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REVIEW

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Emerging roles of intratumor microbiota in cancer: tumorigenesis and management strategies

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Abstract

The intricate interplay between the host and its microbiota has garnered increasing attention in the past decade. Specifically, the emerging recognition of microorganisms within diverse cancer tissues, previously presumed sterile, has ignited a resurgence of enthusiasm and research endeavors. Four potential migratory routes have been identified as the sources of intratumoral microbial "dark matter," including direct invasion of mucosal barriers, spreading from normal adjacent tissue, hematogenous spread, and lymphatic drainage, which contribute to the highly heterogeneous features of intratumor microbiota. Importantly, multitudes of studies delineated the roles of intratumor microbiota in cancer initiation and progression, elucidating underlying mechanisms such as genetic alterations, epigenetic modifications, immune dysfunctions, activating oncogenic pathways, and inducing metastasis. With the deepening understanding of intratumoral microbial composition, novel microbiota-based strategies for early cancer diagnosis and prognostic stratification continue to emerge. Furthermore, intratumor microbiota exerts significant influence on the efficacy of cancer therapeutics, particularly immunotherapy, making it an enticing target for intervention in cancer treatment. In this review, we present a comprehensive discussion of the current understanding pertaining to the developmental history, heterogeneous profiles, underlying originations, and carcinogenic mechanisms of intratumor microbiota, and uncover its potential predictive and intervention values, as well as several inevitable challenges as a target for personalized cancer management strategies.

Keywords Intratumor microbiota, Cancer, Tumorigenesis, Immunotherapy, Biomarker, Personalized cancer therapy

Introduction

The human microbiota encompasses a vast number of interacting microorganisms, including bacteria, viruses, fungi, and archaea, inhabiting various locations both on human surfaces and within the body [1]. Thereinto, the gastrointestinal tract, particularly the colon, stands out as the largest microecosystem, harboring several trillion microbial cells [2]. Moreover, the gene set among the resident microbes surpasses that of the human host by more than 100 times [3]. Once as a "forgotten organ", the gut microflora has been extensively demonstrated over the past decade to play a pivotal role in upholding host homeostasis, which serves as a significant determinant in the variability of disease occurrence, progression, and therapeutic response [4, 5]. Thanks to advancements in modern sequencing and metagenomics techniques, it has become evident that human tissues and organs, including tumor tissues (excluding those on body surfaces or in cavities), are not entirely sterile but rather harbor

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low biomass microbial communities [6]. Specifically, the tumor tissue-resident microbes in both extracellular and intracellular spaces, termed as intratumor microbiota, has gained significant attention as a novel and crucial component within the tumor microenvironment (TME) across various cancer types [7]. Despite their eminently lower abundance compared to gut microbiota, recent studies have uncovered tangible findings regarding the impact of intratumor microbiota on cancer development and treatment [8].

As of now, only 11 carcinogenic microbes have been identified in humans, including seven viruses (*Epstein-Barr* [EBV], *Hepatitis B* [HBV], and *Hepatitis C* [HCV] viruses; *Kaposi sarcoma herpesvirus*; *human immunodeficiency virus-1*; *human papillomavirus* [HPV]; *human T cell lymphotropic virus type 1* [HTLV]), one bacterium (*Helicobacter pylori*), and three platyhelminths (*Opisthorchis viverrini*; *Clonorchis sinensis*; *Schistosoma haematobium*) [9]. However, it is noteworthy that an estimated 13% of global cancers could be attributable to these pro-tumorigenic "oncomicrobes" [10]. Actually, the renewed topic of intratumor microbiota has been under discussion for thousands of years (Fig. 1). As early as 2600 BC, the esteemed Egyptian physician Imhotep advocated an anti-tumor regimen that entailed applying poultices, followed by incising swellings and then causing infection [11]. In the fourteenth century, the Italian priest Peregrine Laziosi (1265–1345) earned recognition as the patron saint of cancer patients when the tumor in his tibia miraculously vanished after the malignant lesion became severely infected [12]. Likely inspired by these, several endeavors were undertaken to shrink cancers through erysipelas infection. The German physician Busch in 1868 pioneered the practice of infecting cancer patients with erysipelas, resulting in a remarkable regression of the malignancy [13]. The next notable advancement in microbiotherapy came from William Coley, now credited as the father of cancer immunotherapy. In 1893, he invented a vaccine incorporating two inactivated bacteria: *Streptococcus pyogenes* and *Serratia marcescens*, known as "Coley's toxins". Coley's vaccine demonstrated universally effectiveness against a variety of malignancies, including sarcomas, melanomas, lymphomas, myelomas, and a broad spectrum of carcinomas [14, 15]. Currently, the sole conventional analogous to Coley's vaccine is bacillus Calmette-Guerin. It is administered directly to the tumor, representing the most effective treatment for superficial bladder cancer [16, 17].

Additionally, following Bloch's discovery of the accumulation of phages in tumor tissue leading to the suppression of cancer growth in 1940s [18], significant progress has been achieved in identifying pro-tumorigenic viruses, such as EBV and HTLV for special lymphoma subtypes [19, 20]. Of note, EBV was the first human oncogenic virus to be discovered by Epstein and Barr in 1964 [21], leading to approximately 143,000 (1.8%) annual deaths globally attributed to EBV-related malignancies [22]. In 2015, the U.S. Food and Drug Administration approved the first oncolytic virus, talimogene laherparepvec (T-VEC), for the treatment of metastatic melanoma [23], signifying a promising emerging category of anti-tumor immunotherapy.

Nevertheless, although the significant milestone achieved with the first successful cultivation of *H. pylori* in 1982 by Marshall and Warren, marking a pivotal moment in human medicine, currently, only this

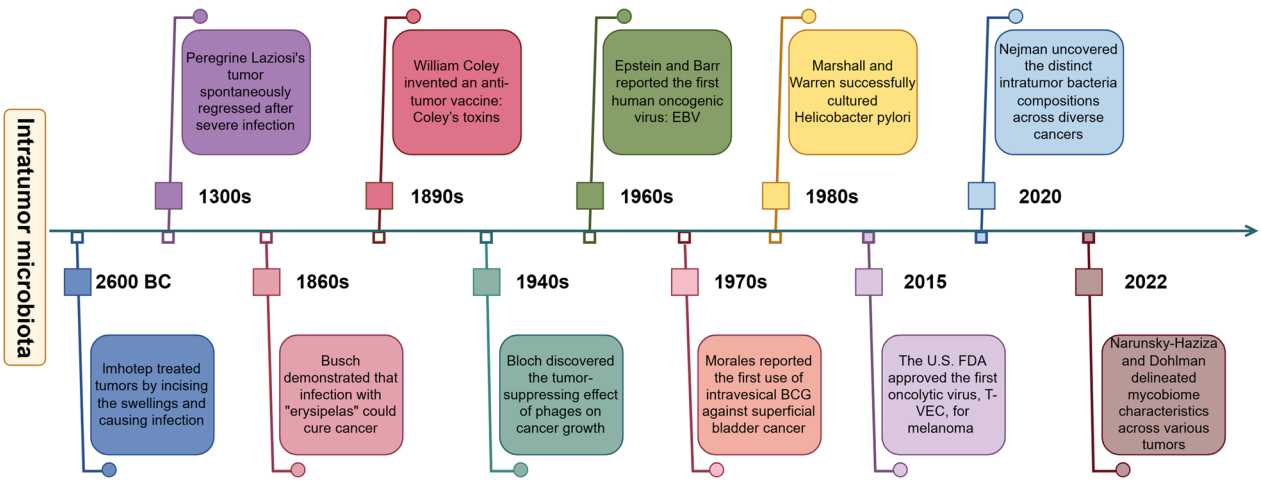

Fig. 1 Significant milestones in intratumor microbiota. Timeline outlining pivotal discoveries regarding intratumor microbiota and major accomplishments in microbial-based anticancer therapy from 2600 BC to the present day. Abbreviations: EBV, *Epstein-Barr* virus; BCG, bacillus Calmette-Guerin

particular carcinogenic bacterium has been firmly established causal relationships with gastric cancer [24, 25]. Notably, through employing real-time quantitative polymerase chain reaction of 16S ribosomal RNA (rRNA) in 2020, Nejman et al. investigated 1010 tumors for bacteria spanning melanoma, glioblastoma, breast, lung, ovary, bone, and pancreas cancers [6], which unveiled unique microbial compositions varying among different cancer types. Subsequently, Narunsky-Haziza [26] and Dohlman [27] have individually revealed the characteristics of cancer mycobiome and its diagnostic as well as prognostic potential in 2022.

Nowadays, technological advancements are greatly expediting the characterization of intratumoral microbial "dark matter," fueling widespread research interest in leveraging intratumor microbiota to influence both the development and treatment of cancer. Here, we present a comprehensive overview of the heterogeneous composition and probable origins of intratumor microbiota, as well as their evolving roles in modulating cancer progression and underlying mechanisms. Furthermore, we elucidate the potential transformative influence of intratumor microbiota in clinical settings, while also highlighting the inherent challenges and future prospects it poses as an intervention target for custom-fit precision cancer management strategies.

Intratumor microbiota

While the intratumor microbiota has been a subject of discussion for a long time, recent years have seen a resurgence of enthusiasm, driven by advancements in modern technology and methodology [28], such as 16S rRNA sequencing, shotgun metagenomic sequencing, electron microscopy, immunohistochemistry, fluorescence in situ hybridization (FISH), and culturomics, allowing for the differentiation of low biomass bacterial DNA from tumor tissue (Table 1). Here, we present recent encouraging findings related to intratumor microorganisms, outlining their distinct compositions and potential migratory trajectories, with the aim to foster an in-depth understanding of the microbe-host interactions within tumors.

Heterogeneity of intratumor microbiota

The abundance, composition, and spatial distribution of intratumor microbial populations vary significantly across different cancer types (Table 2). By analyzing seven tumor types—lung, breast, pancreas, ovary, bone, melanoma, and brain tumors—using a platform with species-level resolution, Nejman and colleagues unveiled cancer type-specific microbial features [6]. Their findings highlighted that breast cancer harbors a notably rich and diverse microbiome compared to other tumor types and normal breast tissues from healthy individuals. Interestingly, tumor-adjacent normal breast samples exhibited an intermediate bacterial load and richness between those of the breast tumors and normal samples. In terms of microbial composition, *Firmicutes* and *Bacteroidetes* were the predominant bacterial species in colorectal tumors, while *Proteobacteria* dominated the microbiome of pancreatic cancer. *Actinobacteria*, including *Corynebacteriaceae* and *Micrococcaceae* families, were notably prevalent in non-gastrointestinal tumors. Following that, the research team further revealed cancer-type-specific fungal localization patterns [26]. Of note, intratumor fungi were predominantly found intracellularly in breast, pancreatic, and ovarian cancers, whereas they primarily localized to macrophages in melanoma and lung cancers. In a sense, this pronounced heterogeneity of intratumor microbiota aligns with the intricate molecular and pathological characteristics of malignancies, which could be crucial for grasping the biological behavior of the tumors and exploiting novel targets for personalized cancer therapy.

Origination of intratumor microbiota

The TME provides conducive niches for microbial habitation owing to its hypoxic microenvironment, nutrients availability, and immune incompetence [72]. Although considerable research on the composition of intratumor microbes, there remains limited understanding regarding the origins of microorganisms inhabiting tumor tissue and their migratory pathways. In this context, we primarily delve into four likely originations or pathways of intratumor microbiota based on recent findings, namely direct mucosal barriers invasion, dissemination from normal adjacent tissue (NAT), hematogenous spread, and lymphatic drainage (Fig. 2).

It is understandable that microorganisms can infiltrate tumors in tissues or organs that are in direct contact with the external environment, such as the gastrointestinal tract, lungs, oral cavity, nasopharynx, and genitourinary organs, due to compromised mucosal barriers caused by tumorigenesis. Among these, *Fusobacterium nucleatum* not only characterized the gut microbial feature of colorectal cancer (CRC) patients [73], but also exhibited enrichment in CRC tumor tissues, particularly in those with certain molecular subtypes such as Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation and microsatellite instability-high [74, 75]. Recently, Zhu et al. demonstrated that *F. nucleatum* could invade cancer cells by binding to DHX15, contributing to colorectal tumorigenesis in *Villin-Cre/Kras*$^{G12D+/-}$ mice [74]. Another analysis of 1697 CRC samples from Australasian revealed a close association between *F. nucleatum* and DNA mismatch repair deficiency (MMRd) in both hereditary and sporadic CRC cases, indicating the

Table 1 Summary of common methodologies used in intratumor microbiota research

Methodologies	Description	Advantages	Disadvantages
16S rRNA sequencing	One of the most frequently employed microbial sequencing methods targeting either single amplicon (V4, etc.) or multiple hypervariable region (V3-V4, V4-V5, etc.) of the 16S rRNA gene [29]	Cost-effective and accessible data analysis	1. Lower coverage and resolution in bacterial species-level; 2. Inability to determine microbial activity
Shotgun metagenomic sequencing	A powerful, untargeted sequencing of all microbial genomes present in a sample [30]	1. Higher resolution than 16S rRNA sequencing; 2. Providing functional insights	1. Overwhelming contamination of human DNA; 2. Higher cost and complexity in data analysis and interpretation
Electron microscopy	Transmission electron microscopy can be utilized to observe the ultrastructure, morphology, and function of microorganisms within tumor tissues [6]	High spatial resolution enables direct visualization of ultrastructure	1. Complex sample preparation; 2. Costly and time-consuming
Immunohistochemistry	Using antibodies against bacterial lipoteichoic acid and lipopolysaccharides to detect Gram-positive and Gram-negative bacteria, respectively [31]	Providing specificity and spatial localization of intra-tumor microbiota	1. Restricted by the specificity of antibodies targeting microbial antigens; 2. Risk of false positives attributed to non-specific binding
Fluorescence in situ hybridization	Using a probe targeting the bacterial 16S rRNA gene or specific to bacteria of interest allows visualization of intratumor microbes [32]	High specificity and intuitive spatial localization	1. Inability to distinguish live from dead cells; 2. Risk of false positives
Culturomics	Involving the extensive isolation of strains through diverse culture conditions, followed by identification using mass spectrometry or sequencing of the 16S rRNA gene [33]	1. Offering insights into the viable microbiota; 2. Facilitating the discovery and isolation of novel species	1. Selective growth bias due to certain microbes either fail to grow or exhibit poor growth under laboratory conditions; 2. Time-consuming

Table 2 Summary of human intratumor microbiota researches in recent three years

Tumor types	Specimen types	Methods	Main findings	Significances
Lung cancer	Surgical tumor and adjacent healthy tissue samples from 29 NSCLC patients [34]	16S rRNA sequencing	1. Bacterial β-diversity in an intra-patient was more similar to those of different patients; 2. *Escherichia-Shigella*, *Faecalibacterium*, *Pseudomonas*, *unclassified Enterobacteriaceae*, *Alloprevotella*, and *Brevundimonas* were only recurrently present in the tumoral tissues	The cancerous tissue was mainly featured by enteric and proinflammatory bacteria
	Surgically resected tissue samples, including adjacent normal regions, from 12 lung cancer patients [35]	Spatial meta-transcriptomic analysis method based on NanoString Digital Spatial Profiler	1. Bacterial burden was significantly higher in tumor cells compared with T cells, macrophages, other immune cells, and stroma; 2. Bacterial burden increased from tumor adjacent normal lung and tertiary lymphoid structures to tumor cells to the airways	1. Supporting a rationale for reducing the local intratumor microbiome in lung cancer for patient benefit; 2. Providing a novel spatial meta-transcriptomic method to capture both microbial and host transcriptome information
	Thirty-seven pieces of tumor tissue through surgery or biopsy from NSCLC patients [36]	Pathogen targeted sequencing	1. Forty-nine pathogenic microorganisms were detected in the tumor samples; 2. There were 11 common pathogens between the tumor and BALF samples	Detection of microbial classification and abundance in the BALF may help to evaluate the severity of NSCLC
	Twenty-four paired tumor and non-tumor tissue by surgery [37]	Fungi-enriched DNA extraction and deep shotgun metagenomic sequencing	1. *Aspergillus sydowii* was significantly enriched in lung adenocarcinoma tumor tissues; 2. *A. sydowii* could promote lung tumor progression via IL-1β-mediated expansion and activation of MDSCs; 3. *A. sydowii* was associated with immunosuppression and poor patient outcome	Intratumor mycobiome promotes lung cancer progression and could be an intervention target for lung adenocarcinoma patients
Breast cancer	Surgically resected human tumor tissue with paired adjacent normal breast tissue and the lymph node metastasis [31]	16S rRNA sequencing and pan-pathogen microarray	1. The tumor microbiota and lymph node metastasis microbiota exhibited significantly reduced alpha diversity, and increased facultative anaerobes compared with human normal breast samples; 2. The tumor tissue showed significant higher abundance of *Enterococcus* and *Streptococcus*	The intratumor microbiota may play a conserved role in the cancer pathogenesis and progression

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
	Formalin-fixed, paraffin-embedded (FFPE) tissue samples [38]	16S rRNA sequencing and liquid chromatography-mass spectrometry untargeted metabolomic analysis	1. *Turicibacter* exhibited a significantly higher abundance in triple-negative breast cancer; 2. Several metabolites such as nutriacholic, pregnanetriol, and cortol exhibited statistically different abundances between tumor group and controls; 3. Three microbial taxa, including *Cytophagaceae*, *Conexibacteraceae*, and *Flavobacteriaceae*, were related to tumor-infiltrating lymphocytes	Intratumor microbiota or metabolites could be promising biomarkers as the diagnostic and therapeutic tools for triple-negative breast cancer
	FFPE and fresh frozen tumor samples from 30 patients [39]	16S rRNA sequencing	1. 78.55% of OTUs in FFPE samples were consistent with fresh frozen samples; 2. There was no significant difference in the alpha diversity and core bacterial composition between FFPE and fresh frozen samples	FFPE tissues could be a potential substitute for fresh tumor tissues in the analysis of breast cancer microbiota
Liver cancer	168 clinical tissue samples from 100 individuals, including 68 paired primary HCC and peritumor HCC tissues (2 cm away from cancerous tissue), 3 additional peritumor tissues, and 29 normal liver tissues [40]	16S rRNA sequencing, immunohistochemistry, FISH, and tissue culture	1. Both alpha and beta diversity in peritumor and HCC microbiota were increased; 2. *Patescibacteria*, *Proteobacteria*, *Bacteroidota*, *Firmicutes*, and *Actinobacteriota* were the most predominant phyla in HCC; 3. Random Forest machine learning based on the top 50 OTUs achieved great discriminative performance in HCC prediction	Specific intratumor microbes represent potential targets for both therapeutic and diagnostic interventions for HCC
	FFPE specimens from 28 primary liver cancer patients [41]	16S rRNA sequencing	1. A significant decrease in the alpha diversity and unique OTUs in combined HCC and intrahepatic cholangiocarcinoma patients; 2. *Pseudomonadaceae* was markedly decreased in tumor areas and positively related to the prognosis of cancer patients	Cancer microbiota represented a potential novel diagnostic biomarker and therapeutic strategy for primary liver tumor

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
	Ninety-nine surgical tissues from intrahepatic cholangiocarcinoma (ICC) patients [42]	16S rRNA sequencing, FISH, HE staining, tissue culture, transmission electron microscopy, and single-cell RNA sequencing	1. *Staphylococcus capitis* was cultured from fresh tissues; 2. Intratumor bacteria could be present in diverse cell types; 3. *Burkholderiales*, *Pseudomonadales*, *Xanthomonadales*, *Bacillales*, and *Clostridiales* constitute the most abundant bacterial orders; 4. *Paraburkholderia fungorum* was significantly depleted in the tumor tissues, which could suppress tumor growth through alanine, aspartate and glutamate metabolism	1. Conforming the presence of intracellular bacteria in ICC; 2. Modulation of intratumor bacteria would be a potential approach for ICC therapy
	Surgically resected 99 HCC and para cancerous tissue samples [43]	FISH and 16S rRNA sequencing	1. Microbial diversity was significantly higher in HCC tissues than in adjacent tissues; 2. *Enterobacteriaceae*, *Fusobacterium*, and *Neisseria* were significantly increased, while certain anti-tumor bacteria such as *Pseudomonas* were decreased; 3. Processes of fatty acid and lipid synthesis were significantly enhanced in the microbiota in HCC tissues	1. There were significant differences in the microbial communities in HCC and para cancerous tissues; 2. The high abundance of *Enterobacteriaceae* and *Fusobacterium* may affect HCC progression
	Three tissue microarray chips, 92 FFPE samples, and 413 tumor tissue samples [44]	Single-molecule RNA FISH, qRT-PCR, and tissue culture	1. Patients with high *Mycoplasma hyorhinis* load exhibited poor prognosis; 2. *M. hyorhinis* could retrogradely infect the liver through the hepatopancreatic ampulla; 3. Intratumor *M. hyorhinis* could promote the initiation and progression of HCC by enhancing nuclear ploidy	*Mycoplasma* clearance with antibiotics or regulating mitochondrial dynamics might have the potential for HCC therapy
	Ninety-one tumor and para tumor tissues from patients underwent hepatectomy [45]	FISH and 16S rRNA sequencing	1. *Actinobacteria* was significantly enriched in tumor tissues, while *Deinococcus-Thermus* was markedly abundant in normal tissues; 2. Hepatotypes of intratumor microbiota significantly impacted the survival of patients with HCC after surgery	Intratumor microbiome could be helpful for predicting patient outcomes

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
Colorectal carcinoma	436 bioptic specimens from CRC (n = 36) or adenoma (n = 32), including tumor and adjacent normal tissues [46]	16S rRNA sequencing	1. Abundances of certain CRC-associated pathobionts, including *Fusobacterium*, *Bacteroides*, *Parvimonas*, and *Prevotella* were highly varied within a single neoplasia; 2. The intratumor microbial variation in abundance changed along the adenoma-carcinoma sequence; 3. Intratumor microbiota was closely associated with CRC-associated genetic alterations of KRAS mutation or microsatellite instability	Intratumor microbiota was significantly heterogeneous and related to colorectal carcinogenesis
	Fresh tumor and adjacent normal samples from 29 CRC cases [47]	16S rRNA sequencing	1. *Fusobacterium* was enriched in tumor tissues while *Bacteroides* was enriched in non-tumor tissues; 2. *Fusobacterium* was linked with many mutated genes and cell cycle-related pathways	The identification of intratumor microbial patterns and their specific genetic alterations might facilitate targeted interventions
	Surgical resection of tumor and adjacent normal tissues from 276 cases [48]	16S rRNA sequencing	1. Young-onset CRC tumors exhibited significantly higher alpha diversity and varied beta diversity than average-onset tumors; 2. Young-onset tumors were enriched with *Akkermansia* and *Bacteroides*, whereas average-onset tumors showed greater relative abundances of *Bacillus*, *Staphylococcus*, *Listeria*, *Enterococcus*, *Pseudomonas*, *Fusobacterium*, and *Escherichia/Shigella*; 3. *Fusobacterium* and *Akkermansia* were correlated with the overall survival of young-onset CRC	Providing prospective preventative and therapeutic targets for guiding the interventional strategies of CRC patients
	1,697 FFPE CRC tumor tissue from the Australasian Colorectal Cancer Family Registry [49]	Quantitative PCR	1. $Pks^{+}E.coli^{+}$ was related to male sex and *APC*:c.*835–8* A > G somatic mutation, specifically for early-onset CRCs; 2. *Fusobacterium nucleatum* was associated with DNA mismatch repair deficiency, *BRAF*:c.1799 T > A p.V600E mutation, CpG island methylator phenotype, proximal tumor location, and high levels of tumor infiltrating lymphocytes	Specific molecular features and pathways of colorectal tumorigenesis were linked with each genotoxic intratumor bacterium

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
Gastric cancer	Fifty-three surgically resected tumor tissues and 30 gastric mucosal tissue from chronic gastritis patients who underwent endoscopy [50]	16S rRNA sequencing	1. The α diversity of intratumor microbiota was significantly reduced and the microbial composition in tumor samples was markedly different from that of chronic gastritis; 2. The genera *Oceanobacter*, *Methylobacterium*, and *Syntrophomonas* were significantly enriched in cancer tissues; 3. Intratumor microbiota was closely associated with the exhausted CD8 + tissue-resident memory T cells in the TME of gastric cancer	Providing novel insights into the diagnosis and therapeutic strategy of gastric cancer
	311 human gastric biopsy tissues, including 110 superficial gastritis, 117 atrophy gastritis, 45 intestinal metaplasia, and 39 gastric cancers [51]	16S rRNA sequencing	1. *Streptococcus anginosus* was significantly enriched in the gastric mucosa of gastric cancer patients; 2. *S. anginosus* could induce gastritis-atrophy-metaplasia dysplasia sequence in mice and promote gastric tumorigenesis by its surface protein TMPC binding through an Annexin-2 and MAPK signaling cascade	Identifying a non-*Helicobacter pylori* pathogen, *S. anginosus*, that could directly contribute to gastric tumorigenesis
Esophageal cancer	FFPE specimens from 306 esophageal cancer patients [52]	Quantitative PCR	1. 21.2% cases were found with intratumor *Fusobacterium nucleatum* DNA; 2. The positivity of *F. nucleatum* DNA in a tumor was significantly linked with LINE-1 hypomethylation	Epigenetic modification induced by intratumor microbiota could serve as a potential mechanism for exploring the pathogenesis of esophageal cancer
	FFPE specimens from 300 esophageal cancer patients [53]	Quantitative PCR	High abundance of *Fusobacterium nucleatum* in cancer microbiota exhibited a much lower level of the peritumoral lymphocytic reaction	Providing a framework for further investigations on the interactive roles between intratumor microbiota and host immunity during tumorigenesis
	Twelve surgical or biopsy tissue samples and 19 paraffin sections [54]	Quantitative PCR	1. *Fusobacterium nucleatum* DNA was enriched in the nonresponder group among cancer patients receiving PD-1 inhibitor; 2. *F. nucleatum* could suppress the proliferation and cytokine secretion of T cells and upregulate PD-L1 expression	Eradication of intratumor *F. nucleatum* could be a critical therapeutic strategy before initiating esophageal cancer immunotherapies
	Forty surgical tissues from patients with esophageal squamous cell carcinoma [55]	16S rRNA sequencing, tissue culture, and transmission electron microscopy	1. The enrichment of *Streptococcus* was positively related to GrzB + and CD8 + T-cell infiltration in tumor tissues; 2. *Streptococcus* abundance could predict prolonged disease-free survival in esophageal squamous cell carcinoma	Intratumor microbiota was closely linked with chemoimmunotherapy response

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
Pancreatic cancer	FFPE samples from pancreatic ductal adenocarcinoma (PDAC) patients, including 13 short-term survivors and 17 long-term survivors [56]	16S rRNA sequencing and quantitative PCR validation	1. The intratumor microbiota was closely linked with the survival of PDAC patients; 2. Administration of *Megasphaera* synergistically inhibited tumor growth when combined with immune checkpoint inhibitor therapy	Specific intratumor microbes could enhance the anti-tumor effect in PDAC patients
	Twenty surgical tumor tissues and paired-normal adjacent tissues (NAT) samples from pancreatic cancer (PC) patients [57]	16S rRNA sequencing and FISH	1. Forty-four genera were found significantly different between PC and NAT; 2. Intratumor *porphyromonas gingivalis* promoted PC progression by increasing the secretion of neutrophilic chemokines and neutrophil elastase	Clearance of *P. gingivalis* could provide a potential strategy for the treatment of PC patients
	Fresh-frozen tissues of 84 resected pancreatic tumors and 41 normal pancreatic tissues [58]	Quantitative PCR	1. Fusobacterium nucleatum was detected in 15.5% of PC patients; 2. *F. nucleatum* was positively associated with the tumor size; 3. *F. nucleatum* promoted CXCL1 secretion from tumor cells and inhibited tumor-infiltrating CD8 + T cells by recruiting myeloid-derived suppressor cells	Blockade of the paracrine mechanisms, CXCL1- CXCR2 axis, might be a potential therapeutic approach for patients with intratumor *F. nucleatum*-positive pancreatic cancer
	FFPE sections from 162 PDAC patients who underwent surgery [59]	Quantitative PCR, in situ hybridization targeting 16S rRNA, and 16S rRNA sequencing	1. The presence of intratumor bacteria was confirmed in 52 tumors (32%); 2. Certain anaerobic bacteria such as *Bacteroides*, *Lactobacillus*, and *Peptoniphilus* were associated with an inferior survival and a decrease in the number of tumor-infiltrating T cells	Intratumor anaerobic bacteria could suppress the anti-PDAC immunity and lead to a poor prognosis
Head and neck cancer	Tissue samples from a first cohort of 122 patients with head and neck squamous cell carcinoma, including 61 oral squamous cell carcinomas (OSCC), and a second cohort of 90 additional OSCC [60]	Quantitative PCR	1. *Fusobacterium nucleatum* was linked with a lower recurrence rate, less frequent lymph node invasion, less metastatic relapses, and significantly longer survival; 2. Gram-negative bacteria load was inversely associated with M2 macrophages	*F. nucleatum*-related OSCC exhibited a specific immune microenvironment and correlated with a favorable prognosis
	802 pretreatment biopsy tissues of nasopharyngeal carcinoma (NPC), including fresh-frozen tissues and FFPE samples, from 2 hospitals [61]	16S rRNA sequencing and FISH	1. *Corynebacterium* and *Staphylococcus* were predominated in NPC tumor tissues; 2. A high bacterial load was positively linked with inferior rates of disease-free survival, distant metastasis-free survival, and overall survival; 3. The NPC intratumor bacteria were mainly derived from nasopharyngeal microbes; 4. A higher intratumoral bacterial load was negatively linked with T-lymphocyte infiltration	Intratumor microbiota exhibited a robust prognostic tool for NPC patients and indicated potential guidance for treatment decisions

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
	Multiple sample types from 27 patients, including 26 saliva, 16 swabs from the surface of tumor tissues, 16 adjacent normal tissues, 22 tumor outer tissue, 22 tumor inner tissues, and 10 lymph nodes [62]	16S rRNA sequencing	1. *Fusobacterium*, *Neisseria*, *Porphyromonas*, and *Alloprevotella* were more abundant in outer tumor tissues, while *Prevotella*, *Selenomonas*, and *Parvimonas* were enriched in inner tumor tissues; 2. *Gemella* and *Bacillales* were enriched in T1/T2-stage patients and the non-lymphatic metastasis group, while *Spirochaetae* and *Flavobacteriia* were enriched in the extranodal extension negative group	Characterizing the oral tumor microbiome associated with different specimens and identifying space specific microbial composition at the invasive front of tumors
	Surgical specimens from 95 subjects, including 11 healthy individuals, 15 precancerous subjects, and 69 cancer patients [63]	16S rRNA sequencing	1. The genera *Capnocytophaga*, *Fusobacterium*, and *Treponema* were significantly enriched in the cancer group, whereas *Streptococcus* and *Rothia* were enriched in the precancer group; 2. Capnocytophaga and Fusobacterium were closely associated with late and early cancer stages, respectively; 3. High abundance of bacteria in the TME was either negatively correlated or not associated with the effector lymphocytes	Intratumor microbiota could serve as late-stage cancer biomarkers and impact the regional immune system of oral cancer, conferring a possibility of immune modulation by microbial manipulation
Urogenital neoplasms	Twenty-four paired renal cell carcinoma (RCC) and adjacent normal tissue samples were collected by surgery [64]	16S rRNA sequencing	1. The microbial diversity was significantly decreased in RCC tissues; 2. Twenty-five enriched taxa and 47 depleted taxa were identified in RCC tissues compared with normal tissues; 3. The class *Chloroplast* and the order *Streptophyta* exhibited higher indication accuracy to discriminate RCC from normal tissues	Revealing the distinct intratumor microbial features in RCC tissues and adjacent normal tissues, which might play a pivotal role in the pathogenesis and development of RCC
	95 hysterectomy samples from endometrial cancer patients [65]	16S rRNA sequencing	1. Microbial diversity was greater in the malignant uterus; 2. *Firmicutes*, *Cyanobacteria*, and *OD1* were enriched in tumors from Black versus White women, while the genus *Dietzia* and *Geobacillus* were depleted in tumors of obese Black versus obese White women	Intratumor microbial dysbiosis could provide novel targets for the treatment and prevention of endometrial cancer patients

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
	Thirty surgical ovarian tissues, including 15 epithelial ovarian cancers (EOCs) and 15 benign ovarian tumors [66]	16S rRNA sequencing and tissue culture	1. EOC tissues exhibited a higher microbial diversity and richness compared with benign ovarian tumors; 2. Pathogens, including *Actinomycetales*, *Acinetobacter*, *Streptococcus*, *Ochrobacterium*, *Pseudomonadaceae*, and *Pseudomonas*, were enriched in the cancer tissues; 3. *Propionibacterium acnes* could promote EOC progression through increased inflammatory response to activate the hedgehog pathway	Intratumor microbiota provide clues for improving EOC treatment
Other cancers	Surgically resected tumor samples from 80 patients with papillary thyroid carcinoma (PTC) [67]	16S rRNA sequencing	1. The tumor bacterial diversity in advanced lesions (T3/T4) was significantly higher than that of relatively mild lesions (T1/T2); 2. Combination of eight tumor bacterial taxa could effectively predict the invasion status of PTC	The microbial host factors might determine tumor behaviors and patient outcomes
	FFPE tissues of neuroendocrine neoplasms (NEN), including 20 pancreatic NEN and 20 intestinal NEN [32]	FISH	1. 90% pancreatic NEN and 75% intestinal NEN exhibited bacterial infiltration, with a homogeneous microbial distribution in pancreatic NEN; 2. A higher bacterial infiltration in the tumoral tissues as compared with non-tumoral tissue	Demonstrating the presence of bacterial infiltrate in the neuroendocrine tumor microenvironment,
	Twenty-six adrenocortical carcinoma (ACC) tissues and 9 healthy adrenals through surgery [68]	16S rRNA sequencing	1. The phylum *Proteobacteria*, especially the *Pseudomonas* and *Serratia* genera, was enriched in ACC tissues; 2. The *Proteobacteria* abundance was negatively associated with tumor size, Ki67 and cortisol secretion; 3. Patients with high abundance of *Proteobacteria*, *Pseudomonas*, and *Serratia* and with low abundance of *Bacteroidota*, *Firmicutes*, and *Streptococcus* exhibited higher levels of mitotane at 9 months after therapy	Identifying the intratumor microbial features, which could impact the circulating mitotane levels in ACC patients
	Fifteen biopsy and resection samples from soft tissue sarcomas (STS) patients [69]	Whole genome shotgun sequencing	1. A small but consistent proportion of bacterial DNA (0.02–0.03%) was found in all tumors, including *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and viral species; 2. There was a strong positive relationship between viral relative abundance and natural killer cell infiltration	Revealing the link between intratumor microbiota and STS TME, which might be a target for STS therapy

Table 2 (continued)

Tumor types	Specimen types	Methods	Main findings	Significances
	Tissue samples from 89 randomly selected patients with primary vulvar squamous cell carcinoma (VSCC) [70]	16S rRNA sequencing, quantitative PCR, and immunohistochemistry	1. *Fusobacterium nucleatum* and *Pseudomonas aeruginosa* were identified as tumor-promoting bacteria, which were linked with an inferior outcome in VSCC patients; 2. Neutrophilic inflammation in the VSCC environment was permissive of tumor bacteria and promoted cancer progression	Targeting neutrophils as a new therapeutic opportunity to be developed for VSCC patients
	Thirty-seven surgical tissue samples from pituitary neuroendocrine tumors (PitNETs) patients [71]	16S rRNA sequencing, immunohistochemistry, and FISH	1. Common and diverse intratumoral bacterial types across the four clinical PitNET subtypes were identified; 2. The pathogenesis and development of tumors were linked with the behavior of intratumoral bacteria	Exhibiting the existence of intra-tumoral bacteria in PitNET

NSCLC, non-small cell lung cancer; BALF, bronchoalveolar fluid; MDSCs, myeloid-derived suppressor cells; FFPE, Formalin-fixed, paraffin-embedded; OTUs, operational taxonomic units; FISH, fluorescence in situ hybridization; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; CRC, Colorectal carcinoma; TME, tumor microenvironment; PDAC, pancreatic ductal adenocarcinoma; NAT, normal adjacent tissues; PC, pancreatic cancer; OSCC, oral squamous cell carcinoma; NPC, nasopharyngeal carcinoma; EOCs, epithelial ovarian cancers; RCC, renal cell carcinoma; PTC, papillary thyroid carcinoma; NEN, neuroendocrine neoplasms; ACC, adrenocortical carcinoma; STS, soft tissue sarcomas; VSCC, vulvar squamous cell carcinoma; PitNETs, pituitary neuroendocrine tumors

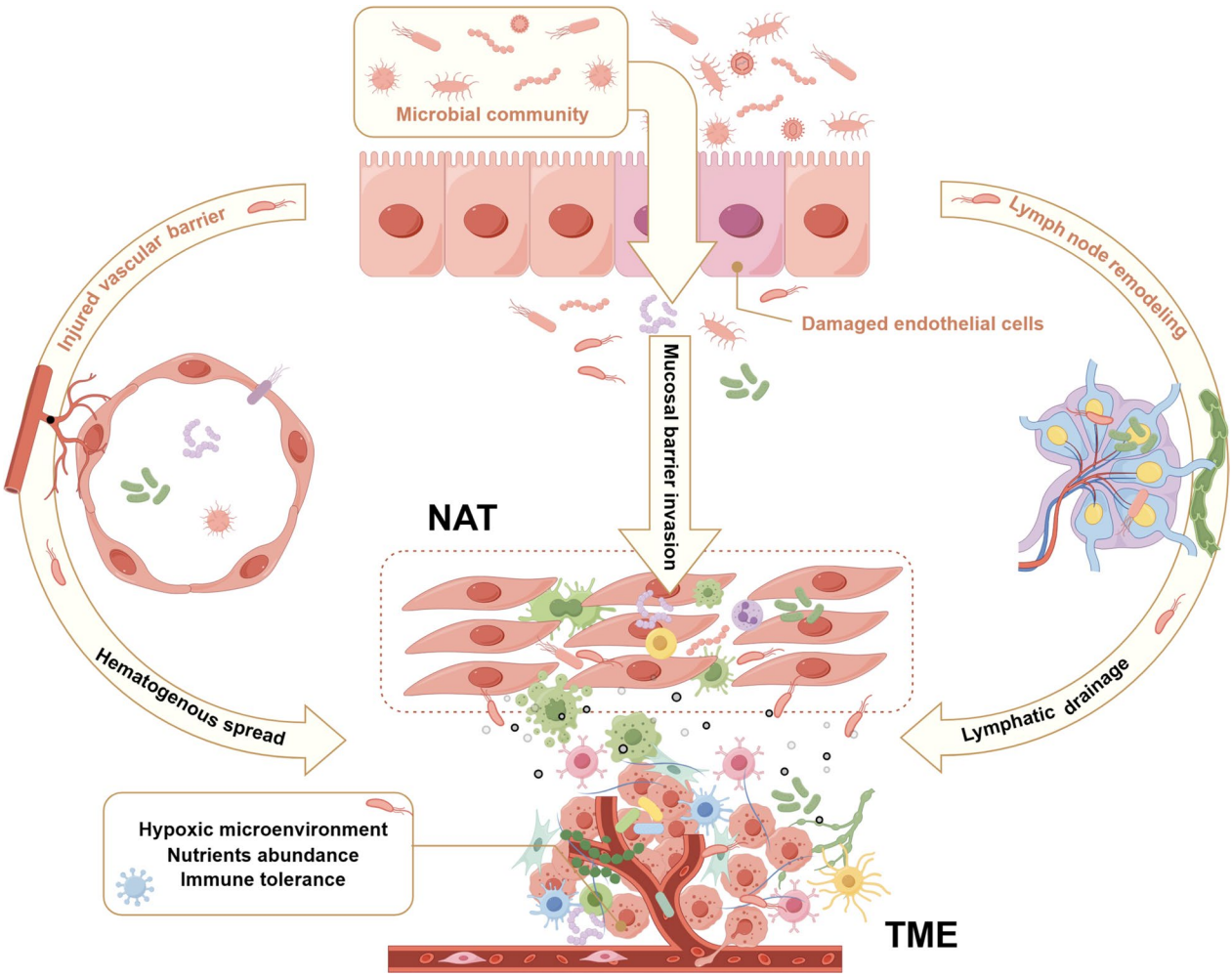

Fig. 2 Potential sources of intratumor microbiota. The hypoxic, nutrient-abundant, and immunoincompetent TME provides a desirable habitat for microorganisms. The initiation for microbial migratory involves direct mucosal barriers invasion, while the NAT would serve as a "transfer station" following microbes' invasions. In addition, hematogenous spread and lymphatic drainage play significant roles as supplementary pathways for microbial migratory trajectories. Abbreviations: NAT, normal adjacent tissue; TME, tumor microenvironment

pivotal role of MMRd-related TME in *F. nucleatum* colonization [49]. Notably, genetic heterogeneity appears to play a pivotal role in determining bacterial colonization. Zepeda-Rivera et al. conducted pan-genomic analyses on 135 *F. nucleatum* strains and found that the *F. nucleatum* subspecies *animalis* (Fna) consists of two distinct clades [76]. Of them, Fna C1 is primarily found in the oral cavity, whereas Fna C2 predominates in human CRC tumor niches and promotes tumorigenesis by altering intestinal metabolism to increase oxidative stress.

Furthermore, efforts are underway to trace bacterial migratory pathways more accurately. Through single-nucleotide variant analysis, Qiao and colleagues unveiled that the predominance of microbes, including *Corynebacterium* and *Staphylococcus*, in nasopharyngeal carcinoma (NPC) tissues largely stemmed from the nasopharyngeal microbiota [61]. Likewise, Liao et al. identified 13 species, such as *F. nucleatum and Prevotella intermedia*, as oral-translocated and enriched in NPC patients through 16S rRNA sequencing on the paired nasopharyngeal-oral microbial samples, further validated by culturomics, clonal strain identification, and metatranscriptomes [77].

Despite the discussion of the NAT as a potential source of intratumor microbiota due to the resemblance in microbial composition between NAT and tumor tissue in a number of Reviews [28, 78, 79], the NAT might function as the "transfer station" subsequent to microorganism invasion of mucosal barriers. A study involving 82 CRCs, 118 adenomas, and 149 matched adjacent normal mucosae found that the abundance of *F. nucleatum* in NAT from cancer cases was significantly higher than

that in tumor tissue and NAT from adenoma cases [80]. In addition, the detection rate of *F. nucleatum* in CRC cases at stage III/IV increased gradually from superficial NAT, deep NAT, to cancer tissue. Likewise, Wong-Rolle et al. showed that the peak bacterial load was observed in the airway, followed by a lower level in tumor cells and further reduction in NAT in early-stage lung cancer [35], suggesting that intratumor microbes might originate from the lower airway through NAT. Hence, we categorized the source of spreading from NAT to be a consequence of mucosal barrier invasion.

Navigating hematogenous or lymphatic migratory routes for microbes presents more stereotypical challenges than direct invasion of mucosal barriers. But actually, the impairment of mucosal barriers consistently constitutes the initial step for microbial migratory. One notable example is the work by Bertocchi and colleagues in 2021, which reported the injured gut vascular barrier by the virulence regulator VirF from *Escherichia coli* could boost microbial dissemination along the gut-liver axis and further induce a premetastatic niche formation of CRC in the liver [81]. Intriguingly, the hematogenous migratory route appears to offer a promising avenue for the microbiota-based cancer modulation. Zhu et al. observed that the abundance of *Akkermansia muciniphila* increased in tumor tissue following the gavage of *A. muciniphila* in a lung cancer mouse model, which has a significant impact on the composition of intratumor microbiota [82]. Crucially, after 2 h of bacterial administration, *A. muciniphila* was notably elevated in blood samples as detected by 16S rDNA sequencing, suggesting a probable crosstalk between intestinal and intratumoral microbiota through systemic circulation. Similarly, the lymphatic drainage route of microbial translocation plays a pivotal role in bolstering extraintestinal anti-tumor immune responses during immune checkpoint blockade (ICB) therapy. Choi and colleagues reported that ICB treatment could stimulate the translocation of specific gut bacteria, such as *Enterococcus faecalis* and *Lactobacillus johnsonii*, to extraintestinal tissues by inducing lymph node remodeling and activating dendritic cell (DC) in a preclinical melanoma model [83], which is conducive to exert optimal anti-tumor T cell responses against extraintestinal tumors.

Impacts of intratumor microbiota on *cancer* occurrence and progression

The roles of microorganisms in tumorigenesis have been extensively explored, with *H. pylori* infection associated with gastritis, gastric ulcer, and gastric cancer serving as a paradigm of bacterium-mediated cancer formation [84]. With advances in detection techniques, accumulating data suggest the presence of tumor type-specific intratumor microbes, which are now recognized as integral components of the TME [85]. Using spatial-profiling technologies, Li and colleagues established a spatial coupling between microbes and T cells in cancer, which plays a critical role in shaping the contexture of TME [86]. Here, we mainly discuss emerging evidence regarding the mechanisms by which intratumor microbiota contribute to cancer development in recent years (Fig. 3), including genetic alterations, epigenetic modifications, immune dysfunctions, activating oncogenic pathways, inducing metastasis, and other relevant mechanisms.

Genetic alterations

Genome alterations, such as DNA damage and gene mutations, play a central role in the genesis and progression of cancer. Members of the microbiota can directly cause DNA damage through the production of certain metabolites or secretions such as toxins [87, 88] and membrane vesicles (MVs) [89], which have the ability to alkylate DNA, induce DNA double-strand breaks, or generate excessive reactive oxygen species (ROS). For instance, Okuda et al. observed the associations between tumor-residing *Fusobacterium* and a series of altered genes in CRC, and they underscored the potential of *Campylobacter* in promoting carcinogenesis by inhibiting double-strand DNA break repairs [47]. Moreover, *Campylobacter jejuni* has been shown to induce CRC pathogenesis in a cytolethal distending toxin-dependent manner in germ-free $Apc^{Min/+}$ mice [90]. MVs, which are nano- or micrometer-sized lipid-bound vesicles released from cells, serve as vehicles for the systemic delivery of a variety of molecular cargoes, including nucleic acids, sugars, lipids, and proteins, to recipient cells [91]. Miyakawa and colleagues discovered that *odontolyticus* secretes lipoteichoic acid-rich MVs, which can induce excessive ROS production by causing mitochondrial dysfunction in colonic epithelial cells, consequently leading to DNA damage and the initiation of CRC [89].

Epigenetic modifications

Epigenetic modifications, including DNA methylation, non-coding RNAs, and histone modifications, constitute another significant mechanism implicated in cancer development through the regulation of gene expression. *F. nucleatum* has been implicated in the alterations of genome-wide methylation levels in esophageal cancer [52]. Additionally, Park et al. observed an intimate relationship between high levels of *F. nucleatum* and hypermethylation of the *CDKN2A* promoter CpG island, potentially linked with M2 macrophages infiltration in CRC [75]. Apart from DNA modifications, Qiao et al. suggested an m6A-dependent mechanisms mediated by the intratumor *Mycoplasma hyorhinis* in boosting the

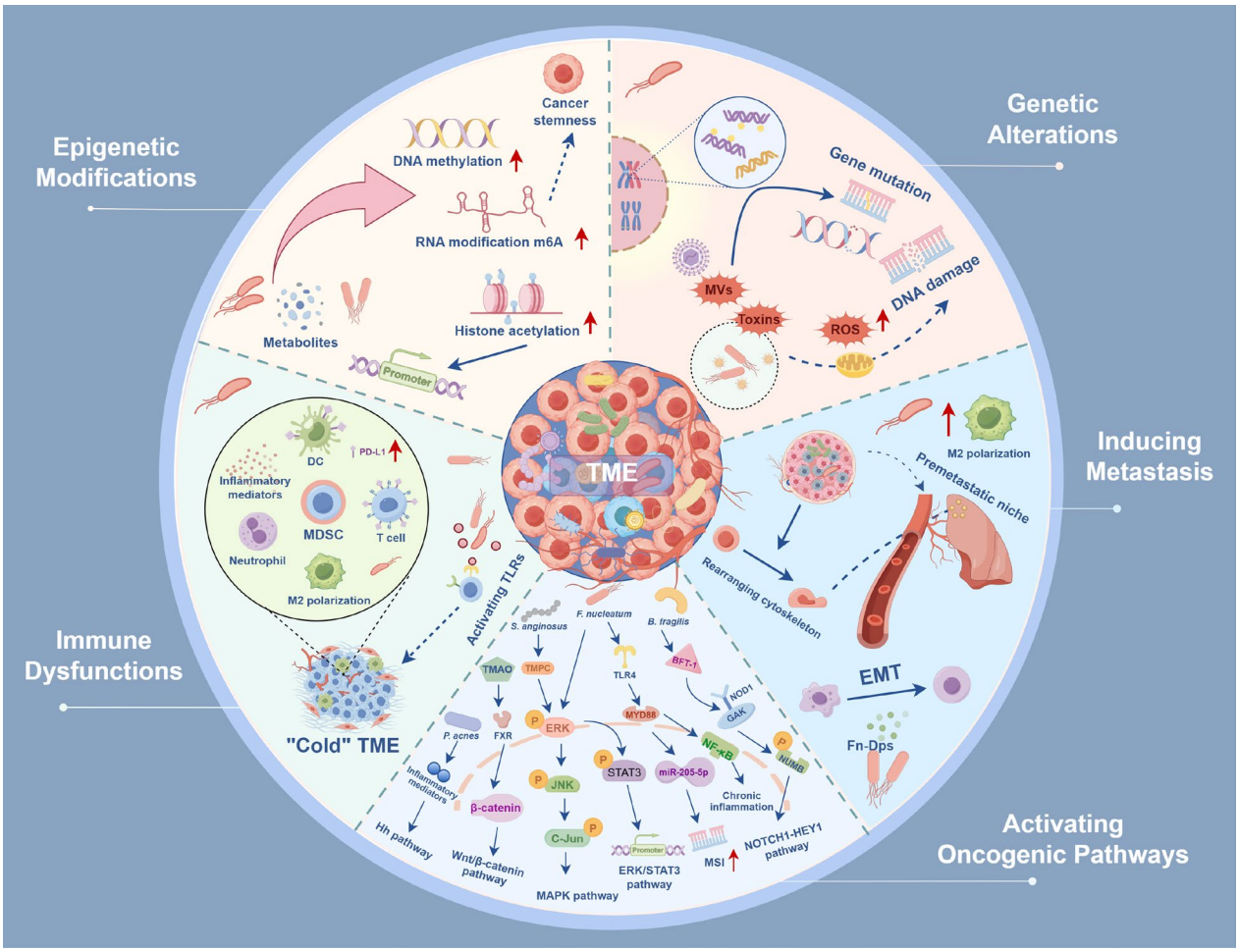

Fig. 3 Underlying mechanisms of intratumoral microbiota-mediated cancer progression. Five putative mechanisms have been proposed to illustrate how intratumor microbiota contribute to cancer formation and development, including genetic alterations, epigenetic modifications, immune dysfunctions, activating oncogenic pathways, and inducing metastasis. Abbreviations: TME, tumor microenvironment; MVs, membrane vesicles; ROS, reactive oxygen species; PD-L1, programmed cell death-ligand 1; DC, dendritic cell; MDSCs, myeloid-derived suppressor cells; TLR, Toll-like receptor; Hh pathway, Hedgehog pathway; TMAO, Trimethylamine-N-oxide; FXR, farnesoid X receptor; TMPC, surface protein of *Streptococcus anginosus*; MSI, microsatellite instability; BFT-1, toxic protein secreted by *Bacteroides fragilis*; EMT, epithelial-mesenchymal transition; Fn-Dps, *F. nucleatum*-DNA hunger/stationary phase protective proteins. (By Figdraw)

initiation and progression of hepatocellular carcinoma [44]. That is, *M. hyorhinis* infection could facilitate the degradation of mitochondrial fusion protein 1 mRNA through increasing the level of RNA modification m6A, thereby promoting mononuclear polyploidy and cancer stemness by enhancing mitochondrial fission. Notably, microbial metabolites often engage in the complex interactions between intratumor microbiota and epigenetic changes. Ma et al. demonstrated that intratumor-bacteria-derived butyrate could fuel lung cancer development by upregulating H19 expression in tumor cells through increasing histone H3 lysine 27 acetylation at the H19 promoter and potentiating M2 macrophage polarization [92]. Interestingly, further studies have pinpointed the beneficial roles of butyrate in preventing the pathogenesis of diverse cancer types by enhancing histone acetylation and activating antitumor immunity [93–95], implying the existence of tumor-specific roles for microbial metabolites in different cancers.

Immune dysfunctions

The immune-oncology-microbiome axis denotes immune-mediated interactions between microbiota and host anti-tumor responses [7], where intratumor microbes and their metabolites or by-products within the TME can exert either immunosuppressive or immune-promoting effects. In pancreatic ductal adenocarcinoma (PDAC), the microbiota contributed to a tolerogenic

immune microenvironment by activating Toll-like receptor (TLR) ligation in monocytic cells [96]. Strikingly, elimination of intratumor bacteria could reverse the immunosuppressive TME, lead to diminished myeloid-derived suppressor cells (MDSCs) and enhanced M1 macrophage differentiation, thereby fostering TH1 differentiation of CD4 + T cells and activating CD8 + T cells. Conversely, Kalaora et al. reported that intracellular bacteria-derived HLA-bound peptides could be presented by tumor cells and elicit immune reactivity in melanoma [97].

The tumor-associated neutrophils tend to confer a boosting effect on the microbiota-mediated tumorigenesis, but warrants further investigation. In a recent pan-cancer analysis of 4,160 metastatic tumor biopsy tissues, Battaglia et al. disclosed potential associations between microbial diversity and tumor-infiltrating neutrophils and macrophages, resulting in an immunosuppressive TME [98]. Similarly, the relationships between neutrophilic inflammation and the growth of tumor-promoting bacteria have been observed in vulvar squamous cell carcinoma [70]. Furthermore, Tan and colleagues found that the intratumor *Porphyromonas gingivalis* could facilitate pancreatic cancer progression through increasing the secretion of neutrophilic chemokines and neutrophil elastase [57]. However, neutrophils have been found to hinder tumor development by restricting the numbers of bacteria and tumor-associated inflammatory responses mediated by interleukin (IL) 17 in colon cancer [99].

To date, various studies have highlighted *F. nucleatum*, a periodontal pathogen, as an oncogenic bacterium capable of initiating tumorigenesis [100]. Within the TME of pancreatic cancer mouse model, intratumor *F. nucleatum* has been shown to inhibit the infiltration of CD8 + T cells by recruiting MDSCs [58]. The virulence protein Fn-Dps (*F. nucleatum*-DNA hunger/stationary phase protective proteins) plays an essential role in favoring the intracellular survival of *F. nucleatum* within macrophages through increasing the expression of chemokine CCL2/CCL7 [101]. Additionally, Li et al. revealed that intracellular infection with *F. nucleatum* could suppress the proliferation and cytokine secretion of T cells and promote programmed cell death-ligand 1 (PD-L1) expression in esophageal squamous cell carcinoma (ESCC) [54]. Likewise, *F. nucleatum* contributed to the progression of oral squamous cell carcinoma via potentiating tumor cell proliferation and inducing M2 macrophage polarization [102]. Another periodontitis pathogen, *Porphyromonas gingivalis*, also exhibited close associations with the phenotype of cancer immune cell [100]. Ren and colleagues found that *P. gingivalis* infection robustly promoted PD-L1 expression on DCs and suppressed antigen-specific CD8 + T cells [103]. Interestingly, the co-culture of *P. gingivalis* and *F. nucleatum* in oral keratinocytes markedly increased the expressions of pro-inflammatory mediators, such as IL-1β, IL-8, IL-6, and TNF-α, implying potential linkages with the pathogenesis of oral cancer [104].

In addition to bacteria, the intratumoral mycobiome and virome also play pivotal roles in cancer progression. Among them, Alam et al. demonstrated that intratumor fungi were capable of accelerating PDAC tumor growth by activating innate lymphoid cells 2 (ILC2) through IL-33, and antifungal treatment reduced the infiltration of T helper 2 and ILC2, thereby extending survival [105]. Moreover, *Aspergillus sydowii* has been shown to foster lung cancer development by inducing the expansion and activation of MDSCs through IL-1β secretion [37]. Viral infections can directly regulate certain immune cells [106]. In human soft tissue sarcomas, Perry et al. reported close associations between the intratumor viral microbiome and NK cell infiltration, with a higher abundance of intratumor *Respirovirus* observed in patients without metastases compared to those with metastases.

Activating oncogenic pathways

Activation of oncogenic signaling pathways is a prevalent mechanism underlying cancer formation and progression. Remarkably, intratumoral *F. nucleatum* can promote tumor growth through modulating multiple pathways. Zhu et al. revealed that in CRC tumor tissues, *F. nucleatum* upregulated the oncoprotein DHX15 by activating the ERK-STAT3 pathway [74]. Similarly, *F. nucleatum* infection activated the mitogen-activated protein kinase (MAPK) pathway, leading to increased expression of Matrix metalloproteinase 7 protein and enhanced CRC cell migration capacity [107]. Hsueh and colleagues demonstrated that *F. nucleatum* facilitated the proliferation of head and neck squamous cell carcinoma through inhibiting the expression of DNA mismatch repair-related genes, including MLH1, MSH2, and MSH6, via the TLR4/MYD88/miR-205-5p signaling pathway [108]. Moreover, the progression of epithelial ovarian cancer induced by *Propionibacterium acnes* was associated with an elevated inflammatory response that activated the hedgehog pathway [66]. Importantly, intratumor microorganisms can regulate oncogenic pathways through their metabolites or toxins. Most recently, Fu and colleagues identified *Streptococcus anginosus*, apart from *H. pylori*, as a pathogen that facilitated gastric tumorigenesis through its surface protein TMPC, leading to activation of the MAPK pathway [51]. Administration of the microbial metabolite trimethylamine-N-oxide could promote CRC tumor cell and stem cell proliferation by activating the Wnt/β-catenin pathway [109]. In addition, intratumor enterotoxigenic *Bacteroides fragilis* enhanced

the stemness and chemoresistance of breast cancer cell by secreting the toxic protein BFT-1, which activated the NOTCH1-HEY1 signaling pathway via directly bounding to NOD1 receptor [110].

In addition to the oncogenic pathways discussed above, several other signaling pathways involve interactions between intratumor microbiota and cancer development. For instance, intracellular bacteria residing in breast cancer suppressed the RhoA/ROCK signaling, thereby enhancing the survival of circulating tumor cells through cytoskeleton reorganization [31]. Kong and colleagues demonstrated the crucial role of the TLR4/Keap1/NRF2 pathway in which *F. nucleatum* regulates CRC metabolism to promote metastasis [111]. Additionally, cytokines such as IL-6 and TNF-α, stimulated by intratumor microbiota, could activate the NF-κB and STAT3 signaling and facilitate tumor proliferation [112]. Remarkably, activation of the stimulator of interferon genes (STING) pathway by intratumor microbes has been shown to confer beneficial roles in enhancing anti-tumor responses [113–115], such as promoting M1 macrophage polarization, as well as activating NK cells and dendritic cells, which presents a promising opportunity to improve the therapeutic outcomes of cancer patients.

Inducing metastasis

Tumor metastasis stands as a primary driver of the limited efficacy seen in cancer therapies, and emerging findings underscore the crucial involvement of intratumor microbiota in this metastatic process [116]. Detectable bacterial DNA within tumors has been noted in various cases of cancer metastasis [98]. Notably, Hilmi et al. reported that the diversity of intratumor microbiota correlated more closely with the biopsy site than with the primary cancer type [117]. Specifically, they observed lower bacterial richness in lymph node metastases compared to metastases in the lung and liver. Intratumor bacteria have the capacity to trigger the formation of a premetastatic niche in the metastatic organ, thereby facilitating the recruitment of metastatic cells [81]. Once entering the circulatory system, metastatic cancer cells can leverage intracellular bacteria to enhance their survival by rearranging the actin cytoskeleton [31]. Furthermore, the virulence proteins or metabolites produced by intracellular microbes play a critical role in the metastatic process. For instance, the virulence factor Fn-Dps from *F. nucleatum* can enhance its survival within macrophages and stimulate the migration of CRC cells through the epithelial-mesenchymal transition (EMT) induced by CCL2/CCL7 [101]. Additionally, low concentrations of butyrate produced by intratumor bacteria have been implicated in driving lung cancer metastasis by promoting H19 expression and enhancing M2 macrophage polarization [92].

Other relevant mechanisms

Exosomes, carrying diverse proteins, lipids, and RNAs, play a crucial role in intercellular communication and regulation [118]. Importantly, increasing evidence suggests intimate interactions between exosomes released by bacteria-infected tumor cells and cancer development (Fig. 4) [119]. Among them, *F. nucleatum* infection could upregulate the expression of microRNA-21 by activating TLR4/MYD88/NF-κB pathway, which indicated a higher risk of inferior outcomes in CRC patients [120]. Tang et al. reported that sustained *F. nucleatum*-induced expression of microRNA-31 enhanced the tumorigenicity of CRC cells by targeting eukaryotic initiation factor 4F-binding protein 1/2 [121]. In addition, a variety of researches have revealed the relationships between exosomes derived from bacteria-infected tumors, including miRNA and long non-coding RNA (lncRNA), and cancer metastasis [122–125], and these exosomes served as an important hinge joint for intratumor microbiota-mediated cancer progression. For instance, *F. nucleatum* has the ability to facilitate CRC metastasis by the miR-1322/CCL20 axis and M2 macrophage polarization [125]. In oral epithelial carcinomas, Zhang and colleagues demonstrated the capacity of *F. nucleatum* in triggering EMT through lncRNA MIR4435-2HG/miR-296-5p/Akt2/SNAI1 pathway [126].

Inducing resistance to anti-tumor drugs represents another critical mechanism for exosomes-induced tumor development. In a recent study, Hui et al. observed that exosomes derived from *F. nucleatum*-infected CRC cells transferred hsa_circ_0004085 between cells and imparting resistance to oxaliplatin/5-fluorouracil via relieving endoplasmic reticulum stress in recipient cells [127]. Likewise, Zeng and colleagues demonstrated that *F. nucleatum*-induced expression of miR-135b could inhibit KLF13 expression and promote cisplatin resistance in CRC [128]. While much of this research has focused on the interactions between *F. nucleatum* infection and CRC, these findings highlight the potential of exosomes as an intervention target for cancer patients, which deserves further exploration.

Metabolic dysregulation, such as heightened glucose metabolism, is a critical hallmark of cancer. Of note, *F. nucleatum* has been shown to promote CRC pathogenesis through enhancing glycolysis in tumor cells via the activation of lncRNA ENO1-IT1 transcription [129]. In oral squamous cell carcinoma, Sun et al. demonstrated that tumor-resident *F. nucleatum* could drive the formation of tumor-associated macrophages and a pro-tumorigenic microenvironment through regulating glycolysis and extracellular lactate deposition [130]. Apart from glucose metabolism, alterations in amino acid and nucleotide metabolism, inositol phosphate metabolism, and

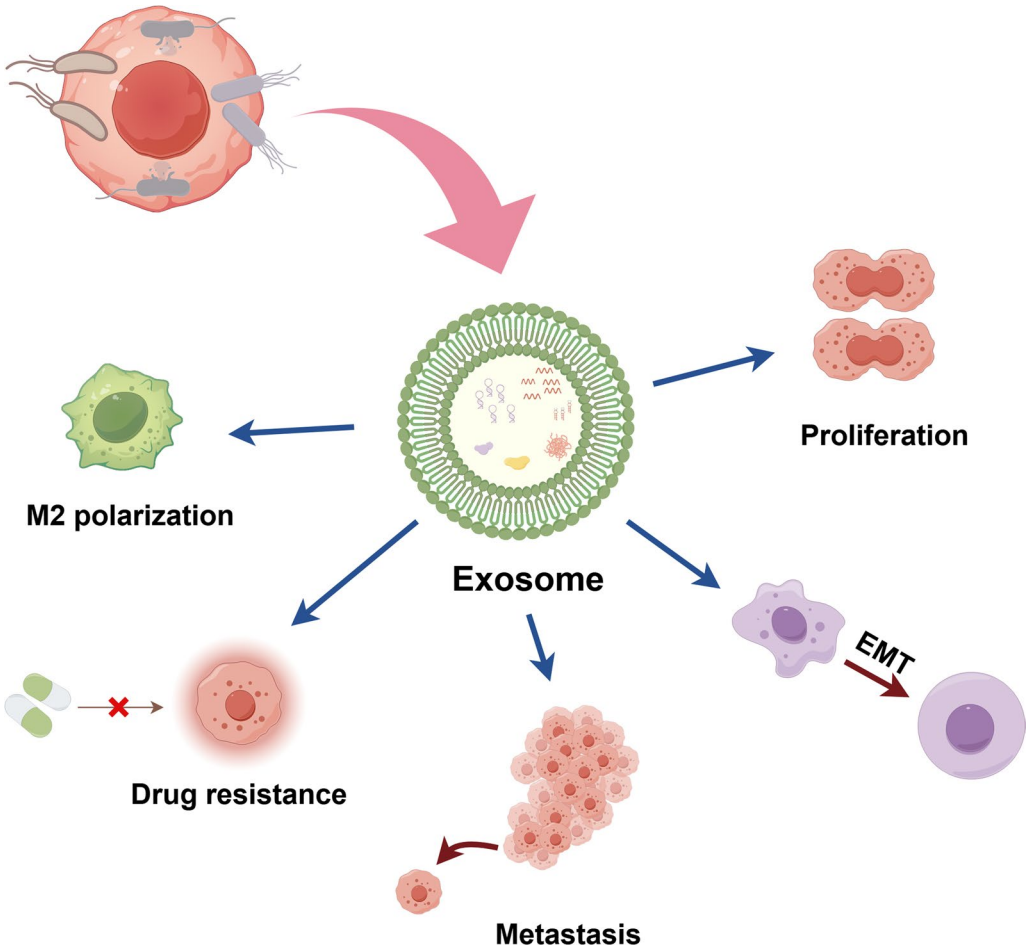

Fig. 4 Roles of exosomes derived from bacteria-infected tumor cells in cancer development. Exosomes released by microbiota-infected tumor cells can promote the progression of tumor by enhancing cell proliferation, EMT, metastasis, anti-tumor drug resistance, and M2 macrophage polarization. M2, M2-type macrophages; EMT, epithelial-mesenchymal transition

fatty acid biosynthesis have also been implicated in intratumor bacteria-mediated tumorigenesis [79].

Clinical implication and therapeutic potential of intratumor microbiota

Diagnostic and prognostic tools

The considerable heterogeneity within the intratumor microbiota, coupled with significant disparities in composition between normal and malignant tissue, emphasizes its potential as a target for cancer diagnosis and risk stratification. Nejman and co-authors elucidated the distinct microbial profiles across multiple cancer types [6]. Specifically, intratumor microbiota of CRC was dominated by *Firmicutes* and *Bacteroidetes*, and *Proteobacteria* was prevalent in pancreatic cancer, while extra-gastrointestinal tumors primarily featured the *Actinobacteria* phylum. Subsequently, Narunsky-Haziza et al. highlighted the diagnostic utility of cancer-type-specific fungal compositions in distinguishing diverse tumors from normal controls [26]. Among these, the most robust diagnostic performance was noted in breast cancer, with an area under receiver operating characteristic curve of 95% CI 81.40–93.53%.

Multiple studies have underscored the prognostic significance of intratumor microbiota across a spectrum of cancer types. Among these, intratumor infection by anaerobic bacteria, including *Bacteroides*, *Peptoniphilus*, and *Lactobacillus*, has been strongly linked to shorter survival time in PDAC [59]. Patients with a higher intratumor bacterial load, compared to those with low bacterial load, exhibited significantly inferior rates of overall survival (hazard ratio [HR], 3.41; 95% CI 1.90–6.11, $P<0.001$), disease-free survival (HR, 2.90; 95% CI 1.72–4.90; $P<0.001$), and distant metastasis-free survival (HR, 3.18; 95% CI 1.58–6.39; $P<0.001$) in nasopharyngeal carcinoma [61]. In addition, a higher abundance of intratumor *F. nucleatum* is frequently indicative of poor survival in various tumor types, such as esophageal

squamous cell carcinoma, cervical carcinoma, and CRC [131–133]. However, Neuzillet et al. noted that *F. nucleatum*-positive cases in oral squamous cell carcinoma exhibited a lower metastatic recurrence rate and longer overall survival when compared to *F. nucleatum*-negative tumors [60], implying the complexity to develop microbiota-based prognostic strategies specific to tumors.

Bracingly, intratumor microbiota-based cancer stratification models demonstrate exceptional capability in demarcating patients with particular clinic-molecular characteristics and prognosis. In an analysis of 423 patients with stage I to IV CRC, Mouradov et al. identified oncomicrobial community subtypes capable of stratifying CRCs into three distinct subgroups with unique features and outcomes, which supports the development of a framework for intratumor microbiota-based stratification of CRC [134]. Another classification system, termed hepatotype, developed through clustering analysis of microbial profiles in HCC, could serve as an independent biomarker for predicting prognosis after surgery [45]. Namely, patients with hepatotype A exhibited remarkably shorter overall survival and recurrence-free survival compared to those with hepatotype B. Furthermore, Zhang et al. constructed a virus-associated prognostic signature based on selected viral compositions in a pan-cancer level, which could classify patients into three prognostic groups, including good, intermediate, and poor survival outcomes [135].

Impacting the efficacy of cancer therapy

Chemo- and radiotherapy

Nowadays, radiotherapy and chemotherapy remain the cornerstone for cancer therapy, and ample evidence indicates the critical roles of intratumor microbiota in modulating the efficacy of chemo- and radiotherapy. According to a metagenomic analysis of biopsy tumoral tissues, five core bacteria, including *Streptococcus equinus*, *Blautia producta*, *Schaalia odontolytica*, *Pseudomonas azotoformans*, and *Clostridium hylemonae*, exhibited close interactions with the resistance of neoadjuvant chemoradiotherapy in locally advanced rectal cancer [136]. Moreover, Colbert et al. demonstrated that the intratumor *Lactobacillus iners* could trigger resistance of chemotherapy and radiation in cervical cancer cells through altering tumor metabolism and lactate signaling pathways [137].

Various intratumor "oncomicrobes" have been documented to influence the therapeutic outcomes of chemotherapy. The heightened abundance of *F. nucleatum* has been intimately linked to chemoresistance in CRC patients, possibly result from its role in preventing pyroptosis by the Hippo pathway [138]. Furthermore, Li et al. delineated how *F. nucleatum* contributed to oxaliplatin resistance in CRC by suppressing ferroptosis [139]. Interestingly, *F. nucleatum* levels may decrease with the administration of the chemotherapeutic agent 5-fluorouracil in CRC [140]. However, when 5-fluorouracil was metabolized by *Escherichia coli*, it no longer effectively curtailed cancer cell growth or the proliferation of *F. nucleatum*, highlighting the intricate relationships between intratumor microbes with chemoresistance. Intratumor *Desulfovibrio desulfuricans* has been found to exhibit increased colonization in CRC tissues among non-responders [141], and further in vivo experiments demonstrated that *Desulfovibrio*, along with its metabolite S-adenosylmethionine, could attenuate the effectiveness of the FOLFOX chemotherapy regimen by upregulating the expression of methyltransferase-like 3. In addition, Colibactin-producing *Escherichia coli* has been shown to induce chemotherapeutic resistance in CRC through facilitating EMT and cancer cell stemness [142].

Immunotherapy

Immunotherapy represents the cutting-edge in cancer treatment methods, such as ICB therapy and chimeric antigen receptor (CAR) T cell therapy. Remarkably, mounting evidence indicates the substantial influence of intratumor microbiota on regulating cancer immunotherapy response in recent years [143]. In a recent analysis of the intratumor microbiota in metastatic cancer, Battaglia et al. indicated the potential relationships between *Fusobacterium* and ICB resistance in lung cancer. Specifically, a higher abundance of *Fusobacterium*, after adjusting for genome-wide mutational burden, was significantly linked with reduced overall survival and progression-free survival in non-small cell lung cancer patients [98]. Moreover, differences in intratumoral microbial composition have been observed between responders and non-responders to ICB therapy in metastatic melanomas [6]. That is, responders were enriched in *Clostridium*, while *Gardnerella vaginalis* was more abundant in tumors of non-responders. Nevertheless, the accumulation of intratumor *Bifidobacterium* can convert the non-responders into responders to anti-CD47 immunotherapy by stimulating STING pathway in CRC- and lymphoma-bearing mouse models [114]. Similarly, Wu and colleagues demonstrated that the enrichment of *Streptococcus* in tumor tissues was conducive to a favorable outcome to anti-PD-1 treatment by inducing CD8 + T cell infiltration in an ESCC mouse model [55]. In addition, Bender et al. elucidated the interactions between intratumoral *Lactobacillus reuteri* and the TME in melanoma, mediated by the metabolite indole-3-aldehyde, using a preclinical mouse model, which simulated antitumor immunity and augmented the efficacy of ICB therapy by amplifying interferon-γ-producing CD8 + T cells [144].

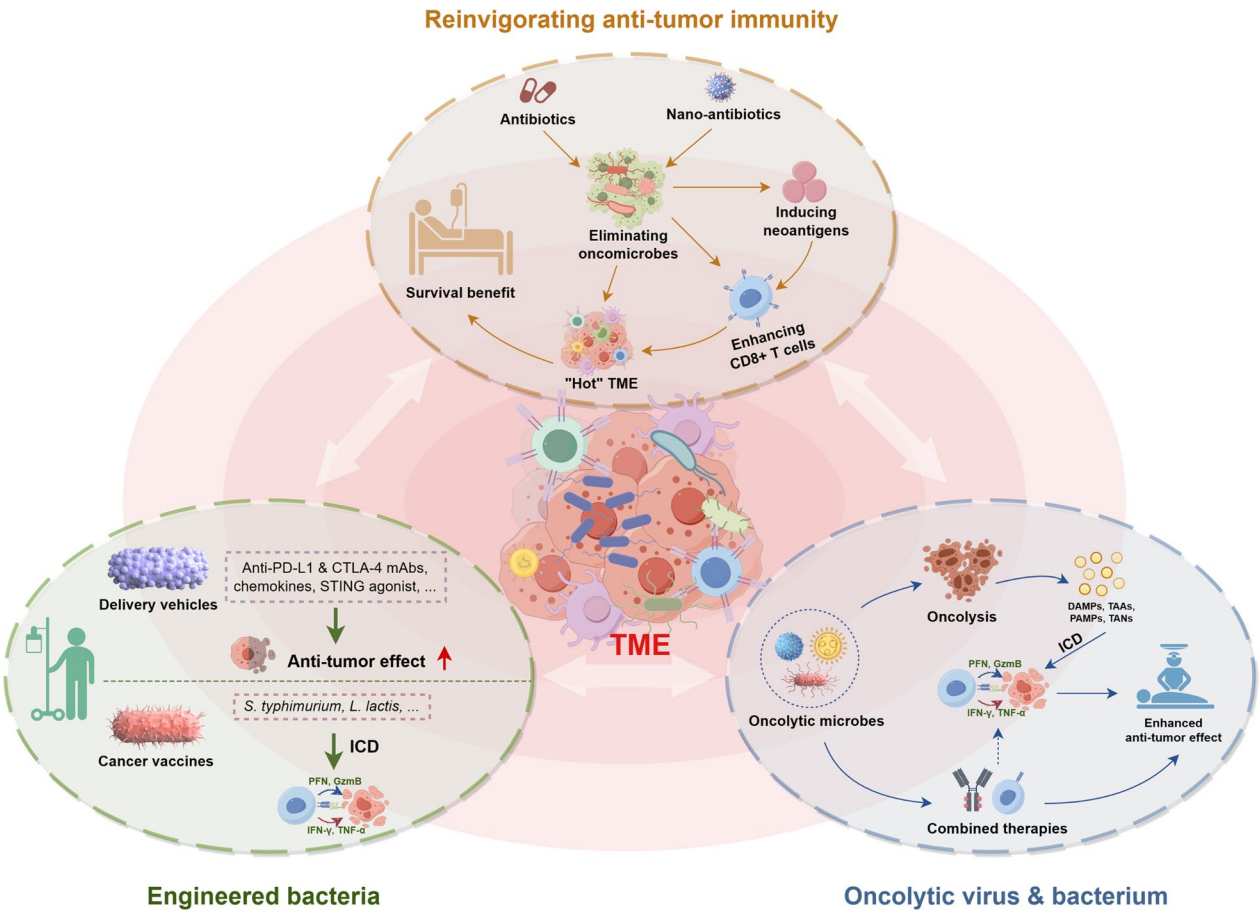

Fig. 5 Targets for intratumoral microbiota-based cancer therapeutic strategies. Modification of intratumor microbiota presents promising avenues for enhancing existing cancer therapies, such as modulating microbial composition by antibiotics or nanoparticle-coupled antibiotics to reinvigorate anti-tumor immunity, engineering bacteria to deliver therapeutic payloads directly to tumors or developing cancer vaccines to trigger immunogenic cell death, and utilizing oncolytic viruses or bacteria to directly eliminate tumor cells. PD-L1, programmed cell death-ligand 1; mAb, monoclonal antibody; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; STING, stimulating stimulator of interferon genes; ICD, immunogenic cell death; PFN, perforin; GzmB, Granzyme B; DAMPs, damage-associated molecular pattern molecules; TAAs, tumor-associated antigens; PAMPs, pathogen-associated molecular pattern molecules; TANs, tumor-associated neoantigens

Although CAR-T cell therapy stands as a monumental breakthrough in cancer treatment, demonstrating remarkable efficacy against multiple lymphohematopoietic malignancies, its application to solid tumors remains challenging due to limited effector cell infiltration into tumors and the highly immunosuppressive TME [145]. Encouragingly, emerging evidence suggests the potential to improve CAR-T efficacy through genetically engineered virus. Wang and colleagues revealed that adenovirus armed with the chemokine CXCL11 could significantly potentiate CAR-T effectiveness and establish a long-lasting anti-tumor response by reshaping the immunosuppressive TME in glioblastoma [146], including elevated infiltration of CD8 + T cells, NK cells, and M1-polarized macrophages, along with reduced frequencies of MDSCs, regulatory T cells, and M2-polarized macrophages.

Targets for cancer therapy

As a leading cause of mortality worldwide, cancer poses a significant global public health challenge. Thus, there is an urgent imperative to explore novel targets for cancer treatment [147]. Considering the profound influence of intratumor microbiota on cancer progression and treatment outcomes, harnessing intratumor microbiota as therapeutic targets would present innovative strategies to complement existing cancer treatments (Fig. 5), such as (1) modulating microbial composition to reinvigorate anti-tumor immunity; (2) engineering bacteria to deliver therapeutic payloads directly to tumors or convert the

immunosuppressive TME to an immunostimulatory one; and (3) exploiting oncolytic viruses or bacteria to directly eliminate tumor cells.

Reinvigorating anti-tumor immunity

The intratumor microbiota emerges as a promising target for revitalizing the anti-tumor immune response that is crucial for advancing cancer treatments. The elimination of intratumor bacterial by antibiotics could potentially reverse the immune tolerance within the TME in PDAC, thereby enhancing the efficacy of ICB therapy [96]. Nevertheless, given the potential influence of oral antibiotics on systemic microbial flora, such as the induction of gut microbial dysbiosis, which often correlates with dampened responses to immunotherapy [148], therefore, a more targeted bacteriolytic approach within the tumor is warranted. Indeed, several studies have demonstrated the effectiveness of nanoparticle-coupled antibiotics in eliminating tumor-promoting intratumor bacteria without disrupting the body's microbiota balance. For instance, Wang et al. devised a liposome-encapsulated antibiotic silver-tinidazole complex (LipoAgTNZ) to target intratumor bacteria in primary CRC tumors and liver metastases with the aim to prevent gut dysbiosis [149]. Significantly, LipoAgTNZ treatment contributed to the generation of microbial neoantigens, thereby potentiating anti-tumor CD8 + T cells and resulting in long-term survival in two *F. nucleatum*-infected CRC models. Additionally, Gao et al. reported that dual-targeting nanoparticles containing metronidazole and fluorouridine accumulated within the TME and released these drugs, achieving a synergistic anti-tumor effect by attacking intratumor bacteria and cancer cells, respectively [150]. This finding underscores the feasibility of "kill two birds with one stone" by targeting intratumor microbes in cancer therapy.

Peptides derived from intracellular bacteria has been observed to be immunogenic and can be presented by the human leukocyte antigen class (HLA)-I and HLA-II molecules on tumor cells to elicit anti-tumor immune reactivity in melanoma [97], highlighting the theoretical foundation for intratumor microbiota-mediated anti-tumor immune activation. Bacterial outer membrane vesicles (OMVs) serve as potent immune adjuvants, capable of transforming the immunosuppressive TME into an immunologically active one. Caproni et al. demonstrated that intratumor injection of OMVs derived from the *E. coli* BL21(DE3)Δ60 strain could induce robust anti-tumor activity, accompanied by rapid infiltrations of DCs and NK cells in a tumor-bearing mouse model [151]. In addition, intratumoral vaccination can trigger significant anti-tumor immunity. Peng and colleagues indicated that intratumoral *Tissue Antigen-Cervical Intraepithelial Neoplasia* vaccination arouse a stronger antigen-specific CD8 + T cell responses and anti-tumor effects when combined with anti-PD-1 blockade therapy in a preclinical HPV model [152].

Engineered bacteria

Bacteria can be genetically engineered to function as therapeutic delivery vehicles in cancer therapy [153]. Intercellular self-replication and translocation represent the primary mechanisms facilitating bacterial penetration into tumors [154]. Gurbatri et al. developed a probiotic bacteria system to meticulously produce and release nanobodies targeting PD-L1 and cytotoxic T lymphocyte-associated protein-4 within tumors [155]. They observed a significantly amplified anti-tumor effect with this probiotic *E. coli* Nissle 1917 (EcN) system, leading to superior tumor eradication and survival benefits compared to analogous clinically relevant antibodies. Moreover, Savage and colleagues engineered bacteria to secrete chemokines, including CXCL16 and CCL20, directly into tumors [156], which could recruit and activate innate (DCs) and adaptive (CD8 + T cells) anti-tumor immune responses, thus providing a novel approach to potentiate cancer immunotherapy. It is worth mentioning that in a phase I clinical trial involving advanced cancer patients, Luke et al. investigated the safety and efficacy of intratumoral injection of engineered EcN expressing a STING agonist, with and without ICB therapy [157]. The researchers observed the well-tolerated and effective nature of this probiotic EcN treatment, with no severe therapy-related adverse events or infections reported.

Notably, beyond serving as a platform for the intratumoral delivery of cancer therapeutics, engineered bacteria also possess the capability to activate the anti-tumor response within the immunologically "cold" TME. Zhou and colleagues demonstrated the activation of CD8 + T cells in the TME of melanoma following the treatment with genetically modified *Salmonella typhimurium*, which induced targeted methionine deprivation in tumor tissues [158]. Additionally, these encoded bacteria could be employed as in situ cancer vaccines with minimal systemic exposure and adverse effects. Among them, calcium carbonate biomineralized *Salmonella* has been shown to function as a cancer vaccine producer, inducing immunogenic cell death (ICD) in cancer cells and promoting the formation of gap junctions between tumor cells and DCs to enhance antigen presentation [159]. On the other hand, the internalization of Ca^{2+} into various immune cells could synergize with *Salmonella* to systematically regulate the immune system, including DCs maturation, macrophages polarization, and T cells activation. Likewise, Zhu et al. displayed that intratumoral delivery of a probiotic, food-grade *Lactococcus lactis*-based in situ vaccination (FOLactis), contributed

to sustained activation of NK cells, cytotoxic T cells, and conventional-type-1-dendritic cells within tumors and tumor-draining lymph nodes by secreting a fusion protein of Fms-like tyrosine kinase 3 ligand and co-stimulator OX40 ligand in a murine colon cancer model [160]. Further, FOLactis established long-term immunological memory and protection lasting over 60 days, significantly prolonged the survival of tumor-bearing mice in combination with anti-PD-1 therapy.

Oncolytic virus and bacteria

Oncolytic viruses (OVs) refer to native viral species or genetically engineered viruses that selectively infect and preferentially lyse tumor cells while sparing non-neoplastic cells [23], making them appealing candidates for precise cancer treatment. Additionally, exposure to OVs can trigger ICD by prompting the release of damage-associated molecular pattern molecules, pathogen-associated molecular pattern molecules, tumor-associated antigens, and tumor-associated neoantigens, leading to the amplification of innate immunity and adaptive antitumor response [161]. So far, a host of preclinical, clinical trials, and real-world clinical studies have highlighted the promise of oncolytic virotherapy. Four commercial OVs, including H101, T-VEC, ECHO-7, and Teserpaturev, have been approved for cancer therapy globally, as extensively reviewed [23, 161–163]. Notably, the combination of OVs with immunotherapeutic strategies, such as ICB and adoptive cellular therapy, has yielded compelling results and manageable safety profiles in tumor intervention in recent years (Table 3). Most recently, Wang et al. evaluated the efficacy of combining an oncolytic vaccinia virus encoding hyaluronidase with diverse cancer therapies, including gemcitabine, doxorubicin, liraglutide, anti-PD-1 and anti-CD47 monoclonal antibodies, or CAR-T cells [164]. The authors found that OVs remarkably improved the therapeutic outcomes of existing cancer therapeutics by degrading hyaluronic acid in the TME, highlighting the alluring foreground of OVs in potentiating the efficacy of cancer treatments. Despite this, several concerns regarding oncolytic adenovirus should be acknowledged, such as the potential safety thereat if "off-target" and dissemination throughout the body, in vivo pre-existing neutralizing antibodies to restrain OVs, and poor targeting delivery efficacy to reach tumor tissues [162, 165].

In addition, particular bacteria have demonstrated oncolytic effects in recent years. Among these, *Clostridium ghonii* has shown oncolytic abilities both in *in-vitro* and in a mouse model of lung cancer by facilitating the apoptosis and necrosis in tumor cells [197]. Notably, Goto et al. were the first to document the safety and tumor-suppressing effects of administering tumor-isolated oncolytic bacteria via intravenous injection in various preclinical models, including CRC, sarcoma, metastatic lung cancer, and drug-resistant breast cancer [198]. Further, the tumor-derived *Cutibacterium acnes* has proven to be an effective anti-tumor "living drug" with oncolytic potential in a mouse model of CRC [199]. Importantly, in the pioneering phase I clinical trial investigating bacteriolytic therapy utilizing attenuated *Clostridium novyi*-NT (non-toxic) for refractory solid tumors [200], Janku and colleagues observed that a sole intratumoral injection of *C. novyi*-NT led to bacterial spore germination, resulting in tumor masse lysis in 42% of patients with manageable toxicities. Despite these findings hint at the potential feasibility of utilizing oncolytic bacteria in cancer therapy, further research is warranted to verify their safety, efficacy, and underling mechanisms of action.

Prospect and challenges

The burgeoning recognition of the roles played by intratumor microbiota in tumorigenesis presents both promising prospects and formidable challenges in cancer management. On one front, the nuanced understanding of the intricate interactions between microbial communities and the processes driving tumor initiation and progression offers novel avenues for innovative therapeutic intervention strategies. Targeting specific cancer-promoting or immunosuppressive oncomicrobes inhabiting tumors holds the potential to disrupt tumorigenic pathways and augment the efficacy of existing cancer treatments. Furthermore, leveraging the tumor microbiome as a discerning biomarker for cancer diagnosis and prognostication exhibits considerable promise for advancing personalized medicine paradigms. Lastly, exploiting microbiota-mediated cancer therapy involves harnessing engineered microorganisms as "living drugs" to rejuvenate anti-tumor immunity, deliver therapeutic payloads, or directly kill cancer cells.

Nevertheless, several challenges must be addressed to fully utilize the potential of intratumor microbiota in cancer management. Foremost among these obstacles is the highly heterogeneity within and between tumor types but low biomass characterizing tumor microbiome, which impede precise characterization and establishment of universal therapeutic approaches. Meanwhile, it is essential to refine and standardize methodologies for profiling intratumor microbial communities while eliminating host genome and environmental contamination, which is imperative for reproducibility and comparability of intratumor microbiota studies. In a recent quantitative microbiome profiling analysis of CRC, Tito et al. did not observe significant associations between the well-established microbial marker *F. nucleatum* and CRC diagnostic groups, including healthy individuals,

Table 3 Summary of oncolytic virotherapy in combination with immunotherapy in recent three years

Synergistic therapies	Combined drugs	OVs and delivery route	Cancer types	Main findings
ICB therapy	Anti-PD-L1 mAb	Intratumoral OVV-MnSOD injection	Lymphoma	Increased proportion of CR rate and prolonged survival time in lymphoma-bearing mouse model [166]
		Intratumoral injection of oncolytic adenovirus OBP-702	Pancreatic cancer	Enhanced anti-tumor efficacy against the gemcitabine-resistant tumors by suppressing myeloid-derived suppressor cells accumulation in preclinical model [167]
		Intratumoral oncolytic Zika virus injection	Glioblastoma	Sensitizing tumors to anti-PD-L1 therapy and obtaining twofold increase in the survival of orthotopic xenografts [168]
		Intratumoral injection of oncolytic JX-594	Soft-tissue sarcomas	Observing a safety profile, but without significant clinical benefit in a phase 2 clinical trial [169]
	Anti-PD-1 mAb	Intratumoral injection of oncolytic measles virus	Glioblastoma	Elevated cure rates in diverse tumor-bearing models, and potentiated activation of CD8 + T cells [170]
		Intratumoral oncolytic adenovirus injection	CRC	Enhancing the anti-tumor efficacy of anti-PD-1 by inhibiting the tumor growth in a tumor-bearing mouse model [171]
		Intratumoral herpes-simplex-virus thymidine-kinase injection	TNBC	Obtaining 21.4% of clinical benefit rate and 6.6 months' median OS with tolerated toxicities in metastatic TNBC patients [172]
		Intravenous injection of Coxsackievirus A21	Multiple solid tumor types[a]	Observing a manageable safety profile, but without significant clinical benefit in a phase 1 trial [173]
		Intravenous adenovirus enadenotucirev administration	CRC and squamous cell carcinoma of the head and neck	Observing16.0 months' median OS and 1.6 months' median progression-free survival with manageable toxicities in a phase I trial of advanced/metastatic epithelial cancer [174]
		Intratumoral oncolytic DNX-2401 injection	Glioblastoma	Obtaining of 56.2% of clinical benefit rate and 12.5 months' median OS without dose-limiting toxicities in a phase 1/2 clinical trial [175]
		Intratumoral oncolytic virus V937 injection	Melanoma	Obtaining 47% of ORR, including 22% of CR rate without dose-limiting toxicities in a phase 1b single-arm trial of advanced melanoma patients [176]
		Intratumoral injection of oncolytic Pseudorabies virus live attenuated vaccine	Kidney cancer	Synergistically enhanced efficacy with complete clearance of mouse tumors and prolonged survival period in tumor-bearing mouse model [177]
		Systemically delivery of OVVs through peritoneum	Renal cell carcinoma	Effectively suppressed primary tumors and the metastatic burden with reduced hepatic injuries in tumor-bearing model [178]
		Intratumoral injection of oncolytic influenza virus	Hepatocellular carcinoma	Potentiated anti-tumor activity against untreated distant tumors and extended survival in preclinical model [179]

Table 3 (continued)

Synergistic therapies	Combined drugs	OVs and delivery route	Cancer types	Main findings
	Anti-CTLA-4 mAb	Intratumoral oncolytic virus V937 injection	Melanoma	Obtaining 30% of objective response rate (ORR) and 45.1 months' median overall survival (OS) in advanced melanoma patients [180]
		Intratumoral injection of T-VEC	Melanoma	Significantly improved ORR and duration of response; Prolonged duration of response and 5-year OS in a multicenter, randomized phase II trial of advanced melanoma [181]
		Intravenous delivery of coxsackievirus A21	Uveal melanoma	Observing a manageable safety profile, but without meaningful clinical benefit in a phase 1b trial [182]
	Anti-PD-1 and anti-LAG-3 mAbs	Intratumoral or intraperitoneal OVV-scFv-TIGIT injections	Breast cancer, colon cancer, and hepatocellular carcinoma	Potentiated antitumor efficacy and increased CR rate in multiple tumor-bearing mouse models [183]
	Anti-CTLA-4 or anti-TIM-3 mAbs	Intratumoral oncolytic herpes simplex virus injection	Hepatocellular carcinoma	Improved therapeutic efficacy by elevating tumor immunogenicity and potentiating anti-tumor adaptive immune responses in various mouse models [184]
	Anti-PD-L1 or anti- CTLA-4 mAbs	Intravenous injection of pexastimogene devacirepvec	CRC	Observing the safety and tolerance profiles in a phase I/II study of refractory metastatic CRC [185]
Adoptive cellular therapy	CAR-T cells	Intratumoral oncolytic adenovirus injection	Pancreatic cancer	Achieving consistent and sustained eradication of all tumors in xenograft mouse model, and increased CR rate in humanized mouse model [186]
		Intratumoral injection of interleukin-7-loaded oncolytic adenovirus	Glioblastoma	Prolonged survival and reduced tumor burden with enhanced proliferation and persistence of tumor-infiltrating CAR-T cells in tumor-bearing xenograft mice [187]
		Intratumoral injection of oncolytic adenovirus encoding CD40L and 4-1BBL	Lymphoma	Boosting CAR T-cell function with increased release of IFN-γ and granzyme B, CD107a expression in preclinical models [188]
		Intratumoral injection of oncolytic herpes virus G47Δ	Glioblastoma	Significant inhibition of tumor growth and survival benefit in a xenograft mouse model [189]
		Intratumoral oncolytic vesicular stomatitis virus injection	Melanoma and glioma	Potentiated CAR-directed anti-tumor function, and extended survival of tumor-bearing mouse models [190]
		Intravenous delivery of tumor-specific immunogene virus NG-347 encoding IFNα, MIP1α and CD80	Lung cancer	Synergizing with CAR T cells to suppress human tumor xenografts and their pulmonary metastases by remodeling the immunosuppressive TME [191]
		Intratumoral injection of oncolytic herpes simplex virus-1	Glioblastoma	Enhanced tumor degradation with elevated proportion of T cells and NK cells, but reduced regulatory T cells and transformed growth factor-β1 in orthotopic tumor-bearing model [192]

Table 3 (continued)

Synergistic therapies	Combined drugs	OVs and delivery route	Cancer types	Main findings
		Intratumoral injection of oncolytic adenovirus carrying chemokine ligand 5 and cytokine interleukin-12	Renal cell carcinoma	Enhanced CAR-T cells infiltration, improved survival, and restrained tumor growth in xenografted tumor-bearing model [193]
		Intratumoral injection oncolytic virus CF33	Pancreatic cancer	Obtaining markedly tumor regression with enhanced T-cell activation and synergistic cell killing in preclinical model [194]
	CAR-NK cells	Intratumoral oncolytic herpes simplex 1 injection	Glioblastoma	Extended survival of tumor-bearing mice with activation of NK and CD8 + T cells, and increased persistence of CAR-NK cells [195]
		Intratumoral or intravenous injection of adeno-associated viral vector	Glioblastoma	Obtaining significant tumor control and senior survival outcomes in the GL261-HER2 mouse model [196]

OVs, oncolytic viruses; T-VEC, Talimogene laherparepvec; TNBC, Triple-negative breast cancer; CR, complete remission; ICB, immune checkpoint blockade; PD-1, programmed cell death 1; mAb, monoclonal antibody; OVV, oncolytic vaccinia virus; MnSOD, manganese superoxide dismutase; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CRC, colorectal cancer; CAR-T, chimeric antigen receptor T cell; LAG-3, lymphocyte-activation gene 3; PD-L1, anti-PD ligand 1; TME, tumor microenvironment

[a] including non-small cell lung cancer, urothelial cancer, prostate cancer, and melanoma

adenoma patients, and those diagnosed with carcinoma, when controlling for several covariates [201], highlighting the challenges and uncertainties inherent in delineating appropriate controls for identifying biomarkers in cancer microbiome research. In addition, deciphering how host factors, including genetics, immune status, and environmental exposures, regulate microbial colonization and activity within tumors necessitates interdisciplinary collaborations and innovative research approaches. Importantly, untangling the causal relationships between specific microbial taxa and tumorigenesis warrants comprehensive longitudinal investigations spanning diverse cancer types and patient cohorts.

Furthermore, the development of cancer therapeutic strategies targeting intratumor microbiota requires meticulous attention to safety and efficacy considerations. Apart from potential off-target effects, manipulating microbial populations within tumors may inadvertently disrupt the symbiotic balance of commensal microbiota in the host, potentially precipitating adverse effects or engendering treatment resistance. Thus, refining precision medicine approaches that selectively target carcinogenic microbiota populations without altering the composition of beneficial microbial communities assumes paramount importance for the success of microbiota-based cancer therapies.

In summary, while the exploration of intratumor microbiota signifies a promisingly revitalized frontier in cancer research, navigating the complexities and uncertainties inherent in this field demands a concerted effort from researchers, clinicians, and policymakers alike. By embracing interdisciplinary collaborations, advancing technological innovations, and upholding ethical standards, we can unlock the full potential of intratumor microbiota as pivotal determinants of cancer pathogenesis and therapeutic response, ultimately improving outcomes for cancer patients.

Abbreviations

TME	Tumor microenvironment
EBV	*Epstein-Barr* Virus
HBV	*Hepatitis B* Virus
HCV	*Hepatitis C* Virus
HPV	*human papillomavirus*
HTLV	*human T cell lymphotropic virus type 1*
HCC	Hepatocellular carcinoma
T-VEC	Talimogene laherparepvec
16S rRNA	16S ribosomal RNA
FISH	Fluorescence in situ hybridization
NAT	Normal adjacent tissue
CRC	Colorectal cancer
KRAS	Kirsten rat sarcoma viral oncogene homolog
MMRd	Mismatch repair deficiency
Fna	*F. nucleatum* Subspecies *animalis*
NPC	Nasopharyngeal carcinoma
ICB	Immune checkpoint blockade
DC	Dendritic cell
MVs	Membrane vesicles
ROS	Reactive oxygen species
PDAC	Pancreatic ductal adenocarcinoma
TLR	Toll-like receptor
MDSCs	Myeloid-derived suppressor cells
IL	Interleukin
PD-L1	Programmed cell death-ligand 1
ESCC	Esophageal squamous cell carcinoma
ILC2	Innate lymphoid cells 2
MAPK	Mitogen-activated protein kinase
STING	Stimulating stimulator of interferon genes
EMT	Epithelial–mesenchymal transition
lncRNA	Long non-coding RNA
HR	Hazard ratio
CAR	Chimeric antigen receptor
LipoAgTNZ	Liposome-encapsulated antibiotic silver-tinidazole complex
HLA	Human leukocyte antigen class
OMVs	Outer membrane vesicles
EcN	*E. coli* Nissle 1917
ICD	Immunogenic cell death
FOLactis	Food-grade *Lactococcus lactis*-based in situ vaccination
OVs	Oncolytic viruses

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MZ, ZL and ZS conceived the study. ZS and ZL collected the data. ZS and ZL drafted the manuscript and prepared the figures. MZ and ZS revised the manuscript.

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