Acta Biochim Biophys Sin 2026, 58(8): 1684–1702
https://cstr.cn/32197.14.abbs.2026098
https://doi.org/10.3724/abbs.2026098
Review

Review

The intratumoral microbiota: from origin and identification to function and therapeutic perspective

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Received 12 March 2026 Accepted 8 May 2026 Published 11 June 2026

Abstract

Tumor tissues, once considered sterile, actually host diverse microbial communities that play key roles in several physiological and pathological processes, closely related to tumorigenesis and progression. Studies have demonstrated that intratumoral microbiota potentially contributes to immune regulation and significantly influences cancer treatment outcomes. Here, we aim to provide an extensive review of the conceptual framework, potential origins, spatial heterogeneity, and analytical methodologies of intratumoral microbiota, explore their carcinogenic mechanisms and potential role in tumor prognosis. In addition, we discuss current therapeutic strategies that target intratumoral microbiota and highlight the research prospects and limitations in this field, although there are some inevitable challenges.

Key words intratumoral microbiota, tumor microenvironment, immune evasion, microbial metabolites, cancer immunotherapy

Introduction

Human microorganisms, including bacteria, fungi, and viruses, constitute microbial communities within the human body, outnumbering human cells by approximately tenfold [1]. While commensal microbes colonize all mucosal surfaces, their highest density resides in the distal gastrointestinal (GI) tract [2]. Human microbiome studies have revealed distinct differences in microbial composition between healthy and diseased individuals. In particular, accumulating evidence has elucidated the significant role of gut microbiota in cancer initiation, progression, and the development of drug resistance. However, the existence and functional role of the intratumoral microbiota, which is distinct from the gut microbiota, remain unclear and incompletely established.

Historically, microbial presence in tumors was hypothesized as early as the 19th century, but was long dismissed due to contamination concerns and the subsequent focus on viral oncogenesis [3]. Interest was revitalized by the definitive characterization of *Helicobacter pylori* in gastric carcinogenesis, and modern high-throughput sequencing has since firmly established that distinct, viable microbial communities reside intracellularly within tumors, influencing chemotherapy response, genomic stability, and anti-tumor immunity (Figure 1).

Despite these advances, the precise origins, functional dynamics, and clinical utility of intratumoral microbiota require systematic synthesis. This review provides a comprehensive overview of the origins and features of intratumoral microbes across diverse tumor types, along with the methodologies employed for their detection. We further discuss how intratumoral microbiota modulates tumor progression and anti-tumor immunity, and assess their potential as biomarkers for cancer prognosis. These findings complement previous studies and may provide theoretical support for future intratumoral microbiota-targeted cancer diagnosis and therapy.

Origins of Intratumoral Microbiota

In this review, the term "intratumoral microbiota" specifically refers to the diverse communities of bacteria, fungi, archaea, and non-oncogenic viruses that colonize established tumor tissues [4]. These microbes primarily originate from mucosal reservoirs, adjacent normal tissues, or systemic circulation, and exert context-dependent modulatory effects on tumor progression, immune evasion, and therapeutic response. This concept is conceptually distinct from classical oncogenic pathogens (*e.g.*, HPV, HBV, HCV, EBV, and KSHV), which are well-established etiological agents that directly initiate malignant transformation through viral oncogene expres-

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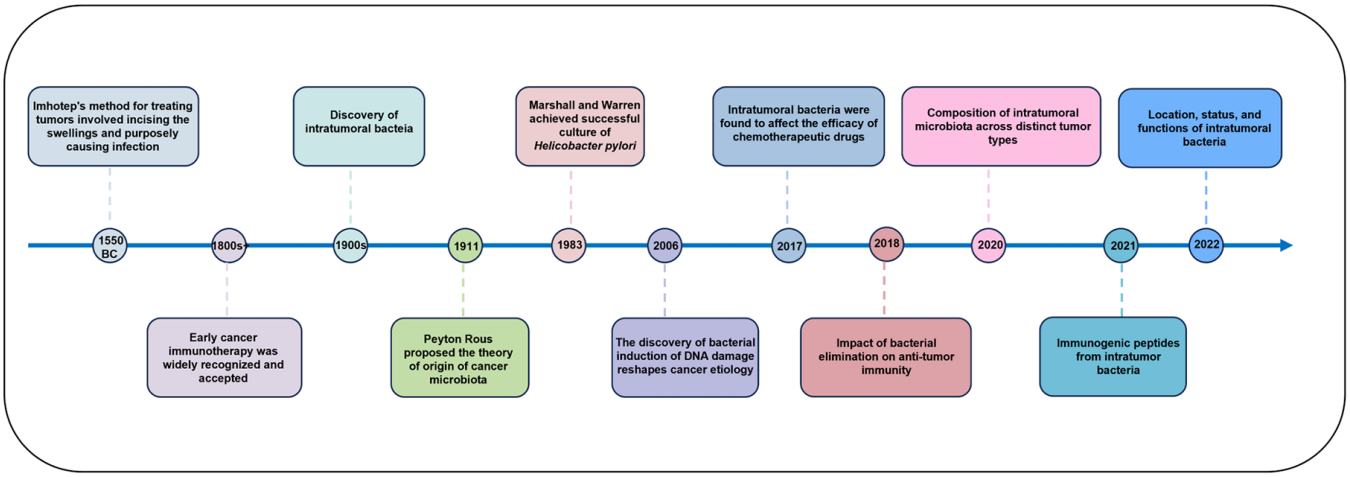


Figure 1. The evolving timeline of intratumoral microbiota

sion, host genome integration, and direct manipulation of cell cycle checkpoints. While oncogenic viruses drive cancer initiation, intratumoral microbiota primarily shapes the tumor microenvironment (TME) after tumorigenesis has been established.

The Earth hosts approximately 10^{12} microbial species, yet only 11 (Table 1) are classified as human carcinogens "oncomicrobiota" by the International Agency for Research on Cancer (IARC) [5]. These microbes are responsible for about 2.2 million cancer cases annually, accounting for roughly 13% of the global cancer burden. Among human-associated microbial habitats, the gastrointestinal tract, particularly the colon, harbors the highest microbial density in the body [6]. However, intratumoral microbiota remains understudied, and its origins are still unclear despite detection in diverse tumor types.

Recent studies suggest that intratumoral microbiota originates from multiple sources. One proposed mechanism involves microbial translocation from native reservoirs, such as the gut or respiratory tract, into tumors through compromised mucosal barriers (Figure 2A). For instance, nitrite-induced disruption of epithelial integrity promotes bacterial colonization and is closely associated with gastric, colorectal, lung, and urothelial cancers, as well as melanoma [7]. A second potential source is direct microbial invasion from normal adjacent tissues (NATs) into the tumor microenvironment (Figure 2B). Although NATs and tumor tissues often share overlapping microbial communities, they exhibit markedly distinct profiles of bacterial abundance and metabolic enzyme expression. For instance, breast tumors typically harbor greater bacterial diversity and display elevated levels of enzymes involved in anaerobic respiration [8] (Table 2). These differences imply functional specialization of the microbiota within the tumor, although the directionality of microbial migration remains to be fully elucidated. Hematogenous dissemination constitutes a third route of microbial entry (Figure 2C). For example, oral *Fusobacterium nucleatum* can disseminate via the bloodstream to colorectal tumors, where its Fap2 binds to Gal-GalNAc on cancer cells [9]. Similarly, gut-derived *Escherichia coli* can disrupt the gut vascular barrier and disseminate to distant sites such as the liver, thereby contributing to premetastatic niche formation [10]. Given the aberrant vasculature characteristic of tumors, microbes from oral, gut, or other reservoirs are likely to infiltrate tumor tissues via leaky blood vessels. Nevertheless, the precise mechanisms underlying their colonization remain poorly understood.

Table 1. Eleven carcinogenic microbes and their associated cancer types

Carcinogenic microbes	Category	Cancer types
Helicobacter pylori	Bacterium	Gastric cancer
		MALT lymphoma
Hepatitis B virus (HBV)	Virus	Hepatocellular carcinoma
Hepatitis C virus (HCV)	Virus	Hepatocellular carcinoma
		Non-Hodgkin lymphoma
Human papillomavirus (HPV)	Virus	Cervical cancer
		Anal carcinoma
		Oropharyngeal cancer
Epstein-Barr virus (EBV)	Virus	Nasopharyngeal carcinoma
		Gastric cancer
		Hodgkin lymphoma
Kaposi sarcoma herpesvirus (KSHV/HHV-8)	Virus	Kaposi's sarcoma
Human T-cell lymphotropic virus type 1 (HTLV-1)	Virus	Primary effusion lymphoma
		Adult T-cell Leukemia/ Lymphoma
Merkel cell polyomavirus (MCPyV)	Virus	Merkel cell carcinoma
Schistosoma haematobium	Parasite	Bladder cancer
Opisthorchis viverrini	Parasite	Cholangiocarcinoma
Clonorchis sinensis	Parasite	Cholangiocarcinoma

Diversity of Intratumoral Microbiota

The multiple potential origins of intratumoral microbes suggest correspondingly diverse microbial communities across cancer types. This was substantiated by Ravid Straussman's pan-cancer analysis of seven tumor types—lung, breast, pancreatic, ovarian, brain, bone, and melanoma—each exhibiting a distinct microbial profile. Moreover, bacteria predominated over fungi, and similar microbial communities were also detected in adjacent normal tissues. Given the profound heterogeneity of the TME, the composition and abundance of intratumoral microbiota vary

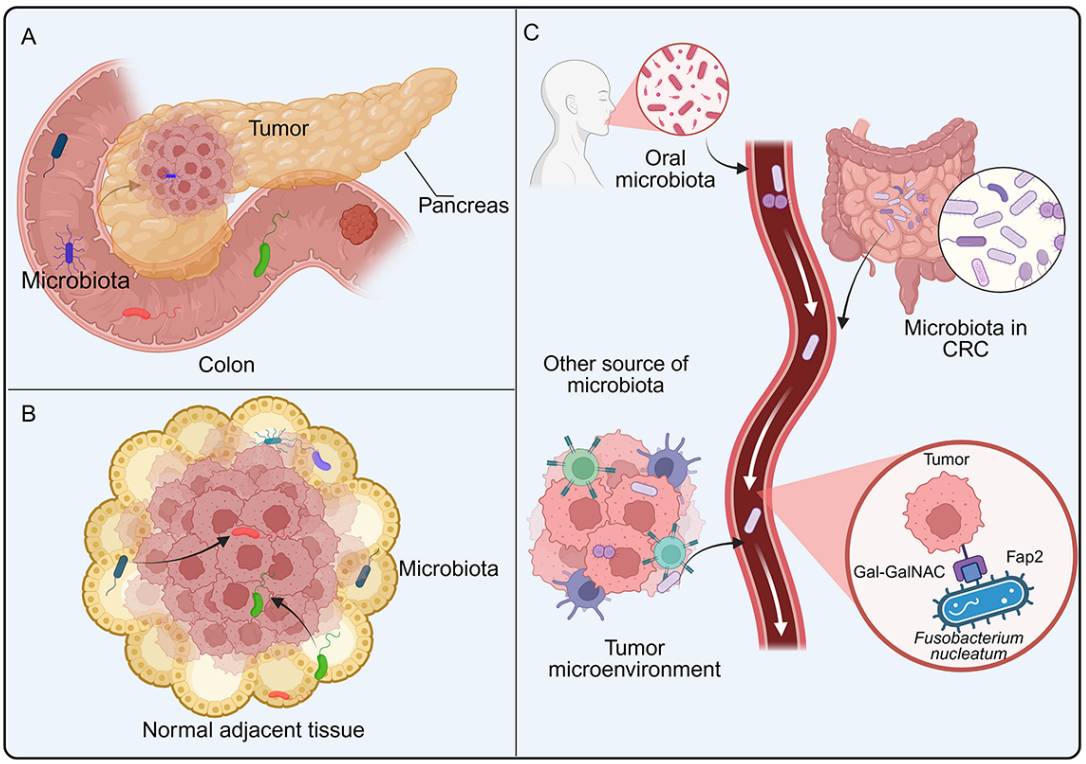


Figure 2. Origins of intratumoral microbiota (A) Translocation from mucosal organs. (B) The normal tissue surrounding a tumor (NAT) is a potential reservoir for microbiota. (C) Circulatory system.

Table 2. Origins of intratumoral microbiota across representative cancer types

Origins of intratumoral microbiota	Cancer types	Representative microbiota	Quantitative dynamics
Translocation from mucosal organs	Gastric cancer	*Helicobacter pylori*	Increase
	Colorectal cancer	*Fusobacterium nucleatum*	Increase
	Lung cancer	*Streptococcus pneumoniae*	Increase
	Melanoma	*Staphylococcus epidermidis*	Increase
Direct invasion from NATs	Breast cancer	*Sphingomonas yanoikuyae*	Increase
		Sphingomonas echinoides	Increase
		Sphingomonas wittichii	Increase
		Pseudomonas aeruginosa	Increase
		Pseudomonas putida	Increase
Hematogenous dissemination	Colorectal cancer	*Fusobacterium nucleatum*	Increase
		Bacteroides fragilis	Increase
	Hepatocellular carcinoma	*Klebsiella pneumoniae*	Increase
		Enterococcus faecalis	Increase

markedly across cancer types, implying distinct functional roles (Figure 3). However, the relationship between microbial variation and TME heterogeneity remains unclear and warrants further pan-cancer investigation.

As the body's largest mucosal interface, the lung remains continuously exposed to environmental microbes [11]. Once considered sterile, the lung is now known to harbor a resident microbiota that may contribute to carcinogenesis. Notably, specific bacterial taxa are associated with the activation of oncogenic pathways such as ERK and PI3K in non-small cell lung cancer (NSCLC). This subtype accounts for more than 80% of lung cancer cases and is frequently preceded by chronic respiratory infections. As shown in Figure 4A, exposure to *Prevotella*, *Streptococcus*, and *Veillonella* activates the PI3K/AKT signaling pathway both *in vitro* and *in vivo* [12]. Additionally, the microbial composition differs between lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC), suggesting subtype-specific functional roles. SCC exhibits higher microbial diversity with enrichment of Proteobacteria (particularly Enterobacteriaceae, *Haemophilus influenzae*, and *Capnocytophaga*), whereas ADC is characterized by *Acinetobacter*, *Propionibacterium*, *Brevundimonas*, and *Prevotella* [13]. Importantly, broad-spectrum antibiotic use has been linked to reduced

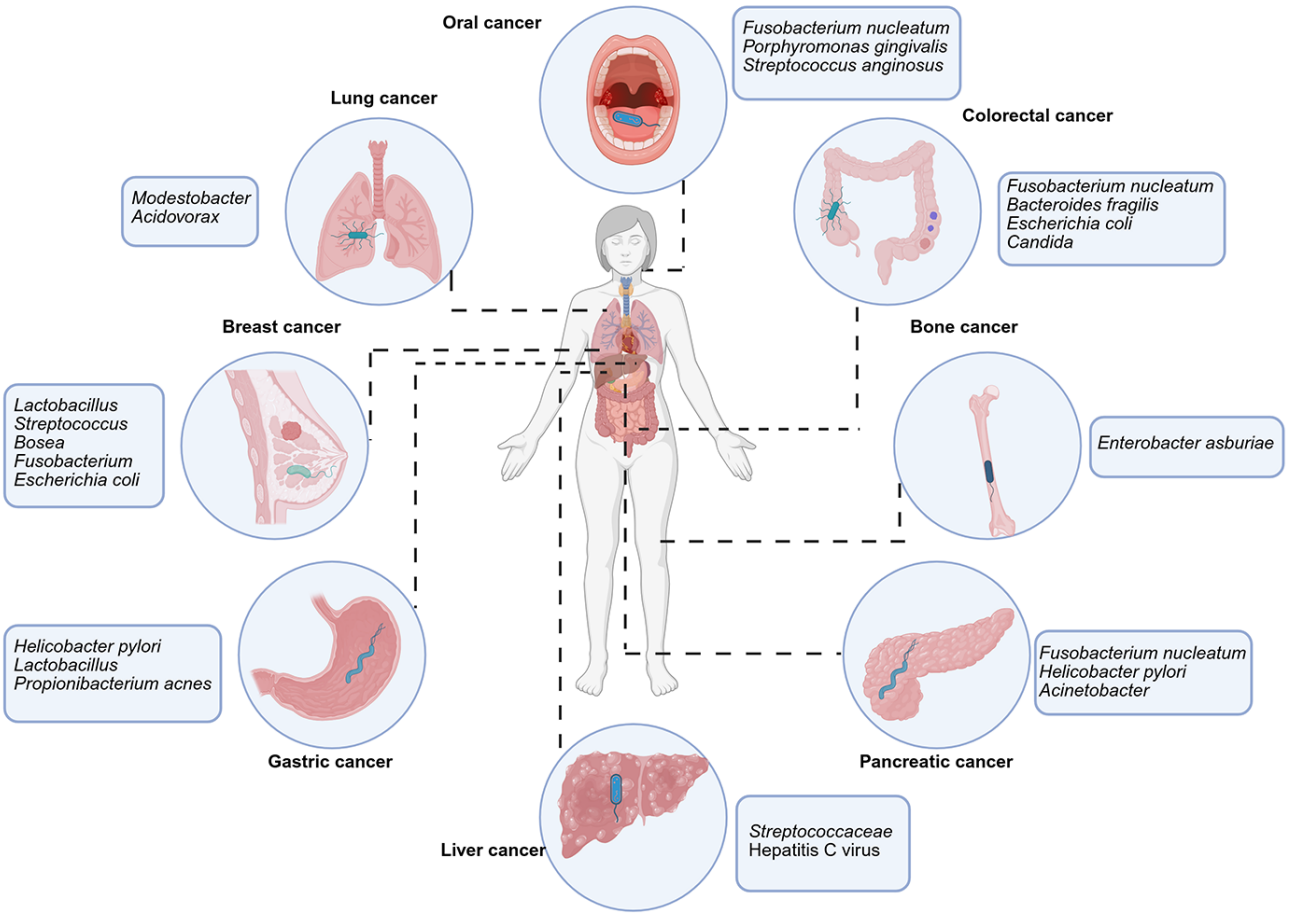


Figure 3. **The diversity of intratumoral microbiota**

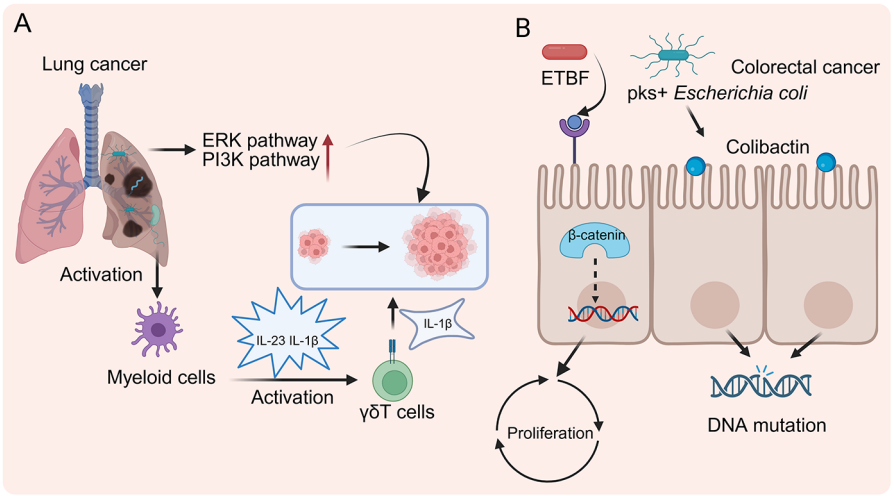


Figure 4. **Heterogeneity of intratumoral microbiota in different tumors** (A) In NSCLC, specific bacterial taxa are linked to oncogenic pathways such as ERK and PI3K, with *Prevotella*, *Streptococcus*, and *Veillonella* experimentally shown to activate PI3K/AKT signaling *in vitro* and *in vivo*. (B) Toxin-producing bacteria such as pks$^+$ *E. coli* and ETBF drive colorectal tumorigenesis.

efficacy of immune checkpoint blockade, suggesting that integrating microbiota-modulating approaches with immunotherapy may enhance therapeutic outcomes.

The skin harbors a highly diverse microbiota. In melanoma, genera such as *Acinetobacter*, *Corynebacterium*, and *Streptococcus* are frequently detected, whereas *Staphylococcus aureus* predominates in non-melanoma skin cancers [14].

Breast tissue harbors a substantial microbiota, although it is not a mucosal site. Intriguingly, breast tumors display the highest microbial diversity among the seven cancer types analyzed [15].

The dominant taxa, including *Bifidobacterium infantis*, *Lactobacillus iners*, *Corynebacterium*, and *Fusobacterium nucleatum*, differ markedly from those in normal breast tissue. In mouse models, *F. nucleatum* promotes tumorigenesis, suppresses T cell infiltration, and enhances metastasis to the lungs.

The gastrointestinal tract exhibits a microbial density gradient, with concentrations peaking in the colon at approximately 10^{12} bacteria per gram, which accounts for the high microbial biomass observed in colorectal cancer (CRC). CRC tissues harbor viable microbial communities associated with poor prognosis. For example, polyketide synthase-positive *E. coli* (pks$^+$ *E. coli*) and enterotoxigenic *B. fragilis* (ETBF) promote tumorigenesis through genotoxins that induce direct DNA damage (Figure 4B) [16]. *F. nucleatum* is enriched in CRC and binds to Gal-GalNAc overexpressed on cancer cells via its Fap2, activating the Wnt/β-catenin pathway. Meanwhile, its lipopolysaccharide (LPS) induces NF-κB-mediated inflammation and miR-21-dependent chemoresistance [17]. *F. nucleatum* consistently exacerbates CRC progression despite lacking canonical toxin-encoding genes. Currently, major technical barriers continue to impede detailed mechanistic studies. These barriers include the difficulty of genetically manipulating clinical isolates and unstable microbial colonization in murine models.

The microbiota constitutes a multi-kingdom ecosystem that extends well beyond bacteria to encompass fungi, viruses, archaea, protists, and mycoplasmas. To date, research exploring microbial contributions to tumorigenesis and progression has been predominantly centered on bacteria. However, emerging evidence indicates that non-bacterial taxa—particularly intratumoral fungi and viruses—also contribute to cancer development.

In PDAC, the fungal burden is ∼3000-fold higher than that in normal tissue, predominantly *Malassezia* spp., which accelerates tumorigenesis by binding to mannose-binding lectin (MBL) to activate the complement cascade; fungal ablation suppresses tumor progression [18]. Gut-to-pancreas translocation of fungi has also been demonstrated in murine models. Candida species may promote oncogenesis via nitrosamine production and secretion of TNF-α/IL-18, modulating proliferation and immune adhesion [19]. Fungi have further been detected in prostate, esophageal, gastric, and colorectal cancers, though mechanistic insights remain limited.

The intratumor virus similarly correlates with multiple malignancies. Established oncogenic viruses—including HPV, EBV, HBV/HCV, KSHV, HTLV, and MCPyV—drive carcinogenesis in cervical, hepatic, esophageal, and other solid tumors [20]. HPV, for instance, is causally linked to cervical cancer and implicated in head/neck and bladder cancer progression. Emerging data also suggest that bacteriophages may indirectly shape the tumor microenvironment, as Streptococcus-specific bacteriophages are enriched in colorectal cancer patients [21].

Collectively, these findings underscore that evaluating bacteria alongside fungi and viruses is essential for a complete understanding of the intratumor microbiota's role in cancer.

Methods Used to Characterize the Intratumoral Microbiota

Whether the intrinsic pancreatic mycobiome influences the initiation and progression of PDAC remains a matter of debate. This uncertainty largely stems from methodological inconsistencies in both the generation and analysis of microbiome sequencing data. Here, we provide an overview of current methodologies for detecting intratumoral microbiota. Although the low biomass of intratumoral microbiota poses challenges, multiple effective approaches have been developed for its characterization. High-throughput sequencing enables culture-independent, comprehensive profiling of microbial communities. This is primarily achieved through deep sequencing of universal marker genes, such as prokaryotic 16S rRNA, eukaryotic 18S rRNA, and internal transcribed spacer (ITS) regions, or via whole-metagenome shotgun sequencing (WMS) for downstream analysis. Collectively, these technological advances now facilitate the in-depth exploration of tumor-associated microbiomes. To facilitate method selection and provide a comprehensive comparison, we summarize the principles, applications, advantages, and limitations of eight major detection strategies in Table 3.

16S rRNA gene sequencing

16S rRNA gene amplicon sequencing remains the most widely used method for profiling intratumoral microbiota. This gene comprises conserved regions and nine hypervariable regions (V1-V9) that enable taxonomic classification [22]. Among these, the V4 region is most commonly targeted for bacterial diversity assessment. Despite its cost-effectiveness and speed, this method exhibits limited resolution in distinguishing closely related species and is susceptible to PCR amplification bias [23]. Recent advances utilize multiplex PCR targeting multiple hypervariable regions to improve taxonomic coverage and resolution over conventional V4 or V3-V4 amplicon strategies. The standard workflow involves PCR amplification, amplicon purification, high-throughput sequencing, and clustering of sequences into operational taxonomic units (OTUs) for taxonomic assignment against reference databases (Figure 5A) [24]. This approach has been successfully applied to characterize intratumoral microbiomes across multiple cancer types, such as PDAC and CRC.

Shotgun metagenomic sequencing

Shotgun metagenomic sequencing randomly fragments and sequences total DNA from a sample, enabling comprehensive profiling of microbial communities [25]. Compared with 16S rRNA gene amplicon sequencing, shotgun metagenomic sequencing provides higher taxonomic resolution and captures functional profiles, including metabolic pathways and antibiotic resistance genes [26]. Additionally, it detects non-bacterial microbes such as fungi and viruses, which are frequently overlooked by targeted approaches (Figure 5B). Huang *et al.* [27] applied this approach to CRC patients and identified *Coprococcus* as a taxon associated with response to neoadjuvant chemoradiotherapy. Key challenges include high computational demands arising from abundant host DNA, elevated sequencing costs, and reduced sensitivity for detecting rare microbial species in low-biomass tumor samples. Optimization of sample preparation and bioinformatics pipelines is critical to ensure reliable and reproducible results. As sequencing technologies advance and costs decline, shotgun metagenomic sequencing is poised to play an increasingly prominent role in tumor microbiome research and precision oncology.

Immunohistochemistry

Immunohistochemistry (IHC) utilizes antigen-antibody interactions to localize bacteria in tissues [28]. Antibodies against surface

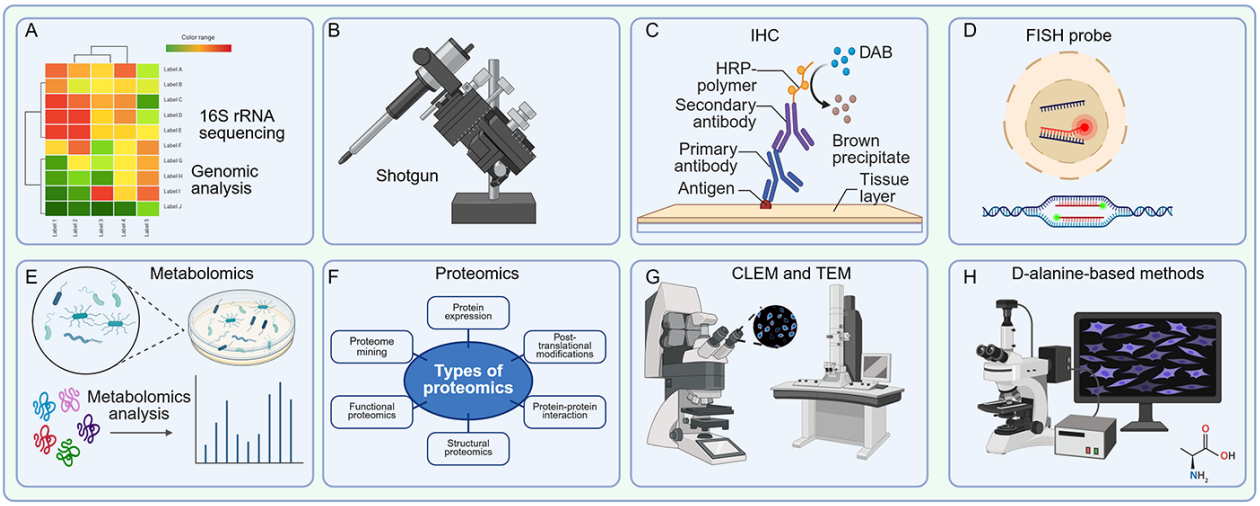


Figure 5. Methods to detect intratumoral microbiota (A) 16S rRNA sequencing. (B) Shotgun. (C) IHC. (D) FISH. (E) Metabolomics. (F) Proteomics. (G) CLEM and TEM. (H) D-alanine-based methods

Table 3. Methodological overview for characterizing the intratumoral microbiota

Method	Principle	Applications	Advantages	Limitations
16S rRNA gene sequencing	PCR amplification and sequencing of conserved 16S rRNA gene regions	Bacterial community profiling	Cost-effective; rapid	PCR amplification bias; cannot detect fungi/viruses; prone to contamination
Shotgun metagenomic sequencing	Random fragmentation and sequencing of total DNA from samples	Comprehensive microbial profiling; detection of bacteria, fungi, and viruses	High taxonomic resolution; detects non-bacterial microbes	High computational demands; elevated costs
Immunohistochemistry (IHC)	Antigen-antibody interactions targeting bacterial surface markers	Spatial localization of bacteria within tumor tissues	High spatial resolution; direct visualization in tissue context	False positives from antibody cross-reactivity; limited sensitivity for low-abundance microbes
Fluorescence *in situ* hybridization (FISH)	Fluorescent probes targeting conserved rRNA sequences	Single-cell visualization	Single-cell resolution; quantitative at cellular level	Limited multiplexing due to spectral overlap
Metabolomics	Systematic identification and quantification of small-molecule metabolites	Functional metabolic profiling; microbial metabolite detection	Reveals functional activity; identifies metabolic crosstalk; links microbes to therapeutic responses	Difficult to attribute metabolites to specific microbes; dynamic and context-dependent
Proteomics	LC-MS-based identification and quantification of proteins	Microbial protein detection; host-microbe interaction analysis	Provides functional insights; identifies candidate biomarkers	High complexity; challenging for low-abundance microbial proteins
CLEM and TEM	Correlative light-electron microscopy combining fluorescence and ultrastructural imaging	Nanoscale localization; intracellular distribution of microbes; subcellular compartment analysis	visualizes microbes in native ultrastructural context; single-particle tracking	Technically demanding; complex sample preparation
D-alanine-based methods	Incorporation of fluorescent D-alanine into peptidoglycan via bacterial alanine racemase	Detection of metabolically active bacteria; viability assessment	Selectively labels live bacteria; provides functional evidence of metabolic activity	Limited to fresh tissues; only detects actively dividing bacteria

markers, such as LPS and lipoteichoic acid (LTA), are employed to enable visualization of Gram-negative and Gram-positive bacteria with high spatial resolution (Figure 5C) [29]. This spatial precision permits direct visualization of bacterial localization within the tumor microenvironment. Combined with molecular methods such as 16S rRNA sequencing and FISH, IHC provides critical evidence for microbe-tumor interactions [30]. However, IHC is prone to false-positive signals due to antibody cross-reactivity or residual bacterial antigens and exhibits limited sensitivity for low-abundance microbes. Cross-validation with complementary techniques and rigorous optimization of antibodies and controls are essential for reliable results [31]. Advances in multiplex fluorescent IHC and automated image analysis hold promise to improve its utility in tumor microbiome research.

FISH

Direct visualization of individual microbial cells in tissues is enabled by FISH using probes targeting conserved ribosomal RNA sequences, such as bacterial 16S rRNA or fungal 28S rRNA [32]. This provides spatial resolution at the single-cell level (Figure 5D). Probe specificity can be tuned to detect microbes at various taxonomic levels [33]. For example, species-specific FISH probes identified *Klebsiella pneumoniae* in intrahepatic cholangiocarcinoma, while Fusobacterium enrichment was detected in CRC tissues [34]. However, a key limitation of conventional FISH lies in its restricted multiplexing capacity, which is primarily due to fluorescence spectral overlap. This issue has been addressed by advanced variants, including catalyzed reporter deposition-FISH (CARD-FISH) and high phylogenetic resolution FISH (HiPR-FISH) [35]. These methods enhance sensitivity for rare taxa and enable single-cell quantification. Furthermore, techniques such as combinatorial labeling and spectral imaging-FISH (CLASI-FISH) improve multiplexing capability [36]. Consequently, FISH is becoming increasingly valuable for the spatial profiling of complex tumor-associated microbiomes.

Metabolomics

Metabolomics enables the systematic identification and quantification of small-molecule metabolites within biological systems at defined time points (Figure 5E) [37]. In tumor microbiome research, metabolomics reveals functional metabolic reprogramming driven by specific intratumoral microbial taxa and their contributions to tumorigenesis and therapeutic responses [38]. Key immunomodulatory metabolites are microbially derived: short-chain fatty acids (SCFAs), such as butyrate and acetate, are primarily produced by commensal anaerobes, including *Faecalibacterium prausnitzii*, *Roseburia* spp., and *Bifidobacterium* spp.; secondary bile acids (*e.g.*, deoxycholic acid) are generated via microbial 7α-dehydroxylation by taxa such as *Clostridium scindens*; inosine is secreted by *Bifidobacterium pseudolongum*; and indole derivatives (*e.g.*, indole-3-aldehyde, indole-3-propionic acid) are synthesized from dietary tryptophan by *Lactobacillus*, *Clostridium sporogenes*, and *Bacteroides* species [39]. These microbe-specific metabolites modulate the TME through immune regulation and signaling cascades, thereby influencing cancer progression and treatment efficacy. Spatially resolved metabolomics has further revealed distinct metabolic signatures linked to particular microbes. For instance, *Akkermansia muciniphila* produces STING-activating cyclic dinucleotides that enhance antitumor immunity, while Yu and colleagues [40] demonstrated that *Clostridium butyricum* selectively colonizes CRC tissues via secD-GRP78 interactions. This bacterium secretes butyrate, which activates cytotoxic T lymphocytes (CTLs) to synergistically enhance antitumor immunity. In preclinical models, *C. butyricum* significantly enhanced the efficacy of anti-PD-1 therapy, underscoring its translational potential. Therefore, metabolomics provides a powerful platform for deciphering the specific metabolic crosstalk between cancer cells and defined intratumoral microbial taxa, facilitating the discovery of targeted therapeutic strategies.

Proteomics

Proteomics systematically characterizes protein interactions and functions within biological systems, offering comprehensive insights into cellular processes at the proteome level (Figure 5F) [41]. In tumor microbiome research, liquid chromatography-mass spectrometry (LC-MS) is employed to identify and quantify differentially expressed proteins derived from both host and intratumoral microbes in healthy versus tumor tissues [42]. This approach has successfully profiled virulence factors and metabolic enzymes produced by specific cancer-associated taxa, such as FadA from *Fusobacterium nucleatum*, colibactin biosynthesis proteins from pks$^+$ *E. coli*, and BFT toxin from enterotoxigenic *B. fragilis*, thereby uncovering microbial pathogenic mechanisms and candidate cancer biomarkers [43]. Furthermore, advanced strategies, such as data-independent acquisition (DIA) and targeted proteomics, enhance analytical precision and reproducibility [44]. Metaproteomics extends this capability to profile thousands of proteins directly synthesized by intratumoral microbial communities, although its application in oncology is still in its early stages. A promising advance is major histocompatibility complex (MHC) peptidomics, which characterizes microbial peptides presented by tumor cells via MHC molecules [45]. Recent studies have identified peptide fragments derived from intratumoral commensals—including *Bifidobacterium*, *Lactobacillus*, and *Staphylococcus* species—that are processed and displayed on melanoma cells through human leukocyte antigen (HLA) complexes [46]. These microbe-derived antigens suggest a direct role for intratumoral microbes in modulating local immune responses and shaping T-cell recognition within the TME. Collectively, these proteomic approaches provide powerful tools to decipher the functional interactions between tumor-infiltrating microbial taxa and host cells, which accelerates the development of microbiota-targeted immunotherapies.

Correlative light-electron microscopy and transmission electron microscopy

Correlative light-electron microscopy (CLEM) combines the complementary advantages of light and electron microscopy to achieve precise subcellular localization of intratumoral microbes at nanoscale resolution (Figure 5G) [47]. Transmission electron microscopy (TEM) reveals ultrastructural features of intratumoral microbes and their intracellular distribution, such as bacterial sequestration within lysosomal compartments [48]. When combined with fluorescent labeling, CLEM enables targeted visualization and single-particle tracking within live cells. A key advantage of CLEM over FISH and immunofluorescence (IF) staining is its capacity to visualize labeled microbes within their native ultrastructural context, thereby yielding deeper biological insights [49]. Advances in super-resolution and intravital CLEM hold promise for real-time characterization of microbial dynamics within tumors, establishing CLEM as an increasingly indispensable tool in tumor microbiome research.

D-alanine-based methods

D-alanine-based labeling exploits a conserved bacterial metabolic pathway to detect metabolically active microbes within tumors (Figure 5H) [50]. Nearly all bacteria express alanine racemase, which converts L-alanine to D-alanine—an essential precursor for peptidoglycan synthesis [51]. By incubating fresh tumor tissues with fluorescently labeled D-alanine, researchers can selectively label live, metabolically active bacteria in the TME [52]. This approach not only confirms bacterial viability but also provides functional evidence of their potential involvement in TME regulation, making it a valuable tool for studying the activity of

intratumoral microbiota.

Effects of the Intratumoral Microbiota on Cancer

Cancer progression involves core hallmarks, including sustained proliferation, evasion of growth suppression and cell death, replicative immortality, angiogenesis, and metastasis. Accumulating evidence suggests that the intratumoral microbiota critically modulates these oncogenic processes. This modulation occurs largely by reshaping crosstalk between malignant cells and key components of the tumor microenvironment, such as immune and stromal cells. Diverse microbial communities, comprising bacteria, viruses, and fungi, have been identified in various solid tumors. Moreover, their composition varies substantially across different cancer types. Notably, the intratumoral microbiota exhibits context-dependent functional duality. Some microbes promote tumorigenesis through chronic inflammation, DNA damage, or immunosuppression. Conversely, others exert anti-tumor effects by activating immunity or modulating metabolism. This functional plasticity establishes the microbiota as a dynamic regulator of tumor fate and a promising target for therapeutic intervention.

DNA mutations

Induction of genomic instability and DNA mutations constitutes a fundamental carcinogenic mechanism mediated by the intratumoral microbiota (Figure 6A) [53]. Oncogenic viruses account for more than 10% of human malignancies. HPV is associated with cervical cancer and head and neck squamous cell carcinoma (HNSCC), while HBV is linked to hepatocellular carcinoma [54]. These viruses drive carcinogenesis through the integration of their genomes into host chromosomes. This genomic integration induces genetic alterations that promote uncontrolled cell proliferation and malignant transformation [55]. Beyond causing direct DNA damage, integrated viruses encode oncoproteins that reprogram host cell biology. The HPV E7 oncoprotein suppresses the cGAS-STING signaling pathway, thereby attenuating type I interferon and pro-inflammatory cytokine production to facilitate immune evasion [56]. Human T-lymphotropic virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia [57]. Its Tax oncoprotein inhibits DNA repair pathways, leading to genomic instability and the accumulation of oncogenic mutations.

Specific bacterial species also contribute significantly to carcinogenesis through diverse DNA-damaging mechanisms. pks$^+$ *E. coli* produces colibactin, a genotoxin that induces DNA double-strand breaks and accelerates colorectal tumorigenesis. *F. nucleatum* employs FadA to activate E-cadherin-β-catenin signaling, leading to CHK2 upregulation and subsequent DNA damage in colorectal cancer models [58]. ETBF and *B. fragilis* frequently co-enrich in the intestinal mucosa of patients with familial adenomatous polyposis [59]. There, they synergistically induce interleukin-17 production, resulting in extensive epithelial DNA damage.

Furthermore, microbial metabolic activities contribute to genomic instability. ETBF upregulates spermine oxidase in colonic epithelial cells, inducing ROS generation and phosphorylation of γ-H2AX [60]. β-Glucuronidase, which is elevated in breast cancer tissues, reactivates dietary carcinogens, such as heterocyclic amines, by releasing DNA-reactive intermediates [61]. Oral pathogens, including *Streptococcus anginosus* and *Porphyromonas gingivalis*, metabolize ethanol to acetaldehyde [62]. This metabolite forms DNA adducts and impairs DNA repair enzymes, potentially contributing to oral carcinogenesis. Taken together, these microbial virulence factors foster pro-inflammatory, DNA-damaging microenvironments that promote tumor initiation [63]. Nevertheless, the precise mechanistic contributions of specific microbes to cancer initiation warrant further investigation. Such studies are needed to

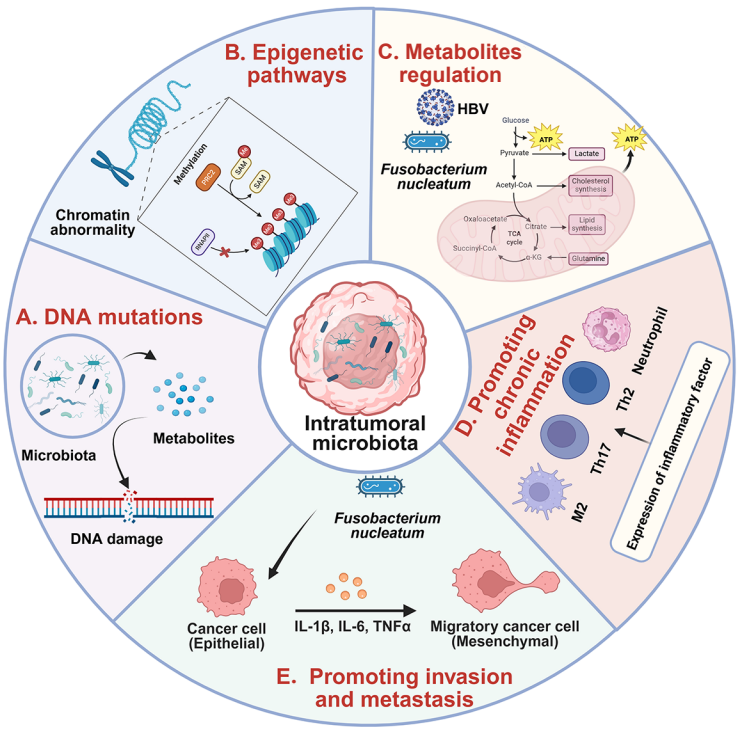


Figure 6. Effects of the intratumoral microbiota on cancer (A) Induction of DNA damage and genomic instability. (B) Epigenetic regulation. (C) Metabolic reprogramming. (D) Promotion of chronic inflammation. (E) Promotion of invasion, metastasis, and cancer stemness.

fully elucidate the complex interplay between microbial factors and host-driven carcinogenesis.

Epigenetic pathways

Aberrant epigenetic regulation constitutes a fundamental mechanism through which intratumoral microorganisms drive oncogenesis by silencing tumor suppressor genes and activating oncogenic pathways. Pathogenic bacteria have evolved sophisticated mechanisms to reprogramme the host epigenome, thereby promoting their survival and facilitating immune evasion. *Helicobacter pylori* drives gastric adenocarcinoma primarily through the induction of aberrant DNA methylation patterns (Figure 6B) [64]. Other gastric microbes, such as *Kytococcus sedentarius* and *Actinomyces oris*, are also involved in this mechanism, promoting gastric cancer progression [65]. Furthermore, the link between microbial colonization and epigenetic alterations extends beyond gastric cancer to other malignancies. In high-cellularity thyroid carcinoma, enriched microbial communities correlate with elevated methylation of tumor suppressor genes. In hepatocellular carcinoma, distinct intratumoral microbiome profiles are significantly associated with methylation-driven alterations in gene expression. In CRC, the abundance of *F. nucleatum* is linked to tumor-associated macrophage infiltration and hypermethylation of the *CDKN2A* promoter [66]. This underscores the multifaceted influence of the microbiota on the tumor epigenetic landscape.

Microorganisms directly modulate the host epigenetic machinery through diverse molecular mechanisms. *F. nucleatum* orchestrates epigenetic reprogramming by upregulating the long non-coding RNA ENO1-IT1 through SP1, which recruits the histone acetyltransferase KAT7 to remodel chromatin and enhance glycolytic metabolism in colorectal cancer [67]. *F. nucleatum* and *Hungatella hathewayi* further promote hypermethylation of tumor suppressor genes by upregulating DNA methyltransferases [68,69]. Indirectly, microbes shape host epigenetic regulation through their metabolic activities. They synthesize essential methyl donors, including folate and B vitamins, which function as cofactors for DNA and histone methyltransferases. Microbe-derived SCFAs modulate the activity of histone acetyltransferases and deacetylases, thereby inducing genome-wide epigenetic alterations. Specific bacteria in lung cancer patients produce methionine, a key methyl donor that fuels methylation of nucleic acids and proteins.

Viral pathogens similarly engage in epigenetic crosstalk with host cells. Seven oncogenic viruses have been shown to drive malignant transformation through host epigenetic modifications. HBV employs RNA m^6A modification to regulate viral replication, mediate immune evasion, and promote hepatocarcinogenesis, highlighting the intricate virus-host epigenetic interplay [70]. Collectively, intratumoral microorganisms spanning bacterial and viral species can modulate all major classes of epigenetic modifications, including DNA methylation, histone modifications, RNA modifications, and non-coding RNA-mediated regulation [71]. However, the precise molecular mechanisms underlying microbiota-induced epigenetic changes in tumorigenesis remain incompletely understood. Further investigation is warranted to elucidate their pathophysiological significance and therapeutic potential.

Metabolite regulation

The intratumoral and gut microbiota shape carcinogenesis by rewiring host metabolism and immune responses. Distinct microbial communities and metabolic signatures characterize various malignancies, from altered inositol phosphate and amino acid metabolism in breast and cervical cancers to enriched glycerophospholipids and iron/tryptophan pathways in gastric and oral tumors. Oncogenic viruses similarly reprogram cellular metabolism via HIF1α/PI3K-Akt-mTOR activation and PKM2 stabilization, synergizing with hypoxia and inflammation to drive virus-associated tumorigenesis (Figure 6C) [72]. Beyond direct microbe–cancer cell interactions, microbial metabolites serve as systemic functional effectors that link the microbiome to tumor progression and immune modulation. Key metabolites—including SCFAs, bile acids (BAs), and tryptophan/adenosine derivatives—orchestrate TME remodeling through receptor signaling, epigenetic regulation, and metabolic reprogramming of both malignant and immune cells.

SCFAs

Microbial fermentation of dietary fiber yields SCFAs (acetate, propionate, butyrate), which exert context-dependent effects on tumorigenesis and immunity. In CRC, SCFA depletion correlates with disease progression, though whether this drives or results from tumorigenesis remains unresolved. SCFAs primarily signal through G-protein-coupled receptors (GPR43/41/109A) and inhibit histone deacetylases (HDACs), collectively maintaining chromatin accessibility to promote cell cycle arrest and apoptosis [73]. These pathways underpin their immunomodulatory roles: SCFAs enhance $CD103^+$ dendritic cell activation via GPR43/109A, driving regulatory T cell differentiation (Figure 7A), while simultaneously boosting $CD8^+$ T-cell cytotoxicity and ILC3-derived IL-22 production through HDAC inhibition and AhR/HIF-1α activation [74]. Butyrate exemplifies metabolic selectivity—the "butyrate paradox"—where it fuels healthy colonocytes but accumulates in glycolytic cancer cells, inducing HDAC inhibition, downregulating the oncogenic miR-17-92a cluster, and upregulating PTEN/p21 [75]. Acetate displays dual functionality: microbiome-derived acetate enhances $CD8^+$ T cell responses and potentiates anti-PD-1 therapy, whereas host-derived acetate can be converted to acetyl-CoA to acetylate c-Myc, upregulate PD-L1, and promote immune evasion. Synergistic combinations of SCFAs with chemotherapy or checkpoint inhibitors highlight their translational potential.

BAs

Gut microbiota convert hepatic primary BAs into secondary metabolites, *e.g.*, deoxycholic acid and lithocholic acid (LCA), which exert dual pro- and anti-tumor effects. Carcinogenic secondary BAs promote DNA damage, Wnt/β-catenin activation, and COX-2 expression, while protective BAs enhance apoptosis and antitumor immunity [76]. BA signaling is mediated primarily through the nuclear receptor FXR and membrane receptor TGR5 (Figure 7B) [77]. FXR maintains epithelial barrier integrity and suppresses pro-tumorigenic inflammation; its downregulation in CRC exacerbates Wnt signaling and tumor progression [78]. Conversely, TGR5 activation on macrophages, dendritic cells (DCs) and T-cells elevates cAMP-PKA signaling, polarizing immune cells toward anti-inflammatory phenotypes and modulating Treg/effector T cell balance [79]. Despite their oncogenic potential, secondary BAs such as LCA can induce caspase-dependent apoptosis in prostate and breast cancer cells and stimulate antitumor immunity via TGR5, underscoring the context-dependent nature of BA-mediated signaling.

Tryptophan and adenosine metabolites

Dietary tryptophan is metabolized by gut microbes into indole

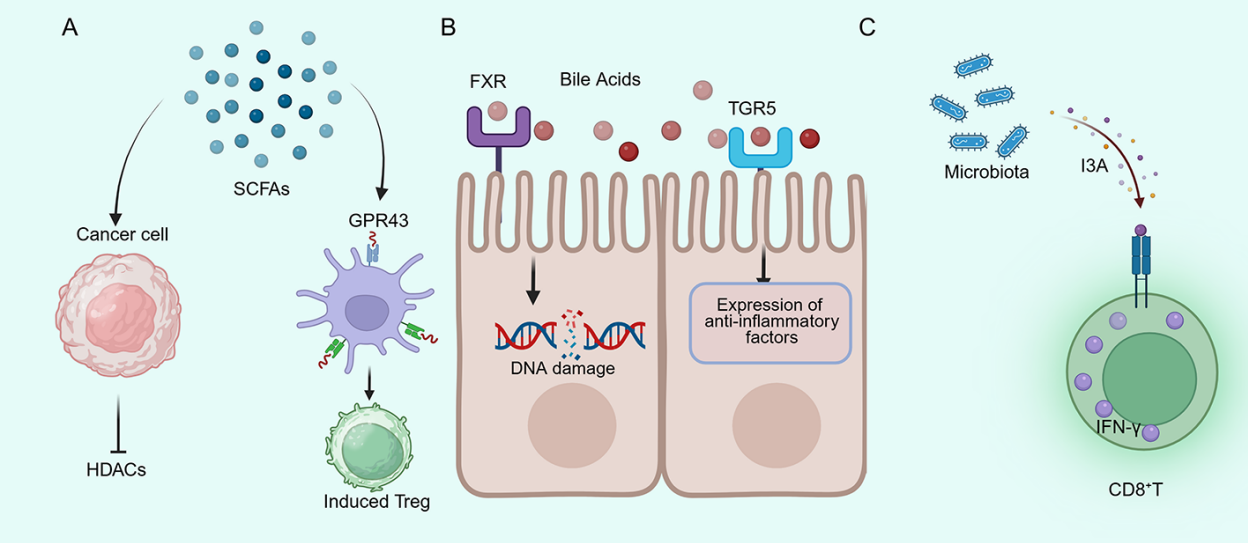


Figure 7. Microbial metabolite-mediated signaling (A) SCFAs inhibit HDACs and activate CD103⁺ DCs through GPR43. (B) BAs act via two key receptors: nuclear FXR (regulating genes and anti-inflammatory responses) and membrane TGR5 (activating cAMP pathways to modulate immune cell function). (C) I3A promotes IFN-γ production in CD8⁺ T cells via AhR signaling and enhances immune checkpoint inhibitor (ICI) efficacy.

derivatives, *e.g.*, indole-3-aldehyde (I3A) and indole-3-propionic acid, which serve as aryl hydrocarbon receptor (AhR) ligands [80]. Microbial I3A directly activates CD8⁺ T cells via AhR, amplifying IFN-γ production and enhancing ICI efficacy (Figure 7C) [81]. A tryptophan-rich diet similarly potentiates checkpoint blockade in an AhR-dependent manner, highlighting the therapeutic promise of targeted microbial metabolite supplementation. In parallel, tumor-associated ATP hydrolysis generates extracellular adenosine, a potent immunosuppressant that accumulates under hypoxia [82]. Adenosine signals through A2aR (broadly expressed on lymphocytes) and A2bR (enriched on myeloid cells) to inhibit effector T cell function, promote Treg activity, and impair antigen presentation. Pharmacological blockade of adenosine receptors or inhibition of upstream ectonucleotidases restores antitumor immunity and synergizes with T cell-based therapies.

Promoting chronic inflammation

Persistent chronic inflammation constitutes a hallmark of cancer progression. Within the TME, the intratumoral microbiota actively drives carcinogenesis by engaging pattern recognition receptors, particularly Toll-like receptors (TLRs), which are expressed on both immune and stromal cells. A key example is TLR4 recognition of *F. nucleatum*, which activates the IL-6/pSTAT3/c-MYC signaling axis to drive M2-like macrophage polarization and accelerate colorectal cancer progression in murine models [83]. Similarly, microbiota associated with pancreatic ductal adenocarcinoma suppress Th1 cell polarization and M1 macrophage differentiation through TLR signaling, thereby fostering immune tolerance.

Microbial LPS binding to TLR4 triggers chemokine-dependent recruitment of monocyte-like macrophages that produce IL-1β, amplifying Th17 cell expansion [84]. This cascade increases intestinal permeability, facilitating microbial product leakage and further promoting M2 macrophage differentiation—a self-reinforcing cycle that establishes an immunosuppressive niche (Figure 6D). Disrupted tumor epithelia release microbial products that activate dendritic cells, inducing γδT17 cell polarization [85]. These cells secrete IL-17, IL-8, GM-CSF, and TNF-α, recruiting polymorphonuclear myeloid-derived suppressor cells to convert inflammatory microenvironments into immunosuppressive hubs that fuel tumor progression [86].

ETBF exemplifies microbial-driven inflammation through its metalloproteinase toxin BFT, which cleaves E-cadherin to activate STAT3-NF-κB signaling, releasing IL-17 and IL-23 to attract pro-tumor myeloid cells. Other pathogens, including *F. nucleatum* and *Aggregatibacter actinomycetemcomitans*, activate TLR2/TLR4–NF-κB signaling in macrophages to stimulate IL-6 production [87]. *Porphyromonas gingivalis* promotes proliferation in pancreatic and colorectal cancers through gingipain-dependent activation of the MAPK pathway [88].

Promotion of invasion and metastasis

Cancer invasion and metastasis are promoted by intratumoral microbes through reshaping the TME. The epithelial-mesenchymal transition (EMT) confers migratory mesenchymal phenotypes on carcinoma cells through TGF-β/SMAD, PI3K/AKT, Wnt/β-catenin, and MAPK signaling pathways. Microorganisms actively regulate EMT: *F. nucleatum* virulence protein Fn-Dps upregulates CCL2/CCL7 to induce EMT and enhance metastasis in colorectal cancer models [89]. In bladder cancer, *E. coli*, butyrate-producing bacteria SM4/1, and *Oscillatoria* correlate with altered expression of EMT markers including E-cadherin and vimentin [90]. *P. gingivalis* elevates EMT transcription factors Slug, Snail, and ZEB1 to promote oral epithelial cell migration [91]. *Candida* colonization in metastatic colon tumors may facilitate dissemination by downregulating cellular adhesion molecules.

The microbiota also enhances cancer stemness during EMT (Figure 6E). *H. pylori* CagA-positive strains induce EMT-like changes and upregulate the gastric cancer stem cell marker CD44, increasing migratory capacity and tumor sphere formation [92]. ETBF toxin promotes stemness and metastasis via E-cadherin cleavage and β-catenin nuclear translocation. In spontaneous breast tumors, enriched *Staphylococcus*, *Lactobacillus*, and *Streptococcus*

suppress RhoA-ROCK signaling to remodel actin cytoskeletons, enhancing tumor cell resistance to fluid shear stress and accelerating metastasis. Additionally, *F. nucleatum* activates ALPK1–NF-κB signaling to upregulate ICAM1, which strengthens cancer cell adhesion to the vascular endothelium [93].

Enhancing antitumor immune responses

While intratumoral microbes frequently drive tumor progression by inducing EMT, enhancing cancer stemness, and facilitating metastatic dissemination, emerging evidence underscores their profound functional duality. In stark contrast to these pro-tumorigenic activities, specific intratumoral microbial communities actively restrain tumor growth by reshaping the local immune system and stimulating robust antitumor responses.

STING signaling activation

Intratumoral bacteria can directly engage the STING pathway to bridge innate and adaptive immunity. For instance, Bifidobacterium species colonize tumor sites and trigger STING-dependent DC maturation (Figure 8A) [94]. Systemic or local administration of Bifidobacterium enhances DC cross-priming capacity and synergizes with anti-CD47 therapy in a STING-dependent manner, revealing a novel mechanism by which intratumoral bacteria augment T cell-targeted immunotherapy. Similarly, *Akkermansia muciniphila*-derived cyclic dinucleotides act as STING agonists, stimulating intratumoral monocytes to produce type I interferons. This cascade reprograms macrophages and fosters NK-DC crosstalk, ultimately improving ICB efficacy in melanoma.

T and NK cell activation

Beyond innate sensing, the tumor microbiota directly potentiates adaptive and cytotoxic lymphocyte responses. In PDAC, the presence of *Saccharopolyspora*, *Pseudoxanthomonas*, and *Streptomyces* correlates with enhanced $CD8^+$ T cell infiltration and activation, which is strongly associated with prolonged patient survival. Microbial metabolites further amplify this axis; trimethylamine N-oxide (TMAO), secreted by *Clostridiales* genera, induces PERK-mediated endoplasmic reticulum stress and tumor cell pyroptosis, thereby sensitizing triple-negative breast cancer to immunotherapy (Figure 8B) [95]. Additionally, dietary interventions such as high salt intake enrich intratumoral *Bifidobacterium*, which elevates hippurate levels to boost NK cell cytotoxicity and drive melanoma regression. Resident microbes, including *Lachnoclostridium* and certain viruses (EBV, HBV, and MCPyV), can also induce chemokine networks that recruit $CD8^+$ T cells, correlating with improved survival in cutaneous melanoma [96].

Intratumoral microbiota-derived antigen presentation

Finally, intracellular bacteria residing within tumor or immune cells provide a reservoir of non-self antigens. Bacterial peptides processed and presented via MHC molecules on tumor cells or antigen-presenting cells can elicit tumor-specific T cell reactivity (Figure 8C) [97]. Studies have demonstrated that melanoma-infiltrating lymphocytes exposed to bacterial peptides exhibit heightened IFN-γ secretion, indicating that microbe-derived antigens function as endogenous adjuvants that sensitize tumors to immune attack. Immunofluorescence analyses further confirm the intracellular localization of bacteria within $CD45^+$ immune cells and tumor cells, supporting their role in priming antitumor immunity and enhancing immunotherapy responsiveness [98].

Intratumoral Microbiota as Diagnostic and Prognostic Biomarkers

In addition to their established roles in metabolic and immune regulation, intratumoral microbiota exhibit cancer-type-specific signatures that hold substantial potential for clinical diagnostics, tumor localization, and treatment response monitoring.

Unlike the relatively stable human genome, which exhibits only minor somatic variation, the microbiota demonstrates pronounced inter-individual and tissue-specific heterogeneity. This variability enables microbial profiles to serve as sensitive indicators for cancer detection and anatomical localization. For instance, while circulating *TP53* mutations lack tissue specificity across >25 malignancies, *Streptococcus gallolyticus* bacteremia strongly indicates a colorectal origin due to its gastrointestinal niche [99]. Despite this promise, microbe-based diagnostics face inherent constraints, including low biomass relative to host DNA, susceptibility to reagent or environmental contamination, and confounding effects from antibiotics or systemic inflammation [100]. Questions regarding the specificity, stability during therapy and clinical utility of these signatures remain unresolved and must be addressed before routine implementation.

Recent multi-omics and spatial imaging studies have validated cancer-type-specific intratumoral microbial signatures across >30 malignancies, extending beyond traditional aerodigestive tumors to include breast, brain, and bone cancers [101]. Rigorous contamination-controlled analyses revealed distinct taxonomic compositions, α-diversity, and inferred metabolic functions across cancer types, alongside heterogeneous spatial distributions and frequent intracellular localization within malignant and immune cells. Although bacterial viability in low-biomass tumors has been debated, successful cultivation across multiple cancer types confirms that intratumoral microbes are metabolically active rather than passive passengers. Concurrently, machine learning applied to large-scale genomic datasets has extracted robust, cancer-specific nucleic acid signatures capable of high-accuracy malignancy classification.

Building on tissue-based discoveries, microbial signatures are increasingly being adapted for liquid biopsy applications. Plasma-derived cell-free microbial DNA has shown promise for non-

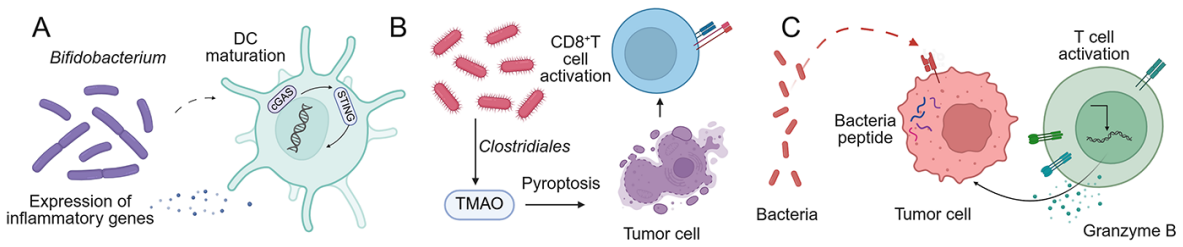


Figure 8. The intratumoral microbiota enhances antitumor immune responses through diverse mechanisms (A) STING signaling activation. (B) T cell activation. (C) Intratumoral microbiota-derived antigen presentation.

invasive cancer detection and for stratifying immunotherapy responders, although the predictive mechanisms remain poorly defined. Translational progress is currently hindered by several unresolved issues: the pathways governing microbial DNA release into circulation are unknown, initial bioinformatics findings often lack orthogonal experimental validation, and distinguishing true tumor-associated signals from transient bacteremia or systemic infection remains challenging. Prospective validation against cohorts with non-malignant infections, sepsis, or concurrent antibiotic exposure is essential to establish clinical specificity.

Collectively, intratumoral microbiota represents a complementary biomarker layer that bridges spatial tumor biology with non-invasive diagnostics. Realizing their clinical potential requires standardized decontamination workflows, longitudinal multi-omics integration, and mechanistic studies to clarify whether microbial alterations drive tumorigenesis or merely reflect disease progression. Addressing these technical and biological gaps will be critical to transition microbial biomarkers from research observations to clinically actionable assays.

Therapeutic Potential of the Intratumoral Microbiota

The microbiome, often called a "second genome", has a key advantage over the human genome: it can be modified. This means that strategies designed to alter the microbiome could become new treatments for cancer and its complications. In the following section, we summarize such experimental approaches and discuss their potential applications in cancer therapy (Figure 9).

Fecal microbiome transplantation

Human fecal microbiome transplantation (FMT) transfers the entire gut microbial community from a donor to a recipient [102]. Although not new—historical records from 4th century BCE China describe its use for diarrhea—FMT gained prominence during the 1990s–2000s *Clostridioides difficile* epidemic. It is now explored as a therapeutic strategy for diverse conditions (Figure 9A). In oncology, accumulating evidence links fecal microbiome composition and function to cancer therapy outcomes, prompting active investigation into FMT's potential to enhance treatments, particularly immunotherapy.

FMT has proven highly effective for recurrent *C. difficile* infection and shows promise in ulcerative colitis. Vaginal microbiota transplantation has yielded encouraging early results for refractory bacterial vaginosis. In cancer research, transferring fecal samples from patients into germ-free or antibiotic-treated tumor-bearing mice receiving ICIs established a causal link between specific microbiome configurations and immunotherapy efficacy. FMT from ICI responders conferred treatment responsiveness to recipient mice, whereas non-responder microbiomes did not [103]. Two pioneering human trials recently demonstrated that FMT from ICI-responsive melanoma patients can overcome resistance in a subset of refractory recipients. Treatment success depended on donor selection and successful engraftment of donor microbes in the recipient gut.

Although therapeutic outcomes remain variable, these findings highlight FMT's potential to improve cancer treatment. Current FDA regulations have mitigated acute risks, but long-term safety requires further evaluation. Future applications of FMT demand rigorously designed prospective trials. These trials should be integrated with comprehensive microbiome and metabolome profiling to assess efficacy and safety and to elucidate underlying mechanisms for developing personalized therapeutic strategies.

Prebiotics, probiotics and postbiotic

Prebiotics and probiotics are capable of modulating the gut microbiota. Although evidence regarding their efficacy remains inconsistent, they are actively being investigated for their potential roles in tumor development and therapy [104]. A self-reported study of 3032 participants revealed that insoluble fiber intake

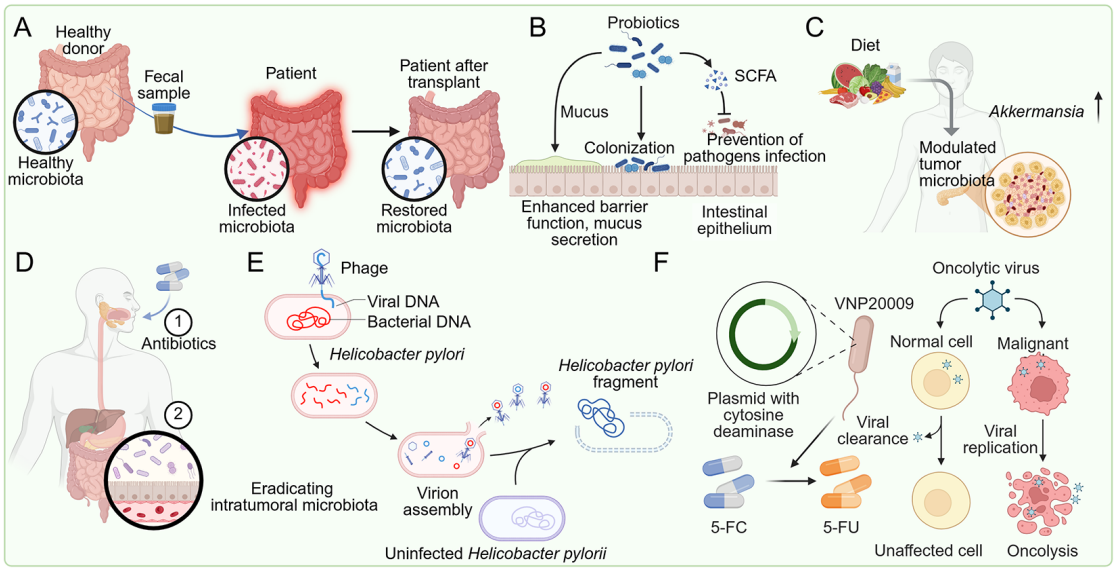


Figure 9. Modulating the intratumoral microbiota for cancer therapy (A) FMT involves transferring healthy gut microbiota to treat diseases and is being studied to enhance cancer immunotherapy efficacy. (B) Probiotics modulate the microbiome and show potential in influencing cancer development and treatment responses. (C) Dietary modifications, such as high-fiber intake, can reshape the gut microbiome and improve the efficacy of cancer therapies. (D) Antibiotics are used to target tumor-associated bacteria. (E) Phage therapy offers a targeted approach to eliminate cancer-promoting bacteria without broadly affecting the microbiome. (F) Engineered bacteria and oncolytic viruses are designed to deliver antitumor agents or stimulate immune responses.

correlated with increased colorectal cancer mortality, yet research on fiber supplementation effects is ongoing. A randomized crossover trial is evaluating dry bean supplementation in individuals with colorectal polyps or cancer history. Fiber intake has also been linked to improved responses to checkpoint inhibitors in melanoma, with high-fiber consumers showing better outcomes than those on low-fiber diets. These encouraging observations require validation through larger prospective studies and mechanistic investigations.

Probiotics deliver beneficial microbes directly to the gastrointestinal tract (Figure 9B) [105]. Despite widespread public interest and numerous clinical trials, many studies report no beneficial effects. Limited data suggest probiotic use may impair checkpoint inhibitor responses in melanoma, potentially by reducing microbial alpha diversity. Bioengineered bacteria, including next-generation probiotics and live biotherapeutic products (LBPs), are being developed for cancer treatment owing to their cytotoxic properties, immunomodulatory capacity, or utility as drug delivery vehicles [106].

Postbiotic therapy refines these approaches by using defined microbial metabolites as adjunctive cancer treatments [107]. Germ-free mice colonized with selected *Bacteroidales*, *Eubacterium*, and *Faecalibacterium* strains exhibited elevated mevalonate and dimethylglycine levels, correlating with enhanced antitumor immunity and improved checkpoint inhibitor efficacy. Indoles, tryptophan-derived bacterial metabolites acting as aryl hydrocarbon receptor ligands, maintain mucosal barrier integrity. Ovarian cancer patients show reduced serum 3-indole propionic acid, while indoles suppress breast cancer cell growth *in vitro* and reduce metastatic burden in mouse models upon dietary supplementation [108].

Dietary modulation of the tumor microbiota

Dietary intervention represents a promising strategy to reshape the gut microbiota toward health-promoting configurations and enhance the efficacy of cancer therapy [108]. Western-style diets, characterized by high intake of saturated fats, refined carbohydrates, and fructose, are associated with an increased risk of colorectal, prostate, and breast cancers. In murine models, high-fat feeding enriches *Firmicutes* and *Proteobacteria* while depleting *Bacteroidetes*. It also activates NF-κB-driven inflammatory signaling, and these alterations are strongly associated with tumorigenesis. Conversely, high-fiber diets promote microbial production of SCFAs, including butyrate, propionate, and acetate. These metabolites exert anti-inflammatory and pro-apoptotic effects in preclinical cancer models. Oral inulin supplementation markedly enhances the efficacy of anti-PD-1 therapy in mice bearing adenocarcinoma. It increases the intestinal abundance of *Akkermansia* and fecal SCFA levels, both of which have been previously associated with improved responses to ICIs [109]. Furthermore, it remodels the tumor immune microenvironment by expanding PD-1^{+}CD8^{+} T cells and reducing regulatory T cells (Figure 9C).

Most evidence comes from animal studies, which fail to capture the interindividual variability in human physiological and disease responses. Recent findings from the Personalized Nutrition Project showed that integrating microbiome and host data from large human cohorts enables machine learning models to predict personalized postprandial glycemic and lipidemic responses. Dietary interventions guided by such predictions reduced the metabolic consequences of glucose spikes in prediabetic individuals. Applying similar frameworks to map dietary responses in cancer contexts could enable personalized nutrition strategies based on an individual's microbiota profile. Such approaches may support cancer prevention, improve treatment outcomes, mitigate complications, and enhance therapeutic responsiveness.

Antibiotic therapy

Antibiotic eradication of *H. pylori* is a clinically established strategy for preventing gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma [110]. Although *H. pylori* colonization is common and often asymptomatic, persistent infection with virulent strains is a major risk factor for gastric carcinogenesis. Emerging evidence suggests that antibiotics may overcome microbiota-mediated chemoresistance in non-gastric cancers [111]. In PDAC, intratumoral Gamma-*Proteobacteria* express cytidine deaminase, which inactivates gemcitabine. In CRC, *F. nucleatum* promotes resistance to oxaliplatin and 5-fluorouracil by suppressing apoptosis [112]. In mouse models, metronidazole reduced *F. nucleatum* abundance, inhibited tumor growth, and restored chemosensitivity (Figure 9D). Preclinical studies report that combining ampicillin with paclitaxel enhances antitumor efficacy in breast cancer models.

Despite their therapeutic benefits, systemic antibiotics disrupt the gut microbiota, which plays a critical role in antitumor immunity and treatment response. Antibiotic exposure during the peri-initiation period of ICIs impairs therapeutic efficacy and increases the risk of immune-related adverse events. These adverse effects result from the loss of beneficial bacteria, such as *Bifidobacterium* and *Akkermansia*, and the depletion of key microbial metabolites, including SCFAs [113].

Current research therefore prioritizes targeted strategies that eliminate tumor-promoting microbes while sparing commensal microbial communities. These include colon-targeted antibiotic adsorbents and metronidazole-fluorouridine nanoparticles. Both enhance intratumoral drug delivery while avoiding systemic disruption of the gut microbiota. Optimizing the selective eradication of pro-tumoral microorganisms while preserving microbial homeostasis is a critical goal for improving cancer therapy outcomes.

Phage therapy

Bacteriophages are viruses that selectively infect bacteria and dynamically modulate gut microbiota composition through phage bloom events [114]. Historically studied as precision antibacterial agents, systemically administered phages can treat infections in preclinical and clinical settings. However, bacterial resistance, immune recognition, and rapid clearance remain significant challenges. Oral delivery offers a promising alternative because it avoids systemic immune activation and enables selective targeting of pathobionts [115]. This approach minimizes broad disruption of the gut microbiota. Bacteriophage-rich transplants have alleviated diet-induced dysbiosis in murine models. They have also shown feasibility in human studies, although engraftment efficiency remains variable.

Phage cocktails engineered to target multiple bacterial surface receptors can improve targeting precision and reduce the emergence of resistance [116]. Additionally, phages are susceptible to inactivation by gastric acidity, intestinal mucus layers, and microbial biofilms in the gastrointestinal tract.

In oncology, phages present a strategy for selectively eliminating tumor-promoting bacteria while preserving beneficial commensals (Figure 9E). Lytic phages have reduced *H. pylori* burden *in vitro*, and engineered phages can deliver therapeutics directly to the tumor microenvironment [117]. For instance, *F. nucleatum*-targeted phages conjugated to irinotecan nanoparticles enhanced drug delivery and efficacy in murine CRC models.

Phages outperform conventional antibiotics in biofilm penetration and bacterial specificity. Emerging synthetic biology approaches reprogram phages to recruit antitumor immune cells or deliver cytotoxic payloads [118]. Although high specificity often necessitates patient-customized formulations, standardized phage cocktails hold therapeutic promise. Current evidence positions phages as adjuvants to antibiotics or salvage therapies rather than standalone replacements, particularly given their immunomodulatory potential. Defining optimal phage selection, dosing regimens, and delivery routes is essential for successful clinical translation in oncology.

Engineered bacteria and oncolytic virus

Advances in synthetic biology now enable the genetic engineering of bacteria to reduce off-target toxicity and improve tumor-targeting precision. These engineered bacteria exert antitumor effects through multiple mechanisms. They deliver prodrug-converting enzymes, express cytotoxic agents, activate immune responses, and modulate the tumor stroma.

Attenuated *Salmonella typhimurium* VNP20009, engineered to express cytosine deaminase, increased intratumoral concentrations of 5-fluorouracil in cancer patients (Figure 9F) [119]. *B. infantis* engineered to deliver herpes simplex virus thymidine kinase in combination with ganciclovir suppressed bladder cancer progression in rat models [120]. Engineered *E. coli* induced localized TNF-α production and inhibited tumor growth in murine models. Separately, bacteria expressing cytolysin A showed specific tumor accumulation and potent antitumor activity.

Engineered bacteria can also enhance anti-tumor immunity through multiple immunomodulatory mechanisms. *E. coli* Nissle 1917 converts tumor-derived ammonia into L-arginine, thereby enhancing T cell function and responses to immunotherapy [121]. Attenuated *S. typhimurium* engineered to release flagellin B promotes M2-to-M1 macrophage repolarization through TLR4 signaling. *Listeria monocytogenes* engineered to express galactosylceramide activates NKT cells and reduces metastatic burden. Targeting the tumor stroma constitutes another therapeutic strategy. *Salmonella* engineered to secrete hyaluronidase degrades hyaluronic acid in pancreatic cancer models. This enhances chemotherapy penetration into tumors. Despite promising preclinical results, bacterial therapies face significant challenges. These include interspecies physiological differences, uncertainties in dosing safety, and potential infection risks.

Oncolytic virus (OV) therapy uses genetically engineered viruses that selectively replicate in and lyse cancer cells. This selectivity arises from dysregulated signaling pathways, such as EGFR/Ras activation and defective interferon responses [122]. Cell lysis releases tumor antigens to stimulate immunity, and some viruses disrupt the tumor vasculature. The oncolytic poxvirus JX-594 selectively replicates in tumors with activated EGFR/Ras signaling [123]. Viruses engineered to express IL-23 remodel the TME and enhance T cell infiltration [124]. OV infection can upregulate PD-L1, sensitizing tumors to checkpoint inhibitors such as pembrolizumab. Combination with radiotherapy, chemotherapy, or immunotherapy generally yields superior therapeutic outcomes. Intratumoral injection remains the standard route of administration [125]. In contrast, intravenous delivery is limited by rapid immune-mediated clearance. Emerging strategies utilize cell-based carriers and polymer-based coatings to improve systemic delivery.

Conclusions and Perspectives

The emerging field of tumor microbiology has yielded critical insights into how tumor microbiota contributes to the physiological and pathological mechanisms of human cancers. As one of the most complex and formidable human diseases, cancer has been increasingly linked not only to specific alterations in tumor microbiota but also to broader changes in microbial community structures. The intratumoral microbiota, which modulates tumor progression and influences therapeutic outcomes, is attracting growing scientific interest. Multiple tumor types are now believed to harbor unique microbial communities. The intratumoral microbiota can promote cancer development through various mechanisms, such as inducing genomic instability and mutation, causing epigenetic modifications, promoting pro-tumor inflammation, regulating metabolism, and activating invasion and metastasis. As research deepens, mucosal organs and NATs are currently recognized as two potential sources of intratumoral microbes. Furthermore, the detection of microbes in tumors of non-mucosal organs such as breast and liver suggests the circulatory system as another potential origin of colonization. Regarding the mechanisms, the interaction between adhesin Fap2 on *F. nucleatum* and overexpressed Gal-GalNAc on tumor cells may mediate this bacterial invasion. However, the entry mechanism for most other intratumoral microbes remains poorly understood.

The study of intratumor microbiota faces several challenges, with environmental contamination posing a major obstacle. Such contamination significantly compromises detection accuracy and has led to ongoing debate regarding the biological relevance and reproducibility of intratumoral microbiota [126]. To improve reliability, researchers typically employ extensive negative controls. Moreover, metagenomic data are susceptible to environmental contamination, reducing the analytical precision. Hence, the development of bioinformatics approaches for filtering spurious signals will be essential for future studies.

The investigation into the role of intratumoral microbiota in tumorigenesis and malignant progression represents a promising yet nascent field of research. Numerous studies have reported divergent or even contradictory conclusions regarding the relationship between intratumoral microorganisms and tumors [127]. Consequently, there is a pressing need to develop standardized experimental and analytical protocols across the field. Moreover, current evidence is largely derived from microbe-based cross-sectional studies. While valuable, this study design inherently limits the ability to establish causality. Thus, although many studies suggest that the intratumoral microbiota can drive tumorigenesis, it remains possible that observed microbial changes are a consequence rather than a cause of cancer development.

Interactions within the tumor microenvironment are complex and bidirectional. The microbiota exerts broad influences on tumor cells, and tumor cells also significantly shape the microbial community through the release of soluble factors, alterations in local pH, oxygen tension, and nutrient availability [128]. For

instance, cytokines secreted by tumor cells may selectively promote or suppress the growth of specific bacterial species. Conversely, certain microbes exhibit adaptations that enable them to thrive in the acidic, hypoxic tumor niche and to evade immune clearance. Although the precise regulatory mechanisms governing these interactions remain under investigation, it is clear that within tumor microecology, both microbes and cancer cells share the fundamental drive of survival [129]. Their relationship may be competitive, symbiotic, or commensal. Critically, any bacteria-associated interaction that can provide advantages to cancer cells may reveal promising new targets for therapeutic intervention.

A significant gap still persists between clinical observations of intratumoral microbiota and their therapeutic application. Although modulation of these microbes in murine immunotherapy models has yielded promising results, such strategies have not yet been translated into commercially available human therapies. Moreover, even after uniform bioinformatics reanalysis, persistent taxonomic discrepancies have been observed in intratumoral microorganisms associated with immunotherapy response identified in both human and murine studies.

Given the considerable heterogeneity of the intratumor microbiota, personalized treatment strategies represent a promising avenue due to their potential for high efficacy and precise targeting. Blood-based microbial biomarkers have already shown diagnostic potential, and bacterial-derived peptides identified in melanoma may serve as novel therapeutic targets. Consequently, advances in detection and identification technologies are expected to facilitate the development of personalized treatments employing antibodies or other biologics tailored to an individual's intratumor microbiota. Similar to other anti-tumor modalities, bacterial therapies and antibiotics can be effectively combined with existing treatments such as immunotherapy and chemotherapy. For instance, engineered bacteria combined with immune checkpoint blockade have entered clinical trials, and antibiotics are being evaluated as adjuvants in several clinical settings. Just as normalizing tumor immunity is central to modern immunotherapy, normalizing the intratumor microbiota may emerge as a viable strategy to improve patient outcomes in the context of microbe-based interventions [130]. Moreover, with the successful application of fecal microbiome transplantation and growing evidence linking specific microorganisms to prognosis in cancers such as pancreatic cancer, the introduction of selected microbial communities into the TME may yield promising therapeutic benefits, although research in this area remains limited. Further investigation into the mechanisms governing microbial colonization and function within tumors will also enhance the design of bacterial delivery systems, enabling more targeted accumulation, reduced infection risks, and improved payload capacity.

In summary, as an integral component of the TME, the intratumor microbiota is poised to become a major focus in oncology research and a rich source of novel therapeutic targets. Despite persistent challenges, elucidating the role of microbiota in cancer could pave the way for powerful new strategies to improve patient care.

Author Contributions

Feiyue Guo: Writing—original draft, formal analysis. Wanru Zhuang: Writing—original draft, formal analysis. Jingyan Huang: Formal analysis. Yuping Dong: Writing-review & editing. Haiyan Xie: Writing—review & editing.

Acknowledgement

The authors thank the State Key Laboratory of Natural and Biomimetic Drugs (Peking University, Beijing, China) and Biological & Medical Engineering Core Facilities (Beijing Institute of Technology, Beijing, China).

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 22293034 to H.X., 32400774 to W.Z.), the National Science Fund for Distinguished Young Scholars (No. 22025401 to H.X.), and the Postdoctoral Fellowship Program of CPSF (No. GZB20240048 to W.Z.).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Sepich-Poore GD, Zitvogel L, Straussman R, Hasty J, Wargo JA, Knight R. The microbiome and human cancer. *Science (New York, NY)* 2021, 371: eabc4552
2. Nobels A, van Marcke C, Jordan BF, Van Hul M, Cani PD. The gut microbiome and cancer: from tumorigenesis to therapy. *Nat Metab* 2025, 7: 895–917
3. Yang L, Li A, Wang Y, Zhang Y. Intratumoral microbiota: roles in cancer initiation, development and therapeutic efficacy. *Sig Transduct Target Ther* 2023, 8: 35
4. Zhang Z, Liao Y, Tang D. Intratumoral microbiota: new frontiers in tumor immunity. *Carcinogenesis* 2022, 43: 719–727
5. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological Agents. Lyon, France: International Agency for Research on Cancer; 2012 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 100B)
6. Queen J, Shaikh F, Sears CL. Understanding the mechanisms and translational implications of the microbiome for cancer therapy innovation. *Nat Cancer* 2023, 4: 1083–1094
7. Cullin N, Azevedo Antunes C, Straussman R, Stein-Thoeringer CK, Elinav E. Microbiome and cancer. *Cancer Cell* 2021, 39: 1317–1341
8. Bernardo G, Le Noci V, Di Modica M, Montanari E, Triulzi T, Pupa SM, Tagliabue E, *et al*. The emerging role of the microbiota in breast cancer progression. *Cells* 2023, 12: 1945
9. Abed J, Emgård JEM, Zamir G, Faroja M, Almogy G, Grenov A, Sol A, *et al*. Fap2 mediates fusobacterium nucleatum colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host Microbe* 2016, 20: 215–225
10. Pleguezuelos-Manzano C, Puschhof J, Rosendahl Huber A, van Hoeck A, Wood HM, Nomburg J, Gurjao C, *et al*. Mutational signature in colorectal cancer caused by genotoxic pks$^+$ *E. coli*. *Nature* 2020, 580: 269–273
11. Charlson ES, Bittinger K, Haas AR, Fitzgerald AS, Frank I, Yadav A, Bushman FD, *et al*. Topographical continuity of bacterial populations in the healthy human respiratory tract. *Am J Respir Crit Care Med* 2011, 184: 957–963
12. Tsay JCJ, Wu BG, Badri MH, Clemente JC, Shen N, Meyn P, Li Y, *et al*. Airway microbiota is associated with upregulation of the PI3K pathway in lung cancer. *Am J Respir Crit Care Med* 2018, 198: 1188–1198
13. Chen JW, Dhahbi J. Lung adenocarcinoma and lung squamous cell carcinoma cancer classification, biomarker identification, and gene expression analysis using overlapping feature selection methods. *Sci Rep* 2021, 11: 13323
14. Mrázek J, Mekadim C, Kučerová P, Švejstil R, Salmonová H, Vlasáková

J, Tarasová R, *et al.* Melanoma-related changes in skin microbiome. *Folia Microbiol* 2019, 64: 435–442
15. Wong AYW, Bicchieraro G, Palumbo I, Ciabattoni A, Aristei C, Spaccapelo R. Microbial signatures in breast cancer: exploring new potentials across body niches. *Int J Mol Sci* 2025, 26: 8654
16. Allen J, Rosendahl Huber A, Pleguezuelos-Manzano C, Puschhof J, Wu S, Wu X, Boot C, *et al.* Colon tumors in enterotoxigenic bacteroides fragilis (ETBF)-colonized mice do not display a unique mutational signature but instead possess host-dependent alterations in the APC gene. *Microbiol Spectr* 2022, 10: e0105522
17. Seong J, Lee J, Lim YK, Yoon WJ, Jung S, Kook JK, Lee TH. Osmunda japonica extract suppresses pro-inflammatory cytokines by downregulating NF-κB activation in periodontal ligament fibroblasts infected with oral pathogenic bacteria. *Int J Mol Sci* 2020, 21: 2453
18. Zou Y, Zhang H, Liu F, Chen ZS, Tang H. Intratumoral microbiota in orchestrating cancer immunotherapy response. *J Transl Internal Med* 2025, 12: 540–542
19. Aykut B, Pushalkar S, Chen R, Li Q, Abengozar R, Kim JI, Shadaloey SA, *et al.* The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. *Nature* 2019, 574: 264–267
20. Honcharyuk I, Caridi B, Pinco P, Ferri S, De Giani A, Baeri A, Amoroso C, *et al.* The intratumor microbiome and cancer immunity: from pathogenesis to therapeutic opportunities through artificial intelligence. *Expert Rev Clin Immunol* 2025, 21: 1755–1768
21. Abril AG, Carrera M, Böhme K, Barros-Velázquez J, Cañas B, Rama JLR, Villa TG, *et al.* Characterization of bacteriophage peptides of pathogenic streptococcus by LC-ESI-MS/MS: bacteriophage phylogenomics and their relationship to their host. *Front Microbiol* 2020, 11: 1241
22. Miyaue N. 16S rRNA gene amplicon analysis of human gut microbiota. *Methods Mol Biol* 2024, 2766: 343–349
23. Sun Y, Liu Y, Li J, Tan Y, An T, Zhuo M, Pan Z, *et al.* Characterization of lung and oral microbiomes in lung cancer patients using culturomics and 16S rRNA gene sequencing. *Microbiol Spectr* 2023, 11: e0031423
24. Zhang T, Li H, Ma S, Cao J, Liao H, Huang Q, Chen W, *et al.* The newest Oxford Nanopore R10.4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling. *Appl Environ Microbiol* 2023, 89: e0060523
25. Frankel AE, Coughlin LA, Kim J, Froehlich TW, Xie Y, Frenkel EP, Koh AY. Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. *Neoplasia* 2017, 19: 848–855
26. Yin Y, Yu R, Chen H. Shotgun metagenome sequencing of Chinese gut microbiota: a review. *Sheng Wu Gong Cheng Xue Bao* 2021, 37: 3717–3733
27. Huang X, Chen C, Xie W, Zhou C, Tian X, Zhang Z, Wang Q, *et al.* Metagenomic analysis of intratumoral microbiome linking to response to neoadjuvant chemoradiotherapy in rectal cancer. *Int J Radiat Oncol Biol Phys* 2023, 117: 1255–1269
28. Tan WCC, Nerurkar SN, Cai HY, Ng HHM, Wu D, Wee YTF, Lim JCT, *et al.* Overview of multiplex immunohistochemistry/immunofluorescence techniques in the era of cancer immunotherapy. *Cancer Commun* 2020, 40: 135–153
29. Mebratie DY, Dagnaw GG. Review of immunohistochemistry techniques: applications, current status, and future perspectives. *Semin Diagnostic Pathol* 2024, 41: 154–160
30. Ramos-Vara JA. Principles and methods of immunohistochemistry. *Methods Mol Biol* 2017, 1641: 115–128
31. Moreno V, Smith EA, Piña-Oviedo S. Fluorescent immunohistochemistry. *Methods Mol Biol* 2022, 2422: 131–146
32. Barbosa A, Miranda S, Azevedo NF, Cerqueira L, Azevedo AS. Imaging biofilms using fluorescence *in situ* hybridization: seeing is believing. *Front Cell Infect Microbiol* 2023, 13: 1195803
33. Chai X, Wang J, Li H, Gao C, Li S, Wei C, Huang J, *et al.* Intratumor microbiome features reveal antitumor potentials of intrahepatic cholangiocarcinoma. *Gut Microbes* 2023, 15: 2156255
34. Gu J, Wang H, Zhang M, Xiong Y, Yang L, Ren B, Huang R. Application of fluorescence in situ hybridization (FISH) in oral microbial detection. *Pathogens* 2022, 11: 1450
35. Piwosz K, Mukherjee I, Salcher MM, Grujčić V, Šimek K. CARD-FISH in the sequencing era: opening a new universe of protistan ecology. *Front Microbiol* 2021, 12: 640066
36. Šimek K, Mukherjee I, Nedoma J, de Paula CCP, Jezberová J, Sirová D, Vrba J. CARD-FISH and prey tracer techniques reveal the role of overlooked flagellate groups as major bacterivores in freshwater hypertrophic shallow lakes. *Environ Microbiol* 2022, 24: 4256–4273
37. Bauermeister A, Mannochio-Russo H, Costa-Lotufo LV, Jarmusch AK, Dorrestein PC. Mass spectrometry-based metabolomics in microbiome investigations. *Nat Rev Microbiol* 2022, 20: 143–160
38. Yang L, Xiang Z, Zou J, Zhang Y, Ni Y, Yang J. Comprehensive analysis of the relationships between the gut microbiota and fecal metabolome in individuals with primary Sjogren's syndrome by 16S rRNA sequencing and LC-MS-based metabolomics. *Front Immunol* 2022, 13: 874021
39. Studer N, Desharnais L, Beutler M, Brugiroux S, Terrazos MA, Menin L, Schürch CM, *et al.* Functional intestinal bile acid 7α-dehydroxylation by clostridium scindens associated with protection from clostridium difficile infection in a gnotobiotic mouse model. *Front Cell Infect Microbiol* 2016, 6: 191
40. Xie M, Yuan K, Zhang Y, Zhang Y, Zhang R, Gao J, Wei W, *et al.* Tumor-resident probiotic *Clostridium butyricum* improves aPD-1 efficacy in colorectal cancer models by inhibiting IL-6-mediated immunosuppression. *Cancer Cell* 2025, 43: 1885–1901.e10
41. Yuan X, Chen B, Duan Z, Xia Z, Ding Y, Chen T, Liu H, *et al.* Depression and anxiety in patients with active ulcerative colitis: crosstalk of gut microbiota, metabolomics and proteomics. *Gut Microbes* 2021, 13: 1987779
42. Dong Y, Fan H, Zhang Z, Jiang F, Li M, Zhou H, Guo W, *et al.* Berberine ameliorates DSS-induced intestinal mucosal barrier dysfunction through microbiota-dependence and Wnt/β-catenin pathway. *Int J Biol Sci* 2022, 18: 1381–1397
43. Li S, Liu J, Zheng X, Ren L, Yang Y, Li W, Fu W, *et al.* Tumorigenic bacteria in colorectal cancer: mechanisms and treatments. *Cancer Biol Med* 2021, 19: 147–162
44. Chen RY, Mostafa I, Hibberd MC, Das S, Mahfuz M, Naila NN, Islam MM, *et al.* A microbiota-directed food intervention for undernourished children. *N Engl J Med* 2021, 384: 1517–1528
45. Ruiz L, Hidalgo C, Blanco-Míguez A, Lourenço A, Sánchez B, Margolles A. Tackling probiotic and gut microbiota functionality through proteomics. *J Proteomics* 2016, 147: 28–39
46. Dubin RF, Rhee EP. Proteomics and metabolomics in kidney disease, including insights into etiology, treatment, and prevention. *Clin J Am Soc Nephrol* 2020, 15: 404–411
47. Oorschot V, Lindsey BW, Kaslin J, Ramm G. TEM, SEM, and STEM-based immuno-CLEM workflows offer complementary advantages. *Sci Rep* 2021, 11: 899
48. Golmohammadzadeh M, Sexton DL, Parmar S, Tocheva EI. Advanced imaging techniques: microscopy. *Adv Appl Microbiol* 2023, 122: 1–25
49. Prabhakar N, Peurla M, Koho S, Deguchi T, Näreoja T, Chang H,

Rosenholm JM, *et al.* STED-TEM correlative microscopy leveraging nanodiamonds as intracellular dual-contrast markers. *Small* 2018, 14: 1701807
50. Kanauchi M. Assaying D-alanine racemase in lactic acid bacteria using NADH oxidoreduction enzymic system. *Methods Mol Biol* 2024, 2851: 115–123
51. Parker MFL, Luu JM, Schulte B, Huynh TL, Stewart MN, Sriram R, Yu MA, *et al.* Sensing living bacteria *in vivo* usingd-alanine-derived ^{11}C radiotracers. *ACS Cent Sci* 2020, 6: 155–165
52. Yang X, Dang Y, Lou J, Shao H, Jiang X. D-alanyl-D-alanine-modified gold nanoparticles form a broad-spectrum sensor for bacteria. *Theranostics* 2018, 8: 1449–1457
53. Smith ALM, Whitehall JC, Greaves LC. Mitochondrial DNA mutations in ageing and cancer. *Mol Oncol* 2022, 16: 3276–3294
54. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. *Int J Cancer* 2017, 141: 664–670
55. Araldi RP, Sant'Ana TA, Módolo DG, de Melo TC, Spadacci-Morena DD, de Cassia Stocco R, Cerutti JM, *et al.* The human papillomavirus (HPV)-related cancer biology: an overview. *Biomed Pharmacother* 2018, 106: 1537–1556
56. Hu C, Liu T, Han C, Xuan Y, Jiang D, Sun Y, Zhang X, *et al.* HPV E6/E7 promotes aerobic glycolysis in cervical cancer by regulating IGF2BP2 to stabilize m^{6}A-MYC expression. *Int J Biol Sci* 2022, 18: 507–521
57. Nakahata S, Enriquez-Vera D, Jahan MI, Sugata K, Satou Y. Understanding the immunopathology of HTLV-1-associated adult T-Cell leukemia/lymphoma: a comprehensive review. *Biomolecules* 2023, 13: 1543
58. Hong M, Li Z, Liu H, Zheng S, Zhang F, Zhu J, Shi H, *et al.* Fusobacterium nucleatum aggravates rheumatoid arthritis through FadA-containing outer membrane vesicles. *Cell Host Microbe* 2023, 31: 798–810.e7
59. Ma W, Zhang L, Chen W, Chang Z, Tu J, Qin Y, Yao Y, *et al.* Microbiota enterotoxigenic *Bacteroides fragilis*-secreted BFT-1 promotes breast cancer cell stemness and chemoresistance through its functional receptor NOD1. *Protein Cell* 2024, 15: 419–440
60. Ko SH, Jeon JI, Woo HA, Kim JM. *Bacteroides fragilis* enterotoxin upregulates heme oxygenase-1 in dendritic cells via reactive oxygen species-, mitogen-activated protein kinase-, and Nrf2-dependent pathway. *World J Gastroenterol* 2020, 26: 291–306
61. Hu S, Ding Q, Zhang W, Kang M, Ma J, Zhao L. Gut microbial beta-glucuronidase: a vital regulator in female estrogen metabolism. *Gut Microbes* 2023, 15: 2236749
62. Marroquin TY, Guauque-Olarte S. Integrative analysis of gene and protein expression in atherosclerosis-related pathways modulated by periodontal pathogens. Systematic review. *Jpn Dent Sci Rev* 2023, 59: 8–22
63. Ionescu VA, Gheorghe G, Bacalbasa N, Chiotoroiu AL, Diaconu C. Colorectal cancer: from risk factors to oncogenesis. *Medicina* 2023, 59: 1646
64. Duan Y, Xu Y, Dou Y, Xu D. Helicobacter pylori and gastric cancer: mechanisms and new perspectives. *J Hematol Oncol* 2025, 18: 10
65. Yue K, Sheng D, Xue X, Zhao L, Zhao G, Jin C, Zhang L, *et al.* Bidirectional mediation effects between intratumoral microbiome and host DNA methylation changes contribute to stomach adenocarcinoma. *Microbiol Spectr* 2023, 11: e0090423
66. Kreuger IZM, Slieker RC, van Groningen T, van Doorn R. Therapeutic strategies for targeting CDKN2A loss in melanoma. *J Investig Dermatol* 2023, 143: 18–25.e1
67. Hong J, Guo F, Lu SY, Shen C, Ma D, Zhang X, Xie Y, *et al. F. nucleatum* targets lncRNA ENO1-IT1 to promote glycolysis and oncogenesis in colorectal cancer. *Gut* 2021, 70: 2123–2137
68. Xia X, Wu WKK, Wong SH, Liu D, Kwong TNY, Nakatsu G, Yan PS, *et al.* Bacteria pathogens drive host colonic epithelial cell promoter hypermethylation of tumor suppressor genes in colorectal cancer. *Microbiome* 2020, 8: 108
69. Tae CH, Ryu KJ, Kim SH, Kim HC, Chun HK, Min BH, Chang DK, *et al.* Alcohol dehydrogenase, iron containing, 1 promoter hypermethylation associated with colorectal cancer differentiation. *BMC Cancer* 2013, 13: 142
70. Feng J, Xu T, He M, Li J, Yao P, Ma C, Yang S, *et al.* NSUN2-mediated m5C modification of HBV RNA positively regulates HBV replication. *PLoS Pathog* 2023, 19: e1011808
71. Zaib S, Rana N, Khan I. Histone modifications and their role in epigenetics of cancer. *Curr Med Chem* 2022, 29: 2399–2411
72. Herbein G. Cellular transformation by human cytomegalovirus. *Cancers* 2024, 16: 1970
73. Parada Venegas D, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, Harmsen HJM, *et al.* Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol* 2019, 10: 277
74. Tan J, McKenzie C, Vuillermin PJ, Goverse G, Vinuesa CG, Mebius RE, Macia L, *et al.* Dietary fiber and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways. *Cell Rep* 2016, 15: 2809–2824
75. Wei Q, Su J, Meng S, Wang Y, Ma K, Li B, Chu Z, *et al.* MiR-17-5p-engineered sEVs encapsulated in GelMA hydrogel facilitated diabetic wound healing by targeting PTEN and p21. *Adv Sci* 2024, 11: 2307761
76. Qu Q, Chen Y, Wang Y, Long S, Wang W, Yang HY, Li M, *et al.* Lithocholic acid phenocopies anti-ageing effects of calorie restriction. *Nature* 2025, 643: 192–200
77. Chiang JYL, Ferrell JM. Bile acid receptors FXR and TGR5 signaling in fatty liver diseases and therapy. *Am J Physiol Gastrointestinal Liver Physiol* 2020, 318: G554–G573
78. Abdulaal WH, Omar UM, Zeyadi M, El-Agamy DS, Alhakamy NA, Ibrahim SRM, Almalki NAR, *et al.* Pirfenidone ameliorates ANIT-induced cholestatic liver injury via modulation of FXR, NF-κB/TNF-α, and Wnt/GSK-3β/β-catenin signaling pathways. *Toxicol Appl Pharmacol* 2024, 490: 117038
79. Zhao C, Wu K, Hao H, Zhao Y, Bao L, Qiu M, He Y, *et al.* Gut microbiota-mediated secondary bile acid alleviates *Staphylococcus aureus*-induced mastitis through the TGR5-cAMP-PKA-NF-κB/NLRP3 pathways in mice. *npj Biofilms Microbiomes* 2023, 9: 8
80. Correia AS, Vale N. Tryptophan metabolism in depression: a narrative review with a focus on serotonin and kynurenine pathways. *Int J Mol Sci* 2022, 23: 8493
81. Fong W, Li Q, Ji F, Liang W, Lau HCH, Kang X, Liu W, *et al. Lactobacillus gallinarum*-derived metabolites boost anti-PD1 efficacy in colorectal cancer by inhibiting regulatory T cells through modulating IDO1/Kyn/AHR axis. *Gut* 2023, 72: 2272–2285
82. Sun C, Wang B, Hao S. Adenosine-A2A receptor pathway in cancer immunotherapy. *Front Immunol* 2022, 13: 837230
83. Huang TQ, Willis MS, Meissner G. IL-6/STAT3 signaling in mice with dysfunctional type-2 ryanodine receptor. *JAKSTAT* 2016, 4: e1158379
84. Kanno T, Nakajima T, Miyako K, Endo Y. Lipid metabolism in Th17 cell function. *Pharmacol Ther* 2023, 245: 108411
85. Han Y, Ling Q, Wu L, Wang X, Wang Z, Chen J, Zheng Z, *et al. Akkermansia muciniphila* inhibits nonalcoholic steatohepatitis by orchestrating TLR2-activated γδT17 cell and macrophage polarization.

Gut Microbes 2023, 15: 2221485
86. Veglia F, Hashimoto A, Dweep H, Sanseviero E, De Leo A, Tcyganov E, Kossenkov A, ***et al.*** Analysis of classical neutrophils and polymorphonuclear myeloid-derived suppressor cells in cancer patients and tumor-bearing mice. *J Exp Med* 2021, 218: e20201803
87. Krueger E, Brown AC. *Aggregatibacter actinomycetemcomitans* leukotoxin: from mechanism to targeted anti-toxin therapeutics. *Mol Oral Microbiol* 2020, 35: 85–105
88. Gasmi Benahmed A, Kumar Mujawdiya P, Noor S, Gasmi A. *Porphyromonas gingivalis* in the development of periodontitis: impact on dysbiosis and inflammation. *Arch Razi Inst* 2022, 77: 1539–1551
89. Wu Y, Guo S, Chen F, Li Y, Huang Y, Liu W, Zhang G, ***et al.*** Fn-Dps, a novel virulence factor of *Fusobacterium nucleatum*, disrupts erythrocytes and promotes metastasis in colorectal cancer. *PLoS Pathog* 2023, 19: e1011096
90. Murray BO, Flores C, Williams C, Flusberg DA, Marr EE, Kwiatkowska KM, Charest JL, ***et al.*** Recurrent urinary tract infection: a mystery in search of better model systems. *Front Cell Infect Microbiol* 2021, 11: 691210
91. Bregaint S, Boyer E, Fong SB, Meuric V, Bonnaure-Mallet M, Jolivet-Gougeon A. *Porphyromonas gingivalis* outside the oral cavity. *Odontology* 2022, 110: 1–19
92. Liu S, Zhang N, Ji X, Yang S, Zhao Z, Li P. *Helicobacter pylo*ri CagA promotes gastric cancer immune escape by upregulating SQLE. *Cell Death Dis* 2025, 16: 17
93. Zhang Y, Zhang L, Zheng S, Li M, Xu C, Jia D, Qi Y, ***et al.*** *Fusobacterium nucleatum* promotes colorectal cancer cells adhesion to endothelial cells and facilitates extravasation and metastasis by inducing ALPK1/NF-κB/ICAM1 axis. *Gut Microbes* 2022, 14: 2038852
94. Shi Y, Zheng W, Yang K, Harris KG, Ni K, Xue L, Lin W, ***et al.*** Intratumoral accumulation of gut microbiota facilitates CD47-based immunotherapy via STING signaling. *J Exp Med* 2020, 217: e20192282
95. Wang H, Rong X, Zhao G, Zhou Y, Xiao Y, Ma D, Jin X, ***et al.*** The microbial metabolite trimethylamine N-oxide promotes antitumor immunity in triple-negative breast cancer. *Cell Metab* 2022, 34: 581–594.e8
96. Zhu G, Su H, Johnson CH, Khan SA, Kluger H, Lu L. Intratumour microbiome associated with the infiltration of cytotoxic CD8+ T cells and patient survival in cutaneous melanoma. *Eur J Cancer* 2021, 151: 25–34
97. Kalaora S, Nagler A, Nejman D, Alon M, Barbolin C, Barnea E, Ketelaars SLC, ***et al.*** Identification of bacteria-derived HLA-bound peptides in melanoma. *Nature* 2021, 592: 138–143
98. Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, Rotter-Maskowitz A, ***et al.*** The human tumor microbiome is composed of tumor type–specific intracellular bacteria. *Science* 2020, 368: 973–980
99. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, Colaprico A, ***et al.*** Comprehensive characterization of cancer driver genes and mutations. *Cell* 2018, 173: 371–385.e18
100. Klein RS, Recco RA, Catalano MT, Edberg SC, Casey JI, Steigbigel NH. Association of *Streptococcus bovis* with carcinoma of the colon. *N Engl J Med* 1977, 297: 800–802
101. Quan L, Rong H, Yue J, Xu J, Zheng Y, Yuan P. Integrative pan-cancer analysis of intratumoral microbial communities for biomarker discovery and diagnostic modeling. *J Transl Med* 2025, 23: 1347.
102. Porcari S, Benech N, Valles-Colomer M, Segata N, Gasbarrini A, Cammarota G, Sokol H, ***et al.*** Key determinants of success in fecal microbiota transplantation: from microbiome to clinic. *Cell Host Microbe* 2023, 31: 712–733
103. Halsey TM, Thomas AS, Hayase T, Ma W, Abu-Sbeih H, Sun B, Parra ER, ***et al.*** Microbiome alteration via fecal microbiota transplantation is effective for refractory immune checkpoint inhibitor–induced colitis. *Sci Transl Med* 2023, 15: eabq4006
104. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, ***et al.*** Expert consensus document: the international Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017, 14: 491–502
105. Legesse Bedada T, Feto TK, Awoke KS, Garedew AD, Yifat FT, Birri DJ. Probiotics for cancer alternative prevention and treatment. *Biomed Pharmacother* 2020, 129: 110409
106. Wang HL, Kajbaf K, Gau BC, Dawdy AW, Edwards R, Bare B, Raymundo G, ***et al.*** A novel *in-vitro* expression assay by LC/MS/MS enables multi-antigen mRNA vaccine characterization. *Sci Rep* 2025, 15: 10336
107. Liu L, Wang H, Chen X, Zhang Y, Zhang H, Xie P. Gut microbiota and its metabolites in depression: from pathogenesis to treatment. *eBioMedicine* 2023, 90: 104527
108. Owumi SE, Arunsi UO, Oyelere AK. The protective effect of 3-indolepropanoic acid on aflatoxin B1-induced systemic perturbation of the liver and kidney function in rats. *Fundam Clin Pharmacol* 2023, 37: 369–384
109. Kalia VC, Gong C, Shanmugam R, Lin H, Zhang L, Lee JK. The emerging biotherapeutic agent: *Akkermansia*. *Indian J Microbiol* 2022, 62: 1–10
110. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, ***et al.*** Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018, 359: 91–97
111. Fernandez E, Wargo JA, Helmink BA. The microbiome and cancer. *JAMA* 2025, 333: 2188–2196
112. Yu TC, Guo F, Yu Y, Sun T, Ma D, Han J, Qian Y, ***et al.*** *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell* 2017, 170: 548–563.e16
113. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nat Rev Gastroenterol Hepatol* 2019, 16: 605–616
114. Saw PE, Song EW. Phage display screening of therapeutic peptide for cancer targeting and therapy. *Protein Cell* 2019, 10: 787–807
115. Thi HV, Ngo AD, Tran LT, Chu DT. Phage for cancer therapy. *Prog Mol Biol Transl Sci* 2023, 201: 225–239
116. Abd El-Moaty HI, Doghish AS, Moustafa HAM, Elkalla WS, Sayed GA, Elshami NH, Mageed SSA, ***et al.*** Phage therapy and its role in cancer treatment and control. *Folia Microbiol* 2025, 70: 941–960
117. Jofre J, Muniesa M. Bacteriophage isolation and characterization: phages of *Escherichia coli*. *Methods Mol Biol* 2020, 2075: 61–79
118. Cui L, Watanabe S, Miyanaga K, Kiga K, Sasahara T, Aiba Y, Tan XE, ***et al.*** A comprehensive review on phage therapy and phage-based drug development. *Antibiotics* 2024, 13: 870
119. Ahmed SG, Oliva G, Shao M, Mekalanos JJ, Brenner GJ. Culture of attenuated *Salmonella* Typhimurium VNP20009 in animal-product-free media does not alter schwannoma growth control. *Hum Vaccines ImmunoTher* 2023, 19: 2262639
120. Funayama E, Hosonuma M, Tajima K, Isobe J, Baba Y, Murayama M, Narikawa Y, ***et al.*** Oral administration of *Bifidobacterium longum* and *Bifidobacterium infantis* ameliorates cefcapene pivoxil-induced attenuation of anti-programmed cell death protein-1 antibody action in mice. *Biomed Pharmacother* 2025, 182: 117749
121. Luo M, Chen Y, Pan X, Chen H, Fan L, Wen Y. *E. coli Nissle 1917* ameliorates mitochondrial injury of granulosa cells in polycystic ovary syndrome through promoting gut immune factor IL-22 via gut microbiota and microbial metabolism. *Front Immunol* 2023, 14: 1137089

122. Leitão JH. *Listeria monocytogenes* as a vector for cancer immunotherapy. *Vaccines* 2020, 8: 439
123. Lin D, Shen Y, Liang T. Oncolytic virotherapy: basic principles, recent advances and future directions. *Sig Transduct Target Ther* 2023, 8: 156
124. Lee WS, Nam KH, Kim JH, Kim WJ, Kim JE, Shin EC, Kim GR, ***et al.*** Alleviating psoriatic skin inflammation through augmentation of Treg cells via CTLA-4 signaling peptide. *Front Immunol* 2023, 14: 1233514
125. Chowaniec H, Ślubowska A, Mroczek M, Borowczyk M, Braszka M, Dworacki G, Dobosz P, ***et al.*** New hopes for the breast cancer treatment: perspectives on the oncolytic virus therapy. *Front Immunol* 2024, 15: 1375433
126. Yamamoto S, Kinugasa H, Hirai M, Terasawa H, Yasutomi E, Oka S, Ohmori M, ***et al.*** Heterogeneous distribution of *Fusobacterium nucleatum* in the progression of colorectal cancer. *J Gastro Hepatol* 2021, 36: 1869–1876
127. Kneis B, Wirtz S, Weber K, Denz A, Gittler M, Geppert C, Brunner M, ***et al.*** Colon cancer microbiome landscaping: differences in right- and left-sided colon cancer and a tumor microbiome-ileal microbiome association. *Int J Mol Sci* 2023, 24: 3265
128. Bullman S. The intratumoral microbiota: from microniches to single cells. *Cell* 2023, 186: 1532–1534
129. Pandey SP, Bender MJ, McPherson AC, Phelps CM, Sanchez LM, Rana M, Hedden L, ***et al.*** Tet2 deficiency drives liver microbiome dysbiosis triggering Tc1 cell autoimmune hepatitis. *Cell Host Microbe* 2022, 30: 1003–1019.e10
130. Dizman N, Meza L, Bergerot P, Alcantara M, Dorff T, Lyou Y, Frankel P, ***et al.*** Nivolumab plus ipilimumab with or without live bacterial supplementation in metastatic renal cell carcinoma: a randomized phase 1 trial. *Nat Med* 2022, 28: 704–712