Opinion

The Cancer Microbiome: Distinguishing Direct and Indirect Effects Requires a Systemic View


The collection of microbes that live in and on the human body—the human microbiome—can impact on cancer initiation, progression, and response to therapy, including cancer immunotherapy. The mechanisms by which microbiomes impact on cancers can yield new diagnostics and treatments, but much remains unknown. The interactions between microbes, diet, host factors, drugs, and cell–cell interactions within the cancer itself likely involve intricate feedbacks, and no single component can explain all the behavior of the system. Understanding the role of host-associated microbial communities in cancer systems will require a multidisciplinary approach combining microbial ecology, immunology, cancer cell biology, and computational biology—a systems biology approach.

The Roles of Microbiomes in Cancer Are Unclear and Require More Investigation

A statistic often cited says that infectious agents cause 20% of cancers worldwide [1]. That fact perhaps explains why cancer biology tends to view microbes such as viruses (Box 1) and bacteria (Box 2) as carcinogens to be eliminated. The microbial communities that make up the human microbiome (see Glossary) impact on many aspects of host biology: nutrient uptake, drug metabolism, inflammation [2], and even behavior [3]. They most likely also influence cancer progression and treatment, and in ways that can range from harmful to beneficial. Understanding how microbiomes impact on cancers—directly and indirectly, harmfully and beneficially—could bring new opportunities for cancer prevention, treatment, and management [4].

Many knowledge gaps remain: microbiomes are complex ecosystems with spatiotemporal dynamics that emerge from interactions between microbes and with the cells of their host. Each cancer is itself an ecosystem in which cancer cells interact with each other and with stromal cells in intricate, dynamic ways. Common factors such as circulating metabolites, systemic immunity, etc. can impact on—and be affected by—the two types of ecosystems simultaneously. The relevant links between microbiomes and cancers may be impossible to reduce to a single component. Exploring the role of microbiomes in cancer will likely require a systems biology approach.

The International Cancer Microbiome Consortium1 made a frank statement in 2019: ‘there is currently no direct evidence that the human commensal microbiome is a key determinant in the aetio-pathogenesis of cancer’ [5]. It will be crucial to distinguish direct microbiome effects on cancer from indirect effects. Direct effects could occur when microbes contact the cancerous tissue directly and impact on their behaviors. Indirect effects could occur when microbiomes impact on tumors at...
distant sites, such as for example, when the gut microbiome influences host physiology or systemic inflammation, which then impacts on cancer progression elsewhere (Figure 1). Although there are few examples so far of microbiomes that impact on cancers directly, the past decade of microbiome research has showed us that microbiomes influence host physiology in many ways. It is likely that some of those indirect effects determine the etiopathogenesis of cancer.

**Indirect Effects of Microbiomes on Cancer through Metabolism**

One aspect of host physiology where the impact of the gut microbiome is well established is the systemic balance of circulating levels of nutrients and metabolites [6]. A seminal study in the microbiome literature showed that, when the gut bacteria of obese mice were transferred into lean germ-free mice, the recipient mice became obese [2]. Importantly, the recipient mice from obese donors gained more fat than control recipient mice from lean donors, even though both groups consumed the same number of calories. The bacteria residing in the gut microbiome catalyze many bioreactions that convert metabolites that reach the intestine – either through the diet or released by the host. The host can absorb or reabsorb some of those microbially converted metabolites which then enter the circulation [7], and from circulation the microbial products can reach distant tissues and impact on cancer progression there. In liver cancer, for example, gut commensals can metabolize bile acids from primary to secondary structures which can recirculate and modulate recruitment of natural killer T cells to cancerous liver cells [3]. Other bacteria in the gut metabolize estrogen, potentially altering the risk of postmenopausal estrogen receptor-positive breast cancer, even though the breast is far from the gut [9,10]. In men, microbes can generate 11-oxygenandrogens from host glucocorticoids in the gut and urinary tract, which may contribute to prostate cancer [11–13].

Antibiotics can damage the gut microbiome and impact on metabolite levels in the plasma [14], which can affect host cells that interact with cancers in distant tissues. Two metabolites affected indirectly by antibiotic use – trimethylamine N-oxide and betaine – impact on peritoneal macrophages in mice, changing their phenotype to display markers associated with atherosclerosis [15]. In that example, the antibiotic treatment impacted on the microbiome, which then affected the levels of circulating metabolites in a way that harmed host health. However, the demonstration that microbiota manipulations can alter macrophage markers could inspire strategies against cancer. Macrophages are phenotypically very versatile, and some of their phenotypes can actually promote tumor growth [16]. Engineering a microbiome that reduces the systemic levels of metabolites, such as lactate that polarizes tumor-associated macrophages [17,18], could potentially turn off the tumor-promoting phenotypes of those macrophages.

The gut microbiome may also influence the central nervous system, mood, and behavior, and potentially impact on cancer. The link between microbiomes and animal behaviors is a hot topic [19], but this link is probably ancient: animals have always coexisted with microbes and have many symbiotic relationships with their commensals which affect behavior [3]. The reward system of the brain, a key circuit in emotional processes, can potentiate an antitumor immune response. In a recent study also in mice, activating the reward system with chemogenetics shrank lung tumors [20]. The mechanism involved a cascade of effects: the reward system attenuated noradrenergic input to a major immunological site, the bone marrow. The myeloid-derived suppressor cells became less immunosuppressive as a result and caused an antitumor response.

The microbiota can indirectly impact on the host reward systems [21] and even the host response to drugs that affect those systems [22]. Therefore, the microbiome could in principle enhance antitumor immunity and decrease cancer progression indirectly by altering the psychological state of the host. A cancer therapy that works on this principle may seem to be science fiction today, but...
the use of probiotics to reduce pain – either directly or indirectly by potentiating analgesics such as opioids – is already under study (Figure 2) [23]. The interaction between microbiomes and the central nervous system could at least be leveraged to control cancer symptoms when cures are unavailable.

Taking full advantage of the potential of the microbiome against cancer requires a better understanding of how commensals function. Fortunately, the microbiome field is rapidly expanding from taxonomic studies to functional studies (Box 3). Some beneficial functions may be pinned
Box 2. The Duplicitous Role of Microbes in Cancer

The best-known bacterial cause of cancer is Helicobacter pylori, which colonizes 50% of people worldwide [77] and is responsible for ~90% of gastric cancers [1]. Stomach cancer led the list of deadly cancers for the first half of the 20th century. Its incidence has decreased in Western countries, possibly thanks to hygiene and antibiotic use, because some antibiotics taken to treat other infections kill H. pylori unintentionally [78]. However, H. pylori exemplifies a paradox: this bacterium may have coevolved with humans because it can be transmitted from person to person and could be considered as a normal component of the stomach microbiome [79]. H. pylori colonization may even benefit human health because decolonization increases the risk of severe gastroesophageal reflux disease and its sequelae, Barrett’s esophagus, and adenocarcinoma of the esophagus [80]. It may also protect against asthma, multiple sclerosis, and IBD [81–83], although it is a risk factor for type 2 diabetes and some other diseases [84,85]. Therefore, the same microbe could both harm (by causing gastric cancer and some diseases) and benefit human health (by preventing other diseases and even cancers in other organs).

This duplicity applies to other microbes [86]. Perhaps the difference between harm and benefit lies in the type of inflammation caused by the microbe. Human immunity is a subtle balance, and a measured immune response is essential for homeostasis: a weak immune response can fail to protect against infection, but an excessive response can destroy tissues or cause autoimmune disorders. IBD, a form of chronic gut inflammation, alters the composition of the luminal microbiota and enriches for Gram-negative bacteria. Patients with IBD have a higher risk of colorectal cancer, which may be due to bacteria enriched in the luminal microbiota such as pks+ E. coli [32]. However, inflammation caused by microbes can be leveraged against cancer. Bacillus Calmette–Guérin (BCG) – a vaccine prepared from an attenuated strain of Mycobacterium bovis – is one of the most successful biotherapies that can treat bladder cancer [87]. Moreover, microbiomes may affect cancers beyond the presence or absence of a specific microbe, and the simpler cases where the effect is attributed to a single organism may be exceptions more than a general rule. Understanding microbiomes in cancer may require knowledge of networks to distinguish a homeostatic network from a carcinogenic network, to understand whether those networks are static or plastic, and to devise methods to convert a carcinogenic network back into a homeostatic network.

down to specific microbes, such as when a specific commensal belonging to the Bifidobacterium genus promotes antitumor immunity in mice [24]. However, other functions, such as metabolic capabilities, may spread across broad phylogenetic clades. In these cases, several microbes could be used to provide the same function, assuming conservation of function within clades [25]. In addition, studying the composition of microbiomes in large populations can reveal new links between commensal members of the microbiome and cancer incidence (Box 4).

Direct Effects of Microbiomes on Cancer

Most studies of direct effects come, perhaps expectedly, from tumors in tissues naturally colonized by commensals: the gut microbiome on colorectal cancer, the lung microbiome on lung cancer, the skin microbiome on melanoma, the vaginal microbiome on cervical and endometrial cancers, etc. However, microbes in unexpected tissues can impact on cancer progression and response to therapy. Pancreatic cancers can have bacteria accidentally introduced by medical intervention [26–28], and colorectal cancers can bring along gut bacteria when they metastasize to other tissues such as the liver [29]. Regardless of whether a tissue is already colonized, the direct interaction between microbes and cancer cells remains poorly understood.

This area of research would benefit greatly from a system that allows modular experiments in vivo, where each component can be changed independently such that the mechanisms of interaction between cancer cells and microbes can be untangled. Colorectal cancer is the second largest cause of cancer death in the USA [30]. The key risk factor is genetic predisposition, but the remaining factors – diet, lifestyle, and microbiota composition – account for the majority of cases [31]. Untangling these risks could benefit from a systems approach. Colorectal cancer already has animal models such as I/10^15 mice, which could provide a modular experimental system. Depending on the genetic background, up to 100% of I/10^15 mice develop colitis; with the addition of the colon-specific carcinogen azoxymethane, 60–80% of these mice develop colon tumors [32]. Colorectal cancer is itself a modular disease that progresses in stages [33]. The tissue is directly colonized by a microbial community and its composition in bacteria, virus, and fungi changes as the disease proceeds through its stages [34–36]. The combination of a well-
established model and a disease with a pathway may enable cancer microbiome studies that discern the roles and mechanisms of microbes in distinct disease stages [37].

Western diets have many nutrients that can increase intestinal tumors [38]. These diets abound in animal protein and fat, and can increase bile acid secretion into the gastrointestinal tract. Gut commensals can conjugate these bile acids before they are reabsorbed into circulation. One species, Bilophila wadsworthia, converts taurine to acetate and ammonia, and releases hydrogen sulfide which is carcinogenic [39]. However, even when specific microbes such as B. wadsworthia contribute to cancer progression, other microbes may also impact on the process, which means that the mechanisms involved must be approached systematically. Spatial structure may also play a key role. For example, microbial biofilm organization has been associated with the location of development of colorectal cancer [40] and was also shown to promote carcinogenesis in preclinical models [41].

The transition from a localized cancer to a disseminated disease – metastasis – is a key turning point when many cancers evolve from a curable to an incurable stage. A recent study showed
that microbes can travel from primary tumor sites to distal sites together with metastatic cells. The study focused on *Fusobacterium nucleatum*, one of the most prevalent bacteria in colorectal cancers. Patient biopsies suggested that *Fusobacterium* and other bacteria from the gut microbiome travel from the primary tumor to distant metastases (Figure 3). Mouse xenografts from primary colorectal adenocarcinomas show that cancer cells can indeed carry viable *Fusobacterium* and transport them to metastatic sites. The ability to carry bacteria was maintained after several passages and was important for malignancy: reducing the bacterial load by treating the mice with antibiotics reduced tumor growth. These results suggested that antimicrobial interventions could help in treating colorectal cancers associated with *Fusobacterium* [42].

However, bacteria that interact directly with cancer cells could actually help to treat cancers by causing localized inflammation [43]. This serves as additional evidence of the duplicitous role of bacteria (discussed in Box 2). The interaction becomes even more complex when we consider that bacteria can drive drug resistance: some bacteria can metabolize the chemotherapeutic cancer drug gemcitabine (2',2'-difluorodeoxycytidine) into its inactive form, 2',2'-difluorodeoxyuridine. The inactivation of the cancer drug requires that bacteria express a long isoform of cytidine deaminase, an enzyme that is predominantly present in gammaproteobacteria. Experiments in a mouse model with subcutaneous syngeneic tumors showed that gammaproteobacteria living inside the tumor can increase tumor resistance to gemcitabine. A combination therapy of gemcitabine and...
The Microbiome and Cancer Immunotherapy

Understanding the intricate feedback between microbes, immunity, and cancer – and their impact on immunotherapy – will likely require multidisciplinary approaches and a systems perspective. Immunotherapy can treat advanced melanoma, and its successes may apply to other cancers. Up to 60% of patients with metastatic melanoma can now achieve durable remissions thanks to the ability to unleash the immune system with checkpoint inhibitors [44]. Three papers published recently suggest that the gut microbiome of a patient can influence their response to immunotherapy [45–47]. These studies found differences in the gut microbiota composition of patients who responded to checkpoint inhibitors and patients who did not respond. Intriguingly, the three studies did not agree on which specific microbes mattered for the success of immunotherapy [48]. In addition to a lack of agreement about which commensal microbes matter the most, the mechanisms by which the gut microbiome influences immunotherapy remain unclear [49]. The microbiomes of responders can induce the production of interferon (IFN)-γ and granzyme B by CD4 and CD8 T cells, which would help to recruit antitumor macrophages. Checkpoint inhibitors are less successful against models of melanoma in germ-free and antibiotic-treated mice compared with mice with intact gut microbiota [24]. Some studies even suggest that specific bacteria enriched in responders, such as Bacteroidetes thetaiotaomicron and Faecalibacterium prausnitzii [50], may be used in precision probiotic treatments to augment the efficacy of checkpoint inhibitors.

A recent study showed that short-chain fatty acids (SCFAs) produced by gut bacteria promote the memory potential of antigen-activated CD8+ T cells [51]. This important finding addresses the healthy skepticism about the disparity in taxa discovered by different groups. F. prausnitzii,.....
which several groups had identified in patients who responded favorably to checkpoint blockade, is a key clostridial producer of SCFAs including butyrate. The literature on IBD and gut graft-versus-host disease suggests that these cluster XIVa and IV clostridia produce SCFAs that induce an anti-inflammatory environment by inducing regulatory T cells and interleukin (IL)-10. It had seemed contradictory that gut microbes such as *F. prausnitzii* would be beneficial in the context of cancer immunotherapy, but this recent study showed that these SCFAs may induce different phenotypes depending on various environmental and host-specific factors [51]. The conditional mechanism explaining why SCFA-producing clostridia impact on immune checkpoint blockade efficacy echoes the point raised earlier that several gut microbiota members may have a common functional output (e.g., SCFA, butyrate production), and the metabolites that they produce are functionally important.
There are other reasons to investigate the gut microbiome of patients receiving immunotherapy: the therapy has side effects and can itself impact on the gut microbiome, causing more complications. Checkpoint inhibition can cause immune-mediated colitis [52]. A prospective study of patients with metastatic melanoma undergoing ipilimumab treatment identified gut bacteria in the precolitis microbiota of patients that later developed colitis [53]. The patients had fewer bacteria from the Bacteroidetes phylum and also lower expression of genes involved in polyamine transport and B vitamin biosynthesis. If these biomarkers indeed explain why some patients developed colitis, then this finding could lead to interventions that ameliorate the microbiota and reduce the risk of inflammatory complications from cancer immunotherapy. More broadly, the prospective study illustrates that immunotherapy can cause gut inflammation that then alters the composition of the microbiome, causing a feedback loop that can lead to more complications. Remarkably, in a case report serial fecal microbiota transplantation (FMT) alleviated programmed cell death protein 1 (PD-1)-induced refractory colitis in patients [54].

Restoring Microbiomes after Therapy
Cancer treatments such as radiation and chemotherapy can damage the microbiome and lead to complications. Patients with prostate, gynecological, or gastrointestinal cancers, for example, pelvic tumors, often receive pelvic radiotherapy which can be extremely effective (e.g., 90% effective for prostate cancer). However, pelvic radiotherapy causes chronic diarrhea for which there is no effective treatment [55-57]. The composition of the gut microbiome at the time a patient receives the radiation may be responsible for this complication. Specifically, low levels of bacterial richness in the lower gut before radiation is linked to a higher risk of diarrhea after radiation.

Figure 3. Metastases Can Carry Bacteria from the Microbiome of Primary Tumors to Distal Tissues. A recent study [42] investigated patient biopsies from colorectal cancer and liver metastases from the same patients. Bacteria found in primary tumors, including Fusobacterium nucleatum, were also found in metastases, suggesting they had traveled with the metastatic cells. Experiments with mice validated these findings and showed that antibiotic treatment could reduce tumor load.
treatment [58]. Understanding how microbiome composition at the time of the treatment may influence post-treatment complications requires more systematic analysis.

Cancer patients receiving bone marrow transplantations experience drastic changes in their gut microbiota as a result of the large doses of antibiotics given to prevent or treat infections. Antibiotics are essential because any infection becomes life-threatening if it occurs before the bone marrow transplant has had time to engraft and the patient is neutropenic. However, the antibiotics cause collateral damage to the gut commensals: the microbiota loses diversity and may become dominated by bacteria such as vancomycin-resistant Enterococcus faecium [59], which increases the risk of bloodstream infections and patient mortality [60]. A recent trial conducted with transplant patients showed that autologous fecal microbiota transplantation (auto-FMT) can reconstitute the microbiota composition in patients after they receive bone marrow transplantation (Figure 4) [61]. The study concluded that auto-FMT restored microbiota composition for the average patient, but the success varied significantly from patient to patient. In the best case, a patient recovered practically 100% of their original microbiota composition but, in the worst case, a patient recovered only 50%. Why successes varied so widely is not known, but could reflect factors such as the actual composition of the microbiota transplanted, the state of the microbiota before the auto-FMT, or even personal factors including patient genetics or underlying disease. Understanding the variable success of FMT is an important direction for future research.

Probiotic therapies also vary markedly depending on the resident microbiota of the host [62], but also depending on differences in genes expressed by the host. This indicates complex interplay between microbiota ecology and host factors. Stable colonization of the gut microbiome by a probiotic strain of Bifidobacterium longum depends on the resident microbiome through unclear

Figure 4. Many Cancer Therapies such as Chemotherapy and Pelvic Irradiation Can Damage Healthy Microbiomes. Transplants of intestinal microbiota such as autologous fecal microbiota transplant (auto-FMT), where a stool sample is collected, cleared for safety, and stored to then be given as a treatment after microbiota damage, could be used to safely remediate the microbiome of a patient. Auto-FMT has been applied to cancer patients receiving hematopoietic cell transplantation. Applications to other cancer patients should proceed with caution because we do not fully understand the link between the gut microbiome and cancer; auto-FMT raises a concern that restoring the pretreatment microbiome composition could increase the odds of cancer recurrence.
In addition, it is important to consider that success of auto-FMT can be at least partially compromised by probiotics [64]. Despite the initial successes of auto-FMT to restore the microbiota of cancer patients receiving hematopoietic cell transplantations, the same idea may not work as well for other cancers. If the microbiota has a crucial role in cancer initiation and progression, performing auto-FMT in those patients could increase the odds of recurrence. Given the current state of our knowledge, auto-FMT cannot be deemed a risk-free approach for every cancer without further understanding the role of the microbiome in a particular disease. We should try to understand better how the microbes residing in the gut interact with each other and with the host to improve the success of probiotics and FMT, and – especially – future personalized cancer therapies based on microbiota cocktails [65].

Concluding Remarks
The many microbes living in and on the human body impact on many aspects of human physiology. It seems logical, then, that human microbiomes can impact on cancer prevention, development, treatment, and management, although the specific mechanisms remain unclear. The departure from physiological homeostasis caused by cancer and by cancer therapies can damage microbiomes, which may then increase the risk of more complications. Cancer, cancer treatment, tumor-associated microbes, and the human microbiome may all be linked through intricate feedback networks. Untangling these networks and their plasticity requires a systems biology approach.

The examples discussed here could be taken as invitations to investigate a scientific frontier that remains largely unexplored (see Outstanding Questions). The field should benefit from recent advances in microbiome research, as a field evolving from cross-sectional studies that first sought to catalogue the human microbiome to longitudinal studies that seek to understand function. The evolution of the microbiome field brings us closer to understanding the dynamic role of microbiomes in carcinogenesis and cancer progression. The human microbiome is an integrated part of a large cancer–host–microbiome system. Although each single component may appear to be insignificant compared with the magnitude of the disease, recent research already shows that microbiome manipulations can nudge cancer systems towards desired states.

Without better ways to prevent, detect, and treat cancer at an early stage, the number of new people diagnosed with cancer will continue to outpace the treatments available – even as new treatments are discovered. That means many of us may need to learn to live with cancer in our lifetime. It is likely that the solution for prolonging quality and fulfilling lives, even when a cancer cure is unavailable, resides in our future ability to leverage the human microbiome to help to provide sustainable, long-term control of the disease. This could help make incurable cancer a chronic but manageable disease. Once better understood, the homeostasis between microbes and cancer systems may ultimately contribute to healthier and longer lives.

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Resources
www.human-microbiome.org/
www.microbiotavault.org/
www.openbiome.org/

Outstanding Questions
What other members of the human microbiome beyond bacteria – archaea, fungi, and viruses (including bacteriophages) – are important for cancer?
How can we integrate microbial composition and function with other relevant data such as habitat, host information, and other variables quantified by omic approaches?
Does the human microbiome play a role in the link between migration and cancer incidence?
Why does the success of microbiota transplants vary from patient to patient?
Can the ability of gut microbes to influence the central nervous system be leveraged to manage pain in cancer patients or even cancer development?
Can we engineer microbiomes to turn immunotherapy nonresponders into responders?
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