Molecular Mechanisms of Gut Microbiota-Associated Colorectal Carcinogenesis

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Abstract
Colorectal cancer (CRC) is the cancer occurring in colon and rectum, and is the fourth leading cause of tumor-associated deaths worldwide. As a multi-etiologic cancer, CRC could be induced by genetic and environmental factors, including unhealthy diet, irregular lifestyle, inappropriate inflammatory, and the dysbiosis of gut microbiota. Since immunotherapy has been the most popular cancer therapy nowadays, the relationships among gut microbiota, host immune cells and CRC pathogenesis are widely investigated. Scientists constantly tried to figure out the underlying mechanisms involved to support the further therapeutic studies. In this review, we discuss the component shifts of gut microbiota in CRC patients compared with healthy people, summarize how immune cells participate in protecting host from pathogenic microbes, elaborate the molecular mechanisms involved in gut microbiota-associated carcinogenesis of colonic epithelial cells and look into how gut microbiota influence the CRC therapy.

Keywords: gut microbiota; colorectal cancer; immune cells

Introduction
Colorectal cancer (CRC), the cancer of the rectum or colon, majority cases of which occur sporadically, is the fourth leading cause of tumor-associated deaths worldwide.¹ Colorectal carcinogenesis is believed to be result from genomic and/or epigenetic instability, leading to the formation of neoplastic lesions. Such instabilities are prone to mutations that induce the inactivation of tumor suppressor genes and/or the activation of oncogenes, causing cancer formation. Indeed, CRC is a disease with multiple etiology, including not only mutation of genes, but also unhealthy diet, irregular lifestyle, inappropriate inflammatory and dysbiosis of gut microbiota.² Of note, more than 95% of CRC patients are demonstrated to have no genetic predisposition.³

The number of bacteria in gastrointestinal tract comes up to cells composing the human body. Maintaining the homeostasis of gut microbiota is crucial for taking advantage of the commensal microbiota to inhibit pathogens from gut colonization.⁴ Interactions between gut microbiota and the host, including colonocytes as well as immune cells, are extensively demonstrated to have great importance to the host to keep in healthy state. The intestinal architecture of germ-free mice has been shown to be apparently different from mice with commensal gut microbiota, including impaired colon barrier, dysfunctional immune system, and activated carcinogenic signaling pathways in colocolonic epithelial cells.

Immunotherapy, such as immune checkpoint inhibitors, has attracted extensive attentions to the treatment of cancer patients. Researchers and clinicians have achieved limited success in applying diverse therapeutic modality of immunotherapy on CRC patients.⁵ To improve the efficacy of immunotherapy, it is of great account to investigate the relationships among gut microbiota, host immunity, and cancer pathogenesis, which serve as the bases of appropriate and accurate application of immunotherapy.⁶ While classic inflammatory-triggering immune cells help to suppress tumor formation and progression, there are also immune cells serve inverse or bilateral functions. Notably, gut microbiota critically shapes the characteristic of immune cells, pro- or anti-inflammatory, subsequently triggers or suppresses carcinogenesis in gut microenvironment.

The participation of gut microbiota into the colorectal carcinogenesis is becoming a common sense in consideration of years of studies on revealing the distinct gut microbiota composition between CRC patients and healthy population.⁷ Researchers further investigated the causing factors accounting for the cancer-inducing dysbiosis and the underlying molecular mechanisms of carcinogenesis of colonic epithelial cells.⁸ Consequently, this review briefly illustrates the diverse constitution of gut microbiota between CRC patients and healthy people showed by the most recent researches. Then, we summarize how immune cells participate in protecting host from pathogenic microbes. Besides, we elaborate the molecular mechanisms involved in gut microbiota-associated carcinogenesis of colonic epithelial cells. Last, we look into how gut microbiota influences the treatment of CRC.
The gut microbiota of CRC patient

As first reported in 1975, there is certain correlation between gut microbiota and CRC.9 Years of studies supported the hypothesis by showing cancer reduction in germ-free mice, colorectal carcinogenesis triggered by dysbiosis, and promising therapeutic effect of antibiotics. In detail, when housed on germ-free condition, interleukin (IL)-10-/- and therapeutic effect of antibiotics. In detail, when housed on germ-

colorectal carcinogenesis triggered by dysbiosis, and promising hypothesis by showing cancer reduction in germ-free mice, 

The latest 5 years' studies of gut microbiota abundance shifts between CRC patients and normal people are summarized in Table 1. The fold-changes in relative abundance of these investigated bacterial species could potentially be used as clinical biomarkers for CRC diagnosis and prognosis. Nevertheless, because of the cross-study interference factors, which mean various sample types, detecting methods and bioinformatics-analyzing approaches, studies might not always show consistent results. Some researchers conducted meta-analyses to make cross-study comparisons and identified the consistent associations between gut microbiota and CRC. For instance, a study identified up to 29 core species of bacteria significantly enriched in CRC metagenomes by conducting a meta-analysis, which included eight diverse fecal shotgun metagenomic studies with geographical and technical variations.14 With further validations, meta-analysis like this could depict the general world-wide map of CRC-associated microbiome for predicting CRC generation and progression.

Some studies focused on finding the association of gut microbiota and the progressive procedure of CRC. A study with samples from stage I to IV CRC patients showed increased abundance of *Fusobacteria, Bacteroidetes*, and *Firmicutes* as well as decreased abundance of *Proteobacteria* at the phylum level when cancer progressed.15 Another study showed a significantly higher relative abundance of *Fusobacterium* in the invasive cancer group.16 These studies supported the possibility of several certain bacteria serving as indicator of the degree of CRC development. Besides, a large cohort study with 616 patients participated in these studies, analyzed taxonomic and functional characteristics of gut microbiota and metabolites and observed dynamic distinctions in microbial composition during multistep CRC progression. Two different types of bacterial growth were

| Table 1 | Summary of the latest 5 years’ studies of gut microbiota abundance shifts between CRC patients and normal people. |
|---------|------------------------------------------------------------------------------------------------------------|
| Increased microorganism | Decreased microorganism | Sample type | Sample size | Method | Reference |
| Bacteroidetes Cluster 2, Firmicutes Cluster 2, Pathogen Cluster, Prevotella Cluster | Bacteroidetes Cluster 1, Firmicutes Cluster 1 | Fecal and mucosal samples | 59 patients undergoing surgery for CRC, 21 individuals with polyps and 56 healthy controls | 16S rRNA | 84 |
| Fusobacterium, Porphyromonas, p-hydroxy-benzaldehyde, palmitoyl-sphingomyelin | Clostridia, Lachnospiraceae, p-aminobenzoxal, conjugated lineolate | Lympholized feces | 42 CRC cases and 89 matched controls | 16S rRNA | 85 |
| Malasseziales | Saccharomycetes, Pneumocystis | Fecal samples | 184 patients with CRC, 197 patients with adenoma and 204 control subjects from Hong Kong were analysed | Shotgun metagenomic sequences | 86 |
| Devosia | Eubacterium | Mucosal-luminal interface | Nine CRC patients and 14 normal controls | 16S rRNA | 87 |
| Fusobacterium nucleatum, Clostridium hathewayi, Fusobacteria | Rosebudia intestinalis, Bacteroides claus | Fecal samples | 203 colorectal cancer and 236 healthy subjects | qPCR | 88 |
| Panivornas micro, Sphingobacterium moorei, F. nucleatum, Peptostreptococcus stomatis | Eubacterium ventriosum | Stool samples | 74 CRC patients and 54 controls | Illumina HSeq 2000 | 90 |
| Fusobacteria, Fusobacterium, Porphyromonas | – | Fecal samples | 52 pre-treatment colorectal cancer cases and 52 matched controls | Whole-genome shotgun sequencing | 91 |
| clbA bacteria, F. nucleatum | – | Stool sample | 174 CRC/dysplasia patients and 66 controls | qPCR | 92 |
| Alcaligenaceae, Enterobacteriaceae | – | Fecal samples | 21 subjects were diagnosed with ADK, 21 with HRA, 18 with LRA, 14 with HP and 18 controls | 16S rRNA | 93 |
| F. varium | – | Colonoscopy aspirates | 47 colorectal adenoma, 24 intramucosal CRC patients, and 10 healthy subjects | 16S rRNA | 94 |

CRC: colorectal cancer.
established: increased continuously from intramucosal carcinomas to more advanced stages, like Fusobacterium nucleatum spp, or increased only in multiple polypoid adenomas and/or intramucosal carcinomas, including Actinomyces odontolyticus and Atopobium parvulum. Though it had not been identified that these species were the direct causations of colon tumorigenesis, constitutive alterations in gut microbiota might induce shifts in oncogenic microenvironment in intestine. Their findings suggested the identification of metagenomic markers to discriminate very early stage colon carcinoma, which was of potential importance in etiological investigation and clinical diagnosis.17

CRC contains several recognized distinct common molecular subtypes, which reasonably has attracted attentions to investigate whether distal and proximal intestinal epithelial cells (IECs) might be involved in distinctively diverse microbial environment. At least 5 microbe genus (Bacteroides, Fusobacterium, Faecalibacterium, Parabacteroides, and Ruminococcus) were showed to have distinctively diverse relative abundance between distal and proximal CRC segments,18 suggesting the possibility of correlation between CRC molecular subtypes and microbial exposures. It underlined the importance of separately analyzing the carcinogenic microenvironment of CRC from different location of colon.

Taken together, many microbes were suggested to have a close correlation with colon tumorigenesis. However, the detailed mechanisms how microbes affect colorectal carcinogenesis are critical for diagnosis, therapy, and prognostic prediction.

Immune system, inflammatory, and gut microbiota

**Pattern recognition receptors mediate gut microbiota-host interactions**

The intestinal barrier between intestinal mucosal epithelium and gut microbiota, together with biochemical substances secreted, protects the host from the impairment of its harmful surroundings, maintaining tissue homeostasis. Barrier dysfunction leads to the exposure of the host to the risk of opportunistic bacterial infections, subsequently results in morphological and functional changes in gut tissues, inducing chronic inflammation and even intestinal carcinogenesis. Pattern-recognition receptor (PRR) is a pivotal component of intestinal barrier in detecting pathogenic bacteria antigens surrounding the colorectal epithelial cells are recognized by PRRs, triggering downstream signaling cascades to activate the intestinal immune system.19 Here we review several most studied PRRs which have been implicated in CRC carcinogenesis.

Toll-like receptor (TLR), a most characterized membrane bound receptor, powerfully stimulates the pro-inflammatory responses. Because of the critical role of microbe-associated molecular patterns for pathogenic survival, they usually would not be mutated to escape immune surveillance, thus could be recognized by TLRs to enable the discrimination between pathogens and commensal microbiota.20 Accumulated studies showed the interaction between TLRs of IECs and microbe-associated molecular patterns contributed to colorectal carcinogenesis. It was showed that CRC-associated bacteria, Peptostreptococcus anaerobius, could activate the TLR2 and/or TLR4 pathways to promote carcinogenesis in mice.21 and F. nucleatum could induce tumorigenesis in mice through TLR-4 signaling pathway.22 Notably, the downstream signaling pathways of TLRs commonly have multiple functions and completed ablation of these pathways affect not only cancer cells but also normal epithelial cells, which otherwise could trigger severe side effects in therapeutic application.

Nucleotide-binding oligomerization-like receptor (NLR) is a well-established cytoplasmic PRR, being characterized by a central NOD domain. A study showed that NOD2-deficient mice had higher susceptibility to bacterial infections, suggesting the important role of NOD in intestinal protection.23 As a component of inflammation, NLRP6-deficiency was showed to trigger decreased level of IL-18, and increased inflammatory reaction in mouse model after treatment with dextran sodium sulfate,24 which subsequently induced CRC development.

Of note, an antigen of the bacteria could bind to different PRRs thus trigger different downstream signaling. Both pro- and anti-inflammatory cytokines were found to be secreted when facing the same pathogen, suggesting the complex functions of PRRs. Interestingly, NOD2 and TLR2 show cooperative interplay in triggering MAPK and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling pathway through diverse upstream signals.18 Given these evidences, intricate relationship between PRRs and carcinogenic process, synergic or conflict, should be intensively investigated in the future.

**Immune cells and gut microbiota**

The host immune system not only holds the tolerance to gut commensal microbiota, but also defends against opportunistic pathogens. Gut microbiota plays a critical role in the inflammatory activity in the intestine, by closely interacting with the host immune system.3 As proved recently, gut microbiota could also stimulate CRC cells to produce and secret chemokines, which could recruit T cells into tumor tissues. The study identified the association between distinct T cell subsets and defined chemokine gene signatures. Furthermore, clinical samples showed the defined microbiota could trigger higher chemokine secretion, increased T cell infiltration and progressed prognosis, partly explaining the underlying mechanisms of using probiosis to help the treatment of cancer patients.26

Among the gut CRC pathogenic procedure, both adaptive immune cells and innate immune cells are involved. Landmark studies showed that tumor-infiltrating adaptive immune cells critically influenced the clinical prognosis, even affected the recurrence of tumor.27 Longer disease-free survival and overall survival were observed within CRC patients with more effector T cells infiltration in tumor tissues, suggesting the vital role of T cells to protect host against cancer.28 So, it is easy to understand why tumors with high density of cytotoxic T cells have a higher rate of response to checkpoint blockade-based immunotherapy, such as PD-1 therapy. However, suppressive immune cells, like regulatory T cells (Tregs), are associated with bad prognosis. Tregs were found to down regulate responses of pro-inflammatory T-helper cells like Th1, Th17 and Th2.29 A recent study found that increased infiltration of Tregs and the decreased involvement of M1 tumor-associated macrophages were associated with suppressed immune response against CRC cells.30

Th17 cells, differentiating from naive CD4+ T cells, not only secret an important pro-inflammatory cytokine IL-17, but also produce CCL5 and CCL20, which promote localization of CCR3/CCR6/CD8+ cytotoxic T cells. But some studies showed that high amount of Th17 cells infiltrating in CRC microenvironment might lead to reduced survival.31 Furthermore, cytokines which could trigger progression of Th17 cells, like IL-17 and IL-22, might serve as indicators of poor prognosis. IL-17 plays intricate roles in microbiota-CRC interaction. On one
hand, IL-17 limits invasion and dissemination of pathogens, including Salmonella typhimurium, by recruiting neutrophils and promoting antimicrobial productions. On the other hand, the prolonged presence of Th17 cells is associated with colorectal carcinogenesis, as shown by sustained mucosal inflammation.32

Another unique kind of immune cells involved in the gut microbiota-related CRC pathogenesis, γδ T cells, recognize the superstructure of antigen without the requirement of MHC molecules. γδ T cells have dual effects in tumor development. On one hand, γδ T cells perform an early protective inflammatory response when barrier dysfunction happens and opportunistic pathogens invade. Notably, γδ T cells show a compelling feature that in early pathogen invasion, γδ T cells produce IL-17, which in turn recruits immune cells to serve clearance effect on infection or over-response-induced inflammatory disease.33 On the other hand, however, IL-17-producing γδ T cells could also induce the communication between myeloid-derived suppressor cells and gut microbiota, subsequently could promote CRC metastasis.34 Indeed, the environmental change could induce remodeling of γδ T cells function, thus the impact of local tumor microenvironment should be seriously considered when γδ T cells are used for immunotherapy in the future.35 Because of the complicated effects of immune cells serving on tumor-associated pathogens, it is necessary to find out appropriate therapeutic strategies that will trigger inhibition of tumor-promoting immune responses and avoid impairment of tumor-suppressing responses.

Classic innate immune cells play an undoubted vital role in interaction with gut microbiota. Represent of innate immune cells, dendritic cells (DCs) have the ability to make polarization of naive T-helper (Th0) lymphocytes to Tregs, which could produce IL-4, IL-10, and transforming growth factor-β (TGF-β). Moreover, DCs could stimulate B cells to produce commensal specific IgA.36 Triggered by bacterial flagellin, CD103+CD11b+ DCs were shown to upregulate their expression of IL-23, which subsequently induce epithelial expression of anti-microbial peptide RegIIIγ through a burst of IL-22.37 DCs keep the balance between responses of Tregs and effector T cells in gut environment, avoiding inadequate response-induced pathogenic infection or over-response-induced inflammatory disease. DCs and mononuclear phagocytes could secret type-I interferon (IFN) to trigger nature killer (NK) cells priming via the trans-presentation of IL-15 when they encounter pathogenic bacteria.38 Activated NK cells produce IFN-γ, which could in turn recruit additional NK cells from peripheral blood and activate myeloid cells to enhance phagocytosis, subsequently trigger better cytotoxic effects on pathogens. Even in tumor microenvironment, NK cells play a protective role of the host, as shown by simultaneously infiltration of NK cells and effector T cells in CRC tissue, which lead to better clinical prognosis compared with infiltration of effector T cells only.39

Immune cells discussed above (summarized in Figure 1) play multiple roles in anti-tumor or pro-tumor immune responses by suppressing/promoting tumor growth and angiogenesis. In summary, main immune responses keep the balance of immune tolerance and pro-inflammatory functions. However, when a chronic inflammation persists, such as prolonged existence of IL-17, it creates a microenvironment that favors suppression of anti-tumor immune responses and promotion of tumor growth.40 As immunotherapy for patients with CRC has attracted increasing attention, underlying associations among the gut microbiota, immune cells, and response of CRC patients to immunotherapy should be investigated. The CRC immune microenvironment might be one of the important directions for further research.

Molecular mechanisms of gut microbiota-associated colorectal carcinogenesis

Substantial studies have been done to investigate the relationship of gut microbiota and colorectal carcinogenesis, especially aetiological and molecular mechanisms that serve as the basements for clinic translation, such as the development of new drugs and the prediction of prognosis for CRC. The aetiological mechanisms have been comprehensively reviewed from different angles, which include immune regulation, inflammation, metabolisms of dietary components, and genotoxic productions.41 The molecular mechanisms, such as signaling pathways involved in carcinogenesis of colonocytes, also require a clear review (mechanisms of how gut microbiota act on colonic inflammation and carcinogens are summarized in Table 2, and a brief summary is showed in Figure 2).

DNA damage

Among the toxins related to gut microbiota, colibactin and cytolethal distending toxin (CDT)42 are considered to exert direct double-strand DNA damage, and are therefore regarded as genotoxin. Colibactin is produced by members of the Enterobacteriaceae family, such as polyketide synthase positive Escherichia coli, and promotes carcinogenesis by interfering cell cycle and inducing overgrowth of IECs through DNA damage, mutation, and genomic instability.42 In vitro experiment showed that IECs co-cultured with polyketide synthase positive E. coli, which could produce colibactin, had chromosomal aberrations and increased mutation frequency rate, as well as affected cell cycle behavior.44 The genotoxin CDT is produced by selective enteric pathogen strains such as Salmonella, Escherichia, and Campylobacter spp. He et al. showed that CDT-producing Campylobacter jejuni changed composition of gut microbiota and affected transcriptomic response via influencing the mammalian target of rapamycin (mTOR) signaling controlled process, a DNA-damaging procedure, subsequently developed CRC.45 Furthermore, a study showed that a small molecular inhibitor of CIBP, an enzyme related to colibactin synthesis, could reduce tumor burden in a mouse model, suggesting that inactivating or blocking these genotoxins is worth further investigation for the therapeutic implications on CRC.46,47 Another genotoxin, named hydrogen sulfide, which is mainly generated by autochthonous sulfidogenic bacteria, is considered to be mutagenic and DNA-damaging. The abundance of this kind of bacteria correlates to dietary habits with high fat, high red meat, and high processed food intake.47 A study found that compared with control group, sulfidogenic bacteria, such as Bilophila wadsworthia, had expanded abundance in CRC patients. However, hydrogen sulfide also showed to trigger pro-inflammatory pathways and hyper proliferation of IECs.48,49 Besides, sulfidogenic bacterial abundance in colonic mucosa was proven to be a potential environmental risk factor contributing to CRC pathogenesis in African Americans, indicating a race-dependent association between sulfidogenic bacteria and CRC.49 People consuming high-fat diet were observed to have a rise in secondary bile acids in their colon.50 Secondary bile acids, including deoxycholic acid (DCA), are de-conjugated by β-glucuronidase bacteria from steroid acids synthesized by the liver.51 Fecal or serum samples of CRC patients showed a significant increase of DCA as compared with healthy controls. Increased exposure of colonocytes to high levels of DCA triggered a promotion of CRC pathogenesis by nitrogen species, oxidative stress and DNA damage.52 DCA was proven to improve
When account with pathogens, DCs secret cytokines, like IFN to make NK cells prime and produce IFN-γ and TNF. DCs not only present antigens to T cells to make naïve T cells turn to stimulated T cells, but also stimulate B cells to secret IgA. Besides, DCs have the ability to make polarization of Th0 cells to Tregs. Macrophages could directly swallow pathogens which have invaded through intestinal barrier. Myeloid cells, activated by interaction with microbiota via TLRs, could trigger an IL-23 and IL-17 pro-carcinogenic signaling pathway. In early pathogen invasion, γδ T cells could produce IL-17 before Th17 cells to protect the host, but γδ T cells could also induce the communication between gut microbiota and myeloid cells, especially myeloid-derived suppressor cells (MDSCs). Non-conventional lymphocytes, like innate lymphoid cells (ILCs), could interact directly (or with the help of other immune cells) with microbiota to secret pro-inflammatory cytokines. Th17 cells, the main producer of IL-17, could also secret CCL5 or CCL20 to trigger cytotoxic T cells priming. IL-17 acts as a bilateral functioning cytokine in immunity and gut microbiota interaction. On one hand, IL-17 limits the invasion and dissemination of pathogens; on the other hand, it has been shown to be a vital driver of colorectal cancer.

### Table 2

Mechanisms of microbiota act on colonic inflammation and carcinogenesis.

| Microorganism                  | Critical carcinogenic character                        | Carcinogenic mechanism involved                                                                 | Reference |
|--------------------------------|--------------------------------------------------------|---------------------------------------------------------------------------------------------------|-----------|
| *Escherichia coli*             | Produce colibactin                                     | DNA damage, mutation and genomic instability                                                     | 42,44,46  |
| *Campylobacter jejuni*         | Produce CDT                                           | DNA damage                                                                                       | 43,45     |
| *Bilophila wadsworthia*        | Produce hydrogen sulfide                               | DNA damage, affect transcriptomic response                                                        | 47–49     |
| *Clostridium genus*            | De-conjugate steroid acids to secondary bile acids, like DCA | DNA damage, oxidative stress, aneuploidy and micronuclei formation                               | 51,52     |
| *Fusobacterium nucleatum*      | Secret FaDA                                           | E-cadherin and β-catenin signaling activation                                                     | 58,59     |
| *Peptostreptococcus anaerobius*| PCWBR2 directly interact with colonic cells            | PI3K-Akt-NF-κB signaling axis activation                                                          | 67,68     |
| *Enterotoxigenic Bacteroides fragilis* | Secret B. fragilis toxin | STAT3 signaling pathway activation Sustained IL-17 expression                                    | 73        |

CTD: cytotoxial distending toxin; DCA: deoxycholic acid; PKS+: polyketide synthase positive; STAT3: signal transducer and activator of transcription 3.
aneuploidy and micronuclei formation, thus was regarded as indicators of genomic instability in colon epithelial cells. Furthermore, in people with high animal protein intake, bile acid was shown to favor taurine conjugation than glycine, inducing hydrogen sulfide generation when metabolized by bacteria-producing enzymes.53

As shown above, substantial gut microbiota-associated substances participate in the DNA damage procedure, ultimately contributing to the colorectal carcinogenesis. Genotoxins (like CDT and colibactin), hydrogen sulphide, and secondary bile acids (especially DCA) are the most studied DNA-damaging factors, which show extensive clinical applicative potential, including prevention, prediction and treatment of CRC. Based on the increasingly distinct oncogenic mechanisms, decreasing the opportunity or degree of DNA damage by precise therapies, like niche targeting antibiotics, tumor-promoting diet interventions, and tumorigenic metabolites inhibitions, could be promising directions for further development.

Wnt-β-catenin signaling

The Wnt signaling pathway, widely proved by molecular studies, is account for approximately 90% of CRC carcinogenesis.54 Wnt pathway signaling changing in CRC is associated with weakened tight junctions, which reduce cellular adhesion and hence promote migration and metastasis of cancer cells.55 Mutations generate a stop codon, which truncates APC protein, leading to failed binding of β-catenin to the cytoplasmic part of the e-cadherin complex.56 β-catenin translocates to nuclear and works together with T-cell factor/lymphoid enhancing factor transcription factors to upregulate Wnt-signaling and might subsequently induce uncontrolled cell growth.57

F. nucleatum, an occasional pathogen identified in CRC, secretes FadA, which binds to E-cadherin to activate Wnt-β-catenin signaling. Pathogenic bacteria antigens, like MAMPs, can trigger activation of NF-κB and STAT3 signaling via PRRs. Gut microbiota closely interact with host immune cells and the latter can secret pro- or anti-inflammatory cytokines to influence intracellular signaling cascades. CDT: cytolethal distending toxin; DCA: deoxycholic acid; H_{2}S: hydrogen sulfide; MAMPs: microbe-associated molecular patterns; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NOS: nitric oxide synthase; PRRs: pattern-recognition receptors; ROS: reactive oxygen species; STAT3: signal transducer and activator of transcription 3.
disruption of synbindin protected mice against tumorigenesis and the protective effect was compromised by gut microbiota depletion, which suggested that synbindin played a vital role in gut microbiota-related colorectal carcinogenesis. Further studies showed that synbindin could stabilize β-catenin and enhance transactivation of Wnt signaling, which critically influenced the promoting and progressing procedure of CRC.61 Synbindin has been used as a biomarker for CRC diagnosis, and with the advance of oncogenic mechanism discoveries, synbindin shows the potential to provide a novel target for therapeutic intervention.

Though β-catenin was proven to be critically involved in the Wnt pathway, it was not generally applied in clinic as a biomarker for prognosis, for the reason that common increase of β-catenin could be detected in CRC cancers and no study suggested more β-catenin turned to worse clinical outcome.62 However, c-Myc,63 another biomarker involved in Wnt signaling pathway, was proved to be useful clinically. For further application of these molecules involved in Wnt signaling pathway, much more detailed and critical mechanisms should be defined.

NF-κB signaling

NF-κB is a direct regulator of a complex of genes, such as bcl-x and survivin, inducing uncontrolled proliferation and reducing apoptosis of tumor cells.64 Oxidative stress induces NF-κB-associated chronic inflammation to cancer by upregulating pro-inflammatory cytokines.65 Loss of the tumor suppressor p53 in azoxymethane-dextran sodium sulfate-treated mice was reported to induce barrier function impairment, which permitted bacteria invasion into epithelial cells and surrounding stromal cells, causing activation of NF-κB.66 P. anaerobius, a Gram-positive anaerobic bacterium, is a commensal resident in the oral cavity and gut.67 In Apcmin/mice, mucosal adhesion of P. anaerobius was associated with accelerated colorectal carcinogenesis. P. anaerobius was also found to increase in the stool and mucosa of patients with CRC. Besides, putative cell wall binding repeat 2 (PCWBR2), a surface protein of P. anaerobius, directly interacts with colonic cell lines via α2β1 integrin, which is a receptor constantly overexpressed in CRC cells. When the PKB—Akt pathway in CRC cells is activated, tumor cell proliferation and NF-κB activation are subsequently triggered, which provoke a pro-inflammatory immune microenvironment-favor tumor growth.68

Over-upregulated NF-κB signaling is considered as a critical factor which induces overgrowth of IECs and overexpression of pro-inflammatory cytokines, such as IL-10 and IFN-γ, leading to cancer pathogenesis. Therefore, NF-κB-mediated inflammatory pathway has attracted researchers to design a variety of therapeutic strategies targeting this pathway. For example, dietary intervention of α-Ketoglutarate, an important intermediary in the NF-κB-mediated inflammatory pathway that maintains intestinal homeostasis and prevents initiation of intestinal inflammation, was proven to have protective effect against inflammatory-related CRC.69

STAT3 signaling

Signal transducer and activator of transcription 3 (STAT3), which is considered to be an oncogene, is a multi-transcriptional factor that regulates immune response and inflammation in the tumor microenvironment. STAT3 activity promotes the production of immunosuppressive factors, alters gene-expression programs, and thereby reduces anti-tumor immune responses.70 Bollrath et al. used loss- and gain-of-function mice in a colitis-associated cancer model and showed when IEC-specific STAT3 was ablated, mutagen-induced carcinogenesis and tumor multiplicity was reduced. Furthermore, they demonstrated that STAT3 had the ability to trigger overgrowth of IECs via G1 and G2/M cell-cycle progression.71 It considered a common tumor cell-autonomous mechanism that explained the underlying molecular links between chronic inflammation and colon cancer pathogenesis. The activation of IL-6—STAT3 signaling axis was found to down-regulate miR-34a, a microRNA suppressing tumor proliferation, thus to promote transition of epithelia cells to mesenchymal cells and to benefit tumor-cell invasion.72 Enteroxotoxic Bacteroides fragilis, which secretes B. fragilis toxin, induces colitis-related carcinogenesis by inducing STAT3 activation. Subsequently, STAT3-regulated factors, such as IL-10 and vascular endothelial growth factor, propagate STAT3 activity from tumor cells to diverse immune cells.73 Li et al. showed that gut microbiota activated the c-Jun/JNK and STAT3 signaling pathways in combination with anemia and accelerated colonic tumor growth in Apcmin mice.74 Based on all previous studies, elevated p-STAT3 has been identified as a prognostic factor for multiple cancers, including CRC.

Gut microbiota influence treatment of CRC

Multiple studies, at mouse model level or clinical trial level, showed that gut microbiota influenced the therapeutic efficiency of CRC chemotherapy or immunotherapy. Along this line, microbiota-based interventions, like probiotics and fecal microbiota transplantation, are conducted aiming to improve the prognosis of CRC patients. Different kinds of influences on treatment of CRC triggered by gut microbiota are summarized in Table 3.

Chemotherapy

Bacterial enzymes affect the absorption and bioavailability of many oral-taken drugs and some commensal microorganisms could physically bind to the drug and decrease drug absorption. On the other hand, injected drugs would also contact with gut microbiota when metabolized by liver and excreted into gut by biliary. For instance, misonidazole could be nitro-reduced by gut microbiota so that its sensitizing effect on radiation was impaired.75 Besides, chemical reactions, like reduction and hydrolysis, toward the drug mediated by gut microbiota could influence the drug effect. Indeed, when E. coli and Listeria welshimeriwas co-incubated with 30 drugs, 10 of the drugs were found to decrease killing efficacies while 6 other drugs helped the killing. In detail, E. coli increased the cytotoxicity of tegadur and decreased the effect of etoposide phosphate, gemcitabine, and vidarabine. Further experiments demonstrated that the modified chemical structures of these drugs induced the difference.76

Beside chemical modification of drugs, gut microbiota could interact with IECs directly through PRRs and influence the intracellular signaling pathways which subsequently impair the chemotherapeutic response. For example, P. mucilaginos found to promote CRC resistance to chemotherapy through activating autophagy pathway via TLR4 targeting and specific microRNAs regulation.77 Thus targeting the associated pathways might yield promising clinical outcome.
Since microbiota play a key role in chemotherapy, scientists tried to restore gut microenvironment by probiotics to improve the chemotherapeutic response. The encouraging data came from animal models. In gut microbiota-depleted mice, the ROS production by tumor-infiltrating myeloid cells were reduced. Therefore, the chemical drugs, like oxaliplatin, which killed tumor cells through this pathway, were not effective. Restoring Lactobacillus acidophilus in gut was successfully shown to recover the antitumor effect of cisplatin in antibiotic-treated mice.78

**Immunotherapy**

As mentioned above, immunotherapeutic efficacy depends not only on the numbers of tumor-infiltrating cytotoxic T cells, but also the cell-activity state which could be suppressed by PD-1 and PD-L1 interaction. Multiple studies found that gut microbiota played critical roles in the immunotherapeutic response. Sivan et al. identified that *Bifidobacterium* was associated with the antitumor effects and combination of *Bifidobacterium* administration and anti-PD-L1 antibody therapy nearly abolished tumor outgrowth.79 Another study defined a consortium of 11 commensal bacteria which had the ability to elicit IFN-γ-producing CD8+ T cells in the tumor microenvironment and to increase tumor-infiltrating DCs that express high levels of MHCI molecules. More importantly, these 11 strains were proven to have the capacity to enhance immune checkpoint inhibitor therapies, including both anti-PD-1 antibody therapy and anti-CTLA-4 antibody therapy.80 Moreover, CpG oligodeoxynucleotides, which are abundant in bacterial DNA, were found to enhance antitumor immunity via binding to TLR9 of CD8+ T cells. In detail, IL-12 signaling pathway was activated and PD-1 expression was reduced. In addition, the combination of CpG and PD-1 blockade showed a synergistic antitumor effect.81

**Table 3**

| Kinds of influence | Typical drug example | Main mechanism | Microorganism involved | Reference |
|--------------------|----------------------|----------------|-----------------------|-----------|
| Chemotherapeutic drug metabolism | Misonidazole | Misonidazole is nitroreduced by gut microbiota to loses its sensitizing effect on radiation | Gut microbiota | 75 |
| Chemotherapeutic drug response | Oxaliplatin, Cisplatin | ROS production by tumor-infiltrating myeloid cells are reduced by gut microbiota-depletion | Gut microbiota | 78 |
| Chemotherapeutic drug toxicity | CPT-11 | CPT-11 is activated by bacterial β-glucuronidase and triggers side effect | β-glucuronidase producing bacteria | 95 |
| Immune checkpoint inhibitor (ICI) therapy response | Anti-PD-L1 antibody | Augmented DC function enhanced CD8+ T cells priming and accumulation in the tumor microenvironment | Bifidobacterium | 79 |
| | Anti-PD-1 antibody | The bacterial consortium increases the frequency of IFN-γ+ CD8 tumor-infiltrating lymphocytes (TILs) | Parabacteroides distasonis, Parabacteroides gordonii, Akkataes senegalensis, Parabacteroides johnsonii, Paraprevotella xylaniphila, Bacteroides dorei, Bacteroides uniformis | 80 |
| | | Produce LPS to induce immunosuppressive microenvironment | Gram-negative bacteria | 96 |
| Anti-CTLA-4 antibody | The microbiota composition affects interleukin 12 (IL-12)–dependent Th1 immune responses | B. fragilis and/or B. thetaiotaomicron and Burkholderiales, Bacteroides | 98,99 |
| | | Increased CD8+ T cell, increased effector memory T cells (CD44+ CD8+ CD62L-), decreased Treg (CD4+ CD25+ Foxp3+) and M2 macrophages (F4/80+ CD206+) in the tumor microenvironment | Lactobacillus acidophilus | 100 |

CRC: colorectal cancer; Treg: regulatory T cell.
characterized and some of them have been applied to improve therapeutic response. Combination of the emerging immunotherapy and gut microbiota modification for cancer therapy undoubtedly would be a hot study direction.

**Conclusions and prospects**

With the progression of the detecting technology and bioinformatics-analyzing method on investigating gut microbiota abundance variation, researchers could accurately obtain the list of several certain bacteria involved in the carcinogenesis. Depending on the well-established results on colorectal carcinogenesis-related gut microbiota, some pre-clinical and clinical trials have taken specific species as predictor or therapeutic target for CRC. An increasingly clear map of molecular signaling pathways in IECs, which undergo cancerization, provides the cornerstone for further new therapeutic strategy development and diagnostic or prognostic biomarkers exploration.

Numerous clinical trials with CRC patients’ participations and experimental evidences with animal models have proven the certain association of gut microbiota and CRC, leading to the identification of specific bacterial species that contribute to the colorectal carcinogenesis. As comprehensively reviewed by others, we do not say much about that here. Nevertheless, an intensive investigation of the unclear relationship between special bacterial species and the possible molecular mechanisms serving on carcinogenesis should be an important researching direction in the future.

Though we review the molecular signaling pathway-mechanisms involved in colorectal carcinogenesis of colonic epithelial cells, we find that there are evident deficiency in studies focusing on the direct relationship of gut microbiota and some of the associated carcinogenic molecules. For example, pro-inflammatory cytokines, especially multifunctional ones, such as INF-γ, TNF-β, are widely considered to be involved in the inflammation procedure and ultimately induce colorectal carcinogenesis. Nevertheless, the direct mechanisms how the gut microbiota influence the variation of these important cytokines is not clear enough.

Since immune cells do not always help to suppress cancer pathogenesis, and it has been shown inconsistency among several biomarkers secreted by immune cells when used in diagnosis and prognosis, more researches are needed, especially in the field of gut microenvironment in immune activities. If attempts on gut microbiota-related CRC therapies had more convincing mechanistic bases, but less speculative components, the field would move forward much more substantially.

While personalized medicine or accurate medicine is gradually becoming an indispensable concept, especially for cancer patients, it is increasingly necessary to perform a comprehensive analysis of the significant features of the tumorigenesis. As for gut microbiota-related CRC patients, current treatment strategies, such as probiotics and fecal microbiota transplantation, have achieved encouraging improvements in patient prognosis. However, patients and oncologists are supposing much more effective therapeutic strategy with improved safety and minimized side effect. That needs intensive investigation of the mechanisms involved.

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