have found that the incidence of IBD is increasing steadily in newly industrialized, non-Western countries.[35] In IBD, mucosal lesions are caused by disorders of the intestinal microbiota.[36] Intestinal microbiota also drive IBD pathogenicity through pro-inflammatory factors or restriction of protective compounds.[37] This hints at the joint induction of intestinal inflammation by a variety of microbes and the possibility of causing CRC.[38] The inflammatory cytokines associated with CRC are primarily intermediate mediators, such as microbial metabolites. A large amount of metabolites in the blood come from the intestines, which support the important role of metabolites in formation of microbial-cytokines and construction of intestinal microenvironment.[39] However, there has been little research in this area. The symbiotic and pathogenic microbiota also induce local inflammation by invading the normal colon tissue, and accelerate tumorigenesis by promoting the genotoxicity of colonic epithelial cells, thus promoting the development of CRC.[40] The DNA in the IECs undergoes modification (including nitration, oxidation, methylation and deamination) by chronic inflammation, which leads to abnormal proliferation. This process may contribute to the activation or progression of CRC.[41] Innate immune cells, such as macrophages, dendritic cells (DCs), and adaptive immune cells are recruited in response to inflammation.[42] The mitotic immune system is further developed with the participation of symbiotic bacteria. Macrophages, DCs, and natural killer (NK) cells proliferate and release proinflammatory cytokines, such as interleukin (IL)-12, IL-23, tumor necrosis factor α (TNF-α), and INF-γ (Fig. 2). These factors activate cells of the adaptive immune system, including T lymphocytes, B lymphocytes, and various inflammatory mediators.[43]The inflamed colorectal epithelial cells do not form an effective surface barrier to reject the invasion of the symbiotic bacteria and their derivatives. As a result of this defect in the barrier function, symbiotic bacteria become the driving force for inducing and maintaining the tumor to promote inflammation.[44] Genes or cells undergo mutations, proliferation, or apoptosis under the action of inflammation and gradually develop carcinogenic phenotypes. Nuclear factor kappa (NF-κB) is the link between inflammation and cancer, which mainly regulates cell survival and immunity.[45] IL-6 is one of the major cytokines induced by NF-κB. IL-6 produced in the lamina propria activates the

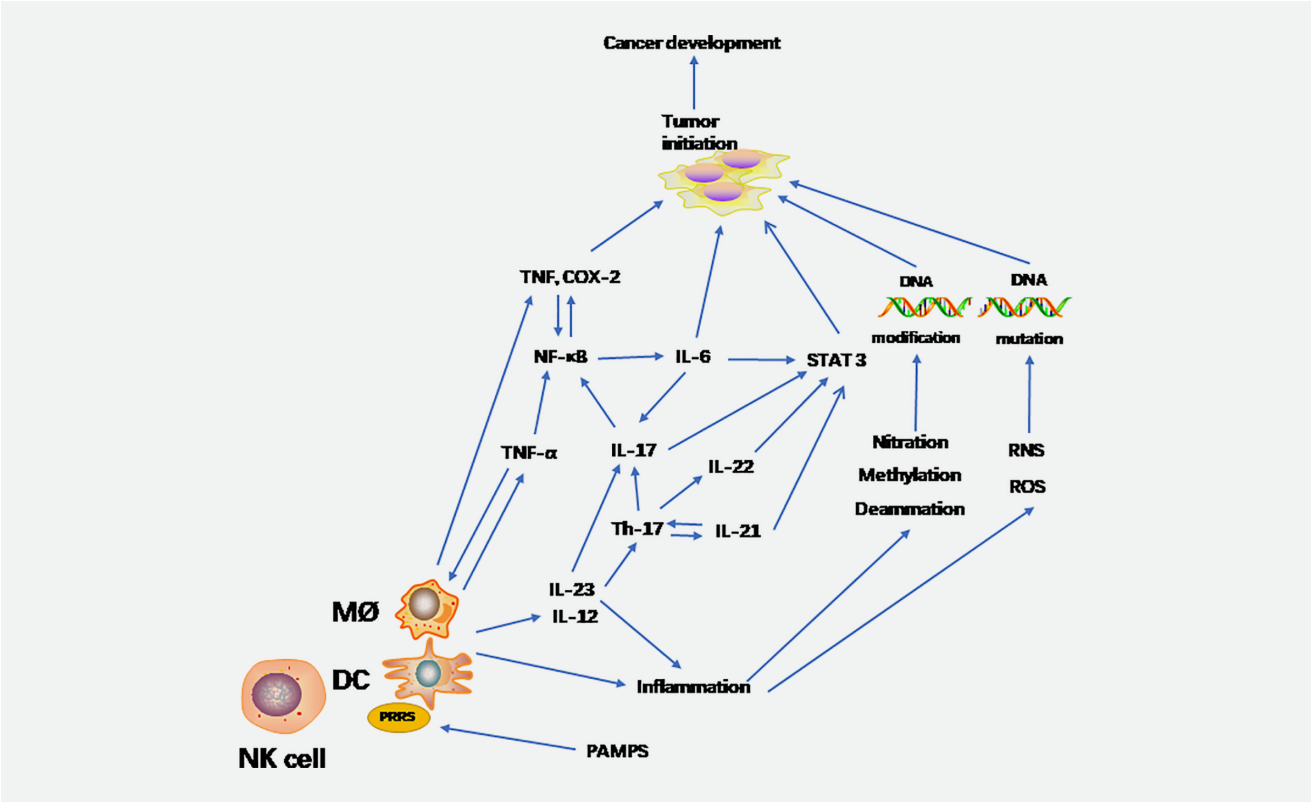

Figure 2. Inflammation oncogenic signaling pathways. PAMPs recognize surface PRRs such as macrophages and dendritic cells. The inflammatory cells release cytokines such as IL-6, IL-23, TNF-α *et al.* IL-6 activates the NF-κB and signal transducer and STAT3 pathways, which in turn induce cancer. IL-17 is an important cytokine that also activates the NF-κB and STAT3 signaling pathways, but requires the help of IL-23. At the same time, the release of ROS and RNS, and chemical modifications such as methylation and amination, can damage DNA and promote the development of cancer.

signal transduction and activator of transcription (STAT3) signaling pathway in IECs. This pathway promotes cell proliferation, inhibits apoptosis and other tumorigenic pathways to promote tumorigenesis.[46] Two transcription factors, NF-κB and STAT3, are essential for inflammation-promoted cancer development and progression. NF-κB signaling pathway is activated by TNF-α and IL-17, and is associated with cytokines. STAT3 is activated with the help of IL-6, IL-21, IL-22, and IL-23.[40] In a mouse model, the development of colitis was shown to require the expression of IL-23. At the same time, IL-23 is crucial in the regulation of T helper cell 17 (Th17)[47] function and IL-17 production.[48] The increase in IL-17 expression appears in the colons of patients with UC and CD.[49] The NF-κB pathway also serves as an important regulator of the genes encoding TNF and Cyclooxygenase-2 (COX-2),[50] which are often highly overexpressed in inflammatory bowel disease, as well as in colorectal adenomas and adenocarcinomas. Other key innate components of the inflammatory response that contribute to CRC progression include reactive nitrogen species (RNS) and reactive oxygen species (ROS), which serve as genotoxic compounds promoting the accumulation of mutations within proliferating epithelial cells.[51]

Typical Microbial Families Contributing to Colorectal Cancer

Enterococcus faecalis

E. faecalis is a gram-positive facultative anaerobic bacterium. Recent research has linked *E. faecalis* with CRC, because the bacterium was found to aggregate at a higher level in fecal specimens from patients with CRC compared to that of healthy controls, and is more abundant in the adjacent tissues of cancer and CRC compared to healthy mucosa.[52] In *Il10* knockout mice, *E. faecalis* promoted colon inflammation, leading to dysplasia and CRC.[53] One study also showed that *E. faecalis*, which can cause colitis after infection, can express TGF-β in the intestinal epithelial cells of wild-type mice, thereby activating the Smad signaling pathway.[54] In this process, the dedifferentiation of TGF-β signal transduction enhances the stem cell characteristics of CRC, thereby promoting its occurrence.[55] This is associated with the deletion of the expression of the TLR2 protein and the inhibition of NF-κB-dependent pro-inflammatory gene expression[54] (Fig. 3). In contrast, *Il10* gene-deficient mice in IECs fail to suppress TLR2-mediated pro-inflammatory gene expression during colonization with *E. faecalis*.[56] In addition, the Smad4 haploid environment not only affects colitis, but also has an impact on

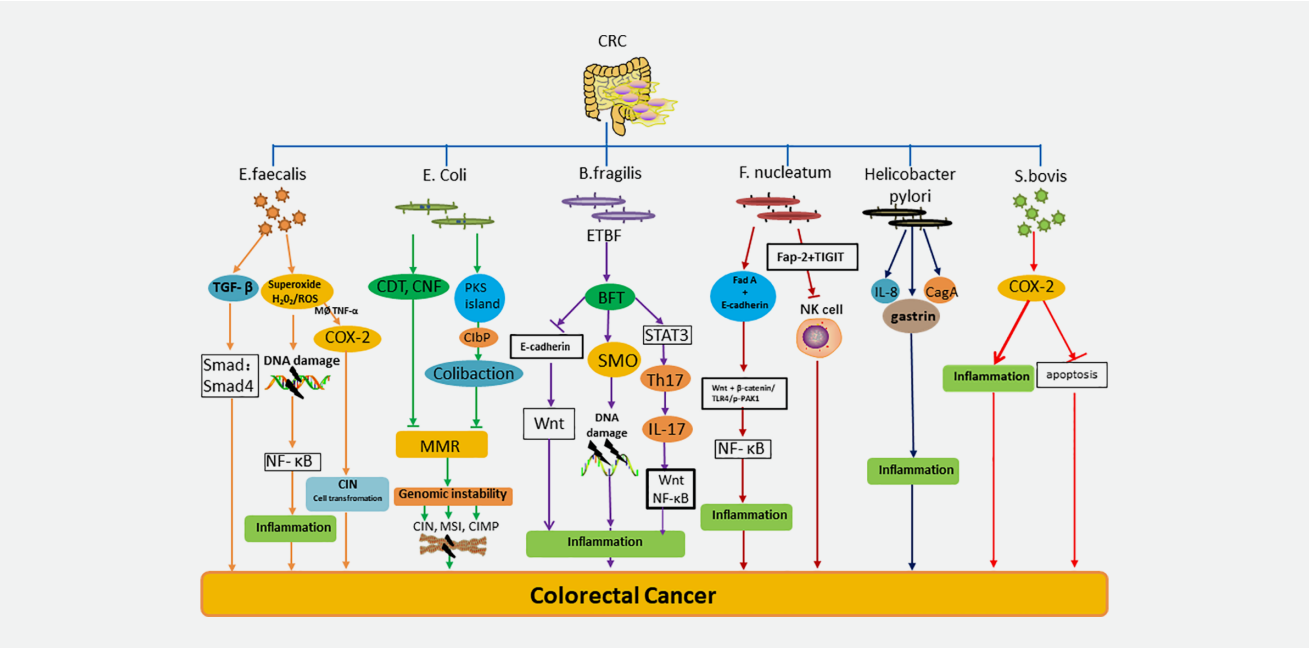

Figure 3. The carcinogenic mechanisms of six major microorganisms. *E. faecalis* causes colitis after infection and expresses TGF-β in intestinal epithelial cells, thereby activating SMAD4 signaling pathway. *E. faecalis* also produces extracellular superoxide and hydrogen peroxide,inducing DNA damage. Meanwhile, it also appears to be involved in the bystander effect of COX-2, the endogenous CIN and cell transformation caused by the release of TNF-α from macrophages, results in cancer. Some strains of *E. coli* B2 produce CDT and CNF, which can directly lead to a DNA damage response and genomic instability. At the same time, the mismatch repair pathway is inhibited, leading to tumorigenesis. *E. coli* can also induce late bridges and chromosomal aberrations by genomic toxin-containing pks islands and catalyze the synthesis of colibactin by clbP. Eventually it leads to CIN, MSI and CIMP, which leads to cancer. ETBF synthesizes BFT. BFT binds to CECs and stimulates the cleavage of E-cadherin, thereby amplifying the Wnt and NF-κB pathway and releasing pro-inflammatory mediators to destroy DNA. At the same time, ETBF activates the signal transducer and activator of STAT3 signaling pathway, induces the production of Th17, releases interleukin IL-17, and promotes colon tumor formation. BFT rapidly causes the expression of SMO and promotes SMO-dependent ROS production and damages DNA in intestinal epithelial cell lines. These reactions cause tumor formation. By complexing with E-cadherin on epithelial cells, *F. nucleatum* stimulates FadA, which activates wnt/β-catenin/TLR4/p-PAK1 signaling and upregulates oncogene expression. By releasing RNA into host cells and activating NF-κB, *F. nucleatum* stimulates inflammation. *F. nucleatum* also inhibits natural killer (NK) cell activity in the tumor microenvironment, leading to colorectal tumorigenesis. *Helicobacter pylori* causes the metastasis of colorectal cancer caused by chronic gastritis. In addition, *H. pylori* infection may cause colorectal epithelial damage through inflammatory reactions such as IL-8 mediated inflammatory responses. *H. pylori* virulent strains induce enhanced inflammatory responses, and expression of the cytotoxin-associated gene A (*CagA*) gene may also lead to colorectal cancer. The *S. bovis* antigen induces the expression of COX-2. With the help of prostaglandins, COX-2 promotes cell proliferation and angiogenesis, and inhibits apoptosis, thus stimulating the cancer pathway.

CRC.[57] In addition to inducing chronic inflammation, *E. faecalis* can also generate extracellular superoxide and hydrogen peroxide. *In vitro*, DNA damage was induced by free radicals generated outside the cells.[58] Infection by *E. faecalis* induced an intracellular ROS reaction that was independent of the oxphos system and impaired the mitochondrial genome in gastric cell culture. Finally, the microbiota can induce NF-κB through DNA damage, leading to inflammation.[59] ROS can cause chromosome instability (CIN), which may be related to the occurrence of CRC. In mammalian cells, *E. faecalis* can induce CIN *via* the production of superoxide. At the same time, *E. faecalis* seems to be involved in the bystander effect of COX-2.[60] These results validate a novel mechanism for CRC induction that involves endogenous CIN and cellular transformation arising through a microbiome-driven bystander effect.[61] At the same time, the extracellular superoxide formation of *E. faecalis* can enhance the expression of COX-2 in macrophages and promote the CIN in IECs. The macrophages polarized by *E. faecalis* can induce malignant tumor aneuploidy or chromosome instability in primary colonic epithelial cells.[62] These findings can explain the effect of *E. faecalis* on the incidence of CRC.

Escherichia coli

E. coli has a predominant place among aerobic-anaerobic gram-negative bacteria in the normal intestinal microbiota. Some virulent strains of *E. coli* that contain pathogenicity islands can infect the human gastrointestinal system and induce disease. *E. coli* is divided into four phylogenic groups (A, B1, B2, and D) according to the acquisition of virulence factors.[63] Interestingly, some strains of *E. coli* from phylo group B2 are associated with CD, a chronic IBD that is a risk

factor for CRC.[64] At the same time, *E. coli* in group B2 can also contain the polyketone acid synthetase (pks) island containing the genotoxin. This hybrid peptide-polyketone genotoxin is generated by a multi-enzyme factory, which is encoded by the 54 kb pks genotoxicity island[65] (Fig. 3). Infection with pks$^+$ *E. coli* induces anaphase bridging, chromosome aberrations, aneuploidy, and tetraploidy.[66] Mucus degradation enables an increase in pks$^+$ *E. coli* adhesion, triggering increased DNA damage in colonic epithelial cells by colibactin.[67] Bacteria-host cell contact is required for the genotoxic effect of pks; therefore, an environment where bacteria can more readily take root in the epithelial layer could deliver more of the pks product colibactin to host cells in the epithelium.[68] Deletion of the pks island decreased tumor number without altering inflammation, suggesting that colibactin contributes to tumor promotion independently of inflammation.[69] Recent evidence shows that colibactin is synthesized *via* the peptidase activity of the editing enzyme, clbP, which is present in the pks island, indicating that the colibactin is synthesized as a prodrug.[69] Vizcaino and others have used nuclear magnetic resonance spectroscopy and bioinformatics-guided isotopic markers to describe the colibactin warhead. The warhead crosslinks duplex DNA *in vitro*, which is evidence for colibactin's DNA-damaging activity.[70] Some toxins can cause DNA damage, and then affect the cell cycle. *E. coli* harboring cytotoxic necrotizing factor (CNF) and cytolethal distending toxin (CDT) are particularly associated with CRC biopsies.[71] In addition, DNA mismatch repair (MMR) plays a key role in sustaining genomic stability,[72] which is a highly conserved biological pathway. MMR acts in the DNA damage response pathway, which degrades severely damaged cells and prevents both short-term mutagenesis and long-term tumorigenesis. Using a method involving depletion of DNA mismatch repair proteins,[73] an *E. coli* effector protein stimulates host mutation. The pathogenic mechanisms that cause this condition included in three pathways: CIN, microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP),[74] which eventually lead to the occurrence of CRC.

Bacteroides fragilis

B. fragilis usually exists symbiotically and is believed to contribute to the host's nutritional status, together with mucosal and systemic immunity. By contrast, *B. fragilis* also induces human disease when colonic integrity is interfered with, which allows it to escape into the sterile peritoneum where it acts as an opportunistic pathogen.[75] Among *B. fragilis* strains, only enterotoxigenic *B. fragilis* (ETBF) is associated with diarrheal disease. Alternatively, nonenterotoxigenic *B. fragilis* (NTBF) strains are believed to be a possible pro-biotics but with the potential to induce colonic inflammation.[76] The central part of the pathogenicity of ETBF is the synthesis of the *B. fragilis* toxin (BFT) (Fig. 3). BFT is a 20-kDa zinc-dependent metalloprotease toxin that binds to colonic epithelial cells (CECs) and stimulates cleavage of the tumor suppressor protein, E-cadherin.[77] E-cadherin cleavage enhances procarcinogenic triggering, including Wnt signaling, CEC proliferation, and epithelial barrier disruption, which stimulate mucosal inflammation and the formation of colon tumors, as illustrated in murine models of colon carcinogenesis.[78] At the molecular level, BFT binds to a specific colonic epithelial receptor, activating the Wnt and NF-κB pathways. ETBF induces rapid activation of STAT3, both in the colonic epithelial cells, which are the targets of transformation in the colon, and in a subset of mucosal immune cells. STAT3 activation is required for Th17 cell development and, consistent with this, ETBF induces a rapid mucosal Th17 inflammatory response within 1 week of colonization.[79] Colon tumors induced by ETBF also have a marked increase in STAT3 activation. IL-17 is produced by a subpopulation of Th17 cells.[80] The IL-17-dependent signaling pathway promotes NF-κB and Wnt activation, and establishes an inflammatory tumor microenvironment in the gut.[81,82] Furthermore, administration of an IL-17-blocking antibody had an inhibitory effect on excess tumor formation, indicating that IL-17 is necessary for tumorigenesis in this model.[83] Spermine oxidase (SMO), a polyamine catabolic enzyme, is an epithelial source of inflammation-induced ROS and DNA damage. BFT rapidly triggers SMO expression and contributes to SMO-dependent ROS and DNA damage in intestinal epithelial cell lines. Mice that are infected with ETBF show elevated intestinal SMO expression.[84] It has been suggested that SMO acts as a potential source of inflammation-associated ROS, which is produced during polyamine catabolism. This causes apoptosis, DNA damage, and consequently, the formation of cancer.[85]

Streptococcus bovis/Streptococcus gallolyticus

S. bovis is implicitly associated with an expanded of a variety of cell and molecular modifications that may be linked with the appearance of CRC.[86] The wall-extracted *S. bovis* antigen induces the expression of COX-2 (Fig. 3). With the help of prostaglandins, COX-2 promotes cell angiogenesis and inhibits apoptosis. Thus, it can stimulate the cancer pathway.[87] Using human CRC patient's feces and mucosa samples, Abdulamir and his colleagues showed enrichment of the bacteria compared to the healthy control group, without gastrointestinal lesions, which strengthened the connection between *S. bovis* and CRC.[88] Thus, it appears that Streptococcus can provide a growth advantage in the tumor microenvironment and induce inflammation to promote carcinogenesis.

Fusobacterium nucleatum

F. nucleatum is an opportunistic commensal obligate anaerobic gram-negative bacterium that is indigenous to the human oral cavity and has a role in periodontal disease. Recent data provide experimental support for tumor induction by *F. nucleatum. F. nucleatum* is more abundant in CRC tumor *versus* normal tissue.[89] This bacterium induces the expansion of bone marrow-derived immune cells, and promotes the

expression of inflammatory genes in the small intestine and colon.[90] Recent studies have uncovered oncogenic mechanisms that support a tumorigenic role of *F. nucleatum*. While Kostic's[90] study unveiled an indirect method of interaction with tumor sites, Rubinstein's[91] study demonstrated a direct interaction between *F. nucleatum* and host cells. *F. nucleatum* and other intestinal microbiota can also directly adhere to enterocytes, altering endothelial integrity, with possible oncogenic consequences. The best-characterized associated factor is the external adhesin, FadA[92] (Fig. 3). The unique FadA adhesin of *F. nucleatum* can bins to E-cadherin, which activates β-catenin signaling, inducing oncogenic responses in CRC cells.[93] This is accompanied by increasing expression of transcription factors, oncogenes, Wnt, and inflammatory genes, together with growth promotion of CRC cells.[94] By releasing RNA into the host cell and activating NF-κB, *F. nucleatum* induced inflammation.[95] NF-κB is an internalized signal of the above process. Rubinstein *et al.* used *FadA*-knock-out mutants to demonstrate the effect of adhesion, and its effect on β-catenin signaling cascades.[65] A series of cytokines trigger the activation of NK cells, including IFN, IL-2, IL-12, IL-15, and IL-18. They recognize uncoordinated ligands to activate and/or suppress receptors. Alternatively, activation can be further accomplished by directly identifying pathogen-associated molecular patterns. However, *F. nucleatum* inhibits NK cell activity in the tumor microenvironment, resulting in colorectal tumorigenesis.[96] Fap-2, which is a lactobacillus-resistant nucleolus, is an inhibitor of lactose binding, and is involved in cell adhesion.[97] Fap-2 defends tumors from host immune attack. Abed *et al.* showed that *F. nucleatum* Fap-2 attaches to beta-D-galactosyl(1–3)-N-acetyl-D-galactosamine (Gal-GalNAc) as a polysaccharide receptor for CRC, reducing the ability of *F. nucleatum* to enhance CRC.[98] T Cell immunoreceptor with Ig and ITIM domains (TIGIT) is an important inhibitory receptor on NK and T cells.[99] Gur *et al.* proved that Fap-2 binds to TIGIT on NK and T cells and interferes with the attack by the host immune system on *F. nucleatum*-associated tumors.[100] At the same time, under the control of TLR4/p-PAK1/p-β-catenin S675 cascade, *F. nucleatum* enhances intestinal tumorigenesis *in vivo*.[101] Invasive *F. nucleatum* activates catenin signaling through the TLR4/p-PAK1/p-β-catenin S675 cascade, leading to CRC.[102]

Helicobacter pylori

H. pylori is a major factor in the development of stomach disorders and gastric cancer (GC).[103] Some reports have indicated a potential relationship between *H. pylori* infection and colorectal neoplasms; however, this has been disputed by others. The pathophysiological mechanism of how *H. pylori* induces colorectal neoplasm remains unclear. Various mechanisms have been proposed to explain the correlation between *H. pylori* infection and CRC. First, *H. pylori* inhibits the gastric mucosal proton pump to reduce the secretion of gastric acid and increase the chances of the proliferation of other microbes.[104] A meta-analysis also showed that *H. pylori* may increase the risk of CRC; however, the evidence is insufficient.[105] Second, *H. pylori* infection may cause damage to the colorectal epithelium through an inflammatory reaction, such as an inflammatory response mediated by IL-8,[49] which is a factor that corresponds with CRC (Fig. 3). In addition, virulent *H. pylori* strains expressing the *CagA* gene could also contribute to CRC by inducing enhanced inflammatory responses.[106] To determine whether there is a correlation between colorectal cancer and CagA, Shmuely *et al.* detected the expression of serum IgG antibodies and CagA protein in 67 cases of colorectal adenocarcinomas. They found that $CagA^+$ seropositivity was related to additional risk of gastric cancer and colonic cancer.[107] Third, *H. pylori* infection promotes the secretion of gastrin, which may induce the proliferation of mucosal cells in the colon, leading to CRC.[108] Increased plasma gastrin levels may induce higher mucosal cell proliferation in the colon and contribute to the development of CRC.[109]

Clinical Applications

We have shown that microbes are essential in the process of CRC development. Therefore, the protection of intestinal microbes can effectively reduce the occurrence of CRC. Although antibiotics and probiotics are frequently used, researchers have developed certain technologies to treat CRC associated with the above-mentioned typical bacterial groups. Fecal microorganism transplantation (FMT) has recently been re-evaluated as a promising method to treat diseases associated with microorganisms. Colonic enema or endoscopy is often used to introduce the distal gut flora from healthy donors into unhealthy recipients.[110] FMT is most commonly used to treat recurrent *Clostridium difficile* infection (CDI) to replace the commensal microbiota that has been eliminated by antibiotic treatment.[111] Reducing the adhesion of *E. coli* and targeting the host metabolism can affect the colonization of *E. coli* and prevent the occurrence of CRC.[112] In the case of *B. fragilis*, researchers developed a biologically active recombinant BFT-2 *in vitro* and found that a low dose of bioactive BFT-2 could inhibit the formation of colorectal tumors when administered gastrically.[113] In the case of *F. nucleatum*, xenografted mice with CRC were treated with antibiotics, which reduced the load of *F. nucleatum* and could reduce the overall growth of the tumor.[114] CRC prediction based on fecal microbiota can provide accurate CRC prediction noninvasively with high accuracy. Although colonoscopy can visually observe the lesion, preoperative preparation is cumbersome. Moreover, the accuracy of the examination results can be affected by the skill and experience of the surgeon, and certain complications may occur. Painless colonoscopy reduces discomfort, but increases the risks associated with anesthesia. In addition, a limitation of short colonoscopy is that the working range is narrow and the examination is not comprehensive. Computed tomography (CT) colonoscopy may cause radiation damage and is expensive. Therefore, FMT,

assisted by fecal DNA testing, could further improve the accuracy of CRC screening, and is a good choice for early screening and prognosis of CRC. We expect that these technologies will move out of the laboratory in the future and be applied in the treatment of colorectal cancer.

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Conflict of interests

The authors declare that there is no conflict of interest.

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