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Lower Gastrointestinal Cancers (AB Benson, Section Editor)

The Microbiome: the Link to Colorectal Cancer and Research Opportunities

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Opinion statement

In recent years, we have seen an increase in the study and interest of the role of the microbiome in the development of malignancies, their progression, and evasion of therapies. This has been particularly fruitful in the case of colorectal cancer; multiple investigators have described correlative observations as well as hypotheses strengthened in preclinical studies that have begun to elucidate the critical role the gut and tumoral microbiome plays in carcinogenesis. Furthermore, these landmark studies lay the groundwork in describing the microbiome's role in carcinogenesis and provide a rich field of future study. Here, we review contemporary understandings of these observations and proposed mechanisms behind them.

Introduction

Harboring 70% of the bacterial load of the gut microbiome, the colon hosts approximately 10^{14} bacteria comprising 10^{3} different species [1]. Bacteria interact with local host tissues and the systemic immune system in a complex system crucial to normal gut physiology, global immunologic, and metabolic functions. Baseline

physiologic gut microbiota typically varies along the gastrointestinal tract due to multiple factors including transit time, enzymatic activity, pH, and fermentation of luminal contents. The most abundant phyla of bacteria have been variously reported to be Bacteroides, Firmicutes, Proteobacteria, Actinobacteria, and Verrucomicrobia [2, 3]. Given the high variability in luminal bacterial communities, it is hypothesized that a homeostatic equilibrium exists between host and gut microbiota that supports health, and perturbations of this equilibrium can elicit local gut injury and alterations in the host immune status. To date, clear markers of a "healthy" gut microbiome remain elusive.

Disruptions in the composition and function of the native microbiota, referred to as dysbiosis, have been implicated in the pathogenesis of a variety of local and systemic inflammatory and autoimmune disorders [4–7]. Over the past decade, evidence has increasingly linked gut dysbiosis to the development and progression of cancer. To date, the gut microbiome has been shown to affect carcinogenesis via direct metabolic effects, systemic immune modulation, and modulation of the tumor immune microenvironment (TIME), both in gastrointestinal and non-GI cancers [5, 8]. New evidence suggests the gut microbiota participates in modulating response to both immune and cytotoxic cancer therapeutics [5, 9–12], opening the door for gut microbial modulation as a promising anti-cancer intervention in combination with existing therapeutics. These observations and proposed mechanisms are especially prescient in the case of colorectal cancer (CRC) given its proximity to the gut microbiome and early supported mechanistic hypotheses behind carcinogenesis, evasion of therapeutics, and progression.

Currently, colorectal cancer (CRC) is the 2nd leading cause of cancer-related death in the USA and worldwide with an increasing incidence, especially in younger patient populations [13]. The development of colonic adenocarcinoma is multifactorial, with contributions from genetic, immunologic, and environmental factors. These tumors represent a heterogenous group of cancers classically presented as four subtypes as defined by their canonical molecular subtype (CMS) [14]. These subtypes, initially identified by transcriptomic differences, are also associated with unique immune cell infiltrates. Beyond subclassifying of tumors themselves, there is an urgent need for more in depth understanding of tumor biology and interactions between the tumor microenvironment and global host response. While CMS classification greatly improves understanding of disease biology, correlations with subtype, outcome, and TIME remain incompletely understood.

Tumor immune infiltrates, which correlate with CMS, have also been shown to correlate with gut microbial and tumor microbial signatures. These associations are in part linked by a patient's "exposome," consisting of environmental factors such as smoking, diet, and antibiotic use, which trigger composition changes of the microbiome, pro-inflammatory pathways, and perturbations of the TIME [15, 16]. The gut microbiome plays an important role as part of and reflecting the broader exposome [17]. The mucosal interface between the colon and this exposome relies on an intricate balance of homeostatic processes. Irregularities in inflammation, immune response, gut microbiome, and host epithelial barrier can potentially result in a tumorigenic cascade. Recent evidence highlights the gut microbiotas' interplay with this barrier function and subsequent immune infiltrates within tumors. Opportunities now exist to test clinical applications in prevention, detection, and management of CRC. Here, we review the current literature behind these associations and opportunities to leverage these results to novel therapeutics.

Gut dysbiosis and colorectal cancer carcinogenesis

Studies have aimed to identify specific bacterial taxa and their metabolites responsible for colonic mucosal injury and tumorigenesis. CRC patients have significantly lower stool bacterial diversity [18] and the fecal microbiota is predominated by pro-carcinogenic species including *Fusobacterium*, *Bacteroides fragilis*, and *Escherichia* [19–21]. Not only are there compositional differences in the gut microbiota of CRC patients, but stage-specific analyses of the gut

microbiome of patients with colorectal adenomas and carcinomas have demonstrated shifts in the relative abundance of taxa between progressive stages of tumor development [22, 23]. This suggests the mechanistic influence of these bacterial communities is dynamic and intricate. In this analysis, *Fusobacterium nucleatum* spp., a predominantly oral symbiotic species, were found to be progressively more abundant from precursor to late stages; meanwhile, *Atopobium parvulum* and *Actinomyces odontolyticus* were only elevated in early stages [22•]. In addition to the hypothesis generating pathologic implications of these findings, this also demonstrates a potential clinical role of species abundance as a biomarker for disease and progression.

A causal role of dysbiosis on CRC development and progression has been demonstrated in murine models through the administration of stool from CRC patients leading to higher rates of high-grade dysplasia and polyp formation [24]. Its impact on the host immune status was evidenced by upregulation of inflammatory pathways and intestinal recruitment of T helper cells. Furthermore, in animal models with induced colorectal carcinogenesis, germ-free (GF) rats grew fewer and smaller tumors when compared to rats with conventional gut microbiota. The interplay between gut dysbiosis and the host mucosa is multifaceted, but suspected mechanisms include genotoxin-driven DNA injury, inflammation from digestion-derived metabolites, and immunity dysregulation [25], which in combination, tip the scales towards a colonic environment favoring dysplastic phenotypes.

To better understand a potentially causal link between the presence of specific bacterial taxa and CRC oncogenesis, metabolomic studies have demonstrated pro-tumorigenic or anti-tumorigenic environments of the colonic mucosa can largely be described by specific bacterial metabolomic signatures. Specific food habits correlate with gut microbiota signatures, and the resulting microbial byproducts can impact the host immune system [26, 27]. This provides a potential mechanistic link with observational data that has long supported associations of CRC with certain dietary factors including red and processed meats [28, 29], and multiple toxins generated from microbial metabolism.

Bacterial production of the short chain fatty acid, butyrate, provides the primary energy source for colonocytes and has been shown to demonstrate anti-inflammatory and immunomodulatory properties. Butyrate can inhibit release of inflammatory cytokines and drive the differentiation of regulatory T cells in vitro and in vivo, creating an anti-inflammatory local environment [30]. Fiber-fermenting bacteria, including *Fusobacterium nucleatum*, can be butyrate producers, and may be involved in normal gut homeostasis. While butyrate is generally considered anti-tumorigenic, given its high levels in at risk populations (African-Americans) and low levels in low risk populations (Native Americans) [29], in vitro studies have demonstrated butyrate's ability to drive colonic epithelial proliferation [31], suggesting the need for homeostasis within the host, bacteria, and metabolite interaction.

Beyond metabolomic interactions, the bacterial driver-passenger theory helps to describe the series of microbial events that drive colorectal carcinogenesis. In this model, colonization of the colonic mucosa by pathogenic bacterial species results in epithelial DNA damage, leading to driver

mutations initiating carcinogenesis [32]. Activation of inflammatory pathways allows opportunistic passenger bacteria to proliferate, outcompete native species, and support disease progression, resulting in a remodeled, dysregulated TIME [33]. The result is a milieu of chronic inflammation, immune dysregulation, and microbial dysbiosis, leading to an accumulation of mutations and tumor progression.

Two microbial oncogenic drivers of CRC are enterotoxigenic *Bacteroides fragilis* and colibactin-producing *Escherichia coli* [34, 35]. *B. fragilis*' enterotoxin has been shown to directly damage DNA strands through the production of reactive oxidative species. This further stimulates a cascade, characterized by T_H17 recruitment, IL-17 production, and NF-κB signaling, creating a pro-inflammatory setting featuring immature myeloid cells [34]. The *B. fragilis* enterotoxin also compromises the protective mucus layer of the colonic epithelium, allowing adhesion of pathogenic opportunistic bacteria [36]. Similarly, colibactin production by *E. coli* is directly genotoxic and has been shown to cause chromosomal instability in murine models [37]. Similar findings have been described across numerous bacterial species (Table 1). Initial work by Cougnoux et al. demonstrated potential therapeutic strategies targeting bacterial genotoxins. Utilizing an inhibitory molecule to the bacterial colibactin-producing enzyme resulted in suppression of DNA damage and cell proliferation in CRC murine models [38].

Chronic inflammation is a common and well-known driver of CRC development. Murine models of colonic epithelial injury have shown that bacterial byproducts and toxins drive IL-23 and IL-17 pathways that lead to tumorigenesis [39]. Similarly, colonic microbiota have been linked to tumorigenesis via activation of Th17 cell responses [40]. Ultimately, gut dysbiosis as well as specific bacterial pathogens has been correlated with intestinal inflammation and epithelial injury that results in gut permeability and immune activation, leading to the chronic inflammation, proliferation, invasion, and cancer development. Given the complexity of these systems,

Table 1. Bacterial taxonomies and proposed mechanisms of carcinogenesis

Species	Mechanism of action	Reference
Bacteroides fragilis	Enterotoxin induces epithelial injury Stimulates NF-KB signaling, increases myeloid cell infiltration	[34, 36]
Escherichia coli	B2- colibactin induced DNA injury cytolethal distending toxin induces dsDNA breaks	[37, 41]
Enterococcus faecalis	DNA oxidative injury from reactive oxygen species	[42]
Fusobacterium nucleatum	Virulent adhesin, Fap2, used for invasion; stimulates wnt/B-catenin signaling Activates NF-KB pathway and drives myeloid cell tumor infiltration	[43–45]
Helicobacter pylori	Increased colonization in CRC CagA, a cytotoxin, induces inflammatory pathways in gastric cancer and may participate in CRC tumorogenesis	[46]
Peptostreptococcus anaerobius	Activates Toll-like receptor pathways in mouse models; increased abundance in human CRC	[47]
Streptococcus bovis/gallolyticus	Colonic upregulation of angiogenic cytokines (IL-8); free radical formation	[48]

specific mechanisms have been proposed but a unifying cause has remained elusive.

Hypotheses of carcinogenesis and progression

Fusobacterium nucleatum is seen in high abundance in the gut microbiome of colorectal cancer patients and has been associated with chemoresistance in CRC [22, 49]. *F. nucleatum* within colorectal tumor itself appears to be critical to tumor development and progression [18, 50]. Multiple hypotheses have been suggested for the mechanism of *F. nucleatum* tumorigenesis, suggesting this is likely a multifactorial effect. This microbe utilizes a virulent adhesin, Fap2, which permits epithelial adhesion to a CRC polysaccharide and invasion [43]. This generates a FadA adhesion complex that stimulates wnt/β-catenin signaling and oncogenic transcription profiles [43, 44]. This mechanism has been established in preclinical murine models, providing mice *F. nucleatum* results in inflammatory and TIME alterations mimicking those seen in *F.nucleatum* associated CRC [50, 51]. Similarly, colorectal cell lines incubated with *F. nucleatum* and injected into mice result in xenograft tumor growth increased in size and rate compared to controls [44, 52]. Among colorectal cancer patients, a high *F. nucleatum* level corresponds with predominantly right sided cancers and a decreased cancer-specific survival when compared to those with low or undetectable levels of *F. nucleatum* [49, 53, 54].

In addition to its role in tumor development, recent evidence has suggested that the microbiota, specifically *F. nucleatum*, is involved in tumor metastasis. Studies have demonstrated the presence of *F. nucleatum* in lymph nodes and liver metastases from *F. nucleatum*-positive primary colorectal tumors, suggesting bacteria travel with cancer cells [55, 56]. In murine models, antibiotic treatment resulted in decreased *F. nucleatum* loads, as well as cancer cell proliferation and tumor cell growth [55]. These results highlight a link between the development of CRC metastases and the tumor microbiome, as well as the potential for microbiota modulation as a treatment strategy for CRC.

Potential mechanisms for the microbiota's involvement in metastasis involve injury to the gut vascular barrier (GVB) and the development of the pre-metastatic niche (PMN) within sites of metastatic spread. Enteric microbes can invade the mucosal barrier and directly injure the GVB, allowing circulatory access for pathogens [57]. Once in circulation, microbes can induce environments rich in innate immune cells and pro-inflammatory signaling, which fosters the settling of migrating cancer cells [58, 59]. Bertocchi et al. recently demonstrated that GVB injury was associated with significantly more bacteria within hepatic metastases, suggesting that bacterial translocation occurs when the GVB is compromised [60••]. Furthermore, in antibiotic-treated mice, hepatic PMN formation was significantly diminished. These telling findings indicate bacterial dissemination to the liver may be responsible for the promotion of this pro-tumorigenic pre-metastatic niche.

Role of the virome in CRC

The bacterial contribution to carcinogenesis has been the large focus of research into the microbiome and cancer, yet nonbacterial entities are known to be involved as well. Human viruses have been shown to be critical to the development of a variety cancers, including hepatocellular, nasopharyngeal, cervical,

and some gastrointestinal cancers, typically with long-standing infection. The host virome, consisting of both eukaryotic viruses and bacteriophages, influences host cellular function and bacterial community composition [61]. In a recent study of shotgun metagenomic analyses of viromes from fecal samples, patients with CRC demonstrated higher diversity of gut bacteriophages [62, 63] suggesting bacteriophages indirectly affect carcinogenesis by altering the composition of gut bacteria. By altering commensal bacterial communities, opportunistic passenger bacteria can migrate in and proliferate. Moreover, others theorize that bacteriophages play a role in biofilm production, which supports proliferation and invasion of opportunistic bacteria [63]. Bacteriophages have also been shown to cross epithelial cell layers in the gut allowing for the possibilities that they play a role in CRC invasiveness [64].

Gut microbiota and cancer therapy

Role of the gut microbiota in checkpoint blockade

Immune checkpoint blockade (ICB) is an effective therapeutic strategy for a subset of CRC patients. This therapy targets the inhibitory signals to anti-tumor T cell activation, enabling appropriate anti-tumor immune responses[65]. Over the last decade, clinically relevant discoveries have been made demonstrating the gut microbiomes' role ICB response across cancer types [9, 11]. Among those receiving ICB, gut microbiota signatures vary between responder and nonresponders to ICB [66]; species shown to be associated with ICB response include *Akkermansia muciniphila*, *B. fragilis*, *Bifidobacterium* spp., *Eubacterium liomosum*, and *Faecalibacterium* spp. [9–11, 67]. Further investigation demonstrated higher abundance of *Faecalibacterium* spp. in anti-PD1 responders, correlating with more robust tumor immune infiltrates, consisting of higher levels of anti-tumor T cells and lower levels of regulatory T cells [67]. To study the role of the gut microbiome in these observations, preclinical melanoma models have demonstrated that responder or non-responder phenotypes could be altered among GF or antibiotic-treated mice through treatment with fecal microbiota transplantation (FMT) or gavage of specific bacterial species [9, 11]. Similarly, in mouse models of colon and melanoma tumors, antibiotic-treated or GF mice did not respond to CTLA-4 blockade, whereas gavage with *B. fragilis* restored response to therapy.[10] The underlying mechanism attributing to this immunologic effect and ICB response is through presentation of bacterial components to antigen-presenting cells (APCs) and innate effectors, ultimately inducing an adaptive immune response [5]. Globally, this is believed to cause a stronger anti-tumor effect, heightening the action of ICB.

Among colorectal cancers, there is a dichotomous response to ICB based on a tumor's microsatellite instability (MSI) status, with MSI-high (MSI-H) tumors having typically robust responses to anti-PD1 therapy [68, 69]. Phenotypic differences exist between MSI-H and microsatellite stable tumors (MSI-S), with significantly more prominent cytotoxic T cells and T_H1 cell infiltrates in MSI-H and upregulated expression of immune checkpoints [70, 71]. Based on this rationale, a phase 2 trial for metastatic or recurrent MSI-H CRC tumors treated with PD-1 inhibition demonstrated improved and durable responses with prolonged survival compared to the expected survival of MSI-H metastatic cancer patients [72]. There is need for future investigations into how MSI-H associated microbiota signatures contribute mechanistically and into the

potential therapeutic role of modulating gut microbe populations to enhance treatment responses.

The gut microbiome plays an important role in priming host immunity. Certain microbial species can stimulate T cell activation and differentiation, although the resulting immune response can vary based on the colonic local environment. *Helicobacter hepaticus* (Hhep) colonization in a healthy colon induces T cell differentiation into regulatory and follicular helper T cells, while in immunodeficient models stimulates Th1 and Th17 cells. In the context of modern anti-cancer strategies, understanding this process could help close an important knowledge- gap, as most anti-tumor immunotherapy relies on activating T cells. In a recent study, *Hhep* colonic colonization in a CRC-induced mouse model limited tumor burden and increased the tumor's immune infiltrate. Furthermore, investigators identified *Hhep*-specific follicular T helper cell (T_{FH}) activation and T_{FH}-induced tertiary lymphoid structures as necessary for this anti-tumor immunity and tied to response to ICB in other histologies [73•].

The impact of the gut microbiota on immunotherapy toxicity, particularly autoimmune colitis, has also been explored. Evaluation of microbiota signatures in patients treated with anti-CTLA4 therapy revealed that increased abundance of *Bacteroides* spp. is protective against autoimmune colitis [74, 75]. Meanwhile, higher abundance of *Firmicutes* correlated with higher rates of anti-PD1-induced colitis [74]. In our experience, immunotherapy-induced colitis was successfully treated in two patients with FMT, by reconstituting the gut microbiome, which led to increased infiltration of regulatory T cells into the colonic mucosa [76]. Ultimately future validation of this work in CRC is needed.

The role of the gut microbiome in chemotherapeutic response

In addition to associations with ICB response, evidence exists demonstrating that the microbiome's influence on the immune system may tailor responses to other forms of cancer therapy as well [77]. In preclinical murine models, cyclophosphamide, an alkylating chemotherapeutic, not only altered gut microbiota composition, but also resulted in migration of Gram positive bacteria into mesenteric lymph nodes where T_H17 cell stimulation and memory T_H1 cell response were visualized [77, 78]. This process resulted in a systemic anti-tumor effect. In this same study, antibiotic treatment suppressed bacterial invasion and immune response, resulting in resistance to cyclophosphamide. Platinum-based chemotherapeutics have similarly been described to have microbiome-dependent responses. Response to oxaliplatin in CRC and lymphoma murine models was diminished in GF and antibiotic-treated mice [79]. Moreover, oxaliplatin response depended on microbe-induced inflammation and ROS production in the TIME.

Specific to CRC, intratumoral *F. nucleatum* has been shown to promote chemoresistance to oxaliplatin and 5-FU, via activation of autophagy pathways by targeting TLR4 and MyD88 receptors for innate immune signaling [49]. In CRC models, *Gammaproteobacteria* have been linked to oxaliplatin and gemcitabine resistance as it harbors an inactivating enzyme, cytidine deaminase [80]. As the immune system's role in response to cytotoxic therapies continues to be

elucidated, the role of tumoral and gut microbes in modulating tumor response to cytotoxics should not be ignored.

Role of gut microbiota on radiation therapy

Radiation therapy (RT) plays a key role in the management of rectal adenocarcinoma. In preclinical models, RT has been shown to alter the normal gut microbiota [81]. Specifically, RT response has been associated with a reduction in Firmicutes abundance and increase in Proteobacteria. The microbiota's effect on radiosensitivity of the intestinal endothelium is highlighted by a preclinical study that demonstrated that GF mice developed less radiation-induced enteritis and less lymphocytic infiltration [82].

This association is strengthened by the observation that antibiotics modulate response to radiation therapy and radiation toxicity. The addition of vancomycin to RT enhanced local and distant RT-induced anti-tumor effects [83]. Furthermore, in melanoma murine models, total body irradiation enhanced intestinal bacterial translocation into the mesenteric LNs leading to stronger anti-cancer response [84]. This may be, in part, due to the abscopal effect, the phenomenon wherein tumor irradiation results in anti-tumor immune activation, thus allowing anti-cancer activity beyond the radiated field. High-dose RT generates tumor cell death, exposing antigens to the innate immune system, which subsequently activate Th1 and cytotoxic T cells which drive anti-cancer activity. It is hypothesized that through this same process, radiation can prime the TIME, and lead to stronger response from immune CPB [85].

Dietary effects

The gut microbiome is shaped by numerous environmental exposures, particularly diet and medication use. Given the microbiomes role in disease development and response to therapy, a new frontier in cancer research revolves around harnessing microbial modulation, in an effort to alter host physiology and support favorable outcomes. Personalized nutrition, in the form of dietary intervention or recommendation, has emerged as an exciting strategy to provide individualized care in various clinical contexts [86] (Figure 1). Dietary habits affect the microbiome's structure and function, but the interaction is complex, and the effects of a particular diet can vary significantly between individuals. Nonetheless, numerous studies have demonstrated successful modulation of the gut microbiome and consequential changes in host metabolism and immune function through changes in dietary inputs, including dietary fiber and fermented foods [26, 87, 88]. In a recent study of melanoma patients treated with ICB, higher fiber diets were associated with significantly improved progression-free survival [89•]. This was recapitulated in preclinical models in which mice treated with low-fiber diets or probiotics had impaired ICB responses, and a less robust cytotoxic T cell infiltration in the TIME. Clinical trials involving gut microbiota alteration through diet intervention are ongoing and will be crucial for understanding the safety and efficacy of this strategy in the context of ICB treatment. Another strategy for microbiome modulation is live biotherapeutics, a new class of microbiome-derived therapeutics under

development. These are distinct from over the counter probiotics in that they are subjected to rigorous clinical testing and regulatory approval. While evidence exists to support probiotic-induced alterations in host metabolism and inflammatory pathways, preclinical models and controlled trials in colitis and colorectal cancer have yielded mixed results [90, 91]. The use of probiotics, especially in the cancer patient, should be undertaken with caution given preliminary findings that they may be deleterious in patients treated with ICB. Ultimately, the composition of probiotics is variable, largely homogenous, and has been inadequately studied. Further development of individualized and targeted, live biotherapeutics are necessary.

Future directions

Prior work has clearly demonstrated the role of microbes in normal gut function, immune response, and therapeutic anti-cancer therapies. Based on these findings, efforts are underway to establish preventative or therapeutic anti-cancer interventions through the modulation of the gut microbiota. FMT has demonstrated significant efficacy in treatment-resistant *C. difficile* infections [92], and in benign disease, an excellent safety profile has been described [93]. A number of studies are underway investigating how FMT may be used in the context of cancer therapy, including its use for modulating responsiveness to immunotherapy and treatment-related toxicities [94, 95]. Also in production

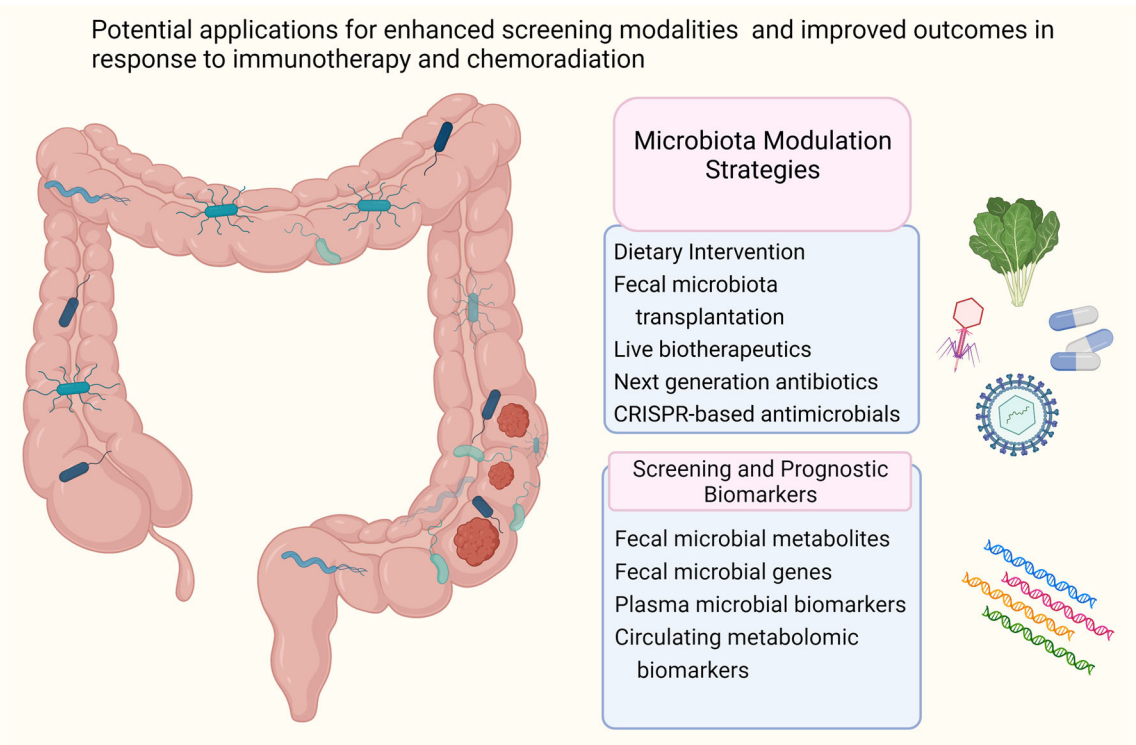

Figure 1. Potential applications for enhanced screening modalities and improved outcomes in response to immunotherapy and chemoradation. Created with BioRender.com

are CRISPR-based antimicrobials that exploit phage delivery of CRISPR-Cas systemics to bacteria leading to genome editing and elimination of specific bacteria at a strain level [96]. Given the challenges of antibiotic resistance and the known effect of antibiotics on the gut microbiome, this development in synthetic biology may impact our ability of targeting deleterious organisms without inducing global gut microbial dysbiosis. This targeted therapy can eradicate pathogenic bacteria, while leaving the rest of the gut microbiota intact, which could potentially be utilized for the treatment of a variety of microbiome-related diseases including infectious diseases, autoimmune disorders, and cancer.

Beyond treatment of CRC, early detection is a cornerstone of management, complicated by the rise in early-onset cancers. Fecal immunochemical tests (FIT) have been posited as an option to screen fecal samples for blood with a sensitivity of 69-86%,but have poor sensitivity for adenomatous precursors [97]. Detection of CRC in early stages is associated with excellent treatment outcomes, thus supporting the need for improved biomarkers for early CRC screening. Microbial signatures associated with various stages of CRC may represent the basis for potential screening strategies. In a metagenomic analysis of patients with CRC, a signature of 20 microbial gene markers was identified that differentiated CRC from normal controls; two of these genes were enriched at early stages, highlighting its potential as a screening biomarker [19]. *F. nucleatum*'s abundance in CRC has been employed in biomarker studies, and its use in conjunction with the FIT test has demonstrated improved sensitivity and specificity compared to FIT alone [98]. As previously described, certain microbial metabolic byproducts are associated with CRC and may also serve as biomarkers for cancer detection.

Conclusion

Over the past decades, enormous strides have been made in understanding the effects of the gut microbiome on normal health-promoting functions, as well as variety of benign and malignant disease processes. Microbial patterns and mechanisms have now been described that clearly associate with cancer development and treatment response. As we continue to treat colorectal cancer in the future, our ability to leverage these observations will be determined by continued engagement by the research community, directed study of mechanisms behind these observations, and early clinical trials to test the translation of these findings to patients at population scale.

Declarations

Conflict of interest

Samuel Cass, Nadim Ajami, and Michael White each declare no potential conflicts of interest.

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Further investigation into the microbiome's role in the development of CMS subtype and response to immunotherapy is needed.

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