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Tumor-resident microbes: the new kids on the microenvironment block

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Abstract

Tumor-resident microbes (TRM) are an integral component of the tumor microenvironment (TME). TRM can influence tumor growth, distant dissemination, and response to therapies by interfering with molecular pathways in tumor cells as well as with other components of the TME. Novel technologies are improving the identification and visualization of cell type-specific microbes in the TME. The mechanisms that mediate the role of TRM at the primary tumors and metastatic sites are being elucidated. This knowledge is providing novel perspectives for targeting microbes or using microbial interventions for cancer interception or therapy.

The complex community of microorganisms that reside within the human body functions as an independent organ that can have profound effects on the metabolic and immune systems of the host in health and disease [1,2]. The gastrointestinal tract contains the highest density of microbes in the human body, but sites such as the skin are also heavily exposed to and colonized by microorganisms [3]. The vast majority of microbes are commensals, defined as those that form a symbiotic relationship with the host, but in the setting of disease some microbes can also be pathogenic. The composition of the microbiota is closely regulated by the host through the local secretion of antimicrobial peptides, hormones, and cytokines by epithelial, stromal, and immune cells [4,5]. The microbiota aids in the digestion of nutrients to influence host and microbial metabolites which interact with the immune system and ultimately modulate its responses [6,7].

Microbes play a critical role influencing the pathogenesis of several diseases, and their role in cancer has been recognized in recent years [8,9]. The TME is a multifactorial ecosystem

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that encompasses cancer cells, immune cells (innate and adaptive), fibroblasts, adipocytes, neurons, blood vessels (endothelial cells), and extracellular matrix, among other components [10]. The crosstalk between the different components of the tumor and the microbiota orchestrates the organization of the TME and ultimately affects its functional processes and responses to therapies.

Tumor-resident microbes versus the tumor microbiome

Microbes that stably colonize tumors are called TRM, whereas the collection of microbial genomes detected in a tumor is known as the intratumoral microbiome (ITM). Based on tumor type and location, the ITM can exhibit higher biomass, such as in colorectal cancer (CRC) and other tumors that are directly exposed to the gastrointestinal lumen, or the ITM can present as a lower biomass, as in pancreatic cancer [11]. TRM are unique and distinct from resident microbes within an organism. The characterization of the ITM is commonly investigated by high-throughput sequencing, including 16S rRNA sequencing and metagenomic sequencing. By contrast, TRM detection is based on microbiological techniques based on live microbial culture and detection, such as *ex vivo* expansion, high-throughput screening, and subsequent microbial sequencing. A comprehensive analysis of the ITM has been more commonplace in the field, but more focus must be diverted to TRM to be able to identify functionally important microbes that influence tumor behavior.

Influence of microbes on tumorigenesis

Epithelial cells that have undergone malignant transformation recruit various types of cells to construct their TME. Most human solid tumors have been found to contain an intratumoral microbiome, and its composition has been extensively catalogued across cancer types [11]. It has been reported that the bacterial content is primarily intracellular and distributed between cancer and immune cells [11]. As mentioned in the previous section, not all microbial genomes detected in a tumor correspond to viable organisms that stably reside in a tumor. Microbial species such as *Helicobacter pylori*, polyketide synthase-positive (*pks*$^{+}$) *Escherichia coli*, enterotoxigenic *Bacteroides fragilis* (ETBF), and *Fusobacterium nucleatum* have been recognized for their oncogenic potential (Figure 1A). These microbes mediate pathogenic effects by exerting genotoxicity, recruiting protumorigenic immune cells, and altering the efficacy of therapeutics through chemical modifications.

H. pylori is a pathogenic microbe that can cause gastritis and peptic ulcers [12], and has been further ratified as a risk factor for gastric cancer [13]. *H. pylori* utilizes the adhesin protein HopQ to bind to CECAM (carcinoembryonic antigen-related cell adhesion molecule) proteins in epithelial cells, resulting in translocation of its virulence factor CagA that triggers proinflammatory signaling pathways in the host [14]. A pathogenic genomic island in *E. coli*, termed *pks*, encodes a genotoxic peptide, colibactin, that promotes CRC in experimental mouse models by increasing adherence to epithelial cells [15] and by forming DNA adducts in adenine-rich regions [16,17]. Genotoxic *pks*$^{+}$ *E. coli* induces a distinct mutational signature in human-derived organoids that can also be found in a subset of human colorectal tumors [18]. ETBF is increased in precancerous CRC lesions such as familial adenomatous polyposis (FAP) [19] and promotes colonic tumorigenesis by

increasing interleukin-17 (IL-17)-dependent signaling in immune and tumor cells [20,21]. ETBF affects colonic epithelial cells by triggering a STAT3/NF-κB-dependent IL-17 proinflammatory signaling cascade that promotes colitis and colonic tumorigenesis, and recruits $CD4^+$ type 17 T helper (Th17) cells that contribute to ETBF-induce tumorigenesis. *F. nucleatum* is highly enriched in human CRC tumors and promotes tumorigenesis in experimental mouse models [22,23] by binding to E-cadherin through an adhesin protein, FadA, that activates the WNT/β-catenin signal transduction pathway in target cells [24]. *F. nucleatum* also utilizes a different adhesin protein, Fap2, to bind to galectins in target tumor cells, and this increases their proliferation through the TLR4/MyD88 signal transduction pathway by upregulating miR-21 [25,26]. *Fusobacterium* spp. have been detected in other tumors such as breast cancer [27] and pancreatic cancer [28], suggesting that *Fusobacterium* spp. may have a universal propensity to colonize tumors.

Overall, tumors appear to selectively enrich for microbes, and upon colonization they can influence the local environment and alter signal transduction in target cells. However, the factors that contribute to the generation of a microbial niche in tumors are not clearly established, and it is not known how microbes differentiate between normal and malignant cells.

Role of tumor microbes in metastasis

Commensal bacteria resident in tumors can affect the dissemination of cancer cells, but the mechanisms remain to be fully understood. Higher intratumoral microbial load enhances the metastatic ability of tumor cells in nasopharyngeal carcinoma [29]. Tumor microbes improve resistance of fluid shear stress by changing the actin cytoskeleton and can accelerate breast cancer metastasis, underscoring the crucial role of bacteria in driving tumor cell seeding at distant sites [30]. In a lung metastasis model, local bacterial depletion by antibiotic aerosolization improved the response to chemotherapy and suppressed melanoma lung metastasis [31]. The lung microbiota is constantly exposed to the respiratory tract microbiome that may mediate different prometastatic mechanisms than other endogenous microbiota in common metastatic sites such as liver, brain, and bone. Thus, the influence of the tumor site should be incorporated when considering the influence of exogenous microbiota on tumor seeding.

Intratumor bacterial signatures have also been identified that can predict local and distant cancer metastasis. An increased abundance of intratumor Neisseriaceae was reported to accelerate lymph node metastasis in tonsil oropharyngeal squamous cell carcinoma [32]. Specific tumor microbes such as *Brevundimonas* and *Staphylococcus* spp. are associated with the development of distant metastases in breast cancer [33]. *F. nucleatum* has been detected in primary tumor and metastatic sites in many cancer types [27,34,35]. *F. nucleatum* controls cell death processes such as apoptosis and autophagy which drive tumor metastasis and development [36,37]. In addition, *F. nucleatum* improves the interactions between tumor cells and endothelial cells that influence the intercellular adhesion and dissemination of cancer cells by activation of the NF-κB pathway and the release of cytokines such as CCL8, CXCL16 and IL-8 [38,39]. Currently, several strategies for targeting *F. nucleatum* are being explored that are based on genomic approaches or chemical

RNA modifications that can lead decreased prometastatic phenotypes and potentially aid in the prevention of tumor spreading [40] (Figure 1C).

Because tumor microbes have been widely described to accelerate tumor metastasis, further investigations will need to explore the similarities and differences between microbes resident in metastatic lesions. Which microbial species enable the spreading of cancer cells? The metastatic organotropism of microbes needs to be studied, and the premetastatic profiles should not be ignored. For the initiation of metastatic tumors, we need to discriminate between bacteria or cancer cells regarding which are the first to arrive. Do bacteria in primary tumors prime cancer cells for migration, or do they promote tumor cell seeding? Do particular bacteria translocate ahead of tumor cell dissemination and remodel the microenvironment of the site of metastasis? Recent studies have demonstrated that TRM can impair the gut vascular barrier and drive CRC dissemination into the liver. Tumor-resident *E. coli* can also migrate to the liver and recruit macrophages, neutrophils, and monocytes, leading to the formation of a premetastatic niche [41,42]. These findings suggest that TRM may traffic ahead of tumor cells and generate a premetastatic microenvironment by increasing vascular permeability. Because different tumors show different preference for metastatic sites, the tissue-dependent orchestration of pathogens and the trajectories of bacterial travel, including lymphatic and vascular routes, need to be fully understood. Further exploration of how microbes break through cellular barriers to enter and colonize distant organs will be essential.

Effect of tumor microbes on immunity

TRM are now being recognized as a crucial component of the TME. Evidence is accumulating about the potential role for bacteria in shaping immunity. TRM can signal through pattern recognition receptors (PRRs) that are critical sensors of microorganisms and are mostly expressed by innate immune cells. TRM can also induce immunostimulatory and immunosuppressive effects through microbe-derived metabolites. Because the interaction between microbes and the TME is dependent on the tissue type, the interplay between tumor microbes and immunity is complex. Distinct tumor microbial diversities and communities can influence cancer patient outcomes, and TRM can determine immune cell heterogeneity [43–46]. TRM can also affect the infiltration of immune cells, such as cytotoxic $CD8^+$ T cells, mucosa-associated invariant T cells, macrophages, and natural killer (NK) cells, which can modulate tumor responses to chemotherapy and immunotherapy [11,46–50].

Previous findings suggest that tumor microbiome diversity can predict outcome in pancreatic ductal adenocarcinoma (PDAC) patients [46]. Tumor microbial diversity in long-term survival (LTS) PDAC patients was significantly higher than that in short-term survival (STS) PDAC patients. The tumor microbiome from LTS PDAC patients induces $CD8^+$ T cell infiltration and activation that improves the antitumor immune response. How do TRM alter the immune response? As an example of specific microbial effect, *Bifidobacterium* has been described to migrate from the gut to the tumors and improve antigen-presenting capacity in dendritic cells through a STING- and IFN-β-dependent mechanism that sensitizes tumors to anti-CD47 immunotherapy [51]. Bacterial metabolism is dynamic and can provoke immune responses. The specific composition of the TRM can also improve the efficiency of immune

checkpoint blockade (ICB) therapy in preclinical models through metabolic reprogramming. Intratumoral *Lactobacillus reuteri* metabolizes dietary tryptophan to indole-3-aldehyde which activates the aryl hydrocarbon receptor in IFN-γ-producing $CD8^+$ T cells [6]. The intratumoral *Lactobacillus*–metabolite interaction can enhance antitumor immunity and improve the response to ICB in melanoma [6]. A recent study discovered that breast cancer patients who responded to immunotherapy had a higher abundance of Clostridiales in the gut and tumor tissues [52]. Intratumoral Clostridiales produce trimethylamine *N*-oxide and improve the efficacy of immunotherapy through $CD8^+$ T cell-dependent antitumor immunity and the induction of pyroptosis in cancer cells. In addition, intratumoral *Bifidobacterium pseudolongum*-derived inosine induces T cell activation and enhances the CRC response to immunotherapy because inosine signaling in T cells via the adenosine 2A receptor drives activation of type 1 T helper (Th1) cells and antitumor immunity [53].

By contrast, TRM can induce the secretion of inflammatory mediators that promote tumor development. They can increase the production of IL-β and IL-23 that drive $V\gamma6^+V\delta1^+$ γδ T cell proliferation and activation, as well as IL-17 release, which can accelerate the development of malignancies such as lung cancer [54]. Intratumoral *Porphyromonas gingivalis* increases the secretion of neutrophilic chemokines and neutrophil elastase which can contribute to PDAC progression [55]. *F. nucleatum* was found to induce tumor cells that secrete CCL20, which can polarize macrophages towards type 2 that are also involved in CRC metastasis [56]. In addition, tumor-resident fungi have been shown to induce IL-33 secretion that activates type 2 T helper (Th2) cells and type 2 innate lymphoid cells (ILC2s) in PDAC [57]. Depletion of fungi leads to diminished activation of Th2 and ILC2 cells, leading to suppression of tumor growth. Furthermore, a recent study showed that intratumoral viruses induce NK cell infiltration and improve the prognosis of soft tissue sarcoma patients [58]. Thus, in addition to bacteria, the immunoregulatory roles of other microorganisms such as fungi, viruses, and bacteriophages in carcinogenesis, metastasis, and cancer therapies should also be explored (Figure 1B).

Identification of the distribution of TRM and precise annotation of bacterial species can improve our understanding of the involvement of TRM in cancer biology. Multi-omic studies have suggested multiple links between TRM and immune modulation in different cancer types, and novel technologies such as spatial profiling and single-cell sequencing have been developed to identify and map bacteria in tumor compartments [59]. However, the crosstalk between cell type-specific bacteria and remodeling of the tumor immune microenvironment are not fully understood. Exploring the interactions between TRM and targeted molecules within complex cellular components such as cancer cells, immune cells, and fibroblasts will offer more clues towards understanding the effects that microbes may have on responses to chemotherapy or immunotherapy.

Microbial therapeutic interventions

Prebiotics, probiotics, and fecal microbiome transfer (FMT) have been considered as beneficial options for stimulating innate and adaptive immune responses which can further improve the efficacy of chemotherapy and immunotherapy. Owing to the low levels of neoantigen and the absence of tumor-infiltrating T cells, several cancers are insensitive

to ICB. Bacteria can target and penetrate tumors through motility, chemotaxis, and manipulation of inflammatory signaling pathways [60]. Could local delivery of bacteria or clearance of pathogenic bacteria alter tumor immunity and cancer regression? Could TRM-derived peptides stimulate T cell responses that improve immune response against tumors [61].

Programmed bacteria can carry nanobodies, release cytokines, and produce metabolites that could be delivered to both primary and metastatic tumor sites, and present promising strategies to trigger antitumor immunity [62–65]. Chemotherapy and radiation therapy can also damage the host microbiota, and, depending on the populations affected, these interventions may lead to undesired effects. Microbial depletion by antibiotics can have broad effects and can interfere with the diversity of the native microbiome of the host, and may thus be a ‘double-edged sword’ as a cancer therapy. Antibiotic treatment has been found to improve the response to chemotherapy and extend survival in PDAC patients, implying that clearance of pathogenic bacteria such as Gammaproteobacteria in the TME of PDAC may overcome drug resistance [66,67]. By contrast, antibiotics cause unfavorable responses to ICB in other type of cancers, suggesting that the microbiome of the host or the tumor is required for the stimulation/mobilization of immune system that is necessary to enhance immunotherapy [68–71]. Future endeavors will need to assess how antibiotics affect TRM and elucidate the microbe-associated molecular mechanisms that influence the priming of immune cells and antitumor immunity.

Microbe–drug interactions

Microbes play a pivotal role in the pathogenesis of cancer and likewise can modulate the host responses to therapies, but the effects are often variable and are dependent on the type of cancer, clinical parameters, and the type of therapy. *F. nucleatum* is a candidate oncomicrobe that can modulate the response to therapy. Yu *et al.* demonstrated that infection of CRC tumor cells with *F. nucleatum* activates the TLR4/MyD88 pathway and induces a switch from an apoptotic to an autophagic cell program by downregulating miR-18a/4802, leading to chemoresistance and cell survival upon treatment with chemotherapeutic agents such as oxaliplatin and 5-fluorouracil (5-FU) [72]. In another study, Zhang *et al.* showed that the efficacy of 5-FU monotherapy was reduced by *F. nucleatum*, and this effect was reversed by tumoral deletion of the apoptosis inhibitory protein BIRC3 (baculoviral IAP repeat-containing 3) [73]. A more recent study by LaCourse *et al.* focused on the antimicrobial properties of 5-FU, especially against *F. nucleatum*, as part of the antitumoral mechanism [74]. They also found that *ex vivo* intratumoral microbiota derived from a subset of CRC patients and specific microbes such as *E. coli* have the capacity to metabolize 5-FU when cocultured [74]. The Gammaproteobacteria class of bacteria, which are highly enriched in PDAC tissue, have also been shown to metabolize the chemotherapeutic drug gemcitabine into its inactive form via the long isoform of bacterial cytidine deaminase in preclinical mouse models of CRC [75].

In the context of immunotherapy, *F. nucleatum* has been linked to variable effects. Gao *et al.* found that CRC patients with higher levels of intratumoral *F. nucleatum* had improved survival when treated with PD-1 blockade compared to patients with lower *F. nucleatum*

burden [76]. They validated these findings in preclinical mouse models and human-derived organoids, and showed that *F. nucleatum* increased tumoral PD-L1 expression by activating the STING signaling pathway and infiltration of cytotoxic T cells [76] (Figure 1D).
In support, a recent clinical trial achieved complete clinical response with neoadjuvant immunotherapy in a cohort of patients with locally advanced rectal adenocarcinoma and MSI (microsatellite instability) status [77], which are known to have a higher tumoral *F. nucleatum* burden compared to tumors with MSS (microsatellite stability) status [45,78]. However, a recent study by Jiang *et al.* showed that a higher tumoral *F. nucleatum* burden is associated with decreased efficacy of PD-1 blockade in two cohorts of CRC patients [79]. Using preclinical models of CRC, they further demonstrated that *F. nucleatum* decreased the cytotoxicity of $CD8^{+}$T cells by producing succinic acid, and this effect was abrogated by antibiotic treatment [79]. Correlative analyses have reported an inverse correlation between intratumoral *F. nucleatum* and enrichment of tumor-infiltrating lymphocytes (TILs) in MSI^{high} CRC patients compared to non-MSI^{high} CRC patients [45,80]. These studies reveal that the role of *Fusobacterium* in modulating therapeutic responses in CRC is variable and appears to depend on whether the therapy targets malignant cells or the immune system. It also remains unclear whether *Fusobacterium* mediates its effects locally in the TME, through its interactions with other microbes, or via systemic effects permeated through the metabolic system. Improved understanding of the downstream mechanisms targeted by intratumoral microbiota could lead to development of directed therapies that could improve antitumor effects.

Tumor neoantigens have also been reported to trigger crossreactive T cells when these neoantigens possess a high degree of similarity to microbial antigens. Fluckiger *et al.* identified a major histocompatibility class I (MHC-I)-restricted intestinal enterococcal bacteriophage that boosted T cell responses and led to improved chemotherapy and immunotherapy responses in melanoma [81]. Another study found homology between T cell receptors (TCRs) and immunogenic infectious disease-derived epitopes in rare long-term survivors of pancreatic cancer [82], suggestive of molecular mimicry.

Overall, the intratumoral microbiota is critical for the cancer patient outcomes because they can modulate host responses to therapies and present a novel avenue for identifying actionable targets.

Concluding remarks

The field of microbiome research in cancer is moving beyond association towards causation and mechanisms. This is being achieved by mechanistic studies that evaluate how microbes, especially those that are viable and reside in the tumors, affect the TME, the development of metastases, and responses to therapies. Studies on the effects of known oncomicrobes have been popular because they can not only be tested using *in vitro* assays but can also be manipulated *in vivo* as a single agent to understand their influence on the tumor ecosystem. However, studying the role of the entire microbiota on tumor progression is much more challenging. Such studies can often only be conducted with *in vivo* preclinical models or clinical specimens. In addition, we are currently limited because the technological tools at

our disposal are unable to independently modulate tumor microbes without affecting other microbial populations and host systems.

Given the importance of tumor microbes during tumorigenesis, tumor progression, and metastasis, efforts are now focused on better understanding the underlying mechanisms (see Outstanding questions). This knowledge will eventually aid in the development of microbe-based therapeutics. Furthermore, improved understanding of the host processes modulated by TRM could identify novel molecules/pathways which could be targeted to promote antitumor responses.

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References

1. Nicholson JK et al. (2012) Host–gut microbiota metabolic interactions. Science 336, 1262–1267 [PubMed: 22674330]
2. Zheng D et al. (2020) Interaction between microbiota and immunity in health and disease. Cell Res. 30, 492–506 [PubMed: 32433595]
3. Turnbaugh PJ et al. (2007) The human microbiome project. Nature 449, 804–810 [PubMed: 17943116]
4. Liu Q et al. (2020) Staphylococcus epidermidis contributes to healthy maturation of the nasal microbiome by stimulating antimicrobial peptide production. Cell Host Microbe 27, 68–78 [PubMed: 31866425]
5. Ivanov II et al. (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139, 485–498 [PubMed: 19836068]
6. Bender MJ et al. (2023) Dietary tryptophan metabolite released by intratumoral *Lactobacillus reuteri* facilitates immune checkpoint inhibitor treatment. Cell 186, 1846–1862 [PubMed: 37028428]
7. Fidelle M et al. (2023) A microbiota-modulated checkpoint directs immunosuppressive intestinal T cells into cancers. Science 380, eabo2296 [PubMed: 37289890]
8. Cullin N et al. (2021) Microbiome and cancer. Cancer Cell 39, 1317–1341 [PubMed: 34506740]
9. Sepich-Poore GD et al. (2021) The microbiome and human cancer. Science 371, eabc4452
10. Anderson NM and Simon MC (2020) The tumor microenvironment. Curr. Biol 30, R921–R925 [PubMed: 32810447]
11. Nejman D et al. (2020) The human tumor microbiome is composed of tumor type-specific intracellular bacteria. Science 368, 973–980 [PubMed: 32467386]
12. Marshall BJ and Warren JR (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1, 1311–1315 [PubMed: 6145023]
13. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (1994) Schistosomes, Liver Flukes and Helicobacter pylori, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol. 61. International Agency for Research on Cancer
14. Javaheri A et al. (2016) Helicobacter *pylori* adhesin HopQ engages in a virulence-enhancing interaction with human CEACAMs. Nat. Microbiol 2, 16189 [PubMed: 27748768]
15. Arthur JC et al. (2012) Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338, 120–123 [PubMed: 22903521]
16. Wilson MR et al. (2019) The human gut bacterial genotoxin colibactin alkylates DNA. Science 363, eaar7785 [PubMed: 30765538]
17. Xue M et al. (2019) Structure elucidation of colibactin and its DNA cross-links. Science 365, eaax2685 [PubMed: 31395743]

18. Pleguezuelos-Manzano C et al. (2020) Mutational signature in colorectal cancer caused by genotoxic pks^{+} *E. coli*. Nature 580, 269–273 [PubMed: 32106218]
19. Dejea CM et al. (2018) Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. Science 359, 592–597 [PubMed: 29420293]
20. Wu S et al. (2009) A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat. Med 15, 1016–1022 [PubMed: 19701202]
21. Chung L et al. (2018) *Bacteroides fragilis* toxin coordinates a pro-carcinogenic inflammatory cascade via targeting of colonic epithelial cells. Cell Host Microbe 23, 203–214 [PubMed: 29398651]
22. Castellarin M et al. (2012) *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. Genome Res. 22, 299–306 [PubMed: 22009989]
23. Kostic AD et al. (2013) *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 14, 207–215 [PubMed: 23954159]
24. Rubinstein MR et al. (2013) *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell Host Microbe 14, 195–206 [PubMed: 23954158]
25. Abed J et al. (2016) Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. Cell Host Microbe 20, 215–225 [PubMed: 27512904]
26. Yang Y et al. (2017) *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating Toll-like receptor 4 signaling to nuclear factor-kappab, and up-regulating expression of microRNA-21. Gastroenterology 152, 851–866 [PubMed: 27876571]
27. Parhi L et al. (2020) Breast cancer colonization by *Fusobacterium nucleatum* accelerates tumor growth and metastatic progression. Nat. Commun 11, 3259 [PubMed: 32591509]
28. Mitsuhashi K et al. (2015) Association of *Fusobacterium* species in pancreatic cancer tissues with molecular features and prognosis. Oncotarget 6, 7209–7220 [PubMed: 25797243]
29. Qiao H et al. (2022) Association of intratumoral microbiota with prognosis in patients with nasopharyngeal carcinoma from 2 hospitals in China. JAMA Oncol. 8, 1301–1309 [PubMed: 35834269]
30. Fu A et al. (2022) Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. Cell 185, 1356–1372 [PubMed: 35395179]
31. Le Noci V et al. (2018) Modulation of pulmonary microbiota by antibiotic or probiotic aerosol therapy: a strategy to promote immunosurveillance against lung metastases. Cell Rep. 24, 3528–3538 [PubMed: 30257213]
32. Rajasekaran K et al. (2021) The microbiome of HPV-positive tonsil squamous cell carcinoma and neck metastasis. Oral Oncol. 117, 105305 [PubMed: 33905914]
33. Chiba A et al. (2020) Neoadjuvant chemotherapy shifts breast tumor microbiota populations to regulate drug responsiveness and the development of metastasis. Mol. Cancer Res 18, 130–139 [PubMed: 31628201]
34. Li Z et al. (2021) *Fusobacterium nucleatum* predicts a high risk of metastasis for esophageal squamous cell carcinoma. BMC Microbiol. 21, 301 [PubMed: 34717543]
35. Bullman S et al. (2017) Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. Science 358, 1443–1448 [PubMed: 29170280]
36. Chen G et al. (2023) *Fusobacterium nucleatum* outer membrane vesicles activate autophagy to promote oral cancer metastasis. J. Adv. Res, Published online April 13, 2023. 10.1016/j.jare.2023.04.002
37. Chen Y et al. (2020) *Fusobacterium nucleatum* promotes metastasis in colorectal cancer by activating autophagy signaling via the upregulation of CARD3 expression. Theranostics 10, 323–339 [PubMed: 31903123]
38. Zhang Y et al. (2022) *Fusobacterium nucleatum* promotes colorectal cancer cells adhesion to endothelial cells and facilitates extravasation and metastasis by inducing ALPK1/NF-κB/ICAM1 axis. Gut Microbes 14, 2038852 [PubMed: 35220887]

39. Guo S et al. (2020) Exosomes derived from *Fusobacterium nucleatum*-infected colorectal cancer cells facilitate tumour metastasis by selectively carrying miR-1246/92b-3p/27a-3p and CXCL16. Gut 70, 1507–1519
40. Yang M et al. (2023) Targeting *Fusobacterium nucleatum* through chemical modifications of host-derived transfer RNA fragments. ISME J. 17, 880–890 [PubMed: 37005460]
41. Bertocchi A et al. (2021) Gut vascular barrier impairment leads to intestinal bacteria dissemination and colorectal cancer metastasis to liver. Cancer Cell 39, 708–724 [PubMed: 33798472]
42. Carloni S et al. (2021) Identification of a choroid plexus vascular barrier closing during intestinal inflammation. Science 374, 439–448 [PubMed: 34672740]
43. Qiao H et al. (2022) Multi-omics integration reveals the crucial role of *Fusobacterium* in the inflammatory immune microenvironment in head and neck squamous cell carcinoma. Microbiol. Spectr 10, e0106822 [PubMed: 35862975]
44. Liu Z et al. (2023) Multi-omics analysis reveals intratumor microbes as immunomodulators in colorectal cancer. Microbiol. Spectr 11, e0503822 [PubMed: 36786568]
45. Mima K et al. (2015) *Fusobacterium nucleatum* and T cells in colorectal carcinoma. JAMA Oncol. 1, 653–661 [PubMed: 26181352]
46. Riquelme E et al. (2019) Tumor microbiome diversity and composition influence pancreatic cancer outcomes. Cell 178, 795–806 [PubMed: 31398337]
47. Zhu G et al. (2021) Intratumour microbiome associated with the infiltration of cytotoxic $CD8^+$ T cells and patient survival in cutaneous melanoma. Eur. J. Cancer 151, 25–34 [PubMed: 33962358]
48. Sheng D et al. (2023) The interaction between intratumoral microbiome and immunity is related to the prognosis of ovarian cancer. Microbiol. Spectr 11, e0354922 [PubMed: 36975828]
49. Serna G et al. (2020) *Fusobacterium nucleatum* persistence and risk of recurrence after preoperative treatment in locally advanced rectal cancer. Ann. Oncol 31, 1366–1375 [PubMed: 32569727]
50. Peng R et al. (2022) Gastric microbiome alterations are associated with decreased $CD8^+$ tissue-resident memory T cells in the tumor microenvironment of gastric cancer. Cancer Immunol. Res 10, 1224–1240 [PubMed: 35881964]
51. Shi Y et al. (2020) Intratumoral accumulation of gut microbiota facilitates CD47-based immunotherapy via STING signaling. J. Exp. Med 217, e20192282 [PubMed: 32142585]
52. Wang H et al. (2022) The microbial metabolite trimethylamine N-oxide promotes antitumor immunity in triple-negative breast cancer. Cell Metab. 34, 581–594 [PubMed: 35278352]
53. Mager LF et al. (2020) Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 369, 1481–1489 [PubMed: 32792462]
54. Jin C et al. (2019) Commensal microbiota promote lung cancer development via γδ T Cells. Cell 176, 998–1013 [PubMed: 30712876]
55. Tan Q et al. (2022) Periodontitis pathogen *Porphyromonas gingivalis* promotes pancreatic tumorigenesis via neutrophil elastase from tumor-associated neutrophils. Gut Microbes 14, 2073785 [PubMed: 35549648]
56. Xu C et al. (2021) *Fusobacterium nucleatum* promotes colorectal cancer metastasis through miR-1322/CCL20 axis and M2 polarization. Gut Microbes 13, 1980347 [PubMed: 34632963]
57. Alam A et al. (2022) Fungal mycobiome drives IL-33 secretion and type 2 immunity in pancreatic cancer. Cancer Cell 40, 153–167 [PubMed: 35120601]
58. Perry LM et al. (2023) Human soft tissue sarcomas harbor an intratumoral viral microbiome which is linked with natural killer cell infiltrate and prognosis. J. Immunother. Cancer 11, e004285 [PubMed: 36599469]
59. Galeano Nino JL et al. (2022) Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. Nature 611, 810–817 [PubMed: 36385528]
60. Fan JX et al. (2022) Progress of engineered bacteria for tumor therapy. Adv. Drug Deliv. Rev 185, 114296 [PubMed: 35439571]
61. Kalaora S et al. (2021) Identification of bacteria-derived HLA-bound peptides in melanoma. Nature 592, 138–143 [PubMed: 33731925]

62. Savage TM et al. (2023) Chemokines expressed by engineered bacteria recruit and orchestrate antitumor immunity. Sci. Adv 9, eadc9436 [PubMed: 36888717]
63. Canale FP et al. (2021) Metabolic modulation of tumours with engineered bacteria for immunotherapy. Nature 598, 662–666 [PubMed: 34616044]
64. Leventhal DS et al. (2020) Immunotherapy with engineered bacteria by targeting the STING pathway for anti-tumor immunity. Nat. Commun 11, 2739 [PubMed: 32483165]
65. Chowdhury S et al. (2019) Programmable bacteria induce durable tumor regression and systemic antitumor immunity. Nat. Med 25, 1057–1063 [PubMed: 31270504]
66. Mohindroo C et al. (2021) Antibiotic use influences outcomes in advanced pancreatic adenocarcinoma patients. Cancer Med. 10, 5041–5050 [PubMed: 34250759]
67. Fulop DJ et al. (2023) Association of antibiotic receipt with survival among patients with metastatic pancreatic ductal adenocarcinoma receiving chemotherapy. JAMA Netw. Open 6, e234254 [PubMed: 36951863]
68. Zhang X et al. (2021) Antibiotics modulate neoadjuvant therapy efficiency in patients with breast cancer: a pilot analysis. Sci. Rep 11, 14024 [PubMed: 34234229]
69. Derosa L et al. (2018) Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Ann. Oncol 29, 1437–1444 [PubMed: 29617710]
70. Pederzoli F et al. (2021) Is there a detrimental effect of antibiotic therapy in patients with muscle-invasive bladder cancer treated with neoadjuvant pembrolizumab? Eur. Urol 80, 319–322 [PubMed: 34053782]
71. Iida N et al. (2013) Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 342, 967–970 [PubMed: 24264989]
72. Yu T et al. (2017) *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. Cell 170, 548–563 [PubMed: 28753429]
73. Zhang S et al. (2019) *Fusobacterium nucleatum* promotes chemoresistance to 5-fluorouracil by upregulation of BIRC3 expression in colorectal cancer. J. Exp. Clin. Cancer Res 38, 14 [PubMed: 30630498]
74. LaCourse KD et al. (2022) The cancer chemotherapeutic 5-fluorouracil is a potent *Fusobacterium nucleatum* inhibitor and its activity is modified by intratumoral microbiota. Cell Rep. 41, 111625 [PubMed: 36384132]
75. Geller LT et al. (2017) Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 357, 1156–1160 [PubMed: 28912244]
76. Gao Y et al. (2021) *Fusobacterium nucleatum* enhances the efficacy of PD-L1 blockade in colorectal cancer. Signal Transduct. Target. Ther 6, 398 [PubMed: 34795206]
77. Cercek A et al. (2022) PD-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. N. Engl. J. Med 386, 2363–2376 [PubMed: 35660797]
78. Nosho K et al. (2016) Association of *Fusobacterium nucleatum* with immunity and molecular alterations in colorectal cancer. World J. Gastroenterol 22, 557–566 [PubMed: 26811607]
79. Jiang SS et al. (2023) Fusobacterium nucleatum-derived succinic acid induces tumor resistance to immunotherapy in colorectal cancer. Cell Host Microbe 31, 781–797 [PubMed: 37130518]
80. Hamada T et al. (2018) *Fusobacterium nucleatum* in colorectal cancer relates to immune response differentially by tumor microsatellite instability status. Cancer Immunol. Res 6, 1327–1336 [PubMed: 30228205]
81. Fluckiger A et al. (2020) Cross-reactivity between tumor MHC class I-restricted antigens and an enterococcal bacteriophage. Science 369, 936–942 [PubMed: 32820119]
82. Balachandran VP et al. (2017) Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature 551, 512–516 [PubMed: 29132146]

Highlights

Tumor resident microbes (TRM) influence the pathogenesis of cancer and metastases.

Antitumor immunity can be modulated by TRM.

The efficacy of chemotherapy or immunotherapy can be affected by TRM.

TRM can be manipulated for anticancer therapy.

Outstanding questions

What is the best strategy for detecting microbes in the TME?

Does manipulation of TRM affect cancer development and therapeutic responses?

What is the interplay between TRM and immune cells?

Is the burden or composition of TRM important for patient outcomes?

What is the role of TRM in cancer cell dissemination and seeding at distant sites?

Can targeting TRM influence the development of metastatic niches?

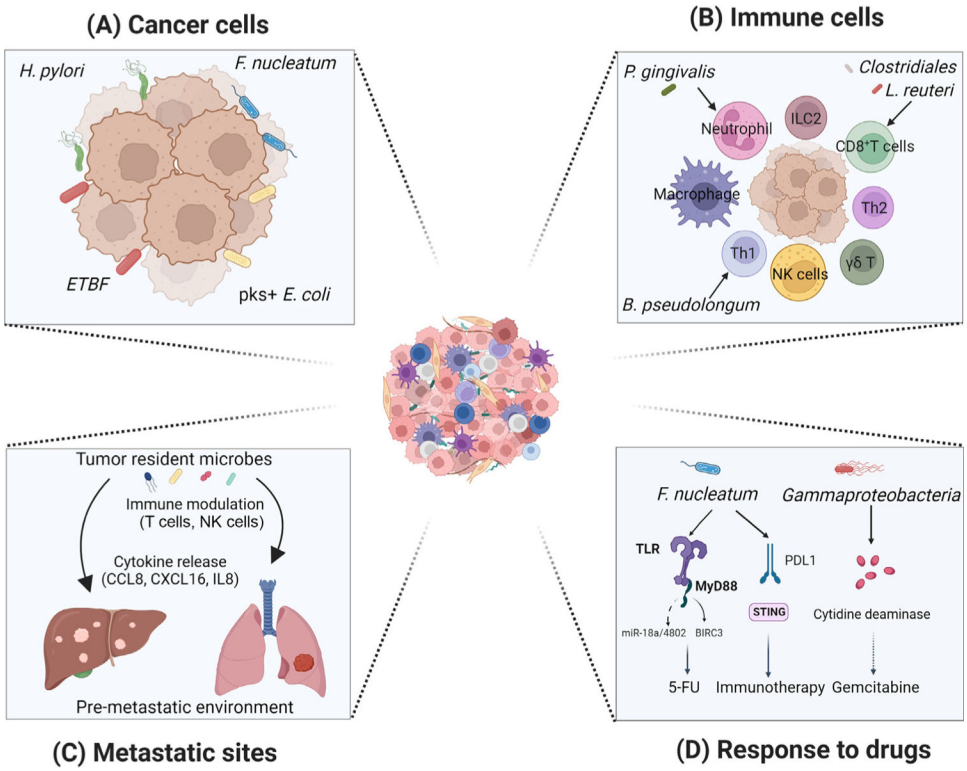

Figure 1. Effects of tumor-resident microbes (TRM) on (A) cancer cells, (B) immunity, (C) metastasis, and (D) responses to drugs.

Abbreviations: *B. pseudolongum, Bifidobacterium pseudolongum; E. coli*, *Escherichia coli*; ETBF, *enterotoxigenic Bacteroides fragilis*; *F. nucleatum*, *Fusobacterium nucleatum*; 5-FU, 5-fluorouracil; *H. pylori, Helicobacter pylori*; ILC2, type 2 innate lymphoid cell; NK cell, natural killer cell; *L. reuteri, Lactobacillus reuteri; P. gingivalis*, *Porphyromonas gingivalis*; Th1/2, type 1/2 T helper; TLR, Toll-like receptor.