The Struggle Within: Microbial Influences on Colorectal Cancer

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Abstract: Recently, an unprecedented effort has been directed at understanding the interplay between chronic inflammation and development of cancer, with the case of inflammatory bowel disease (IBD)-associated colorectal cancer at the forefront of this research endeavor. The last decade has been particularly fertile, with the discovery of numerous innovative paradigms linking various inflammatory, proliferative, and innate and adaptive immune signaling pathways to the development of colorectal cancer. Because of the preponderant role of the intestinal microbiota in the initiation and progression of IBD, recent efforts have been directed at understanding the relationship between bacteria and colorectal cancer. The microbiota and its collective genome, the microbiome, form a diverse and complex ecological community that profoundly impacts intestinal homeostasis and disease states. This review will discuss the differential influence of the microbiota on the development of IBD-associated colorectal cancer and highlight the role of innate immune sensor-dependent as well as -independent mechanisms in this pathology.

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Key Words: colorectal cancer, inflammatory bowel disease, intestinal microbiota

A significant risk of morbidity experienced by patients with inflammatory bowel disease (IBD) is the development of colitis-associated colorectal cancer. The risk of developing colitis-associated colorectal cancer increases with the duration and severity of inflammation and may confer a 10-fold greater risk than that observed in the healthy population. Thus, understanding the etiology of IBD represents a key step in elucidating the events leading to colitis-associated colorectal cancer and the elaboration of strategies aimed at preventing the pathology.

Genome-wide association studies and experimental models of IBD underscore the importance of the innate immune system and the intestinal microbiota in the pathogenesis of IBD.[1–6] Numerous innate immune sensors and their associated signaling proteins have been linked to the maintenance of intestinal homeostasis through their effects on cellular proliferation, inflammation, and wound healing. An emerging body of literature highlights the impact of these innate immune sensors in protecting or promoting the development of colitis-associated colorectal cancer and much of the focus lies in the role of the complex intestinal microbiota. Indeed, the human gastrointestinal tract is colonized by thousands of bacterial species, amounting to more than 100 trillion bacteria. This complex microbial community carries a rich and diverse microbial genome, the microbiome, with a tremendous metabolic potential that influences intestinal homeostasis. Recent reports indicate that microbial composition may impact the status of various pathologic conditions including IBD and colorectal cancer. In this review we will focus on the relationship between innate sensors, the microbiota and development of colitis-associated colorectal cancer.

MICROBIAL SYMBIONTS OF THE MAMMALIAN INTESTINE

The human intestinal microbiota is a community of 10^{13}–10^{14} microorganisms that reside in the intestine and normally participate in a symbiotic relationship with their eukaryotic host.[7–9] While IBD has been recognized as a disease influenced by the intestinal microbiota, difficulty in culturing these complex microorganisms has precluded the formal identification of colitogenic bacteria. Moreover, IBD is likely not caused by a single microbial entity but rather through the action of a complex consortium of microorganisms that render identification of these bacteria even more challenging using classical microbiology culturing techniques. However, characterization of this complex ecological system has made an extensive leap forward through the use of newly developed molecular approaches focused on differentiating specific genetic signatures in ribosomal 16S genes.[10,11] Unbiased metagenomics

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sequencing has revealed that the human distal intestinal microbiota comprises two predominant phyla, the Firmicutes and Bacteroidetes, with lesser contributions from Proteobacteria and Actinobacteria, and minor contributions from Fusobacteria, Verrucomicrobia, and Cyanobacteria.[12,13] Importantly, the murine microbiota is remarkably similar[14]; thus studies performed in murine models will likely have translational implications relevant to human disease.

The microbiome contains a wealth of information, encompassing 150-fold as many genes as the human genome.[15,16] Accordingly, a considerable worldwide effort is under way to identify both the constituents and unique traits of this complex microbial community. One such endeavor is the International Human Microbiome Consortium, which includes the Human Microbiome Project and the Metagenomics of the Human Intestinal Tract project (MetaHIT).[17,18] A primary goal of these projects is to determine the presence, composition, and function of a putative core microbiome shared among all humans. Already these studies have hinted that a core human microbiome exists at the gene level, with a large number of microbial genes and pathways shared among individuals.[13,18] Deviations from this core microbiome could potentially affect human health and promote disease. Thus, characterizing the vast intestinal microbial community present in healthy as well as pathologic conditions will likely revolutionize our conception of bacteria/host interactions and identify new physiological processes influenced by the microbiota. Already the microbiota has been linked to cardiac development,[19] angiogenesis,[20] innate and adaptive immunity,[21–24] metabolism,[25] nutrient acquisition,[26] and gastrointestinal development and homeostasis.[27] Furthermore, alterations in the microbial community are associated with multiple diseases, including obesity,[13,14,28–30] fatty liver disease,[31] type 1 and type 2 diabetes,[32,33] kidney disease,[34] arthritis,[35] IBD,[16,36] and colorectal cancer.[37] In IBD, analyses of the fecal and mucosally adherent microbiota have revealed significant differences in microbial community structure between patients and healthy controls. For example, studies have consistently identified reductions in total gut microbial concentration, decreases in the richness and diversity of the microbiota, and changes in the proportion of the two dominant phyla, the Firmicutes and Bacteroidetes.[16,38–40] At the moment, though, the relationship between alterations in the microbiota and development of IBD and colorectal cancer is unclear.

MICROBIOTA CAN CONFER HOST TRAITS

The major limitation of total microbial gene pool analysis in health and disease, through what is known as metagenomic studies, relates to the correlative nature of this experimental approach. In essence, do changes in the microbial composition and metagenome directly influence the development of disease? The ability to transfer host traits through microbiota transplants suggests that a dysbiotic microbial community plays an active, rather than passive role in disease process. Glimcher and colleagues[41] have described a transferable trait in colitis-prone TRUC mice (*Tbx21*$^{-/-}$; *Rag2*$^{-/-}$ ulcerative colitis). TRUC mice develop highly penetrable colitis that progresses to colorectal cancer.[42] Housing either newborn or adult wildtype (WT) mice with TRUC mice induces aggressive colitis in this normally colitis-resistant strain.[41] Although microbiome analyses were not performed in these mice, these observations suggest that the colitis phenotype is transferred through a dysbiotic microbiota present in TRUC mice. Analysis of the TRUC microbiome and microbiota transplants into germ-free mice may address this question and provide further insight into the transmissibility of such traits.

The impact of the microbial community on host traits is not limited to an intestinal phenotype. For example, obesity alters the composition of the gut microbiota and increases its metabolic potential to harvest energy from the host diet.[13,14,28–30] Microbiota transplantation from obese mice into lean germ-free recipients induces weight gain and increases adiposity, demonstrating that these traits can be conferred through a microbial dependent mechanism.[29,30] More recently, *Tlr5*-deficient mice were observed to become hyperphagic and develop metabolic syndrome comprising hyperlipidemia, hypertension, insulin resistance, and enhanced adiposity.[43] In these mice, specific bacterial phylotypes were expanded and contracted compared to WT mice. Interestingly, hyperphagia and metabolic syndrome are transferable to WT mice upon fecal transplantation of microbiota obtained from *Tlr5*$^{-/-}$ mice.[43] These fascinating experiments demonstrate that the microbiota has the potential to profoundly impact the host health status.

HOST RECOGNITION OF MICROBIAL SPECIES AFFECTS COLITIS AND COLORECTAL CANCER

Germ-free studies have revealed a key role for the microbiota in driving colorectal cancer. Hereditary colorectal cancer is commonly modeled with the *Apc*$^{Min/+}$ (multiple intestinal neoplasia allele of the adenomatous polyposis coli gene) mouse, a model of human familial adenomatous polyposis. When raised in conventional or specific pathogen-free (SPF) conditions, a mutation in the tumor suppressor *Apc* causes this mouse strain to develop tens to hundreds of intestinal adenomas.[44,45] However, mice raised in germ-free conditions exhibit ≈50% reduction in intestinal tumors compared to those housed in SPF conditions.[46]

More recently, the microbial composition has been found to influence the development of colitis-associated colorectal cancer. In the newly developed AOM/IL-10$^{-/-}$

model, intestinal inflammation occurs spontaneously from lack of immunosuppressive IL-10, and tumorigenesis is initiated with the colon-specific carcinogen azoxymethane (AOM).[47] When raised in conventional or SPF conditions, these mice develop extensive intestinal inflammation and colonic adenomas. However, when mono-associated with *Bacteroides vulgatus* these mice exhibit fewer tumors. Remarkably, mice raised in germ-free conditions are devoid of intestinal inflammation and tumors.[47] These findings highlight the critical role of specific members of the commensal microbiota in the development of colitis-associated colorectal cancer.[47]

Although the microbiota influences the development of colorectal cancer, colitis-associated colorectal cancer, and other forms of cancer (Table 1),[48–97] the extent of host microbial recognition in this process is still unclear. Humans, mice, and other eukaryotes are equipped with an elegant repertoire of receptors, and each recognize specific conserved microbial patterns, such as components of bacterial cell walls or nucleic acids. These microbial sensors are termed pattern recognition receptors (PRR) and include retinoic acid inducible gene-I like RNA helicases (RLH), C-type lectin receptors (CLR), nucleotide-binding domain leucine-rich repeat proteins (NLR; also known as Nod-like receptors), and Toll-like receptors (TLR).[98–101] Many of these receptors are expressed on intestinal epithelial cells (IEC) as well as on various mucosal immune cells. Upon engagement, these receptors initiate signaling cascades that activate numerous downstream effector systems including mitogen-activated protein kinases (MAPK), nuclear factor of kappa B (NF-κB), and interferon regulatory factors (IRFs) that then modulate apoptosis, proliferation, cell migration, and inflammation.[102,103]

The most well-characterized family of PRRs is the TLRs. With the exception of TLR3, these extracellular or endosomal PRRs signal through the adaptor protein myeloid differentiation factor 88 (MyD88).[104,105] Accordingly, investigators have utilized *Myd88*$^{-/-}$ mice to link host innate signaling with the microbiota and development of colitis-associated colorectal cancer. Development of colitis and colorectal cancer is strongly reduced in *Il10*$^{-/-}$ mice deficient in MyD88 signaling.[1,47,106] Importantly, deletion of *Myd88* also diminishes development of spontaneous colorectal cancer in *Apc*$^{Min/+}$ mice, reducing both intestinal tumor size and multiplicity.[107] The cellular compartment responsible for MyD88-dependent proinflammatory and procarcinogenic signaling is not completely understood. However, MyD88 signaling in macrophages promotes colonic epithelial progenitor cell proliferation and cellular organization within colonic crypts,[108,109] raising the possibility that disrupted MyD88 signaling may affect the balance between proliferation and apoptosis. Indeed, bone marrow transplant experiments in *Tlr4*$^{-/-}$ mice indicate that immune cells are critical in promoting the development of colitis-associated colorectal cancer.[110,111] These data suggest that host recognition of microbial species is necessary for the development of colitis-associated colon cancer.[112–115]

Because host PRRs distinguish between a wide spectrum of microorganisms, it is important to establish their individual contribution to the development of colitis and colorectal cancer. Like TLR4,[116] PRRs of the NLR family including NOD1, NOD2, and NLRP3 have been genetically linked to the development of IBD.[112–115] However, in contrast to TLR4/MyD88, these intracellular sensors appear to play a protective role in gastrointestinal disease.[117,118] Interestingly, *Nod2*-deficient mice harbored increased numbers of gut commensal bacteria and impaired bacterial killing and antimicrobial peptide secretion in the intestine,[119] providing a potential mechanism by which *Nod2* mutations may perturb intestinal homeostasis. Recent findings indicate a link between *Nod2* polymorphisms and development of colorectal cancer.[120,121] However, the lack of data generated from experimental models of colorectal cancer and colitis-associated colorectal cancer precludes a direct assessment of the function of NOD2 in the carcinogenic processes.

Similarly, NOD1, a close relative of NOD2, has been shown recently to play a protective role against development of colitis and colorectal cancer. Compared to WT mice, *Nod1*-deficient mice displayed exacerbated colitis in response to dextran sulfate sodium (DSS) exposure.[122] Likewise, AOM/DSS-induced colitis-associated colorectal cancer and spontaneous colorectal cancer (*Apc*$^{Min/+}$) is augmented in *Nod1*$^{-/-}$ mice, a phenomenon attenuated with broad-spectrum antibiotic treatment.[122] These findings suggest a role for NOD1 and microbial signaling in the development of colorectal cancer. Furthermore, the related intracellular sensor NLRP3 also appears protective against colitis and colitis-associated colorectal cancer, a role attributed to its expression in immune cells.[123] These findings suggest a protective role for NLRP3, NOD1, and microbial signaling in the development of colorectal cancer. The fact that TLR/MyD88 signaling promotes the development of colorectal cancer, whereas NLR signaling appears to prevent this pathology, highlights the complex interaction between the host and the microbiota. These differential responses may be dictated by the bacterial motif recognized by a particular PRR and/or the location of the PRR, i.e., intracellular versus extracellular. Since NOD1, NOD2, and NLRP3 reside in an intracellular location, perhaps bacterial invasion activates these microbial sensors, which then initiate a protective response to clear invading bacteria and restore intestinal homeostasis.

Although the above studies clearly indicate the importance of PRRs in modulating the development of colitis-associated colorectal cancer, the interplay between

TABLE 1. Bacterial Associations with Carcinogenesis

Bacteria	Cancer	*	Evidence	Refs.
Helicobacter hepaticus	Colorectal	A	Augments AOM-induced, and spontaneous colorectal cancer in *Smad3*$^{-/-}$, *Rag2*$^{-/-}$ and *Apc*$^{Min/+}$ mice	(48–50,179,180,213)
H. hepaticus + *H. bilis*	Colorectal	A	Dual infection induces colorectal cancer in *Mdr1a*$^{-/-}$ mice	(51,52)
H. typhlonius + *H. rodentium*	Colorectal	A	Dual infection in neonates induces colorectal cancer in *Il10*$^{-/-}$ mice	(53,181)
Streptococcus bovis	Colorectal	H	*S. bovis* bacteremia and endocarditis associated with human colorectal cancer	(54–58)
	Colorectal	A	Augments AOM-induced cancer in rats	(59)
Bacteroides fragilis	Colorectal	A	Enterotoxigenic *B. fragilis* augments spontaneous (*Apc*$^{Min/+}$) colorectal cancer in mice	(60)
	Colorectal	H	Increased prevalence of enterotoxigenic *B. fragilis* in human colorectal cancer	(148)
B. vulgatus	Colorectal	A	Induces mild AOM-induced colorectal cancer in *Il10*$^{-/-}$ mice	(47)
Escherichia coli	Colorectal	H	Increased mucosa-associated *E. coli* in human Crohn's and colorectal cancer	(61)
Citrobacter rodentium and C. freundii	Colorectal	A	Etiologic agent of transmissible murine colonic hyperplasia	(62)
	Colorectal	A	Augments spontaneous (*Apc*$^{Min/+}$) and DMH-induced colorectal cancer in mice	(63,64)
H. pylori	MALT lymphoma	H	Lymphoma regression after eradication of *H. pylori* in humans	(65–68)
H. mustelae	MALT lymphoma	A	Induces MALT-like lymphoma in ferrets	(69)
H. pylori	Gastric	H	Causative agent of human peptic ulcer disease; Predisposes to gastric cancer	(70,212)
	Gastric	A	Induces gastric cancer in gerbils	(71)
H. felis	Gastric	A	Induces gastric cancer in insulin-gastrin transgenic mice	(72)
S. anginosus	Oral and esophageal	H	Found in human oral and esophageal cancer tissue	(73–77)
H. hepaticus	Hepatobiliary	A	Induces hepatitis and liver cancer in A/JCr mice	(78–80)
Salmonella typhi	Hepatobiliary	H	Chronic infection increases risk of hepatobiliary cancer by up to 8-fold in humans	(81–85)
Chlamydia pneumoniae	Lung	H	Chronic infection raises lung cancer risk; renders epithelial cells resistant to apoptosis	(86–91)
H. hepaticus	Mammary	A	Promotes mammary cancer in *Apc*$^{Min/+}$ and *Rag2*$^{-/-}$; *Apc*$^{Min/+}$ mice	(48)
Lawsonia intracellularis	Proliferative enteropathy	A	Induces proliferative intestinal lesions in animals resembling human IBD lesions	(92)
Mycoplasma fermentans and M. penetrans	General	C	Induce malignant transformation and independence from growth factors *in vitro*	(93–95)
		A	Infected cells induce tumors with high *H-ras* and *c-myc* expression in mice	(96)
Bartonella sp.	General	A	Induces tumor-like structures and angiogenesis through VEGF	(97)

*H, evidence in humans; A, evidence in animal models; C, cell lines.

inflammation and colorectal cancer is not always clear. For example, development of colitis-associated colorectal is strongly reduced in AOM/DSS-treated *Tlr4*$^{-/-}$ mice compared to WT mice, whereas the inflammatory status is marginally affected in these mice.[110] This incomplete relationship between inflammatory status and tumorigenesis is also observed in AOM/DSS-treated *Il6*$^{-/-}$ and *Stat3*$^{EC-/-}$ mice.[124] The IL-6/STAT3 signaling axis profoundly

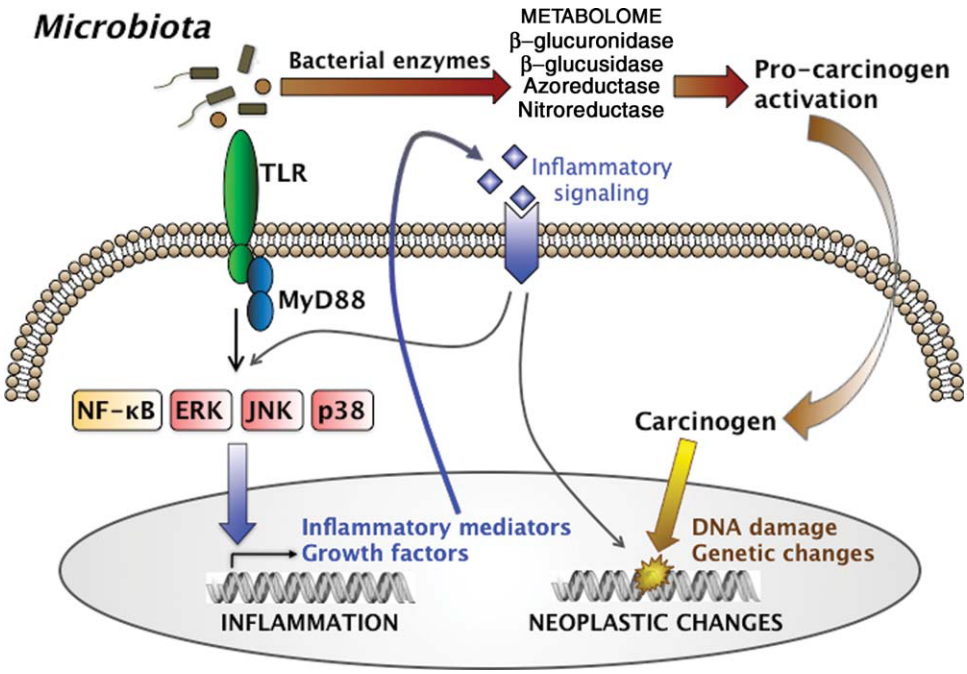

FIGURE 1. The intestinal microbiota promotes inflammation and neoplasia in the colon. Conserved microbial signatures are recognized by pattern recognition receptors such as TLRs, which trigger downstream signaling pathways leading to the expression of various genes including growth factors and inflammatory mediators. Autocrine and paracrine signaling from these mediators amplifies inflammation and promotes neoplasia. Independent of inflammation, microbial enzymes of the metabolome process latent dietary procarcinogens to their biologically active form and elicit neoplastic changes.

modulates intestinal homeostasis through pro-proliferative and antiapoptotic effects. Remarkably, while AOM/DSS-exposed *Il6*$^{-/-}$ or *Stat3*$^{IEC-/-}$ mice display increased intestinal inflammation and proinflammatory cytokine secretion, they exhibit significantly fewer intestinal tumors than WT mice. In addition, *Enterococcus faecalis* mono-associated *Il10*$^{-/-}$ mice develop inflammation to the same extent as conventionalized *Il10*$^{-/-}$ mice; however, tumors fail to develop in the mono-associated mice.[125] These observations suggest that mechanisms other than inflammation can influence the development of colorectal cancer. Although this concept is at odds with epidemiologic data in humans showing a correlation between inflammatory status and colorectal cancer,[126] an intriguing possibility is that microbial status could impact tumorigenesis without directly affecting the inflammatory status (Fig. 1). Indeed, up to 80% of patients with long-standing IBD (<30 years) do not develop colitis-associated colorectal cancer,[127] suggesting that inflammation alone is not sufficient to promote colorectal cancer.

MICROBIAL INFLUENCE ON CARCINOGENESIS

The impact of microorganisms on the development of colorectal cancer has been mostly studied from the point of view of PRR signaling and inflammatory responses. However, it has become apparent that the tremendous metabolic capacity offered by the microbiota (referred to as the metabolome) likely plays an important role during the carcinogenesis process, independently of overt inflammation (Table 2).

The activation or detoxification of carcinogens that modulate the tumorigenic process is strongly influenced by the abundant enzymatic activities contributed by the intestinal microbiota.[128–168] In the 1960s it was observed that germ-free rats did not develop intestinal tumors from exposure to the carcinogenic plant glycoside cyasin. However, intestinal tumors developed in germ-free rats directly administered methylazoxymethanol, the downstream active metabolite of cyasin.[169] The generation of this metabolite is dependent on bacterial β-glucosidase enzyme activities,[169] strongly implying that the microbiota influences the production of bioactive carcinogenic compounds. Further investigation has revealed that the intestinal microbiota can convert latent carcinogens into bioactive forms through the action of various enzymes such as β-glucuronidase, β-glucosidase, azoreductase, and nitroreductase.[170] The most commonly used experimental colon carcinogen, AOM, is first hydrolyzed in the liver to methylazoxymethanol and conjugated with glucuronic acid before transport to the intestine through bile secretion.[171] Further metabolism through bacterial β-glucuronidase converts the glucuronic acid-conjugated methylazoxymethanol to its carcinogenic form that spontaneously yields the highly reactive methyl carbonium ion.[170,172,173] Interestingly, β-glucuronidase inhibition significantly reduces the ability of AOM to induce tumors in rats.[149] In addition, the heterocyclic amines 2-amino-3-methylimidazo-[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) that form when meat is cooked to high temperatures must be

TABLE 2. Bacterial Products Linked to Carcinogenesis

Bacterial Toxins	Prominent Producers	Cancer Relevance	Refs.
Bacillus fragilis enterotoxin	Enterotoxic *Bacillus fragilis*	Induces Stat3 activation and Th17 cells that promote colorectal tumorigenesis; Increased prevalence in human colorectal cancer patients	(60,128)
Cytolethal distending toxin	*Helicobacter hepaticus*	Induces progression of hepatitis to dysplasia in mice	(129)
	Escherichia coli, Campylobacter sp., *Salmonella typhi*,	Acts as a DNase to create double-strand DNA breaks; arrests host cell cycle at G2/M transition through inactivation of Cdk1	(130–133)
Cycle inhibiting factor	Enteropathogenic *E. coli*	Disrupts cell cycle through stabilization of Cdk inhibitors p21 and p27, cells replicate DNA without dividing, results in hyperploidy	(134–137)
Cytotoxic necrotizing factor	*E. coli*	Prevents apoptosis via Bcl-2 upregulation in epithelial cells; promotes motility in uroepithelial cells	(138–140)
Pasteurella multocida toxin	*Pasteurella multicida*	Promotes anchorage-independent growth of enterocytes and fibroblasts	(141,142)
Epidermal differentiation inhibiting factor	*Staphylococcus aureus*	Induces transient hyperplasia in the epidermis of mice	(143,144)
Cytotoxin-associated antigen A	*H. pylori*	Induces rapid progression through cell cycle and morphological changes that promote invasion; Augments the risk of human gastric cancer	(145–148)
Bacterial Enzymes	**Enzymatic Action**	**Cancer Relevance**	**Refs.**
β-glucuronidase	Hydrolyzes glucuronic acid conjugates in bile	Converts AOM and heterocyclic amines from cooked meat to active carcinogens; induces colon cancer in rodents	(149–150)
		High levels associated with a higher risk of human colorectal cancer	(151)
β-glucosidase	Hydrolyzes plant glycosides	Converts cyasin to its active carcinogen; induces colon cancer in rats	(169)
Nitroreductase	Reduces nitrates to nitrites, forming N-nitroso compounds	Nitrates in red meat and processed foods linked to cancer	(152,153)
		N-nitroso compounds act as DNA alkylating agents	(154)
Azoreductase	Reduces azo compounds, may produce mutagenic amines	Human intestinal microbiota can convert azo compounds in some food products to carcinogenic aromatic amines	(155–156)
Mucinase	Degrades protective mucins	Areas of colonic dysplasia located at mucin-depleted foci	(157–159)
Catalase	Converts hydrogen peroxide to water and oxygen	Produced by lactic acid bacteria; protective against DMH-induced colorectal cancer	(160)
		Catalase attenuates *E. faecalis*-induced aneuploidy	(161)
7-α/β dehydroxylation enzymes	Catalyze the formation of secondary bile acids	Levels of deoxycholic acid (DCA) correlate with risk of colorectal cancer; high DCA found in colon cancer patients	(162–165)
		DCA activates beta-catenin, promotes proliferation and invasion; promotes carcinogenesis in animal models of colorectal cancer	(166–168)

metabolically activated by the enzymatic activities of intestinal microbes to exert full mutagenic potential.[150,174–177] These important observations have a profound implication for the field of colorectal cancer research since animal facilities at various institutions and even commercial vendors likely harbor different microbial communities.[178] Presumably, their microbiota contain differential metabolic capacities that could influence their responses to procarcinogenic agents such as AOM. Likewise, phenotypic variability observed in $Il10^{-/-}$ and $Smad3^{-/-}$ mice appears to correlate with the presence of specific opportunistic microorganisms such as *Helicobacter* sp. and *Citrobacter* sp.[179–182]

Interestingly, probiotic bacteria such as *Lactobacillus* sp. and *Bifidobacterium* sp. exert anticarcinogenic effects, in part by inactivating microbial enzymes important for procarcinogen activation.[183] For example, probiotic lactic acid bacteria including *L. casei* and *L. acidophilus* can decrease

the activity of β-glucuronidase, azoreductase, and nitroreductase.[184,185] In fact, *B. longum* reduces AOM-induced aberrant crypt formation, which correlates with a decrease in AOM-activating β-glucuronidase activity.[186] In addition, other *Lactobacillus* sp. and *Bifidobacterium* sp. can inhibit DNA damage and tumorigenesis induced by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), 1,2-dimethylhydrazine (DMH), AOM, and heterocyclic amines IQ, MeIQx, and PhIP,[187–191] although the extent to which inactivation of carcinogen-activating enzymes is involved in these processes remains unknown. This balance of activation and detoxification is reminiscent of the balance between host oncogenes and tumor suppressors and suggests that microbial community structure and its associated metabolomic capacity play a significant role in the initiating step of carcinogenesis (Fig. 1).

DNA damage and chromosomal instability are early genetic events involved in the development of colorectal cancer. Chromosomal instability, such as aneuploidy, is associated with long-standing IBD and frequently predicts the future development of colorectal cancer.[192–194] A common commensal intestinal bacterium, *E. faecalis*, induces aneuploidy in colonic epithelial cells and aggressive colitis in mono-associated $Il10^{-/-}$ mice.[161,195,196] Inhibitors of reactive oxygen and nitrogen species (RONS) prevent *E. faecalis*-induced aneuploidy,[161,197] suggesting that the unique ability of this bacterium to induce RONS can lead to chromosomal instability in a susceptible host. Experimentation in mice deficient in the antioxidant enzymes glutathione peroxidase -1 and -2 ($Gpx1/2^{-/-}$) further supports the role of the microbiota and RONS in carcinogenesis. $Gpx1/2^{-/-}$ mice spontaneously develop intestinal tumors with ≈25% penetrance when raised in conventional conditions, whereas this is reduced to <9% in SPF conditions and abolished in germ-free animals.[198] These findings strongly indicate that the microbiota—and most likely specific members of the microbiota—induce RONS that promote carcinogenesis.

The protective role for the microbiota in intestinal homeostasis includes their ability to influence epithelial cell proliferation and apoptosis. A major mechanism by which this is accomplished involves microbial fermentation of dietary fibers to the short chain fatty acids (SCFA) acetate, propionate, and butyrate. These SCFAs, butyrate in particular, are avidly absorbed by colonocytes and used as a primary source of energy. In addition to their important antiinflammatory role,[199,200] SCFAs support intestinal homeostasis and the resolution of intestinal injury by promoting cellular proliferation and differentiation in the normal nonneoplastic colon.[200,201] Remarkably, SCFAs exert an opposite effect on cancerous cells. Butyrate in particular can induce apoptosis in colon cancer cell lines by a variety of mechanisms, most of which are associated with its role as a histone deacetylase inhibitor. This can involve hyperactivation of Wnt, sensitization to Fas-mediated apoptosis, and activation of the intrinsic/mitochondrial apoptosis pathway through upregulation of Bak, downregulation of Bcl-xL, cytochrome-c release, and caspase-9 activation.[202–207] Nonetheless, butyrate may only provide protection during the early stages of tumorigenesis, as the two major receptors for butyrate, SLC5A8 and GPR109A, are frequently silenced in human cancers.[208–211]

Although colorectal cancer has not been linked to any specific microorganisms, some species have been identified as cancer-promoting bacteria (Table 1). For example, *Helicobacter pylori* infection in humans predisposes to gastric cancer.[212] In addition, *H. hepaticus* augments both experimental colitis-associated colon cancer and spontaneous colorectal cancer in mice.[179,213] *Bacteroides fragilis* is a common intestinal commensal, yet an enterotoxigenic variant induces spontaneous colonic tumorigenesis in $Apc^{Min/+}$ mice.[60] Therefore, exclusion of opportunistic pathogens by commensal bacteria may represent a natural defense against gastrointestinal diseases, including colorectal cancer. This is exemplified in the therapeutic use of probiotics, live bacteria that confer health benefits to the host. Probiotic bacteria engage the host to produce biofilms that prevent adhesion or invasion of pathogenic species, maintain gut barrier function by preventing redistribution of host tight junction proteins, induce host cytokines to modulate inflammation an immunity, and neutralize carcinogens and toxins.[214–219] Commensal bacteria may also exert selective pressure against potential intruders as a mechanism to maintain a niche. For example, conditioned medium derived from a human intestinal microbiota directly inhibits synthesis of an enterohemorrhagic *E. coli* O157:H7 toxin.[220] Communication with the commensal microbiota provokes antimicrobial responses from the host epithelium including the release of antibacterial lectins like RegIIIγ, angiogenins, and α-defensins.[221–223] Remarkably, these antibacterial products not only deplete subsets of potentially pathogenic bacteria, but also protect against subsequent aberrant immune responses. For example, the intestinal microbiota of mice expressing a human enteric α-defensin, DEFA5, is depleted of segmented filamentous bacteria.[222] Intriguingly, this class of bacteria induces IL-17-producing T-helper (Th17) cells, which have been strongly linked to IBD and colorectal cancer.[60,178,222,224,225] In fact, *B. fragilis* enterotoxin promotes colorectal carcinogenesis through IL-17,[60] and *B. fragilis* prostate-specific antigen (PSA) exerts protection by suppressing Th17 differentiation.[226]

These numerous reports highlight the critical role of the intestinal microbiota in shaping intestinal health. Members of this microbial community can modulate immune and inflammatory responses, activate and detoxify carcinogens, promote DNA damage and chromosomal instability, shift the balance of proliferation and apoptosis, and thwart pathogen invasion.

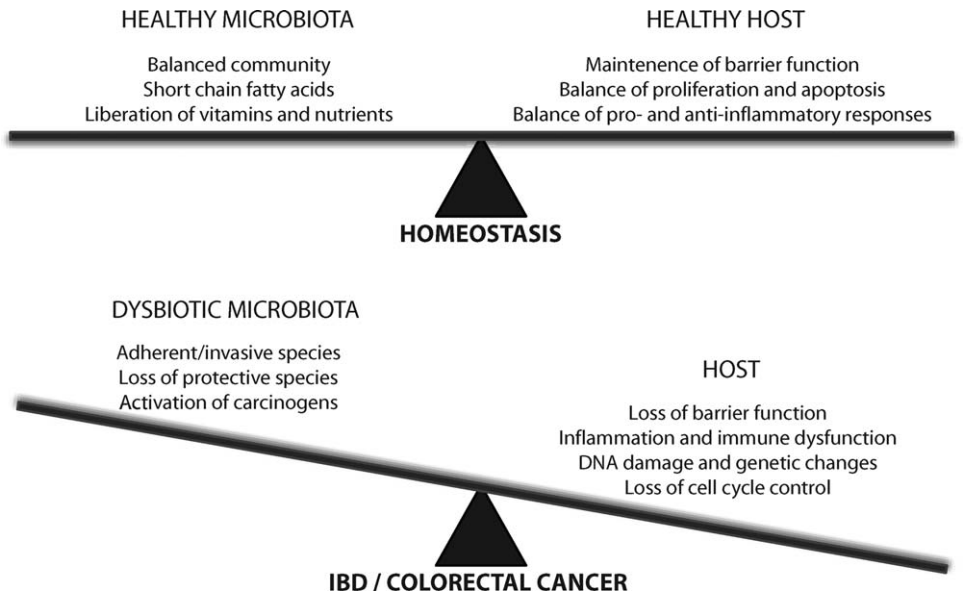

FIGURE 2. Microbial dysbiosis is associated with a variety of diseases including IBD and colorectal cancer. In a healthy individual a balanced microbial community structure promotes the maintenance of intestinal homeostasis. The presence of a dysbiotic microbiota that is associated with loss of protective species and predominance of adherent/invasive species promotes inflammation, activation of innate and adaptive immunity, and loss of barrier function in a susceptible host. Longstanding IBD and persistence of microbial dysbiosis may encourage genetic changes, loss of cell cycle control, and immune dysfunction that promote neoplastic changes and development of colorectal cancer.

FACTORS INFLUENCING MICROBIAL COMMUNITY STRUCTURE

The intestinal microbiota is a dynamic community strongly influenced by external forces including genetic factors, inflammation and infection, antibiotic treatment, and diet.[16,29,30,227–231] Metagenomics studies have indicated greater similarity between the intestinal microbiota of family members versus unrelated individuals.[14,229] Yet analyses of congenic mice bred from heterozygotes and raised in the same cage have revealed that deficiency of even one host gene can significantly change intestinal microbial community composition. For example, deficiency of the Crohn's disease-associated *Nod2* gene increases the total number of commensal bacteria and reduces the ability of these mice to clear *H. hepaticus*.[119] NOD1-deficient mice harbor an increased total number of commensal bacteria, yet a decreased number of protective *Lactobacillus* species.[232] MyD88-deficient NOD diabetic mice exhibit microbiota changes at the family level and in the proportion of the two prominent phyla, the Firmicutes and Bacteroidetes. Furthermore, deficiency of MyD88 allows bacterial penetration of the intestinal epithelial barrier and systemic dissemination.[233,234] Taken together, these data suggest that host innate signaling influences the microbial community structure, which in turn shapes intestinal homeostasis.

Alterations in microbial community structure occur rapidly upon changes in dietary habits.[29,30,235,236] Gordon and colleagues found that the intestinal microbial community structure of mice maintained on standard low-fat chow could be changed significantly within weeks of switching to a high-fat diet (HFD). Consumption of HFD was associated with a shift in the balance of the two dominant phyla, the Bacteroidetes and Firmicutes.[29,30] In fact, the most prominent change was an expansion in one group of Firmicutes, the Mollicutes, which again contracted when mice were switched back to standard low-fat chow. The Mollicutes expansion was associated with an increased ability for energy harvest, and this trait could be transferred by microbiota transplant into lean recipients.[29,30] Importantly, similar changes in the proportion of Firmicutes and Bacteroidetes have been identified in overweight and obese humans, genetically obese mice, and obesity-resistant mice fed HFD diet.[13,14,28–30] As human colorectal cancer is tightly linked to diet,[237,238] it will be interesting to see if diet-induced changes in the microbiota also influence the development of colorectal cancer.

CONCLUSIONS AND FUTURE DIRECTIONS

The events leading to the development of colorectal cancer are complex. Adding to the complexity of the disease is the growing evidence that the microbiota and its associated microbiome are active participants in the pathology. Within recent years, we have experienced exponential growth in our understanding of how the microbiota affects intestinal health and diseases. Gastrointestinal diseases including esophagitis/Barrett's esophagus, gastric cancer, IBD, and colorectal cancer have been strongly linked to

alterations in the composition of the gut microbiota.[16,37,47,212,239] Although tantalizing, these observations have not moved beyond the descriptive phase and into functional and malleable territory. Consequently, numerous questions remain unanswered.

The most pressing question is if the shift in the microbiota directly alters the course of disease. Functional studies using dysbiotic microbiota obtained from germ-free animals and various disease states—inflammation, colorectal cancer, etc.—will help address this important question.

Another key question relates to the identity of the microorganisms promoting health and/or disease. Continuous progress in next-generation gene sequencing technology and more affordable cost associated with this high-throughput technique will likely yield important information regarding the composition of healthy and dysbiotic microbiota. This information may be sufficient to initiate functional experiments where a cause/effect relationship could be established using animal models. The fact that a "core microbiome" was recently identified in healthy individuals and that deviation from this core is associated with IBD[16–18] indicate that a potential dysbiotic microbiota could be found in colorectal cancer patients and tested in experimental models.

Although identification of microbial consortia associated with particular pathological conditions represents an important milestone, this critical step is not sufficient to fully comprehend the role of the microbiota in health and disease. Indeed, the in-depth characterization of the metabolic capacity contributed by the microbiota in relation to the maintenance of intestinal homeostasis and development of disease states is undeniably a central piece of the metagenomic puzzle. With this knowledge will come the possibility to manipulate the human microbiota and its metabolic capacity as an innovative approach to treating and preventing IBD and colorectal cancer.

In summary, the microbiota can no longer be considered a bystander in the complex biological events regulating intestinal homeostasis. Both the composition of the microbiota and its associated metabolic capacity likely influence host susceptibility to developing various pathological conditions, including IBD and colorectal cancer (Fig. 2). Harnessing the power of the microbiota holds tremendous promise in medicine and could represent a novel means to alleviate these devastating diseases.

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