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REVIEW

Tumor-resident bacteria in gastrointestinal cancers: from regulatory mechanisms to clinical implications

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ABSTRACT The human microbiome is increasingly recognized as a key contributor to the tumor microenvironment (TME). Advances in sequencing technologies have revealed the pivotal role of intratumoral microbiota in the development of gastrointestinal cancers. This review summarizes current knowledge on the characteristics and functional mechanisms of tumor-resident bacteria in colorectal, gastric, and hepatocellular carcinoma. We describe how these microbes affect carcinogenesis and disease progression through multiple pathways, including interactions between the microbiota and the host gut barrier, tumor cells, anti-tumor immunity, and other non-cellular components of the TME. In addition, the translational potential of intratumoral microbiota as diagnostic and prognostic biomarkers, as well as the influence on therapeutic responses, is discussed. A growing understanding of tumor-microbe interactions not only deepens insight into cancer biology but also opens new directions for innovative diagnostic and therapeutic strategies, highlighting the potential of targeting the intratumoral microbiome to improve patient outcomes in gastrointestinal oncology.

KEYWORDS Intratumoral microbiota; gastrointestinal cancers; tumor microenvironment; microbiome-host interactions

Introduction

The human gut microbiome is comprised of communities of bacteria, fungi, viruses, archaea, and protozoa, together with their metabolites. These microorganisms inhabit different anatomic regions of the gastrointestinal tract and have co-evolved with the host for tens of millions of years, playing an essential role in maintaining human health. Dysbiosis of the gut microbiome contributes to the complex mechanisms underlying tumorigenesis in gastrointestinal cancers.

Although bacteria were observed in tumor tissues >100 years ago, tumors were long considered sterile. Owing to the extremely low microbial burden in tumors and the lack of sensitive detection methods to exclude contamination, the presence of intratumoral bacteria was not widely acknowledged or studied. Over the past decade, progress in next-generation sequencing (NGS) technologies, including 16S rRNA amplicon and metagenomic shotgun sequencing, has provided new insights into intratumoral microbial communities. Using 16S rDNA PCR analysis of tissues from seven human cancer types (bone, brain, breast, lung, melanoma, ovary, and pancreas), Nejman et al.[1] reported in 2020 that most tumors and adjacent normal tissues harbor diverse bacteria that are primarily localized within cancer and immune cells. Since then, intratumoral bacteria have become a major focus of research with the potential to reshape current concepts of tumor biology.

This article reviews the characteristics of intratumoral microorganisms in major gastrointestinal cancers, particularly colorectal cancer (CRC), gastric cancer (GC), and hepatocellular carcinoma (HCC), together with the mechanisms of action and clinical relevance. Although many other microorganisms are present, this review mainly focuses on intratumoral bacteria in gastrointestinal tumors. The aim of this work is to provide a reference for investigating intratumoral microorganisms as potential biomarkers for the diagnosis, treatment, and prognosis of gastrointestinal cancers.

The characteristics of intratumoral microbiota in gastrointestinal cancers

With continued advances in microbial sequencing approaches, an increasing number of studies have demonstrated close

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associations between tumor-resident microbiota and gastrointestinal cancer carcinogenesis. Here, recently reported features of intratumoral microorganisms in CRC, GC, and HCC are outlined, including the composition and diversity. **Table 1** summarizes published reports on intratumoral microorganisms in gastrointestinal tumors.

CRC

Overall, the tumor-associated microbiota in CRC is characterized by reduced diversity and an altered community structure. Most sequences (>95%) detected in CRC tissues and adjacent normal mucosa belong to *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Fusobacteria*[2]. Dysbiosis in CRC is reflected by depletion of commensal bacteria, such as *Bifidobacterium* and *Roseburia*, along with enrichment of pro-oncogenic taxa (e.g., *Fusobacterium*, *Bacteroides*, and *Campylobacter*) and bacteria of oral origin[3]. Liu et al.[4] reported that the abundance of several CRC-associated pathobionts (e.g., *Fusobacterium*, *Bacteroides*, *Parvimonas*, and *Prevotella*) vary markedly within a single tumor, suggesting substantial intratumoral microbial heterogeneity. A whole-transcriptome analysis of 807 CRC samples further showed that associations between bacteria and tumor gene expression are context- and species-dependent[5]. When comparing young-onset CRC (yoCRC) with average-onset CRC (aoCRC), *Akkermansia* and *Bacteroides* were enriched in yoCRC tumors, which also displayed significantly higher microbial alpha diversity and distinct beta diversity[6].

Fusobacterium nucleatum is regarded as a potential risk factor for CRC progression. The levels of *F. nucleatum* in tumor tissues and fecal samples from CRC patients are significantly elevated compared to normal controls[18]. In parallel, a higher abundance of *F. nucleatum* in CRC is associated with a shorter survival time[19]. Recent genomic analyses have revealed that the CRC tumor niche is predominantly dominated by the *F. nucleatum* subspecies *animalis* (*Fna*) C2 clade, rather than *Fna* C1, within the *Fna*. Enrichment of this specific lineage appears to directly drive the increased *F. nucleatum* levels observed in CRC[7].

GC

GC involves multiple risk factors, including *Helicobacter pylori (H. pylori)* infection, diet, cigarette smoking, and inherited and non-inherited genetic factors[20]. The commensal microbiota, mainly composed of *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, and *Fusobacteria*, have an important role in maintaining gastric homeostasis[21]. Niche-specific interactions develop during gastric carcinogenesis with marked enrichment of oral microbes, such as *Peptostreptococcus stomatis*, *Streptococcus anginosus*, *Parvimonas micra*, *Slackia exigua*, and *Dialister pneumosintes*, which become progressively more prominent as disease advances[8].

There is currently no consensus on the relationship between microbial diversity and gastric tumor mucosal tissues. Liu et al.[9] reported reduced diversity and richness in peritumoral and tumoral tissues compared to non-tumor tissues in a cohort of 276 GC patients. *S. anginosus* was similarly increased, whereas *H. pylori*, *Prevotella copri*, and *Bacteroides uniformis* were significantly decreased in GC tumor tissues. In contrast, other studies have reported increased bacterial richness and diversity in GC tissues without a parallel increase in community uniformity. Wang et al.[10] recently identified increased α-diversity as a characteristic feature of GC-associated microbiota. The abundance of the *Helicobacter* genus was reduced, while *Streptococcus*, *Peptostreptococcus*, *Fusobacterium*, and *Clostridium* were significantly enriched in GC samples.

HCC

Owing to the close anatomic and functional connections between the intestine and the liver, a leading hypothesis regarding the origin of tumor-associated microbes proposes that damage to the intestinal barrier, together with increased gut permeability, allows gut microbes to translocate through the portal vein to the liver, thereby contributing to the development of liver cancer[22]. In addition to the gut, intratumoral bacteria may also originate from adjacent tissues or be transported through the circulatory system.

Findings related to changes in intratumoral microbiota diversity remain inconsistent in HCC. Several studies have reported higher alpha diversity in the microbiota of HCC tumors compared to peri-tumor tissues[11,12]. Specific bacterial genera, including *Firmicutes*, *Streptococcus*, *Fusobacteriota*, and *Lactobacillus*, are enriched within tumors. In contrast, other studies have documented reduced microbial diversity in tumor tissues. Li et al.[13] analyzed 29 pairs of Hepatitis B virus (HBV)-related HCC tumors and adjacent normal tissues and noted lower alpha diversity in the tumor microenvironment (TME). Li et al.[13] further revealed a clear shift in bacterial composition, characterized by increased abundance of *Methylobacterium* spp. and a concomitant decrease in *Klebsiella variicola*. In addition, several studies have reported no significant differences in microbial diversity between tumor tissues and adjacent normal tissues[14-16]. These discrepancies are likely related to differences in HCC etiology [e.g., hepatitis virus infection, alcohol intake, and non-alcoholic fatty liver disease (NAFLD)], as well as dietary patterns, underscoring the need for more detailed investigations to clarify underlying trends.

Lu et al.[17] performed an in-depth analysis of intratumoral microbiota in multifocal HCC and identified marked differences in microbial characteristics between intrahepatic metastasis (IM)–HCC and multicentric occurrence (MO)–HCC. Lu et al.[17] identified specific bacteria, including *Enterococcus faecalis* and *S. anginosus*, which were associated with HCC metastasis.

Table 1 Characterization of the intratumoral microbiota in gastrointestinal cancers

Tumor	Region/Race	Cohort size	Detection method	Associated microbiota	Ref.
CRC	Australia	423 tumor and 339 adjacent normal mucosa samples from patients with stage I–IV CRC	16S rRNA gene sequencing	OCS1 (*Fusobacterium/oral pathogens*), OCS2 (*Firmicutes/Bacteroidetes*), OCS3 (*Escherichia/Pseudescherichia/Shigella*)	2
CRC	United States, Malaysia	MAL1 cohort: 54 samples MAL2 cohort: 46 samples	16S rRNA gene sequencing	*Bacteroides fragilis*, *F. nucleatum*, *P. micra*, *P. stomatis*	3
CRC	China	436 tissue biopsies from patients with CRC (*n* = 36) or adenoma (*n* = 32)	16S rRNA gene sequencing	*Fusobacterium*, *Bacteroides*, *Parvimonas*, *Prevotella*	4
CRC	33 regions	807 tumor tissues from patients with CRC	Whole-transcriptome RNA sequencing	*Fusobacterium animalis F. polymorphum*, *F. vincentii*, *F. nucleatum*	5
CRC	United States	Tumor and paired adjacent non-malignant fresh frozen tissue specimens prospectively collected from 136 yoCRC and 140 aoCRC patients	16S rRNA gene sequencing	*Akkermansia and Bacteroides*	6
CRC	North America and Europe	130 human CRC tumors	qPCR	*F. nucleatum*	7
GC	China	Gastric mucosal samples from 81 cases, including SG, AG, IM and GC	16S rRNA gene sequencing	*Peptostreptococcus stomatis*, *Streptococcus anginosus*, *P. micra*, *Slackia exigua*, *Dialister pneumosintes*	8
GC	China	229 tumoral tissues, 247 peritumoral tissues and 230 normal tissues	16S rRNA gene sequencing	*P. melaninogenica*, *S. anginosus*, *P. acnes*	9
GC	China	251 GC samples and paired NATs	16S rRNA gene sequencing	*Streptococcus*, *Prevotella*, *Fusobacterium*, *Parvimonas*, *Lactobacillus*	10
HCC	China	46 HCC samples, 28 adjacent tissues, and 33 samples from normal controls	16S rRNA gene sequencing	*Firmicutes*, *Streptococcus*, *Helicobacter*, *Bifidobacterium*, *Lactobacillus*, *Bacillus*	11
HCC	China	99 HCC and para-cancerous tissue samples	FISH, MiSeq	*Enterobacteriaceae and Fusobacterium*	12
HCC	China	29 pairs of HBV-related HCC and adjacent tissues and 12 CHB liver tissues	Metagenomic sequencing	*Methylobacterium* spp. *XJLW*	13
HCC	China	HCC and adjacent tissues from 91 patients	16S rRNA gene sequencing	*Actinobacteria*, *Nesterenkonia*, *Rubrobacter*, *Prauserella*	14
HCC	China	72 tumor tissues and adjacent normal tissues	16S rRNA gene sequencing	*Intestinimonas*, *Brachybacterium*, *Rothia*	15
HCC	China	19 pairs of HCC and adjacent tissues	16S rRNA gene sequencing	*Lactobacillales*, *Veillonellaceae*, *Rhodobacter*, *Megasphaera*	16
HCC	China	242 tumor nodules and 58 adjacent non-tumor tissues from 58 patients with multifocal HCC	16S rRNA gene sequencing	*Streptococcus*, *Enterococcus*, *Fusobacterium*	17

AG, atrophic gastritis; aoCRC, age-onset CRC; yoCRC, young-onset CRC; CRC, colorectal cancer; FISH, fluorescence in situ hybridization; GC, gastric cancer; HBV, Hepatitis B virus; HCC, hepatocellular carcinoma; IM, intestinal metaplasia; SG, superficial gastritis.

The composition and spatial distribution of the gut microbiota are highly organ-specific and heterogeneous, shaped by anatomic structure, physiologic conditions, and host-microbe interactions[23]. Microbial communities directly colonize the mucosal surface and are regulated by local microenvironmental factors in luminal/mucosal organs. For example, the stomach, which is characterized by high acidity, elevated oxygen tension, and rapid transit, harbors a low-biomass, low-diversity microbiota dominated by acid-tolerant or niche-adapted bacteria, such as *H. pylori*[24-26]. In contrast, the colon, an anaerobic environment with slow transit, a thick mucus layer, and abundant complex polysaccharides, contains the densest and most diverse microbial community in the body and is largely composed of obligate anaerobes from *Bacteroidota*, *Pseudomonadota*, and *Bacillota*. Specifically, the colonic microbiota is dominated by *Bacteroidaceae* and *Enterobacteriaceae* with additional representation from *Lachnospiraceae*, *Enterococcaceae*, and *Ruminococcaceae* and organized into spatially stratified niches, including the outer mucus layer, crypts, and lumen[27,28]. As a solid organ, intratumoral bacteria in HCC are thought to arise mainly from translocation across a compromised gut-vascular barrier *via* the portal vein or less frequently from the biliary tract or systemic circulation[29,30]. This fundamental distinction between local colonization in mucosal organs and translocation or dissemination in solid organs largely determines the origin, composition, and interaction patterns of intratumoral microbiota across different gastrointestinal cancers. Understanding the spatial ecology and source of tumor-resident bacteria is therefore essential before examining the mechanistic and clinical implications.

Researchers use an integrated set of molecular and spatial profiling approaches to accurately characterize these intratumoral communities and trace the ecologic origins. 16S rRNA gene amplicon sequencing remains a core method for profiling microbial composition and diversity. Analyzing conserved regions enables genus-level taxonomic classification and has been widely used in microbial surveys of tissue samples from patients with CRC, GC, and HCC. However, the resolution is constrained by primer selection and database completeness and generally provides limited functional information[31]. In contrast, shotgun metagenomic sequencing performs unbiased sequencing of total DNA extracted from tumor tissues. This strategy allows species- or strain-level identification and supports reconstruction of microbial metabolic pathways, as well as detection of functional features, such as antimicrobial resistance genes. Shotgun metagenomic sequencing is particularly useful for defining genomic characteristics and potential pathogenic mechanisms of key bacterial strains in gastrointestinal tumors, such as *Fusobacterium nucleatum*[32].

Techniques, such as spatial transcriptomics and fluorescence *in situ* hybridization (FISH), are increasingly applied to investigate the spatial distribution of intratumoral microbes and the interaction with host cells. FISH enables visualization of microbes within specific tumor regions using targeted probes, linking spatial heterogeneity to the immune and metabolic microenvironment of the tumor. Lu et al.[17] showed that *E. faecalis*, *S. anginosus*, and *Fusobacterium* spp. are enriched in HCC with intrahepatic metastatic nodules using multi-omics profiling and are correlated with upregulated epithelial-mesenchymal transition (EMT) drivers (SNAI2 and CEACAM6). Emerging single-cell RNA sequencing (scRNA-seq) approaches can simultaneously resolve host cell transcriptomes (e.g., epithelial and immune cells) and microbial signals, thereby revealing microbe-host interactions at the single-cell resolution. Galeano Niño et al. developed an innovative approach by incorporating bacterial 16S rRNA gene-targeting primers into the gel bead-in-emulsions of the standard 10× Genomics 5′ scRNA-seq platform (Boston, MA, USA), enabling simultaneous capture of eukaryotic and bacterial transcripts at the single-cell resolution[33]. This strategy elucidated microbial preferences for specific micro-niches and cell types with respect to adhesion and invasion, and identified bacteria-associated alterations in host signaling pathways at the single-cell level[33]. Strict sterile procedures and appropriate negative controls are essential throughout experimental workflows, while bioinformatic strategies are applied to distinguish true intratumoral colonizers from external contaminants. Integration of these methods allows clarification of the taxonomic identity, functional potential, and spatial organization of intratumoral microbes, providing a foundation for exploring the roles in gastrointestinal oncology.

Functional mechanisms of intratumoral microbiota-host interactions in gastrointestinal cancers

It is generally accepted that gut microbiota translocate across a compromised gut barrier, colonize tumor tissues in gastrointestinal cancers and subsequently interact with tumor cells and the TME. Here, interaction mechanisms between microbiota and the host gut barrier, tumor cells, anti-tumor immunity, as well as non-cellular components (e.g., extracellular matrix, soluble factors) of the TME are discussed.

The impaired gut barrier

Colonization by intratumoral microbiota is commonly accompanied by an impaired gut barrier in gastrointestinal cancers. The gut barrier is a complex system consisting of gut microbiota, two layers of surface mucus, epithelial cells, immune cells, and an endothelium. Commensal bacteria occupy physical space and ecological niches in the outer mucus layer, thereby

competing with and limiting access for pathogenic invaders. This outer layer is relatively loose and contains abundant bacteria, whereas the inner mucus layer is firmly attached to the epithelium and is dense with an absence of bacteria.

In cancers of mucosal organs with an externally exposed cavity, such as GC, CRC, and pancreatic cancer, damage to the mucosal barrier represents a major route through which microorganisms colonizing the mucosa may invade the tumor[34]. Some bacteria can directly disrupt the mucus layer. For example, *Ruminococcus gnavus* is a mucolytic bacterium that can utilize carbon sources from the mucus layer as raw material to sustain growth, leading to degradation of the mucus barrier[35]. In contrast, microbiota-derived short-chain fatty acids (SCFAs) can strengthen gut barrier defenses by upregulating the expression of mucin proteins, the main structural components of the mucus layer[36,37]. These observations partly explain how gut dysbiosis regulates microbial invasion into the TME. Tjalsma et al.[38] further proposed a bacterial driver-passenger model that partially explains alterations in intratumoral microbiota composition during gastrointestinal tumorigenesis. "Driver" bacteria, such as enterotoxigenic *B. fragilis* and *H. pylori*, initiate tumorigenesis (as discussed in the next section) and create conditions that permit "passenger" bacteria to enter and colonize the TME. Notably, "passenger" bacteria may include tumor-foraging opportunistic pathogens as well as commensal or probiotic species. Therefore, deeper investigation of the mechanisms by which microorganisms enter the TME from mucosal organs may improve the efficiency of therapeutic strategies aimed at delivering bacteria to mucosal sites, such as fecal microbiota transplantation (FMT).

In addition, microorganisms are also detected in tumors of non-mucosal organs, such as the liver, where microorganisms spread *via* the gut vascular barrier (i.e., the portal vein) and subsequently reach the tumor site[34]. In this context, microbiota must penetrate not only the mucus layer but also the epithelial, immune, and endothelial cell barriers. Tight junctions of the gut epithelium are tightly regulated by the proximal colonizing microbiome. Ethanol produced by bacterial fermentation of carbohydrates is oxidized to acetaldehyde, which can disrupt tight junction proteins, such as occludin and ZO-1 between intestinal epithelial cells, thereby damaging the intestinal mucosal barrier[39]. In contrast, microbiota-derived SCFAs induce the expression of tight junction proteins, such as ZO-1 and occludin, and accelerate assembly through activation of AMP-activated protein kinase[40]. Notably, signals generated by gut microbiota beyond small-molecule metabolites can also modulate gut barrier permeability. Extracellular vesicles derived from *Akkermansia muciniphila* upregulate tight junction proteins and restore mucus layer thinning in High-Fat Diet (HFD)-fed mice[41]. At the same time, commensal gut microbiota limit pathogen colonization by stimulating cell-mediated immunity. Plasma and T cells are markedly reduced in the intestinal lamina propria of germ-free mice. B and T cells from Peyer's patches and the lamina propria can be stimulated by antigens or antigen-presenting cells, differentiate into plasma and effector T cells that secrete immunoglobulin A, and participate in local immune responses of the intestinal tract after returning to the lamina propria[42,43]. Wang et al.[44] recently demonstrated that translocation of the pathogen, *Klebsiella pneumoniae*, to liver tumors depends on MMP2/9-secreting macrophages that degrade the extracellular matrix of the gut barrier, revealing a novel mechanism by which gut pathogens translocate and colonize the liver, an organ long regarded as sterile. Furthermore, Virf1 from *Escherichia coli* strain C17 was shown to induce the endothelial marker, plasmalemma vesicle-associated protein-1 (PV-1), and increase vascular permeability in CRC, promoting formation of a pre-metastatic microenvironment in liver metastases[45]. Taken together, gastrointestinal microbiome homeostasis profoundly influences the translocation and functional roles of intratumoral microbiota.

Tumor cells

Interaction mechanisms between intratumoral microbiota and cancer cells are discussed below based on current evidence, focusing on genetic alterations and non-genetic regulatory pathways, including post-translational modification and metabolic signaling.

Modulating genetic materials

Disruption of genetic material through DNA damage and epigenetic alterations is a key driver of cancer malignancy and represents one of the carcinogenic mechanisms of the intratumoral microbiome. Microbes, particularly oncoviruses responsible for 15%–20% of human cancers[46], can integrate their genomes into host cells, as occurs for EBV in gastric and nasopharyngeal carcinomas and HBV in liver cancer, thereby inducing mutations that promote malignant progression[47,48]. Genomic DNA damage in host cells can also be triggered by intratumoral microbes through production of specific toxins, enzymes, and reactive oxygen species (ROS). Genotoxic pks$^+$ *E. coli* encode and secrete the carcinogenic toxin, colibactin, which alkylates DNA and induces DNA damage, leading to colorectal carcinogenesis[49,50]. Enterotoxigenic *B. fragilis* (ETBF) secretes *B. fragilis* toxin (BFT), which increases expression of spermine oxidase (SMO) in colonic epithelial cells, triggering SMO-dependent ROS production and activation of γ-H2A[51]. In addition, several oral microbiota, such as *Streptococcus mitis and Prevotella histicola*, can convert ethanol into acetaldehyde, a highly reactive compound classified as a "group 1 carcinogen," resulting in formation of DNA adducts that distort and disrupt the double-helical structure[52,53].

Studies have also linked tumor-resident microbiota to tumorigenesis through modulation of the epigenetic

landscape, including DNA methylation and histone modifications. *H. pylori* infection induces aberrant DNA methylation and contributes to GC risk, although the underlying mechanisms remain debated. It has been reported that DNA methylation changes in gastric mucosa following *H. pylori* infection depend on infection-associated inflammatory responses[54], whereas *H. pylori* can also directly regulate host epigenetic modifications *via* the nuclear factor-kappa B (NF-κB)–TET1–GNB4 demethylation–YAP1 axis in tumor cells[55]. *F. nucleatum* and *Hungatella hathewayi* upregulate expression and nuclear activity of DNA methyltransferases (DNMT1 and DNMT3A) in CRC, inducing promoter hypermethylation of tumor suppressor genes[56]. Microorganisms can additionally influence host epigenetic modifications indirectly by supplying epigenetic substrates and cofactors[57]. Probiotic bacteria, such as *Bifidobacterium adolescentis*, *B. pseudocatenulatum*, and *Lactobacillus plantarum*, produce folate[58], which serves as a key component of methionine and folate cycles that generate S-adenosylmethionine for DNA and histone methylation. Similarly, regulation of histone modifications in tumor cells by SCFAs, particularly butyrate, involves two complementary mechanisms[59]. Butyrate acts as a potent inhibitor of histone deacetylases (HDACs), leading to accumulation of acetylated histones[59]. In contrast, butyrate enhances histone acetylation by being metabolized to acetyl-CoA and stimulating histone acetyltransferase (HAT) activity[60].

Modulating non-genetic carcinogenic signaling pathways

Signaling pathways in tumor tissues exhibit marked differences compared to normal tissues. Dysregulation of these pathways represent a central oncogenic mechanism involved in cancer initiation, progression, and therapeutic response. Intratumoral bacteria can regulate signaling pathways in tumor cells through direct attachment (**Figure 1**) and indirect mechanisms mediated by microbial metabolites.

Direct binding between bacteria and tumor cells

Recent studies indicated that direct binding of intratumoral bacteria to tumor cell receptors *via* surface adhesins (e.g., membrane proteins or polysaccharides) is an important mechanism for activating downstream oncogenic signaling. Among intratumoral bacteria associated with gastrointestinal tumors, *F. nucleatum* is the most extensively studied with respect to host cell adhesion. *F. nucleatum* was initially identified in CRC by tumor tissue whole-genome and RNA sequencing, quantitative PCR, FISH, and culture-based validation across multiple independent studies[61-63]. Infection with *F. nucleatum* upregulates Toll-like receptor 4 (TLR4) and myeloid differentiation primary response 88 (MYD88) expression, thereby promoting proliferation, autophagy, and chemoresistance in CRC cells[64,65]. In parallel, activation of the *F. nucleatum*-mediated TLR4/NF-κB pathway has been shown to induce BIRC3 expression and resistance to 5-fluorouracil (5-FU) in CRC[66]. However, these studies did not clarify the underlying direct binding mechanisms. In 2013 Rubinstein et al.[67] reported that *F. nucleatum* adheres to tumor cells through FadA-mediated binding to E-cadherin, resulting in modulation of β-catenin signaling. More recently, Shen et al.[68] identified the surface adhesin, CbpF, which binds human CEACAM1 and CEACAM5 receptors *via* a multivalent, Velcro®-like adhesion mechanism. Taken together, these findings elucidate how *F. nucleatum* drives CRC progression through direct bacterium-host interactions and provide a basis for developing targeted therapeutic interventions.

Adhesion-based mechanisms have also been reported for other pathogenic bacteria involved in gastrointestinal cancers. *Peptostreptococcus anaerobius* interacts with CRC cells through the surface protein, putative cell wall binding repeat 2 (PCWBR2). Binding to α2/β1 integrin activates the PI3K–AKT pathway, leading to enhanced cell proliferation and NF-κB-mediated pro-inflammatory responses. *P. stomatis* promotes colorectal tumorigenesis by binding the surface protein, fructose-1,6-bisphosphate aldolase (FBA), to the integrin α6/β4 receptor on CRC cells, thereby activating ERBB2 and the downstream MEK–ERK–p90 signaling cascade[69]. Similarly, *B. fragilis* colonizes colorectal tumors and exerts effects through binding of the surface protein, SusD/RagB, to the Notch1 receptor on cancer cells, activating Notch1 signaling, inducing EMT, and suppressing chemotherapy-induced apoptosis[70]. LOX-1/OLR1 has been identified as a membrane receptor in GC mediating *H. pylori* adhesion and catalase activity. Upon adhesion, *H. pylori* delivers the effector protein, CagA, into host cells, leading to activation of the β-catenin pathway and progression toward gastric adenocarcinoma[71]. Furthermore, Fu et al.[72] reported that the surface protein, TMPC of *S. anginosus*, interacts with the annexin A2 (ANXA2) receptor on gastric epithelial cells. This interaction facilitates bacterial attachment and colonization, while activating the oncogenic MAPK signaling pathway, resulting in persistent gastric barrier dysfunction, increased cell proliferation, and reduced apoptosis. These findings extend the understanding of GC pathogenesis by identifying *S. anginosus* as an important bacterial carcinogen beyond the well-established role of *H. pylori*[72]. *Fusobacterium periodonticum* activates the PI3K–AKT/FASN signaling pathway in esophageal squamous cell carcinoma (ESCC) through interaction between the surface adhesin, FadAL, and the host tumor protein, FLOT1. This activation promotes intracellular accumulation of triglycerides and palmitic acid (PA), enhancing palmitoylation of Wnt3a at the conserved cysteine residue, Cys-77. This lipid modification drives membrane localization of Wnt3a and nuclear translocation of β-catenin, ultimately activating the Wnt/β-catenin axis and promoting an EMT phenotype. This process may be further amplified by N-nitrosamine-mediated upregulation of FadAL in *F. periodonticum*[73]. Notably, intratumoral bacterial colonization and metabolite-driven tumor progression are

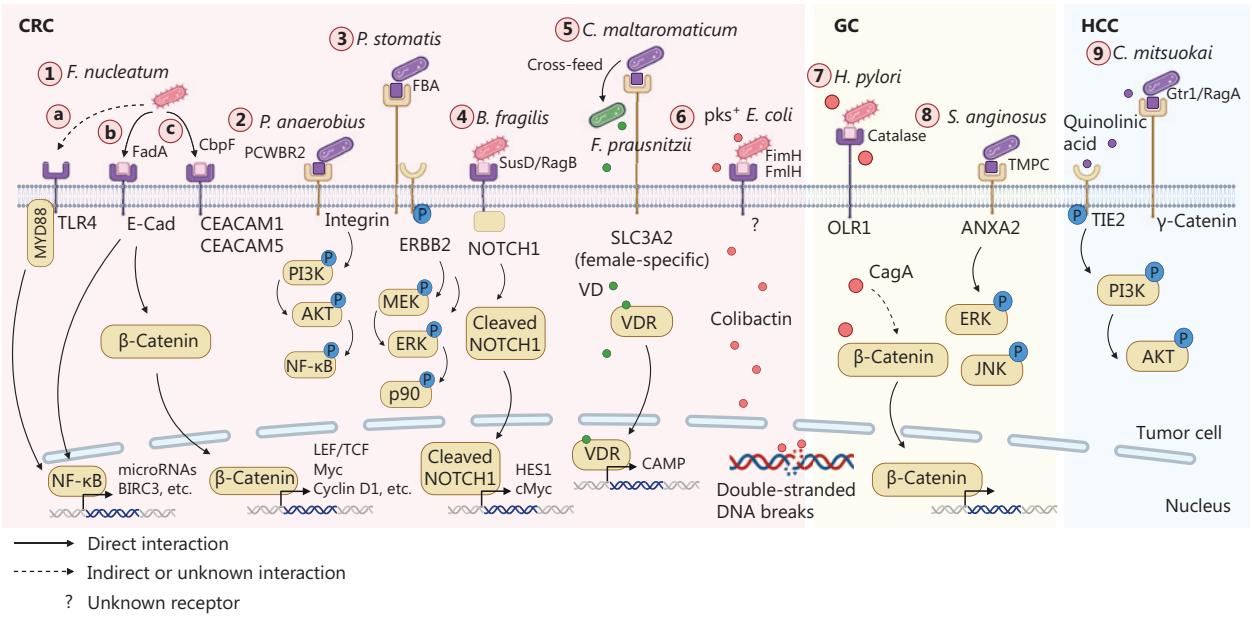


Figure 1 Modulation of carcinogenic signaling pathways *via* attachment between intratumoral bacteria and host tumor cells. This schematic summarizes selected evidence of how intratumoral bacteria directly bind to host tumor cells to activate key carcinogenic pathways in CRC, GC, and HCC. The figure is organized by cancer type and numbered sequentially to illustrate distinct bacterial–host interaction mechanisms. **CRC**: ① *F. nucleatum* attaches to human CRC cells *via* multiple mechanisms (A–C). A. *F. nucleatum* activates NF-κB through TLR4/MYD88 signaling *via* unknown factor. B. The surface protein, FadA, binds to E-cadherin, thus activating β-catenin pathways and inducing NF-κB expression. C. *F. nucleatum* can also adhere to CEACAM1 and CEACAM5 receptors *via* the surface adhesin, CbpF protein. *Peptostreptococcus anaerobius* and *P. stomatis* are frequently detected in CRC tumors. ② *P. anaerobius* activates the PI3K/AKT/NF-κB pathway by directly binding to integrin on host tumor cells *via* PCWBR2 protein. ③ *P. stomatis* binds to ERBB2 and activates the ERBB2/MEK/ERK/p90 pathway *via* FBA. ④ *B. fragilis* colonizes colorectal tumors and activates NOTCH1 signaling through direct interaction *via* SusD/RagB, which leads to tumor cell EMT and drug resistance. In some instances, bacterial attachment to tumor cells facilitates metabolite-mediated indirect interactions. For example, ⑤ *C. maltaromaticum* attaches to CRC cells and cross-feeds *F. prausnitzii*, resulting in VD accumulation and reduced CRC tumorigenesis. ⑥ pks+ *E. coli* attaches to CRC cells *via* FimH/FmlH and induces DNA damage through intracellular accumulation of colibactin. **GC:** ⑦ *H. pylori* attaches to gastric epithelial cells *via* catalase and delivers effector CagA into host cells. This process leads to activation of the β-catenin pathway, although the direct binding of CagA to β-catenin remains to be clarified. ⑧ *S. anginosus* interacts with the ANXA2 receptor on gastric epithelial cells and activates oncogenic MAPK signaling. This interaction results in sustained impairment of the gastric barrier, enhanced cellular proliferation, and inhibition of apoptosis. **HCC**: ⑨ *C. mitsuokai* binds host γ-catenin through the surface protein, Gtr1/RagA, to enable hepatic colonization, while the secreted metabolite, quinolinic acid, activates the PI3K/AKT pathway in HCC cells and promotes tumor progression. ANXA2, annexin A2; AKT, AKT serine/threonine kinase; CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CRC, colorectal cancer; EMT, epithelial-mesenchymal transition; ERBB2, Erb-B2 receptor tyrosine kinase 2; ERK, extracellular signal-regulated kinase; FBA, fructose-1,6-bisphosphate aldolase; GC, gastric cancer; HCC, hepatocellular carcinoma; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MYD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-B; NOTCH1, Notch receptor 1; p90, p90 ribosomal S6 kinase; PI3K, phosphoinositide 3-kinase; TLR4, Toll-like receptor 4; VD, vitamin D. Figure created using BioRender (www.biorender.com).

not mutually exclusive and may instead reinforce each other. pks+ *E. coli* adheres to host epithelial cells *via* the adhesins, FimH (type 1 pili) and FmlH (F9 pili), enabling localized production of the highly unstable but genotoxic colibactin near host cells and ultimately driving carcinogenesis[74]. Recent findings involving *Catenibacterium mitsuokai* further illustrate this dual mechanism, as follows: the bacterium binds host γ-catenin/*CTNNB1* through the surface protein, Gtr1/RagA, to facilitate hepatic colonization, while the secreted metabolite, quinolinic acid, activates the TIE2 receptor and downstream PI3K/AKT signaling in HCC cells, together promoting liver cancer progression[75].

Finally, heterogeneity in host receptor expression can influence intratumoral bacterial binding and thereby modulate cancer progression. Yu et al.[76] reported that a single nucleotide polymorphism (SNP rs2355016) in an intronic region of the *KCNJ11* gene reduces *KCNJ11* expression in a cohort of 748 Chinese patients with CRC. This reduction increases

the surface levels of Gal–GalNAc on CRC cells, facilitating enhanced binding and invasion of *F. nucleatum via* the Fap2 protein and accelerating colorectal tumor growth. *Carnobacterium maltaromaticum* markedly suppresses colorectal tumorigenesis in female mice but shows weaker protective effects in males. This gender-specific difference may be related to estrogen-mediated upregulation of SLC3A2, which could promote bacterial anchoring and colonization *via* the DD-CPase protein[77]. In human CRC with *KRAS* mutations, fecal abundance of bacteroides is increased and ETBF levels are higher within xenograft tumors harboring the *KRAS G12D* mutation compared to wild-type tumors. It has been proposed that indirect modulation of the miR-3655/SURF6/IRF7/IFNβ axis creates a permissive niche for ETBF survival and colonization[78].

Regulation by bacteria-derived metabolites

The impact of tumor-resident bacteria on tumor metabolomic profiles has been demonstrated in various gastrointestinal cancers[79,80]. Enrichment of arginine-to-ornithine metabolism was shown in *S. anginosus*-abundant GC samples. Notably, these samples showed no significant changes in human arginine-related enzymes, suggesting a potential role of bacterial arginine metabolism mediated by *S. anginosus* in GC development[81]. *P. anaerobius*-derived *trans*-3-indoleacrylic acid (IDA) inhibits ferroptosis through the AHR–ALDH1A3–FSP1–CoQ10 axis in CRC[82]. *Streptococcus thermophilus* accumulates galactose within tumors *via* secretion of β-galactosidase, thereby suppressing Hippo signaling and exerting anti-cancer effects[83]. It should be noted that intratumoral metabolic profiles are strongly shaped by tumor cells, immune cells and extracellular matrix. Therefore, the specific contribution of primary metabolites derived from intratumoral bacteria within this complex network, particularly the direct effects on tumor cells, requires further validation.

In addition, several bacteria-derived secondary metabolites and proteins have been reported to regulate tumor progression in CRC. *Bacillus toyonensis* BV-17, isolated from fecal samples of healthy donors, secretes hemolysin BL, which exhibits anti-tumor activity *in vitro* and *in vivo*[84]. Reuterin, also known as 3-hydroxypropionaldehyde and primarily produced by the commensal bacterium, *Lactobacillus reuteri*, was identified among microbial metabolites that suppress colon cancer growth *in vivo*. Reuterin exerts cytotoxic and growth-inhibitory effects by altering redox homeostasis and inhibiting ribosomal biogenesis in colon cancer cells[85]. Biliverdin was identified as a key metabolite produced by pathogenic *E. faecalis* that promotes cell proliferation and inhibits cell cycle arrest in CRC cells. In addition, biliverdin markedly increases expression of the angiogenic factors, IL-8 and VEGFA, by activating the PI3K/AKT/mTOR signaling pathway in CRC[86]. Cytolethal distending toxin (CDT), derived from *Campylobacter jejuni*, promotes liver or pulmonary metastasis of CRC cells through JAK2–STAT3–MMP9 signaling in murine models[87]. Moreover, the *Salmonella* effector, AvrA, regulates β-catenin post-translational modifications and the upstream regulator, AKT, potentially promoting intestinal proliferation[88]. The virulence factor, gingipain, mediates *Porphyromonas gingivalis*-induced proliferation and activation of the MAPK/ERK signaling pathway in CRC cells[89]. It will be of interest to determine whether similar secondary metabolites or proteins have comparable roles in GC and other gastrointestinal tumors, such as HCC and pancreatic ductal adenocarcinoma (PDAC).

In conclusion, current evidence has established that intratumoral microorganisms, primarily viruses and bacteria, can directly or indirectly regulate host DNA damage, epigenetic modifications, and signaling pathways. However, the molecular mechanisms underlying these effects are complex and highly interconnected and warrant further exploration.

Anti-tumor immunity

While previous sections have described how intratumoral microbes influence tumor cells through direct binding and metabolite secretion, the role in shaping the local immune landscape (the tumor immune microenvironment) is equally important yet mechanistically distinct. This section focuses on the local immunomodulatory mechanisms by which tumor-resident bacteria regulate anti-tumor immunity, beyond the direct carcinogenic effects on epithelial cells.

In recent years, accumulating evidence suggested that intratumoral microbiota regulate the composition and function of intratumoral $CD8^+$ T cells[90], natural killer (NK) cells[91], neutrophils[92,93], myeloid-derived suppressor cells and macrophage[94,95], thereby affecting immunotherapy efficacy[96,97]. Although many correlative associations have been identified, most underlying mechanisms are incompletely understood. Herein, recent findings on regulatory mechanisms of intratumoral microbiota in anti-tumor immunity are reviewed with emphasis on microbe-derived small molecules, macromolecules, and direct bacterial adhesion (**Figure 2**). Evidence for these mechanisms is synthesized, while acknowledging that this field is still evolving and many pathways require further clarification.

Microbiota-derived small molecule metabolites

This subsection highlights key microbial metabolites with demonstrated immunomodulatory roles within the TME, such as SCFAs and tryptophan derivatives, distinguishing the immune-focused actions from the direct tumor cell effects discussed earlier.

SCFA

Concentrations of fecal or plasma SCFAs (e.g., acetate, propionate, and butyrate) correlate with immunotherapy responses in solid tumors[98], although the intratumoral concentrations remain less clearly defined. Studies have indicated that butyrate and pentanoate enhance mTOR signaling, while inhibiting

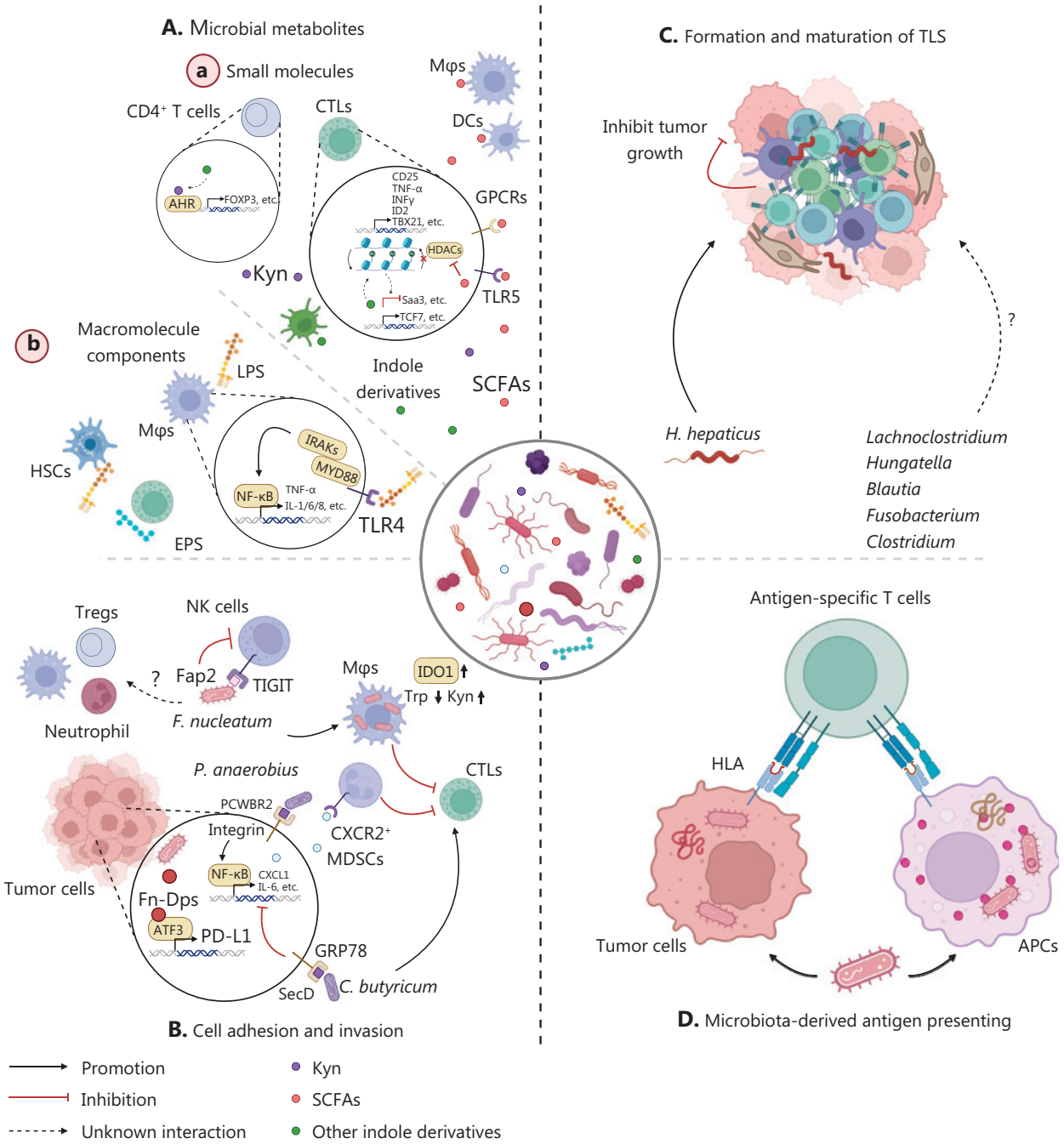


Figure 2 The impact of intratumoral microbiota on anti-tumor immune surveillance in gastrointestinal cancers. This schematic illustrates the well-established mechanisms by which intratumoral bacteria modulate anti-tumor immunity through direct interaction with host cells (Panels A and B) and highlights critical knowledge gaps requiring further investigation (Panels C and D). A. **Microbial metabolites**: Specific examples of how intratumoral microbiota regulate anti-tumor immunity through small and macromolecules. a. **Small molecules**: SCFAs, such as butyrate, enhance the anti-tumor efficacy of cytotoxic T cells by inhibiting HDACs, thereby increasing transcription of immune regulators involved in T-cell differentiation and function. Butyrate also binds GPCRs on cytotoxic T cells and DCs to augment anti-tumor activity and can enhance anti-PD-1 therapy efficacy in CRC by binding to TLR5 and activating NF-κB signaling in $CD8^+$ T cells. Kyn, an indole derivative frequently linked to pathologic processes, binds to and activates AHR, inducing FOXP3 expression in $CD4^+$ regulatory T cells in CRC. Other indole derivatives, such as ILA and IPA, enhance H3K27 acetylation and promote $CD8^+$ effector T-cell function. b. **Macromolecule components**: LPSs from Gram-negative bacteria activate TLR4 on macrophages and HSCs, triggering the MYD88–IRAK–NF-κB pathway and inducing inflammatory

cytokines (TNF-α, IL-1, IL-6, and IL-8). EPSs, synthesized by both Gram-positive and -negative bacteria, interact with $CD8^+$ T cells *via* binding of phosphorylated EPS structures to lysophosphatidic acid receptors. B. **Cell adhesion and invasion:** Regulatory mechanisms of intratumoral microbiota in anti-tumor immunity involving direct bacterial adhesion. *F. nucleatum* adheres to NK cells through the outer membrane protein, Fap2, which binds the inhibitory receptor, TIGIT, thereby suppressing NK cell activity and facilitating tumor immune evasion. *F. nucleatum* can also invade and persist within macrophages, inducing IDO expression and suppressing lymphocyte proliferation. Although intratumoral *F. nucleatum* abundance correlates with neutrophils and Tregs, the underlying mechanisms are unknown. Bacterial adhesion to tumor cells indirectly modulates immune surveillance, as follows: *F. nucleatum*-derived Dps binds ATF3 to upregulate PD-L1 expression; *P. anaerobius* binds integrin α2β1 and activates NF-κB signaling in CRC cells, inducing CXCL1 secretion and recruitment of $CXCR2^+$ MDSCs; and *Clostridium butyricum* binds GRP78 on CRC cells and inhibits downstream IL-6-mediated immunosuppression. C and D: Significant gaps remain in understanding the role of intratumoral microbiota in TLS formation and antigen presentation. C. **Formation and maturation of TLS:** Oral administration of *Helicobacter hepaticus* induces Tfh cell- and B cell-dependent anti-tumor immune responses, promoting formation of proximal TLSs that suppress colon cancer growth. While gut microbiota, such as *Lachnoclostridium*, are associated with intratumoral TLS presence in HCC patients, the causal relationships and mechanisms have not been established. D. **Microbiota-derived antigen presenting:** Intratumoral bacteria represent an important source of tumor antigens. However, the utilization of these non-human tumor antigens to enhance cancer immunotherapy efficacy has not been thoroughly investigated. AHR, aryl hydrocarbon receptor; APCs, antigen-presenting cells; ATF3, activating transcription factor 3; CRC, colorectal cancer; CTLs, cytotoxic T lymphocytes; CXCL1, C-X-C motif chemokine ligand 1; CXCR2, C-X-C motif chemokine receptor 2; DCs, dendritic cells; EPS, exopolysaccharides; FOXP3, Forkhead box P3; GPCRs, G protein-coupled receptors; GRP78, glucose-regulated protein 78; HDACs, class I histone deacetylases; HLA, human leukocyte antigen; HSCs, hepatic stellate cells; IDO, indoleamine 2,3-dioxygenase; ILA, indole-3-lactic acid; IPA, indole-3-propionic acid; Kyn, kynurenine; LPS, lipopolysaccharide; MDSCs, myeloid-derived suppressor cells; Mφs, macrophages; NF-κB, nuclear factor kappa-B; NKs, natural killer cells; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; SCFAs, short-chain fatty acids; Tfh, T follicular helper; TLR5, Toll-like receptor 5; TLRs, Toll-like receptors; TLS, tertiary lymphoid structures; Trp, tryptophan. Figure created using BioRender (www.biorender.com).

histone deacetylases, resulting in increased effector molecule expression and improved T-cell cytotoxicity[99]. In addition, butyrate epigenetically upregulates TBX21 in $CD8^+$ T cells *via* HDAC inhibition, suppressing PD-1 expression and alleviating T-cell exhaustion[100]. Butyrate also promotes anti-tumor cytotoxic responses in $CD8^+$ T cells in an ID2-dependent manner through activation of IL-12 signaling[101]. Butyrate induces expression of IL-10 and ALDH1A1 in macrophages and dendritic cells *via* GPR109A in CRC[102], whereas butyrate enhances $CD8^+$ T-cell function through GPR109A/HOPX signaling in GC[103]. Notably, butyrate produced by the probiotic, *Roseburia intestinalis*, enhances the efficacy of anti-PD-1 therapy in CRC by binding TLR5 and activating NF-κB signaling in $CD8^+$ T cells[104]. Conversely, butyrate can inhibit STING-activated type I IFN expression in dendritic cells, thereby attenuating radiation-induced tumor-specific cytotoxic T-cell responses in CRC[105]. These opposing effects highlight the context-dependent nature of SCFA signaling and emphasize the need for precise therapeutic targeting. In addition, butyrate has a very short half-life, making development of targeted delivery strategies a key direction for future research aimed at harnessing the beneficial effects in cancer therapy.

Tryptophan (Trp) and derivatives

Trp metabolism generates a range of bioactive indole derivatives [e.g., indole-3-lactic acid (ILA), indole-3-propionic acid (IPA), indole-3-carboxylic acid (ICA), indole-3-acetic acid (IAA), and kynurenine (Kyn)] that regulate important physiologic processes, including inflammation, metabolism, immune responses, and neurologic function[106]. *Lactobacillus plantarum* produces ILA, which enhances IL-12a production in DCs *via* histone acetylation, thereby promoting $CD8^+$ T-cell priming[106]. *L. johnsonii* cooperates with *Clostridium sporogenes* to produce IPA, which enhances H3K27 acetylation at the super-enhancer region of the *Tcf7* gene and promotes effector $CD8^+$ T-cell differentiation[106]. ICA derived from *L. gallinarum* competes with Kyn for binding to the aryl hydrocarbon receptor (AHR) on $CD4^+$ T cells, thereby inhibiting regulatory T cell differentiation and strengthening anti-PD-1 therapy in CRC[107]. In contrast, reduced production of indole derivatives may contribute to immunotherapy resistance. Enrichment of *Phocaeicola vulgatus* in HCC non-responders impairs $CD8^+$ T-cell function, an effect that can be reversed by IAA supplementation[108]. Kyn represents another major metabolic route of Trp. Both gut bacteria and the host express indoleamine 2,3-dioxygenase (IDO) enzymes that convert Trp into Kyn. IDO expression in tumor tissues correlates with reduced infiltration of $CD3^+$ T cells[109]. The IDO inhibitor, INCB023483, suppresses tumor growth by lowering Kyn levels and enhancing $IFN\gamma^+$ T cells in pancreatic cancer[109,110]. Kyn can directly bind to and activate AHR, inducing FOXP3 expression in $CD4^+$ regulatory T cells in CRC[107,111].

Other microbial metabolites

Bile acids (BAs) also participate in immune regulation. The secondary BA, 3β-hydroxydeoxycholic acid (isoDCA), increases

peripheral Foxp3$^+$ regulatory T cells by inhibiting FXR activity in DCs, thereby reducing the immunostimulatory capacity[112]. *Bacteroides*-derived secondary BAs, including deoxycholic acid and lithocholic acid, activate the β-catenin/chemokine (C-C motif) ligand 28 (CCL28) axis in tumor cells and subsequently increase intratumoral immunosuppressive regulatory T cells in CRC[113]. Gut microbiota-mediated conversion of primary-to-secondary BAs, mainly depletion of taurocholic acid (TCA) and chenodeoxycholic acid (CDCA), correlates with CXCL16 expression in sinusoidal endothelial cells, accumulation of NKT cells in the liver, and corresponding tumor growth in liver cancer[114].

Lactate can serve as an energy source for rapidly proliferating cancer cells and impair anti-tumor immune cell function[115]. Although *Lactobacillus* spp. are commonly regarded as beneficial probiotics excessive lactic acid production in the cancer setting may be detrimental. Colonization of the stomach with *H. pylori* with a limited commensal microbiota (decreased *Clostridium* and *Bacteroides*, and increased *Lactobacillus*) in the INS-GAS GC mouse model promotes neoplastic lesion formation, although the underlying mechanism has not been established[116]. Tumor-resident *L. iners* alters lactate signaling in tumor metabolism and contributes to therapeutic resistance without affecting antigen-specific T-cell repertoires[117]. In contrast, intratumoral *E. coli* enhances lactate production and induces retinoic acid-inducible gene 1 (RIG-I) lactylation, suppressing NF-κB binding and promoting M2 macrophage polarization[118]. While most studies have focused on host-derived lactate in cancer metabolism, these findings emphasize the need to consider exogenous bacterial lactate.

Vitamins also have important roles in host-microbiota interactions. Vitamin availability significantly influences gut microbiota composition; conversely, the gut microbiota affects vitamin production and metabolism[118]. Free vitamin D binds to the gut epithelium and alters microbiome composition, particularly favoring *B. fragilis*, which is positively associated with favorable anti-tumor immune responses in CRC[119]. As a precursor of coenzyme A, pantothenic acid (vitamin B5) enhances differentiation of IL-22-producing Tc22 cells from CD8$^+$ cytotoxic T cells and improves immunotherapy responses in pancreatic tumors[120].

Collectively, microbiota-derived metabolites, especially those enriched within tumors, constitute a largely untapped reservoir of potential anti-tumor drugs or therapeutic targets. Clarifying the functions of these metabolites in the TME may enable development of new strategies to enhance immunotherapy efficacy and improve outcomes in patients with gastrointestinal cancers.

Microbiota-derived macromolecules

Microbiota-derived macromolecular components, including lipopolysaccharide (LPS), peptidoglycan, DNA, and extracellular vesicles, are collectively referred to as pathogen-associated molecular patterns (PAMPs). LPS, a major outer membrane component of Gram-negative bacteria, is a well-characterized activator of innate immunity. LPS activates pattern-recognition receptors (PRRs), such as TLR4 on Kupffer cells and hepatic stellate cells (HSCs), in HCC[121-123]. This activation triggers myeloid differentiation primary response 88 (MYD88), a canonical adaptor for TLR family members that links the IL-1 receptor (IL-1R) to the IL-1R-associated kinase (IRAK) family kinases[124,125]. Activation of IRAK kinases leads to multiple downstream effects, including activation and nuclear translocation of NF-κB and phosphorylation of activator protein 1 (AP-1), thereby promoting transcription of inflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-8[126,127]. Consequently, cytokine release by LPS-activated immune cells is increased, generating an excessive and uncontrolled pro-inflammatory environment. TLR4 activation also promotes cell proliferation and NF-κB-mediated resistance to apoptosis in HSCs, thereby exacerbating liver injury, fibrosis, and carcinogenesis[128]. Associations between the LPS–TLR axis and infiltration of pro-inflammatory immune cells have also been observed in other gastrointestinal cancers[129-131], indicating a central role for this signaling pathway in shaping an inflammatory milieu that facilitates tumorigenesis. Unlike LPS, which is produced by Gram-negative bacteria, exopolysaccharides (EPSs) are synthesized by Gram-positive and -negative bacteria. EPS produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* induces CCR6$^+$ CD8$^+$ T cells and enhances the efficacy of anti-CTLA-4 or anti-PD-1 immunotherapy. Induction of CCR6 expression is mediated by binding of phosphorylated EPS structures to lysophosphatidic acid receptors on CD8$^+$ T cells.

Regulating the immune response via bacteria-cell adhesion and invasion

In addition to tumor cell adhesion (Section 2.2.2), intratumoral bacteria can directly bind to or invade immune cells, thereby altering function. *F. nucleatum* adheres to NK cells *via* outer membrane protein Fap2, which binds the inhibitory receptor, TIGIT, suppressing NK cell activity and facilitating tumor immune evasion[132]. *F. nucleatum* can also survive within macrophages, inducing IDO expression and impairing lymphocyte proliferation and cytotoxic activity[133,134]. In addition, intratumoral *F. nucleatum* abundance has been reported to correlate with neutrophils[129,130], macrophages[131], and regulatory T cells (FoxP3$^+$ Tregs)[135]. The *F. nucleatum* virulence factor, Dps, binds activating transcription factor 3 (ATF3) in invaded tumor cells in ESCC, upregulating PD-L1 expression and reducing the efficacy of αPD-L1 therapy[136]. Similarly, *Peptostreptococcus anaerobius* activates integrin α2β1–NF-κB signaling in CRC cells, inducing CXCL1 secretion and recruitment of CXCR2$^+$ MDSCs, thereby abolishing anti-PD-1 therapeutic efficacy[137]. Conversely, some bacteria enhance

immunotherapy responses. Specifically, *Clostridium butyricum* binds to GRP78 on CRC cells and suppresses downstream IL-6-mediated immunosuppression, enhancing responses to anti-PD-1 therapy[138]. These examples demonstrate that bacterial adhesion and invasion extend beyond epithelial cells to multiple immune populations with effects that may suppress, or in some situations, potentiate anti-tumor immunity.

Intratumoral microbiota may also support beneficial immune structures. Intratumoral bacteria can promote formation and maturation of tertiary lymphoid structures (TLSs), facilitating lymphocyte infiltration and activation. TLSs are organized aggregates of immune cells that form in non-lymphoid tissues after birth and consist of T cells, B cells, and additional immune cell types, such as DCs. The oral administration of *Helicobacter hepaticus* induces T follicular helper (Tfh) cell- and B cell-dependent anti-tumor immune responses, promoting development of proximal TLSs that suppress colon cancer growth[139]. *Lachnoclostridium*-dominated gut microbiota has been associated with intratumoral TLS presence in HCC patients[140]. Moreover, intratumoral bacteria constitute an important source of tumor antigens. Earlier studies showed that infection-derived microbial antigens can stimulate selective $CD8^+$ T-cell expansion and degranulation in pancreatic cancer[141]. More recently, combined tumor tissue 16S rRNA sequencing and HLA bacterial peptidomics revealed that peptides derived from intratumoral bacteria can be presented by cancer and antigen-presenting cells (APCs), thereby activating tumor-specific T cells[142], particularly for gastrointestinal-associated bacteria, such as *F. nucleatum*[143]. These findings not only provide a methodologic advance, demonstrating that 16S-based taxonomic information can guide peptidomic searches without the need for bacterial cultivation, but also identify a novel class of non-human tumor antigens that may enhance cancer immunotherapy efficacy[144].

Non-cellular components

Other components of the TME include the extracellular matrix, soluble factors, and biophysical and chemical conditions. Bacteria have been reported to regulate local drug metabolism as well as the acidic and hypoxic tumor core.

Drug metabolism

In addition to the mechanisms of tumor drug resistance mediated by interactions between intratumoral bacteria and tumor or immune cells, other contributing factors have not been thoroughly explored. It has been reported that intratumoral bacteria can enhance drug resistance by directly degrading chemotherapeutic agents within the TME. Intratumoral *Gammaproteobacteria* express cytidine deaminase (CDD), an enzyme that can directly inactivate gemcitabine, reducing the local concentration in tumors and thereby diminishing chemotherapeutic efficacy. In PDAC tumor tissues, 51.7% of all reads from 16S rDNA sequencing belonged to the class *Gammaproteobacteria* and 14 of 15 (93%) cultured bacteria conferred resistance to gemcitabine in CRC cells[145-147]. In addition, some intratumoral bacteria harbor homologs of human enzymes involved in drug metabolism. For example, LaCourse et al.[148] identified an *E. coli* strain in CRC tissues that expresses enzymes (*pre*T and *pre*A) highly homologous to human dihydropyrimidine dehydrogenase. These enzymes convert 5-FU into inactive dihydrofluorouracil, facilitating survival of the 5-FU-sensitive pathogen, *F. nucleatum*, and reducing drug efficacy in CRC cells[147]. Therefore, targeting these drug-resistance-conferring bacteria or improving drug performance through targeted modifications may represent effective strategies to overcome intratumoral bacteria-mediated chemoresistance.

The acidic and hypoxic TME

Rapid tumor growth leads to intratumoral hypoxia, which stabilizes and activates the key regulatory factor, HIF-1α. HIF-1α induces the Warburg effect, increasing glycolytic flux in tumor cells, generating lactate as an end product, and resulting in acidification of the microenvironment. The extracellular pH of the TME is typically maintained between 6.7 and 7.1, whereas cancer cells exhibit a relatively higher intracellular pH of approximately 7.4[149]. Lactate accumulation is accompanied by marked reductions in butyrate and propionate production and by shifts in microbiota composition, with *Bacteroidetes* and *Firmicutes* being replaced by *Actinobacteria*, *Lactobacilli*, and *Proteobacteria*[150]. Interestingly, microbiota can alter the transcriptomic profiles to resist the acidic microenvironment in CRC[151], suggesting that selective pressure within the TME shapes not only aggressive cancer cells but also adaptive intratumoral microbiota. In turn, intratumoral microbiota may influence acidic and hypoxic TMEs through multiple mechanisms. First, anaerobic bacteria, like tumor cells, utilize lactate dehydrogenase (LDH) to convert pyruvate into lactate, further contributing to environmental acidification[152]. *F. nucleatum* indirectly activates GalNAc-autophagy–TBC1D5 signaling, promoting GLUT1 aggregation at the plasma membrane and subsequent extracellular lactate accumulation[153]. In addition, butyrate has been shown to stabilize and activate HIF-1α in intestinal epithelial cells under normal and colitic conditions[40,154], suggesting that bacteria-derived metabolites may participate in pathways that support epithelial adaptation to hypoxia. Whether intratumoral bacteria are directly involved in this specific process is unclear.

These observations highlight the complex cross-talk between tumor-resident bacteria and non-cellular components of the TME. The gut microbiome represents a promising target for intervention through dietary modulation, probiotics, and FMT. Accordingly, further investigation into the mechanisms by which gut microbes translocate and colonize tumors, intratumoral bacteria influence cellular tumor components, and intratumoral bacteria modulate non-cellular TME features is clearly warranted.

The clinical implications of intratumoral microbiota in gastrointestinal cancers

Diagnosis and prognosis

As an integral component of the TME, intratumoral microbiota provides a promising avenue for cancer diagnosis and prognosis. The distinctive signatures, reflected in composition, metabolism, and spatial organization, offer important biological insights. When systematically characterized, these features may be translated into diagnostic and prognostic tools, enabling a more personalized approach to gastrointestinal cancer management (**Table 2**).

A recent study analyzed tissue microbiota from 53 GC patients and 30 patients with chronic gastritis using 16S rRNA gene sequencing to investigate microbiota alterations within the GC TME[81]. Intratumoral *Methylobacterium* abundance was shown to be significantly associated with poor prognosis in GC patients. In addition, *Methylobacterium* abundance correlated with reduced frequencies of $CD8^+$ tissue-resident memory T cells and TGFβ. These findings suggested that *Methylobacterium* may contribute to GC progression and could serve as a marker of unfavorable prognosis. Analyses of The Cancer Genome Atlas database have further identified the *Klebsiella* genus, *Kytococcus sedentarius*, and *Actinomyces oris* as being associated with poor outcomes in GC[155,156]. Gao and colleagues reported that intratumoral bacteria, including *Pseudomonas* and *Leptospira*, recruit diverse immune cell populations by regulating chemokine expression, thereby influencing the prognosis of GC[157].

Several intratumoral bacteria have been linked to patient prognosis in CRC with *F. nucleatum*, *Hungatella*, and *Selenomonas* associated with poorer outcomes[158,159]. Intratumoral microbiota also shows potential as a predictor of therapeutic response. One study demonstrated that the presence of intratumoral *Bifidobacterium* is associated with improved immunotherapy responses[160]. Another study established a predictive signature combining 22 intratumoral bacterial taxa (including *Akkermansia*, *Clostridium*, and *Flavonifractor*) with 3 immune cell types, achieving an area under the curve (AUC) of 0.888 for predicting immunotherapy response in CRC patients[164]. High *F. nucleatum* abundance was associated with favorable disease-free survival in stage III or high-risk stage II CRC patients receiving adjuvant FOLFOX or CAPOX[161]. The presence of *Ruminococcus* was associated with complete response in patients with locally advanced rectal cancer undergoing neoadjuvant chemoradiotherapy (nCRT), whereas *Fusobacterium*, *Porphyromonas*, and *Oscillibacter* spp. were linked to non-complete responses[162]. Additional analyses identified a distinct resistome comprising *Streptococcus equinus*, *Schaalia odontolytica*, *Clostridium*

Table 2 Key prognosis-associated microbes in gastrointestinal cancers and the proposed mechanisms

Tumor	Microbiota	Prognosis	Mechanism	Ref.
GC	*Methylobacterium*	Poor	Correlate with a decrease in the frequency of $CD8^+$ tissue-resident memory T cells and the expression of TGFβ	90
GC	*Klebsiella*	Better	Associated with an increased gamma-delta T cell infiltrate	155
GC	*Kytococcus sedentarius*, *Actinomyces oris*	Poor	Interacted strongly with methylation changes in immune genes	156
GC	*Pseudomonas*, *Leptospira*	Poor Better	Regulating different chemokines accumulation to recruit multiple types of immune cells	157
CRC	*Hungatella*, *Selenomonas*	Poor	–	158
CRC	*Fusobacterium nucleatum*	Poor	Independently associated with dMMR status	159
CRC	*Bifidobacteria*	Better	Associated with the extent of signet ring cells	160
CRC	*Fusobacterium nucleatum*	Better	–	161
CRC	*Fusobacterium*, *Porhpyromonas*, *Oscillibacter*	Poor	–	162
CRC	*Streptococcus equinus*, *Schaalia odontolytica*, *Clostridium hylemonae*, *Blautia producta*, *Pseudomonas azotoformans*	Poor	Linked nitrate and sulfate-sulfur assimilation, histidine catabolic process, and resistance to cephamycin to the reduced therapeutic response	163
HCC	*Intestinimonas*, *Brachybacterium*, *Rothia*	Poor Better	Associated with pro-inflammatory and cell proliferation pathways Associated with metabolism and cytokine–cytokine receptor interaction pathways	15

CRC, colorectal cancer; dMMR, deficient MMR; GC, gastric cancer; HCC, hepatocellular carcinoma.

hylemonae, *Blautia producta*, and *Pseudomonas azotoformans*, which was also associated with nCRT resistance[163].

Multiple studies have confirmed the presence of intratumoral bacteria in HCC using diverse approaches, including tissue culture and FISH[17,75,100]. Huang et al.[165] applied 16S rRNA sequencing to analyze the diversity and structure of intratumoral microbiota in HCC and developed a random forest machine learning model that accurately predicted HCC based on intratumoral bacterial features. Another study involving 172 surgically treated HCC patients compared intratumoral microbiota between patients with long and short overall survival (OS)[15]. The presence of *Intestinimonas*, *Brachybacterium*, and *Rothia* was identified as an independent risk factor for OS in HCC patients.

In addition to individual bacterial taxa, several studies have integrated multi-omics data to classify GC, CRC, or HCC into subtypes characterized by distinct microbial community structures. Wang et al.[10] performed 16S rRNA sequencing on tumor tissues from 251 GC patients and identified 3 subtypes (M1, M2, and M3). The M3 subtype, enriched in *Sphingobium*, *Delftia*, *Comamonas*, and *Stenotrophomonas*, also showed enrichment of Wnt and EMT pathways and was associated with the poorest prognosis and lowest chemotherapy efficacy. In another study, Yuan et al.[81] conducted 16S rRNA sequencing on 290 GC tumors and paired adjacent normal tissues, identifying group III and IV tumors enriched in *Streptococcus* or *Pseudomonas*, respectively, with the worst prognosis. Notably, *S. anginosus* was successfully cultured from GC tumor tissues and shown to simultaneously promote tumor cell proliferation while suppressing $CD8^+$ T-cell differentiation.

Yan et al.[166] compared the intratumoral microbiota of CRC patients with and without liver metastases. Yan et al.[166] identified four genera (*Methylobacterium*, *Agrobacterium*, *Faecalibacterium*, and *Fusobacterium*) that were significantly less abundant in the metastasis group. A signature combining these four genera accurately predicted the occurrence of liver metastasis in CRC patients (AUC = 0.78). Subsequently, the researchers classified CRC liver metastasis (CRLM) samples into three distinct subtypes based on microbial community features. Subtype 1, characterized by detectable *Methylobacterium* alone or the absence of all four genera, was associated with the longest disease-free survival (DFS). In contrast, subtype 3, defined by the presence of three or all four identified intratumoral pathogens, exhibited the shortest DFS. This study established a novel prognostic stratification system for CRLM based on intramicrobial characteristics.

A study involving 29 HBV-related cases in HCC classified tumors into bacteria- and virus-dominant subtypes based on intramicrobial signatures[13]. *E. coli*, *Shigella dysenteriae*, *Babesia bigemina*, and *Pannonibacter phragmitetus* were predominantly detected in the bacteria subtype, whereas HBV was mainly observed in the virus subtype. The bacteria subtype displayed a more aggressive phenotype, characterized by larger tumor size, poorer differentiation, a higher incidence of vascular invasion and satellite nodules, and earlier recurrence. Another study categorized 91 HCC cases into 2 hepatotypes based on microbial signatures[14]. LEfSe analysis showed that *Proteobacteria* and *Actinobacteria* were significantly enriched in hepatotype A, whereas *Firmicutes*, *Fusobacteria*, and *Bacteroidetes* were more abundant in hepatotype B. Patients in the hepatotype B group had a significantly shorter OS and hepatotype was identified as an independent prognostic risk factor. Collectively, these findings demonstrated that intratumoral microbial features in HCC are closely associated with clinicopathologic characteristics and treatment outcomes, supporting the potential utility as prognostic biomarkers.

Treatment

Although substantial progress has been achieved in gastrointestinal cancer therapy, the efficacy of current treatments still requires improvement. One promising strategy involves fostering beneficial host-microbiota symbiosis and exploiting the intratumoral microbiome to complement conventional therapies. While microbiota modulation approaches, such as FMT, probiotics, or antibiotics, are known to influence tumor growth and therapeutic[100,167,168], these interventions may indiscriminately disrupt gut barrier homeostasis. Refining therapeutic strategies to enable precise targeting of intratumoral microbiota therefore represents an important and promising direction in microbiome-based oncology.

Multiple studies have shown that advanced drug delivery carriers allow precise delivery of antibiotics and anti-tumor agents to tumor sites. This strategy eliminates intratumoral pathogenic bacteria with minimal disturbance to the gut microbiota while improving the bioavailability and efficacy of anticancer drugs. Hu et al.[169] developed an oral drug carrier incorporating tellurium by complexing tellurium-containing polycarbonate with cisplatin (PTE@CDDP). PTE@CDDP effectively eliminated *F. nucleatum*, reduced tumor cell proliferation and local inflammation, and significantly enhanced the oral bioavailability of cisplatin. Similarly, other groups have developed delivery systems co-loaded with oxaliplatin and metronidazole, metronidazole-fluorouridine nanoparticles, and *F. nucleatum* nanovaccines[170-172]. These platforms simultaneously target intratumoral microbes and cancer cells, resulting in synergistic antitumor effects.

Alternatively, microbes can be directly exploited as antitumor agents. Scientists have engineered bacteria that combat tumors through several mechanisms, including the production of cytotoxic substances, enhancement of anti-tumor immune responses, and modulation of the TME. Bacteria possess an intrinsic ability to colonize tumors and release therapeutic molecules in a regulated manner. In one study, non-pathogenic *E. coli* was engineered as a drug delivery carrier by introducing a TNF-α expression vector[173]. Animal experiments

confirmed that these engineered bacteria enabled robust and sustained TNF-α production, thereby significantly inhibiting tumor growth. In another investigation, researchers isolated naturally occurring purple photosynthetic bacteria[174], which display inherent biocompatibility and strong immunogenic anti-cancer properties. These bacteria selectively colonize and proliferate within the TME, effectively enhancing immune cell infiltration and inducing potent anti-cancer responses across multiple syngeneic mouse models.

Collectively, these studies highlight the safety profiles and translational potential of engineered bacterial systems and nanocarrier platforms. Genetically engineered bacteria designed for tumor-specific colonization and localized therapeutic molecule production (e.g., TNF-α) demonstrate precise intratumoral targeting with minimal systemic toxicity. Preclinical evidence supports the biosafety and specificity. In parallel, nanocarrier systems, including colon-targeted liposomes, metronidazole-fluorouridine nanoparticles, antibacterial nanovaccines, and tellurium-incorporated polymeric carriers, improve delivery accuracy through microenvironment-responsive release and enhanced tumor accumulation. These platforms rely on biocompatible materials and context-sensitive designs to reduce off-target effects while supporting synergistic chemo-immunotherapeutic efficacy.

Taken together, these approaches reflect a shift in cancer treatment paradigms by leveraging interactions between intratumoral microbiota and host immunity. The demonstrated safety and feasibility provide a strong foundation for future clinical development in GC, CRC, and HCC.

Future perspectives

The understanding of intratumoral microbiota is fundamentally reshaping current views of gastrointestinal cancer biology. Tumor-associated microbes exhibit diverse origins, substantial inter-individual variability, and distinct organ- and tissue-specific distribution patterns. However, based on available evidence, knowledge of intratumoral microbiota remains incomplete. This emerging field holds considerable promise but is also accompanied by significant challenges. Rational utilization of intratumoral microbiota may enable the development of new frameworks for early cancer diagnosis, multimodal treatment strategies, and assessment of prognosis and therapeutic response, which represent central directions for future research.

At present, many studies rely on relatively low-resolution detection approaches and limited sample sizes to characterize disease-associated changes in intratumoral microbiota. As a result, conclusions drawn from such studies may not fully capture the true biological landscape. Future investigations should apply advanced technologies, including metagenomic sequencing, spatial transcriptomics, and microbial *in situ* imaging, to achieve high-resolution and comprehensive profiling of intratumoral microbiota and the interactions with host cells in large-scale, multicenter cohorts.

Many existing studies primarily describe associations between intratumoral microbiota composition and clinical phenotypes or outcomes, while underlying mechanisms remain largely unexplored. Future work should integrate experimental models, such as organoids and xenograft systems with germ-free animals, to mechanistically define how specific microbes or the components drive tumorigenesis, immune modulation or evasion, and therapy resistance. Such approaches will be essential for identifying concrete molecular mechanisms and actionable therapeutic targets.

The ultimate objective is to develop precise and personalized therapies targeting intratumoral microbiota. With deeper functional insight into intratumoral microbial activity, a new generation of precision microecologic therapies may emerge. Potential strategies include engineered phages designed to selectively eliminate pro-carcinogenic bacteria, as well as bacteria-drug combination therapies aimed at reversing treatment resistance through microbiota modulation. In addition, intratumoral microbiota shows promise as a biomarker for predicting therapeutic response and guiding patient stratification. Nevertheless, translation of these approaches into clinical practice will require high-quality prospective studies with larger cohorts and extended follow-up periods.

Conclusions

In summary, intratumoral microbiota has emerged as a fundamental and active component of the gastrointestinal cancer ecosystem, substantially reshaping understanding of tumor biology. The intratumoral microbiota is now recognized as a dynamic contributor to carcinogenesis, tumor progression, and therapeutic response. This review has integrated current evidence demonstrating how specific microbial communities and their metabolites influence key cancer hallmarks, including sustained proliferative signaling, immune evasion, and metabolic reprogramming. While associations between microbial dysbiosis and clinical outcomes are increasingly apparent, the field must now move beyond correlation toward causation. Future efforts are needed to delineate precise mechanistic pathways, validate findings in large-scale prospective cohorts, and translate these insights into clinical applications. The translational potential includes the use of intratumoral microbes as diagnostic and prognostic biomarkers, engineered bacteria for targeted drug delivery, microbiota modulation in combination with immunotherapy, and precision interventions, such as phage therapy and nanocarrier systems. However, critical gaps remain, including the need to establish causal relationships, map spatial and temporal microbial heterogeneity within tumors, standardize analytical methodologies, and rigorously evaluate safety and efficacy in human studies prior to clinical adoption.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contribution

Conceived and designed the analysis: Lixia Xu, Lei Zhou, Ying Zhang.
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