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Authors

Sholl, Jonathan
Sepich-Poore, Gregory D
Knight, Rob
et al.

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Redrawing Therapeutic Boundaries: Microbiota and Cancer

Jonathan Sholl[*],
Univ. Bordeaux, CNRS, ImmunoConcEpT, UMR 5164, 33000 Bordeaux, France

Gregory D. Sepich-Poore,
Department of Bioengineering, University of California San Diego, La Jolla, CA, USA

Rob Knight,
Departments of Bioengineering, Pediatrics, and Computer Science and Engineering, and Center for Microbiome Innovation, University of California San Diego, La Jolla, CA, USA

Thomas Pradeu[*]
Univ. Bordeaux, CNRS, ImmunoConcEpT, UMR 5164, 33000 Bordeaux, France

Abstract

The unexpected roles of the microbiota in cancer challenge explanations of carcinogenesis that focus on tumor-intrinsic properties. Most tumors contain bacteria and viruses, and the host's proximal and distal microbiota influence both cancer incidence and therapeutic responsiveness. Continuing the history of cancer-microbe research, these findings raise a key question: to what extent is the microbiota relevant for clinical oncology? We approach this by critically evaluating three issues: how the microbiota provides a predictive biomarker of cancer growth and therapeutic responsiveness, the microbiota's causal role(s) in cancer development, and how therapeutic manipulations of the microbiota improve patient outcomes in cancer. Clarifying the conceptual and empirical aspects of the cancer-associated microbiota can orient future research and guide its implementation in clinical oncology.

Keywords

Cancer; microbiota; biomarkers; causality; therapeutic modulation; network medicine

[*]Correspondence: jonathan.sholl@u-bordeaux.fr (J. Sholl), thomas.pradeu@u-bordeaux.fr (T. Pradeu).

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G.D.S.-P. and R.K. are inventors on a US patent application (PCT/US2019/059647) submitted by The Regents of the University of California and licensed by Micronoma; that application covers methods of diagnosing and treating cancer using microbial biomarkers in blood and cancer tissues. G.D.S.-P. and R.K. are founders of and report stock interest in Micronoma. G.D.S.-P. has filed several additional US patent applications on cancer microbiome diagnostics that are owned by The Regents of the University of California. R.K. additionally is a member of the scientific advisory board for GenCirq, holds an equity interest in GenCirq, and can receive reimbursements for expenses up to US $5,000 per year.

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Introducing the microbiota to oncology

Recent work elucidating the microbiota's various roles in cancer initiation, progression and treatment challenges the predominant view that cancer can be explained primarily in terms of tumor-intrinsic properties [1]. Not only do tumors contain bacteria [2,3], viruses [4] and sometimes fungi [5], but both cancer incidence and response to various cancer therapies are influenced by the host's microbiota [6]. Despite the overall enthusiasm around the microbiota in most areas of current biology and medicine [7–10], these discoveries came as a surprise because they expanded the scope of what was considered relevant for oncology. This research has also been met with skepticism; amidst the controversial history of using microbes to explain or treat cancer, it has often been difficult to establish the reproducibility, efficacy and safety of these approaches [11]. Moreover, in certain cancer types, the relative importance of microbial versus tumor cell mechanisms and their interactions in carcinogenesis remains debated [12]. It is thus still an open question to what extent the microbiota is relevant for clinical oncology [13–16], and whether the role of the microbiota in cancer requires an explanatory shift beyond tumor-intrinsic features.

After providing some brief historical background on the use of microbes/infections in cancer research and therapy, we illustrate how data on cancer-associated microbiota question several tightly held assumptions in oncology [17–19]. We then evaluate the relevance of the microbiota for oncology by clarifying three complementary perspectives. First, can the microbiota be used as a *biomarker* of cancer growth and therapeutic responsiveness? Second, can the microbiota be *causally* linked to cancer development? Finally, can the microbiota be *therapeutically manipulated* to improve the treatment and course of cancer, and if so, how? By clarifying several conceptual and empirical challenges at the intersection of oncology and microbiology, this conceptual review proposes the oncological utility of cancer-associated microbiota in patient diagnosis, prognosis, and treatment.

Historical background: from microbes to mutations

There is a long tradition of using microbes—particularly bacteria and viruses—in cancer *therapy*. In a broad sense, this may date back to observations in ancient Egypt and Greece that tumor regression followed infections and/or fevers [11,20,21]. The first scientific attempts at modulating the immune system to treat cancer appear to emerge in the late 19th Century with the German physicians Wilhelm Busch and Friedrich Fehleisen who independently noticed tumor regression in several patients following erysipelas infections caused by *Streptococcus pyogenes*. In the early 20th Century, this tradition was advanced by one of the 'fathers' of immunotherapy, William Coley, who, after tracking down a patient in New York who experienced spontaneous regression of an egg-sized sarcoma following erysipelas, started controversially injecting live and later heat-inactivated bacteria ("Coley's toxins") into his patients with inoperable cancer [21]. Despite the apparent success of Coley's method to achieve ~30% long-term remission in 11 types of malignancies across ~210 patients before 1940 [22], his findings and methods were not well received by the oncology community due to poorly understood mechanisms, painful fevers (often associated with therapeutic responses), difficult reproducibility, and non-trivial risks of death in immunocompromised patients. Some suggest that as surgery and radiotherapy

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advanced, and antibiotics and antipyretics were commonly used to enhance hygiene or suppress undesirable immune responses, this sterilizing environment may have obscured the potential anti-tumoral roles for microbes and immune or febrile reactions [21].

Throughout the 20th Century, there was a parallel tradition of considering whether microbes play a *causal role* in carcinogenesis and progression [23]. Amidst interest for cancer vaccines, first Thomas Glover and later Virginia Livingston-Wheeler argued that bacteria could be cultivated from tumors and that cancer had a bacterial origin [24,25]. However, Glover's findings were not reproducible by researchers at the National Institutes of Health and Livingston-Wheeler's research was criticized for not controlling for contamination [24]. As this bacterial research was being abandoned (though not entirely [26]), Peyton Rous made the tentative observation in 1911 that specific 'agents' from a chicken tumor could be transmitted to healthy chickens, thereby reproducing an avian tumor resembling human neoplasms [27]. Initially met with outright rejection or considerable skepticism [28], Rous's findings were eventually vindicated, opening the field of tumor virology [23]—canonized by his receipt of the Nobel Prize in 1966. While a variety of viruses have been linked to several cancers, such as Epstein-Barr, hepatitis viruses, and human papilloma—the most recent being the Merkel cell polyomavirus described in 2008—the viral origins of cancer proved limited and ultimately gave way to a focus on *internal* etiologies such as cellular mechanisms and mutations. Notably, the 1989 Nobel Prize to Michael Bishop and Harold Varmus signaled this major shift in thinking, for they found that many retroviral oncogenes, including from Rous's sarcoma virus, had a cellular (non-viral) origin and were found in many animal species [29]. As the presumed origin of cancer moved from external to internal factors, it engendered research into cancer genomics.

Together, these historical traditions exhibit the persistent challenges, and even reluctance, for evaluating the importance of microbes in oncology. One interesting tension running through this history concerns the relative importance of microbes for conceptualizing tumorigenesis: is cancer formation and progression primarily intrinsic or extrinsic [28]? We have seen that throughout the 20th Century microbes were viewed as an *extrinsic* factor that might have a direct causal or therapeutic role, but paradoxically, this perspective eventually catalyzed the study of cancer genetics [30] and the focus on cell-*intrinsic* mechanisms (while clearly acknowledging extrinsic triggers).

Recent research is not only examining specific microbes but also the *microbiota*, or communities of microbes that inhabit and influence the human body and their potential role in cancer progression and treatment [18]. The microbiota colonizes tumors and even individual tumor cells [18], prompting us to once again ask, for instance, in what sense the microbiota is an extrinsic or intrinsic causal and therapeutic factor. The additional findings that patient responsiveness to cancer therapies (e.g., immune checkpoint inhibitors (ICI) or chemotherapies) *depends on* the microbiota in the host appear to blur the intrinsic-extrinsic distinction [14,31,32]. As advances continue to uncover complex interactions among the microbiota, cancer cells, and the host's immune responses [33], the microbiota have become a manipulable tool at once external to and part of the host and its tumor(s). As such, the microbiota is no longer an accidental environmental factor to be suppressed but appears necessary for understanding and effectively treating cancer.

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The microbiota provides distinct biomarkers in oncology

A key area in oncology comprises the search for cancer-specific *biomarkers* that enable accurate predictions about patient diagnosis, prognosis, and treatment. Going beyond tumor-intrinsic factors, many have begun investigating whether the microbiota, and its functions or metabolites, can alone serve as non-human biomarkers for cancer [34–36]. Here, we discuss how the microbiota constitute distinct types of biomarkers [37].

First, blood-derived microbial DNA and specific alterations in localized microbial communities have recently been put forth as possible *diagnostic* biomarkers for various cancers [2,3,38], though their real-world performance remains to be seen [18]. As *prognostic* and *risk* biomarkers for evaluating cancer progression, patterns in microbial communities in patients may help explain inter-individual variation in which mutations become cancers [39], personal risk of acquiring cancer [40], and the anti- or pro-tumor function of specific mutations [12]. These various biomarkers can be derived by evaluating the microbiota specific to a given cancer type (the ‘intratumoral microbiome’), but also the host microbiota at distant sites. For instance, alpha diversity in the intratumoral microbiota is predictive of short versus long-term survival in pancreatic cancer [15], patterns of microbial community changes in lung tissues may be markers of lung cancer progression [41], and gut microbiota composition can inform the risk of developing colorectal cancer [42]. Distally, gut microbiota can help assess the risk of progression from liver disease to cancer [43] and determine patients at risk of tumor metastasis in breast cancer [35], and periodontal microbes appear to stratify risk for developing pancreatic cancer [44].

Next, as *therapeutic response* biomarkers, the microbiota can surprisingly determine a patient’s likelihood of responding to treatment [45]. Bourgeoning evidence suggests how clinical antibiotics is a predictor of poor survival in various cancers (with some exceptions [14,46]) and might undermine cancer therapies [47]. Conversely, increasing attention is given to the ability of the microbiota to support the efficacy of immune checkpoint inhibitors [48,49], as well as traditional chemotherapies [14,50]. Researchers have extracted specific microbial species from the feces of responders and non-responders and have shown how these phenotypes are replicated in mice or humans upon receipt of concomitant microbes [6,51,52]. These data provide persuasive examples of how the microbiota is linked to therapeutic efficacy and in some cases may outperform traditional biomarkers such as tumor mutational load [31]. Some researchers also suggest that the functional traits they exhibit could have more explanatory value than the taxa compositions alone, although this remains to be rigorously tested [53].

Ultimately, predictions about cancer risks and progression are not solely tied to tumor-intrinsic properties but are coupled with, and in some cases superseded by, local and distant microbial signatures [41,43]. As such, the microbiota may eventually allow for better diagnoses and appears crucial for predicting the health and survival of the cancer-bearing host, highlighting its relevance for oncology. However, the transition from correlative signatures and biomarkers to causative factors raises challenges since many of these microbial biomarkers comprise entire communities of organisms in comparison to individually assayed host biomarkers [54].

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Establishing causality in cancer-microbiota interactions

Although experts agree that a handful of microbes have causal roles in carcinogenesis [55], many complexities exist, including how particular microbes and communities can aid in tumorigenesis without being direct causal agents, or can even protect against tumorigenesis [18]. Determining causality requires a careful analysis of the relevant context and the distinct roles that microbes play under various physiological conditions. Box 1 addresses some of the mechanisms identified for the roles discussed below and Figure 1 offers a visual representation.

Pathogenic microbes in cancer

From the perspective of microbes as *pathogens*—generally considered extrinsic causes—it is well accepted that key bacteria and viruses have oncogenic effects in humans. There are currently 11 agents recognized as bona fide 'oncomicrobes' in humans (IARC Working Group 2012), including *Helicobacter pylori*, human papillomavirus (HPV), hepatitis B virus (HBV), and hepatitis C virus (HCV), Epstein-Barr, herpesviruses, and various polyomaviruses [57]. Moreover, several seemingly pro-carcinogenic bacteria, such as *Bacteroides fragilis*, *Enterococcus faecalis*, *Fusobacterium* and *pks+* strains of *Escherichia coli,* all appear to have carcinogenic capacities through their effects on the host immune system, mutagenesis, and inflammation [48,58,59].

However, even where specific microbes are shown to have cell-transforming abilities, they often occur alongside inflammation or specific changes to the microbial milieu, such that "causal" microbes may be necessary yet insufficient for tumorigenesis and progression [58,60]. Thus, while causal links exist, the influence of the broader microbial community and the physiological responses of the host should also be considered.

Microbes living in and traveling with the tumor

Recent data supports the perspective that microbes pervasively *colonize* tumors—evidence that falsifies the assumption of cancer's sterility [2–5,61], a theory possibly kept alive by sterility assumptions of various organs, such as the lungs or bladder [62]. In fact, distinct cancers have cancer type-specific microbial signatures [2,3]: a microbiome unique to each cancer with varying ratios of, e.g., *Proteobacteria* and *Firmicutes*, appearing in higher loads in tumors than in adjacent normal tissue. It is perhaps not surprising to find microbes in gastrointestinal cancers, which are anatomically proximal to the gut microbiota, but intratumoral microbiomes have also been found in tissues distal from the gut, such as in breast, lung, ovary, melanoma, bone, and brain tumors. In these studies, many bacteria detected in tumors appear to be live, cell-wall deficient bacteria, which are exclusively *intra*cellular bacteria and are mainly found in cancer cells and immune cells [2]. It remains unknown how many of these bacteria are merely *passengers* rather than active participants in a nutrient-rich and immunosuppressed environment.

Additionally, the microbiota can be seen as *migrators*. For instance, microbes found in primary colorectal tumors have also been found in matched metastatic liver tumors [61]. Although originally thought to hitchhike within metastatic cancer cells [61], new evidence

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suggests that colorectal cancer bacteria may actually travel to the liver ahead of metastatic cells and prepare a pro-tumoral, pro-inflammatory environment for them to later seed [63]. These metastatic processes remain uncharacterized in most cancer types.

While specific taxa were identified in these studies, their functional repertoire and spatial distribution within tumors remain poorly characterized, obscuring causal roles they may play in cancer progression [53]. Determining the pathogenicity of these tumoral colonizers is complicated by the observation that, in some cases, the composition of specific microbes in tumors may improve patient outcomes [15] or enhance immunotherapy response [2].

The role of the resident microbiota in promoting or inhibiting cancer

Another perspective studies the microbiota as an oncological *regulator*. This idea is supported by research showing how the resident microbiota can play a dual role in *promoting* or *inhibiting* cancers.

There are multiple ways in which the microbiota promotes cancer growth and progression. Bacterial infections trigger inflammation and innate immunity pathways, which in turn create a tumor-promoting microenvironment [63–65]. A second pathway is through dysbiosis, a widely discussed and sometimes contested concept, generally referring either to a loss of beneficial microbes, an expansion of pathobionts, or reduced diversity [66–68]. While in some cases it is unclear whether dysbiosis is driving inflammation and thus tumorigenesis, or whether inflammation drives the dysbiosis, there are nevertheless strong links between the microbial community changes, inflammation, and tumor promotion [58]. Furthermore, when barrier epithelial cells are damaged (an innate immune defense), the “normal” resident microbiota can further damage these cells or underlying tissues, induce genetic instability via DNA-damaging reactive oxygen and nitrogen species [48], or translocate into circulation causing systemic inflammatory responses [69].

While certain microbes are pathogenic, others support the body’s antitumoral responses. The microbiota can epigenetically prime myeloid cells, such as dendritic cells and macrophages, for optimal responsiveness to tumors [65], an effect that is significantly reduced in germ-free mice models. Several bacterial species have also been associated with anticancer immunosurveillance [70], with specific intratumoral microbial compositions linked to *better* chances of survival [15] or response to immunotherapy [2]. It remains undetermined whether the diversity of gut microbes facilitating positive outcomes can be explained by shared functional output of specific metabolites (e.g., short-chain fatty acids) that contribute to reducing inflammation and/or ensuring intestinal barrier integrity [71,72].

Reevaluating causality

Several challenges remain for evaluating causal claims of the cancer-associated microbiota. These include the primary way researchers conclude causality in microbiome studies: rodent models. There are limitations of uneven colonization in recipient animals, the prevention of novel communities forming upon colonization, the lack of ecological factors in these models that were important for producing host disease states, and the difficulty of getting recipient animals to adapt to microbes with which they did not co-evolve [84]. Although some cancer studies have performed human fecal microbiota transplants [51,52], these are rare. Causal

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claims are further complicated due to some infections having long latency periods (e.g., human T-cell leukaemia virus type 1), the fact that many microbes are widespread and yet their associated cancer is rare, and that causal mechanisms may vary during the time course of carcinogenesis [23].

Moreover, this research faces the immense challenge of specifying causality amidst the complex variations of host-microbe and microbe-microbe dynamics [60]. Microbes exist within ecosystems, and even if single microbes can be linked to or associated with various cancers, they also tend to be accompanied by shifts in other microbial taxa [72]. This is further complicated when considering that phages preying on bacteria may influence these dynamics [83]. Finally, there is the issue of context-dependency [53,70,85], such as whether specific microbes contributing to cancer depends on host physiology (e.g., inflammation).

One proposed way forward is to incorporate ideas from systems medicine or ecology [72,86–92]. For instance, we can track how perturbations in one part of the microbe-host ecology will result in adjustments, compensations, or disruptions to other parts of this system [93]. The challenge is to determine how to accurately define these systems and their relevant causal factors without sacrificing explanatory precision or clinical utility.

Manipulating the microbiota for cancer therapy

While establishing etiology is central to this research, there is a closely related issue of investigating the effects of host-microbiota interactions on cancer therapies. Evidence that the microbiota or its metabolites modulate, or in some cases enable, the outcomes of cancer therapies is increasingly well-supported [6]. As microbes and the microbiota play the role of *therapeutic mediators*, this suggests that host physiology is not solely responsible for whether a cancer treatment is effective. Microbes are not just accidental aspects of host physiology that can be ignored or even eradicated, as is commonly done to treat or prevent opportunistic infections [94], possibly obscuring their beneficial roles in cancer [21]. In fact, they may sometimes be necessary for treating cancer. Set against the above history, the microbiota is perhaps less of a medical ‘breakthrough’ [95] than an increasingly promising and better understood therapeutic target.

What the microbiota can do to cancer therapies

While modulating host immune responses to tumors has long been a target of immunotherapies, it was only recently possible to ask whether a patient’s microbiota might undergird treatment efficacy. This intriguing role of the microbiota as a mediator has been confirmed, in part, by showing that antibiotic treatments reduce the efficacy of various cancer therapies: in the absence of commensal microbiota, the immune system will either not, or to a lesser degree, be activated by immunotherapies [70,71,96]. Returning to the research on immune checkpoint inhibitors (ICIs), antibiotic-induced gut dysbiosis can inactivate the antitumoral T cell responses, and re-introduction of several bacterial species, their proteins, and/or their metabolites appears to restore the activity of these therapies [71,97–99]. Moreover, while gut commensals seem to enhance some ICIs (anti-PD-1 or anti-PD-L1), they appear to render others possible (anti-CTLA-4) [100]. The exact mechanisms

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by which microbiota and/or their metabolites support immunotherapy efficacy remains under investigation [101].

The microbiota also appears to mediate conventional cancer therapies. For instance, alterations in gut commensals support the efficacy of total body irradiation (TBI) as a conditioning regime for adoptive T-cell transfer therapy [58], and the beneficial effects of TBI are reduced by antibiotics. Conversely, certain commensals may be important for patient survivability of whole body radiotherapy [102], and intestinal fungi appear to modulate antitumoral responses to radiation therapy in mice [103]. Synergistic antitumor effects seem to be driven by translocating bacteria from the gut into neighboring tissues and subsequently inducing immunostimulation [58], or by priming tumor-infiltrating myeloid cells [65]. Similar antitumor effects have been found with chemotherapies using oxaliplatin or cyclophosphamide: the antitumor immune responses are primed and/or enhanced by commensal bacteria [50,97,104]. For instance, cyclophosphamide disrupts gut mucosal integrity, thereby inducing the translocation of specific gram-positive bacteria into secondary lymphoid organs, which then stimulate the production of antitumoral Th17 cells and Th1 immune responses.

One key question is whether these responses are due less to the individual species found in the so-called ‘responders’ than to specific communities, ‘consortia’, or even the entire *ecosystem* with which these species are associated [70]. Deconvolving microbe-microbe and host-microbe interactions are necessary to address this in detail.

Redrawing the boundaries of therapeutic intervention

Using microbes to treat and better understand cancer fits within the longer historical traditions while expanding the current one [17,105–107]. A nuanced development, though, concerns the shift from using *exogeneous* microbes (e.g., Coley’s treatments) to manipulating *endogenous* ones to stimulate an antitumor immune response. In this sense, the extrinsic-intrinsic distinction concerning the microbiota’s relation to cancer is dissolving: the mechanism is at once external and internal, depending on one’s perspective.

However, a large problem for redrawing the therapeutic boundary becomes apparent when considering the context-dependency of microbes in carcinogenesis. For instance, while bacteria such as *H. pylori* and viruses such as herpesvirus and Epstein-Barr can all be carcinogenic, they can also cooperate with commensals and thereby offer some protection against other diseases and infections [48,108]. Evolutionary trade-offs will thus have to be carefully considered.

This ecological perspective motivates a systems-based approach for interrogating and mapping the interactions between microbes and cancer or immune cells, similar to how systems biologists have sought to systematically map protein-protein interactions and gene knockout effects on cell phenotypes. Understanding the manipulable nodes and edges in this multi-species network (including the host) can then guide rational interventions directed towards a specific goal (e.g., cancer eradication). For example, researchers are investigating how modulation of the estrobolome, or the aggregate of enteric bacteria that metabolize estrogen, can influence hormonal or metabolic pathways that promote

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tumorigenesis [109]. Microbial species with β-glucuronidase and β-glucuronide activities are manipulable ‘nodes’ in this network that facilitate estrogen metabolism and secretion. Targeting these microbial ‘nodes’ can modify the network so as to reduce the emergence of hormone-dependent cancers (Type I (endometrioid) endometrial cancer, estrogen receptor-positive breast cancer, and some ovarian cancers) [60]. However, what is needed to enable this form of systems medicine are precision tools that alter individual or groups of microbes rather than whole communities, which is currently limited by most antibiotics, prebiotics, postbiotics, dietary interventions, and fecal microbiota transplants [34,51,110]. Nonetheless, as the microbiota expands the cancer therapeutic armamentarium, oncologists are no longer simply manipulating or targeting host-centric, tumor-intrinsic properties.

Concluding remarks and future perspectives

This review aimed to elucidate the historical, conceptual, and scientific implications of recent data on the cancer-associated microbiota for oncology. We started with the notion that the microbiota comprises not simply another environmental factor among others that influences cancer growth and treatment but is intimately bound up with it. We then evaluated the relevance of this intimacy for oncology and explored its implications for establishing novel biomarkers, determining causality, and manipulating the microbiota to enhance or hinder treatments (see Outstanding Questions). We strove to capture the distinct conceptual aspects and therapeutic implications of this on-going research (Figure 2) while acknowledging its limitations.

Going forward, we encourage further refinement of the proposals that articulate microbiota-cancer-host ‘axes’ [18,92,111], ‘networks’ [72,86], and ‘systems’ [88–91]. Clarifying the precise components of these networks and how they interact holds promise to help contextualize novel biomarkers, to specify other causal mechanisms, and to rationally guide therapeutic interventions that focus on the most effective targets.

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Box 1: Microbiota-Cancer Causal Mechanisms

When viewed as cancer *pathogens*, we can distinguish direct and indirect mechanisms:

- Direct pathogenic mechanisms [18,73–75]: genotoxic or cytotoxic mutagenesis (colibactin, cytolethal distending toxins) via DNA alkylation, DNAse activity, and ROS/NOS production; activating β-catenin or PI3K/AKT pathways.
- Indirect pathogenic mechanisms [48,65,72,76,77]: following translocation, microbiota promote inflammatory γδ T cells, DNA damaging ROS-producing neutrophils; their metabolites can hinder immunosurveillance of human NK and T cell activity; following mucosal damage in gut, microbes regulate cytokines (e.g., IL-6, 11, 18, 22).

As tumoral *colonizers* and *migrators*, there are various potential mechanisms:

- Intratumoral TME-colonizing mechanisms [15,18,46,65,78]: producing genotoxins, T-cell mediated inflammation, suppressing local antitumor immunity (short-chain fatty acids (SCFAs) inducing Treg production), enzymatically aiding chemoresistance (bacterial cytidine deaminase degrading gemcitabine), activating the host's MBL-C3 axis.
- Mechanisms of migrators [61,63]: potentially intracellular migration with metastatic cancer cells; bacteria such as *E. coli* can open the gut vascular barrier and thereby translocate to the liver where they recruit immune cells (macrophages and inflammatory monocytes) and aid in the maturation of a premetastatic niche.

When viewed as cancer *regulators*, we find mechanisms for *promoting* and *inhibiting* tumorigenesis:

- Promoter mechanisms [18,33,48,65,77,78]: contributing to a pro-inflammatory microenvironment, stimulating IL-1 and IL23 from myeloid cells or IL-17 from Th17 cells; tumor-promoting pathogens escaping immune control (dysbiosis); activating inflammasomes (NLRP3,6) via SCFAs, in turn increasing tumor promoting IL-22 (positive feedback loop); promoting metastasis by upregulating tumor matrix metalloproteinases.
- Inhibiting mechanisms [79–83]: The microbes growing in and around tumors can inhibit growth through the production of anti-inflammatory metabolites, particularly SCFAs such as butyrate and propionate, which have been shown to affect gene expression, cell proliferation and cell death; mimicry between microbial or phage antigens and cancer antigens thereby causing antitumor immune response.

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Outstanding Questions Box

- Do tumor and blood microbial biomarkers vary between patients as much as the gut microbiota? If so, is it still possible to develop microbial meta-signatures that demonstrate robust performance across various cohorts?
- Are taxonomic compositions better biomarkers than microbial functional repertoires or metabolites in terms of predictive performance or generalizability?
- How should the field define causality when communities of microbes and/or their crosstalk with host pathways are frequently implicated?
- What kind of biological models can the field test or develop to demonstrate longitudinal effects of intratumoral microbes?
- How are microbes spatially distributed within tumors, and is it consistent between cancer types?
- How can we develop antimicrobial therapies that are tissue- or cancer type-specific?
- What explains the variation in microbial species between studies that appear to discriminate responders from non-responders to either conventional cancer therapies or immunotherapies?
- When is it more advantageous, if at all, to transplant whole communities of microbes rather than single species or small consortia from one host to another?
- To what extent do gut microbiota affect the metabolism of orally-delivered cancer medications and can their compositions be used as a companion diagnostic?
- To what extent do chemotherapies, radiotherapies, and immunotherapies change microbial metabolism and function within the gut and tumor?
- What will it take to standardize the design and use of prebiotics, probiotics, and other synergistic therapeutics as adjuvants in oncology?
- How can the field harness multi-omics experiments to further clarify the role(s) of the microbiota as passengers, causal agents, and/or therapeutic regulators?

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Highlights

- The role of cancer-associated microbes in carcinogenesis and oncological treatment has a long and controversial history.
- The existence and influence of proximal (intratumoral) and distal (various body sites) microbiota on cancer development and treatment challenge traditional representations of cancer that focus on tumor-intrinsic properties.
- We evaluate the current claims and challenges of the cancer-associated microbiota in terms of diagnosis, causality, and therapeutics.
- We propose a variety of conceptual and empirical distinctions to guide future research on the cancer-associated microbiota and its role in oncology.

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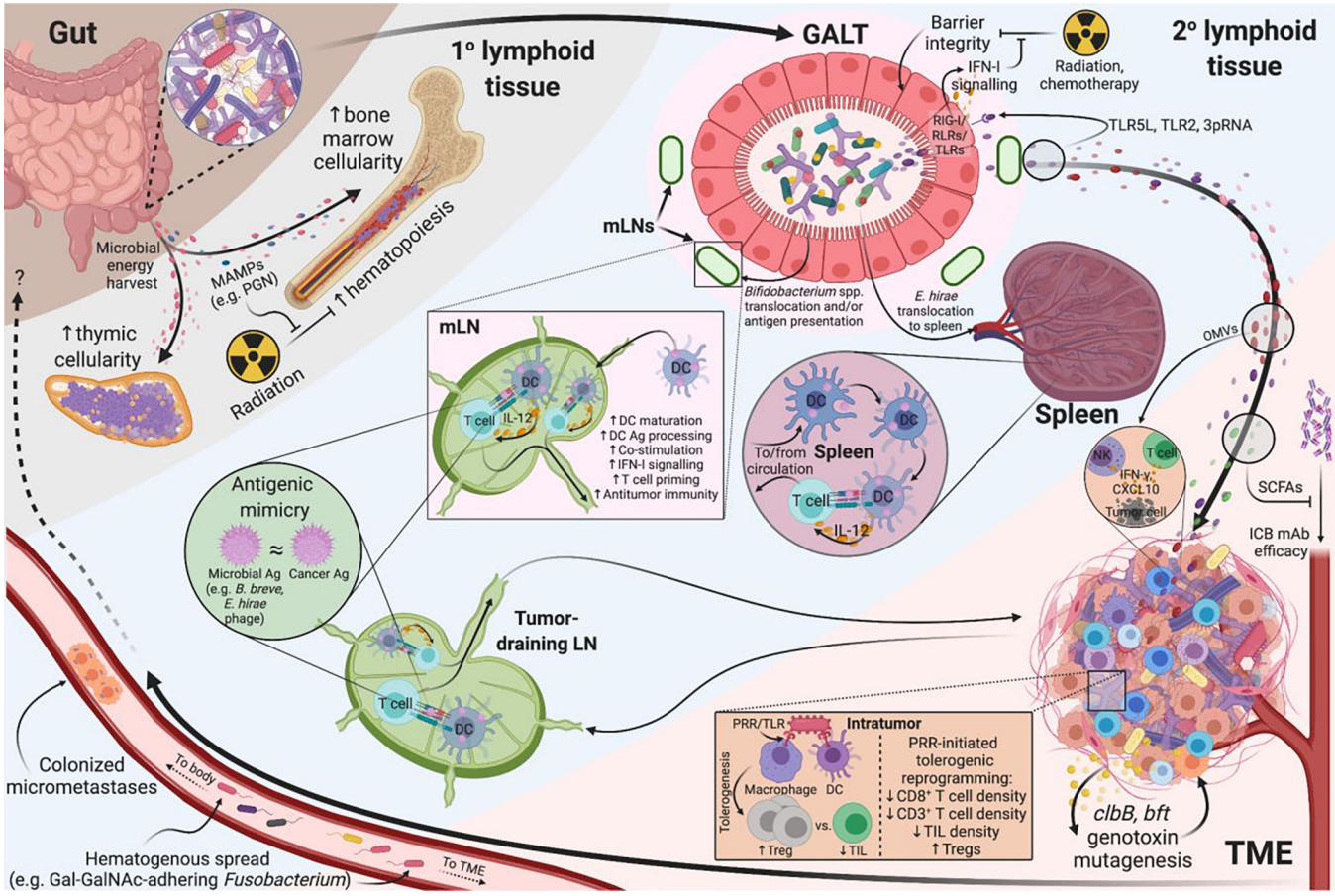

Figure 1. Microbiota-tumor-host interactions.

Visual depiction of some key mechanisms and causal pathways being uncovered within the host-tumor-microbiota network.

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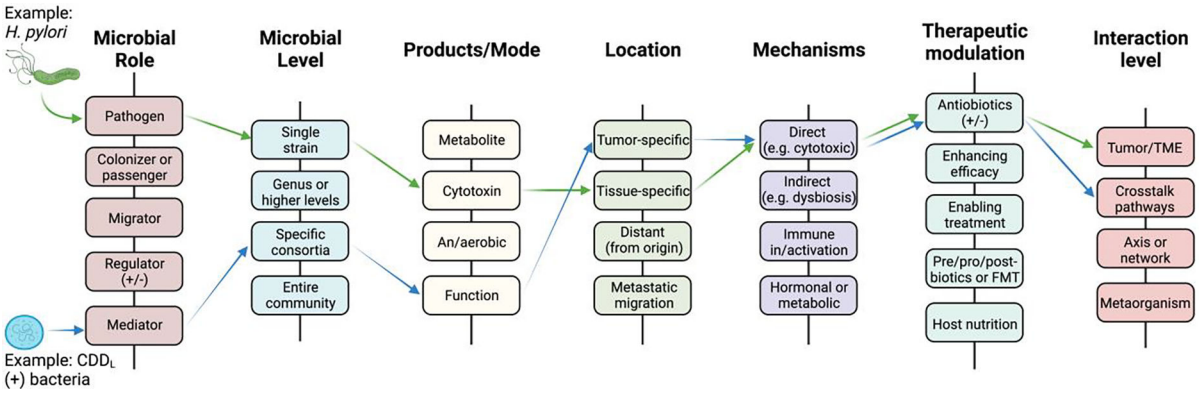

Figure 2. Microbiota-Cancer Conceptual Matrix.

This matrix, depicted as a parallel coordinates plot, reflects various conceptual and empirical issues in current microbiota-cancer research while engendering novel questions. For instance, research has shown how the microbiota can play different roles in tumorigenesis and treatment, but it remains an open question whether these roles are based on a particular microbial species, function, or anatomical location, and the precise mechanisms involved are often tentative due to the complexity of the cancer-host interactions. It further remains important to elucidate how these roles and levels intersect with immune-microbiota pathways and whether they are conserved amongst multiple microbes in a particular environment. Next, we can modulate treatments through the targeted use of antibiotics or host nutritional interventions and explore their efficacy in altering not only tumor- or tissue-specific microbiota, but also preventing microbial translocation and the formation of premetastatic niches. Finally, the complexity of causal mechanisms and therapeutic interventions increases as we shift analysis from the TME level to interacting networks or even the entire metaorganism.