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SUPPLEMENT ARTICLE

The Role of the Gut Microbiome in Cancer: A Review, With Special Focus on Colorectal Neoplasia and *Clostridioides difficile*

Sean M. Anderson and Cynthia L. Sears

Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

The gut microbiome has coevolved with humans to aid in physiologic functions and prevent disease. An increasing prevalence of gut dysbiosis in modern society exists and has strong linkages to multiple disease processes common in the developed world. Mechanisms for microbiome-human interactions that impact host homeostasis include bacterial metabolite/toxin production, biofilm formation with mucous layer infiltration, and host immune system modulation. Most of this crosstalk occurs at the epithelial layer of the gut, and as such the role of these interactions in the induction of colorectal cancer—a highly prevalent disease globally and one undergoing significant epidemiologic shifts—is under increasing scrutiny. Although multiple individual gut bacteria have been hypothesized as possible driver organisms in the oncogenic process, no bacterium has been definitively identified as a causal agent of colorectal cancer, suggesting that host lifestyle factors, microbiome community interactions, and the mucosal and/or systemic immune response may play a critical role in the process. Recent evidence has emerged implicating the ubiquitous human pathogen *Clostridioides difficile* as a possible promoter of colorectal cancer through chronic toxin-mediated cellular changes. Although much remains to be defined regarding the natural history of infections caused by this pathogen and its potential for oncogenesis, it provides a strong model for the role of both individual bacteria and of the gut microbial community as a whole in the development of colorectal cancer.

Keywords. microbiome; cancer; dysbiosis; *Clostridioides difficile*; biofilms.

The communities of bacteria, archaea, fungi, parasites, and viruses colonizing the human body are collectively referred to as the microbiota or microbiome (ie, the living microbes and their gene content, respectively). Functionally, the microbiota, through its virulence factors, cell adhesins, and associated gene products, intimately impacts human physiology, primarily to aid in homeostatic function but also with modulation of local and systemic diseases. The gut contains the highest density and species numbers of bacteria, whereas the skin is a close second [1]. A robust, ever-expanding literature implicates the colonic microbiome as critical to the development of a diverse array of disease processes ranging from local gut disorders (eg, inflammatory bowel disease) to distant pathologies (eg, neuropsychiatric diseases) [2]. In particular, the role of gut dysbiosis in cancer pathogenesis—especially colorectal cancer (CRC)—is considered increasingly important [3], particularly with the emergence of early-onset CRC (EO-CRC, ie, at ages under 45–50 years). In this review, we focus on CRC to introduce key determinants of human gut microbiome composition; whether community structure contributes to colon oncogenesis; and the mechanisms by which individual gut bacteria may sway the balance between health and disease. We close by discussing new evidence for *Clostridioides difficile* (*C. difficile*) association with human CRC.

BROAD LINKAGES CONNECTING THE MICROBIOME and CRC

Microbiome Composition and Dysbiosis

The composition of an individual's microbiome is influenced by many factors. Dietary patterns and social structures (eg, agricultural vs cosmopolitan communities) markedly influence gut microbial signatures [4]. Prior to the advent of modern society, infrequent shifts in lifestyle factors appeared to allow for coevolution on both sides of this interaction that grew into an increasingly dependent symbiosis (ie, an intimate interaction between two distinct organisms, as vs commensals that do not significantly interact with the host). Namely, humans grew dependent on the gene repertoire of their symbionts to fulfill certain physiologic functions (eg, mucosal barrier integrity, food digestion, and immunologic development) and prevent disease processes

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Correspondence: C. L. Sears, Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, 1550 Orleans St, CRB2 Bldg, Ste 1M.05, Baltimore, MD 21231 (csears@jhmi.edu); S. M. Anderson, Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, 1550 Orleans St, CRB2 Bldg, Ste 1M.05, Baltimore, MD 21231 (sande145@jh.edu).

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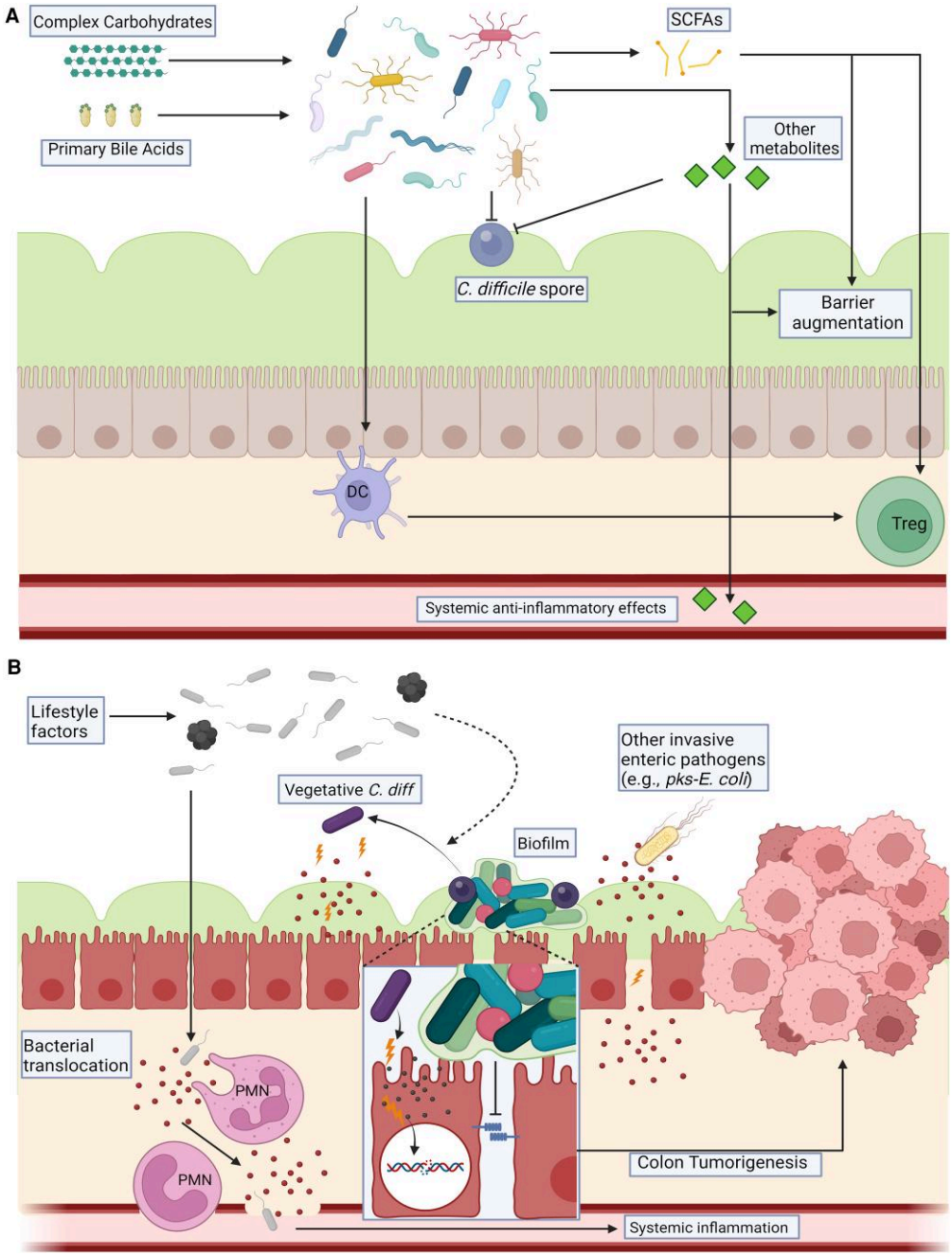

Figure 1. The Microbiome's role in cancer suppression (*A*) and development (*B*). *A*, A "healthy" gut microbiome is characterized by high species richness and a diverse gene and gene product pool. It is considered to be associated with consumption of a diet high in complex carbohydrates and a lifestyle devoid of high-risk behaviors. In this setting, we propose the microbiome suppresses cancer development through mechanisms such as digestion of complex carbohydrates and primary bile acids to create SCFAs that augment the mucosal barrier and may directly inhibit the growth of cancerous cells. SCFAs and other metabolites produced by healthy microbiomes also can stimulate an anti-inflammatory mucosal immune response (including Treg development partially mediated through interactions with dendritic cells [DC]). Direct competition by endogenous symbionts for nutrients helps protect against invading pro-inflammatory pathogens (eg, *C. difficile*, shown here in dormant spore phase restricted to the outer mucous layer). Recent data further suggest that the systemic release of certain small molecules synthesized or modulated by gut microbes can serve anti-neoplastic effects at sites distant from the gut. *B*, In hosts displaying chronic dysbiosis due to lifestyle factors and/or other local or systemic diseases, the species richness and gene pool of the gut microbiome is typically reduced. In these settings, the mucosal barrier is predicted to be more fragile and susceptible to specific pathogen impacts and/or invasion due to release of toxins or other virulence factors (in addition to a relatively reduced production of anti-inflammatory small molecules such as SCFAs) that can contribute to chronic clinical or subclinical intestinal inflammation and further barrier breakdown, predisposing to persistent bacterial translocation. These changes result in the production of pro-inflammatory cytokines and neutrophil (PMN) recruitment, further effecting a cycle of inflammation. A dysbiotic gut microbial community also enables the germination of *C. difficile* leading to toxin production and mucosal damage (see details in text). Alternatively, microbiota communities such as bacterial biofilms that invade the normally sterile inner mucus layer may activate signaling cascades to additionally impair barrier function and promote chronic mucosal inflammation. These cumulative inflammatory stressors are one potential trigger for colonic stem cell DNA mutation that can foster the development of CRC. The figures in (*A*) and (*B*) were created using a paid subscription to BioRender. Abbreviations: *C. difficile*, *Clostridioides difficile*; CRC, colorectal cancer; *pks-E. coli*, *Escherichia coli*; SCFA, short chain fatty acids; Treg, regulatory T-cell.

(eg, infectious diarrhea or auto-inflammation) (Figure 1*A*) [5]. However, this delicate balance has been immensely disrupted by improved hygiene, antimicrobial medications, and dietary changes that occurred over the past 2 centuries, leading to marked, persistent shifts in gut microbiome composition especially amongst urban populations [4]. Although the implications of these changes still require exploration, the observation that mode of delivery (ie, vaginal vs Caesarian section) impacts the infant microbiome and may be associated with early onset of atopic diseases in childhood is only one example of how microbial context may result in human pathology in a short timeframe [6].

The definition of a "healthy microbiome" and, in turn, "dysbiosis' remain poorly delineated, as functional redundancy allows for astounding inter-individual variability in microbiome composition to accomplish the same physiologic functions. However, there are emerging descriptions of microbiome signatures somewhat consistently associated with specific disease processes, including CRC [7, 8]. The epidemiologic factors associated with sporadic CRC include lifestyle parameters such as obesity, diabetes, a Western diet, and alcohol and tobacco use [9], each alone known to disrupt the microbiota, adding strength to the thesis that the colon microbiota is a crucial environmental risk factor for the development of CRC [10]. Medications, particularly antibiotics, are potent microbiome disrupters with associations now drawn between the quantity and type of oral antibiotic exposure and the development of colonic polyps [11] and colon cancer [12], observations supporting the concept that colon oncogenesis is, at least in part, mediated by disturbances in microbial community structure [13].

Bacteria-Human Interface and Colon Oncogenesis

The mechanisms of microbial impacts on human physiologic function are many. There exists strong evidence demonstrating a causative effect by individual microbes in the induction of human cancers; these organisms range from helminths such as *Schistosoma haematobium* [14] to viruses such as human papillomavirus [15], and they accomplish oncogenesis in their respective tissues (bladder and cervical or head and neck cancer, respectively) through a wide variety of active and passive mechanisms. In contrast, beyond *Helicobacter pylori* [16], attempts to definitively implicate gut bacteria in the development of cancer have been less successful, likely in large part due to the sheer number of species, metabolites, and interdependent ecosystems that surround these organisms and confound both controlled experimentation and human studies. These rich communities engage in constant communication amongst themselves and with nearby human cells. Although some microbe:host interactions exhibit far-reaching effects within the human body (eg, trimethylamine [TMA] production in the colon and cardiovascular disease) [17], the majority of crosstalk occurs within the gut. As such, the colon epithelial barrier represents the first line of defense against colon microbiome disruption.

Sporadic CRC pathogenesis including EO-CRC involves slow accumulation of genetic changes in colon epithelial cells (CECs) mediated by repeated, and likely heterogeneous, environmental exposures [9]. Microbial toxins and metabolites that directly damage CEC DNA, bypass cellular controls to activate oncogenic pathways, and/or induce a robust host mucosal immune response likely serve to directly or indirectly contribute to the initiation and/or progression of colon cancer (Figure 1*B*). Several prominent microecological theories exist regarding the hypothesized community-level mechanisms of colonic tumorigenesis, ranging from the driver-passenger model of more long-term colonization effects to the hit-and-run model of transient impacts that leave permanent genetic changes in CECs [10]. The general concept in many of these models is that, beyond an "instigator" bacterium, microbial community enables "bad actor" bacteria to effect critical human cell changes that lead to the development of disease processes such as CRC.

Despite a preponderance of preclinical evidence supporting these models in colon tumor development, no individual microbes are yet proven as causative in CRC [10]. This underscores the likely importance of community and abiotic factors in the oncogenic process. Nonetheless, understanding the mechanisms by which specific bacteria may contribute to pre-cancerous changes in vitro has potential to inform the development of more efficient screening or effective therapeutics. We will next discuss mechanisms utilized by select bacteria hypothesized to foster colon oncogenesis and will also explore the potential for certain gut bacteria to aid the host in its campaign to counteract cancer development and progression, both colonic and otherwise.

INDIVIDUAL AND COMMUNITY BACTERIAL IMPACTS ON CANCER

Individual Bacterial Implicated in Colon Oncogenesis

Although ubiquitous lifestyle factors contributing to dysbiosis and CRC development may be slowly beginning mitigation in Western society [18], approaches to intervene and disrupt the contributions of individual oncogenic gut bacteria are unknown. Linkages between CRC and specific bacteria trace back decades to the initial recognition that endovascular infection with *Streptococcus bovis* type I (now *Streptococcus gallolyticus*) demanded colonoscopy to rule out colon neoplasia [19]. For some time, this and other similar bacterial associations were felt to represent more of a "bystander" effect as opposed to a causal one. However, strong evidence is mounting that individual bacteria (including potentially *Streptococcus gallolyticus* [20]) may be active in the oncogenic process. A well-studied example is *Bacteroides fragilis*, a commensal microbe with 2 molecular subgroups, nontoxigenic (NTBF) and toxigenic (ETBF). The latter strains harbor a pathogenicity island and produce a

metalloprotease toxin (*B. fragilis* toxin [BFT]) that is required to induce colonic inflammation and colon tumorigenesis in mouse models through interleukin (IL)-17-dependent and other mechanisms [21]. ETBF is identified at a disproportionally high level in patients with CRC when compared to controls [10]. Similarly, the acquisition of a pathogenicity island (termed the *pks* island) by strains of another commensal organism, *Escherichia coli* (*pks-E. coli*), also drives tumorigenesis in mouse models through DNA-damaging secondary metabolite (colibactin) production [22] and has strong human CRC linkages [10].

Outside of acquired virulence factors, individual bacteria can utilize other mechanisms to cause pro-inflammatory mucosal changes. Some strains of *Enterococcus faecalis* produce extracellular superoxide that, in certain contexts, leads to genomic instability in nearby human cells [23, 24] and microenvironmental changes favoring "blooms" of additional inflammatory bacteria such as *Ruminococcus gnavus*, a bug implicated in flares of inflammatory bowel disease (IBD) [25]. More recently, high-throughput techniques revealed detection of microbial metabolites such as small-molecule genotoxins called indolimines, produced by CRC-associated bacteria like *Morganella morganii*. These bacterial genotoxins may contribute to direct colonocyte DNA damage and increased intestinal inflammation, known precursors to CRC formation [26]. Both proteomics and metabolomics of colon bacteria involve a staggering amount of data analysis and, as such, the field remains in its infancy but with significant potential for paradigm-shifting discoveries. This list is not comprehensive; detailed and updated reviews of individual bacteria implicated in colon oncogenesis are catalogued elsewhere [10].

Community Structure and Communication

Although the activities of individual bacteria are anticipated to be important in the pathogenesis of CRC, the role of community structure and environmental factors in enabling bacterial behavior may be just as crucial. It is now recognized that at least 50% of human CRC are covered with mucus-invasive bacterial biofilms, bringing dense polymicrobial communities in direct CEC contact in both the cancers and normal tissue [7, 27]. Bacterial biofilms on normal tissue diminish barrier function and induce inflammatory signals and Stat3 activation that contribute to the onset of pro-carcinogenic IL-17 mucosal inflammation [27]. Diminished barrier function is considered an early step in colon carcinogenesis that is permissive to bacterial translocation and activation of innate immune receptors creating a positive feedback loop to further inflammatory cell infiltration as well as dysbiosis [28]. Which communities consistently foster pro-carcinogenesis versus anti-carcinogenesis is unclear. However, increasing attention is focused on the metabolic output of both individual bacteria and of communities in colon in systemic disease pathogenesis linked to the microbiota. For example, early analysis identified N_1, N_{12}-diacetylspermine, a polyamine product, as associated with colon biofilm formation and cancer with contributions by both the bacterial biofilm and host cells [29]. However, further investigation is needed to, for example, contrast biofilm community function on normal tissue versus CRC. Such analyses might provide insight into pro-carcinogenic microbial community functions that could assist in biomarker development for CRC prevention.

Quorum sensing (QS) is a likely mechanism governing both biofilm and fecal microbial community function in this context. QS refers to complex molecular circuits that mediate communication between bacteria within the same community but not necessarily in physical contact with one another. QS acts via small-molecule autoinducers that diffuse through the cell membranes of other bacteria—frequently, but not exclusively, of the same species—and can influence gene expression designed to enhance microbial fitness, increase virulence factor production, and/or otherwise modify function [30, 31]. Additional mechanisms by which bacteria influence bacterial and host cell functions are extracellular vesicles [32] and miRNA production [33].

Anti-Carcinogenic Activity of Gut Bacteria

Although much focus has been on identifying microbes contributing to cancer development risk, the human microbiome has immense genetic reservoir that has been toned over millennia to coexist with and/or facilitate human development and physiology. Even with recent drastic shifts in human gut microbiome composition, there undoubtedly remains significant potential in gut microbial communities to buffer against disease development. For example, most data available on bacterial metabolites such as short-chain fatty acids (SCFAs) suggest these small molecules largely reinforce the colon mucosal epithelial barrier and act to limit colonic/systemic inflammation and cancer risk. Specifically, the SCFA butyrate, the key substrate for epithelial cell energy and present in micromolar concentrations in human blood, is synthesized by commensal microbes as a product of dietary complex carbohydrate metabolism (eg, fiber) with the capacity to inhibit the proliferation of colorectal cancer cells [34] and induce cancer cell apoptosis [35]. Thus, dietary approaches to augment intracolonic/systemic SCFAs may impact cancer biology, including both CRC [35] and melanoma [36].

The colon microbiome may also promote anti-tumor effects outside of its local environment, especially through modulation of the immune response. A recent study [37] demonstrated that the production of trimethylamine by colon microbes with systemic release of the metabolite trimethylamine *N*-oxide (TMAO) potentiated the response to immune checkpoint inhibitor (ICI) therapy in patients with pancreatic ductal adenocarcinoma through the induction of type I interferon pathways and the subsequent activation of effector T-cell responses within the tumor microenvironment (TiME). Similarly, the

translocation and subsequent tumor microenvironmental habitation of gut-derived *Lactobacillus reuteri* in melanoma patients was recently described [38]; this microbe released a dietary tryptophan catabolite into the TiME, leading to the stimulation of interferon-γ-producing CD8 T cells, response to immune checkpoint inhibitor therapy, and increased survival in advanced melanoma patients.

The potential for linkages between specific bacteria, bacterial products and impacts on cancer, colorectal or otherwise, is vast. Teasing apart the relative contributions that individual bacteria have to cancer development or other disease processes versus the role of that bacterium's local environment and community effects is challenging. Nonetheless, it is anticipated that these types of investigations will inform our understanding of disease genesis with the potential of identifying microbes, pathways, and molecules to guide new prevention and therapy approaches.

INVESTIGATING THE MICROBIOME: *CLOSTRIDIOIDES DIFFICILE* AS A MODEL BACTERIUM FOR DISCERNING MICROBIOME-CANCER INTERACTIONS

C. difficile and Colorectal Cancer

C. difficile is a gram-positive, spore-forming anaerobic bacterium that colonizes the large intestine. Toxigenic strains release protein exotoxins (TcdA, TcdB) that induce local inflammation leading to the clinical signs of diarrhea and/or colitis in susceptible individuals [39]. Its hardy spores are immensely difficult to eradicate in the colon as evidenced by disease recurrence rates ranging from 20% to 65% even after current standard-of-care treatments [40]. Since the early 2000s, there has been a dramatic increase in rates of severe *C. difficile* infection [40], as well as community-acquired infection [41]. This trend is not limited to the developed world, as comparable or even elevated prevalence rates have been reported in epidemiologic studies of countries such as India [42] and Indonesia [43].

Along roughly the same time period as this rise in *C. difficile* cases, the global epidemiology of CRC has undergone significant shifts. Specifically, the incidence of CRC has been increasing in individuals under the age of 50 since the early 1990s [44] as well as in areas of the world previously considered to have low CRC risk [45]. This is especially true for Asian countries, which now account for nearly 50% of the worldwide CRC mortality as they have increasingly adopted a "Westernized" lifestyle [46]. Despite these parallel trends in evolving epidemiology and the data supporting that antibiotic exposure is a risk factor for both diseases, no strong mechanistic link between *C. difficile* infection and CRC had been drawn prior to a recent study [3].

In this study, the slurry or polymicrobial cultures of biofilm-positive Stage 1 colon cancers induced colon tumors in a murine model. Notably, removal of *C. difficile* from the cultured bacterial community demonstrated that *C. difficile* was both necessary and sufficient to convert the bacterial community from non-tumorigenic to tumorigenic, raising the specter that *C. difficile* could act, at least in a mouse model, as a colon pro-carcinogenic bacterium. In fact, 5 of 6 colon cancers evaluated in the study were positive for *C. difficile*, and 2 were toxigenic, tumor-inducing strains. Important nuances included the fact that prolonged mucosal colonization with exposure to TcdB (but not TcdA) appeared necessary for colon tumor induction; pro-carcinogenic *C. difficile* strains were recovered from both biofilm-positive and biofilm-negative CRCs; and limited *C. difficile* abundance was tumor-inducing (alluding to a possible keystone role in gut ecology [10]). At present, human data on longitudinal colonization in stool or the mucosa with *C. difficile* strains are lacking. In addition, *C. difficile* is genetically a very pleomorphic bacterium [47], and preliminary data suggest that not all *C. difficile* strains possess procarcinogenic properties. In consideration of this possibility, understanding the "life cycle" of *C. difficile* has merit.

C. difficile Microbiology and Microecology

C. difficile lives a complex, binary life cycle that remains suboptimally understood; a variety of environmental stimuli induce genetic regulatory factors that force switches between the incredibly durable spore phase and a metabolically active vegetative phase, the latter of which is capable of the toxin production necessary for human disease [48]. Although much remains to be defined regarding the inducers and repressors of the *C. difficile* life cycle, local environmental factors likely play a key role. The cascade of sporulation, which transforms the bacterium into the aerotolerant, transmissible form, appears driven by stimuli such as nutrient depletion and QS as it is in other anaerobic gram-positive bacteria [49, 50]. The spore phase "dormant" state is highly resistant to elimination due to its low metabolic activity. A germination phase transforming the bacterium into a more active vegetative form completes the life cycle. In the case of *C. difficile*, it is thought that the key germinants are primary bile acids produced by the host [48]. Interestingly, secondary bile acids synthesized by other commensal gut bacteria inhibit the germination of *C. difficile* and may yield colonization resistance against this bacterium [51], providing one of the main mechanistic hypotheses regarding how the endogenous microbiota protects against *C. difficile* infection.

Germination is a critical step required for the synthesis of the toxins crucial to human disease and, potentially, CRC promotion [3]. A recent study [52] suggests that toxin production may be designed to induce host inflammation to alter the bacterium's nutrient landscape through targeted degradation of collagen and other connective tissue components; this shredding of the extracellular matrix and the obligatory severe host inflammatory response that follows may liberate key nutrients

to sustain long-term *C. difficile* colonization and toxin production while also selecting against certain competitor species, such as members of the Bacteroidaceae family. Host inflammation may further impact toxin production by *C. difficile*, as instability in iron balance, common in inflammatory conditions, can impact the bacterium's synthesis of toxins [53] and augment its defense against host antimicrobial compounds [54]. QS also appears to be an important variable in toxin production, as autoinducers and toxins are synthesized during the same window of the *C. difficile* life cycle [31]. Although biofilms are a feature of many CRCs (discussed above), a study of *C. difficile*-dominant biofilms suggested a predominance of highly resistant spores with reduced germination potential and relatively low toxin production, overall projecting a "hunker-down" as opposed to a symptomatic disease phenotype [55]. However, based on Drewes, Chen et al [3]., we would hypothesize that low level, persistent TcdB release in proximity to CECs, whether within biofilms or not, is critical to colon neoplasia development in this context.

Gut Microbial Evolution and Niche-Seeking Behavior

Establishment of *C. difficile* colonization in the host colon appears to require particular microecological criteria—potentially including but not limited to macro- and micro-nutrient disturbances, host primary bile acid synthesis, an absence of secondary bile acid production by commensal gut microbes, and an appropriate autoinducer milieu—to facilitate germination and the subsequent toxin production critical for human disease and, possibly, the induction of colonic neoplasia. In line with ubiquitous clinical observations, depletion of the endogenous microbiota and stimulation of colon mucosal inflammation are the triggers for symptomatic *C. difficile* infection. To this end, the idea that toxigenic *C. difficile* may be obligately pathogenic in human hosts to support its life style [52] is intriguing. It is interesting to consider whether this hypothesis may extend to all other gut bacteria suspected to play a role in CRC development, such as ETBF and *pks-E. coli*.

CRC development is a chronic process—estimated ≥10 years from onset of neoplastic clonal expansion in mutated CECs to visible neoplasia observable at colonoscopy [56]. At present, little is known about the durability of fecal or mucosal colonic *C. difficile* colonization and the mechanisms that may promote chronic pathogenicity. A recent publication described a model of divergent within-host evolution of certain gut bacteria (ie, *Enterococcus gallinarum* and *Lactobacillus reuteri*) to adapt to fill distinct microecological niches within the host gastrointestinal tract [57]. In a murine model, isolates evolved into 2 broad, coexisting phenotypes, mucosal and luminal; the former developed a heightened ability to translocate through the gut wall, induce hepatoenteric inflammation, and evade immune clearance when compared with its counterpart, hypothesized to be equipped for long-term colonization and higher transmission potential. This identification of apparent targeted, time-dependent functional advantages achieved through within-host evolution suggests that bacterial phenotype may be strongly influenced by the opening of ecologic niches within the host, a finding with potential consequences for other enteric bacteria capable of developing pathogenicity. Importantly, the data suggested that the mucosal strain phenotype was also influenced by host genetics and the neighboring microbiome.

Although within-host evolution of *C difficile* remains to be investigated, studies of the broad evolutionary signatures on its genome suggest that it is designed for survival within gut niches and thus is primed for long-term co-habitation within a host [47], although notably it is unclear whether the forces of selection on its genome imply an obligately pathogenic relationship [58]. Additionally, the genetic architecture of *C. difficile's* QS circuit(s) and its ability to uptake genetic information readily from neighboring organisms may be important factors in its toxin production capability and virulence evolution potential, suggesting a predilection for prolonged community interactions in settings such as the microbiome [59].

Pursuing the *C. difficile* Pro-carcinogenesis Hypothesis

Murine model data support that persistent colonization and production of TcdB in the colon lumen are required for *C. difficile* procarcinogenesis. However, critical gaps in knowledge exist including a dearth of information regarding persistent human *C. difficile* fecal or mucosal colonization, the duration of colon TcdB production, the impact of differing *C. difficile* toxinotypes, and whether persistent colon mucosal inflammation (a critical contributor to oncogenesis) occurs. Prior studies suggest that asymptomatic *C difficile* colonization may occur in 5%–15% of community adults [60], but linkages to inflammation and/or toxin production are unknown. A competent adaptive immune response to TcdA and TcdB through antibody production seems important to diminish the likelihood of clinical disease but does little to protect against colonization, suggesting that a relative loss of humoral immunity may impact bacterial toxin production and the inflammation that results [61]. Key steps to study the oncogenic potential of *C. difficile* will involve detailing epidemiological links between *C. difficile* infection and the rates of colonic neoplasia in humans, as well as determining the frequency of and factors contributing to persistent colonization and toxin production in certain individuals. In addition, mapping the within-host evolution of *C. difficile* in humans deemed persistent carriers may help shed light on how intimate the associations between colonization, toxin production, gut inflammation, and the immune response may be.

CONCLUDING THOUGHTS AND FUTURE DIRECTIONS

As yet, unlike the causal role of *Helicobacter pylori* in gastric cancer [16], no causal linkages between gut bacteria and the

development of human CRC are proven. However, there is a preponderance of evidence connecting disruptions in microbiome community structure and function with the induction of an oncogenic environment at the crucial interface of the microbe-human crosstalk, the epithelial layer of the colon. Possible mechanistic explanations for these findings, both at the level of bacterial pathogenicity and community dysfunction, are rapidly emerging. Despite this, the potential for the gut microbiome to buffer against and even reverse the development of neoplasia, within the colon or distant from it, remains a tantalizing area for future research; after all, only a small number of colon polyps progress to CRC [56]. Although many different individual bacteria have been implicated as potential promoters in the colon oncogenic process, few have the clinical ubiquity and resilience of toxigenic *C. difficile*, a bacterium whose evolving epidemiology, at least in part, mirrors that of the epidemic of EO-CRC. Further studies aimed at defining the true burden and persistence of *C. difficile* following treatment in humans; identifying and manipulating the ecological factors contributing to this bacterium's ability to proliferate and produce pathogenic toxins; and identifying approaches to either durably eliminate and/or neutralize the bacterial-mediated induction of colonic inflammation may inform the approach to understanding colon bacterial oncogenesis with resounding consequences for unraveling microbiota-cancer connections. More importantly, as microbiota-associated disease mechanisms emerge, clinically relevant approaches to disease prevention and therapy will be introduced. There are now two FDA-approved targeted microbiota therapies (Rebiota® and Vowst®) based on studies of *C. difficile* disease with many additional products in development. Although the field of microbiota-host interactions is still young, clinicians can anticipate the introduction of microbiota biomarkers for disease detection and monitoring and microbiota-modifying approaches to assist in disease prevention and therapy.

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