The gastrointestinal microbiota and colorectal cancer

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1Department of Medicine, School of Medicine, University of North Carolina, Chapel Hill, North Carolina; 2Center for Gastrointestinal Biology and Disease, School of Medicine, University of North Carolina, Chapel Hill, North Carolina; and 3Surveillance and Health Services Research, American Cancer Society, Atlanta, Georgia

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Keku TO, Dulal S, Deveaux A, Jovov B, Han X. The gastrointestinal microbiota and colorectal cancer. Am J Physiol Gastrointest Liver Physiol 308: G351–G363, 2015. First published December 24, 2014; doi:10.1152/ajpgi.00360.2012.—The human gut is home to a complex and diverse microbiota that contributes to the overall homeostasis of the host. Increasingly, the intestinal microbiota is recognized as an important player in human illness such as colorectal cancer (CRC), inflammatory bowel diseases, and obesity. CRC in itself is one of the major causes of cancer mortality in the Western world. The mechanisms by which bacteria contribute to CRC are complex and not fully understood, but increasing evidence suggests a link between the intestinal microbiota and CRC as well as diet and inflammation, which are believed to play a role in carcinogenesis. It is thought that the gut microbiota interact with dietary factors to promote chronic inflammation and CRC through direct influence on host cell physiology, cellular homeostasis, energy regulation, and/or metabolism of xenobiotics. This review provides an overview on the role of commensal gut microbiota in the development of human CRC and explores its association with diet and inflammation.

colorectal adenoma; diet; inflammation; bacterial metabolites

COLORECTAL CANCER (CRC) has the third highest cancer incidence in the world. In 2012 CRC accounted for ~1,361,000 new cases (9.7% of total cancer incidence) and ~694,000 deaths (8.5% of total cancer deaths) globally (45). In the United States, CRC is the second leading cause of cancer mortality and the third most commonly diagnosed cancer. It is estimated to account for roughly 50,310 deaths and about 136,830 new cases in 2014 (142).

Despite extensive research, the precise etiology for CRC is still unknown, but genetic, epigenetic, and environmental factors such as diet have been implicated. CRC arises as part of a multistep process associated with the accumulation of a series of genetic and epigenetic alterations (49) that influence fitness and clonal expansion of altered cells in the transformation of normal colonic epithelium to adenomas and cancer.

The first model depicting key genetic alterations in CRC included the activation of K-ras oncogene (44, 84, 163) and the inactivation of tumor suppressor genes such as adenomatous polyposis coli (104, 133) and p53 (encoded by TP53 gene in human) (164, 169). Following this initial model, a number of acquired genetic mutations including PIK3CA, FBXW7, SMAD4, TCF7L2, NRAS, FAM123B, CTNNB1, SMAD2, alterations in pathways of chromosomal and microsatellite instability, mismatch repair (23, 25), and epigenetic CpG island methylation (3, 128), have also been observed to contribute to colorectal carcinogenesis.

In addition to genetic factors, an environmental component to CRC is also strongly implicated. Studies of immigrants show that cancer rates for migrants quickly match those of their current country of residence, even when they originated from a country with a lower baseline cancer rate (46, 102). Because genes do not change over such a short period, diet, a component that changes with migration, is thought to be an important contributor to CRC development. However, studies of dietary elements, such as fiber and CRC, to date are largely inconsistent with some studies reporting protective effects of fiber and others showing no effects (6, 118). This is likely because of an overlooked component of the large bowel, the gut microbiome.

There is mounting evidence that the intestinal gut bacteria (microbiota) play an important role in colorectal carcinogenesis (37, 69, 126, 157, 174).

Intestinal Microbiota

The human large bowel is home to complex and diverse communities of microbiota that play important roles in health and disease. Several studies suggest that, depending on the route of delivery, the human gastrointestinal tract is colonized at birth, at least with limited microbes (73, 134) that gradually become diverse to reach more than 10^{14} microbes comprising 1,000 or more heterogeneous species of bacteria, viruses, archaea, and fungi (135). It is estimated that the number of bacterial cells of the human gut exceeds the number of human cells by 10-fold (48). The collective bacterial genome, referred to as the gut microbiota, harbors ~150-fold more genes than the human genome (120, 171).

Within the large bowel, bacteria are present in two compartments: a luminal compartment and a mucosal adherent compartment. The microbiota in the luminal compartment, while reflective of the bacterial communities in the colonic lumen, may not reflect the composition and localization of epithelial
and cryptal communities (135). The colonic mucosa harbors dense cohesive communities of bacteria that adhere to surface-associated polysaccharide matrices and resist hydrodynamic shear forces (146). Some of these bacteria establish themselves as entrenched residents of the mucosa (146) despite the rapid turnover and propulsion of debris and water through the gut. These adherent resident bacteria interact with the mucosal immune system and as such may be more relevant to diseases such as inflammatory bowel diseases (IBD) and CRC.

Although the gut bacteria have long been considered commensal residents, it is now recognized that they serve diverse and important functions (18) some of which may contribute to CRC etiology. The intestinal bacteria are essential in digestion and absorption of indigestible carbohydrates (fibers), production of vitamins B and K, metabolism of endogenous and exogenous compounds, immune potentiation, are actively involved in innate and cell-mediated immunity, help to maintain intestinal barrier function, and assist with an appropriate immune response against pathogenic microbes (9, 74). Their symbiotic relationship (“normobiosis”) with the host is critical to maintaining a balance (homeostasis) in the gut. A shift in this balance (“dysbiosis”) under abnormal conditions can lead to detrimental consequences for the host. For instance, dysbiosis of the normal microbiota is associated with overgrowth of opportunistic pathogens that are normally inhibited by commensal bacteria (12). Moreover, microbial dysbiosis has been observed in IBD (32, 61, 80, 152), obesity (11, 20, 140, 154, 159), colorectal adenomas, and cancer (26, 37, 92, 99, 109, 116, 130, 138, 144, 145, 171). One cause of dysbiosis may be antibiotic treatment, which has been linked to development of Clostridium difficile colitis (59, 64, 139, 170).

The composition of intestinal microbiota is determined by various factors, including host genetics (159), environment (16, 21), diet (26, 43, 103, 160, 176), and hygiene (7). Recent findings demonstrated that paneth cell defensins, innate antimicrobial peptides that contribute to mucosal host defense, can regulate the composition of the intestinal bacteria (129). Although host genetics may have a significant impact on the microbiota composition, discovery of specific genetic factors that interact with the gut microbiota is in the beginning stages. α1,2-Fucosyltransferases (FUT2) secretor genotype is correlated with the abundance of bifidobacteria in the colon (165). FUT2 gene is responsible for the histo-blood group antigens, and polymorphisms in this gene have been associated with IBD and CRC (56, 76).

**Intestinal Microbiota and CRC**

Several human studies have demonstrated a link between the gut bacteria and CRC. One of the earliest studies to correlate gut bacteria with colorectal neoplasm was conducted by Moore and Moore (101) who assessed fecal samples from polyp patients using culture methods. They observed that the abundance of *Bacteroides* and bifidobacteria was associated with increased risk of colon polyps, whereas *Lactobacillus* and *Eubacterium aerofaciens* were protective. Early studies by Swidsinski et al. (150) also reported an association between the abundance of Escherichia coli and colorectal adenomas and cancer. O’Keefe et al. (113) observed that high abundance of hydrogen sulfide (H₂S)- and bile salt-producing bacteria was associated with increased risk of colon cancer. The majority of bacteria are not culturable, therefore these early studies evaluated only bacteria that are culturable. However, the advances in molecular biology and sequencing technology have revolutionized the microbiome field such that it is now possible to characterize bacteria without culturing. Human studies using high-throughput molecular sequencing methods reveal changes in fecal microbiota composition in CRC subjects compared with healthy controls (145, 166). Although the ease of collection facilitates the evaluation of fecal samples, the luminal contents may not accurately represent the adherent microbiome, since they contain many transient organisms. Several studies have used high-throughput molecular 16S-based methods to examine either the fecal or mucosal microbiome in relation to colorectal adenomas and cancer. Compared with control subjects without adenomas, case subjects with adenomas had significantly increased diversity and richness of bacterial species (130, 141). Chen et al. (26) observed reduced abundance of Clostridium, Roseburia, Eubacteria spp., and other butyrate-producing bacteria in fecal samples of adenoma subjects compared with healthy controls. Marchesi et al. (92) profiled the microbiota in colon tumors and matching normal colon tissues and observed very different microbial patterns and signatures between the two sites. Specifically, they observed an overabundance of Fusobacterium on the tumor compared with matching normal tissue. Sobhani et al. (145) observed that altered fecal bacterial profile was linked with elevated IL-17 in CRC patients compared with healthy controls. A summary of the findings from human studies on gut bacteria and CRC is presented in Table 1. Together, these studies suggest that alterations that favor increased abundance of potentially pathogenic bacteria and reduction of beneficial bacteria are associated with colorectal adenomas and cancer. Although bacterial dysbiosis is associated with CRC, there is limited information on the contribution of specific bacteria. This is currently an area of intense investigation.

To fully understand the role of gut bacteria in CRC, mechanistic studies in animal models are critical. Vital studies of genetically engineered rodents raised in germ-free environments support the role of bacteria in CRC. For example, IL-10 or transforming growth factor (TGF)-β/Rag2 knockout mice maintained under germ-free conditions do not develop tumors and have significantly lower levels of inflammation (29, 41, 137, 172). Findings from studies in murine models of chemically induced colon cancer indicate that intestinal bacteria promote colon carcinogenesis by increasing proliferation and formation of aberrant crypt foci (40, 115). Horie et al. (65) found that the incidence of tumors in germ-free mice mono-associated with bacteria ranged from 30 to 68% depending on the colonizing bacteria and thus supports the notion that the intestinal microflora has potent effects on cancer development. Another study showed that infection with *Helicobacter* spp. promoted colon cancer in SMAD3-deficient mice, suggesting that gut bacteria in combination with genetic alterations in the TGF-β pathway may contribute to colon carcinogenesis (90). More recent studies in animal models suggest that the bacterial dysbiosis phenotype is transferable. Transplantation of feces from tumor-bearing mice to conventionalized germ-free mice resulted in an increased colon inflammation and tumorigenesis (175). Similarly, fecal transplants from human CRC patients into germ-free mice resulted in increased tumor burden in mice (14). A summary of the findings from animal studies on gut...
Table 1. Human studies of gut bacteria associated with adenoma and CRC

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<td>Chen et al. 2013 (26)</td>
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<td>Geng et al. 2013 (46b)</td>
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bacteria and CRC is presented in Table 2. Although these studies in animal models provide compelling evidence to support the contribution of intestinal microbiota to CRC, the relationship is likely to be more complex and may involve interactions between the gut bacteria, chronic inflammation, mutations in oncogenic pathways, and diet to promote colorectal carcinogenesis (63, 69, 108).

Although the exact role of the microbiota and the mechanisms by which it promotes CRC has yet to be fully elucidated, potential mechanisms have been described as follows.

**Cell wall antigens and bacterial colicins.** Klein et al. and Huycke et al. (67, 78) showed that Streptococcus galloptycis (formerly S. bovis) antigens promote premalignant lesions through aberrant crypt formation. Colibactin produced by E. coli strains have been shown to induce DNA double-strand breaks in intestinal cells and trigger chromosomal instability, gene mutations, and cell transformation (4, 14, 26, 108). Cougnoux et al. (33) reported that colibactin-producing E. coli enhanced colon tumor growth in both xenograft and azoxymethane/dextran sodium sulfate IL-10−/− mouse models by inducing a senescence-associated secretory phenotype (4).

**Inflammation.** The long-standing presence of infection with potential pathogenic bacteria can induce chronic inflammation. Chronic inflammation in the colorectal mucosa has been linked to CRC development (77, 79, 95, 161).

**Production of toxic metabolic byproducts from dietary carbohydrates, protein, bile, and mutagenic precursors.** H2S produced by bacteria in the gut are related to CRC etiology. H2S and reactive oxygen radicals are toxic to the epithelium (5a, 35, 50, 68, 112, 122). Phenolic compounds such as amines, N-nitroso compounds from meat consumption, can also be toxic to the host (91, 143).

This review will focus on inflammation and diet as potential mechanisms, since these risk factors for CRC are well established in the literature.

**Inflammation, Intestinal Microbiota, and CRC**

The normal colon maintains a continuous state of low-grade inflammation in response to the presence of endogenous bacteria and dietary antigens. Under normal physiological conditions, this low-grade inflammation is maintained by components of the innate and adaptive immune systems that contribute to the homeostasis between proinflammatory mediators [e.g., IL-1β, interferon-γ (IFNγ), IL-8, TNF-α, IL-23, IL-17, and IL-6] (24, 100, 111) and anti-inflammatory mediators (e.g., IL-10 and TGF-β). The specific components of the innate immune system that regulate this state of homeostasis include monocytes, macrophages, dendritic cells, and natural killer cells, whereas the adaptive immune system lymphocytes (B cells, T cells, and helper cells) and regulatory cells serve a similar role. Disruption of such inflammatory mediator homeostasis may contribute to chronic inflammation and increased risk of colorectal adenomas and cancer (28, 97, 168).

There is evidence to suggest that chronic inflammation increases colon cancer risk (52, 106, 155). Evidence comes from studies in IBD (34, 86) and colitis in animal models (17, 123). Studies in animal models support the importance of IL-10 in the suppression of chronic inflammation and CRC. In addition, anti-inflammatory agents, such as aspirin and non-steroidal anti-inflammatory drugs, have been repeatedly shown to decrease the risk of colorectal adenomas and cancer (10, 13, 123).

An important aspect to consider is the resident gut microbiota and their role in inflammation and cancer. Under normal conditions, commensal gut bacteria maintain a symbiotic relationship with the mucosal immune system and are thought to be in a state of tolerance. The presence of commensal bacteria is vital to the development of the gut immune system. For instance, the immune systems of conventionalized animals are highly developed compared with germ-free animals (17, 30,
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<td>Uronis et al. 2009 (161a)</td>
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GF, germ free; AvrA, antivirulence gene; SPF, specific pathogen free; WT, wild type; TLR, Toll-like receptor; MyD88, myeloid differentiation primary response gene 88; BFT, Bacteroides fragilis toxin; KO, knockout; DMH, dimethylhydrazine.
57, 72). Disruption of the symbiotic relationship between the intestinal microbiota and the host is thought to trigger chronic inflammation. For example, the gut microbiota is a source of antigens for the inflammatory processes associated with IBD (18, 151) and the pathogenesis of enterocolitis and colon cancer in IL-10 knockout mice (114). Recent studies have shown reduced inflammation in IBD through manipulation of the gut microbiota with probiotics (2, 15, 114).

Alterations in the ratio of “harmful” and “beneficial” commensal bacteria contribute to increased mucosal permeability, bacterial translocation, and increased activation of components of the innate and adaptive immune system to promote chronic inflammation (71, 94, 132). Activation of the innate immune system by commensal bacteria (Fig. 1) leads to increased production of proinflammatory cytokines such as IL-12, IL-23, TNF-α, and IFN-γ by macrophages, dendritic cells, and natural killer cells, with subsequent induction of the acquired immune system that includes lymphocytes such as T cells, B cells, and various inflammatory mediators. Bacteria may also be responsible in regulating Th17 cells and IL-17 in the lamina propria (71). For example, the presence of segmented filamentous bacteria was shown to promote the accumulation of Th17 cells and increase expression of inflammatory genes in the intestinal mucosa (5, 70). A major consequence of the inflammatory response to commensal bacteria is the activation of key pro-survival and proliferative signaling pathways by transcription factors such as NF-κB and signal transducer and activator of transcription 3 in epithelial cells (51, 53, 62, 75, 87, 156) as well as generation of reactive oxygen or nitrogen species that leads to oxidative stress, DNA damage, aberrant proliferation, and, ultimately, development of colorectal adenomas and cancer (Fig. 1). Colonization of germ-free animals with Enterococcus faecalis and Bacteroides vulgatus has been observed to induce NF-κB signaling (75, 127) in epithelial cells. Thus, studies to date suggest that disruption of the normal homeostasis between the host and its commensal bacteria community is crucial to inducing inflammation and further downstream changes that promote colon carcinogenesis.

Intestinal Microbiota and Diet

Diet has been extensively studied in relation to colorectal adenomas and cancer. Diet has a major impact on the composition and activity of intestinal bacteria (38, 119). As such, the link between diet and CRC may be explained, in part, by the activities of the intestinal microbiota. The principal components of dietary intake, carbohydrates, protein, and fat, undergo bacterial digestion to generate byproducts, some of which have potential carcinogenic activity (Fig. 2A). Humans lack the enzymes to digest fiber, but bacteria have glycoside hydrolases and polysaccharide lysases that can ferment plant cell wall polysaccharides (147). One of the major functions of bacteria in the colon is fermentation of nondigestible dietary fiber residues and carbohydrates such as resistant starches, pectins, gums, and cellulose. Fermentation leads to generation of short-chain fatty acids (SCFA) such as butyrate, which serves as a

Fig. 1. Mechanisms of gut microbiome to chronic inflammation, adenoma, and colorectal cancer (CRC). The gut microbiome involves several mechanisms to modulate colorectal carcinogenesis. Bacterial dysbiosis weakens the host defense mechanism of barrier function disruption of epithelial layer tight junctions and the mucus film covering. The action causes increased permeability of the intestinal epithelium and favors bacterial translocation through mucosal epithelium (173). Macrophages on recognition of microorganism-associated molecular patterns (MAMPs) expressed by commensal microbiota, through molecular pattern-recognition receptors (TLRs), trigger various signal pathways to produce inflammatory responses (55, 96). Activation of nuclear factor-κB (NF-κB) pathways stimulates the transcription of proinflammatory genes, resulting in increased production of proinflammatory cytokines (TNF, IL-1, IL-23). Proinflammatory cytokines induce signal transducer and activation of transcription 3 (STAT3) and NF-κB signaling, which leads to the suppression of apoptosis and the promotion of cell cycle progression that progress to chronic inflammation and carcinogenic activity (52, 83, 105, 155). During chronic inflammation, there is an overall imbalance in the gut due to production of toxic chemicals or procarcinogens. The production of such chemicals as reactive oxygen species (ROS)/reactive nitrogen species (RNS) from inflammatory cells such as macrophages, as well as bacterial genotoxins (colibactin) and hydrogen sulfide (H2S) from the bacteria, results in oxidative stress which in the target tissue exerts DNA damage and upshoots DNA repair (46, 167). TLR, Toll-like receptor; TH17, T helper 17; Th, T helper. STAT3, signal transducer and activator of transcription 3.
source of energy for colonocytes (121) and demonstrates a protective effect against the development of CRC (22, 36, 124, 125, 148). Butyrate promotes large bowel functions such as modulation of colonic motility, enhanced visceral blood flow, and prevention of potential pathogen overgrowth (19, 54, 85, 89, 158). Butyrate has also been shown to reduce colonic inflammation, induce apoptosis, inhibit tumor cell progression, and protect against development of CRC (19, 54, 60, 85, 117, 136, 177). Increased production of SCFAs significantly lowers the intestinal pH, which promotes colonic fermentation and reduced absorption of carcinogens (89), all of which have a potential to reduce CRC risk (88, 153). Diets rich in fiber and...
complex carbohydrates are thought to have preventive effects against CRC because of the effect of polyphenol metabolites produced by colonic microbiota on cyclooxygenase-2 and glutathione S-transferase 2 expression in colon cells (98), promotion of colonocyte homeostasis, and DNA repair. The chemopreventive effects of butyrate are mediated through induction of p21 (66) as shown in Fig. 2B.

With increased protein ingestion, the colonic residue contains more sulfur, nitrates, ammonia, amines, branched-chain amino acids, and H₂S. Residues from protein digestion can stimulate growth of sulfur-reducing bacteria such as Desulfovibrio and Desulmononas spp. (110). H₂S, an end product of protein metabolism, is proinflammatory and genotoxic (50). Many of the end products of protein catabolism are mutagens and genotoxic and are associated with development of CRC via genetic mutations summarized in Fig. 2C. Increased intake of red or processed meats has generally been shown to be a risk factor for development of colorectal adenomas and cancer (81, 107, 131). Bacteria metabolize meat proteins to produce nitrosamines, known promoters of colon tumors in animal models (31).

Bile acids are byproducts of fat metabolism. Secondary bile acids such as deoxycholic acid (DCA) contribute to increased reactive oxygen species (ROS), DNA damage, genomic instability, and tumor growth (39, 47) (Fig. 2A). Bacteria such as Clostridium spp. convert bile acids into secondary bile acids such as DCA to generate free radicals and induce chronic inflammation, ROS, and CRC (8). The high-fat, low-fiber (low protein) diet in Western countries is thought to increase the risk of colon cancer possibly through increased secondary bile acids and ROS, which induces DNA damage and genomic instability. Consumers of a Western diet rich in fat and protein have a higher proportion of inflammatory 7-α-dehydroxylating bacteria and sulfur-reducing bacteria producing H₂S and secondary bile acids, respectively (50).

Together, studies suggest that byproducts of carbohydrate and fat metabolism may contribute to colorectal carcinogenesis through a variety of mechanisms. Additional studies in humans and animal models are needed to fully understand the mechanisms underlying the interactions of diet, microbiota, and colorectal adenomas and cancer. In particular, studies are needed to characterize the functional aspects of the microbiome. In a recent study Faith et al. (42), employed this approach to evaluate the interrelationship between diet and human gut bacteria. They evaluated germ-free mice colonized with a model bacterial community (10 defined human bacteria phylotypes) and four different diets. They found that certain factors in the diet influenced bacterial community membership. This type of approach will not only help to define underlying mechanisms but also identify metabolic functions, host-microbe, and microbe-microbe interactions as well as develop ways to manipulate the gut microbiota to prevent or treat CRC.

Summary and Future Directions

Until recently, much of our understanding of the complexity and diversity of the microorganisms in the gastrointestinal tract relied on observations from microbiological culture. However, the development of culture-independent molecular methods based on the highly conserved bacterial 16S ribosomal RNA gene and advances in high-throughput sequencing technology have allowed more in-depth probing of the human gut microbiota (58, 82, 93, 149). Several studies have demonstrated an association of bacterial dysbiosis with development of colorectal adenomas and cancer. However, specific causative bacteria have yet to be identified. Microbial signatures of colorectal adenomas and cancer identified in human studies will need to be verified in animal studies using humanized and gnotobiotic mice. Similarly, mouse models can be employed to investigate the potential protective role of specific microbial groups on CRC onset.

A better understanding of the complex interactions between the microbiome and development of CRC will require systems-based approaches that incorporate metagenomics, metabolomics, proteomics, and transcriptomics studies. Such an approach will facilitate the identification of possible interactions between the intestinal microbiota, procarcinogenic factors, and CRC.

Challenges in CRC include identifying at-risk individuals and early detection. While no specific bacteria have been identified as a causative agent for CRC, ascertaining a bacterial signature for development of adenomas and cancer holds great promise for providing novel preventive and therapeutic strategies. It is conceivable that an intestinal bacterial profile might 1) predict an individual’s risk of developing adenomas or CRC and 2) allow manipulation of the microbiota for prevention or treatment. Modulation of the human gut microbiota will likely be accomplished with pre- and probiotics, although other agents may also be beneficial. Large-scale clinical trials will be required to introduce such therapeutic or preventive interventions.

To understand the relationship between intestinal microbiota and development of CRC in humans, epidemiological studies are needed to verify the findings from animal models in humans. Ideally, these studies should be conducted in various populations, with a sufficient sample size that would allow meaningful examination of the contribution of the microbiota to disease after taking into account confounders (e.g., age and gender) and interaction effects with diet. The studies should be prospective to provide information on temporal sequence. Moreover, these studies will require interdisciplinary collaborations with experts in biostatistics, bioinformatics, microbiology, molecular biology epidemiology, and others to analyze and interpret the massive microbial data generated by the high-throughput sequencing technologies.

Conclusions

The human gut microbiome and its role in CRC and other diseases is an active field of research. While initial correlational studies have informed us on the question of: “Who is there?” they also raise questions concerning the functional aspects of the gut microbiota and the exact means by which they influence human disease. These initial studies are likely to give rise to mechanistic studies that will explore host-microbe interactions, microbe-microbe interactions, as well as studies on modulation of the gut microbiota to prevent disease. Continued funding in microbiome research could potentially increase the number of significant discoveries in the microbiome field and provide much needed mechanistic insights that could contribute to disease prevention and treatment.
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