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REVIEW

Emerging roles of the microbiome in cancer

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Gene–environment interactions underlie cancer susceptibility and progression. Yet, we still have limited knowledge of which environmental factors are important and how they function during tumorigenesis. In this respect, the microbial communities that inhabit our gastrointestinal tract and other body sites have been unappreciated until recently. However, our microbiota are environmental factors that we are exposed to continuously, and human microbiome studies have revealed significant differences in the relative abundance of certain microbes in cancer cases compared with controls. To characterize the function of microbiota in carcinogenesis, mouse models of cancer have been treated with antibiotics. They have also been maintained in a germfree state or have been colonized with specific bacteria in specialized (gnotobiotic) facilities. These studies demonstrate that microbiota can increase or decrease cancer susceptibility and progression by diverse mechanisms such as by modulating inflammation, influencing the genomic stability of host cells and producing metabolites that function as histone deacetylase inhibitors to epigenetically regulate host gene expression. One might consider microbiota as tractable environmental factors because they are highly quantifiable and relatively stable within an individual compared with our exposures to external agents. At the same time, however, diet can modulate the composition of microbial communities within our gut, and this supports the idea that probiotics and prebiotics can be effective chemoprevention strategies. The trajectory of where the current work is headed suggests that microbiota will continue to provide insight into the basic mechanisms of carcinogenesis and that microbiota will also become targets for therapeutic intervention.

Introduction

Cancer is a leading cause of death that is associated with tremendous social and economic burdens. In the USA, the number of cancer survivors is projected to increase from 13.8 to 18.1 million over this decade and to cost $125 and $158 billion in healthcare expenses, respectively (1). In many developing countries, cancer incidence is increasing as a result of demographics (population aging) and the adoption of cancer-associated lifestyle choices such as smoking, 'westernized' diets and physical inactivity (2). Increased exposure to known carcinogens or cancer suspect agents might also be a contributing factor, especially in countries with minimal regulatory oversight.

Similar to many other complex diseases, cancer susceptibility and progression are primarily influenced by gene–environment interactions. On the one hand, we have made considerable progress understanding the genetics and the molecular/cell biological mechanisms that underlie carcinogenesis (3). Most recently, our knowledge has been advanced by the advent of next-generation sequencing platforms to catalog recurrent mutations and epigenetic changes (*via* chromatin immunoprecipitation sequencing and bisulfite sequencing) that arise during tumorigenesis (4). Yet, on the other hand, we know relatively little about which environmental factors influence cancer susceptibility and how they impact mechanisms of carcinogenesis. There have been some notable exceptions such as smoking and lung cancers as well as ultraviolet light exposure and melanomas, but this still remains an important challenge. Until recently, the microbiota that inhabit our body were unappreciated in this regard (unknown-unknowns in 'Rumsfeldian speak'). However, these bacteria, archaea, eukaryotes (such as yeast and other fungi) and viruses (including bacteriophage) are environmental factors that we are continuously 'exposed' to, and recent work demonstrates that they can, in fact, influence carcinogenesis. Our microbiota might be considered known-unknowns at the present time because of our limited knowledge, but they are likely to become known-knowns considering the rapid pace of research in this area.

Abbreviations: DCA, deoxycholic acid; GI, gastrointestinal; IL, interleukin; pks, polyketide synthase; TLR, toll-like receptor.

The human microbiome as revealed by metagenomic studies

The human body harbors ≥10^{14} microbial cells, which is ~10-fold greater than all of our somatic and germ cells combined. These microbiota and their collective genomes, referred to as the microbiome, are being characterized by metagenomics approaches that combine next-generation sequencing platforms with the computational analysis and assembly of targeted (16S ribosomal RNA hypervariable region) and random (whole genome shotgun) DNA sequence reads (5,6). Based on these studies, we know that the composition of microbial communities varies across different anatomical sites (7,8). We also know that the vast majority of these microbes are bacteria that reside within the lumen of our gastrointestinal (GI) tract. The commensal and symbiotic bacteria that live within are gut are protected from predators such as nematode roundworms, and they also benefit from a consistent supply of nutrients provided by our carbohydrate-rich diets. In return, many of the symbiotic bacteria digest glycans into disaccharides and monosaccharides for the human host as well as for their own energy utilization. To carry out this function, the gut microbiome is highly enriched for genes involved in carbohydrate metabolism including ≥115 families of glycoside hydrolases and ≥21 families of polysaccharide lyases (9,10). In contrast, the human genome has relatively few genes that encode carbohydrate-metabolizing enzymes, presumably because mammals (and their genomes) coevolved with gut microbiota (and the gut microbiome). As a result of this symbiotic relationship, gut microbiota are believed to improve our ability to absorb nutrients and extract calories from our diets (11,12).

Although microbial populations are conserved at higher taxonomic levels (13), metagenomic studies have documented substantial variation at the genus and species levels among human individuals (8,14). This interindividual variation is due to both host genetics and environmental factors. In support of host genetics, a genome-wide association study combined with 16S ribosomal RNA metagenomics data on a large (n = 645) panel of mouse lines identified a number of quantitative trait loci that influence the relative abundance of specific microbial taxa (15). In support of environmental factors, there is also microbial variation within a human individual over their lifespan (particularly from infancy to adolescence) and in response to diet, stress, household pets, antibiotics, chemotherapeutics and exposure to probably many other agents (16–23).

Human disease susceptibility is primarily influenced by gene–environment interactions, and the microbiome is now believed to be an important factor. Indeed, microbiome differences are evident between cases and controls for a growing list of human diseases including Crohn's disease, type-2 diabetes, autism and chronic allergies (24–26). The microbial imbalances associated with these complex disease states,

which is referred to as dysbiosis, usually involves shifts in the relative abundance of many commensal microbes rather than a simple 'one microbe-one disease' relationship that can exist for certain pathogens such as human immunodeficiency virus and acquired immunodeficiency syndrome or *Helicobacter pylori* and gastric ulcers. This dichotomy is analogous to the difference in genetic complexity between quantitative (polygenic) and Mendelian (monogenic) traits. In the context of gene–environment interactions, it is tempting to speculate that single-nucleotide polymorphisms, mutations or epigenetic perturbations of human disease genes (including tumor suppressors and proto-oncogenes) might influence an individual's microbiome. This would represent a novel biological function and provide mechanistic insight or have translational potential for the diagnosis and treatment of the disease state. One candidate is the Dectin-1 gene because a human single-nucleotide polymorphism is associated with a severe form of ulcerative colits and the corresponding knockout mice exhibit increased sensitivity to dextran sodium sulfate-induced colitis associated with differences in indigenous gut fungi (27).

Differences in microbiota are also associated with several types of cancer (Table I), and the advent of microbiome sequencing projects means that more types of cancer will undoubtedly be added to this list in the near future. This might be particularly true for certain carcinomas because the bacterial density is highest near mucous membranes of gut, lung and urogenital tract epithelia. Mucous membranes form barriers between our bodies' tissues and microbial communities and also have important immunoregulatory functions (28). The mucosa is compromised in certain disease states such as Crohn's disease, and these permeability defects arise because of perturbed tight junctions. Furthermore, some components of tight junctions such as E-cadherin and β-catenin are dysregulated in epithelial tumors (28). It should be mentioned that our knowledge of mucosal-associated microbial communities is more limited than might be expected. This is because most gut microbiome studies have been performed on stool samples since they can be readily obtained in a non-invasive manner. However, the composition of fecal-associated bacterial communities in stool samples is distinct from mucosal-associated communities obtained during colonoscopies (29–31).

Most of the observed microbiome differences that have been identified thus far involve many changes in the microbiota where any one alteration may have a subtle or modest effect, whereas the combined effect in aggregate is more robust. In addition, as with other diseases, there are pathogens capable of driving 'one microbe-one disease' neoplasms such as human papillomavirus for cervical cancer and *H. pylori* for gastric cancer (Table I). Tumor-associated viruses are noteworthy because of their previous role in the discovery of cellular proto-oncogenes. Another analogy is that the difference in microbial complexity between these scenarios (in Table II) is similar to the difference in genetic complexity between sporadic and familial cancers. It is also possible that both situations coexist during tumorigenesis. For example, two recent studies performed metagenomic sequencing on a combined total of 20 pairs of colorectal tumors matched with normal adjacent colonic tissues from the same individuals (32,33). Members of two dominant phyla associated with healthy individuals, Bacteroidetes and Firmicutes, were underrepresented in the tumors, whereas an invasive anaerobe (*Fusobacterium nuculeatum*) previously associated with periodontitis and appendicitis was significantly overrepresented in the tumors. Both groups confirmed their findings on a larger cohort of paired specimen ($n = 95$ and $n = 99$) using PCR analyses (32,33). Despite these consistent differences; however, the overall microbial communities of a tumor and matched non-cancerous colon from one individual were more similar to each other than were tumors or non-affected colon samples from different individuals. This supports the idea that the microbiome will be an important factor in personalized medicine.

Functional studies using gnotobiotic mouse models

A limitation of metagenomic studies is that they are correlative so it is difficult or impossible to know whether a particular microbiome difference is a cause or a consequence of a corresponding disease state. To establish cause-and-effect relationships, it is necessary to manipulate the microbiota in a rigorously controlled manner. For this reason, experiments with gnotobiotic mouse models are crucial, and this is why these models will likely play a more prominent role in future biomedical research. Maintained in a specialized gnotobiotic facility where sterility is maintained, laboratory mice can be raised in a completely germfree state (i.e. without any microbiota) if their diets are autoclaved and fortified with certain essential vitamins that are normally synthesized by microbiota (34). A number of studies have compared germfree mice to controls that are genetically identical but that have been colonized with a complete, albeit undefined, microbiota (referred to as conventionalized or conventionally derived) or that always have been maintained in a specific pathogen-free facility (referred to as conventionally raised). Compared with conventionalized or conventionally derived or conventionally raised controls, germfree mice consume more food, yet they are leaner with ~35% less adipose tissue (35). Germfree mice exhibit other metabolic anomalies (e.g. hypoglycemia and decreased levels of insulin and glycogen) and are resistant to obesity when provided a high-fat diet (35). These experiments support the metagenomic data suggesting that the gut microbiome increases calorie extraction of the host. This principle is exploited by the agricultural industry, which uses subtherapeutic

Table I. Microbiota changes that have been observed in human cancer cases

Cancer type	Sampling site	Microbiome changes in cases compared with controls	References
Oral squamous cell carcinoma	Saliva	Incr: *Capnocytophaga gingivalis*, *C. ochracea*, *Eubacterium saburreum*, *Leptotrichia buccalis*, *Streptococcus mitis*	(100–102)
Barrett's esophagus and esophageal cancer	Saliva, biopsied tissue	Incr: *Campylobacter consisus*, *C. rectus*, *Treponema denticola*, *S. anginosus*, *S. mitis*; Decr: *Helicobacter pylori*	(101,103–105)
Pancreatic cancer	Saliva	Incr: $n = 31$ including *S. mitis* and *Neisseria elongata*; Decr: $n = 25$	(101,106)
Gall bladder cancer	Bile culture	Incr: *Salmonella typhi*, *S. paratyphi*; bile usually free from bacteria but infected in cases	(101,107)
Colorectal cancer	Feces, biopsied tissue	Incr: *S. bovis*, Streptococcus spp., *Escherichia coli*, *Fusobacterium nucleatum*, Clostridium, Bacteroides; Decr: Lactobacillus, butyrate-producing bacteria (including Roseburia and Fecalibacterium), Microbacterium, Anoxybacillus, *Akkermansia muciniphilia* (a mucin-degrading species)	(25,32,33,83–87,101,108–110)

Table II. Single microbes that can drive human cancer

Microbe	Cancer type(s)
Helicobacter pylori	Gastric adenocarcinoma, gastric lymphoma, esophageal adenocarcinoma
Human papillomavirus (HPV)	Anogenital carcinomas, oropharyngeal carcinoma
Epstein-Barr virus (EBV)	Lymphomas, nasopharyngeal carcinoma
Human immunodeficiency virus (HIV)	Lymphomas, Kaposi's sarcoma
Hepatitis B virus	Hepatocellular carcinoma
Hepatitis C virus	Hepatocellular carcinoma, lymphomas
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell leukemia/lymphoma
Human herpesvirus 8 (HHV-8)	Kaposi's sarcoma

Adapted from reference (94).

doses of antibiotics to alter the microbiome in a manner that promotes the growth of farm animals. This effect has been recapitulated in mice where subtherapeutic doses resulted in increased adiposity without affecting caloric intake or hormones that regulate satiety (36). Furthermore, specific microbiome changes were documented in mice, and the juvenile stage of postnatal development was a sensitive developmental window because adiposity was increased even when early-life exposures were followed by a curtailment of the antibiotic regimen. This may have implications in the obesity epidemic considering the pervasive use of antibiotics and the fact that low doses of antibiotics can be detected in drinking water.

Germfree mice have also been used to demonstrate that microbiota play an important role regulating our immune system and inflammatory responses (37). For example, interleukin (IL)-10 is a potent, immunosuppressive cytokine, and IL-10 knockout mice exhibit a colitis phenotype that is rescued by maintaining them in germfree state (38). This observation suggests that a primary function of IL-10 is to prevent an inappropriate inflammatory response against commensal gut microbiota. Similarly, transforming growth factor-β1 knockout mice exhibit colorectal inflammation and cancer, and both of these phenotypes are rescued on a germfree background (39). These results are consistent with the observation that germfree mice have fewer proinflammatory T helper 17 cells in the lamina propria of their gut than specific pathogen-free controls (40,41). The role of microbiota in both cellular energetics and the immune response/inflammation is relevant to cancer because these functional categories are recognized as hallmarks of cancer (3). In fact, it is tempting to speculate that microbiota may be added as a hallmark in the future.

Germfree mice can be colonized with specific microbial communities to assess their role in host physiology. For example, a number of studies have demonstrated that differences in microbial composition can influence malnutrition susceptibility at one extreme and obesity at the other extreme without affecting food intake (12,42–44). Some of these and other studies have used 'humanized mice', which are colonized with microbiota isolated from human cases versus controls, to interrogate the function of human microbes. Germfree mice can also be monoassociated or polyassociated with a small number of microbes. This reductionist approach makes it possible to assign function to a specific microbe(s) and sometimes a specific microbial gene or metabolite. These types of studies are beginning to provide mechanistic insight into how microbes promote or inhibit a variety of disease states including cancer as exemplified below.

Microbiota, inflammation and genomic instability

As mentioned above, inflammation is an important factor in carcinogenesis. For example, colitis patients have a 10-fold increased risk of colorectal cancer (45), and non-steroidal anti-inflammatory drugs such as aspirin have been effective for cancer prevention and as adjuvant therapies (46,47). A recent study demonstrated that members of the Enterobacteriaceae family are upregulated by up to ≥100-fold in the colons of IL-10 knockout mice with colitis compared with wild-type control mice without colitis (48). Monoassociation of IL-10 knockout mice with two of these commensal bacteria (strains of *Escherichia coli* and *Enterococcus faecalis*) indicated that each one was competent to induce colitis. However, only one of the two, the *E. coli* strain but not *E. faecalis*, was capable of driving colorectal cancer in the same mice after they were treated with the procarcinogen azoxymethane. These results suggest that inflammation may have been necessary but was not sufficient for carcinogenesis in this model. Therefore, the next objective was to identify other bacterial-triggered events that contribute. Further analysis revealed that the *E. coli* strain harbors a ~54 kb polyketide synthases (*pks*) pathogenicity island, which encodes enzymes that synthesize a genotoxin named colibactin, that is lacking in the *E. faecalis* strain. To demonstrate that colibactin is functionally important for cancer, IL-10 knockout mice were monoassociated with an isogenic strain of *E. coli* in which *pks* had been deleted. The Δ*pks* strain colonized mice and stimulated inflammation as well as the +*pks* strain but was unable to induce tumor formation. Consistent with colibactin being a genotoxin, the +*pks E. coli* strain was associated with DNA damage in colorectal tumors based on γH2AX as a marker, whereas the Δ*pks* strain did not induce DNA damage. These findings have human relevance because the same study reported that +*pks E. coli* strains are more highly enriched in patients with inflammatory bowel disease and colorectal cancer than controls (48).

A functional link between bacterial-mediated inflammation and DNA damage was also revealed by a study of RAG-2 knockout mice infected with *H. hepaticus* in a specific pathogen-free facility (49). Infiltration of activated macrophages and neutrophils resulted in increased production of cytokines and chemokines as well as nitric oxide, superoxide and a number of reactive species that led to DNA and RNA damage products and culminated in colorectal cancer.

DNA damage and cancer risk are likely to be affected by food–microbiota interactions more often than microbiota in isolation (50). This is particularly true for red meat, which is a significant risk factor for several types of cancer (51). Heterocyclic amines from charred meat are fermented by colonic bacteria, and some of the metabolites are electrophilic and believed to damage DNA and contribute to colorectal cancer (52). Red meat and other high-protein foods are also metabolized by sulfate-reducing gut bacteria to yield hydrogen sulfide, which is also believed to contribute to cancer, particularly when DNA repair mechanisms are perturbed (50,52).

Based on epidemiology studies, obesity is a significant risk factor for several common types of cancer (53), and the worldwide obesity epidemic portends an increased incidence of cancer. Numerous factors including inflammation contribute to obesity-associated cancer (53,54), and evidence has recently emerged that bacterial metabolites can play a role. In one such study, tumorigenesis was initiated in mice with the carcinogen 7,12-dimethylbenz(a)anthracene, which induces *Ras* mutations at a high frequency (55). Obese mice that were either leptin deficient (*ob/ob*) or that were wild-type but provided a high-fat diet developed hepatocellular carcinoma, whereas lean mice that were genetically wild-type and provided a standard diet did not. The obese mice had increased representation of bacteria in the Firmicutes phylum, which is consistent with previous studies and their proposed role in increasing calorie extraction of the host. Bacteria in cluster IX of the genus Clostridium were particularly overrepresented, and they are able to convert primary bile acids into a secondary bile acid named deoxycholic acid (DCA). DCA is a carcinogen that can cause DNA damage *via* the production of free radicals, and it has been implicated in liver and colorectal cancers. The obese mice did, in fact, have higher serum levels of DCA, which made it a plausible candidate. To demonstrate that Clostridium and DCA are functionally important, a couple of experiments were performed. First, when 7,12-dimethylbenz(a) anthracene-treated obese mice were treated with a couple different antibiotic regimens, serum DCA levels and the liver cancer phenotype were significantly attenuated. This was the case for vancomycin, which preferentially kills Gram-positive bacteria including the Clostridium

species that had been overrepresented. Second, DCA production was diminished by two different strategies without the use of antibiotics (by either decreasing 7α-dehydroxylation activity with difructose anhydride III or stimulating bile acid secretion with ursodeoxycholic acid), and this also attenuated the tumor phenotype.

Other inflammatory mechanisms

Microbial-induced inflammation can also contribute to cancer by stimulating the production of cytokines and chemokines that promote cell proliferation and/or inhibit apoptosis. In another mouse model of hepatocellular carcinoma, where diethylnitrosoamine and CCl_4 are used to induce chronic liver injury, intestinal microbiota were shown to be dispensable for tumor initiation but important for tumor promotion (56). This was demonstrated by significantly decreasing the liver tumor burden when the mice were treated with antibiotics or when they were analyzed on a germfree background. Mice with a knockout of toll-like receptor 4 (TLR4), which is a cell surface receptor that senses bacterial lipopolysaccharides, exhibited a similar decrease in tumor burden. In wild-type mice that were treated with diethylnitrosoamine and CCl_4, TLR4 signaling led to increased expression of the hepatomitogen epiregulin, which may have mediated some of the proliferative and antiapoptotic effects that were observed in the tumors. However, TLRs signals through MyD88 and nuclear factor-κB to regulate the expression of many cytokines and other genes.

The TLR-signaling pathway is crucial for the innate immune system, and, in turn, this can modulate microbial composition and other host functions. TLR5 and TLR2 serve as examples. TLR5 knockout mice exhibit signs of metabolic syndrome such as hyperphagia, insulin resistance and increased adiposity, and this phenotype is exacerbated by a high-fat diet as expected (57). The lack of a similar phenotype in MyD88 knockout mice suggests that a non-canonical signaling pathway might be utilized. TLR5 is highly expressed in the intestine and recognizes bacterial flagellin, and the metabolic syndrome phenotype was associated with 116 microbiota changes at the species level (i.e. phylotypes) without significant changes at the overall phylum level. To demonstrate that these microbiota changes are significant, antibiotics were used to rescue the phenotype. More importantly, when microbiota from TLR5 knockout mice were transplanted into wild-type recipients that had been germfree, certain features of metabolic syndrome were recapitulated. This experiment demonstrates that microbiota can cause an altered metabolic state rather than simply being a consequence. In the case of TLR2, it is expressed on the surface of Treg cells and recognizes polysaccharide A from *Bacteroides fragilis* (58). Activation of the TLR2 pathway in these cells promotes immunologic tolerance and allows this commensal bacterium to colonize the gut rather than being eliminated. In contrast, *B. fragilis* lacking polysaccharide A does not receive immunological tolerance and is eliminated *via* the T helper 17 response. This type of mechanism may enable the immune system to discriminate between commensal bacteria and pathogens and to respond accordingly. One possible complication regarding these experiments is that there is evidence that differences in microbial composition are not apparent immediately following intercrosses but require $TLR^{+/+}$ and $TLR^{-/-}$ mice to be bred separately for multiple generations (59,60).

Immune cells in the tumor microenvironment can respond to lipopolysaccharide, flagellin and other bacterial products by producing a battery of cytokines such as IL-23, IL-17 and IL-22BP that modulate tumorigenesis in the colon and other tissues (28,61,62). Furthermore, there is a two-way flow of information as human metabolites can influence microbial populations. For example, in response to intestinal inflammation, mammalian cells produce nitrate, *S*-oxides and *N*-oxides, which can be used by a subset of microbiota (facultative anaerobes but not obligate anaerobes) as electron acceptors for anaerobic respiration (63). This leads to a growth advantage of facultative anaerobes and is due to nitrate respiration because an *E. coli* strain that had several nitrate reductase genes inactivated was outcompeted by an isogenic, wild-type strain. The facultative anaerobes that had a growth advantage were using host-derived nitrate based on the effect being negated in mice with a knockout of inducible nitric oxide synthase.

Probiotics and prebiotics in cancer prevention

Some of the experiments described above used antibiotics to decrease the tumor burden in mouse models. However, antibiotics are not good candidates for chemotherapy or chemotherapeutic adjuvants in the clinic. Not only would this approach make the problem of antibiotic-resistant bacterial strains even worse, but this would also kill many commensal bacteria including some that likely promote homeostasis and protect against carcinogenesis. It has been proposed that increased antibiotics usage is altering our microbiota and contributing to the increased incidence of obesity, inflammatory bowel disease, allergies and asthma (64), and this may also apply to certain cancers. In fact, as *H. pylori* has been eradicated to a large extent in western countries, gastric cancer has decreased but esophageal cancer has become more common. One possibility is that *H. pylori* can alter stomach pH and acid reflux in a manner that protects against Barrett's esophagus and esophageal cancer (64).

Instead of using antibiotics to kill bacteria indiscriminately, it would be better to take steps that restore a beneficial microbial composition. This is the basis for fecal microbiota transplantations that are effective for the treatment of diarrhea in people with severe *Clostridium difficile* infections, which usually arise because antibiotics eliminated commensal bacteria that are capable of displacing or suppressing *C. difficile*. To make these transplantations more uniform, pathogen free and aesthetically appealing, it should be possible to inoculate people with a beneficial mixture of gut bacteria. As a proof of principle, a recent study transplanted a culture of six phylogenetically diverse gut microbes into mice with *C. difficile* infections, and this restored a normal microbial community, displaced the *C. difficile* and resolved the disease as well as homogenized feces (65).

Probiotics and prebiotics are more common ways to establish and maintain healthy microbiomes (66). Probiotics refers to live microorganisms present in foods or dietary supplements that confer a health benefit. Lactobacilli in yogurt is the best known example, but Streptococci and Bifidobacteria in cheeses or other foods and drinks are also common. One well-known benefit of Lactobacilli in yogurt is improved digestion of dairy products in individuals who are lactose intolerant. This allows some people to increase their intake of calcium and is noteworthy because the prevalence of lactose intolerance ranges from 5 to 15% in northern European countries and the USA to >50% in African and Asian countries (66). The beneficial effect is due to live bacteria, which provide β-galactosidase (lactase) activity, because heated or pasteurized yogurt is not effective (67,68). It should also be possible to improve probiotics by supplementing foods with bacteria engineered to either have stronger beneficial effects or to more stably colonize the human GI tract. For example, a strain of *Lactobacillus acidophilus* with a deletion in the phosphoglycerol transferase gene is unable to synthesize lipoteichoic acid, and oral administration of this bacterium to $Apc^{\Delta floxed}$ mice resulted in the regression of already established colonic polyps (69). Strains of *L. casei* and *Lactococcus lactis* have been engineered to produce a protein called elafin that diminished inflammation in a mouse model of colitis (70). When these bacteria were added to inflamed epithelial cells from human colitis patients *ex vivo*, they attenuated cytokine production and cell permeability. A final example is a strain of *L. gasseri*, which was engineered to overexpress the antioxidant superoxide dismutase and decreased colitis in IL-10 knockout mice (71).

Prebiotics refers to indigestible food ingredients that selectively stimulate the growth and/or activity of certain gut microbiota that confer a health benefit. Common prebiotics include dietary fiber sources such as inulin that promote the growth of Bifidobacteria. A number of prebiotics have been implicated in cancer prevention (72). Dietary polyphenols are among the most studied prebiotics. They include flavonoids, phenolic acids, and lignins present in tea, wine, fruits, nuts, and vegetables. Ellagic acid is a polyphenol present in certain berries and nuts that is an antioxidant with cancer-preventive properties. Ellagic acid is metabolized by colonic microbiota into urolithins that have proestrogenic and antiestrogenic activities in a context-dependent manner (73). Urolithins can also downregulate COX-2-mediated inflammation so the anticancer effects might involve multiple pathways (74).

Another polyphenol is daidzein, which is a soy isoflavone metabolized by gut microbiota into equol (75). Equol can be detected in only 30–40% of individuals, and, although the reason for this is not understood, it could be due, in part, to the relative abundance of specific bacteria (72). The ability to produce equol is positively correlated with sulfate-reducing bacteria and negatively correlated with *C. coccoides* and *Eubacterium rectale* (76). Some epidemiological studies have reported correlations between equol or equol-producing bacteria and diminished breast cancer risk in women and diminished prostate cancer risk in men. However, these correlations have been observed in Asian populations but not European populations (77). It is not clear whether these ethnic disparities are due to differences in genetics, microbiota or diet (e.g. soy consumption), and more work will be required to strengthen the link between equol and cancer prevention. Generally speaking, based on situations like this, it would be advantageous to combine prospective epidemiology studies with genome-wide association studies and microbiome studies. This kind of integrated approach might allow a combination of factors to be identified that have a significant and reproducible effect regarding diet and chemoprevention. In addition, it would be cost effective and useful to assess relatively short-term probiotic and prebiotic regimens by performing metabolomics or analyzing cancer-related biomarkers as surrogates and then use this information to direct more expensive, longer term chemoprevention or intervention trials.

Dietary fiber, gut microbiota and butyrate in colorectal cancer prevention

Fiber is a probiotic fermented by bacteria into short-chain fatty acids that can reach very high (mM) levels in the lumen of the colon (78). Butyrate is one of the most abundant of these short-chain fatty acids and is selectively transported into the colonic epithelium. Unlike most cells in the body, which utilize glucose as their primary energy source, colonocytes rely on butyrate for ~70% of their energy needs (79,80). As a fatty acid, butyrate is an oxidative energy source that undergoes β-oxidation followed by the trichloroacetic acid cycle inside of mitochondria. Because virtually all butyrate is derived from bacteria rather than directly from food, colonocytes from germfree mice have suboptimal energetics including ~50% lower levels of adenosine triphosphate and consequently undergo autophagy (81,82). To demonstrate that these metabolic defects are due to butyrate deficiency, when butyrate is added to germfree colonocytes *ex vivo*, normal metabolic parameters are restored (81).

Fiber has been implicated in the prevention of colorectal cancer, and butyrate is one of the most plausible tumor-suppressive molecules. This is consistent with microbiome studies that have reported fewer butyrate-producing bacteria in colorectal cancer cases than controls (83–87). Although butyrate promotes the growth of normal colonocytes, it inhibits the growth of colorectal cancer cell lines *via* decreased cell proliferation coupled with increased apoptosis and/or cell differentiation. The observation that butyrate has opposing effects on normal versus cancerous colonocytes has been referred to as the butyrate paradox (88), and recent work has shown that this is due to the Warburg effect (89). Unlike normal colonocytes, cancerous colonocytes undergo the Warburg effect by upregulating glucose intake (*via* increased GLUT expression) and aerobic glycolysis with a concomitant decrease in oxidative metabolism. As a result, less butyrate is metabolized in the mitochondria of cancer cells and more of it accumulates in the nucleus where it acts as an endogenous histone deacetylase inhibitor (90). Butyrate increases histone acetylation and transcription of cancer-related genes important for cell proliferation (e.g. p21), apoptosis (e.g. BAX, BAK, FAS) and cell differentiation (e.g. mucins).

Butyrate has potent anti-inflammatory properties so it probably also has tumor-suppressive properties that are not cancer cell autonomous. For example, butyrate ameliorates the inflammation associated with colitis in both rodent models and human patients (91). Some of these effects are probably due to histone deacetylase inhibition and epigenetic regulation of gene expression, but there is also evidence that it can signal though G-protein-coupled receptors to stimulate the expansion of T_{reg} cells (92).

Other metabolomic properties of the microbiome in cancer

The metabolic capacity of the microbiome is so prodigious that it has been referred to as our second liver, and it is known to influence human health and disease susceptibility in a variety of ways (93). This applies to cancer, ranging from cancer prevention at the earliest stages of disease to how individuals respond to chemotherapy at the latest stages of disease. Regarding the earliest stages of cancer, the role of microbiota in promoting or inhibiting tumor initiation and progression is not restricted to direct effects on tumorigenesis. For example, gut microbiota play an important role in estrogen metabolism, and higher estrogen levels are a major risk factor for endometrial and breast cancers (94). This is dependent on bioactive food components as discussed above for the connection between ellagic acid, bacterial urolithins and their proestrogenic and antiestrogenic activities (73).

Regarding later stages of cancer, camptothecin is a topoisomerase I inhibitor commonly used for chemotherapy, and microbiota influence the occurrence of diarrhea as an adverse side effect (95). Camptothecin-11 is a prodrug that is converted to SN-38 as the active chemotherapeutic. In the liver, SN-38 is inactivated by glucuronosyltransferases, and inactive SN-38G is excreted *via* the bile ducts into the GI tract. However, commensal bacteria in the intestines express glucuronidases that remove the glucuronide group to reactivate SN-38, which harms rapidly dividing intestinal epithelial cells and causes diarrhea (95). Antibiotics were evaluated as a treatment option but not pursued because the indiscriminate killing of bacteria results in *C. difficile* infections and other events that would have been particularly deleterious to cancer patients. As an alternative approach, pharmacologic inhibitors of bacterial β-glucuronidases were identified that prevent reactivation of SN-38, and oral administration of these inhibitors to camptothecin-11-treated mice protected them from diarrhea without killing bacteria or harming mammalian cells (95). This and other recent studies establish a precedent for drug discovery where the activity of bacterial targets are modulated to improve the outcome of disease or to minimize side effects (96–98).

Future directions

Metagenomics will continue to catalog microbial communities, but this approach does have a couple of limitations. First, metagenomics does not evaluate microbial gene expression, and it is the composite gene expression profiles of microbial communities that will determine microbiome function. To address this issue, it is now possible to move from metagenomics to metatranscriptomics by performing RNA-seq on samples from different anatomical sites (99). In the future, metatranscriptomics projects will provide insight into how diet and other environmental factors influence the expression of each gene within the microbiome. Metatranscriptomics will also be incorporated into case–control studies to identify microbial genes and genetic pathways that are altered in cancer and other disease states. Second, there is considerable functional redundancy between bacterial species that is commensurate with taxonomic relationships (7). Therefore, the microbial diversity or differences identified in microbiome studies is not equivalent to functional diversity or differences. For this reason, the metabolic capacity of different bacterial communities will need to be characterized in an accurate, high-throughput and cost-effective manner. The microbial meta-metabolome is determined, in part, by the relative abundance of microbiota (as determined by metagenomics) and their corresponding gene expression profiles (as determined by metatranscriptomics), and this will bring us a step closer to understanding the translational implications for human health. However, the metatranscriptome and meta-metabolome are probably influenced by diet and other factors to an even greater extent than the metagenome, and this will increase variability and present challenges.

We have not yet developed culture conditions that support the growth of most microbes that inhabit the human body, particularly anaerobic bacteria that reside deep within our GI tract. This has not prevented us from using metagenomics to characterize microbial populations and to identify microbiome differences between individuals with certain diseases including cancer compared with

controls. It is important that these studies continue and that they be integrated with epidemiology studies (especially with respect to diet), genome-wide association studies and metabolomics. However, it will become increasingly important to culture specific bacteria so they can be analyzed in gnotobiotic mouse models. Genetically engineered mouse models of cancer and other diseases are known to be influenced by genetic background, and it will be important to learn about the role of microbiota in modifying these phenotypes. The ability to culture and manipulate specific bacteria and introduce them into gnotobiotic mouse models will allow us to move from correlation to causation and will provide insight into molecular mechanisms, which may lead to improved probiotic/prebiotic strategies of disease prevention as well as therapeutic interventions.

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