

Tumor-associated microbiota: Multi-cancer landscape, mechanistic insights and clinical translation (Review)

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Abstract. Intratumoral microbiota, a critical component of the tumor microenvironment, notably impacts tumor progression through various complex mechanisms such as metabolic regulation, immune system remodeling and genotoxicity. The present review focuses on eight prevalent solid tumors (breast, colorectal, lung, pancreatic, gastric, ovarian, prostate cancers and melanoma), detailing the intratumoral microbial compositional heterogeneity within these malignancies. The present review analyzes the heterogeneous carcinogenic mechanisms mediated by these microorganisms, including gene instability, immune microenvironment remodeling and metabolic intervention. The diagnostic value of microbial markers in liquid biopsy and *in situ* tissue detection is explored, and the potential for synergistic strategies combining microbial-targeted therapy and immunotherapy is discussed. Finally, the review suggests future research directions, such as spatiotemporal dynamic analysis and organoid-microorganism co-culture, offering new insights for precise cancer treatment.

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1. Introduction

The human microbiota, consisting of bacteria, fungi, viruses and protists, numbers in the tens of trillions, matching the count of human cells (1). The traditional theory is that human microorganisms mainly colonize mucosa-associated niches, including the gut, skin, oral cavity and reproductive tract (2). Previous studies have reported that previously considered sterile deep tissues (such as the lungs, mammary glands, pancreas, prostate and kidneys) also harbor low-biomass microbial communities (3). In normal physiological states, these microorganisms support the host's microecological balance by modulating local immunity, metabolism and barrier functions (4).

The intratumoral microbiota refers to the microbial community colonizing the tumor tissue (5). Previous ground-breaking research has demonstrated the presence of diverse microorganisms within tumor tissues, which were previously considered sterile environments, and their abundance is notably associated with tumorigenesis (6,7). These microorganisms colonize the tumor microenvironment (TME) through various routes, including mucosal disruption, migration from adjacent tissues and hematogenous invasion (8). The TME, composed of immune cells, stromal cells, cytokines and hypoxic regions, provides a colonization niche for intratumoral microorganisms due to its pathological characteristics (9). The colonization of intratumoral microorganisms depends on the TME's abnormal vascular leakage, immunosuppressive state, local hypoxic microdomains and eutrophic niches (10). As an endogenous component of the TME, tumor microorganisms drive malignant progression through mechanisms such as cell phenotype reprogramming, paracrine signal mediation and immune microenvironment regulation (8,11).

Beyond microbial influences, intrinsic host factors such as RNA helicases also critically shape the TME. A recent pan-cancer analysis identified DEAD-box helicase 1 (DDX1), an RNA helicase, as a prognostic marker with dual roles across cancers: Low DDX1 expression is associated with improved survival in renal carcinomas, while high expression drives poor prognosis in breast, liver and adrenal cancers by modulating immune infiltration, DNA repair pathways and phosphorylation-dependent signaling (12). This highlights the complex interplay between host-derived molecular machinery and microbial ecosystems in tumor evolution. Components in the

TME can promote tumor invasion and metastasis by inducing epithelial-mesenchymal transition, enhancing the proteolytic activity of matrix metalloproteinases and inhibiting antitumor immunity (13). The interaction between tumor cells and the TME creates a vicious cycle that facilitates both local invasion and metastasis (14). Tumor-associated microorganisms influence tumor susceptibility, treatment response and malignant progression via host cell-microorganism interactions (15). The core carcinogenic mechanisms of intratumoral microorganisms include genetic instability, immune microenvironment remodeling and metabolic intervention (16,17).

The study of intratumoral microorganisms has a long history, dating back to the mid-20th century. Microorganisms were first detected in tumor tissues in the 1950s (5). In 1911, Rous (18) discovered that the Rous sarcoma virus could induce malignant tumors, and the Epstein-Barr virus was later identified in Burkitt lymphoma. A notable milestone was reached in 1983 when Warren and Marshall (19) successfully cultured *Helicobacter pylori* and demonstrated its pathogenic role in gastric cancer. The advent of high-throughput sequencing technology at the start of the 21st century has propelled rapid advancements in this field. In 2020, Nejman *et al* (7) analyzed 1,526 samples to reveal tumor-type-specific microbial compositions. More recently, in 2022, Narunsky-Haziza *et al* (20) mapped the fungal distribution across 17 different tumor types. During this period, microbial intervention strategies have evolved from early erysipelas therapy to engineered strain treatments (21,22).

The present review examines eight prevalent solid tumors: Breast, colorectal, lung, pancreatic, gastric, ovarian, prostate cancers and melanoma. These cancers are pivotal in tumor microbiome research due to their epidemiological impact, sample availability, defined mechanisms and clinical relevance. This selectivity not only reflects the current development status of the field, but also reflects the efficiency of academic communication. These eight types of cancer cover a notable body of the global cancer burden, and the research results have far-reaching impact on public health and have clear evidence of microbial associations. Investigating tumor-associated microorganisms has challenged the notion of tumors as sterile environments, introducing a paradigm shift from targeting cancer cells alone to regulating the host-microbe symbiotic network. Consequently, tumor-associated microorganisms have emerged as a crucial area in cancer therapy research, with numerous studies exploring their diagnostic value and therapeutic potential.

2. Differences in microbial communities among different cancer types

The intratumoral microbiota displays considerable heterogeneity across cancer types, with compositional variations primarily influenced by the TME, host characteristics, dietary habits, environmental exposures and the intrinsic properties of the microorganisms (23). *Proteobacteria*, *Firmicutes* and *Actinobacteria* are commonly enriched in most tumors (7,24,25). However, the same microbial genus may have opposite effects in different cancer types. For example, *Pseudomonas* serves a pro-tumorigenic role in breast cancer, colorectal cancer (CRC), lung cancer and gastric cancer, but

colonizes the prostate as a commensal bacterium (7,26,27). *Acinetobacter spp.* promotes melanoma progression, exhibits commensalism in lung tissue and is enriched in prostate cancer where it appear to suppress tumorigenesis (28,29). Notably, commensal *Veillonella spp.* species from healthy lung tissues exhibit aberrant enrichment in gastric cancer tissues, with elevated abundance notable associating with adverse patient prognosis, which may facilitate gastric mucosal barrier disruption through lipopolysaccharide-mediated activation of the toll-like receptor (TLR) 2/4-NF- κ B signaling pathway (27,30).

Breast cancer. Previous research has revealed that the microbial diversity and abundance in breast cancer tissues surpass those in corresponding normal breast tissues, with adjacent normal tissues exhibiting an intermediate composition (7). Various factors such as ethnicity, tumor stage and molecular subtype contribute to the heterogeneity of the breast microbiota (31). *Methylobacterium radiotolerans* and *Staphylococcus* consistently emerge as the predominant bacteria in breast tumors based on multi-omics analyses (7,32-34). Additionally, Tzeng *et al* (26) further demonstrated that *Lacibacter* and *Ezakiella* are notably enriched in breast cancer tissues, and their abundance increases with the elevation of tumor stage. By contrast, the relative abundances of *Sphingomonas yanoikuyae* and *Acetobacter aceti* are higher in normal breast tissues, while *Anaerococcus*, *Caulobacter* and *Streptococcus* are notably absent in breast cancer (33,34). Despite these insights into microbial community characteristics, further research is required to elucidate the precise mechanisms by which these microorganisms influence processes such as breast cancer cell dissemination, intravasation and extravasation.

CRC. Meta-genomic research has revealed substantial alterations in the microbial community composition within tumor tissues of patients with CRC compared with those of healthy individuals (35). *Fusobacterium nucleatum* has consistently emerged as a prominent member of the microbiota associated with CRC (36). Additionally, *Bacteroides fragilis*, *Parvimonas* and *Bacteroides* have been notably observed to be more abundant in CRC tissues (37,38). Nevertheless, Repass (39) performed a comparative analysis of tumor and adjacent normal tissues from the same cohort of patients, demonstrating that *Fusobacterium nucleatum* was not prevalent in the majority of CRC samples. Moreover, the authors found no marked distinction in the overall microbiota composition between the two tissue types. This suggests that discrepancies in findings may be attributed to variations in sample origins, population diversity and methodological disparities.

Lung cancer. The lungs undergo continuous gas exchange with the external environment, making their microbial communities vulnerable to influences from the oral, nasal and gut microbiota through 'ascending' or 'hematogenous' routes (40). Meta-genomic studies have verified marked dysbiosis in the lung tissues of individuals with lung cancer, characterized by heightened local inflammatory responses, elevated microbial quantities and the proliferation of specific pro-inflammatory bacteria (41). Notably, potential pathogens such as *Brevundimonas* and *Escherichia* are markedly more abundant in cancerous tissues compared with adjacent normal

tissues, whereas *Corynebacterium*, *Lachnoanaerobaculum* and *Halomonas*, prevalent in healthy individuals, are markedly diminished (28). Subsequent investigations indicated that *Streptococcus* and *Peptoniphilus* are associated with an increased lung cancer risk, whereas the presence of *Aggregatibacter* offers a protective effect (42).

Pancreatic cancer. The local pancreatic microbiota is closely associated with the susceptibility, progression and treatment response of pancreatic ductal adenocarcinoma (PDAC) (43,44). *Propionibacterium acnes*, one of the earliest bacteria isolated from pancreatic cancer tissues, can induce chronic inflammation and provide an inflammatory microenvironment for tumorigenesis (45). Meta-genomic analysis has revealed that *Gammaproteobacteria* is the dominant bacterial class in human pancreatic tumors (46). In a PDAC mouse model, *Bacteroides* and *Parabacteroides* were notably enriched in tumor tissues, suggesting their potential involvement in cancer progression (47).

It was further found that intratumoral microbial diversity is associated with prognosis in patients with PDAC. Riquelme *et al* (48) reported that long-term survivors exhibited markedly higher tumor microbial diversity compared with short-term survivors. Moreover, the co-existence and high abundance of *Pseudoxanthomonas*, *Streptomyces*, *Saccharopolyspora* and *Bacillus clausii* could serve as an independent marker for predicting long-term survival.

Gastric cancer. Gastric microbiota dysbiosis serves a crucial role in the progression of gastric cancer. *Helicobacter pylori* (*H. pylori*) has been designated as a Group I carcinogen by the World Health Organization. However, only ~3% of individuals infected with *H. pylori* ultimately develop gastric cancer (49,50). Apart from *H. pylori*, various meta-genomic studies have identified specific microbiota associated with gastric cancer, including *Peptostreptococcus stomatis*, *Streptococcus anginosus*, *Parvimonas micra*, *Slackia exigua* and *Dialister pneumosintes* (51). Throughout the continuum from gastritis-gastric adenoma-early-stage gastric cancer-advanced stage gastric cancer, the microbiota composition undergoes dynamic changes. *Akkermansia* and *Lachnospiraceae* NK4A136 dominate the gastritis stage, whereas *Lactobacillus* and *Veillonella* markedly increase in abundance in gastric cancer tissues compared with normal tissues (30,52). The aforementioned distinctive microbial dysbiosis may expedite the carcinogenic process through mechanisms such as inflammation promotion, metabolic reprogramming and immune suppression. Therefore, monitoring the composition and function of the gastric microbiota holds notable clinical value for the early diagnosis and risk assessment of gastric cancer.

Ovarian cancer. Ovarian cancer ranks among the most aggressive gynecological malignancies. High-grade serous ovarian cancer is considered to originate from either the ovarian surface epithelium or fallopian tubes (53). Previous studies have revealed that dysregulation of the local microbiota may serve a regulatory role in the development and progression of ovarian cancer (54,55). Meta-genomic analyses have identified *Roseomonas mucosa* and *Sphingomonas* US_602

as being notably enriched in ovarian tumor tissues, indicating their potential as key microbial players in this disease (24). Additionally, human papillomavirus (HPV) infection has been proposed as a risk factor for ovarian cancer, with higher expression levels of high-risk HPV observed in malignant tissues compared with adjacent normal tissues (56). However, a study by Ingerslev *et al* (57) found no notable association between high-risk HPV and epithelial ovarian cancer in Caucasian patients, suggesting it may not be related to ethnic differences.

Prostate cancer. Prostate cancer is the most prevalent malignant tumor in men (58). Previous research has demonstrated that prostate tissue is not sterile, with microbial DNA identified in tumor samples from 87% of patients with prostate cancer, implicating the local microbiota in the disease's onset and progression (59,60). Multi-omics analyses reveal elevated levels of *Escherichia* and *Propionibacterium* in prostate cancer, suggesting their potential role in tumor development (61). Additionally, liquid biopsy indicates markedly increased levels of *Streptococcus*, *Peptostreptococcus* and *Haemophilus* in the urine of patients with prostate cancer (62). Furthermore, markers of parasites such as *Toxoplasma* and *Plasmodium* are detected in prostate cancer tissues, potentially contributing to tumorigenesis through chromosomal damage and reactive oxygen species generation (63).

Melanoma. Melanoma arises from the malignant transformation of melanocytes and is the deadliest form of skin cancer (64). The skin microbiota varies notably by location: *Staphylococcus* dominates sebaceous regions, *Corynebacterium* is prevalent in moist areas and β -*Proteobacteria* are found in dry regions (65,66). In melanoma tissues, *Enterobacter* and *Streptococcus* are notably enriched, facilitating tumor immune evasion by inhibiting CD8⁺ T cell infiltration and reducing chemokine expression (29,67). Notably, Kozmin *et al* (68) discovered that a commensal *Staphylococcus epidermidis* strain producing 6-N-hydroxyaminopurine could markedly inhibit B16F10 melanoma growth and decrease ultraviolet-induced tumor incidence, suggesting a novel approach for microbiota-driven prevention and treatment of skin cancer.

3. Mechanisms of heterogeneity of different cancer microorganisms

Intratumoral microbiota varies markedly across tumor types, affecting key biological functions such as metabolic reprogramming, immune regulation, metastatic potential and epigenetic modification. These microorganisms can either promote or inhibit tumor progression by activating or suppressing specific signaling pathways, modulating the immunosuppressive microenvironment, enhancing inflammatory responses and altering metabolic processes.

Genetic instability. Intratumoral microorganisms can drive genomic instability through a dual-pathway mechanism involving direct genotoxicity and indirect activation, with the specific mechanisms being strain-dependent (23). In CRC, *Escherichia coli* carrying the pks gene island secretes the

genotoxin colibactin, inducing somatic base substitutions and insertion or deletion mutations, while the cyto-lethal distending toxin produced by *Campylobacter* can cause DNA double-strand breaks (69). Aflatoxin B₁ (AFB₁) is metabolized by cytochrome p450 to the reactive AFBO metabolite, which blocks nucleotide excision repair and induces TP53 mutations, promoting carcinogenesis in multiple organs (70). In CRC, *Fusobacterium nucleatum* activates the E-cadherin/ β -catenin signaling pathway via the FadA adhesin, upregulates checkpoint kinase 2 and induces DNA damage (71). Elevated β -glucuronidase in the breast cancer microenvironment can catalyze the release of reactive intermediates, indirectly causing DNA damage (8). In the intestinal mucosa of patients with familial adenomatous polyposis, *enterotoxigenic Bacteroides fragilis* and *Escherichia coli* were found to colonize synergistically, accelerating early-stage CRC development through DNA damage and inflammatory pathways (72).

Remodeling of the immune microenvironment. Intratumoral microorganisms colonize TME, influencing immune remodeling and accelerating tumor progression (73). Their viable bacteria, residues and metabolites collectively regulate immune cell functions and inflammatory responses, enhancing immune suppression and promoting pro-tumor phenotypes (74). *Enterobacteriaceae* and *Pseudomonadaceae* in breast and pancreatic cancers directly promote tumor cell proliferation by activating the NF- κ B/TLR4 inflammatory axis and upregulating the PI3K/Akt signaling pathway (34,75). In CRC, *Fusobacterium nucleatum* increases intracellular Ca²⁺, facilitates E-cadherin and Krüppel-like factor 4 (KLF4) interaction, promotes KLF4 nuclear translocation and upregulates integrin α 5 transcription, driving proliferation, invasion and metastasis (76). In pancreatic cancer, *Campylobacter*, *Selenomonas* and *Clostridium difficile* inhibit immune cell activity via the MET proto-oncogene, receptor tyrosine kinase-protein tyrosine kinase 2 and programmed cell death protein 1 (PD-1) pathways, enhancing tumor invasiveness; these genera, rare in normal tissues, are potential diagnostic markers (77). *Fusobacterium* upregulates pathways such as cytotoxic T-lymphocyte associated protein 4, JAK-STAT, TNF and PI3K-AKT-mTOR, creating an immunosuppressive microenvironment and remodeling the transcriptome to promote oral squamous cell carcinoma progression (11). Conversely, microorganisms can inhibit tumor progression; for instance, *Propionibacterium* in normal breast tissue directly inhibits tumor growth by secreting antimicrobial peptides and produces short-chain fatty acids to activate free fatty acid receptors 2 and 3, reducing inflammation and tumorigenesis risk (34).

Metabolic reprogramming. Intratumoral microorganisms modify the metabolic networks of glucose, lipids, and amino acids within tumors via the ‘metabolite-receptor-signal axis’, influencing the immune microenvironment and thereby affecting tumor progression. In CRC, the anaerobic bacteria *Fusobacterium nucleatum*, *Clostridium spp.* and *Bacteroides spp.* produce short-chain fatty acids that activate pro-inflammatory pathways such as NF- κ B, elevating tumorigenesis risk (38). In gastric cancer, *Peptostreptococcus spp.*

enhance phosphatidylcholine hydrolysis and triglyceride synthesis by upregulating phospholipase C and 1-acyl-sn-glycerol-3-phosphate acyltransferase, driving lipid metabolic reprogramming and tumor progression (78). Additionally, the study by Flores-García *et al* (79) demonstrated that long non-coding RNAs boost glycolysis by upregulating 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4, phosphoglycerate kinase 1 and lactate dehydrogenase A, while promoting glutamine catabolism through increased glutamate dehydrogenase 1 and Golgi transport 1A expression, supplying extra energy to tumor cells. These enzymes further activate the hypoxia inducible factor 1 α /PI3K/Akt/mTOR pathway, enhancing tumor cell proliferation, survival and invasion. Table I summarizes the compositional differences and action mechanisms of the tumor microbiome (80,81).

4. Diagnosis of tumor microbial biomarkers

New dimensions of liquid biopsy. Liquid biopsy, a non-invasive technique, utilizes peripheral blood samples to monitor tumor characteristics. Its core detection targets include circulating tumor cells, circulating tumor DNA (ctDNA), cell-free RNA and extracellular vesicles, which have demonstrated applicability in the early diagnosis and dynamic monitoring of various cancers (82). Specifically, ctDNA enables real-time assessment of tumor burden by identifying tumor-specific somatic mutations, copy number variations and epigenetic modifications, such as methylation (83). Furthermore, ctDNA analysis can predict postoperative recurrence in patients with CRC (84).

The bacteremia microbial profile in patients with CRC was closely associated with tumor stage, as reported by Kwong *et al* (85). Specifically, the positive inflection point of *Clostridium perfringens* appeared 100 days after the diagnosis of bacteremia, while the peaks of *Peptostreptococcus spp.* and *Fusobacterium spp.* occurred at 121 days and 127 days, respectively. Notably, *Fusobacterium spp.* and *Peptostreptococcus spp.* were associated with the diagnosis of early-stage CRC, whereas *Streptococcus spp.* was associated with late-stage CRC. Furthermore, the abundances of bacterial and fungal DNA in peripheral blood were highly consistent with the characteristics of the intratumoral microbiome, suggesting their potential as circulating microbial biomarkers (20). The integration of high-throughput sequencing and machine learning algorithms has enabled the multi-cancer early detection technology based on cell-free circulating DNA to identify molecular signals shared by multiple tumors in a single blood sample (86). Wang *et al* (87) demonstrated that *Lactobacillus* and *Streptococcus* could serve as microbial markers for gastric cancer, aiding in its early non-invasive diagnosis. Collectively, these studies indicate that circulating tumor microbiome markers have the potential to become non-invasive and highly sensitive diagnostic tools, which could expand the application of liquid biopsy in early tumor screening (88).

In situ tissue detection. *In situ* tissue detection technology, including immunohistochemistry, immunofluorescence and fluorescence *in situ* hybridization (FISH), serves a crucial role in directly identifying microbial biomarkers within tumor tissues. These techniques are valuable for elucidating the spatial distribution of microorganisms in relation to tumor cells

Table I. Intratumoral microorganisms and their mechanisms of action in multiple cancers.

Tumor types	Microorganisms	Functions	Mechanisms	(Refs.)
Breast cancer	<i>Methylobacterium radiotolerans</i>	Pro-tumor	Suppression of T-cell infiltration and promotion of inflammatory responses	(7)
	<i>Staphylococcus</i>	Pro-tumor	Inhibition of the RhoA-ROCK signaling pathway	(31)
	<i>Fusobacterium</i>	Pro-tumor	Modulation of estrogen metabolism	(26)
	<i>Anaerococcus</i>	Antitumor	Suppression of M2 macrophage polarization, reducing immunosuppressive tumor-associated macrophages and enhancing CD8 ⁺ T-cell infiltration	(33,34)
Colorectal cancer	<i>Caulobacter</i>	Antitumor	Secretion of antimicrobial peptides that inhibit pro-carcinogenic bacteria	(34)
	<i>Streptococcus</i>	Antitumor	Induction of early apoptosis in MCF-7 breast cancer cells, with activation of the PI3K-AKT-mTOR pathway and inhibition of the JAK2-STAT3 pathway	
	<i>Fusobacterium nucleatum</i>	Pro-tumor	Activation of the E-cadherin/ β -catenin axis, up-regulation of CHK2 and induction of DNA damage	(71)
	<i>Escherichia coli</i>	Pro-tumor	Base substitutions or deletions provoking DNA damage	(69)
Lung cancer	<i>Enterotoxigenic Bacteroides fragilis</i>	Pro-tumor	DNA damage elicited via base substitution	(72)
	<i>Firmicutes</i>	Pro-tumor	Upregulation of ERK and PI3K signaling pathways to induce inflammatory responses	(28)
	<i>Prevotella</i>	Pro-tumor	Attenuation of immune surveillance	(42)
	<i>Peptoniphilus</i>	Pro-tumor	Augmented CD8 ⁺ T-cell infiltration	
Pancreatic cancer	<i>Aggregatibacter</i>	Antitumor		(42)
	<i>Propionibacterium acnes</i>	Pro-tumor	Activation of NF- κ B- and TLR4-mediated inflammatory pathways, thereby amplifying inflammation	(28,45)
	<i>Gammaproteobacteria</i>	Pro-tumor		
	<i>Clostridium difficile</i>	Pro-tumor	Activation of MET-PTK2 and PD-1 signaling pathways	(77)
Gastric cancer	<i>Porphyromonas gingivalis</i>	Pro-tumor	MAPK pathway activation inducing inflammatory responses	(80)
	<i>Bacillus clausii</i>	Pro-tumor	Promotion of CD8 ⁺ T-cell infiltration alongside down-regulation of MDSCs and Tregs, abrogating immunosuppression	(48)
	<i>Pseudoxanthomonas</i>	Pro-tumor	Activation of the NF- κ B/TLR4 inflammatory axis coupled with PI3K/Akt upregulation	(34,75)
	<i>Helicobacter pylori</i>	Pro-tumor	TLR2/4-NF- κ B pathway activation and concurrent YAP/ β -catenin stimulation	(49,50)
Prostate cancer	<i>Fusobacterium nucleatum</i>	Pro-tumor	Activation of β -catenin/Wnt signaling pathways and impaired CD3 ⁺ T-cell infiltration	(51)
	<i>Lactobacillus</i>	Pro-tumor	Induction of DNA alkylation, gene mutation and hypermethylation of tumor-suppressor genes	(30,52)
	<i>Veillonella</i>	Pro-tumor	LPS-triggers TLR2/4-NF- κ B activation, leading to barrier disruption	(51)
	<i>Actinobacteria</i>	Antitumor	Induction of apoptosis, necrosis, autophagy and G ₂ /M cell-cycle arrest	(62)
Prostate cancer	<i>Streptococcus</i>	Antitumor	activation of TLR2/4-NF- κ B pathway and enhancement of CD8 ⁺ T cell cytotoxicity	(62)
	<i>Propionibacterium</i>	Pro-tumor	TLR4-PI3K-Akt axis activation to induce COX-2 expression and sustain chronic inflammation	(61)
	<i>Escherichia</i>	Pro-tumor	TLR4-NF- κ B pathway activation eliciting pro-inflammatory cytokines and ROS burst, resulting in DNA damage	(61)
	<i>Acinetobacter</i>	Antitumor	Competitive inhibition of pro-inflammatory pathobiont colonization to preserve local micro-ecological homeostasis	(28,29)

Table I. Continued.

Tumor types	Microorganisms	Functions	Mechanisms	(Refs.)
Melanoma	<i>Acinetobacter</i>	Pro-tumor	IL-17 axis activation to potentiate STAT3 signaling and induce PD-L1 upregulation with Tregs	(23)
	<i>Corynebacterium</i>	Pro-tumor		(30)
	<i>Lachnospira</i>	Pro-tumor		(68)
	<i>Staphylococcus epidermidis</i>	Antitumor	Suppression of Th17 responses and blockade of the TLR4-NF- κ B-ROS inflammatory cascade, diminishing DNA-damage-associated mutagenesis	
Liver cancer	<i>Bacteroides ovatus</i>	Pro-tumor	Metabolite iso-lithocholic acid-mediated impairment of NK-cell-dependent antitumor immunity, accelerating hepatocellular carcinoma progression	(81)
	<i>Pseudomonadaceae</i>	Antitumor	Blockade of EGFR phosphorylation and downstream Akt/I κ B β /NF- κ B signaling, driving cancer cell apoptosis	(25)
Pro-tumor, microbes or mechanisms that promote oncogenesis or accelerate progression; antitumor, microbes or mechanisms that suppress malignant phenotypes or enhance anticancer immunity; ROCK, Rho associated coiled-coil containing protein kinase 1; CHK2, checkpoint kinase 2; TLR, toll-like receptor; MET, MET proto-oncogene, receptor tyrosine kinase; PD-1, programmed cell death protein 1; PTK2, protein tyrosine kinase 2; MDSC, myeloid-derived suppressor cell; Treg, regulator T cell; LPS, lipopolysaccharide; COX-2, cyclooxygenase-2; ROS, reactive oxygen species; PD-L1, programmed death-ligand 1; NK, natural killer; I κ B β , inhibitor of nuclear factor- κ B.				

and their involvement in tumorigenesis and progression (89). Notably, FISH stands out as a well-established molecular cytogenetic approach that employs highly complementary fluorescent DNA probes to sensitively and specifically detect and localize bacterial 16S rRNA genes (90).

Studies have shown that analyzing the tumor-associated microbiome using various techniques such as 16S rRNA gene amplicon sequencing and metagenomic sequencing has the potential to target and eliminate tumor-promoting microorganisms or enrich microorganisms that enhance anti-tumor immunity, thereby regulating the composition of the microbiome and providing new strategies for the diagnosis, prevention and treatment of tumors (91). Chai *et al* (92) identified specific bacteria, such as *Klebsiella pneumoniae* and the fungus *Paraburkholderia*, in intrahepatic cholangiocarcinoma tissues using FISH. In pancreatic cancer, *Fusobacterium* was independently associated with poor prognosis, suggesting it has a role as a prognostic biomarker (93). Zhang *et al* (94) showed that the tumor microbiome influences treatment outcomes and survival rates. By examining microbial changes in patients with stage III-IV non-small cell lung cancer, the authors identified *Haemophilus parainfluenzae*, *Serratia marcescens*, *Acinetobacter junii* and *Streptococcus constellatus* as markers for predicting 2-year survival rates. Yamamura *et al* (95) reported that high *Fusobacterium nucleatum* levels in esophageal squamous cell carcinoma indicated poor prognosis, serving as a potential biomarker. Intratumoral microbiota thus holds promise for tumor diagnosis and prognosis, although large-scale, multi-center studies are necessary to confirm their clinical utility.

5. Treatment strategies based on the tumor microbiota

The effectiveness of traditional tumor treatments such as immunotherapy, chemotherapy and radiotherapy is notably affected by microorganisms. Consequently, innovative microorganism-based therapies, including probiotics, prebiotics, synbiotics, fecal microbiota transplantation (FMT), engineered microbiota, phage therapy and oncolytic virus therapy, are gaining traction in tumor prevention and treatment (72). Contemporary strategies frequently integrate these novel microbial therapies with conventional approaches to enhance therapeutic outcomes.

Microbial targeted therapy. Microorganism-based adjuvant therapies have demonstrated notable potential to enhance the efficacy of tumor immunotherapy. A study has shown that in patients with CRC receiving anti-PD-1 monoclonal antibody (mAb) immune checkpoint blockade, the abundance of *Fusobacterium nucleatum* is markedly increased in non-responders, and its metabolite succinate can induce CRC cells to develop resistance to immunotherapy (96). A clinical trial on locally advanced rectal cancer found that the anti-PD-1 mAb dostarlimab exhibited promising therapeutic effects, with some patients avoiding chemotherapy, radiotherapy or surgery (97). Furthermore, a phase I trial reported complete remission in a metastatic melanoma patient resistant to anti-PD-1 therapy after FMT (98).

Emerging strategies, such as oncolytic viruses (OVs) and engineered bacteria, have shown promise in overcoming the

limitations of CAR-T cell therapy for solid tumors, owing to their tumor selectivity and programmable immunogenicity (99). For instance, the OV CD19t can induce CD19 expression on tumor cells, thereby enhancing the antitumor response of CD19-CAR-T cells in mouse models (100). Additionally, the combined use of attenuated *Brucella* strains and adoptive transfer of antigen-specific CD8⁺ CAR-T cells has demonstrated near-complete elimination of tumor growth and proliferation, achieving a 100% host survival rate and effectively overcoming CAR-T cell resistance (101). However, intratumoral microorganisms may also weaken the efficacy of traditional chemotherapy. 5-fluorouracil (5-FU) is a first-line chemotherapy drug for CRC, but *Fusobacterium nucleatum* and *Escherichia coli* can metabolically deplete 5-FU and activate the autophagy pathway, reducing the local drug concentration and efficacy (102). Therefore, precise regulation of the intratumoral microbiota is of great importance for optimizing comprehensive tumor treatment.

Enhancement of immunotherapy efficacy. The commensal microbiota and essential nutrients have demonstrated notable potential in cancer treatment. Multiple studies have revealed the promoting effects of commensal bacteria and their metabolites on the efficacy of immune checkpoint blockade (ICB). For example, Jia *et al* (103) found that the abundance of commensal *Lactobacillus spp.* was positively associated with ICB responsiveness, and its metabolite, indolepropionic acid, could improve the immunotherapy outcomes of melanoma, breast cancer and CRC by regulating the stemness program of intratumoral CD8⁺ T cells. *Bifidobacterium spp.* has been shown to enhance the immunotherapy response by activating the STING signaling pathway (104). Oral administration of live *Lactobacillus rhamnosus* GG increased the numbers of tumor-infiltrating dendritic cells and T cells, thereby enhancing the antitumor activity of anti-PD-1 therapy (105). Kalaora *et al* (29) identified human leukocyte antigen-presenting peptide fragments derived from intratumoral bacteria in melanoma, which could be co-presented by antigen-presenting cells and tumor cells. This can increase the diversity of immunogenic antigens and promote T cell activation, thus enhancing the benefits of immune checkpoint inhibitors.

Probiotics can mitigate the side effects of conventional cancer treatments. Linn *et al* (106) demonstrated that a probiotic mix of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis subsp. lactis* BB-12 reduces acute diarrhea in patients with cervical cancer undergoing radiotherapy. Certain commensal microbiota also have direct therapeutic potential. Montalban-Arques *et al* (107) showed that oral administration of four *Clostridium spp.* strains prevented and effectively treated CRC in a mouse model, outperforming anti-PD-1 monotherapy. Engineered microbial therapy is an emerging cancer treatment strategy. Canale *et al* (108) developed an engineered *Escherichia coli* Nissle1917 that converts ammonia-nitrogen to L-arginine within tumors, enhancing CD8⁺ T cell infiltration and achieving antitumor effects when combined with programmed death-ligand 1 antibodies. Commensal bacteria, their metabolites and engineered strains can reshape the TME through various pathways, offering new strategies and targets for combined immunotherapy.

6. Limitations

The present review discusses foundational knowledge on intratumoral microbiota, while acknowledging several limitations. Firstly, cancer type coverage remains incomplete, and while eight major solid tumors were analyzed, emerging models such as hepatocellular carcinoma (HCC) and renal cell carcinoma were underrepresented, reflecting current research disparities. HCC exclusion, for instance, stems from unresolved challenges in discriminating true tumoral microbiota from hepatic translocation of gut bacteria (24). Furthermore, methodological heterogeneity across studies, including inconsistent sample processing, DNA extraction protocols and bioinformatic approaches (such as 16S rRNA gene sequencing vs. shotgun metagenomics) compromises the comparability of reported microbial profiles (4). Critically, the majority of cited evidence establishes associative rather than causal relationships, leaving underlying mechanisms largely unvalidated; observed microbial enrichments, such as *Veillonella* in gastric tumors, could potentially be a consequence rather than a driver of tumor-induced microenvironmental changes (30). Finally, notable translational barriers exist: Promising microbial biomarkers (such as *Fusobacterium nucleatum* in CRC) lack validation of clinical utility in large prospective trials, and microbiota-targeted therapeutic strategies, including engineered bacteria, currently lack phase III efficacy data.

7. Challenges and prospects

Bottlenecks in key technologies. The differences in the local microenvironments of microbial colonization and the heterogeneity of host cells form a complex interaction network, because both microbial communities and host cells exhibit notable spatiotemporal dynamic changes (109,110). However, current technological constraints hinder the concurrent acquisition of microbial spatial distribution and host single-cell transcriptome information (111,112).

The causal relationship between microbial colonization and tumor progression is difficult to clarify, mainly due to unclear temporal relationships and interference from confounding factors (111). Most existing studies are based on cross-sectional analyses, making it difficult to distinguish whether microbial colonization is a driving factor, a concomitant phenomenon or a secondary result of tumors (8). The dynamic changes in the TME (such as hypoxia or immune suppression) may reversely shape the microbial community, forming a complex feedback loop of bidirectional interaction (70). In addition, individual differences (including genetic background, diet and antibiotic use) and the spatiotemporal heterogeneity of sample collection (such as differences in intratumoral/peritumoral microbiota) need further investigation (70,113).

Research on microbe-host interactions faces a notable lack of standardization, mainly manifested in the absence of unified specifications for sampling methods and bioinformatics analysis, resulting in poor data comparability (69). The complexity of clinical sample collection poses challenges in guaranteeing the quality and quantity of tumor samples (114). Ensuring sample purity and devising strategies to effectively mitigate environmental microbial contamination are crucial objectives during the detection process (4).

Future research directions. Firstly, future research should prioritize the integration of tumor-microbiome-immune multi-omics through systematic analysis of interaction mechanisms using dynamic network modeling (4). To achieve this, advanced technologies such as single-cell transcriptomics, spatial metabolomics and high-throughput sequencing should be combined for synchronous data collection across different scales (115). Machine learning algorithms should be employed to integrate metagenomic, metabolomic and immune cell interaction data to identify key hub molecules (116). Integrating organoid models with dynamic perturbation experiments could validate the predicted networks. This approach aims to develop a quantifiable and controllable ‘microbiome-metabolism-immunity’ computational model, serving as a predictive platform for targeted interventions and advancing research from association to causal mechanisms (117).

Secondly, future research should focus on the development of precision intervention strategies based on the characteristics of the tumor microbiome. Through the integration of multi-omics data such as intratumoral microbiota sequencing, metabolomics and immune microenvironment analysis, a system of predictive biomarkers should be established to guide individualized treatment (118). Utilizing machine learning to analyze tumor-specific microorganisms and validating the interaction between microorganisms and drugs through organoid co-cultures and high-throughput drug screening are essential (119). Moreover, the advancement of microorganism-targeted delivery technologies such as nano-carriers and the design of microbiome combination therapies are crucial (97,120). Ultimately, the goal is to establish a closed-loop transition from microbial diagnostic profiling to treatment decision-making, thereby shifting the paradigm of tumor treatment from a generalized approach to precise microbiota regulation.

Finally, future research should also focus on establishing a highly biomimetic organoid-microbe co-culture system to construct a standardized and high-throughput research and translation platform for the interaction between microorganisms and the host (121,122). Long-term co-culture systems that pair specific organ-derived organoids with complex microbial communities should be developed, and micro-fluidic-chip technologies should be integrated to simulate key *in vivo* microenvironmental parameters such as oxygen gradients (123,124). Building on these systems, real-time multimodal monitoring should be employed to analyze the regulatory mechanisms by which microbial colonization influences barrier function, immune responses and drug sensitivity in organoids (125). Additionally, an automated culture-and-detection platform should be established and coupled with artificial-intelligence algorithms to predict the therapeutic effects of microbiota-targeted interventions, thereby accelerating the translation of mechanistic discoveries into clinical treatment regimens (126). This platform will bridge the technological gap between *in vitro* models and clinical trials and provide accurate prediction tools for microbial targeted therapy.

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Authors' contributions

JY drafted the manuscript and summarized the tables. QL was involved in the conception of the study. HL supervised methodology and theoretical framework and critically reviewed and edited the manuscript for intellectual content and language accuracy. YD, YC, SR and ZL reviewed the manuscript. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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