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Review

Unveiling the intratumoral microbiota within cancer landscapes

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SUMMARY

Recent advances in cancer research have unveiled a significant yet previously underappreciated aspect of oncology: the presence and role of intratumoral microbiota. These microbial residents, encompassing bacteria, fungi, and viruses within tumor tissues, have been found to exert considerable influence on tumor development, progression, and the efficacy of therapeutic interventions. This review aims to synthesize these groundbreaking discoveries, providing an integrated overview of the identification, characterization, and functional roles of intratumoral microbiota in cancer biology. We focus on elucidating the complex interactions between these microorganisms and the tumor microenvironment, highlighting their potential as novel biomarkers and therapeutic targets. The purpose of this review is to offer a comprehensive understanding of the microbial dimension in cancer, paving the way for innovative approaches in cancer diagnosis and treatment.

INTRODUCTION

Cancer arises as a multifactorial disease influenced by a synergy of genetic factors, environmental conditions, and individual lifestyle choices. Recent advancements in cancer research have marked significant progress, particularly in the modulation of the cancer immune microenvironment, the exploration of immune checkpoints such as PD-1, PD-L1, CTLA-4, CD47, CD24, and CD39, and the application of CAR-T therapy.[1–3] These developments highlight a pivotal shift toward targeting the complex interplay between cancer cells and the immune system, aiming to enhance the body ability to recognize and combat malignancies. Additionally, novel research into mechanisms of cell death, including ferroptosis and cuproptosis, has also opened new avenues for therapeutic intervention.[4–6] Together, these cutting-edge approaches reflect the dynamic and rapidly evolving landscape of oncology research, with the potential to significantly improve patient outcomes, and pave the way for next-generation cancer therapies.[7,8] Variability in tumor bacterial content, shaped by genetic makeup and external environmental influences, contributes to divergent tumor structural and functional properties, complicating treatment approaches. The tumor microenvironment constitutes the ecological niche enveloping the tumor, encompassing adjacent blood vessels, immune cells, fibroblasts, bone-marrow-derived inflammatory cells, a range of signaling molecules, and the extracellular matrix (ECM). This microenvironment is pivotal in the initiation and advancement of cancer. Variability in tumor bacterial content, shaped by genetic makeup and external environmental influences, contributes to divergent tumor structural and functional properties, complicating treatment approaches.[9] The tumor microenvironment constitutes the ecological niche enveloping the tumor, encompassing adjacent blood vessels, immune cells, fibroblasts, bone-marrow-derived inflammatory cells, a range of signaling molecules, and the extracellular matrix (ECM). This microenvironment is pivotal in the initiation and advancement of cancer.

The intricate relationship between cancer and its microenvironment has long been a subject of intense scientific scrutiny. Recently, this exploration has expanded beyond the traditional focus on cancer cells and their immediate surroundings to include a less visible, yet potentially pivotal component—the intratumoral microbiota.[10–13] The evolution from focusing on the tumor microenvironment to the tumor microbe microenvironment (TMEM) marks a pivotal development in our understanding of cancer (Figure 1). This shift emphasizes the integral role of microbial communities, comprising bacteria, viruses, and fungi, which reside within tumors and significantly influence cancer progression, metastasis, and therapeutic outcomes. It highlights the intricate interactions between these microbes and the tumor's cellular components, including immune and stromal cells, fundamentally altering our perception of tumor behavior and potential treatment strategies. Moreover, this expanded view acknowledges how microbial presence within tumors can modulate the immune response, potentially impacting the effectiveness of immunotherapies. It also opens new avenues for research, suggesting that targeting the tumor microbe microenvironment could lead to innovative approaches in cancer treatment, including the development of microbiome-based therapies and diagnostics. This holistic understanding of the tumor microbe microenvironment is reshaping the landscape of cancer research, offering novel insights and promising directions for future therapeutic interventions. This review delves into the impact of intratumoral microbiota on cancer onset and progression, examining its potential in therapeutic and diagnostic applications. The review aims to underscore the potential of intratumoral microbiota as a diagnostic and prognostic tool in cancer patients, with the goal of improving cancer treatment outcomes.

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https://doi.org/10.1016/j.isci.2024.109893

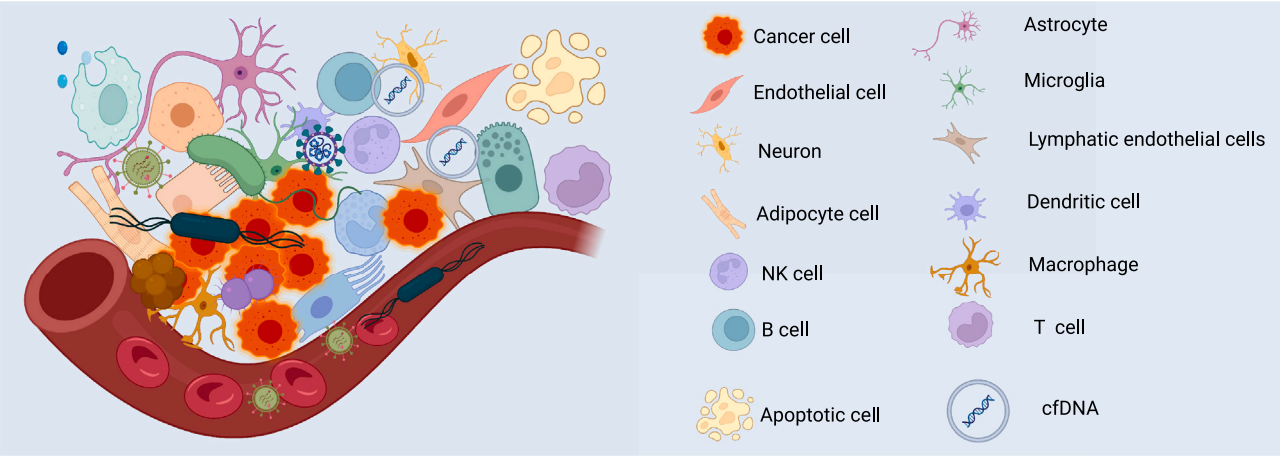

Figure 1. Components of the tumor microbe microenvironment (TMEM)

The traditional tumor microenvironment constitutes a multifaceted ecosystem composed of diverse tumor cells, stromal cells, and numerous immune cells, all situated within a network of irregular vasculature and collagen. The shift from understanding the tumor microenvironment to the tumor microbe microenvironment represents a significant advancement in cancer biology. This transition highlights the critical role of microbial communities, including bacteria, viruses, and fungi, within tumors, influencing cancer progression, metastasis, and response to therapy. It underscores the complex interactions between these microbes and other components of the tumor microenvironment, such as immune and stromal cells, altering traditional views of tumor dynamics and treatment approaches. Created with BioRender.com.

HISTORICAL PERSPECTIVE

The exploration of the role of microorganisms in cancer has a storied history, marked by periods of keen interest interspersed with episodes of skepticism. In the late 19th and early 20th centuries, the initial observations of bacteria in tumor tissues sparked a wave of speculation about their potential role in cancer development. Pioneers like William Russell (1852–1940) observed what he termed as "cancer parasites," igniting early debates on the microbial etiology of cancer.[14,15] However, due to the limitations in technology and understanding of cancer biology at that time, these ideas were not thoroughly pursued. The mid-20th century saw a resurgence of interest with the discovery of oncogenic viruses, lending credibility to the notion that microbes could be implicated in cancer. Studies on viruses such as the Epstein-Barr virus and human papillomavirus established a clear link between viral infections and certain types of cancer. Despite this progress, the focus remained largely on viruses, with bacteria and fungi receiving comparatively less attention.[16,17]

It was not until the advent of advanced genomic and molecular techniques in the late 20th and early 21st centuries that a more comprehensive picture began to emerge. The Human Microbiome Project and other similar initiatives have been instrumental in unraveling the complex interactions between the human body and its resident microorganisms. These developments have enabled scientists to detect and characterize microbial communities within tumor tissues with unprecedented precision, reigniting interest in the bacterial and fungal components of the tumor microenvironment.[18–26]

The 21st century has witnessed an explosion of research in this area, fueled by advancements in sequencing technologies. Studies have increasingly reported the presence of microbiota in tumors and their critical role in the tumor microenvironment and treatment outcomes.[27–29] The advent of next-generation sequencing has propelled intratumoral microbiota studies forward. In 2020, two significant studies made groundbreaking contributions: Poore et al. explored the diverse intratumoral microbiota across over 30 cancer types, suggesting a novel diagnostic approach based on microbiota.[27] Concurrently, Ravid Straussman's team conducted an extensive analysis of seven tumor microbiomes, revealing their spatial distribution and intracellular localization.[30] In 2022, this team further uncovered the distribution and synergistic effects of fungi in 35 cancers.[31] Simultaneously, Dohlman et al. analyzed The Cancer Genome Atlas data, identifying disease-related fungi in various cancers and investigating the role of fungal DNA in diagnosis and prognosis[32] (Figure 2).

ORIGINS AND DIVERSITY OF INTRATUMORAL MICROBIOTA

In recent times, the study of microorganisms within tumors has uncovered that a significant number of these microbes, part of the extensive 3.8 × 10^13 bacteria in the human intestine, can migrate to tumor sites via the bloodstream.[33] However, not all intratumoral microorganisms originate from the gut. Some other articles have discussed the origins, diversity, and the interrelationship between intratumoral and gut microbiota.[33–36] The sources of intratumoral microorganisms are diverse. They can infiltrate tumors through mucosal barriers in cancers like colorectal, pancreatic, lung, and cervical cancer, where mucosal destruction during tumorigenesis allows microbial invasion.[9,35,37–40] Another source is adjacent normal tissues, where bacteria found in organs previously considered sterile can be similar to those in tumor tissues.[41–43] Additionally, hematogenous spread can transport microorganisms from the mouth and intestines to tumor sites.[44] For example, *Escherichia coli* from the gut can enter the bloodstream and colonize the liver, promoting metastasis.[45] Intriguingly, tumor-related bacteria and fungi are predominantly located within cells, suggesting they may be transported as cellular fragments or intact cells.[31,34]

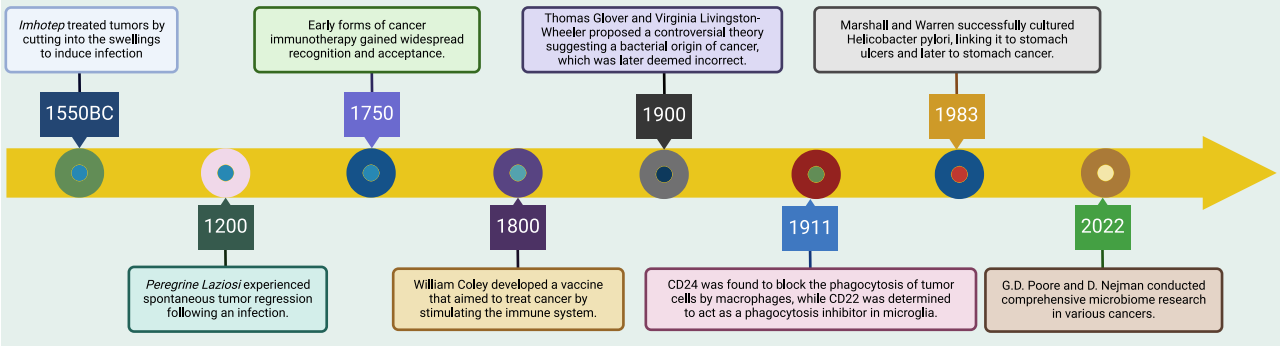

Figure 2. Overview of key research achievements of intratumoral microbiota, tracing back from 1550 BC to the current era

Today, the field stands on the threshold of a new era, where intratumoral bacteria and fungi are recognized not just as passive inhabitants, but as active influencers of tumor dynamics. This historical journey from early observations to current understandings not only highlights the evolution of scientific thought but also underscores the importance of interdisciplinary approaches in unraveling the complexities of cancer. Created with BioRender.com.

The composition of intratumoral microbiota varies across cancer types.[30] Ravid Straussman's team conducted extensive studies on various tumor microbiomes, revealing unique microbial compositions in each cancer type.[30,40,46,47] This diversity extends to fungal microbiomes in different cancers.[31,32,48,49] Although bacteria are more prevalent, fungi are also present in various tumors, with their species and localization varying by cancer type.[25,32,49–58] For instance, studies have shown distinct microbial communities in normal and tumor breast tissues and higher abundance of specific oral microbes in esophageal and gastric cancers than in adjacent tissues.[59,60]

Glioma

Traditionally, the microbiota associated with tumor tissues were thought to reside solely in tumors directly exposed to external environments, such as those in gastrointestinal cancers. Emerging evidence, however, suggests that cancers originating from organs considered "sterile" might also contain microbial populations.

Identifying the origins of intratumoral microbiota remains crucial for understanding their detection, association with tumorigenesis, and physiological roles. The precise sources of glioma microbiome are yet to be determined, with several hypotheses suggesting possible bacterial entry points into the brain, including pre-existing brain tissues, changes in the local microenvironment by gliomas, or microbial migration through compromised barriers post-tumorigenesis.[30,61–64]

A recent analysis provided an exhaustive characterization of the intratumoral microbiota across various cancer types, employing meticulous methods like histological staining, DNA sequencing, and tissue culture.[30] Significantly, this research marked the first identification of bacteria within glioblastoma multiforme (GBM) using histological evidence, revealing that these bacteria predominantly inhabit the cytoplasm of immune and tumor cells.

Moreover, this research illuminated the glioma intratumoral microbiome composition using advanced 16s rDNA sequencing, revealing a notable diversity within the GBM microbiome. Despite limited studies on the intratumoral microbiota in gliomas, emerging research underscores its potential significance in understanding glioma pathogenesis and exploring novel treatment avenues.

Oral cancer

The oral microbiome is host to over 750 commonly identified species, predominantly aerobes, with the proportion of anaerobes escalating in association with oral cancer (OC) development.[65] Oral squamous cell carcinoma, making up 90% of oral epithelial cancers, is often linked to human papillomavirus type 16, implicated in 25%–35% of cases.[66] Additional oncogenic viruses, such as Epstein-Barr virus (EBV) and herpes simplex virus 1 (HSV-1), have been identified as contributing factors to OSCC.[67,68] The presence of *Fusobacterium nucleatum*, part of the natural oral mucosal flora, has been associated with oral malignancies, with increased levels observed in OSCC compared to normal mucosa.[69] *Porphyromonas gingivalis* represents another independent significant risk factor for OC, with heightened levels noted in gingival squamous cell carcinoma and OSCC tissues, correlating with advanced-stage disease, poor differentiation, and lymph node metastasis in OSCC patients.[70,71] *Prevotella* species enrichment in OSCC tissue has also been reported, with *Prevotella intermedia* showing a notable increase and association with carcinogenesis.[72] Similarly, *Treponema denticola* has been closely associated with OSCC and oropharyngeal squamous cell carcinoma (OPSCC).[73,74] *Aerobic bacteria*, including *Streptococcus* species, have been linked to OC with elevated levels of *Streptococcus anginosus* in OC patients.[75] However, contrasting findings have been reported, such as an enrichment of Fusobacterium at tumor sites, with *Streptococcus* presenting opposite patterns.[76,77] Furthermore, *Pseudomonas aeruginosa* and *Campylobacter* sp. oral taxon 44 have been found in abundance in OSCC, along with *Candida albicans*-, *Candida etchellsii*-, and *Hannaella luteola*-like species.[78] The composition of the oral microbiota shifts with OC progression, featuring an increase in *Fusobacterium* and a decrease in *Streptococcus*, *Hemophilus*, *Porphyromonas*, and *Actinomyces* as the cancer advances.[79]

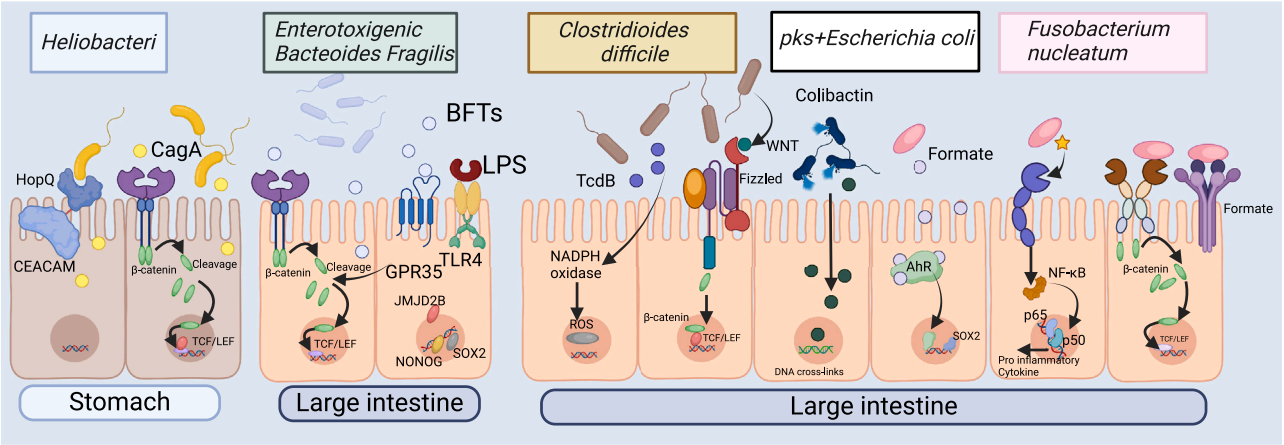

Figure 3. Proposed mechanisms by which dysbiosis contributes to the pathogenesis of non-small cell lung cancer (NSCLC)

An increased presence of specific bacterial species including Streptococcus, Prevotella, Veillonella, and *Chlamydia pneumoniae* reside in the lung TMEM. These bacteria may interact with epithelial cells and immune components, leading to activation of signaling pathways that favor tumor cell survival and proliferation. Bacterial antigens may activate Toll-like receptors (TLRs), which in turn can upregulate pro-inflammatory cytokines such as IL-1β and IL-23. The release of these cytokines could stimulate the production of IL-17 by T helper 17 (Th17) cells, contributing to a state of chronic inflammation that is conducive to tumorigenesis. This integrates the concepts of microbial influence on cellular signaling within the tumor microenvironment, emphasizing the potential for intratumoral bacteria to modulate immune responses and support the survival of malignant cells in NSCLC. Created with BioRender.com.

Breast cancer

Breast cancer is the most common cancer among women, and the role of microbiota in its development and progression has been extensively studied.[80–82] Breast tumors are known for their high bacterial diversity and abundance compared to other tumors. Research by Tzeng and others found a significant presence of *Pseudomonas* and *Proteus* in breast cancer tissues.[83] Xuan's team noted an increase of *Methylobacterium radiotolerans* in tumor tissues, contrasting with the prevalence of *Sphingomonas yanoikuyae* in normal tissues.[84] Interestingly, an inverse relationship was observed between the overall bacterial load at the tumor site and the stage of the tumor, hinting at potential diagnostic markers for breast cancer.[85] Further studies also indicated a heightened presence of *Methylobacterium radiotolerans* in tumor sentinel lymph nodes.[85] In contrast, Wang's findings showed a decrease in *Methylobacterium* abundance within breast cancer tissues.[86] Narunsky-Haziza et al. reported an increase in the *Cladosporium* genus in breast cancer patients aged 50 years and older.[31] The distinct intratumoral microbiota in breast cancer, predominantly including *Lactobacillus*, *Streptococcus*, and *Staphylococcus*, has been identified as a possible factor in tumor metastasis.[87] Additionally, research indicates that the microbial community composition varies by cancer subtype, with the Streptococcaceae family being more prevalent in triple-negative breast cancer and the genus Bosea becoming more abundant as the tumor progresses.[88,89]

Lung microbiota and NSCLC

Non-small cell lung cancer (NSCLC) is the most prevalent lung cancer and a leading cause of cancer-related mortality globally, making the understanding of all contributing factors, including the lung microbiome, crucial for public health. Although the precise impact of the lung microbiome on NSCLC is under-researched, recent findings indicate a correlation between bacterial infections, such as *Chlamydia pneumoniae*, chronic inflammation, and lung cancer development.[27,30] Specifically, in NSCLC, the presence of certain bacterial communities is linked to cancer-related gene activation patterns, including the ERK and PI3K signaling pathways. Preclinical models have been instrumental in revealing how microbiota may enhance lung cancer development.[90,91] Studies demonstrate that microbiota depletion reduces lung tumor growth in certain mouse models, suggesting that an imbalanced lung microbiome can create a pro-inflammatory, cancer-promoting environment, particularly through the activation of interleukin-17 (IL-17)-producing γδ T cells (Figure 3).[92]

Moreover, the detection of microbes in organs traditionally viewed as sterile suggests that a disruption in immune-microbial equilibrium could lead to chronic inflammation, which is a recognized risk factor for cancer. Persistent dysbiosis during cancer evolution could influence the immune system and patient outcomes. In NSCLC, where chronic inflammation is a known risk, further studies are necessary to understand the microbiota's role in cancer initiation and progression.

Gastrointestinal cancer

Specially, the relationship between gut microbiota and cancer progression has received considerable attention in scientific research. The gut microbiota is known to influence the production of specific metabolites and modulate the immune system, thereby affecting the microenvironment of distant organs.[45,93–97] An imbalance in this microbial community, known as dysbiosis, can disrupt the balance of the intestinal environment and is associated with both localized gastrointestinal and widespread systemic diseases. The intriguing links between fecal microbiota and numerous diseases have led to a heightened focus on the microbiota within oncological research. In the last two decades,

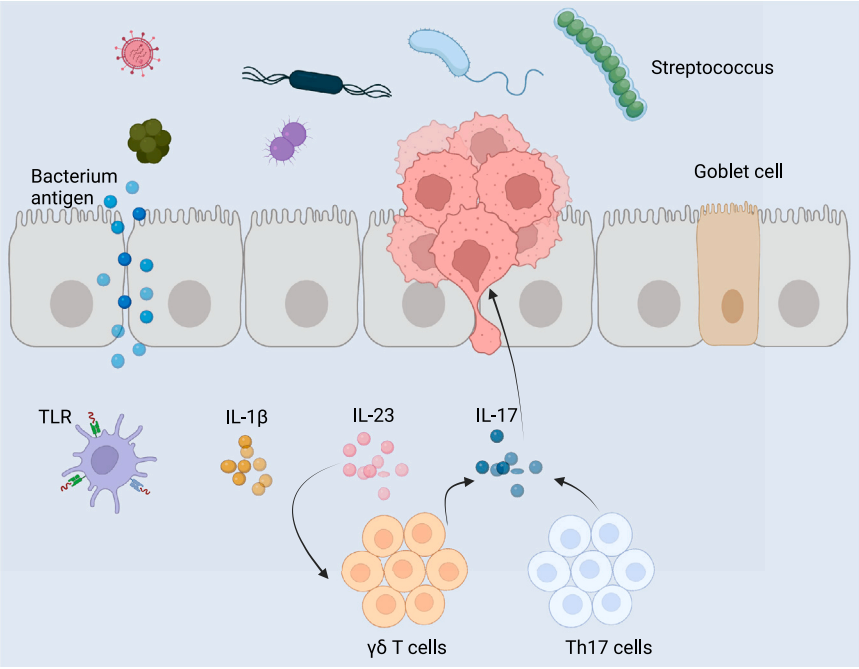

Figure 4. Processes of bacterial tumorigenesis in the gastrointestinal system include several key interactions

(Left) *Helicobacter pylori* attaches to gastric epithelial cells with the aid of the HopQ receptor and interacts with carcinoembryonic-antigen-related cell adhesion molecules, including CEACAM1, CEACAM3, CEACAM5, and CEACAM6. Its virulent component, CagA, generated by a type IV secretion system, influences the Wnt/β-catenin signaling pathway that governs cell division and cell death. When β-catenin enters the nucleus, it activates a suite of genes under the control of TCF/LEF transcription factors. (Middle) Enterotoxigenic *Bacteroides fragilis* (ETBF) produces a metalloproteinase toxin, BFT, that disrupts the tight junctions of intestinal cells, leading to the cleavage of E-cadherin, initiating a series of signals that result in MYC expression and continuous cell proliferation. ETBF's lipopolysaccharide boosts genes responsible for several key transcription factors like SOX2 and NANOG through TLR4 signaling and enhances JMJD2B expression. (Right) TcdB from *Clostridioides difficile* activates the Wnt/β-catenin pathway, though the full mechanism is not entirely understood. TcdB's glucosyltransferase domain causes cell death and triggers the NOX complex, which produces high levels of reactive oxygen species. Strains of *Escherichia coli* with the pks gene synthesize colibactin, leading to DNA damage that is distinct and mutagenic. *Fusobacterium nucleatum* uses the Fap2 adhesin to bind to sugars on cells and the FadA adhesin to interact with E-cadherin, encouraging cell growth via the Wnt/β-catenin pathway and elevating MYC levels, creating an inflammatory environment. *F. nucleatum*'s lipopolysaccharide promotes cancer cell growth and activates the pro-inflammatory nuclear factor κB (NF-κB) pathway. Additionally, nucleatum generates formate, stimulating the AhR pathway, which fosters tumor invasion and cancer cell renewal by increasing ALDH activity and SOX2 expression. CRC, colorectal cancer. Created with BioRender.com.

studies using preclinical models have shed light on how certain microbes, which are found in increased numbers within human cancerous tissues, promote the development of cancer through their direct impact on the transformation of epithelial cells into cancerous cells (Figure 4).

The relationship between intratumoral and gut microbiota is complex. Gut microbes can influence the tumor microenvironment, and intratumoral microbes can regulate host immune responses, impacting therapies like immune checkpoint blockade.[34,98–100] Studies indicate a positive correlation between fungal and bacterial abundance in several tumors, suggesting symbiotic relationships among fungi, bacteria, and immune cells within tumors.[34,101–103] These findings imply that gut microbes might interact with intratumoral microbes, possibly affecting each other's composition and function. However, further research is needed to fully understand these interactions and their implications for the tumor microenvironment (Table 1).

Current research has highlighted the influence of microbiota on the onset and advancement of cancer at sites like the lungs, which were previously thought to be low in microbial biomass in the absence of infection. The lungs, continuously exposed to the external environment, are prone to inflammation due to infections, allergens, pollutants, and smoking.

Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC), a lethal cancer with a low 5-year survival rate, prompts the search for early diagnostic and treatment methods, including the study of gut and tumor microbiota.[117] The pancreas may contain its own microbiota and produce antimicrobial peptides. These peptides can affect the microbiota within the pancreas as well as in the adjacent gut. Additionally, microbiota from the mouth, duodenum, and gut could seed the pancreatic microbiota. The impact of this on pancreatic function and disease susceptibility is still unclear.[118] Oral microbiota, linked to PDAC through various studies and associated with periodontitis, may contribute to PDAC pathogenesis, particularly in conditions like intraductal papillary mucinous neoplasms (IPMN), where oral bacteria and inflammatory signals are prevalent in

Table 1. Research associating gut microbiota profiles with the effectiveness of cancer immunotherapy treatments

Type of cancer	Microbiota	Immunotherapy	Effectors/ Targets/Pathway	Mouse/Human	Reference
Colon Cancer	*Bifidobacterium pseudolongum* *Lactobacillus johnsonii* *Olsenella* spp.*(inosine)*	PD-1, CTLA-4	A2AR signaling	Mouse	Mager et al.[104]
Melanoma	*Bifidobacterium longum*, *Collinsella aerofaciens* *Enterococcus faecium*	PD-1, PD-L1	DC, CD8+T cell, Treg	Mouse, human	Matson et al.; Sivan et al.[18,105]
Melanoma	*B. caccae* *B. thetaiotaomicron*, *Faecalibacterium prausnitzii* *Holdemania filiformis* *D. formicigenerans*	PD-1, CTLA-4	DC, Th1	Mouse, human	Frankel et al.[106]
Melanoma	*Clostridiales* *Ruminococcaceae*	PD-1	CD8+T cell	Mouse, human	Gopalakrishnan et al.[107]
Melanoma	*Bacillus fragilis*	CTLA-4	Th1, intratumoral DC	Mouse, human	Vétizou et al.[108]
Colon Cancer Melanoma		IL-2	TLR2	Mouse	Shi et al.[109]
Lung Cancer	*Akkermansia muciniphila*	PD-1, CTLA-4	CCR9+CXCR3+CD4+T cell, IL-2, IL- 12, IFN-γ	Mouse, human	Routy et al.; Derrien et al.; Derosa et al.[110–112]
Prostate Cancer		PD-1	GZMB+CD8+T cell, IFN-γ+CD8+T cell, M1-like macrophage	Mouse	Luo et al.[113]
Colon Cancer Melanoma	*Lactobacillus rhamnosus GG*	PD-1	DC, cGAS/STING,IFN-1, CD8+T cell	Mouse	Si et al.[114]
Pancreatic Cancer	*Short-chain fatty acids*	CAR-T	CD25,IFN-γ,TNF-α,ROR1	Mouse	Luu et al.[115]
Breast Cancer	*Staphylococcal enterotoxin B*	CAR-T	CAR-T cell	Mouse	von Scheidt et al.[116]

more severe disease stages.[118–120] Diverse oral microbiota, including both potentially protective and harmful bacteria, have been identified in PDAC tissues, with changes in fecal microbiota also observed in PDAC patients, suggesting the role of microbiota in disease progression but with high interindividual variability limiting early detection utility.[121] Translational and preclinical studies show the impact of microbiome on PDAC survival and treatment response, with intratumoral microbiota, particularly *Proteobacteria*, potentially promoting PDAC progression and therapeutic resistance, while certain bacteria consortia in long-term survivors may enhance survival through tumor microenvironment modulation.[22,24,40,104,105,122] The assessment of PDAC microbiome at therapy initiation may provide prognostic insights and guide experimental studies aimed at improving survival via microbiome modulation.

Others

Beyond the previously mentioned cancers, research has identified microbiota in various other tumors.

Intracranial tumors such as pituitary neuroendocrine tumors displayed varied microbial abundance by subtype, with different families enriched in specific PitNET types.[123,124] Studies on head-and-neck squamous cell carcinomas revealed a decrease in *Actinomyces* and an increase in *Parvimonas* compared to normal tissues, with HPV-16 also detected in HNSCCs, showing a notable exclusivity with mutations in TP53, CDKN2A, and telomerase reverse transcriptase within the tumor.[125,126] Nasopharyngeal carcinoma studies found *Corynebacterium* and *Staphylococcus*, with a negative correlation between total intratumoral bacterial load and prognosis.[123,127] Additionally, EBV was found in blood system tumors like Burkitt lymphoma, with human endogenous retroviruses expressed in chronic lymphocytic leukemia.[128–131]

The genitourinary tract cancers, including cancers of the adrenal, bladder, kidney, penile, prostate, and testicular, show emerging data suggesting the role of microbiome in disease etiology, with urine now recognized to host a microbiome influencing these cancers.[132] Bladder cancer's association with *Schistosoma haematobium* infection highlights a microbial role in genitourinary (GU) cancers, with additional evidence pointing to the contribution of urinary tract infections and diverse bacterial genera in bladder cancer development in non-endemic regions.[133–135]Prostate cancer studies reveal differentially abundant microbes with minimal overlap between studies, while kidney cancer research is less developed but indicates a potential link between urinary tract infections and increased risk, especially among male smokers.[136–138]

Ovarian cancer tissue showed higher levels of *Aquificae* and *Planctomycetes* but lower *Crenarchaeota*, with high-risk HPV types linked to advanced-stage tumors. *Bacteroides* and *Faecalibacterium* were associated with endometrial cancer, while certain bacteria, like *Cutibacterium acnes*, persisted in prostatic tissue. These findings underscore the diverse presence of microbiota in tumors, suggesting a need for further investigation into their role across different cancer types.

METHODOLOGICAL ADVANCES IN MICROBIOME ANALYSIS

Advancements in next-generation sequencing (NGS) techniques have precipitated a paradigm shift in our examination of the intratumoral microbiota.[9,139] This quantum leap in genomics has facilitated the intricate decoding of microbial communities residing within oncological environments.[140] The deployment of shotgun metagenomic sequencing has afforded us an unparalleled resolution in cataloging the genetic landscape of microbiome, thereby unmasking the functional attributes of microbial consortia within neoplastic tissues.[141] These techniques transcend traditional 16S rRNA gene sequencing by capturing a more extensive array of genetic material, permitting a granular assessment of microbial diversity.[142]

Furthermore, the utility of NGS in fostering functional analyses of the microbiome, encompassing metatranscriptomic, metaproteomic, and metabolomic studies, has yielded a multidimensional understanding of the metabolic pathways and bioactive compounds within the tumor microenvironment, providing insights into the intricate dialog between microbial inhabitants and oncogenic processes.[141,143,144]

The historical trajectory of experimental systems, marked by the evolution from rudimentary molecular techniques to sophisticated NGS platforms, has dramatically augmented our comprehension of the role of microbiome in carcinogenesis. From the seminal utilization of 16S rRNA gene sequencing to the advent of whole-genome shotgun sequencing, each technological advance has incrementally unveiled the complex interplay between the host and its endogenous microbial milieu. Such evolution is indispensable for the nuanced interpretation of the influence of microbiome on tumorigenesis and its prospective utility in the realms of cancer diagnosis and targeted therapy. As we venture forth, the onus lies in the refinement of these methodologies, which is anticipated to enhance the precision of our insights into the oncobiome and, by extension, to catalyze the development of novel diagnostic and therapeutic modalities.

INFLUENCE OF INTRATUMORAL MICROBIOTA ON TUMOR BIOLOGY AND IMMUNE RESPONSE

Role of intratumoral microbiota in cancer biology

The identification and characterization of intratumoral bacteria have been revolutionized by advances in genomic and molecular technologies. These advancements have enabled researchers to not only detect the presence of bacteria within tumor tissues but also understand their diversity and potential roles in cancer biology.

Tumors driven by microbial influences are estimated to constitute about 20% of all cancers globally.[145] Recent advancements in highly sensitive technologies have significantly enhanced the investigation of microbiomes in tissue samples, using contemporary sequencing methods.[9,42,46] Various studies employing metagenomic techniques have identified new pathogens enriched in different cancer types, compared to adjacent non-tumorous tissues or tissues from healthy individuals.[146,147] These studies have revealed microbial DNA signatures in tumors located in areas previously believed to be sterile, leading to the development of concepts such as tumor-specific colonic and laryngeal microbiomes.[30,148–152]

However, these associative studies often leave open the question of whether the detected microorganisms are merely coexisting with the tumor or actively contributing to its development and persistence. The complexity of these metagenomic studies and their association with cancers have sparked debates. Issues such as differing gut microbiome representations in fecal versus biopsy samples, the challenge of accurately attributing genes in metagenomic analyses, and identifying the origin of microbial genes in samples from paraffin-embedded tissues exemplify the difficulties faced.

Moreover, the low bacterial biomass in tumor samples poses a challenge in distinguishing true microbial signals from background contamination during DNA extraction. Variations in methodologies across different labs for sample extraction, processing, and data analysis can significantly influence results. For instance, the "kitome"—contaminants from different lots of DNA extraction kits—has been found to account for a significant portion of variance in metagenomic sequencing studies.[153]

The necessity of replicating findings across various studies and laboratories is crucial for establishing reliability in this field. Efforts to standardize and validate optimal sequencing protocols are ongoing and vital. Despite the field being in its early stages, it is evident that a range of organisms, potentially originating from various sources like oral plaque microbiomes, can be found in both metastatic and primary cancer sites. These organisms might contribute to tumor inflammation, either through hematogenous spread or through local migration.

This emerging field of research not only challenges our traditional understanding of cancer but also offers novel perspectives on how intratumoral bacteria might be leveraged for diagnostic, prognostic, and therapeutic purposes. The continued exploration and understanding of these microbial residents promise to add a new dimension to cancer biology and treatment.

The mammalian gut, hosting trillions of commensal bacteria, stands as one of the most sophisticated bacterial communities. This diverse ecosystem includes not only bacteria but also archaea, protists, fungi, and viruses, with bacteria being predominant. These microorganisms impact human health by producing essential metabolites, processing nutrients, and generating substances that inhibit pathogenic invaders and support beneficial microbes, further influencing the absorption of nutrients and neutralization of harmful agents. The gut microbiota's interaction with stromal and epithelial cells regulates numerous functions, including controlling pathogen invasion and growth, maintaining symbiosis and mucosal immune balance, managing metabolism, and serving as a protective barrier.[154,155]

Various body sites, including the skin, nasal passages, respiratory tract, breast ducts, vagina, and gastrointestinal tract, harbor diverse microbial communities. Bacteria regularly traverse the gastrointestinal (GI) mucosal barrier, entering the enterohepatic system. Some bacteria are even found concentrated in tumors, likely due to abnormal tumor vasculature allowing residency.[12] Advances in next-generation sequencing have greatly enhanced our understanding of these microbiotas, overcoming biases of traditional culture methods. The human gut microbiome, comprising as many organisms as human cells, has a vast genome, substantially larger than the human genome. Most gut microbiomes have co-evolved with their hosts, featuring metabolic pathways absent in host DNA.[156,157] The host's diet, immune system, and epithelial interactions shape the microbiome to meet nutritional needs. The human gut is predominantly populated by *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*.[158] Early attempts to categorize individuals based on gut microbiome composition led to the concept of "enterotypes," influenced by diet, geography, and individual factors. However, most microbiome variations seem to follow a continuum related to dietary patterns.[159,160]

While the individual microbiome is generally resilient, antibiotics can cause significant disruptions, and the effects of deliberate dietary changes are underexplored. Interactions among less abundant species may be crucial for maintaining the overall microbiome structure. Diet-related bacteria are linked to colon cancer risk, with plant-based diets associated with lower risk.[161,162] The microbiota-produced short-chain fatty acids, like acetate, propionate, and butyrate, have anti-inflammatory effects in the colon.[163–166]

Interplay between intratumoral microbiota and cancer development

The intricate mechanisms through which intratumoral bacteria interact with their host and influence cancer dynamics are central to understanding their role in oncology. This section delves into the various ways bacteria within tumors can affect cancer development, progression, and treatment outcomes.

Role of intratumoral microbiome in cancer initiation

Recent studies have solidified the critical link between the intratumoral microbiome and cancer development, though the precise mechanisms remain partially elusive. Three key mechanisms are proposed: direct promotion of tumorigenesis through increased mutation rates, manipulation of oncogenic signaling pathways, and the induction of inflammation and alteration of the host local immune microenvironment[10,46,167,168] (Figure 5).

Microbial metabolites like cytolethal distending toxin (CDT), colibactin, and *Bacteroides fragilis* toxin (BFT) are known to directly inflict DNA damage, thereby triggering mutations.[169,170] Notably, a substantial proportion of group *B2 Escherichia coli* isolates contain genomic islands responsible for producing colibactin, which can lead to double-strand DNA breaks, thus fostering genomic instability and hastening cancer development.[171–173] CDT, produced by certain gram-negative bacteria, is a multi-subunit protein causing DNA damage, with its subunit CdtB being particularly potent in inducing DNA breaks in a dose-dependent manner.[173–176] BFT, secreted by *Bacteroides fragilis*, enhances reactive oxygen species and DNA damage, thereby promoting tumorigenesis, and is also known to induce an inflammatory response crucial in colon and breast cancer development.[177–181]

Beyond merely damaging DNA, various microorganisms harbor proteins that influence host cellular processes, leading to alterations in host cell signaling and fostering cancer development. The Wnt/β-catenin signaling pathway, pivotal in controlling cellular attributes and implicated in many cancers, can be modulated by certain bacteria linked to cancer.[182–184] For instance, *Fusobacterium nucleatum* can produce FadA, a bacterial adhesion molecule, which activates β-catenin signaling by interacting with E-cadherin. This activation can selectively influence immune, inflammatory, and cancerous responses, thus promoting colorectal cancer.[183,185] Similarly, *Salmonella* produces *AvrA*, which, upon host cell invasion, modifies eukaryotic signaling pathways. It enhances β-catenin signaling by reducing β-catenin ubiquitination, increasing its phosphorylation, and amplifying its nuclear presence.[186–188] Enterotoxigenic *B. fragilis*, through its toxin BFT, specifically cleaves calreticulin, triggering nuclear β-catenin signaling. This activation boosts the expression and activity of the c-Myc proto-oncogene, contributing to colon tumor formation.[189] Besides the Wnt/β-catenin pathway, microorganisms can also promote cancer by affecting ERK and PI3K signaling pathways.[190] Tsay and colleagues found that lung cancer patients exhibited an increase in oral bacteria (*Streptococcus and Veillonella*) in their lower airways, correlated with the activation of the PI3K and ERK pathways.[191]

Inflammation is another critical link between microbiota and cancer. The dynamic balance between the commensal microbiota and the human immune system, when disrupted, can lead to proinflammatory or immunosuppressive responses that foster tumorigenesis.[192–195] For example, *Helicobacter pylori*'s CagA protein can activate STAT3, promoting cell proliferation and cancer development.[192,195,196] Similarly, lung commensal microbiota can stimulate inflammatory responses, contributing to neoplastic hyperplasia.[92] *Fusobacterium nucleatum* creates an inflammatory microenvironment conducive to intestinal tumorigenesis and can also inhibit natural killer (NK) and T cell activity, weakening anti-tumor immune responses.[197]

The dynamic interactions between intratumoral bacteria and the tumor microenvironment underscore the complexity of cancer as a disease. These microbial residents are not isolated entities; rather, they are an integral part of the cancer ecosystem, playing a significant role in the disease's pathophysiology. As our understanding of these interactions deepens, it paves the way for novel therapeutic approaches that target these microbial components of tumors (Figure 6).

Intratumor microbiota in cancer metastasis

Recent findings indicate that microorganisms within tumors can trigger cancer metastasis. Fu et al. identified that specific bacteria like *Staphylococcus*, *Lactobacillus*, and *Streptococcus*, prevalent in breast cancer cells, can disrupt the RhoA-ROCK signaling pathway. This disruption

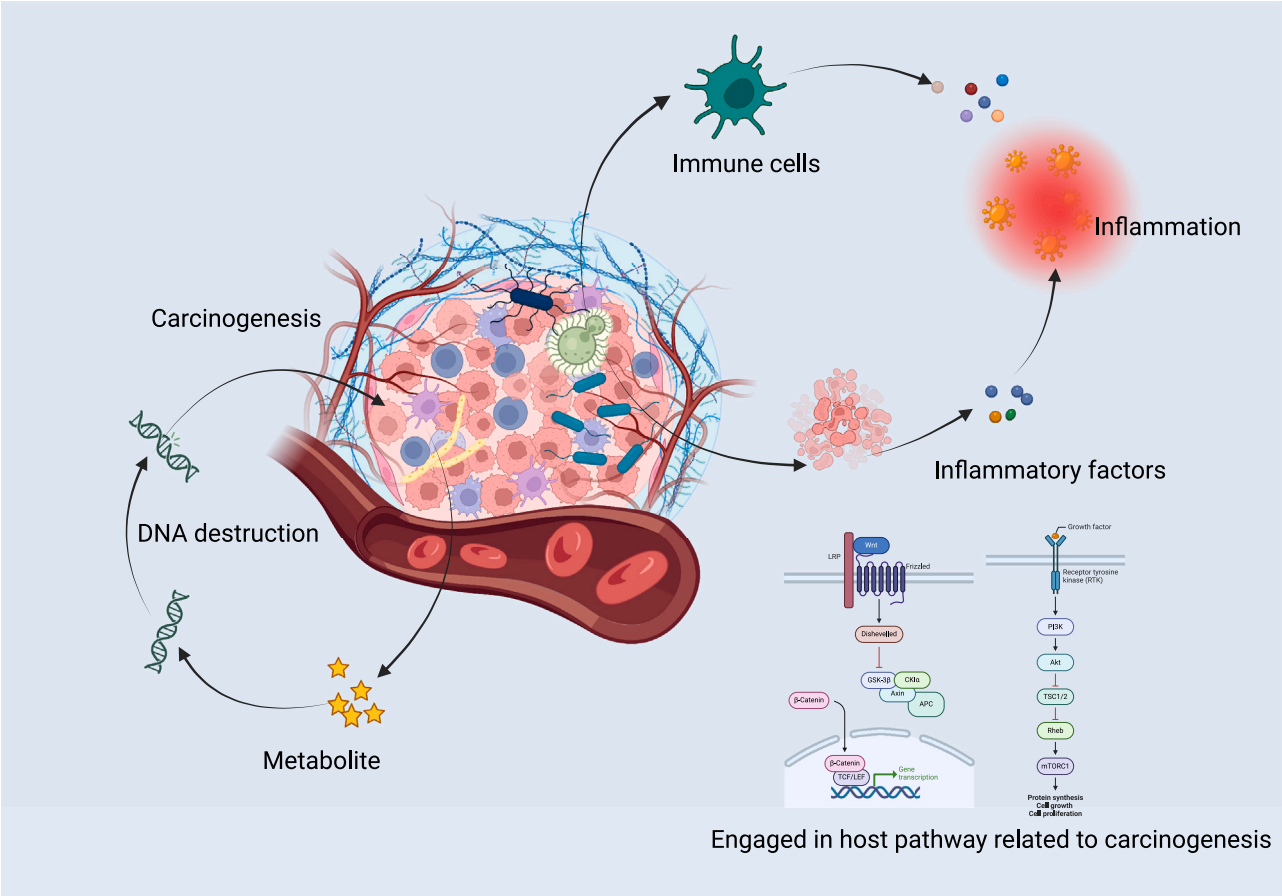

Figure 5. The connection between bacteria within tumors and cancer cells

While the exact processes by which these intratumoral bacteria contribute to cancer development are not fully understood, three main pathways are thought to be involved: first, the secretion of substances that promote genetic mutations; second, the interaction with host cellular pathways that play a role in the onset of cancer; and third, the induction of inflammation and modulation of the immune response, which can lead to the initiation of cancer. Created with BioRender.com.

alters the cell structure, helping cancer cells withstand the mechanical forces in blood vessels, thus aiding in metastasis.[87] Additionally, studies using germ-free and immunodeficient mice have shown that these intratumoral bacteria can promote cancer spread independently of gut flora and the immune system.[87]

This evidence suggests that various microorganisms present in different tumor types can drive cancer initiation, growth, and spread via multiple signaling pathways. These pathways are interconnected. Some microbes induce the production of toxins or reactive oxygen species (ROS), causing DNA mutations in host cells, leading to cancer.[81,198–200] Concurrently, the tumor-intrinsic β-catenin signaling pathway becomes activated, furthering malignant cell transformation.[201,202] This deterioration is exacerbated as this pathway is active in both host and tumor cells. Moreover, other tumor-specific pathways, such as MAPK, may be activated by intratumoral microbes either directly or indirectly, through upstream signaling or by activating elements downstream of TLRs.[203–206] This leads to NF-κB activation and cytokine production, forming a feedback loop that sustains chronic inflammation conducive to tumor growth.[207,208]

While the exact process through which intratumoral bacteria influence cancer metastasis remains uncertain, recent research suggests that exosomes released by bacteria-infected cancer cells could be a key mechanism (Figure 7). Exosomes, which are 40–100 nm vesicles exhibiting 5′-nucleotidase activity, are produced by various cell types in culture. Containing a diverse array of proteins, lipids, and RNAs, these vesicles facilitate cellular communication among different cell types, thereby impacting both normal and diseased states.[209–211] Exosomes originating from tumors are capable of transferring miRNAs and proteins to healthy tissues, and they contribute to cancer spread through several pathways. These include altering the tumor microenvironment, enhancing tumor cell growth while inhibiting programmed cell death, promoting the transition from epithelial to mesenchymal cell states, weakening anti-cancer immune responses, and supporting the spread of cancer via blood and new blood vessel formation.[212,213] It is particularly noteworthy that numerous studies have identified that cancer cells infected with bacteria tend to release more exosomes, thereby potentially increasing the speed of tumor metastasis.[213–215]

These intratumoral microorganisms also influence tumor cell metabolism, leading to epithelial-mesenchymal transition (EMT) and migration.[87,216] During hematogenous metastasis, they regulate the cytoskeleton, helping tumor cells withstand blood flow pressure, enabling

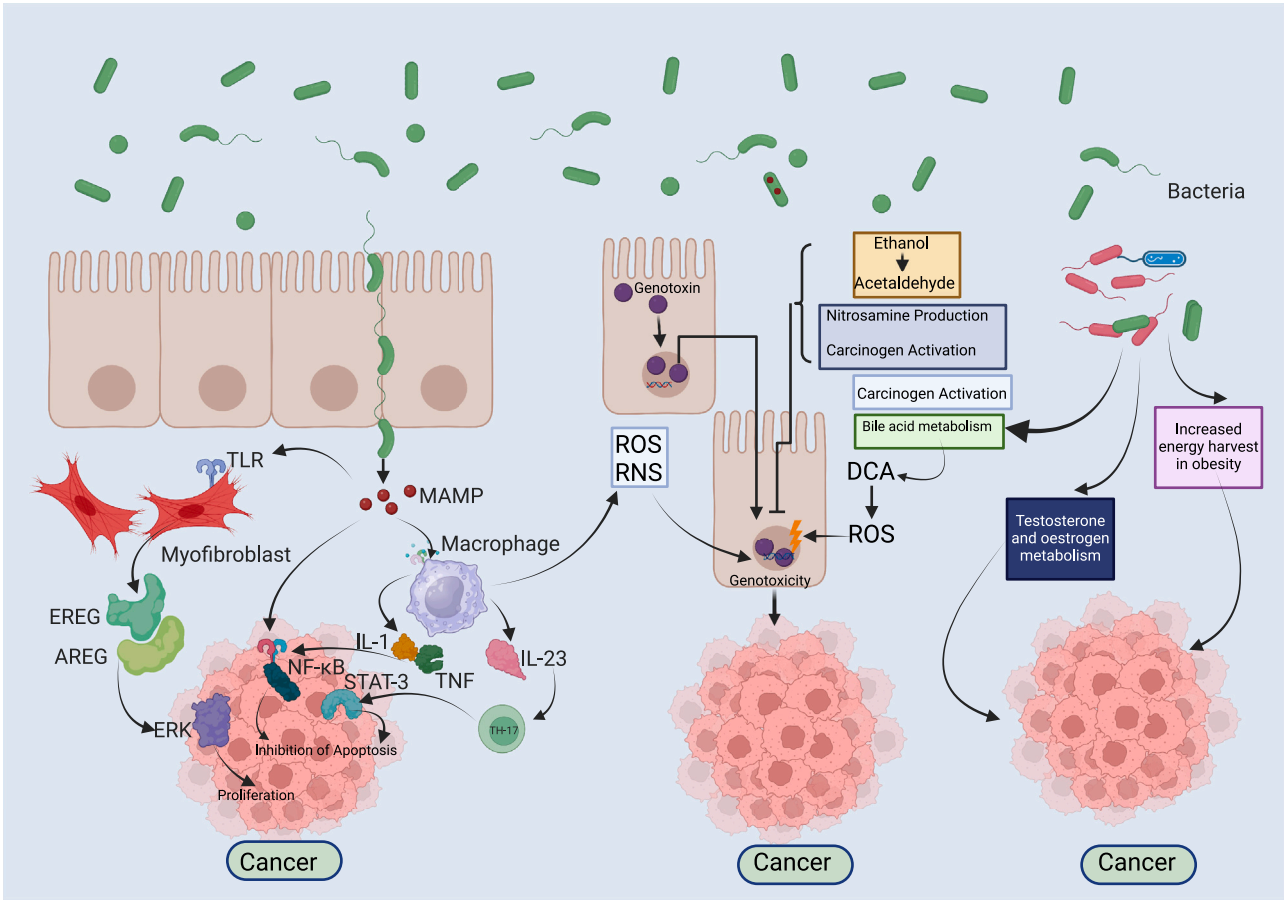

Figure 6. The bacterial microbiome influences cancer development via various pathways

(Left) Alterations in the microbiome alongside compromised host defenses might lead to heightened bacterial movement across barriers, resulting in amplified inflammation. This process is driven by microorganism-associated molecular patterns (MAMPs) that stimulate Toll-like receptors (TLRs) across diverse cell types, including macrophages, myofibroblasts, epithelial cells, and cancer cells. These impacts can manifest both within the immediate vicinity and systemically across different organs. (Middle) The genotoxic impact is exerted by bacterial toxins like colibactin and cytolethal distending toxin (CDT), which, once inside the host cell nucleus, directly cause DNA damage, particularly within organs directly exposed to the microbiome such as the gastrointestinal tract. Additionally, inflammatory cells like macrophages release reactive oxygen species (ROS) and reactive nitrogen species (RNS), and bacterial microbiota produce hydrogen sulfide (H2S), all of which can contribute to genotoxicity. (Right) The microbiome's metabolic functions can activate genotoxins such as acetaldehyde, convert dietary nitrosamines and other carcinogens, and influence the metabolism of hormones like estrogen and testosterone, as well as bile acids, and can lead to changes in energy extraction. Conversely, the microbiota can exert anti-tumorigenic effects by neutralizing carcinogens, producing short-chain fatty acids such as butyrate, and activating cancer-preventive phytochemicals. These mediators of cancer promotion and suppression can have localized as well as systemic, long-distance effects. Created with BioRender.com.

distant colonization. Thus, the role of intratumoral microbiota in cancer biology is multifaceted and complex, representing just the surface of our understanding. The specificity of microbial effects on regulatory pathways and their tumor-type specificity remain largely unexplored. Furthermore, sequential impacts might exist, with various microorganisms influencing different stages of tumor development, collectively advancing tumor progression from different angles.

Notably, research specifically focusing on the role of intratumor microbiota in cancer metastasis is relatively nascent and faces methodological challenges.[216] The main difficulty lies in manipulating the intratumor microbiota without affecting the commensal bacteria in other body parts. Potential solutions to this problem include targeted antibiotic treatments, the use of germ-free mice models, and the re-administration of bacteria directly into the tumor site.

Emerging evidence supports the idea that intratumor bacteria can alter both the inherent characteristics of cancer cells and their surrounding environment.[30,217] This alteration potentially enables cancer cells to thrive and facilitates the process of cancer metastasis. The field, while still developing, is uncovering how intratumor microbiota might directly contribute to the advancement of cancer, offering new perspectives on the complex interactions within the tumor microenvironment and their implications for cancer progression and treatment. Intratumor microbiota are known to influence the inherent properties of cancer cells, assisting them in adapting to the various challenges encountered

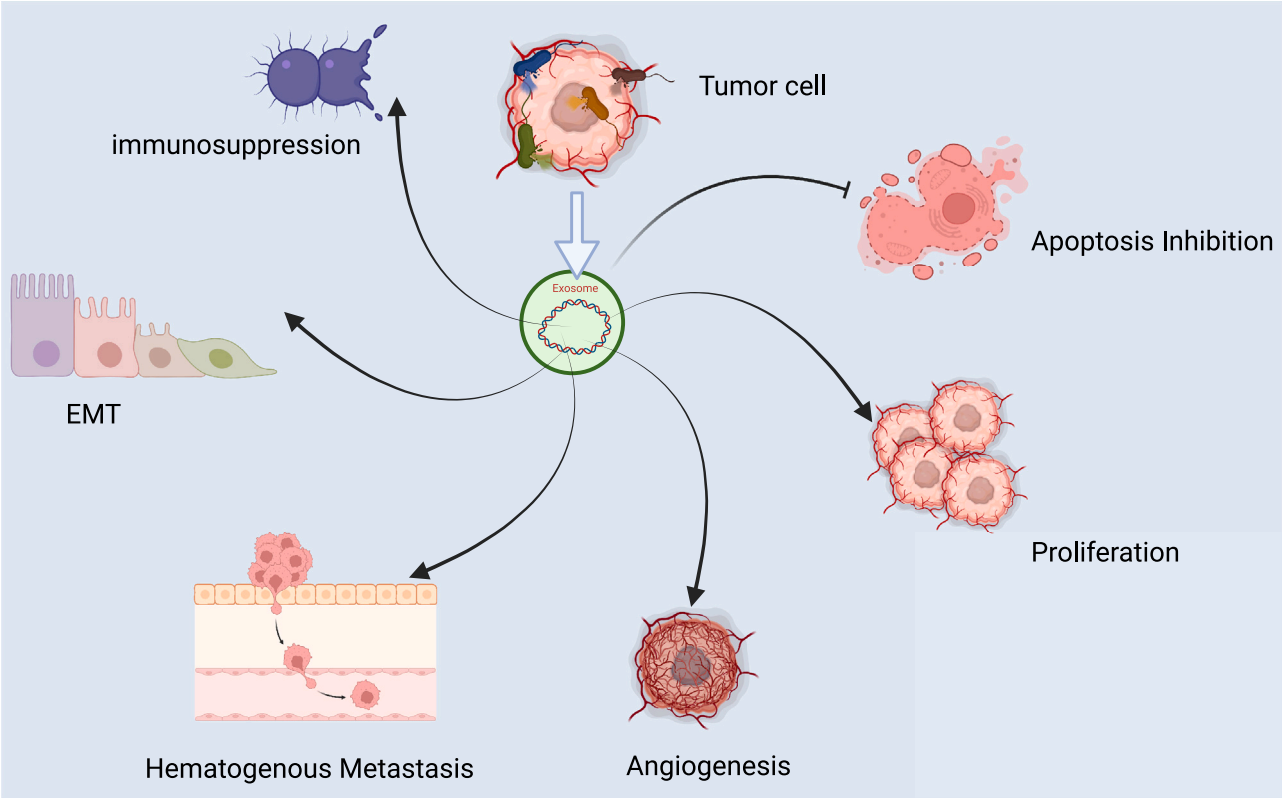

Figure 7. The microbiome within tumors aids in spreading cancer by boosting the release of exosomes produced by the tumor

These exosomes, dynamic carriers filled with diverse DNAs, miRNAs, and proteins, are secreted in greater quantities by tumor cells infected with the intratumoral microbiome. They facilitate cancer spread through multiple pathways: reshaping the environment around the tumor, enhancing the growth of tumor cells while preventing their programmed cell death, encouraging the conversion of epithelial cells to a more mobile mesenchymal form, weakening the immune system's ability to fight the tumor, and supporting the spread of cancer through blood and the formation of new blood vessels. Created with BioRender.com.

during metastasis. This includes modulation of programs related to stem cell behavior and plasticity, the EMT program, adhesion programs, and mechanical stress response programs.

Intratumor microbiota and anti-tumor immunity

In the complex interplay between cancer and the host biology, intratumoral microbiota and their influence on the immune response emerge as pivotal factors in tumor progression.[40,149] This intricate relationship underscores not only the microbial capacity to inhabit tumor microenvironments but also their significant role in modulating host immune mechanisms. The presence of intratumoral microbiota can dramatically alter the immune landscape within tumors. Numerous investigations have highlighted the critical function of the intestinal microbiota in modulating host immune system reactions. Intratumoral microbiota have been shown to bolster antitumor immunity and the effectiveness of immunotherapies through several mechanisms, such as STING signaling pathway activation, T and NK cell stimulation, tertiary lymphoid structure (TLS) formation, and the presentation of microbiota-derived antigens. (1) **STING signaling pathway activation:** Bifidobacterium within tumors can trigger dendritic cell (DC) activation via the STING signaling pathway.[100] *Akkermansia muciniphila* is capable of generating STING agonists, leading to type I interferon (IFN-I) production by intratumoral monocytes, which in turn facilitates macrophage reprogramming and enhances interactions between NK cells and DCs.[218] (2) **T and NK cell stimulation**: intratumoral *Saccharopolyspora*, *Lachnoclostridium*, *EBV*, and *HBV* can improve antitumor immunity by fostering the recruitment and activation of $CD8^+$ T cells through microbiota-derived chemokines such as CXCL9, CXCL10, and CCL5, thereby extending patient survival. Trimethylamine N-oxide (TMAO) produced by Clostridiales may activate the PERK-mediated endoplasmic reticulum stress response, leading to tumor cell pyroptosis and enhancing $CD8^+$ T-cell-mediated antitumor immunity.[219–223] A diet high in salt can increase intratumoral Bifidobacterium, boosting NK cell functionality and leading to tumor regression via the increased production of hippurate, a metabolic by-product.[224] (3) **Tertiary lymphoid structure formation:** *Helicobacter hepaticus* within the tumor environment can stimulate T follicular helper (Tfh) cell and B-cell-dependent antitumor immune responses, promoting the development of tertiary lymphoid structures crucial for an effective immune response.[225] (4) **Presentation of microbiota-derived antigens**: additionally, bacterial antigens within the tumor can be captured by tumor cells or DCs, stimulating the activity of tumor-specific T cells and further enhancing the antitumor immune response.[30,149]

Additionally, intratumoral microbiota can promote cancer progression through various mechanisms including upregulation of ROS, promoting an anti-inflammatory environment, T cell inactivation, and immunosuppression. (1) **ROS upregulation**: *B. fragilis* and *Fusobacterium* may drive tumor advancement through ROS generation, which modulates immune reactions and local inflammation, aiding tumor progression.[168,226,227] (2) **Anti-inflammatory environment promotion**: intratumoral-bacteria-derived IL-17 may encourage intratumoral B cell infiltration that supports tumor growth.[228] Bacterial presence in tumor sites can alter the local anti-inflammatory tumor microenvironment via IL-1β and IL-23 production from myeloid cells, leading to elevated IL-17 levels from γδT cells, thus propelling tumor advancement.[92] Fungal presence in tumor sites is known to boost IL-33 secretion from cancer cells, inviting Th2 and ILC2 cell infiltration, culminating in tumor progression.[229] (3) **T cell inactivation**: furthermore, intratumoral *F. nucleatum* and *Methylobacterium* presence may reduce the presence of tumor-infiltrating T cells and induce T cell dysfunction in tumor sites, thereby facilitating tumor progression.[230–233] (4) **Immunosuppression enhancement**: lastly, intratumoral *N. ramosa, S. aureus, HBV*, and *HCV* can intensify immunosuppression via Tregs to support cancer development.[234,235] Bacteria can direct TAM programming through the TLR signaling pathway, augment MDSC levels, and hinder Th1 cell differentiation to foster immune tolerance.[236] Commensal fungi may increase TAM levels and reduce T cells, impairing antitumor immune responses.[237] (5) **Microbial metabolites** also play a crucial role in this context, acting as messengers that can either promote or inhibit immune responses.[238,239] Short-chain fatty acids (SCFAs), produced through the fermentation of dietary fibers by gut microbiota, have been shown to exert anti-inflammatory effects and enhance the differentiation of regulatory T cells, contributing to an immune environment that may either support or hinder tumor growth.[240–242]

These findings underscore the multifaceted role of intratumoral microbiota in modulating the immune landscape within tumors, offering new avenues for enhancing the efficacy of cancer immunotherapies. Understanding these interactions offers promising avenues for novel therapeutic strategies that aim to manipulate the tumor microbiome to boost the host immune response against cancer (Figure 8).

MICROBIAL INSIGHTS IN CANCER DIAGNOSIS, PROGNOSIS, AND THERAPY DEVELOPMENT

Cancer patient diagnosis and prognosis

The exploration of intratumoral microbiota as diagnostic and prognostic tools represents a significant shift in cancer management strategies. By integrating microbial profiling into clinical practice, there is the potential not only to enhance the accuracy of cancer diagnoses and prognostic predictions but also to pave the way for more personalized and effective treatment approaches (Figure 9).

The microbial community within tumors differs markedly from that in healthy tissues, with certain bacteria having a causal relationship with cancer development. This indicates the potential of using intratumoral microbiota as a biomarker for cancer detection.[9,83,243–245] Nejman et al.'s extensive study on tumor microbiota across various cancers and corresponding healthy tissues revealed distinct microbial compositions for each tumor type.[30] For instance, microbial sequencing in different subtypes of papillary thyroid carcinoma showed unique microbial profiles with varying abundance between tumor and normal tissues. In breast cancer patients, there was an increased presence of bacteria capable of causing DNA damage, like Bacillus, Enterobacteriaceae, and Staphylococcus, while the count of health-promoting lactic acid bacteria declined.[97] Torres et al. found altered oral microbiota in pancreatic cancer patients, with increased Leptospirillum and Porphyria.[246] Familial adenomatous polyposis, a precursor to colorectal cancer (CRC), showed potential for early CRC detection through intratumoral *E. coli*, indicating a pre-cancerous inflammatory state[247] (Figure 10). These insights highlight the potential of tumor microbiomes as markers for cancer screening and diagnosis. However, current research predominantly relies on surgically obtained samples, underscoring the need for more non-invasive diagnostic methods.

Intratumoral microbiota has been shown to play a role in predicting cancer prognosis. In esophageal squamous cell carcinoma, higher levels of intratumoral *Fusobacterium nucleatum* (Fn) correlate with advanced tumor stages and reduced survival and may indicate a likelihood of recurrence-free survival.[248] Contrastingly, in oral squamous cell carcinoma, patients with Fn-positive tumors exhibited better outcomes compared to those with Fn-negative tumors. Fn presence also relates to metastatic recurrence, being more frequent in Fn-negative patient groups.[249] In primary liver cancer, patient prognosis positively correlated with increased relative abundance of *Pseudomonas* at family and genus levels. Long-term survivors (LTS) post-surgery showed a predominance of *Pseudomonas* and other bacteria, unlike short-term survivors (STS), who had different bacterial community dominances. Pancreatic ductal adenocarcinoma research, using bacterial DNA extracted from resected tissues and classified by 16S rRNA gene sequencing, revealed distinct microbiomes in LTS patients compared to STS patients.[40,250] Notably, the presence of intratumoral bacteria like *Saccharopolyspora*, *Pseudoxanthomonas*, and *Streptomyces* strongly correlated with LTS in pancreatic cancer patients.[40]

Furthermore, intratumoral microbiomes have been identified as potential prognostic indicators in various types of thyroid cancer.[97] These findings underline the significance of intratumoral bacterial diversity and uniqueness in impacting patient survival, suggesting a crucial link between intratumoral microbiota and clinical prognosis, and potentially providing a method for determining prognostic status in tumor patients. However, further research is necessary to confirm the accuracy of this method. While current studies offer initial evidence, more comprehensive investigations are required for validation. Precise and detailed data are essential to ascertain the feasibility of using this method for assessing the prognostic status of tumor patients.

Therapeutic implications

The discovery of intratumoral microbiota has profound implications for the development of new cancer therapies. By understanding the roles these microorganisms play within tumors, we can explore novel treatment strategies that target or utilize these microbial residents.

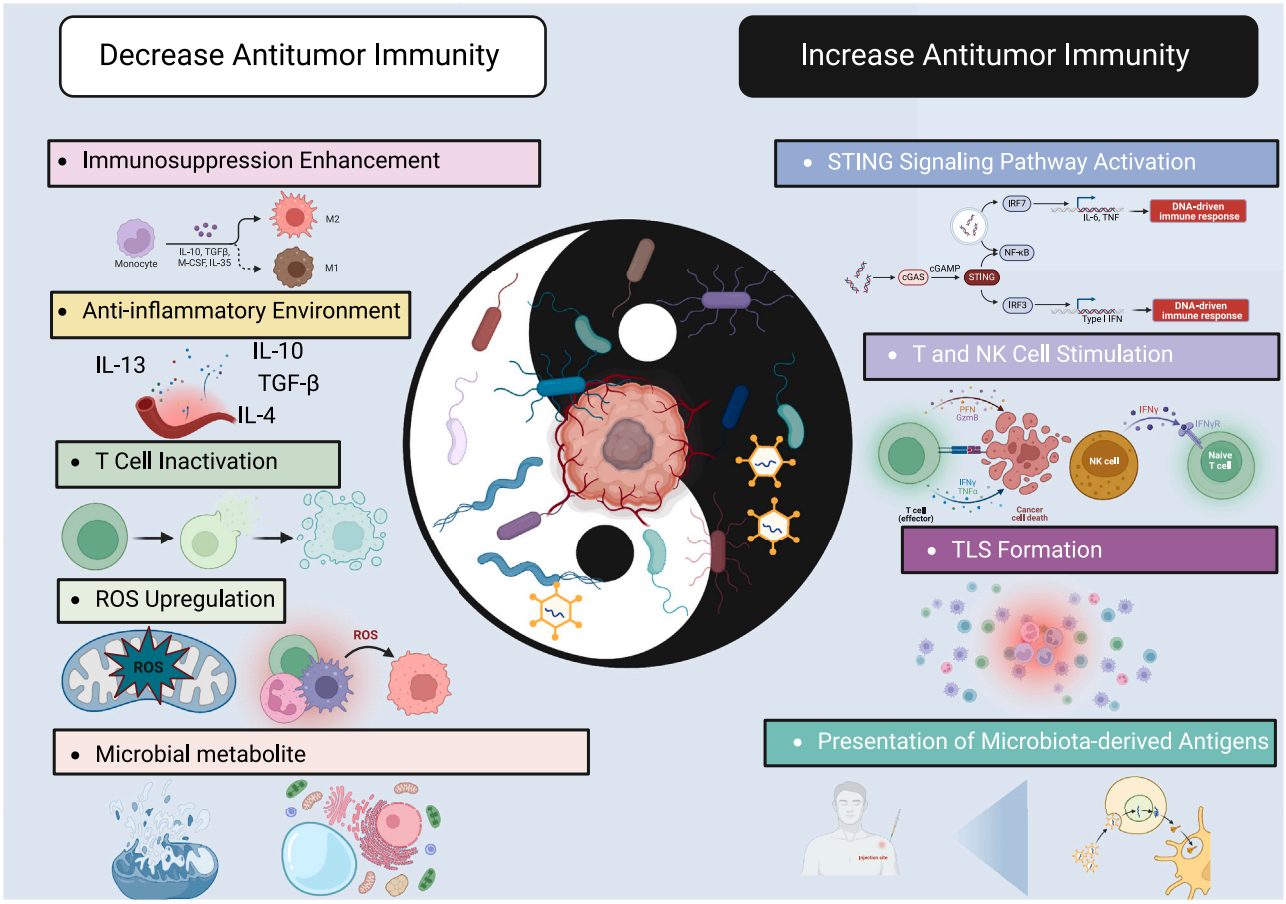

Figure 8. The Tai Chi Principle of Intratumoral Microbiota and Immune Interactions

In the intricate dynamics of tumor progression, the influence of intratumoral microbiota on the host's immune response embodies the dualistic essence reminiscent of Tai Chi, exhibiting both enhancement and suppression of antitumor immunity. This complex interaction not only reveals the capability of microbes to inhabit the tumor microenvironment but also accentuates their pivotal role in modulating the host's immune mechanisms. The intratumoral microbiota are instrumental in both augmenting antitumor immune responses through mechanisms such as activation of the STING signaling pathway, stimulation of T and NK cells, and presentation of microbiota-derived antigens, as well as in impeding these responses by promoting an anti-inflammatory milieu, inducing T cell dysfunction, and fostering immunosuppression, thereby presenting a multifaceted influence on cancer immunotherapies. Created with BioRender.com.

Cancer prognosis remains poor, and both modern and traditional therapies have shown limited improvement in outcomes. The discovery of intratumor microbiota and their interactions with the host presents new opportunities for cancer intervention. Recent evidence suggests that modulating the microbiota is a novel and crucial approach to enhancing cancer therapies. Historically, attempts to use microbiota for cancer treatment were unsuccessful.[251] However, recent extensive investigations into the intratumor microbiome have shown that certain bacteria, like *Salmonella typhimurium* strain VNP20009, *Listeria monocytogenes*, and other Listeria spp., can selectively target and eliminate tumor cells.[30,252–254] These bacteria, due to their ability to selectively colonize tumors or tumor-driven lymph nodes and inhibit tumor growth, have shown promise in preclinical studies using mouse models.[255,256] However, clinical outcomes have been less successful, with only minor or temporary effects observed in trials.[257] This discrepancy may be due to differences in tumor structures and growth rates, affecting bacterial penetration and efficacy.

The use of live bacteria, engineered to produce and deliver anticancer agents, offers several advantages, including enhanced tumor penetration, maximized therapeutic activity, and reduced systemic toxicity.[258,259] Strategies for delivering tumor-targeting bacteria include cytokines, chemotherapeutic agents, prodrug-converting enzymes, siRNAs, and immunomodulators. These methods have shown enhanced antitumor responses in tumor models. The intratumor microbiota's role in modulating host immunity suggests it could influence responses to various cancer therapies. However, direct control over intratumor microbiome modulation is challenging, with obstacles like toxicity control, accessibility to tumor sites, and precision in delivery.

The oral cavity and intestine, as primary sources of the intratumor microbiome, suggest that gut microbial modulation could reshape tumor microbiomes and affect cancer therapies. Modulating the gut microbiota through antibiotics, diet, and fecal microbiota transplantation

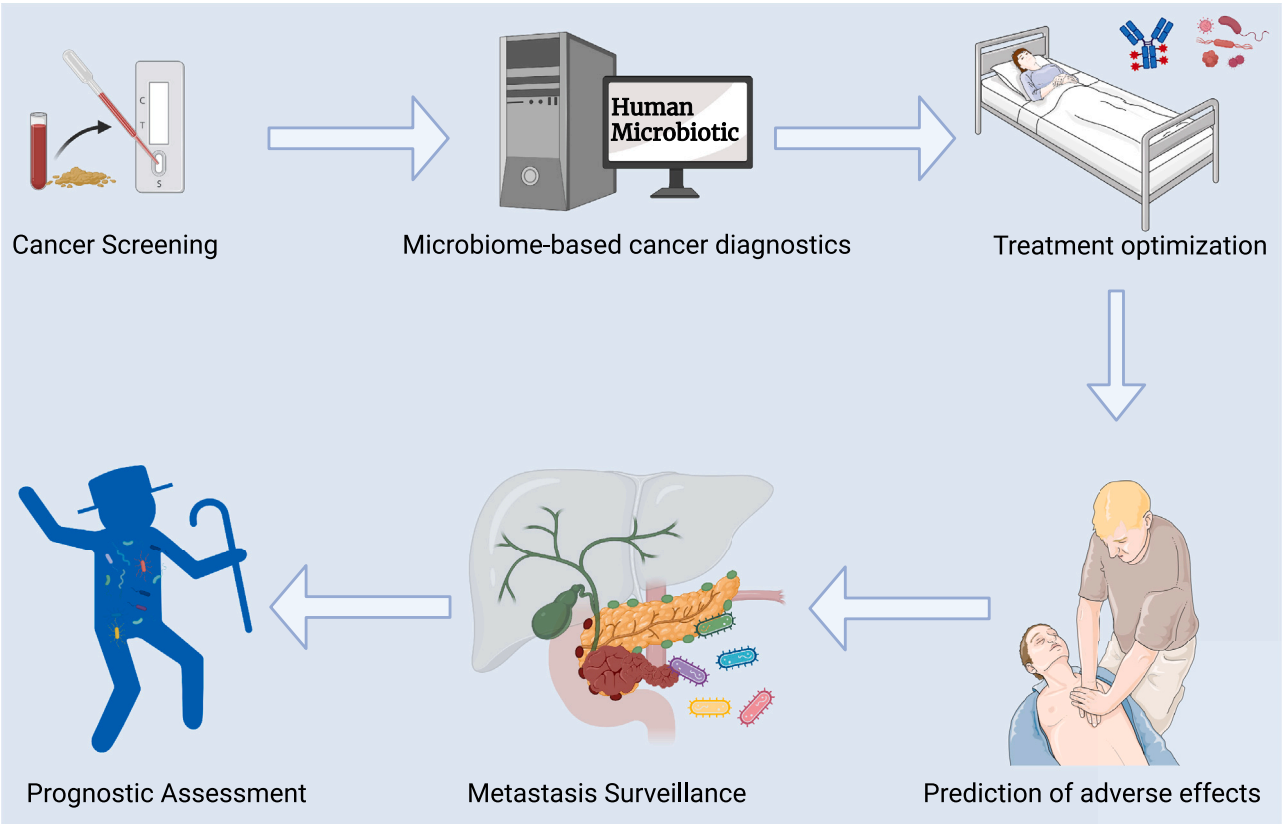

Figure 9. The human microbiome and cancer: a diagnostic and therapeutic perspective

Microbiome analysis data could significantly enhance cancer diagnostics. This includes detecting cancer by identifying microbial DNA and RNA in the peripheral blood, monitoring micro-metastatic progression in cancers, evaluating prognosis, customizing treatment plans for individuals, and employing artificial intelligence algorithms to predict patient responses to treatments and the likelihood of adverse effects. Created with BioRender.com.

(FMT) could be a powerful immunotherapeutic approach. However, systemic antibiotics can weaken immune checkpoint blockade and lead to poor prognosis.[260,261] Dietary interventions, prebiotics, and postbiotics are promising strategies for enhancing antitumor immunity and therapy responses in both mouse models and clinical trials (NCT03870607, NCT03950635).[218,262,263] Metabolomic data may offer insights into the mechanisms behind these strategies. FMT has been used to boost immune checkpoint inhibitor efficacy, with promising results in mouse models and clinical trials.[18,218,264–267] However, the long-term effectiveness and stability of FMT in cancer treatment remain to be evaluated, with complexities involving bacterial strain selection, administration methods, and dietary recommendations.

CHALLENGES AND FUTURE DIRECTIONS

Despite recent progress in understanding the potential existence and activities of microbes in cancer, this field is still emerging and faces significant challenges. The existence of microbial communities comprising bacteria and fungi in traditionally considered sterile tumors remains controversial and requires definitive characterization in future studies.[268,269] Characterizing these low-biomass tumor microbiomes is complicated by technical and biological challenges, including contamination risks, batch effects, erroneous read allocation, and imperfections in analytical pipelines, with limited capacity to pinpoint intracellular bacteria in relation to tumor phenotypes.

The use of animal models and organoids in studying bacterial invasion will deepen our understanding of the extent of intratumoral bacterial colonization and its functions. Rigorous control and analytical methods are crucial for addressing experimental and computational contaminations, as highlighted by the recent resolution of the debate over the existence of a placental microbiome.[270]

Differentiating the effects of extracellular microbes from intracellular bacteria that penetrate tumor cells will help quantify their true impact. However, even with advancements in bacterial identification, decoding variations at species and subspecies levels in low-biomass microbial environments remains challenging, with strains often grouped only by genus, overlooking their diversity and specific virulence factors.

Another major challenge is moving from correlation, association, and prediction to experimentally determining causation and molecular mechanisms. For example, conflicting reports about *F. nucleatum* in colorectal cancer suggest that some strains may not stably colonize the gut or promote colorectal cancer.[271] Comprehensive evaluation across multiple experimental platforms is needed to draw robust conclusions about the causative roles of intratumoral or intracellular microbes in cancer.

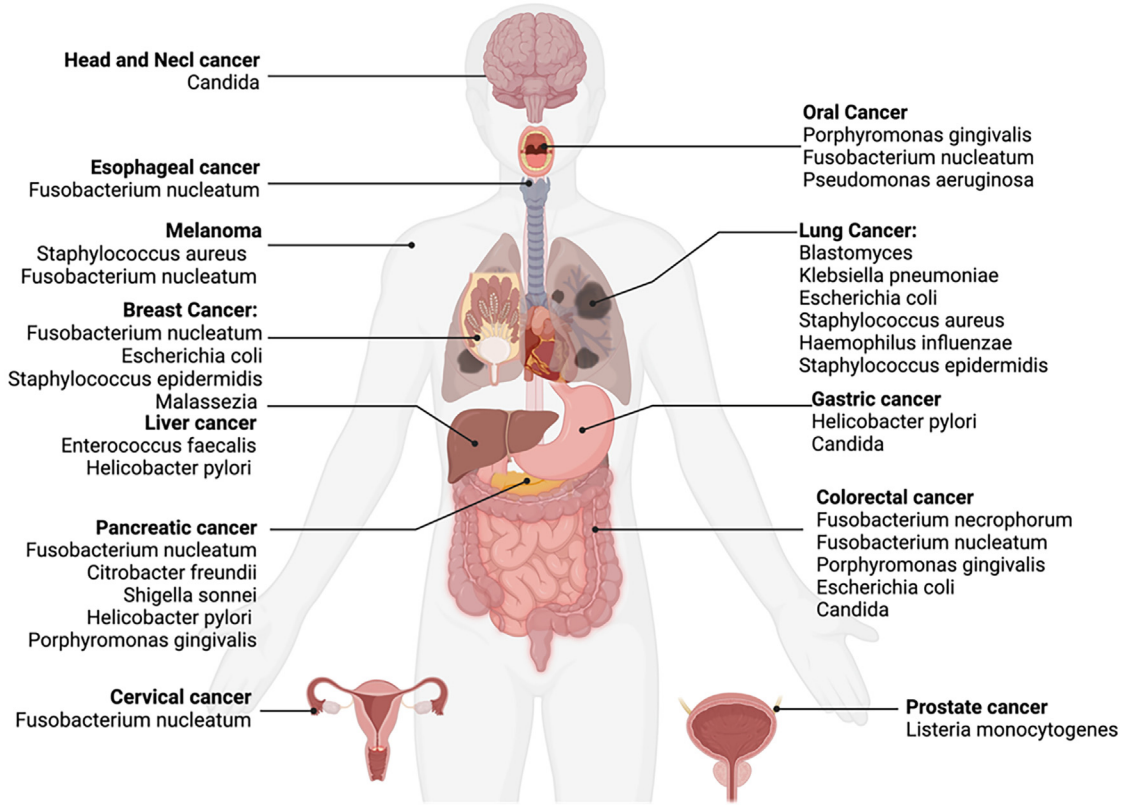

Figure 10. A summary of bacterial species linked to tumors, believed to be abundant in various types of cancer and capable of penetrating host cells

Additionally, two recent studies, referenced as (24) and (23), propose the possibility that fungal populations with low biomass exist within the microenvironment of tumors. Created with BioRender.com.

Future research will also explore bacterial interactions, as well as *trans*-kingdom interactions with viruses, fungi, and eukaryotic microorganisms. Progressing from cellular systems and animal models to clinical applications is complex but offers opportunities to utilize this knowledge in developing novel diagnostics and therapeutics.

CONCLUSION

Understanding of the microbiome's components has deepened our knowledge of immunological interactions. Bacteria, phages, and fungi educate both innate and adaptive immune systems through direct interactions and metabolite regulation. The exploration of intratumoral microbiota represents a burgeoning frontier in cancer research, offering novel insights into the complexity of cancer biology and potential new avenues for diagnosis and treatment.

The tumor microbe microenvironment plays a complex role in modulating the tumor immune environment, presenting opportunities for enhancing immunotherapy. The identification and characterization of intratumoral microbiota have opened the door to innovative diagnostic and therapeutic strategies. The potential for using microbial signatures as biomarkers for cancer diagnosis and prognosis is particularly promising, potentially leading to more personalized and effective approaches to cancer care. Similarly, the exploration of microbiota-targeted therapies, either as standalone treatments or in combination with existing modalities, holds great promise. This review has highlighted the significant strides made in understanding the presence, diversity, and functional impact of microbial residents within cancer ecosystems. These findings challenge traditional views of cancer as purely a disease of human cells, revealing a multifaceted interplay between host, tumor, and microbial elements.

However, the journey from discovery to clinical application is fraught with challenges. The field requires ongoing advancements in technological and methodological approaches, a deeper understanding of the intricate relationships within the tumor microenvironment, and careful consideration of ethical and safety implications. Moreover, the variability and complexity inherent in the microbial composition of tumors necessitate personalized approaches, which may be challenging to implement broadly.

The realization that cancer landscapes are not merely cellular but also microbial in nature marks a paradigm shift in our understanding of oncogenesis. This new perspective not only enriches our comprehension of cancer biology but also propels us toward potentially groundbreaking therapeutic interventions. As we embark on this journey of exploration, it becomes increasingly clear that the microbial inhabitants of tumors are not mere bystanders but are integral to the oncological narrative.

The therapeutic implications of intratumoral microbiota are vast and represent a frontier in cancer treatment. While this field is still in its infancy, it holds promise for enhancing existing treatments and developing entirely new therapeutic strategies. The ongoing research and clinical trials in this area are crucial for unlocking the full potential of these microbial residents in the fight against cancer.

ACKNOWLEDGMENTS

Not applicable.

Funding: this work was supported by the Shandong Province Natural Science Foundation grant (grant no. ZR2022QH372).

AUTHOR CONTRIBUTIONS

S.C., Z.Y., and Y.F. did the literature search and wrote the original manuscript. H.Z. conceived the hypothesis and revised the manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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