# Gut Microbiota, Inflammation, and Colorectal Cancer

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## Keywords

colorectal cancer, inflammation, colitis, mucosal immunology, microbiota/microbiome, *Fusobacterium*

## Abstract

Colorectal cancer is the second-leading cause of cancer-related deaths in the United States and fourth-leading cause of cancer-related deaths worldwide. While cancer is largely considered to be a disease of genetic and environmental factors, increasing evidence has demonstrated a role for the microbiota (the microorganisms associated with the human body) in shaping inflammatory environments and promoting tumor growth and spread. Herein, we discuss both human data from meta'omics analyses and data from mechanistic studies in cell culture and animal models that support specific bacterial agents as potentiators of tumorigenesis—including *Fusobacterium nucleatum*, enterotoxigenic *Bacteroides fragilis*, and colibactin-producing *Escherichia coli*. Further, we consider how microbes can be used in diagnosing colorectal cancer and manipulating the tumor environment to encourage better patient outcomes in response to immunotherapy treatments.

## Contents



## INTRODUCTION

Cancer is a multifaceted disease influenced by both environmental and genetic factors. The microorganisms associated with the human body—collectively referred to as the microbiota—lie at the intersection of these factors. Aging and dietary patterns not only influence cancer susceptibility but also have profound effects on microbiota composition (17, 18, 21, 41, 47, 90). The microbiota plays a myriad of roles in human health and disease, from entraining immune system development and maintaining homeostasis to influencing autoimmune diseases and allergies, that cannot simply be parsed into strict pathogenesis and commensalism (9). How these organisms may influence a disease like cancer, which can develop over the course of decades, is similarly unclear. To survive in a human body tissue over the time frame in which solid tumors develop and influence cancer progression, a bacterium would need to identify metabolic substrates (carbon sources) to sustain growth, avoid immune-mediated destruction, and effectively compete with other microbes (if any) in that environment. These requirements necessitate features or effectors that shape a developing tumor microenvironment. Despite the seemingly insurmountable selective pressures exerted by evolving tumors, the concept of microbe-driven cancer is longstanding, given the well-described roles of *Helicobacter pylori* in gastric cancer (70) and human papillomavirus in cervical cancer (13), among others (**Figure 1**).

The gut microbiota is particularly well suited to influence cancer, as it has already evolved to survive and thrive in the intestinal environment. The gut microbiota, either as individual microbes (34) or as a microbial community exerting a collective effect, may potentiate or mitigate colorectal cancer (CRC) risk. The high bacterial density in the colon and the observation that bacteremias with certain microbes like *Streptococcus gallolyticus* can be clinical indicators of occult colonic adenomas (precancerous tumors) and CRC underscore the importance of studying the roles of gut microbes in CRC (11). The mechanisms by which microbes influence tumorigenesis in the intestine, a particularly microbially rich and immunologically complex environment in the human body, remain to be fully clarified.

## DELINEATING HOW INFLAMMATION AND THE MICROBIOTA INFLUENCE CRC PROGRESSION

Tumor formation in the colon begins with the transition of a normal epithelium to a state of hyperplasia, in which cell proliferation is increased (**Figure 2**). As this occurs, epithelial architecture loses its characteristic shape and organization and becomes dysplastic. This dysplasia has the

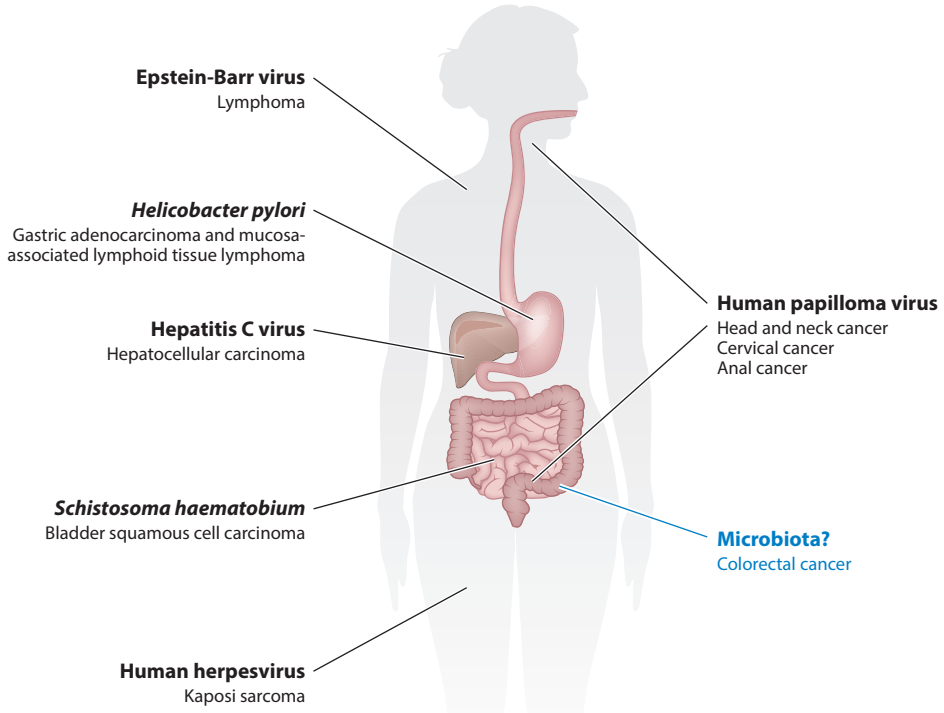


**Figure 1**

Microbes with well-characterized roles in the development of specific cancers (34, 73, 74).

potential to develop into a nonmalignant adenoma, which usually is a polyp that grows from this region of hyperproliferative epithelium and protrudes into the colonic lumen. In response to other changes in the tumoral genetic and immunological microenvironment, adenomas can invade into the submucosa and become cancerous. With continued malignant growth, these tumors develop the potential to spread beyond the colon. For those interested in the microbial world, the microbial communities of the colonic lumen and of tumors offer rich opportunities for discovery efforts, ranging from whether microbes can block CRC development to how microbes can contribute to colorectal carcinogenesis.

The development of CRC from normal colonic epithelia requires a series of genetic and inflammatory-immunological factors to enable and shape a tumorigenic milieu. The initial formation of regions of hyperplasia and polyps can occur in response to the loss of tumor-suppressor genes like *APC* (adenomatous polyposis coli), a component of the Wnt/β-catenin pathway that is important for controlling cell proliferation. In addition, mutations in genes that encode the machinery for DNA repair, such as *hMSH2*, can also contribute to colorectal tumorigenesis. These genetic alterations can be inherited, as in familial adenomatous polyposis or in Lynch syndrome, respectively. Such hereditary forms of CRC account for approximately 5–10% of all cases. Furthermore, the development of dysplasia and CRC is strongly influenced by the inflammatory state of the colon. In patients with inflammatory bowel disease (IBD), chronic, severe inflammation of

**IBD:** inflammatory bowel disease; includes ulcerative colitis and Crohn disease

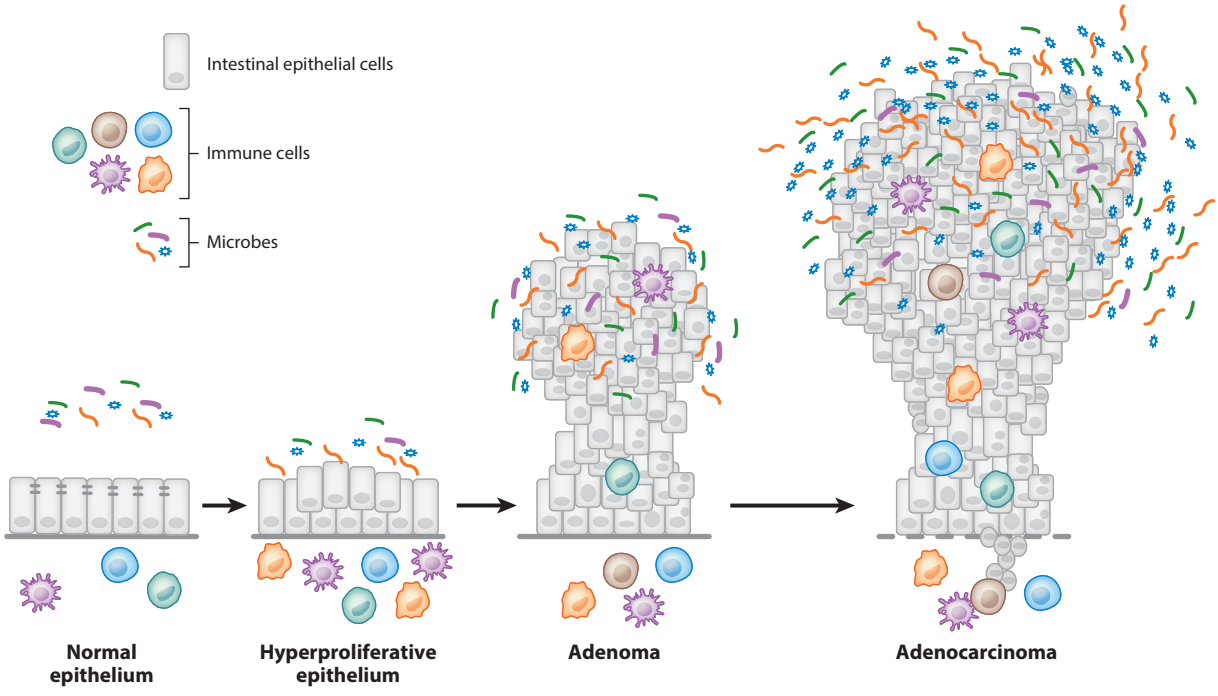


**Figure 2**

Progression of colorectal cancer development from normal epithelium to an invasive carcinoma.

the colon increases the likelihood of developing CRC (6). More subtle inflammation in otherwise healthy colonic tissues plays a major role in the conversion of a healthy colon to a dysplastic colon as well. As crypts become dysplastic, the barriers between the epithelium that aid in separating the microbiota from the immune cells in the lamina propria begin to break down. Barrier disruption facilitates bacterial translocation and, ultimately, exposure of immunogenic microbial compounds to both epithelial cells and antigen-presenting cells.

Activation of immune signaling pathways by bacterial stimuli results in a loss of homeostasis that drives a proneoplastic inflammatory environment. The role of bacterial products and their recognition by host cells in carcinogenesis has been thoroughly reviewed elsewhere (53). Below, we briefly revisit how this recognition results in inflammation and a protumorigenic milieu in CRC. Inflammatory signatures implicated in colorectal carcinogenesis studies include inflammasome activation (28) and activation of the NF-κB pathway (46), both of which can occur by changes in the mutational landscape or in response to either microbial stimuli or cytokines. NF-κB pathway activation mediates production of proinflammatory cytokines like IL-6, which has a pathogenic role in CRC by allowing survival and proliferation of intestinal epithelial cells, especially in colitis-associated cancer. The NF-κB pathway also serves as an important regulator of the genes encoding tumor necrosis factor (TNF) and cyclooxygenase 2 (COX-2), which are often highly overexpressed in inflammatory bowel disease as well as in colorectal adenomas and adenocarcinomas (7, 26, 54, 91). TNF is a cytokine that can drive activation of the NF-κB pathway, thereby driving a feed-forward loop that promotes cell proliferation and survival. COX-2 is an enzyme that produces prostaglandins, bioreactive lipids that influence both colonic inflammatory state and tumor progression through multiple mechanisms. Other key innate components of

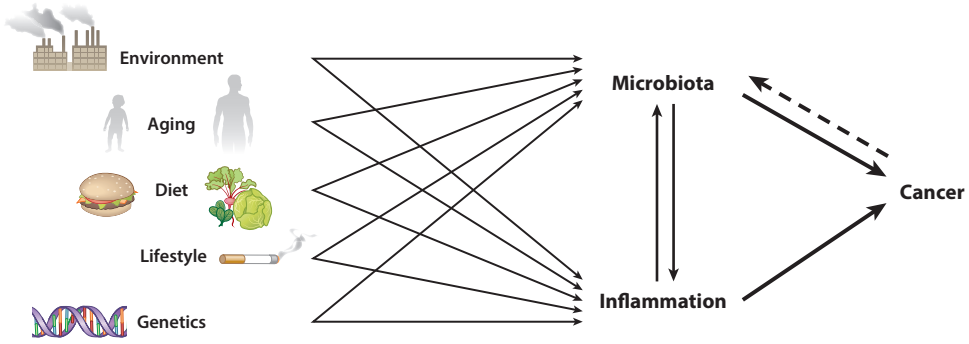


**Figure 3**

Cross talk among environmental and genetic factors with the microbe-inflammation-cancer axis.

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. Beyond the innate immune system, both regulatory T cells and a subset of T helper cells known as Th17 cells modulate inflammation within the colon and contribute to inflammation-associated CRC. The development and function of these cells are influenced by microbes or microbial products, highlighting the dependence of inflammation and the microbiota in shaping the pretumor environment.

The microbiota and specific constituents and/or functions thereof are important drivers of the immune response. Supporting data come from clinical observations of patients with severe, protracted intestinal inflammation, as can occur in IBD, from molecular epidemiologic studies (64), and from rodent models. In the absence of microbes (i.e., germfree), mouse and rat models of intestinal tumorigenesis display reduced tumor loads as compared to those reared under conventional conditions (25, 52, 86). Such studies raise the question of whether specific microorganisms are necessary for the development of the proinflammatory tumor environment or the mere presence of bacterial products produced by any microorganisms is sufficient. Recent metagenomic analyses provided robust evidence not only that microbial communities in CRC tissues differ from the microbiota of healthy host tissues, but also that specific members of the microbiota may contribute to the development of a proinflammatory milieu and CRC. Inflammation, diet, and host genetics, among other considerations, further complicate this interpretation, as these factors can influence microbiota composition and function. Additionally, the tumor environment is populated by immune cells, which serve to provide both pro- and antitumor immunity and can be shaped by the resident microbiota even after progression to CRC. Rather than a straightforward causal relationship, the interactions between the microbiota, immune system, and CRC are a multifactorial web that merits deep consideration, as their connectivities remain to be fully disentangled (**Figure 3**).

**RNS/ROS:** reactive nitrogen/oxygen species that cause nitrosative and oxidative stress, respectively, that can damage cells; they include such molecules as nitric oxide and superoxide

## USING BIG DATA TO IDENTIFY POTENTIAL MICROBIAL DRIVERS OF CRC

Given the difficulties of parsing out the tripartite exchange between host genetics, environmental exposures, and the microbiota, it is important to identify the microbial members that may be a part of the conversation. As CRC develops over the course of 30 years or more, the microbial

community that shaped the pretumor environment from a state of hyperplasia no longer exists and is rarely, if ever, obtainable from human sampling in sporadic colorectal cancer, given the need for longitudinal sampling over many decades. However, in order to have an effect on driving neoplasia, a microbe would need to localize to the region of interest (the evolving tumor) and remain for a long enough period of time for any procancerous functions to influence the colonic environment. This requirement for extended colonization enables the use of samples from later stages of CRC progression, for which human samples are more readily available from colonoscopic examinations and resections.

**Meta'omics:** high-throughput, unbiased research approaches targeting DNA (metagenomics), RNA (metatranscriptomics) and metabolites (metabolomics), among others, from a microbial community

***Fusobacterium nucleatum*:** a CRC-associated microbe whose normal reservoir is the human oral cavity

Following this logic, unbiased meta'omic analyses examined the microbial communities of human colonic adenocarcinoma samples and nearby normal colonic tissues. CRC tissues had decreased microbial diversity, including a reduction of certain bacterial genera like *Clostridium* and *Bacteroides* (49). This major shift in microbial community structure may be due to the inhospitable tumor environment, in which the rapidly growing tumor cells are competing for nutrients and the infiltrating immune cells are producing inflammatory compounds, like RNS and ROS, that can be toxic to microbes. In independent studies, CRC-involved tissues were specifically enriched in *Fusobacterium* spp., predominantly *Fusobacterium nucleatum*, relative to adjacent non-neoplastic tissue (15, 49, 55). *F. nucleatum* is a gram-negative bacterium and a normal constituent of the human oral cavity. As a resident member of the oral microbiota, *F. nucleatum* has been largely studied for its role in periodontal health (75) and its many adhesins that mediate binding to abiotic surfaces, host cells or other microorganisms (19, 40, 45). While *Fusobacterium* spp. are rarely detected in the gut microbiota of healthy individuals, they can be isolated from patients with IBD (80), further supporting a link between fusobacteria and an inflamed colonic environment.

Establishing direct relationships between the presence of *F. nucleatum* and increased CRC risk is a challenge in the absence of longitudinal data. However, identification of *F. nucleatum* enrichments during the premalignant stage of colorectal carcinogenesis begins to build a case for *F. nucleatum* as a biomarker for colonic pathology. Several studies now show *F. nucleatum* enrichments in colorectal tissues with high-grade dysplasia and adenomas (30, 55). Further, so-called big data studies are not limited to the microbial side of the equation, as cancer genomic and epigenetic research use similar techniques to define many molecular features of CRC. If *F. nucleatum* were mediating specific changes to the pretumor milieu, *F. nucleatum* enrichments might correlate with specific molecular phenotypes. Indeed, such correlations have been observed between fusobacterial enrichment and both genomic and epigenetic subsets of CRC, including microsatellite instability (MSI; a marker of mismatch repair)-high lesions and CpG island methylator phenotype (CIMP)-high lesions (42, 59, 82). While several data sets support CRC enrichments of *F. nucleatum*, such data are not proof positive that *F. nucleatum* is a direct protagonist or lone actor in CRC. Deep sequencing efforts in human CRC samples have revealed that *Fusobacterium* species often co-occur with other gram-negative anaerobes, including *Campylobacter* species (94). Given *F. nucleatum*'s role as an important organizer of biofilms in the oral cavity, it may be a pioneer microbe that creates physical and metabolic scaffolds that support broader polymicrobial shifts in evolving tumors over time. Indeed, recent work examining microbial community changes across CRC progression has sought to identify strong microbial networks that might function together at the different stages of tumor formation (63).

Although meta'omic surveys have provided vast amounts of data that were unimaginable ten years ago, these approaches have important limitations that require an understanding of the methods used in these studies (see sidebar about Who's Doing What in the Tumor Microenvironment?). Because of recent data supporting *F. nucleatum* as an important CRC-associated microbe in numerous independent studies, we have focused our attention on it here. However, other

## WHO'S DOING WHAT IN THE TUMOR MICROENVIRONMENT?

Understanding the limitations of methods used in microbiome research is important for interpreting such studies. The approaches used in Kostic et al. (49) and Castellarin et al. (15) demonstrate these differences. Bacterial 16S ribosomal amplicon DNAseq (14) and Pathseq (50) can be used to define the microbial DNA present but furnish no information regarding viability or function. RNAseq (60, 95) can be used to identify transcriptionally active microbial constituents, but it can be limited in providing functional information because of the preponderance of both bacterial and host ribosomal RNA sequences. Ideally, metagenomic approaches would be coupled to a metatranscriptomic analysis (31) of the microbes in colonic adenomas and adenocarcinomas to provide the greatest insight, but the methods to do so are currently limited by sequencing depth and the low microbial abundance relative to host cell number. To improve the bacterial transcriptional information provided by such studies, many technical development efforts focus on approaches to differentially isolate or selectively target host versus microbial nucleic acids (29, 36, 57). Additionally, tumor-associated microorganisms may modulate their functions based on their precise localization and structure within the colon. From a spatial perspective, the organization of the luminal microbiota in patients with CRC appears distinct from that of individuals without CRC (23), which may further affect how these microbes function and are able to influence the host epithelium and CRC development (44).

microbes that have been identified as potential drivers of CRC in both humans and mouse studies—enterotoxigenic *Bacteroides fragilis* (ETBF) (35, 38, 96) and colibactin-producing *Escherichia coli* (2)—have been highlighted in some (63, 89), but not all, of these studies. For colibactin-producing *E. coli*, species-level enrichment may not be necessary—the oncopathogenicity of this organism depends specifically on levels of the *pks* island that encodes the colibactin toxin rather than the total *E. coli* abundance as measured in the 16S rRNA surveys that underlie most metagenomic studies. Direct measurement of the levels of the *pks* island has demonstrated an enrichment of these bacteria in both IBD and CRC colonic mucosa samples (2). Similarly, for ETBF, the presence of the enterotoxin-encoding gene *bft*, rather than *B. fragilis* 16S rRNA levels, is the more relevant assessment; measurements of its prevalence have suggested enrichment of this microorganism in CRC tissues (10, 84, 89).

## SHAPING THE TUMOR MICROENVIRONMENT: MECHANISMS USED BY MICROBES TO POTENTIATE CRC

Several models of microbe-mediated carcinogenesis have provided insight into how different bacteria may influence tumor formation (**Table 1**). While these models have limitations in their applicability to human CRC (e.g., using a microbe that, while able to induce tumor formation in a mouse model, is not prevalent in human colonic tumor tissues), they inform our understanding of the different mechanisms by which microbes influence the pretumor environment, including mediating DNA damage, inducing specific signaling pathways, promoting immune cell infiltration, and blocking antitumor immunity (recently reviewed in 34, 37, 53, 73, 74). Arguably the most important part of CRC development is the accumulation of multiple mutations within the epithelial cells, which results in uncontrolled proliferation. Some microbes, like *Enterococcus faecalis*, are able to indirectly influence DNA damage in the epithelium by eliciting high levels of ROS (92, 93), the same compounds produced by host cells during inflammation. Colibactin-producing *E. coli* attacks host DNA more directly, by introducing double-stranded DNA breaks

**Table 1 Colorectal cancer–associated microbes**

| Organism | Natural reservoir | Evidence of association with CRC[a] | | | Mechanisms identified in models[b] | Effectors[b] |
|---|---|---|---|---|---|---|
| | | Epidemiology | Microbial enrichment | Immune responses | | |
| *Streptococcus gallolyticus* | GI tract | + | – | + | Unknown | Unknown |
| *Enterococcus faecalis* | GI tract | – | – | – | ROS-mediated DNA damage | Unknown |
| Colibactin-producing *Escherichia coli* | GI tract | + | + | – | Toxin-mediated DNA damage | Colibactin (Pks) |
| Enterotoxigenic *Bacteroides fragilis* | GI tract | + | + | – | Inflammation and immune-cell infiltration | Bft toxin |
| *Fusobacterium nucleatum* | Oral cavity | + | + | – | Inflammation and immune-cell infiltration; disruption of antitumor immunity | FadA, Fap2 |

Abbreviations: CRC, colorectal cancer; GI, gastrointestinal; ROS, reactive oxygen species.
[a]Evidence of association with CRC is based on microbial enrichment as described in the main text and epidemiological and human immune responses as described and classified in Reference 74.
[b]As reported in References 2, 39, 48, 72, 92, 93, and 96.

that give rise to genomic instability and increased mutation frequency (20, 66). In the absence of the *pks* island, monoassociated *Il10*-deficient mice treated with the genotoxic agent azoxymethane develop comparable levels of inflammation, but fewer intestinal tumors than similarly treated mice monoassociated with $pks^+$ *E. coli* (2). This observation highlights the importance of DNA damage by microbes in contributing directly to the proneoplastic environment, independent of inflammation. A less obvious example of the microbiota promoting CRC through DNA damage comes from work in mice with two genetic susceptibilities for intestinal tumorigenesis ($Apc^{Min/+}$ and loss of *Msh2*; see sidebar about Addressing Causation: Animal Models of CRC). In this study,

## ADDRESSING CAUSATION: ANIMAL MODELS OF CRC

To determine mechanisms underlying CRC, researchers use many different animal models of CRC that differ in their genetic basis and based on the investigators' scientific objectives. The $Apc^{Min/+}$ model, in which mice bear a point mutation in one copy of the *Apc* tumor suppressor gene, spontaneously forms adenomas along the intestinal tract, but most frequently in the small intestine (81). This model most resembles familial adenomatous polyposis. In comparison, *Il10*-deficient mice lack an important anti-inflammatory cytokine and develop spontaneous colitis; when these mice are treated with the carcinogen azoxymethane, they develop tumors that resemble the pathology seen in colitis-associated CRC (85). Finally, xenograft and allograft models of CRC (56) are generated when either primary or immortalized cancer cells from humans or mice are injected into recipient mice that are often immunocompromised to allow tumor growth. These injections can be either orthotopic, in this case into the distal colon or rectum, or subcutaneous. These models have utility in their ability to use primary human cancer cells and the relatively short time frame over which tumors develop.

the authors found that in the absence of a functional mismatch repair system in the $Apc^{Min/+}$ intestine, the fiber-derived microbial metabolite butyrate promoted cell hyperproliferation and resulted in increased tumor abundance (8). Antibiotic treatment or a low-carbohydrate dietary intervention was sufficient to disrupt this phenotype, highlighting that, even indirectly, the microbiota can have strong effects on the tumor environment.

Beyond microbe-influenced DNA damage or microbial effects on cell proliferation in genetically susceptible hosts, intratumoral microbes can have effects on the tumor immune microenvironment that influence tumor growth and spread. In $Apc^{Min/+}$ mice, exposure to *F. nucleatum* was sufficient to drive increased small intestinal and colonic adenoma formation and accelerate small intestinal adenocarcinoma development (48). Concurrent with an increase in adenoma formation, *F. nucleatum* treatment was associated with myeloid cell infiltration (predominantly dendritic cells, macrophages, and myeloid-derived suppressor cells) and an NF-κB proinflammatory transcriptional profile within mouse intestinal tumors, consistent with the progression of human CRC. Taken together, these data support that *F. nucleatum* serves to contribute to the development of the tumor environment itself. However, the molecular mechanisms of action underpinning *F. nucleatum*'s effects on intratumoral myeloid cell populations remain unclear. Other work using a xenograft model of tumorigenesis and in vitro carcinoma cell culture lines demonstrated that *F. nucleatum* can activate the Wnt/β-catenin pathway, which in turn led to NF-κB activation and stimulated tumor cell proliferation (72). This observation was dependent on the presence of FadA, an adhesin unique to fusobacteria, suggesting the importance of *F. nucleatum* adherence to and invasion of host cells in its promotion of CRC.

A microorganism may not only shape the tumor immune environment where it lives, but also teach us something about immune system function in cancer. The roles for many T cells in cancer are well characterized; for example, T regulatory cells function to suppress tumor immunity, while T helper type 1 cells promote antitumor immunity (33, 65). How T helper type 17 (Th17) cells, which function in inflammation and protection against extracellular microorganisms, influence tumorigenesis remained unclear until 2009. An important breakthrough came from work with enterotoxigenic *B. fragilis*, which demonstrated that ETBF activated the Stat3 transcription factor in the colon in $Apc^{Min/+}$ mice (96). In a toxin-dependent manner, ETBF induced Th17 cell infiltration into the colon, and these Th17 cells mediated tumor formation. These data suggested that Th17 cells, at least in this model of CRC, were involved in the development of a protumorigenic microenvironment. More recent studies have further linked this important cell type to the promotion of CRC (22, 79), demonstrating the importance of microbiota research not just for elucidating how microbes contribute to carcinogenesis but also for defining cancer immunology principles.

By developing effective strategies to avoid immune-mediated destruction, microbes can enable tumor growth and spread by configuring an evolving tumor microenvironment into a milieu that is permissive to the survival of not only bacteria but also tumor cells. We term this the "live and let live" hypothesis. *F. nucleatum* manipulates the tumor microenvironment by using its Fap2 adhesin to engage the immune system and block natural killer (NK) cell–mediated killing (39). In doing so, *F. nucleatum* blocks a potent arm of antitumor immunity. *F. nucleatum* cells were shown to bind to tumor cells and inhibit killing of these cells by NK cells. Interaction of *F. nucleatum* with the NK cells occurs through interactions between Fap2 and human TIGIT [T cell immunoreceptor with Ig and ITIM (immunoreceptor tyrosine-based inhibitory motif) domains], an immune receptor found on NK and T cells and expressed by these immune cells in CRC. By engaging TIGIT, *F. nucleatum* avoids immune-mediated killing, as do the tumor cells to which they are bound. Thus, an interaction that likely evolved to protect *F. nucleatum* from the immune system is coopted by tumor cells to evade antitumor immunity.

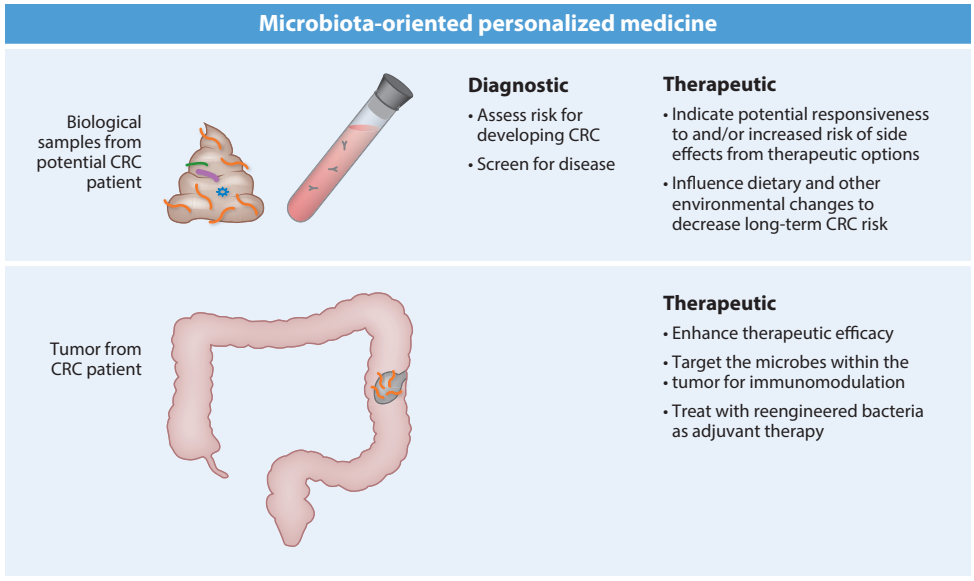


**Figure 4**

The future of microbiota theranostics.

## EXPLOITING INTERACTIONS BETWEEN THE MICROBIOTA AND CRC FOR CANCER THERANOSTICS

Much of the focus on the microbiota in CRC has been on microbes as pathogenic drivers of CRC, and hopefully these efforts can be leveraged for preventive, diagnostic, and therapeutic purposes (**Figure 4**); however, microbes may also reduce CRC risk. Several microbes, including some used widely in the food industry or as supplements, are able to reduce chronic inflammation under some conditions (5, 71, 87). Since inflammation drives CRC in a subset of cases, such beneficial microbes are one possibility for manipulating the intestinal environment that leads to CRC progression. Additionally, given the increasingly recognized role for members of the human body's microbiota and their metabolites in shaping immune development and altering immune response (1, 3, 4, 43, 61, 62, 78), human gastrointestinal tracts may offer a pharmacopoeia of bacterial cancer immunotherapies ripe for development. Microbes are also being mined for their xenobiotic and vaccination potentials as they relate to cancer treatment. Furthermore, research like that in the *Msh2*-deficient mice mentioned above suggests that simple dietary interventions are sufficient to block the microbiota from shaping a proneoplastic environment (see sidebar about Feeding the Gut: The Effect of Diet on the Microbiota and CRC). Below, we use *F. nucleatum* as a model organism to discuss some potential avenues by which the microbiota can be used in cancer theranostics in more detail.

From a diagnostics perspective, the identification of novel biomarkers—that is, reliable noninvasive indicators of cancer within the body—is a challenging endeavor. Fecal samples, which can provide a periscopic view of the luminal and mucosal microbial environments (97) without the need for invasive procedures like colonoscopies, seem like an ideal approach (98). Studies have already shown increasing *F. nucleatum* levels in the fecal samples of patients with CRC (30, 32, 48, 98). However, in these studies fusobacterial levels are observed along a gradient, rather than being present at a standard level or else absent, thereby limiting efficacy of immediate usage of

## FEEDING THE GUT: THE EFFECT OF DIET ON THE MICROBIOTA AND CRC

The gut microbiota is greatly affected by dietary changes, in a matter of days (21, 90). Both the innate properties of the microbiota (i.e., its capacity to break down food into secondary metabolites with both pro- and anti-inflammatory properties) and the shifts that occur in these communities after dietary intervention can contribute to CRC (67–69). Dietary fiber provides perhaps the most interesting example of the relationship among diet, microbiota, immune system, and CRC. The microbiota can convert fiber to short-chain fatty acids, including acetic acid, propionic acid, and butyric acid, that then (*a*) shape immune system function and protect against colitis (1, 78), (*b*) drive anti-inflammatory responses and tumor-suppression (24, 27, 76), and (*c*) promote tumorigenesis in some models of CRC (8). Such seemingly contradictory effects underlie the need to better understand these multipartite associations.

fecal fusobacterial load as a biomarker. Furthermore, the differences are far more subtle between adenoma patients and healthy subjects (no adenoma, CRC, or other pathology detected) than between CRC patients and healthy subjects. Earlier identification (i.e., at the adenoma stage) would be more valuable from a cancer prevention standpoint. Use of *F. nucleatum* as a prognostic marker in CRC may hold potential, given a recent study demonstrating a negative association between *F. nucleatum* levels and survival (58). However, these findings require further validation and a greater understanding of the mechanisms by which *F. nucleatum* shapes a tumorigenic milieu. Consideration of how *F. nucleatum* functions in its normal capacity (i.e., in the oral cavity) as compared to in a preneoplastic or neoplastic colon will provide insight into other biomarker targets, such as detection of tumor-specific gene products. Similarly, other screening approaches, such as detection of antibodies that may arise in response to *F. nucleatum*–specific antigens, may allow greater differentiation among *F. nucleatum* present in its distinct niches.

Another way to utilize an individual patient's microbiota profiles—whether acquired broadly by 16S rRNA sequencing methods or directly by assessing the presence of a specific microbial marker with a directed assay like quantitative PCR—is in the treatment, rather than identification, of CRC. Many of the microbes associated with tumorigenesis in the colon do so by shaping the immune cell environment within the tumor. With *F. nucleatum*, one of these mechanisms is Fap2 engagement of TIGIT to protect against NK cell killing, thereby subverting antitumor immunity and allowing unrestricted tumor growth. Development of anti-Fap2 antibodies that could be used to treat an *F. nucleatum*–positive tumor may allow restoration of antitumoral immune detection and response. Alternatively, one could take a more general approach to correcting the intratumoral immune cell dysregulation in patients known to have *F. nucleatum*–positive tumors. As *F. nucleatum*–enriched tumors demonstrate increased myeloid cells, a treatment that would block myeloid cell migration and differentiation, such as an inhibitor of the chemokine CCL2 that can drive myeloid infiltration and intratumoral function (16), would be another approach. Personalized medicine honing in on immune-microbiota interactions could be used extensively once there are more data on the mechanisms by which microbes influence responsiveness to different cancer therapies.

Another therapeutic avenue to consider is using other, non-CRC-associated microbes to alter the tumor microenvironment response to immunotherapy treatments, which stimulate one's own immune system to fight tumor cells but have had limited efficacy in CRC (see sidebar about Treating Colorectal Cancer). Recent advances in CRC treatment have focused on immune checkpoint blockade inhibitors that target the cytotoxic T lymphocyte–associated protein 4 (CTLA-4) and programmed death protein 1 (PD-1) pathways to enable T cell–mediated antitumor immunity. In two recent studies, particular microbes in the intestinal microbiota were shown to mediate efficacy

**Immune checkpoint:** important molecules modulating the level of immune system response; frequently uncontrolled in tumors

## TREATING COLORECTAL CANCER—WHAT'S THE RIGHT APPROACH?

CRC has proven to be a difficult disease to treat. Surgery remains the most common treatment, with radiotherapy and/or chemotherapy administered to patients with invasive tumors or metastatic disease. Recent advances in immunotherapies, such as immune checkpoint blockade inhibitors, have provided new hope to patients who are not adequately responding to chemotherapy (83). Despite the success of immunotherapies in treating other cancers, to date PD-1 blockade has had limited success in CRC treatment (12), mostly in a small trial of 41 patients that focused on one subset of CRC notable for a mismatch repair deficiency (51). Anti-CTLA-4 immunotherapy and checkpoint inhibitor combinations are in clinical trials for CRC. As such, the potential for personalized medicine to improve CRC outcomes depends on consideration of all relevant information at play, including cancer genomics and epigenetics as well as the microbial constituents that may be shaping the tumor immune environment.

of immunotherapies targeting these pathways in mouse models of cancer (77, 88). Using germfree mouse models, Vétizou and colleagues (88) demonstrated that the microbiota—specifically, *Bacteroides* spp.—was required for the therapeutic effects of the anti-CTLA-4 treatment to drive antitumor immunity in sarcomas. Similarly, Sivan et al. (77) identified *Bifidobacterium* as an important mediator of the effects of anti-PD-L1 therapy in melanoma. Such effects certainly have potential in CRC, where targeting microbes directly to the tumor could shape antitumor immunity and immunotherapy responses on a more local level. Much like the microbial drivers of CRC discussed earlier, such an organism would require a mechanism for localizing to a tumor and surviving in it long enough to affect the immunological milieu; then, ideally, it could be genetically or pharmacologically disarmed. An ideal microbe for such an approach would have much in common with a protumorigenic organism like *F. nucleatum*, further confounding the line between microbes as positive or negative actors in shaping immune response in the tumor microenvironment.

While such possibilities may influence translational approaches to CRC prevention and treatment, understanding the underlying biology of microbe-mediated CRC is essential before considering diagnostics or therapeutic strategies based on using the microbiota to manipulate the tumor microenvironment.

### SUMMARY POINTS

1. Members of the intestinal microbiota are ideally suited to influence CRC, as the tools used by these microorganisms to survive, thrive, and avoid immune detection in the colonic mucosa are capable of becoming tumor-promoting weapons in a dysplastic precancerous environment.
2. CRC development is a sequential process with multiple stages at which both inflammation and the microbiota have important roles that are difficult to disentangle because of their intimate and intertwined relationship.
3. Meta'omics studies provide great insight into the microbes associated with CRC progression, but better tools are needed to parse the individual functions of these microbes in the tumor microenvironment.

4. Modulation of the tumor immune environment and promotion of DNA damage are common proneoplastic mechanisms by the best-studied CRC-associated microbes, including *F. nucleatum*, enterotoxigenic *B. fragilis*, and colibactin-producing *E. coli*.
5. Both the microbiota as a whole and specific CRC-associated microbes have enormous potential to enable much-needed new approaches to diagnose and treat CRC.

### FUTURE ISSUES

1. What about the pretumor environment (e.g., surface molecules/lectins, available metabolites, abiotic factors) leads to specific microbial targeting and/or enrichment?
2. What are the microbial molecules and/or pathways that influence the distinct stages of tumorigenesis?
3. How do the interactions between co-occurring microorganisms in colonic tumors shape the tumor microenvironment?
4. How can understanding the microbiota's role in colorectal cancer direct personalized medicine?

## DISCLOSURE STATEMENT

W.S.G. is a member of the scientific advisory boards of Evelo Biosciences and Synlogic.

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**88. With Sivan et al. (77), implicated the microbiota in effective responses to (CTLA-4) immunotherapy against CRC.**

**96. Demonstrated ETBF as driver of Th17 responses in a Bft-dependent manner in colitis-associated tumorigenesis.**