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1. Describe the role of microbiota that inhabits our gastrointestinal (GI) tract and other anatomical site as an environmental factor that influences cancer risk.
2. Relate the differences in the composition of microbial communities between healthy and diseased individuals.
3. Highlight emerging evidence on how microbiota can be manipulated for the treatment of various disease states including cancer.

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The Role of the Microbiome in Cancer Development and Therapy

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Abstract: The human body harbors enormous numbers of microbiota that influence cancer susceptibility, in part through their prodigious metabolic capacity and their profound influence on immune cell function. Microbial pathogens drive tumorigenesis in 15% to 20% of cancer cases. Even larger numbers of malignancies are associated with an altered composition of commensal microbiota (dysbiosis) based on microbiome studies using metagenomic sequencing. Although association studies cannot distinguish whether changes in microbiota are causes or effects of cancer, a causative role is supported by rigorously controlled preclinical studies using gnotobiotic mouse models colonized with one or more specific bacteria. These studies demonstrate that microbiota can alter cancer susceptibility and progression by diverse mechanisms, such as modulating inflammation, inducing DNA damage, and producing metabolites involved in oncogenesis or tumor suppression. Evidence is emerging that microbiota can be manipulated for improving cancer treatment. By incorporating probiotics as adjuvants for checkpoint immunotherapy or by designing small molecules that target microbial enzymes, microbiota can be harnessed to improve cancer care. **CA Cancer J Clin 2017;000:000-000. © 2017 American Cancer Society.**

Keywords: cancer, dysbiosis, microbiome, prebiotics, probiotics

Practical Implications for Continuing Education

- **Maintenance of microbial diversity is critical for human health. Steps should be taken to prevent indiscriminate antibiotic usage. Furthermore, encouraging a diverse, plant-based diet facilitates microbial diversity.**
- **Precision medicine approaches should incorporate microbiome differences in addition to differences in genetic background.**
- **The efficacy of chemotherapy/immunotherapy likely depends on an individual's microbiota.**

Introduction

Cancer is a leading cause of morbidity and mortality, with approximately 1.7 million newly diagnosed cancer cases and approximately 600,000 cancer deaths this year in the United States alone.[1] In addition to the tremendous suffering it inflicts, cancer is a significant economic burden, with health care costs exceeding $125 billion per year in the United States.[2] Despite a recent, high-impact report that cancer is primarily stochastic or "bad luck" because of the accumulation of spontaneous mutations during DNA replication in tissues where stem cells undergo a relatively large number of cell divisions,[3] it is widely believed that the environment significantly influences cancer risk.[4,5] Numerous epidemiologic and occupational health studies support the importance of lifestyle factors and exposure to known or suspected carcinogens in the development of cancer. In fact, it is estimated that 15% to 20% of cancers are driven by infectious agents[6]; 20% to 30% are largely caused by tobacco use; and 30% to 35% are associated with diet, physical activity, and/or energy balance (eg, obesity).[7,8] Ultraviolet (UV) radiation from sunlight, alcohol,

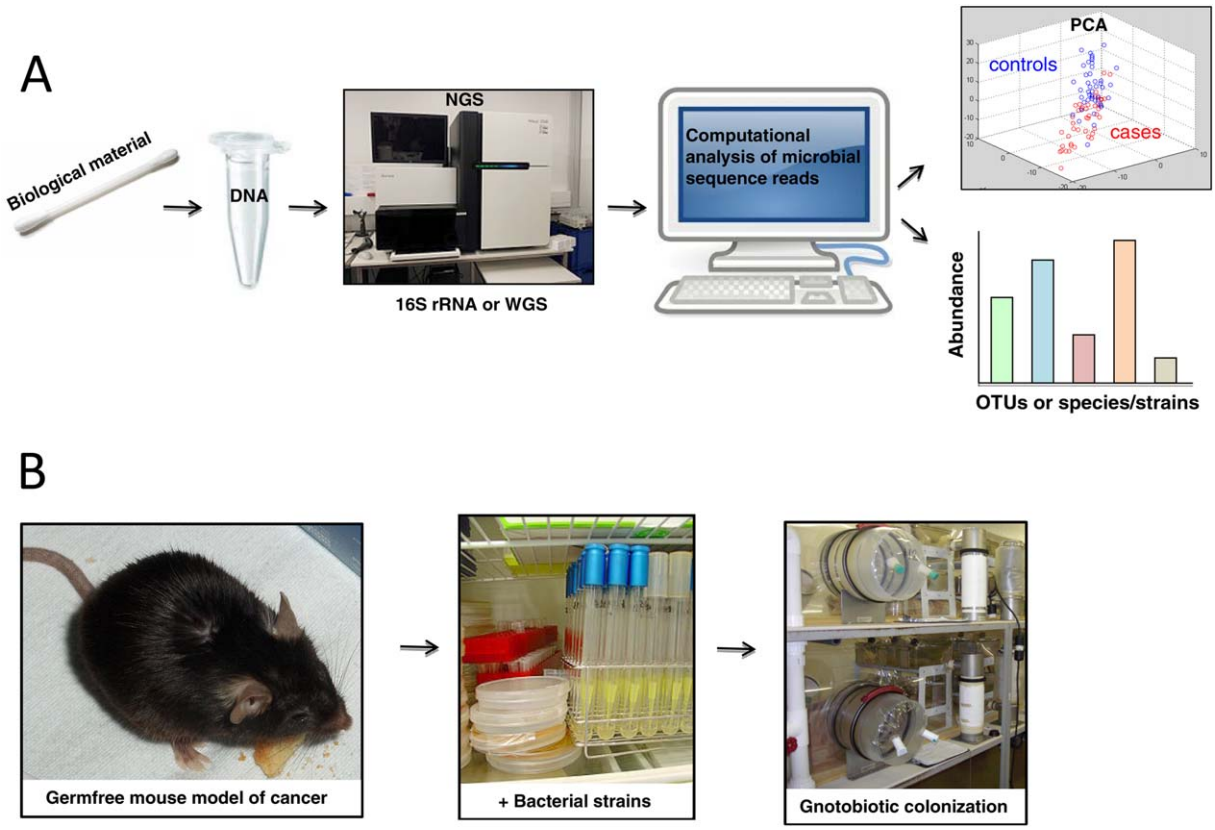

FIGURE 1. Microbiome Research Strategy. (A) This flow chart of metagenomic sequence analysis illustrates the process in which (from left to right) biological materials (buccal swabs, fecal samples, tissue biopsies, saliva) are procured from patients (cases) and healthy controls. DNA is prepared from each sample, next-generation DNA sequencing (NGS) is performed to obtain targeted (16S ribosomal RNA [rRNA] hypervariable regions) or whole-genome shotgun (WGS) sequence reads, computational assembly and analysis of microbial sequence reads allows the microbial community structure to be assessed for each sample, and (*Top*) principal component analysis (PCA) is a statistical procedure that compares the degree of relatedness of sequence reads between samples and illustrates the relationship between cases (red circles) and controls (blue circles), which often form distinct clusters with minimal overlap. (*Bottom*) Other computational methods allow the abundance of different microbial taxa to be quantified when compared with databases. Analysis of 16S data yields the relative abundance of operational taxonomic units (OTUs) and their phylogenetic relationships. Analysis of WGS data provides greater taxonomic resolution, down to the abundance of specific strains within a single species that varies with respect to gene content, including virulence factors and single-nucleotide polymorphisms, and provides more insight into pathways. WGS provides much more information but is more expensive and computationally intensive with less complete database resources, in part because of a limited number of reference genomes. Further details can be found in other reviews (eg, see Goodrich et al[9] and Morgan and Hutenhower[11]). (B) Because a microbiome change between cases and controls can be either a cause or a consequence of disease, gnotobiotic mouse models are used to evaluate the function of specific microbiota in the host. *Left:* Germ-free mouse models, which were originally obtained via C-section delivery but are now obtained by embryo transfer into germfree surrogate females, are colonized by (*Middle*) oral gavage with one bacterial strain (monoassociated), a consortium of specific bacteria (polyassociated), or complex microbial communities (eg, fecal microbiota transplants), while (*Right*) maintained in gnotobiotic isolators.

and many other substances (eg, asbestos, benzene, radon) also play a role, both alone and in combination (ie, mixed exposures), although relative risk depends on the dose and duration of each exposure and the genetic background of each individual.

The microbiota that inhabit our gastrointestinal (GI) tract and other anatomic sites can be considered environmental factors to which we are continuously exposed at high doses throughout life. Most of these microbes are commensal bacteria and, until recently, have been difficult to culture, which has limited our understanding. However, during the past decade, the advent of metagenomic sequencing approaches that combine next-generation DNA sequencing technologies with the computational analysis of targeted (16S ribosomal RNA hypervariable regions) or whole-genome shotgun sequence reads have documented the diversity and abundance of microbes at different body sites in a culture-independent manner[9,10] (Fig. 1A).[6,9,11] The complexity of microbiota can be described using α and β diversity as 2 metrics borrowed from environmental microbial ecology: α diversity describes the richness (ie, the number of organisms and evenness of distribution of those organisms) in a given sample, whereas β diversity defines the extent of absolute or relative overlap in shared taxa between samples.[11] There is a wide range of microbial β diversity in the microbiota that exists between individuals. Some individuals are enriched for a particular organism, which may be minimally represented in others. The overall community structure, or enterotype, varies between individuals to different extents based on genetics, where each person lives, body mass index, diet, and other environmental and lifestyle factors.[12]

Numerous metagenomic sequencing studies have revealed significant differences in the composition of microbial communities between healthy and diseased individuals (Fig. 1A).[6,9,11] As a corollary, microbiota have been implicated in causing or preventing a variety of disease states, including cancer, and this idea is supported by rigorously controlled experiments using gnotobiotic mouse models colonized with one or more specific bacteria (Fig. 1B). There is also emerging evidence that microbiota can be manipulated for the treatment of various disease states, including cancer. In this review, we discuss these topics in the context of cancer prevention and treatment.

The Human Microbiome

The human body harbors as many microbial cells as all of our somatic and germ cells combined.[13] Furthermore, the collective genome of our microbiota, referred to as the microbiome, encodes approximately 100-fold more genes than the human genome.[14] The vast majority of these microbiota are bacteria that reside within our GI tract, although archaea, viruses, and eukaryotes (such as yeast and protozoans) are also represented within the GI tract and at other body sites.[14] Like most other mammals, humans first acquire significant amounts of microbiota from their mother during birth. The composition of microbiota is highly dynamic during the first 3 years of life and then becomes relatively stable and more adult-like with increased complexity, although many smaller changes constantly occur throughout childhood, adolescence, middle age, and old age.[15-19]

Host genetics influence the composition of an individual's microbiome based on twin studies demonstrating that the β diversity of unrelated individuals exceeds that of dizygotic twins, which, in turn, is more diverse than that of monozygotic twins.[20] Not unexpectedly, some taxa are more heritable than others. By considering microbiome composition as a complex trait, genome-wide association studies have begun to map loci in humans and mice.[21] Some of the human loci associated with microbiome traits are in close proximity to loci that have effects on disease risk. Although linkage disequilibrium makes it difficult to distinguish between causative and linked single-nucleotide polymorphisms, some candidate genes, such as the vitamin D receptor, are currently being assessed.[22] However, the overall genetic architecture underlying microbiome traits are complicated with relatively small effects sizes that have been difficult to replicate.[21] Perhaps this is not surprising considering the large effect that diet and other environmental factors exert, thereby representing "noise" that masks modest genetic effects. To address this constraint, it might be useful to integrate dietary intervention studies and genome-wide association studies, as exemplified by one recent study demonstrating that only individuals with a specific genotype have a correlation between milk consumption and *Bifidobacterium* abundance.[21,23]

As mentioned above, our diets influence the composition of our microbiota although long-term dietary patterns outweigh short-term changes in diet.[24,25] It is not surprising that a particular diet selects for certain microbiota at the expense of others, considering that different taxa of gut microbiota have distinct metabolic capacities. A recent study suggests that certain microbiota can even go extinct.[26] In that study, mice were provided a low-fiber diet underwent microbiome changes that were reversible, consistent with previously published studies. But after providing the low-fiber diet for several successive generations, the maternally transmitted microbiome underwent a progressive loss of diversity, with some taxa becoming undetectable. This finding identifies a transgenerational mechanism mediated by the microbiota, rather than epigenetics, and may be relevant for families that consume much less fiber than is recommended, which is not uncommon in the United States and other industrialized countries. A plethora of other factors affects the microbiome, including international travel, infections, and pharmaceuticals.[27] Subsequent to such changes, or after an infection is resolved, most, but not all, commensal microbiota return to their baseline levels. This type of incomplete recovery complicates risk assessment, because a transient event may affect a subset of microbiota in a long-term manner that influences disease risk later in life.

Changes in lifestyles and societal norms influence the microbiome at each stage of life. Vaginal versus cesarean section methods of delivery and breast milk versus formula feeding significantly affect the infant microbiota.[28] Some of these microbiota differences persist beyond infancy and into adulthood, although most do not. Nevertheless, even transient differences in the infant are potentially important, because infancy represents a developmental window of susceptibility for a variety of disease states, in part because various cell types (eg, neurons, lymphocytes) are still developing. This idea is supported by the finding that compositional differences in the microbiota of 3-month-old infants were associated with the development of asthma later in life.[22] On the basis of animal studies, infants and children may be particularly sensitive to low doses of antibiotics in the food supply that can induce obesity via alterations in the microbiota.[29] These examples of asthma and obesity are related to the hygiene hypothesis, which posits that diminished exposure to microbiota during early childhood impairs immune tolerance, predisposing individuals to allergies and other chronic disease states. Much later in life, the microbiome of the elderly is influenced by lifestyle, with less diversity among individuals living at long-term residential care centers than among individuals living independently in

the community.[30] These compositional differences are correlated with dietary differences, increased inflammation, and frailty of individuals at long-term residential care centers, but the issue of causation versus correlation has not been addressed.

Despite the preponderance of microbial cells in the human body, they have small, mitochondria-like dimensions and collectively account for only several pounds of each person's body weight, corresponding to 2% to 7% of an individual's biomass, excluding water weight. However, our microbiota exert an outsized effect on human biology because of their prodigious metabolic capacity and profound effects on the immune system. The relationship between commensal microbiota and the human host is a complicated one that is largely beneficial but sometimes detrimental to human health. On the one hand, our gut microbiota increase our ability to absorb nutrients and extract calories from our diets. For example, the gut microbiome is highly enriched for genes involved in carbohydrate metabolism, including ≥115 families of glycoside hydrolases and ≥21 families of polysaccharide lyases.[31,32] There is a dearth of corresponding genes in the human genome due to a lack of selective pressure, because mammals (and all animals) and their genomes coevolved with gut microbiota and the microbiome. Commensal gut microbiota also play a crucial role in the development and homeostasis of the innate and adaptive immune systems. These beneficial functions are contingent on eubiosis, wherein microbiota remain either commensal or symbiotic with their hosts. However, it is difficult to define a standardized, ideal eubiosis because of the enormous population variation, and what is optimal eubiosis in one individual may differ in another. Changes in diet, antibiotic administration, and invasion of pathogens cause variable changes in microbiota composition among different individuals. Nevertheless, an individual's microbiota remains largely resilient to perturbation and can return to baseline levels over time.[33] In contrast to eubiosis, there is an altered community structure in various disease states that is referred to as dysbiosis. For example, obesity is associated with an altered ratio of the 2 dominant phyla of GI bacteria, Bacteroidetes and Firmicutes, and this taxonomic shift increases calorie extraction and adiposity in mice.[34,35] Dysbiosis can increase the representation of deleterious microbiota that produce harmful metabolites and antigens, leading to maladaptive immune responses. These disturbances are particularly relevant to oncology, considering that deregulated metabolism and inflammation are recognized as hallmarks of cancer.[36]

Microbial Pathogens Drive Certain Cancers

Perhaps the best evidence that microbiota are not passengers or bystanders comes from *Helicobacter pylori* and several oncogenic viruses that drive cancer (Table 1). *H. pylori* infections are strongly linked to gastric adenocarcinoma, and this is mediated by inflammation, with *H. pylori*-induced gastritis considered a precursor of cancer.[37] In work that led to the 2005 Nobel Prize in Physiology or Medicine, Dr. Barry Marshall infected himself with *H. pylori* to fulfill Koch's postulates and demonstrated that *H. pylori* is an etiologic agent of gastritis and gastric ulcers.[37] For this reason, *H. pylori* is in the process of being exterminated from human populations throughout the world. However, *H. pylori* protects against Barrett esophagus and esophageal adenocarcinoma, possibly by affecting stomach pH and ameliorating acid reflux.[38,39] This demonstrates that the relationship between so-called pathogenic microbes and the human host can be considerably more complicated than initially assumed. This is particularly so with bacterial drivers of carcinogenesis. Unlike viruses, which express constitutively active viral mimics of cellular proto-oncogenes,[40] tumor initiation and progression associated with microbial dysbiosis is a multifactorial event and arises after "multiple hits." Not all individuals infected with oncogenic microorganisms develop cancer. Genetic heterogeneity in the microbe as well as the host, in addition to environmental

TABLE 1. Microbes Designated as Class 1 (Carcinogens) by the International Agency for Research on Cancer (IARC)[a]

MICROBE	SITE OF CANCER
Helicobacter pylori	Stomach
Hepatitis B virus (HBV) Hepatitis C virus (HCV) *Opisthorchis viverrini* *Clonorchis sinensis*	Liver
Human papillomavirus (HPV)	Cervix Vagina Vulva Anus Penis Oropharynx
Epstein-Barr virus (EBV)	Nasopharynx Non-Hodgkin lymphoma Hodgkin lymphoma
Kaposi sarcoma-associated herpesvirus (KSHV or HHV8)	Kaposi sarcoma Primary effusion lymphoma
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell lymphoma
Schistosoma haematobium	Bladder

[a]IARC 2012.[6]

factors, determines cancer prevalence and severity. For example, only *H. pylori* strains containing the cytotoxin-associated gene A (*cagA*) virulence factor efficiently trigger gastritis and gastric cancer. Host genetics, which influence the immune response, are another important determinant of whether an infected individual develops cancer. Furthermore, diet and lifestyle factors, such as alcohol, tobacco use, and obesity, play important roles, and chronic inflammation is believed to be a particularly critical risk factor.

Metagenomic Sequencing Studies Reveal Associations Between Commensal Bacteria and Cancer Incidence

Microbial pathogens are the etiologic agents for 15% to 20% of cancers, but commensal microbiota have a more widespread influence on the initiation and progression of tumorigenesis. Metagenomic sequencing studies have detected significant differences in the composition of microbial communities in numerous human cancer cases compared with controls (Fig. 1A).[6,9,11] Many of these studies analyzed fecal samples obtained from patients with colorectal cancer (CRC) and controls, although biopsied tissues, saliva, and other biological materials have been analyzed for multiple types of cancer. Table 2 lists some of the studies that have been published along with cancer type, sampling site, and observed microbiome changes.[41-52] A central theme arising from these studies is that cancers are associated with a dysbiosis that includes a marked decrease in both microbial diversity and community stability. Yet the observed microbiome differences vary on a case-by-case basis and usually involve relatively modest quantitative differences in the abundance of specific taxa of bacteria. Although the combined effects in aggregate are believed to be more robust, the relationship between dysbiosis and cancer is nuanced compared with *H. pylori* and oncogenic viruses that drive cancer in a highly penetrant manner, as discussed in the section above.

Gut dysbiosis primarily involves shifts in the abundance of commensal bacteria, including some that function as opportunistic pathogens. For example, in several studies that compared colorectal tumors with normal adjacent colonic tissues from the same individuals,[41,42] the tumor samples had an underrepresentation of the 2 dominant phyla, Bacteroidetes and Firmicutes, but an overrepresentation of *Fusobacterium sp.*[48,53-55] *Fusobacterium* is an invasive anaerobe that was previously associated with periodontitis and appendicitis, but not cancer. Despite the consistent results that were observed, the overall microbial communities of a tumor and a matched noncancerous colon sample from one individual were more similar to each other than were tumors or noncancerous samples from different individuals. This highlights one of the challenges of this approach and supports the idea that the microbiome will be an important factor in precision medicine.

Metagenomic sequencing studies have limitations, however. They are association studies and cannot determine whether a particular microbiota change is a cause or a consequence of cancer. Very few studies are longitudinal and sample the microbiota at different stages of tumorigenesis. In fact, most studies are conducted at a relatively late stage after immune cell infiltration, altered tumor cell metabolism (including hypoxia and lower pH), and other changes have occurred that increase the likelihood of microbiome changes being secondary to tumorigenesis. In addition, many studies analyze the fecal microbiome, which is different from the mucosal-associated microbiome and less likely to be relevant to disease.[56] Metagenomic sequencing also does not provide insight into the spatial distribution of microbes, including the organization of microbial communities into biofilms, which might be just as important as the composition of the community. For example, colonoscopies have demonstrated that biofilms are present in nearly all right-sided (proximal) CRCs compared with 15% of healthy controls.[57] Finally, current 16S ribosomal RNA-based techniques lack the resolution to detect strain-level differences, including the ability to distinguish between commensal and pathogenic isolates. However, whole-genome shotgun sequencing, coupled with rapidly evolving bioinformatics approaches, can now resolve this limitation.[58,59]

Gnotobiotic Mouse Models Demonstrate Causality and Provide Mechanistic Insights

To demonstrate the functional importance of microbiota in carcinogenesis, mouse models of cancer maintained germ free (ie, devoid of all microbiota) in gnotobiotic isolators are colonized with one or more specific bacteria (Fig. 1B). For example, human *Escherichia coli* strains harboring the *pks* (polyketide synthase) pathogenicity island are enriched in the colonic mucosa of patients who have CRC with an incidence of 67% compared with 21% in healthy controls.[60,61] To demonstrate that *pks* plays a causal role in tumorigenesis, interleukin 10 (IL-10) knockout mice were monoassociated with 2 strains of *E. coli* that were either *pks*+ or Δ*pks* (containing and deleted of *pks*, respectively) and treated with the procarcinogen azoxymethane (AOM) to induce colorectal tumors.[60] Although both *E. coli* strains stimulated inflammation to a similar extent, there was a significant difference in tumor progression, with all of the tumors in the *pks*+ group becoming malignant while all of the tumors in the Δ*pks* group remained benign. It was demonstrated that *pks*, which encodes a genotoxin called colibactin, induces DNA damage in colonocytes based on the γ-histone-2AX (γH2AX) marker.[60]

TABLE 2. A Sample of Published Metagenomic Studies Analyzing Cases and Controls

TYPE OF CANCER	SAMPLING MATERIAL AND SITE	CONCLUSION	FINDINGS			REFERENCE
			ENRICHED IN CASES	REDUCED IN CASES	ENRICHED IN CONTROLS	
Colorectal adenoma	Mucosal adherent bacteria	Higher diversity and richness in cases compared with controls	Proteobacteria, *Dorea* spp., *Faecalibacterium* spp.	Bacteroidetes, *Coprococcus* spp.		Shen 2010[41]
Colorectal adenoma	Mucosal adherent bacteria	Higher diversity and richness in cases compared with controls; similar evenness	30 Genera, including: *Acidovorax*, *Aquabacterium*, *Cloacibacterium*, *Helicobacter*, *Lactococcus*, *Lactobacillus*, *Pseudomonas*		*Streptococcus*	Sanapareddy 2012[42]
Colorectal adenoma	Preneoplastic colon polyps from African American patients	No statistically significant differences	Slight increases in Proteobacteria (*K. pneumoniae*, *E. coli*), Verrucomicrobia, Firmicutes	*Bacteroides*	Slightly higher abundance of *Oscillospira guillermondii*, *Subdoligranulum*	Brim 2013[43]
Colorectal adenoma	Adenomatous tissues		*Bifidobacterium* sp, Eubacteria			Nugent 2014[44]
Colorectal tubular adenoma, adenocarcinoma	Mucosal adherent bacteria	Dysbiosis in cases compared with healthy controls	*Fusobacterium nucleatum*, *Enterobacteriaceae*, *Methanobrevibacter* (Archaea, Methanobacteriales)			Mira-Pascual 2015[45]
Colorectal adenoma, carcinoma	Feces	Progressive dysbiosis concurrent with progressive disease	ADENOMA: *Blautia*, *Ruminococcus*, *Clostridium*, *Lachnospiraceae*; CARCINOMA: *Fusobacterium*, *Bacteroides*, *Phascolarctobacterium*, *Porphyromonas*			Zackular 2014[46]
Colorectal adenoma	Feces	No significant differences; underpowered study confounded by antibiotics treatment	Proteobacteria, TM7			Goedert 2015[47]
Colorectal carcinoma	Mucosal tissues	Increased abundance of *Fusobacterium*	*F. nucleatum*, *F. mortiferum*, *F. necrophorum*	Bacteroidetes, Firmicutes		Kostic 2012[48]
Colorectal Adenoma	Feces	Compositional shifts occur in adenomatous tissues that correlate with alterations in bacterial metabolism	*Bilophila*, *Desulfovibrio*, *Mogibacterium*, Bacteroidetes. *Streptococcus*, *Veillonella*, *Mogibacterium*, and *Sutterella* predict presence of adenomatous polyps			Hale 2017[49]
Colorectal polyps, carcinoma	Fecal and mucosal samples, from tumor and tumor-adjacent regions	Mucosal microbiota differs in cases and controls, particularly if lesion is proximal or distal; fecal and mucosal microbiota differ in CRC; analyses suggest that microbiota shifts are not secondary to the cancer	*Bacteroides*, *Roseburia*, *Ruminococcus*, *Oscillibacter*; ORAL PATHOGENS: *Porphyromonas*, *Peptostreptococcus*, *Parvimonas*, *Fusobacterium*; CLUSTERS OF COABUNDANCE GROUPS: Bacteroidetes cluster 2, Firmictues cluster 2, pathogen cluster, Prevotella cluster	ON MUCOSA: Clusters of coabundance groups (Bacteroidestes cluster 1, Firmicutes cluster 1); IN FECES: Lachnospiraceae incertae sedis and Coprococcus		Flemer 2017[50]

TABLE 2. *Continued*

TYPE OF CANCER	SAMPLING MATERIAL AND SITE	CONCLUSION	FINDINGS			REFERENCE
			ENRICHED IN CASES	REDUCED IN CASES	ENRICHED IN CONTROLS	
Breast cancer	Tumor and adjacent normal breast tissue; healthy tissue from controls	Compositional differences between healthy controls and tumor-adjacent tissue from patients; similar compositional profiles between tumor and tumor-adjacent normal tissue within the same patient; strains isolated from tumors induced DNA double strand breaks in vitro	*Bacillus*, *Enterobacteriaceae*, *Staphylococcus*, *Comamonadaceae*, unclassified *Bacteroidetes*	*Prevotella*, *Lactococcus*, *Corynebacterium*, *Streptococcus*, *Micrococcus*		Urbaniak 2016[51]
Breast cancer	NAF of survivors and healthy controls	No compositional differences on areolar skin; ductal microbiota are significantly different between survivors and healthy controls; microbiota profiles are similar for paired areolar and NAF from the same individual	*Alistepes*		Unclassified *Sphingomonadaceae* family member	Chan 2016[52]

Abbreviation: CRC, colorectal cancer; NAF, nipple aspirate fluid.

Microbiota can be either oncogenic, as described above, or tumor suppressive, as described below. Several metagenomic sequencing studies have identified a significant enrichment of butyrate-producing bacteria in healthy controls compared with patients who have CRC.[62] Butyrate is a short-chain fatty acid produced by bacterial fermentation of fiber in the colon and has tumor-suppressive properties in CRC cell lines.[62] To demonstrate that butyrate is tumor suppressive in vivo, gnotobiotic mice were colonized with a consortium of 4 or 5 commensal bacteria, including the presence or absence of *Butyrivibrio fibrisolvens*, a prodigious butyrate producer, then provided high-fiber or low-fiber diets, and treated with AOM to induce colorectal tumors.[63] Only the combination of a high-fiber diet and *B. fibrisolvens* yielded high levels of butyrate in the lumen and reduced tumor burden, and neither intervention was individually effective. Tumor suppression was attenuated when a mutant *B. fibrisolvens* strain with diminished butyrate production was introduced. In addition, the protective effects of high fiber and *B. fibrisolvens* were recapitulated by directly providing the mice with a butyrate-fortified diet, confirming this is a bacterial-derived, tumor-suppressive metabolite. Furthermore, Warburg metabolism drove the intratumoral accumulation of butyrate, which functions as a histone deacetylase (HDAC) inhibitor, thus epigenetically regulating genes involved in cell proliferation and apoptosis.[63] The findings have translational potential by hypothesizing that the conflicting results from prospective cohort studies that investigate fiber in colorectal prevention could be resolved by evaluating microbiome differences among the participants.

Gnotobiotic mouse models have limitations as well. Germ-free mouse models of cancer can be colonized with complex microbiota (eg, fecal microbiota transplants from patients vs controls), but it is often necessary for them to be monoassociated or polyassociated with specific microbiota to identify which microbes influence tumor initiation and progression in the host. Utilization of genetically modified bacterial strains, as described above for *E. coli* and *B. fibrisolvens*, is particularly useful for elucidating molecular mechanisms. However, although this reductionist approach is necessary for basic mechanistic studies, the lack of microbial diversity in monoassociated and polyassociated mouse models limits their translational relevance. Gnotobiotic mouse models also do not receive the diverse and varied diets consumed by humans. Furthermore, many gut microbiota are obligate anaerobes that have not yet been cultured, which limits the repertoire of specific bacterial isolates that can be studied. Most human gut bacteria have long been considered unculturable, even under anaerobic conditions, but recent reports suggest that this is not the case and that many previously "unculturable" taxa, in fact, can be cultured.[64] The prospect of culturing diverse bacteria and modifying their functional output using clustered regularly interspaced short palindromic repeats (CRISPR)-mediated gene editing[65] will undoubtedly increase the utility of gnotobiotic mouse cancer models in the future.

Microbial Mechanisms of Oncogenesis and Tumor Suppression

Our commensal bacteria influence cancer largely through their metabolic capacity and their effects on immune cells and inflammation. Therefore, it is not surprising that the GI tract has received the most attention and is particularly important. The GI tract is where the vast majority of commensal bacteria reside and is the primary site of metabolism and nutrient absorption. The GI tract also harbors more immune cells than all other mucosal and lymphoid tissues and is crucial for immune cell development and function. Several microbial-mediated mechanisms have been elucidated that either promote or inhibit tumorigenesis, as depicted in Figures 2 and 3 and described in the subsections below.

Immune System and Inflammation

The association between inflammation and cancer is particularly strong for CRC. Patients who have inflammatory bowel disease with chronic colonic inflammation have a 2-fold to 10-fold increased risk of CRC,[66] while aspirin and other nonsteroidal anti-inflammatory drugs have a stronger protective effect for CRC than other cancers.[67,68] The association between inflammation and CRC mediated by gut microbiota is supported by preclinical research using mouse models. IL-10 knockout mice have healthy colons when maintained in a germ-free environment, but they develop colitis shortly after conventionalizing by receiving fecal microbiota transplants from specific pathogen-free mice.[69] This finding supports the idea that IL-10 is an immune-suppressive cytokine that prevents inappropriate immune responses directed against commensal gut microbiota. The inflammatory phenotype of IL-10 knockout mice maintained with conventional microbiota significantly increases the penetrance and multiplicity of colonic tumors in response to AOM treatment compared with wild-type mice.[70] To demonstrate that the extent of inflammation correlates with tumor burden, IL-10 knockout mice monoassociated with a mildly colitogenic strain of *Bacteroides vulgatus* have an intermediate AOM-induced tumor phenotype. The nuclear factor κ light-chain-enhancer of activated B cells (NF-κB) pathway, which is critical for mediating the innate immune response, links microbiota-induced inflammation and CRC. Toll-like receptors (TLRs) detect bacterial antigens, including endotoxins (eg, lipopolysaccharides, flagellin) and signal through the myeloid differentiation primary response gene 88

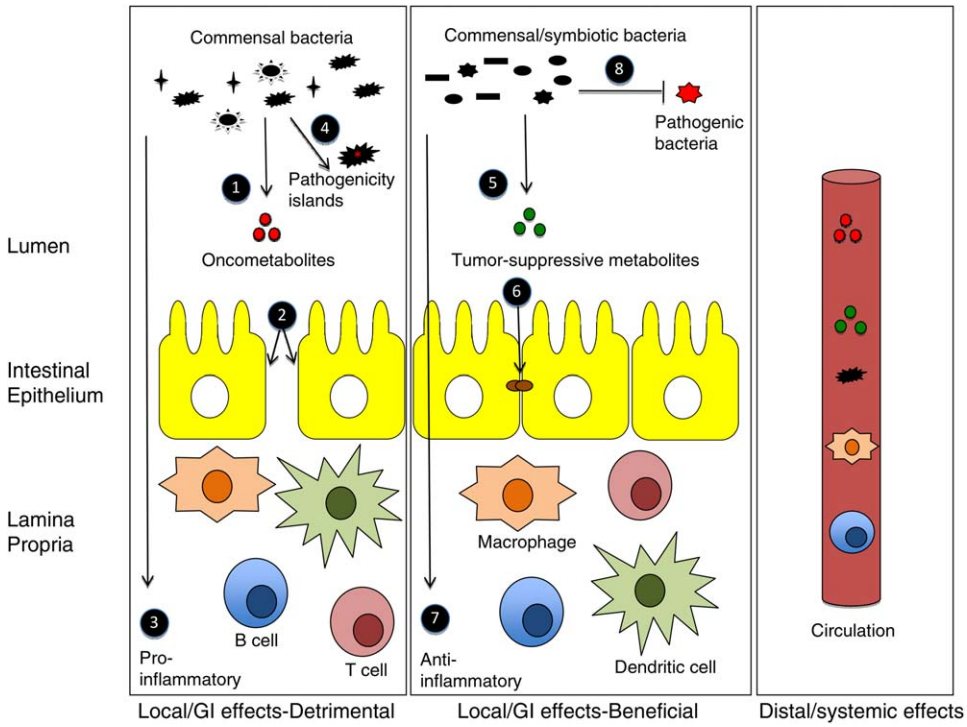

FIGURE 2. Gut Microbiota Have Differential Effects on Tumorigenesis in the Gastrointestinal (GI) Tract and at Distant Sites. The colon is depicted with a single layer of intestinal epithelial cells (yellow) separating commensal bacteria (black shapes) in the lumen above from immune cells (4 different colors) in the underlying lamina propria. The bacteria can have local effects that are either (*Left box*) oncogenic or (*Center box*) tumor suppressive for colorectal cancer, or (*Right box*) they can have distal effects mediated by the circulation that are oncogenic or tumor suppressive for cancer at other anatomical sites. Some of the general effects that gut microbiota can have on tumorigenesis are numbered, including (*Left box*): 1) production of putative oncometabolites, such as hydrogen sulfide; 2) impairment of barrier function, which increases the exposure of immune cells to bacterial endotoxins (eg, lipopolysaccharides) and antigens; 3) direct effects of bacterial metabolites and antigens on immune cells to stimulate inflammation by altering immune cell subsets (eg, the effect of segmented filamentous bacteria or segmented filamentous bacteria on T-helper 17 [TH_{17}] cells) and hyperactivating immune cell responses via proinflammatory cytokines (eg, interleukin 6 [IL-6]); 4) the presence of virulence factors, including pathogenicity islands, which distinguish pathogens from commensals, such as *Escherichia coli* polyketide synthase, can exert multiple effects, including the induction of DNA damage and aberrant Wnt signaling; and (*Center box*) 5) the production of putative tumor-suppressive metabolites, such as butyrate, which functions via multiple mechanisms; 6) maintenance of barrier function; 7) direct effects on immune cells to prevent inflammation by altering immune cells subsets (eg, the ability of butyrate to induce regulatory T-cells) and dampening the immune cell response via immunosuppressive cytokines (eg, IL-10); and 8) competitive exclusion of pathogenic bacteria similar to the prevention of lethal *Clostridium difficile* infections. *Right box:* Gut microbiota can also have oncogenic or tumor-suppressive effects at distal sites in the body via circulation of microbiota, microbial metabolites, activated or suppressed immune cells, and cytokines.

(MyD88) adaptor and NF-κB transcription factors to trigger an inflammatory response. *MyD88* knockout prevents colonic tumors in AOM-treated, IL-10 knockout mice maintained with microbiota in a specific pathogen-free facility.[70]

It is important to distinguish chronic, widespread inflammation, which is generally tumor promoting, from a local immune response where inflammation is restricted to the tumor microenvironment, which can be tumor-suppressive. Proinflammatory T-helper 17 (TH_{17}) cells are dependent on microbiota, because they are absent in germ-free mice and are induced by certain subsets of GI microbiota, such as segmented filamentous bacteria.[71] TH_{17} cells have an unsettled role with respect to tumor immunity, as reports indicate their ability to infiltrate and eradicate some tumors, while also being correlated with poor prognosis in other instances of cancer.[72] Enterotoxigenic *Bacteroides fragilis* (ETBF) encodes a pathogenic toxin that can trigger TH_{17}-mediated colitis, with concurrent colon-specific signal transducer and activator of transcription 3 (STAT3) activation and tumor induction in susceptible Apc^{Min} (adenomatous polyposis coli [Apc] multiple intestinal neoplasia) mice, which is reversed by IL-17 antibody blockade.[73]

Microbial-derived butyrate can induce naive T cells and dendritic cells into a regulatory T-cell (T_{Reg} cell) fate.[74–76] Butyrate-mediated HDAC inhibition can epigenetically activate the forkhead box P3 (FOXP3) master regulator; while signaling through G protein-coupled receptors (GPRs), such as GPR43 and GPR109a, can expand the pool of T_{Reg} cells. T_{Reg} cells have an ambiguous role in cancer.[77] On the one hand, their anti-inflammatory function may mitigate inflammation-driven tumorigenesis; and, on the other, being immunosuppressive, T_{Reg} cell infiltration into the tumor microenvironment may attenuate antitumor responses.

Intestinal microbiota alter gut barrier function, thus indirectly altering immune cell responses. The colonic epithelium is a single cell layer that separates myriad microbiota in

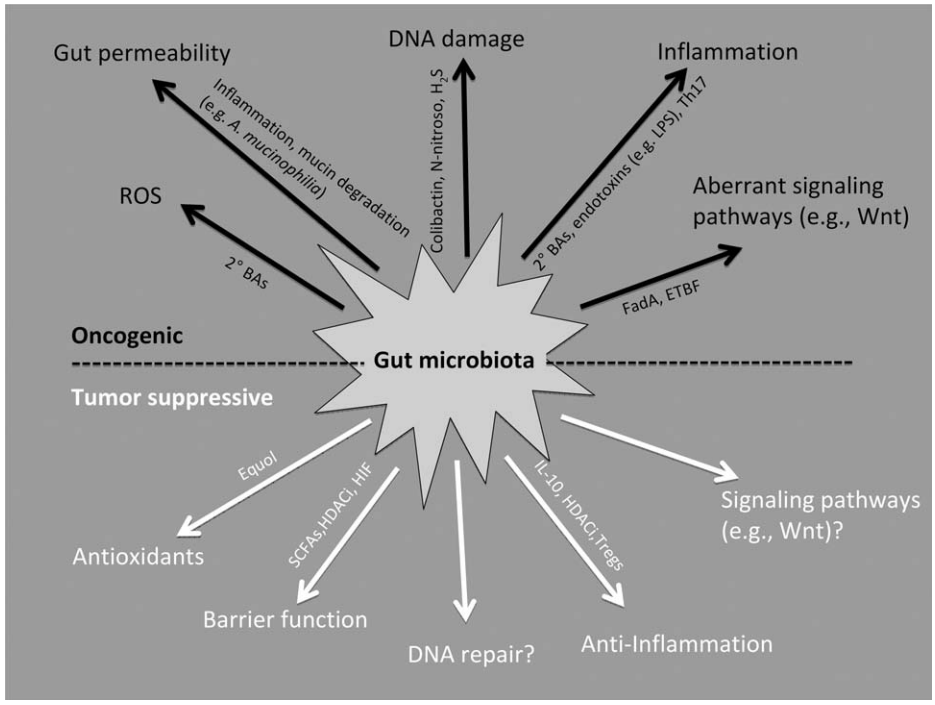

FIGURE 3. Microbial Mechanisms of Oncogenesis and Tumor Suppression. Microbiota can contribute to oncogenesis (*Top*, black arrows) or tumor suppression (*Bottom*, white arrows) by a variety of molecular mechanisms, which are listed at the end of each line. The mechanisms are listed from left to right in a symmetrical manner (from top to bottom) to make it easier to appreciate that some are diametrically opposed. The mechanisms are carried out by a variety of microbial gene products, metabolites, and immune modulators, some of which are indicated in smaller font along each arrow. See text for details. Question marks indicate speculative mechanisms that have not yet been characterized. BF indicates *Bacteroides fragilis*; ETBF, enterotoxigenic *Bacteroides fragilis*; FadA, fusobacterium adhesion A; HDAC, histone deacetylase; IL, interleukin; LPS, lipopolysaccharides.

the lumen from intraepithelial lymphocytes and cells of the innate and adaptive immune system in the lamina propria. A thick (approximately 100-micron) layer of mucus, which is produced by goblet cells, covers the colonic epithelium and prevents most microbes from coming into direct contact with the epithelium and breaching the barrier. A breach is not even required to activate intraepithelial lymphocytes, which do not require priming like other T cells, and secrete proinflammatory cytokines in immediate response to encountering antigens. Diet and gut microbiota were recently shown to maintain mucus and barrier function in a mouse model.[78] A fiber-free diet resulted in dysbiosis with diminished fiber-fermenting bacteria, including butyrate producers, and increased representation of 2 mucus-degrading bacteria (*Akkermansia muciniphilia* and *Bacteroides caccae*). Mucus degradation led to increased susceptibility to a mucosal pathogen, *Citrobacter rodentium*, resulting in a "leaky gut" condition and colitis, which is a risk factor for CRC. The depletion of butyrate-producing bacteria is also likely to be important, as described in the next section, based on their ability to promote barrier function by up-regulating claudins and occludins that comprise tight junctions between epithelial cells. Several other beneficial microbiota, including *Lactobacillus* and *Bifidobacterium* used as probiotics, have been reported to improve barrier function and diminish permeability.[79]

Diet and Microbial Metabolites

Many dietary and digestive components are metabolized by bacteria in the GI tract, yielding putative oncometabolites and tumor-suppressive metabolites.[80] Excessive consumption of red meat is a risk factor for CRC and several other cancers by a variety of mechanisms, including some that are dependent on gut bacteria. High levels of protein intake can lead to increased protein levels in the colon, where many types of bacteria, including some Firmicutes and *Bacteroides sp.*, ferment amino acids into *N*-nitroso compounds, which induce DNA alkylation and mutations in the host.[81] Proteobacteria encode nitroreductases and nitrate reductases that play a role in this process, and they are also strongly associated with inflammation.[82] Charred meat is a particular concern, because it gives rise to carcinogenic heterocyclic amines, which are metabolized by colonic bacteria, yielding electrophilic metabolites that are suspected of inflicting DNA damage.[83]

To digest saturated fat associated with red meat consumption, bile acids are produced in the liver, conjugated to taurine or glycine, and secreted into the GI tract. Approximately 5% of these primary bile acids escape enterohepatic circulation and reach the colon, where they are converted by bacteria into secondary bile acids. This is carried out in 2 steps, with deconjugation of the taurine or glycine moieties

followed by a dehydrogenation or dehydroxylation reaction. For example, primary cholic acid is converted by certain bacteria including *Clostridium scindens* into secondary deoxycholic acid (DCA). DCA functions as a tumor promoter by perturbing cell membranes to release arachidonic acid, which is converted by cyclooxygenase-2 and lipooxygenase into prostaglandins and reactive oxygen species (ROS) that trigger inflammation and DNA damage.[84] Taurine also functions as a tumor promoter by generating genotoxic hydrogen sulfide while also stimulating the growth of certain inflammatory bacteria, such as *Bilophilia wadsworthia*.[84] *F. nucleatum*, which is enriched in human CRC, as described above, produces hydrogen sulfide in response to red meat consumption.[85,86]

GI bacteria metabolize other dietary factors into putative tumor-suppressive metabolites. Dietary fibers are fermented by certain clades of colonic bacteria, such as *Clostridium* clusters IV and XIVa, into short-chain fatty acids. Butyrate, among the 3 most abundant short-chain fatty acids, serves as the primary energy source of colonocytes and has been implicated in CRC prevention based on human metagenomic sequencing studies and gnotobiotic mouse models, as discussed above. A pleiotropic molecule, butyrate likely exerts its tumor-suppressive properties by multiple mechanisms. As an HDAC inhibitor, butyrate epigenetically regulates the expression of genes involved in cell proliferation and apoptosis.[63] Butyrate is also a ligand for certain GPRs that also have been implicated in tumor suppression.[87] Both of these mechanisms are believed to be important for butyrate's ability to induce T_{Reg} cells, as discussed above. Finally, butyrate helps maintain epithelial barrier function, which is also important for preventing inflammation, and this too may involve dual mechanisms. Multiple studies have shown that butyrate up-regulates the expression of tight junction genes, including claudins and zonula occludens, through HDAC inhibition,[88] while another study demonstrated that butyrate is oxidized as an energy source to such an extent that it triggers a hypoxia-inducible factor 1α-based mechanism to maintain barrier function.[89] Other examples of whole foods and dietary components converted by gut microbiota into metabolites with potential tumor-suppressive functions include: daidzein in soy-based products is converted to equol, which functions as an antioxidant; glucosinolates in cruciferous vegetables, such as broccoli, are converted to sulforaphane and other isothiocyanates that function as HDAC inhibitors with anti-inflammatory effects; ellagic acid in certain berries is metabolized to urolithins, which alter estrogens and inhibit cyclooxygenase-2 and inflammation.[90,91] Finally, it should be emphasized that most commensal bacteria are neither "good" nor "bad" per se; rather, our diets dictate whether microbiota produce metabolites that exacerbate or ameliorate tumor progression. For example, *Clostridium scindens* produces secondary bile acids in response to dietary fat, but it is also a member of *Clostridium* cluster XIVa, which produces butyrate in response to fiber.

Cell Signaling Pathways

The *APC* tumor-suppressor gene is mutated in CRC more frequently than any other gene.[92,93] Many familial and sporadic CRCs are initiated by homozygous, loss-of-function *APC* mutations that result in nuclear β-catenin accumulation, aberrant Wnt signaling, and altered expression of downstream target genes, such as *c-MYC* to increase cell proliferation. The Wnt pathway is also perturbed in several mouse models of CRC including AOM-induced tumors. Furthermore, Wnt signaling can also be deregulated by epigenetic silencing of *APC* (eg, DNA hypermethylation of the *APC* promoter) or by perturbation by an opportunistic pathogen. For example, *F. nucleatum* encodes FadA, an adhesin that binds to lectins and E-cadherin on the surface of host epithelial cells and activates β-catenin signaling.[94] ETBF, an opportunistic pathogen enriched in CRC, secretes a zinc-dependent metalloprotease that cleaves and degrades the extracellular domains of E-cadherin, facilitating the intracellular release of β-catenin that is normally inactivated via binding to intracellular E-cadherins. Nuclear translocation of β-catenin leads to the activation of downstream target genes, such as *c-MYC* (avian myelocytomatosis viral oncogene homolog), which promote proliferation.[95] Some *Salmonella typhi* strains secrete *AvrA* to activate β-catenin and are associated with hepatobiliary cancers.[96,97]

Janus kinase/signal transducer and activator of transcription (JAK-STAT) is another important signaling pathway that is inappropriately activated in CRC and other cancers. ETBF constitutively activates STAT3 via phosphorylation and nuclear translocation in colorectal tumors.[73] It is also possible for cellular signaling pathways to modify bacterial virulence factors. For example, the *H. pylori* cagA (cytotoxin-associated gene A) is an important virulence factor that is widely phosphorylated by cellular Src and Abl kinases. Unphosphorylated CagA and phosphorylated CagA have different interactions with a broad repertoire of cellular signaling proteins, many of which are involved in regulating cellular proliferation pathways.[98]

DNA Damage

DNA damage is a major driver of carcinogenesis. Genotoxins are damaging either by forming adducts or by causing double-stranded breaks in DNA, which, when unresolved by normal DNA repair processes, can introduce point mutations, insertions, deletions, or chromosomal rearrangements, such as inversions and translocations. Microbial genotoxins can directly damage host cell DNA. Colibactin is expressed by several Enterobacteriaceae in addition to *E. coli*[99] and

induces double-strand breaks in host DNA.[60,100] Similar DNA damage induction has been observed for the cytolethal distending toxin (CDT) produced by certain Proteobacteria.[101]

Bacterial metabolites can also be indirectly genotoxic by producing free radicals and affecting ROS. For example, *Enterococcus faecalis* is a commensal strain known to produce large amounts of extracellular superoxide (O_2^-) at the luminal side of the colonic mucosa.[102] H_2O_2 resulting from the rapid O_2^- degradation can broadly damage eukaryotic cellular DNA by forming DNA-protein crosslinks, DNA breaks, and point mutations. The ETBF *B. fragilis* toxin is a virulence factor that up-regulates bacterial polyamine catabolism pathways, generating ROS species that can also damage host DNA, leading to colon tumors.[103]

Bile production increases in individuals who consume an excessively fatty diet. Several studies indicate that bile acids rapidly induce both ROS and reactive nitrogen species collectively, which can damage host cell DNA (reviewed by Bernstein et al[104]). Furthermore, diets enriched in fats induce blooms of *B. wadsworthia*, a sulfite-reducing bacterium that is frequently associated with inflammatory bowel disease.[105]

In contrast to the deleterious effects of ROS, the repair of injured intestinal mucosa relies upon redox signaling. Formylated peptides produced and excreted by microbiota activate colonic epithelial formyl peptide receptors, which induce localized ROS generation that activates redox signaling pathways and migration-associated proteins, thereby facilitating mucosal epithelial wound healing.[106] Symbiotic *Lactobacilli* are particularly adept at stimulating ROS generation via nicotinamide adenine dinucleotide phosphate oxidase 1, thus enhancing epithelial cell proliferation.[107]

Distant Sites

Gut microbiota, metabolites, and immune cells can exit the gut via the circulation and influence tumorigenesis at distant sites in the body (Fig. 2, Right). They reach the liver through the enterohepatic circulation and hepatic portal vein before entering the systemic circulation. This is noteworthy, because the liver serves as the primary site for the recognition of potentially harmful endobiotic and xenobiotic compounds, which are excreted after detoxification by hepatic enzymes. A range of endogenous chemicals, including hormones, bile acids, and cholesterol metabolites, as well as ingested or inhaled toxins are first functionalized by phase 1 cytochrome P450s and then often conjugated with glucuronic acid or sulfate by phase 2 uridine diphosphate-glucuronosyltransferases or sulfotransferases, respectively. Although numerous detoxified compounds are filtered through the kidneys, many are eliminated via the bile duct into the GI tract, where they are substrates for a variety of microbial enzyme systems that convert them back into chemicals, which can be reabsorbed, circulated systemically to influence distant sites, and then returned to the liver for reprocessing and reelimination. Such enterohepatic recirculation often involves both mammalian and microbial pathways and plays important roles in normal systemic physiology as well as intestinal and extraintestinal states of disease.

To demonstrate the impact of the microbiome on circulating metabolite levels, a metabolomics study compared serum from germ-free and conventional mice and reported that microbiota affect the abundance of 10% of the metabolites by a magnitude of $\geq$50%.[108] Some of these metabolites influence tumorigenesis at various sites in the body. For example, the secondary bile acid DCA promotes a condition similar to nonalcoholic steatohepatitis and obesity-associated hepatocellular carcinoma in a mouse model.[109] Other gut microbiota-derived metabolites implicated in cancer prevention, such as equol, have been detected in a variety of tissues (eg, breast) and biological fluids, such as blood, urine, and prostatic fluid.[91] Gut bacteria participate in the metabolism of endogenous estrogens, potentially affecting breast cancer.[92,93] Gut inflammatory responses can also affect breast cancer progression, based on studies in which *Helicobacter hepaticus* in the GI tract promoted mammary carcinoma in mouse models via a tumor necrosis factor α-dependent mechanism.[110,111] In mice bearing mutant *K-ras* and *p53,* commensal bacteria induce TLR5 and NF-κB signaling to promote systemic inflammation and enhance tumor growth at multiple distant sites.[112] These results are consistent with a TLR5 single-nucleotide polymorphism in >7% of humans, which abrogates the immune response to flagellin in the gut and is correlated with long-term survival in patients with ovarian cancer.[112]

Finally, it should be highlighted that each of the above-described mechanisms undoubtedly works in combination rather than in isolation. For example, whereas the *E. coli pks* pathogenicity island induces DNA damage, it is enabled by chronic inflammation, as demonstrated by the lack of difference between *pks*+ and Δ*pks* strains in tumor progression on a wild-type genetic background.[60] In other words, the chronic inflammation of IL-10 knockout mice apparently increases *pks* oncogenesis. Combinatorial mechanisms may potentiate oncogenesis after an initiating event that may be insufficient to drive transformation in isolation.

Cancer Treatment

Recent preclinical studies using cell culture and animal models, human clinical studies, as well as meta-analyses of clinical studies have revealed that gut microbiota alter the host response to a variety of anticancer drugs, with immunomodulation emerging as one of the central

mechanisms facilitating these differential responses. Dysbiosis is not only the consequence but often is also the cause for differential responses to therapy. As a prime example, increased intestinal diversity was predictive of decreased mortality in patients who underwent allogeneic hematopoietic stem cell transplantation for the treatment of hematopoietic malignancies.[113] The finding that immune modulation resulting from enhanced microbial diversity governs the intensity of graft-versus-host disease is an important consideration for patients beginning allogeneic hematopoietic stem cell transplantation. Moreover, compositional shifts resulting from treatment may themselves be responsible for some side effects of chemotherapy.

Immunotherapy

The adaptive immune system plays a vital role in the detection and clearance of cancer cells, and T lymphocytes are the central regulator of this response. T-cell activation occurs in a series of steps and relies on the presence of a second costimulatory or coinhibitory signal, which is provided by additional surface molecules on antigen-presenting cells. Coinhibitory molecules, such as programmed cell death 1 (PD-1), PD-1 ligand (PD-L1), and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), serve as immune checkpoints that dampen the immune response to prevent autoimmune diseases. However, coinhibitory ligands and receptors are often overexpressed in cancer cells and stromal cells within the tumor microenvironment and help the cancer evade immune-mediated destruction. Monoclonal antibodies against CTLA-4 (ipilimumab), PD-1, (nivolumab), and PD-L1 (pembrolizumab) are US Food and Drug Administration-approved immune checkpoint inhibitors that unleash the patient's own immune responses against tumors. They have proven highly effective for treating melanomas, Hodgkin lymphoma, lung cancer, kidney cancer, and bladder cancer.

Similar to other cancer therapies, there is considerable interindividual variation in patients' responses to checkpoint inhibitors.[114-116] Interestingly, the efficacy of checkpoint inhibitors appears to depend on the patient's gut microbiome, which itself closely interacts with the immune system. Therefore, it is not unexpected that interaction between the gut microbiota and immune checkpoint inhibitors may explain the observed variation in clinical responses. Two independent studies recently demonstrated that gut microbiota reconcile different responses to immune checkpoint inhibitors in mouse models of melanoma. Sivan et al noted that tumor growth varied, depending on whether the mice were obtained from The Jackson Laboratory (JAX) or Taconic vendors.[117] These mice were on the same genetic background (C57BL/6) but had distinct microbial compositions. Tumors grew slower and responded more robustly to anti–PD-L1 immunotherapy in JAX mice compared with Taconic mice. Fecal microbiota transplants from JAX donors into Taconic recipients enhanced the anti–PD-L1 antitumor efficacy. The authors identified *Bifidobacterium* as crucial, and "therapeutic feeding" (ie, probiotics) of *Bifidobacterium* alone was able to mediate anti–PD-L1 efficacy by altering dendritic cell activity that enhanced CD8-positive T-cell responses to eradicate tumors.

In the other study, Vetizou et al observed a rapid shift in the microbiome upon anti–CTLA-4 administration, characterized by a reduction in *Bacteroidales* and *Burkholderiales* and an increase in the abundance in *Clostridiales*.[114] Anti–CTLA-4 immunotherapy failed to reduce tumor burden in a germ-free state, but this defect was overcome by introducing *B. fragilis* and/or *B. thetaiotaomicron*. Overall, introduction of these bacteria enhanced tumor specificity by triggering dendritic cell maturation and modulating IL-12–dependent TH_1 responses. Although the 2 studies identified different microbiota and used different checkpoint blockades, their mechanisms of action were quite similar, with dendritic cell maturation/activation and improved function of tumor-infiltrating effector T cells.

The utility of immune checkpoint inhibitors comes at the price of GI and hepatic complications.[118] Hepatitis, diarrhea, and enterocolitis are characteristic side effects of immune checkpoint inhibitors that result from a complex interplay of host genetics, immune responses, environment, and the microbiota. Patients who develop new-onset, immune-mediated colitis resulting from anti–CTLA-4 monoclonal antibody therapy have a reduced abundance of Bacteroidetes compared with colitis-free individuals also receiving ipilimumab.[119] Microbial modules associated with polyamine transport and vitamin B (B1, B2, and B5) synthesis conferred protection, as their relative abundance was highly associated with colitis-free individuals.

Synthetic CpG oligonucleotides (CpG-ON) are ligands for TLR9 on immune cells and enhance immune responses. When combined with peptide vaccines, CpG-ON and inhibitory IL-10 receptor antibodies confer a therapeutic benefit, with reduced tumor volume and extended survival time in humans.[120] When CpG-ON and IL-10R antibodies are injected into mouse tumors, they diminish tumor burden via proinflammatory cytokines. They are ineffectual when mice are treated with antibiotics or rendered germ-free.[120,121]

Chemotherapy

Not unexpectedly, chemotherapy alters the composition of microbial communities in patients, although the significance of the altered microbiome with respect to prognosis is unclear.[122-126] Perhaps more importantly, the specific composition of microbiota can influence the anticancer response of a variety of conventional chemotherapeutics based on

work conducted in mouse models. The platinum chemotherapeutic oxaliplatin exerts its tumor-retardation effects in a microbiota-dependent manner. Eliminating microbiota with a regimen of broad-spectrum antibiotics significantly altered host gene expression: genes promoting cancer metabolism and cancer development were up-regulated with a concomitant down-regulation of inflammatory, phagocytic, and antigen-presenting pathways. Moreover, antibiotic treatment decreased the recruitment of immune cells important for mediating tumor regression with a corresponding decrease in their proinflammatory potential. Oxaliplatin efficacy depended on the intratumoral production of ROS, which is attenuated in germ-free mice, and reduced ROS generation corresponded with diminished intratumoral DNA damage.[121] This finding suggests that immunomodulatory effects mediated by the microbiota in response to chemotherapeutic compounds blur the distinction between chemotherapy and immunotherapy.

Cyclophosphamide (CP) is an alkylating agent commonly used for chemotherapy that reduces small intestinal villus height and disrupts the intestinal barrier, causing translocation of commensals to secondary lymphoid organs along with accumulation of inflammatory cells. Viaud et al discovered that the antitumor effects of CP were attenuated in mice raised to be germ-free or made so using antibiotics.[127] In the latter case, antibiotics selectively targeting gram-positive bacteria, compared with gram-negative–targeted antibiotics, significantly reduced CP efficacy. Thus, specific gram-positive bacteria (*Lactobacillus* johnsonii, L. murinus, *Enterococcus hirae*, and segmented filamentous bacteria) were identified as essential to mediate CP's antitumor response in a mouse model of nonmetastasizing sarcoma. A follow-up study from the same group reported that *E. hirae* translocation increased the intratumoral CD8/T_{Reg} ratio.[121] Furthermore, the gram-negative *Barnesiella intestihominis* was identified as an important effector of the antitumor effects of CP via increased infiltration of interferon-γ–producing T cells within cancer lesions.[128] Interestingly, patients who had advanced lung and ovarian cancer with *E. hirae*-specific and *B. intestinihominis*-specific (but not other bacteria) TH_1 cell memory responses were predicted to have lengthened progression-free survival. Collectively, the onus is on these studies to incorporate particular species of *Enterococcus* and *Barnesiella* into an optimized microbiota cocktail to be administered concurrently with CP and possibly other alkylating agents. In the future, these bacteria or their specific immunomodulatory products/metabolites may be incorporated as adjuvants to improve the efficacy of existing chemotherapeutics.

Microbial Drug Targets in Oncology

Currently, the pharmaceutical and biotechnology industries focus on cellular targets for developing chemotherapies and targeted therapies. However, in the not-too-distant future, microbiota might also be drug targets. Microbial drug targets also have the potential to ameliorate the damaging side effects that many chemotherapeutics have on the GI tract. Some side effects, such as those resulting from irinotecan (camptothecin), are serious enough that they limit the dose or duration of therapy. Irinotecan is a topoisomerase I inhibitor that blocks DNA replication preferentially in rapidly dividing cells and is used to treat CRC and pancreatic cancer. Administered as a prodrug, irinotecan is metabolized into the active chemotherapeutic agent SN38; it is subsequently glucuronidated in the liver to form the inactive SN38-G and is excreted via the GI tract. Microbiota express β-glucuronidase enzymes that hydrolyze the glucuronic acid moiety, which bacteria scavenge as an energy source, thereby reactivating SN38 in the GI lumen. Increased SN38 levels in the intestines cause severe and sometimes life-threatening diarrhea, often requiring dose de-escalation and frequent dose adjustment.

Germ-free mice exhibit less GI damage and tolerate higher doses of irinotecan compared with conventional mice that have intact microbiota.[129] A clinical trial noted a slight clinical benefit from administering neomycin concurrent with irinotecan to reduce side effects.[130] However, administering broad-spectrum antibiotics can indiscriminately kill a wide number of GI commensals and open up niches for pathogens, such as *Clostridium difficile*. As an alternative, small-molecule inhibitors targeting bacterial β-glucuronidases have been developed that do not cross-react with human β-glucuronidases and are nontoxic to either mammalian cells or bacteria.[131-133] In preclinical studies, mice receiving concurrent treatment with β-glucuronidase inhibitors were protected from irinotecan-induced diarrhea.[133] Other chemotherapeutic agents also have adverse effects in the GI tract. For example, doxorubicin is similar to irinotecan, in that GI damage requires microbiota.[134] These findings suggest that targeting microbiota may diminish the toxicity of multiple chemotherapeutics.

Future Directions

As the adage goes, an ounce of prevention is better than a pound of cure. Numerous studies have demonstrated that short-chain fatty acids synthesized during bacterial fermentation of plant-based fibers broadly protect against the development of cancer. Incorporating fiber-rich, prebiotic foods into the diet early in life, as well as limiting red meat consumption and decreasing the incidence of obesity, should help to reduce global tumor burden in the long run. Moreover, burgeoning gene-editing technologies using CRISPR-Cas9[135-138] should allow engineering of probiotic bacteria with specific capabilities (eg, expression of superoxide dismutase to counteract superoxide-producing ETBF) or, conversely, to delete pathogenic components of bacterial genomes (eg, *pks* pathogenicity island deletion in *E. coli*).

Dysbiosis appears to be a harbinger of tumorigenesis and not only precedes disease onset but also propagates throughout the course of tumor progression. Maintaining eubiosis, or an optimal microbiota composition, is key to preventing events that may initiate disease. Therefore, there is clearly an onus to develop more specific, narrow-range antibiotics that selectively target pathogens or pathobionts while preserving eubiosis.

Randomized clinical trials strongly demonstrate the utility of fecal microbiota transplants (FMTs) in resolving recurrent and refractory *C. difficile* infections.[139] Instances of improved clinical outcomes after FMTs have also been reported for celiac disease[140] and irritable bowel syndrome,[141] and preclinical studies suggest that FMTs protect against colitis.[139] However, these positive findings have been mixed with negative results. Therefore, randomized clinical trials are necessary to establish therapeutic efficacy for each disease state. Continual efforts should be made to develop capsule-based, synthetic FMTs that contain rationally selected consortia of cultured bacteria. In addition to infinitely increased palatability, this approach should allow for regular, even daily, consumption, which may be necessary for disease states in which reconstruction of the microbial community takes precedence over pathogen exclusion, as in the case of *C. difficile* infection. Synthetic FMTs may also prevent certain drawbacks associated with traditional FMTs, such as the potential acquisition of unwanted phenotypes, antibiotic-resistant bacteria, or viruses that evade screening protocols.[142]

Metabolic syndrome is increasingly associated with cancer development and resulting mortality.[143] Insulin resistance is the linchpin in the development of metabolic syndrome and has been observed in many different forms of cancer such, as prostate, breast, and colorectal cancers.[144-146] Gut microbiota can regulate various metabolic features, such as nutrient harvesting,[147] hepatic metabolism of lipids and cholesterol,[148] and fat storage,[149] and can also compromise the intestinal mucus barrier when diets low in dietary fiber are introduced.[78] Intermittent fasting, or caloric restriction, is known to improve insulin sensitivity along with reduction of other vital markers, such as blood pressure and inflammation.[150] In mouse models, cycles of starvation alternating with a variety of chemotherapeutic agents result in long-term, cancer-free survival compared with either modality alone.[151] Whether the microbiota can mediate the enhanced response to chemotherapeutics during cycles of nutrient deprivation remains to be determined.

Several recent, sophisticated cell culture systems feature the in vitro propagation of organoids derived from wild-type, diseased, or genetically recombined tissues.[152-154] Coupling these advancements with genetic screens that use transposon systems provide the ability to distinguish between factors that either cause ("drive") or minimally influence ("passenger") genetic or epigenetic alterations in host cells.[155] Coculture of microbes and microbial derivatives with colonoids will provide mechanistic insight into host-microbe interactions.[156]

Precision medicine promises medical treatments that are optimized to account for individual patients' genetic makeup and differences in lifestyle and environment. Given the broad range of effects that microbiota exert on human health, compositional differences between patients should also factor into deciding who would benefit from a particular treatment modality. As mentioned above, the presence or absence of specific bacterial community members, or even their metabolites, can alter the prevalence, severity, and treatment of cancer and may serve as prognostic biomarkers. For example, patients receiving immunotherapy treatments may benefit from *B. intestinihominis* or *E. hirae* species to improve efficacy[127]; patients slated to receive irinotecan treatment may benefit from bacterial β-glucuronidase–targeting drugs.[133] Translating these cutting-edge innovations into clinical interventions will benefit from reduced costs for whole genome and transcriptome sequencing, as will simplified inquiry and interpretation by developing standardized bioinformatics analysis pipelines. Furthermore, increasing the access to centralized, cloud-based repositories for whole genome and transcriptome sequencing databases will facilitate data mining approaches by computational scientists. In the future, it is likely that combining pharmacogenomics information with custom microbial organisms or their specific metabolites will allow for precise dosing, symptom management, and improved therapeutic responses. ■

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