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Intratumoral microbiota in colorectal cancer: focus on specific distribution and potential mechanisms

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Abstract

Colorectal cancer (CRC) is one of the most prevalent and lethal malignant tumors globally, posing significant health risks and societal burdens. Recently, advancements in next-generation sequencing technology have identified CRC intratumoral microbiota, thereby opening up novel avenues for further research. This review synthesizes the current advancements in CRC intratumoral microbiota and their impact on CRC progression and discusses the disparities in the relative abundance and community composition of CRC intratumoral microbiota across various colorectal tumors based on their anatomical location and molecular subtypes, as well as the tumor stages, and spatial tumor distribution. Intratumoral microbiota predominantly influence CRC development by modulating colonic epithelial cells, tumor cells, and the tumor microenvironment. Mechanistically, they can cause DNA damage, apoptosis and epithelial-mesenchymal transition. The effects of different intratumoral microbiota on CRC have been shown to be two-fold. In the future, to address the limitations of existing studies, it is important to develop comprehensive experimental protocols and suitable in vitro models for elucidating more mechanisms of intratumoral microbiota on CRC, which will facilitate the clinical application of microbe-related therapeutic strategies in CRC and potentially other tumors.

Keywords Intratumoral microbiota, Colorectal cancer, Specific distribution, Tumor microenvironment

Colorectal cancer (CRC) is the third most prevalent malignant tumor globally, with the third highest incidence and second highest mortality rates, thereby representing the most prevalent malignancy in the digestive system [1]. Due to significant lifestyle alterations impacting microorganisms, with a notable trend towards a younger demographic affected by CRC [2]. The hypoxic and nutritionally rich immunosuppressive milieu within CRC tissues provides an opportune environment for microbial survival. Microorganisms can coexist with host cells in various bodily regions, such as the skin, mouth and gastrointestinal tract, exerting influence on both physiological and pathological processes. Previous investigations have underscored the pivotal role of microorganisms in influencing tumorigenesis, progression, and prognosis [3, 4].

Bacteria were initially identified in tumor tissues over a century ago. However, the recognition of intratumoral microbes has been limited by challenges such as ineffective decontamination methods, the extremely low abundance of microbes within tumors, and substantial interference from host DNA. The advent of next-generation sequencing technologies has revolutionized research

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on intratumoral microbes. In 2020, Nejman et al. [5] examined 1,526 tumor tissues and adjacent normal tissues across seven cancer types and observed characteristic intratumoral microorganisms in different tumors, primarily located within tumor cells and immune cells. Similarly, in 2022, Narunsky-Haziza et al. [6] detected fungi in all 35 tumor tissues, predominantly within tumor cells. Anders B et al. [7] also reported the presence of fungi in gastrointestinal tumors, with a high abundance of *Candida* and *Yeasts* notably associated with gastrointestinal cancers. With the rapid advancement of intratumor microbial detection and analysis techniques, recent studies have elucidated the spatial distribution and localized effects of intratumoral microbes and explored interactions between host cells and microbes at spatial, cellular, and molecular levels. These investigations suggest that microbial distribution within tumors is not random but rather organized into microecological niches that influence CRC progression by modulating functions such as immune and epithelial cells [8].

This review synthesizes recent research on intratumoral microorganisms enriched in CRC and their impact on the disease to stimulate further exploration of microorganisms in colorectal tissues as potential biomarkers for CRC diagnosis, treatment, and prognosis.

Potential sources and detection methods of intratumoral microbiota in CRC

A-diversity and β-diversity are two important measures of microbial community diversity. α-diversity refers to the species richness of microorganisms within a single sample, while β-diversity refers to the differences in microbial composition between samples. CRC intratumoral microorganisms exhibit decreased α-diversity and increased β-diversity compared to normal controls [9], which is characterized by an increased abundance of Proteobacteria, Fusobacteria, Campylobacteria, and Spirochaetes, alongside a decreased abundance of Bacteroidetes, Firmicutes, Verrucomicrobia, Actinobacteria, and Euarchaeota in tumor tissues [10].

Potential sources of intratumoral microbiota in CRC

Colorectal intratumoral microorganisms primarily originate from the intestines, the oral cavity and adjacent normal tissues, where intestinal mucosal damage and blood circulation facilitate their colonization within tumors (Fig. 1). The large number of microorganisms in the gut serves as an important source of intratumoral microorganisms in CRC. Research indicates that disruptions in the intestinal mucosal barrier, induced by various factors, facilitate the infiltration of intestinal microorganisms into

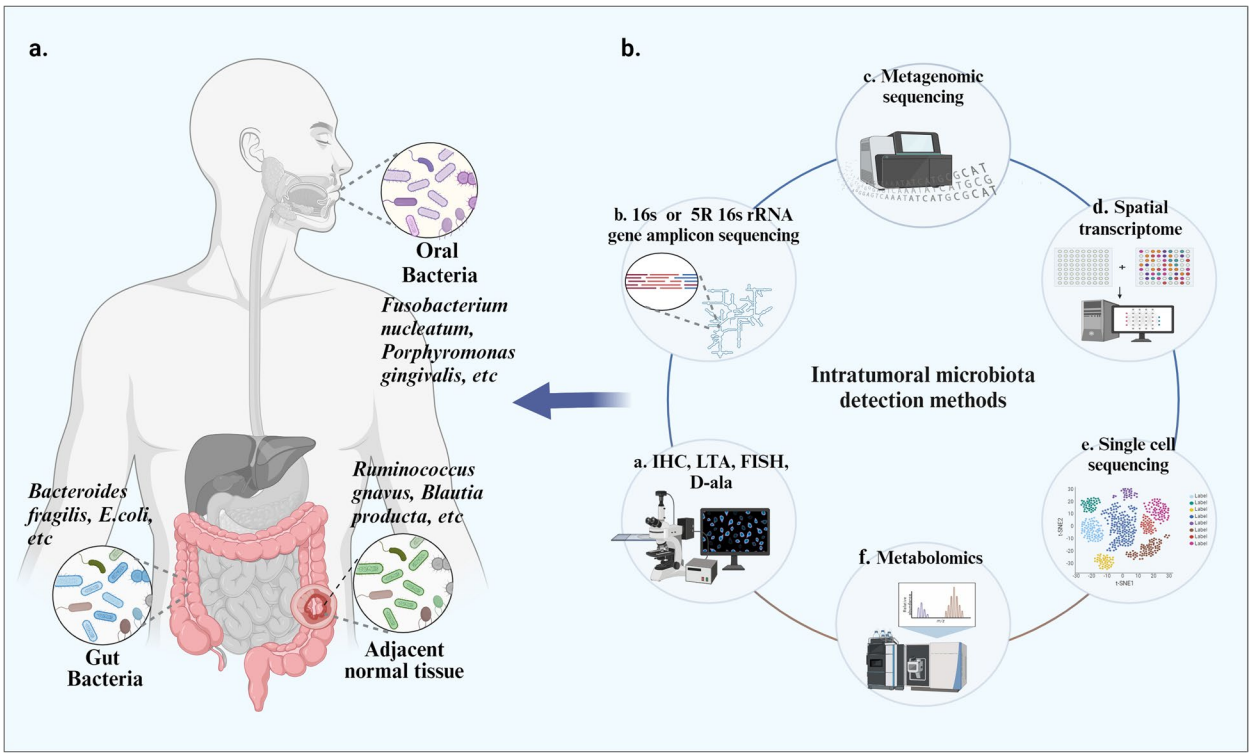

Fig. 1 Potential sources and detection methods of intratumoral microbiota in CRC. **a** Three potential source of intratumoral microbiota in CRC; **b** The detection methods of intratumoral microbiota in CRC. Graphics created using BioRender.com

colonic tissues [4]. Additionally, blood circulation helps transfer oral microorganisms to CRC tissues. Younginger et al. [11] conducted transcriptome analysis on 807 CRC samples, revealing 17 intratumoral bacteria originating from the oral flora, including *Fusobacterium, morbillorum*, *Parvimonas micra*, and *Peptostreptococcus stomatis (P. stomatis)*. In addition, studies have revealed that intratumoral *Escherichia coli* (*E. coli*) can disseminate to the liver via the compromised intestinal vascular barrier, promoting the establishment of a hepatic "pre-metastatic ecological niche" and thereby facilitating hepatic metastasis from primary CRC [4]. Thus, blood circulation serves not only as an important conduit for microorganisms to colonize cancerous tissues but also as an essential route for mediating tumor cell metastasis to distant sites. Thus, investigating the microbial composition of various sites aids in identifying microorganisms significantly associated with CRC development. Circulating microbial DNA (cmDNA) in tumor patients mainly originates from microbial translocation such as oral cavity or intestinal tract, passive release of endogenous microbial DNA following cell death, and active secretion by cells. cmDNA has specific biological characteristics and has the potential to become a biomarker for tumors, and various researches have shown that early screening for tumors can be performed through the detection of cmDNA [12, 13]

The process by which microbes invading tumors is intricate and involves a multitude of pathways. Intestinal microorganisms can penetrate tumor tissues through compromised intestinal mucosa. For example, Tjalsma et al. [14] proposed the "Driver-passenger" model, according to which *Enterotoxigenic Bacteroides fragilis* (*ETBF*) serves as the "driver" bacteria, actively colonizing and invading the colon, causing damage and creating conducive conditions for "passenger" bacteria to infiltrate and establish within the tumor microenvironment. For oral microorganisms, it has been observed that *Fusobacterium nucleatum* (*Fn*) is significantly enriched in CRC. Further studies revealed that Galactose-N-acetylgalactosamine (Gal-GalNAc), which is overexpressed in CRC cells, can be recognized by bacterial fibroblast activation protein (Fap) and promote *Fn* colonization in CRC tissue through the blood-borne transmission pathway [15]. Another study suggests that *P stomatis* can bind to integrin α6/β4 receptors on the surface of CRC cells through its surface protein fructose-1,6-diphosphate aldolase, promoting the adhesion and colonization of *P. stomatis* in the intestine [16].

Detection methods of intratumoral microbiota in CRC

Advancements in detection methods have enabled a comprehensive analysis of all microorganisms without the necessity for culture (Fig. 1). Techniques such as immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) and D-alanine-based assays are commonly used for detecting intratumoral microorganisms [17]. 16S rRNA sequencing and macrogenomic sequencing are the primary methods utilized to characterize microbial composition and abundance. While 16S rRNA sequencing is the main tool for microbiome characterization, its utility in tumor tissues, where microbial abundance is notably low, remains limited. Nejman et al. [5] addressed this limitation by developing the 5R 16 s sequencing protocol for amplifying five regions on the 16S rRNA gene. By amplifying 68% of the bacterial 16S rRNA gene using short amplicons, this method enhances the coverage and resolution of bacterial species detection and proves to be more advantageous for intratumoral microbial detection compared to the conventional V4 or V3-V4 amplification commonly employed for 16S rRNA analysis. Macrogenomics employs high-throughput sequencing of genomic DNA from all microorganisms in a sample, enabling not only the analysis of microbial abundance and diversity but also of colony function and associated metabolic pathways. Additionally, the integration of multi-omics represents the primary research approach for functional analysis of intratumoral microorganisms [8, 18–20]. For instance, SAHMI, a single-cell analytical method, investigates host-microbe interactions at the single-cell level by denoising single-cell sequencing data and recovering microbial signals [19]. Additionally, INVADE seq can identify bacteria in host cells by targeting conserved regions of 16S rRNA with primers. When coupled with spatial genomics, it reveals microbial distribution in the tumor microenvironment, providing insights into host cell-microbe interactions across spatial, cellular, and molecular levels [20].

Intratumoral microbiota in colorectal tumors

Intratumoral microbiota in different molecular subtypes of CRC

Consensus molecular subtypes (CMSs) of CRC is a classification based on the molecular characteristics of tumors, which classifies CRC into four distinct subtypes, each with different biological characteristics, clinical manifestations and therapeutic responses [21]: CMS1 immune subtype: characterized by histology exhibiting massive lymphocytic infiltration, with microsatellite instability being common. CMS2 tumorigenic subtype: exhibits activation of WNT and MYC signal pathways and higher chromosomal instability. CMS3 metabolic subtype: demonstrates significant metabolic dysregulation, often accompanied by a high frequency of K-RAS mutations. CMS4 mesenchymal subtype: shows enhancements in both immunosuppressive and fibrotic responses. The

different molecular subtypes of CRC display variations in intratumoral microbial composition and abundance. Younginger et al. [11] observed that the relationship between *Fusobacterium animalis* (*Fa*) and tumor gene expression varied by CMS through transcriptome analysis of colon tumor tissues. Differentially expressed genes in CMS1 and CMS2 tumors are strongly associated with *Fa*. Another study performed 16S rRNA sequencing of tumor and paracancerous tissues from 423 patients with stage I-IV CRC to categorize CRC in terms of oncomicrobial community subtypes (OCS) [10]. The results showed that 21% were OCS1, enriched for oral pathogens such as *Fusobacteria*, exhibiting high CRC grade, positive MSI-H, CpG island methylation phenotypes, and commonly containing BRAF V600E and FBXM7 mutations. Additionally, 44% were OCS2, enriched with *Firmicutes* and *Bacteroidetes*, and 35% were OCS3, enriched with *Escherichia* and *Shigella*, associated with chromosomal instability. Byrd et al. [22] also found higher abundance of *Alisterella*, *Casatella*, and *Fusobacteriaceae* in tumor tissues with high microsatellite instability. *Fn* is also preferentially enriched in CRC tumor tissues with KRAS p.G12D mutations, and it promotes tumor progression of KRAS p.G12D mutant CRC by binding to DEAH-box helicase 15 [23].

Dynamic changes of Intratumoral microbiota during the evolution of CRC

Dynamic changes in intratumoral microbial composition are closely associated with CRC progression. During colorectal adenoma-carcinoma evolution, the adenoma stage high-variable microbe (HVM) was found to be mainly *Ascomycota* (43.14%). However, the proportion of *Ascomycota* gradually declined with adenoma-carcinoma progression, being supplanted by the *Firmicutes* (54.0%). Additionally, the number of HVMs within the tumor decreased along the adenoma-carcinoma evolution [24]. This study underscores the heterogeneity of microbial communities within colorectal tumors or precancerous adenomas, highlighting their significant association with adenoma-carcinoma sequence changes. The "Driver passenger" model divides CRC into four stages of development: normal, hyperplasia, adenoma, and tumor. During the four developmental stages of CRC, "driver" bacteria predominantly manifest in the early phases, where they alter the intestinal microenvironment and facilitate the colonization of "passenger" bacteria. These "passenger" bacteria become enriched in the later stages of CRC progression, encompassing some opportunistic pathogens and even probiotics [14]. Therefore, intratumoral microbial detection has the potential to differentiate the developmental stages of CRC. For instance, Geng et al. [25] used 454 pyrosequencing of bacterial 16S rRNA genes associated with normal, adenoma, and tumor biopsy samples, and found 7 potential "driver" bacterial genera and 12 potential "passenger" bacterial genera.

Furthermore, the research also revealed that the composition and abundance of microbes in tumor tissues vary at different stages of malignancy. The ratio of *Candida* to *yeast* is usually lower in early-stage CRC, but it significantly increases in stage IV tumors [26]. In addition, the abundance and composition of CRC intratumoural microorganisms differed in TNM staging. Mira-Pascual et al. [27] analyzed the relationship between tumor stage and microbiota composition. They found that the abundance of *Staphylococcus* was lower in T2 and T3 tumors, while the abundance of *Streptococcus* was higher in T3 tumors. *Fn* is notably enriched in CRC tissues, closely linked to CRC proliferation and metastasis, and can serve as a predictive molecule for the course of CRC disease [28]. *Fn* was found to account for 5.9% of adenoma tissue, escalating to 81.8% as CRC progressed to III/IV stages [29].

Distribution of the intratumoural microbiota in right and left colon and rectum tumors

Left and right colon tumors differ significantly in a number of ways, these include epidemiological features, clinical presentation, molecular features, response to treatment, and prognosis. It was found that the composition and abundance of intratumoural microbial vary among tumors in right and left colon. Studies have shown that right-sided tumors are more homogeneous in microbial composition and more likely to lead to a poor prognosis [30, 31]. Nardelli et al. [11] found that *Alistipes spp.*, *Bacteroides spp.* and *Parabacteroides distasonis* were significantly more abundant in the right colon [32]. Mouradov et al. [10] found that tumors enriched with oral pathogens such as Fusobacteria occurred more often in the right side of the colon, whereas tumors enriched with Firmicutes, Bacteroidetes, *Escherichia*, or *Shigella* developed in the left side of the colon. Due to the differences in microbes within tumors of the right and left colon, some researchers have identified important microbial and genomic biomarkers by constructing mathematical models to distinguish between right and left CRCs [33]. In their model, it was also found that *Ruminococcus gnavus*, *Clostridium acetereducens*, *Lachnospiraceae*, and *Ruminococcus sp.* were enriched in the right colon, and *Akkermansia muciniphila* were enriched in the left colon. In addition, Alves et al. [34] found that *pks*$^{+}$*E.coli*, which belongs to Proteobacteria, was enriched in right-sided colon and that patients with *pks*$^{+}$*E.coli* had lower survival. Similarly, it was also found that patients with *Fn*-enriched right-sided colon had shorter progression-free survival times [35]. Research has indicated that in rectal

tumors, certain bacterial genera are more prevalent. Specifically, rectal-cancer samples exhibit higher levels of *Bacteroides*, *Phascolarctobacterium*, *Parabacteroides*, *Desulfovibrio*, and *Odoribacter*. In contrast, non-cancerous samples show a higher prevalence of *Pseudomonas*, *Escherichia*, *Acinetobacter*, *Lactobacillus*, and *Bacillus* [36].

Distribution of CRC intratumoral microbiota in different spatial locations of the tumor

Li et al. [37] observed significant differences between microbiota in tumor tissues and normal tissues from the same patient source but no notable differences in microorganisms between tumor and paracancerous tissues, or between paracancerous and normal tissues. These findings suggest that paracancerous microorganisms may represent a transitional state between tumor and normal tissue microorganisms. Thus, the microbial composition varies heterogeneously across different sites within the same tumor. Several studies on CRC intratumoral microorganisms have sampled multiple sites within the same tumor tissue and paired normal tissues for testing and analysis due to differences in microbial composition at various tissue sites [24, 38, 39]. Nonetheless, it has also been demonstrated that the variation in microbiome α-diversity among individual samples exceeds the variation within individual samples. Due to the extremely low levels of intratumoural microorganisms and the influence of environmental microorganisms, the existence of differences in microorganisms at different sites of the same sample needs to be confirmed with more and more precise clinical samples.

Currently, the existing research on the enrichment of intratumoral microbes in left-sided and right-sided CRC are relatively common and consistent. However, the evidence of associations between types of intratumoral microorganisms and different cancer subtypes and stages is still limited. In addition, current studies tend to concentrate on the disparities in the abundance of single intratumoral microorganism in different molecular subtypes or the abundance of intratumoral microbes in a single molecular subtype, lacking comprehensive and systematic research. Particularly, the trends in the changes of intratumoral microbiota during the process of transformation from colorectal adenoma to carcinoma or inflammatory carcinoma may be inconsistent. During tumor staging, the methodologies vary, some studies categorize based on stages I-IV, while others utilize the TNM staging system. This diversity in staging criteria has also influenced the synthesis and generalization of the overall patterns of intratumoral microbial abundance changes throughout the progression of CRC, as gleaned from an already limited body of literature.

CRC intratumoral microorganisms vary in composition and abundance across different parameters such as tumor subtypes, stages of adenoma-carcinoma progression, tumor tissues of distinct segments, and colon tumor tissues (Fig. 2). Additionally, these variations extend to factors like age of onset [40] and gender [41]. Consequently, a multitude of factors must be taken into account when sampling intratumoral microorganisms, particularly regarding the timing and location of the sampling Table 1 provide a characterization of intratumoral microorganisms in CRC.

Influence of intratumoral microbiota on CRC

The main microorganisms enriched in CRC tumors include *Fn*, *pks*$^{+}$*E.coli* and *ETBF*. Among these, *ETBF* mainly affects the early stage of CRC and promotes its progression. *ETBF* contributes to CRC by modulating CAC immune cells [62], inducing epigenetic changes [63]. *pks*$^{+}$*E.coli* causes DNA damage in colonic epithelial cells primarily through the production of the genotoxin colibactin, which leads to carcinogenesis. The effects of *Fn* on CRC are mainly focused on the development of CRC, including the formation of tumor microenvironment and tumor metastasis. In addition, other intratumoral microorganisms promote or inhibit the development of CRC through different biological pathways. Overall, intratumoral microorganisms exert their influence on CRC development by impacting intestinal epithelial cells, tumor cells, and the tumor microenvironment. Their mechanisms of action involve processes such as DNA damage, apoptosis, and epithelial-mesenchymal transition. Notably, different intratumoral microorganisms exhibit dual characteristics in their effects on CRC.

Effect of intratumoral microbiota on intestinal epithelial cells

DNA damage is a key driver in CRC development, and bacterial toxin production by intratumoral microorganisms acts directly on cells to cause DNA damage. *pks*$^{+}$*E. coli* is closely associated with DNA damage in CRC and produces the genotoxin colibactin, which causes the cells to undergo two types of characteristic DNA mutations: single base substitutions and indels. Using human intestinal organoids as a model, some researchers have found that single-base substitutions (T > N) induced by colibactin are frequently observed in A<u>T</u>N and T<u>T</u>T sequences [64]. Moreover, Chen et al. [65] observed enrichment of *pks*$^{+}$*E.coli* in both tumor tissues and paired normal tissues compared to healthy individuals. CRC patients exhibited a higher incidence of indels in genes closely associated with many driver mutations in CRC. Another study revealed increased DNA damage in colonic epithelial cells, accelerated tumorigenesis, and higher mortality

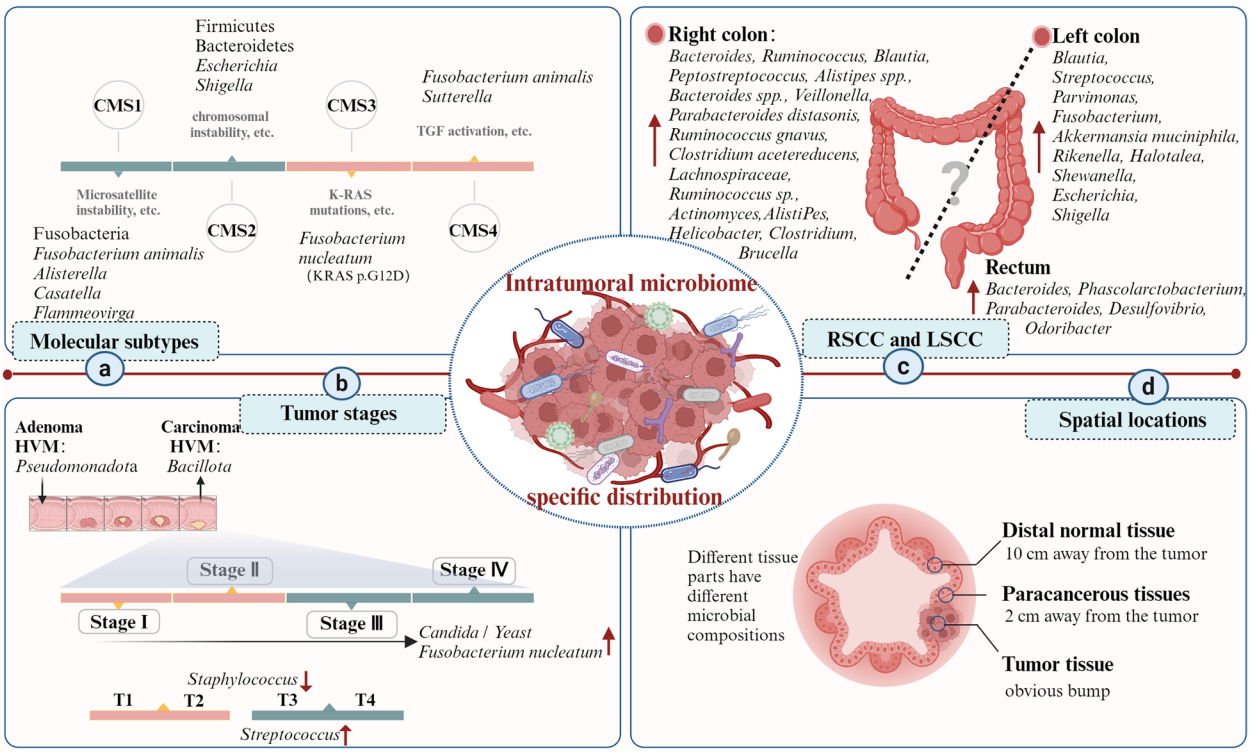

Fig. 2 Intratumoral microorganisms enriched in colorectal tumors. **a** Intratumoral microbiota in different molecular subtypes of CRC; **b** Dynamic changes of Intratumoral microbiota during the evolution of CRC; **c** Distribution of the intratumoural microbiota in right and left colon and rectum tumors; **d** Sampling of CRC intratumoral microbiota in different spatial locations of the tumor. Graphics created using BioRender.com

in tumor-susceptible mice harboring both *pks*$^{+}$*E.coli* and *ETBF* compared to mice with either *pks*$^{+}$*E.coli* and *ETBF* alone. These results suggest that *ETBF* enhanced *pks*$^{+}$*E. coli* colonization and DNA damage in colonic epithelial cells [66]. Additionally, research indicates that *Campylobacter* produces a cell-lethal expansion toxin leading to host DNA double-strand breaks [67]. Shujiro et al. [54] classified tumor samples into *Campylobacter* high and *Campylobacter* low groups based on *Campylobacter* abundance, revealing higher levels of host DNA mutations in the *Campylobacter* high group.

Effect of intratumoral microbiota on tumor cells

Regulating non-coding RNA

Interactions between intratumoural microbes and non-coding RNAs play an important role in CRC progression, which can affect tumor cell proliferation, apoptosis and metastasis (Fig. 3a). Unlike normal cells, tumor cells proliferate uncontrollably, and intratumoral microorganisms promote tumor cell proliferation. *ETBF* can down-regulate miR-149-3p, thereby promoting PHF5A-mediated selective splicing of KAT2A RNA in cells, leading to the trans-activation of SOD2 and ultimately contributing to CRC progression [62]. Moreover, *Porphyromonas gingivalis* can invade tumor cells and enhance their proliferation by activating the MAPK/ERK signaling pathway [68]. In CRC, tumor cell apoptosis is predominantly mediated by *Fn*. Yu et al. [69] discovered that *Fn* targets the TLR4/MYD88/MiR-18a*/ULK1 and TLR4/MYD88/miR-4802/ATG7 autophagy networks, activating the autophagy pathway to inhibit chemotherapy-induced apoptosis in CRC cells. Moreover, *Fn* can inhibit CRC cell apoptosis by impeding autophagic flux through miR-31 [70]. Intratumoral microorganisms not only promote tumor progression but also influence treatment response. Sonodynamic therapy (SDT) exhibits promising therapeutic potential as a complementary technique to conventional cancer treatment due to its deeper tissue penetration and safety profile. Qu et al. [71] developed antimicrobial nanoplatforms (Au@BSA-CuPpIX) capable of generating reactive oxygen species (ROS) and exhibiting potent anti-*Fn* activity under ultrasound. Au@BSA-CuPpIX induced ROS-mediated apoptosis by inhibiting *Fn* and reducing the level of apoptosis inhibitory protein. Furthermore, the treatment of CRC by designing nanomedicines to target and eliminate intratumoral microorganisms has emerged as a prominent research area in tumor therapy in recent years [72–75].

EMT serves as the pathological foundation for epithelial-origin malignant tumor cells to acquire migratory

Table 1 Characterization of the intratumoral microbiota in CRC

No	Number of clinical samples	Research methods	Major finding
1 [42]	14 pairs of CRC tissues and adjacent normal tissues	16 s rRNA, Metagenomics	Firmicutes, Bacteroidota, Proteobacteria and Actinobacteria are abundant in CRC samples
2 [43]	533 tissue microbiome samples of CRC patients and the corresponding metadata	TCMA	The relative abundance of dominant phyla, including Proteobacteria, Firmicutes, and Bacteroidetes, was significantly associated with survival in CRC patients
3 [44]	11 fresh-frozen primary colorectal cancer and paired liver metastasis samples; 77 fresh-frozen primary colorectal cancer samples	PCR, 16S rRNA, Metagenomics	*Fusobacterium* co-occurs with other Gram-negative anaerobes in primary and matched metastatic tumors
4 [45]	65 pairs of CRC tissues and adjacent normal tissues	Metagenomics	*Fusobacterium*, *Campylobacter* and *Leptotrichia* show patterns of co-occurrence in CRC. However, tumor isolates for *Fusobacterium* and *Campylobacter* are genetically diverged from their oral counterparts and carry potential virulence genes
5 [9]	353 pairs of CRC tissues and adjacent normal samples	16S data comes from the database	The normal microbiota (*Clostridia* and *Bacteroidetes*) continues to decrease, and oral-derived microorganisms (*Fusobacterium nucleatum* and *Parvomonas*) are significantly enriched in CRC
6 [46]	30 tumor tissue samples and 30 biopsy tissue samples from healthy people	16S rRNA	The CRC group had significantly different salivary and mucosal microbiome diversity than the HC group, but the fecal microbiome did not
7 [47]	44 pairs of CRC tissues and adjacent normal tissues	16S rRNA, PCR	*Fusobacterium, Providencia*
8 [48]	1313 CRC patient tissue samples, 50 pairs of CRC tissue and adjacent normal tissue	16S rRNA, PCR	*Bifidobacterium*
9 [49]	14 pairs of CRC tissues and adjacent normal tissues	Metagenomics	*Myroides odoratimimus*, *Cellulophaga baltica*
10 [36]	18 rectal-cancer subjects and 18 non-cancer controls	16S rRNA	*B. fragilis, phylum Parcubacteria*
11 [50]	31 pairs of CRC tissues and adjacent normal tissues;62 formalin-fixed paraffin-embedded samples (20 normal, 20 adenomas, and 22 colorectal cancer samples)	qPCR, IHC, FISH	*Porphyromonas gingivalis*
12 [51]	116 CRC samples	16S rRNA	*Prevotella intermedia*
13 [52]	96 CRC samples, 82 adenomas samples and 77 normal controls samples	PCR	*Peptostreptococcus anaerobius*
14 [53]	71 pairs of CRC tissues and adjacent normal tissues	16S rDNA	*Parvimonas micra*
15 [54]	29 CRC samples	16S rRNA	*Campylobacter*
16 [26]	172 CRC samples	16S rRNA	*Candida albicans*
17 [55]	32 pairs of CRC tissues, adjacent normal tissues and normal tissue	16S rRNA	*Ruminococcus, gnavus* 、 *Blautia producta*
18 [56]	24 CRC patients and 18 healthy controls	Metagenomics	Torque teno virus
19 [57]	41 CRC adenocarcinoma samples, 16 adenomatous polyp samples, and 9 non-tumor control samples	PCR	JC polyomavirus
20 [58]	107 CRC samples	PCR 、 IHC	Human Papillomaviruses, Epstein–Barr Virus
21 [11]	835 CRC samples	Whole transcriptome sequencing	*Fa* has the most significant associations in mesenchymal CMS4 tumors. Within CMS4, the prevalence of *Fa* is uniquely associated with collagen and immune-related pathways

Table 1 (continued)

No	Number of clinical samples	Research methods	Major finding
22 [59]	594 CRC samples	TCGA	The TME in CMS1 has inflammation-related HSF1 activation and interactions between endothelin pathway genes and *Flammeovirga*. Integrin-related genes in CMS2 are positively correlated with *Sutterell*, and biosynthetic and metabolic pathways in CMS3 are correlated with microorganisms. Genes in collagen biosynthesis are positively correlated with *Sutterella*, leading to disturbances in CMS4 homeostasis
23 [10]	Tumor and normal mucosa from 423 patients with stage I to IV CRC	16S rRNA	OCS1 (*Fusobacterium*, proteolytic, 21%), right-sided, high-grade, MSI-high, CIMP-positive, CMS1, BRAF V600E, and FBXW7 mutated. OCS2 (*Firmicutes/Bacteroidetes*, saccharolytic, 44%) and OCS3 (*Escherichia/Pseudescherichia/Shigella*, fatty acid β-oxidation, 35%) were located on the left and exhibiting CIN. OCS1 was associated with MSI-related mutation signatures (SBS15, SBS20, ID2, and ID7), and OCS2 and OCS3 are associated with SBS18-related reactive oxygen species damage
24 [22]	451 CRC samples	TCGA	*Alisterella*, *Casatella*, and *Fusobacteriaceae* are more abundant in MSI-H
25 [24]	36 CRC patients,32 adenoma patients (For each sample, 2–6 tumor site tissues and 2–5 adjacent normal tissues were taken.)	16S rRNA	The abundance of some CRC-associated microorganisms (*Fusobacterium*, *Bacteroides*, *Parvimonas* and *Prevotella*) varies greatly within individual tumors. Changes in the abundance of individual microbes within tumors follow the adenoma-carcinoma sequence
26 [29]	200 colorectal neoplasms (118 adenomas and 82 cancers) and 149 matched adjacent normal tissues	qPCR	*Fusobacterium nucleatum* presence in the tumor increased according to the pathological stage (5.9% [adenoma] to 81.8% [stage III/IV])
27 [30]	41 CRC samples	16S rRNA	In the right colon, *Bacteroides*, *Ruminococcus*, *Blautia*, *Peptostreptococcus* and *Veillonella* were enriched; in the left colon, *Blautia*, *Streptococcus*, *Parvimonas* and *Fusobacterium* were enriched
28 [32]	20 CRC samples and 20 healthy controls	16S rRNA	*Alistipes spp*., *Bacteroides spp*. And *Parabacteroides distasonis* were significantly more abundant in the right colon
29 [33]	308 CRC samples	16S rRNA	*Ruminococcus gnavus, Clostridium acetereducens*, *Lachnospiraceae*, and *Ruminococcus sp*. were enriched in the right colon, and *Akkermansia muciniphila* were enriched in the left colon
30 [60]	329 matched samples (329 tumor tissue and 329 adjacent non-tumor tissue)	16S rRNA	Compared with the left colon, at the hilus level, Bacteroidetes, Chloroflexi, Cyanobacteria, Deferribacteres, Elusimicrobia, Firmicutes and Fusobacteria were enriched in the right colon. At the genus level, *Actinomyces, AlistiPes* and *Helicobacter* were enriched in the right colon
31 [61]	59 CRC patient samples, 21 polyp patient samples, and 56 healthy control samples	PCR, 16S rRNA	In the right colon, *Clostridium and Brucella* were enriched; in the left colon, *Rikenella, Halotalea*, and *Shewanella* were enriched
32 [37]	93 matched samples (93 tumor tissue, 93 adjacent non-tumor tissue, and 93 normal tissue)	16S rRNA	The relative abundance of *Fusobacterium*, *Gemella*, *Campylobacter*, *Peptostreptococcus*, and *Parvimonas* steadily decreased along with the tumor, para-cancerous to normal mucosa
33 [40]	Tumors and paired adjacent normal tissues from 136 young-onset CRC and 140 average-onset CRC patients	16S rRNA	Compared with young-onset CRC, average-onset CRC has a richer microbial composition, and *Akkermansia* and *Bacteroidetes* are the main bacterial genera associated with young-onset CRC tumors

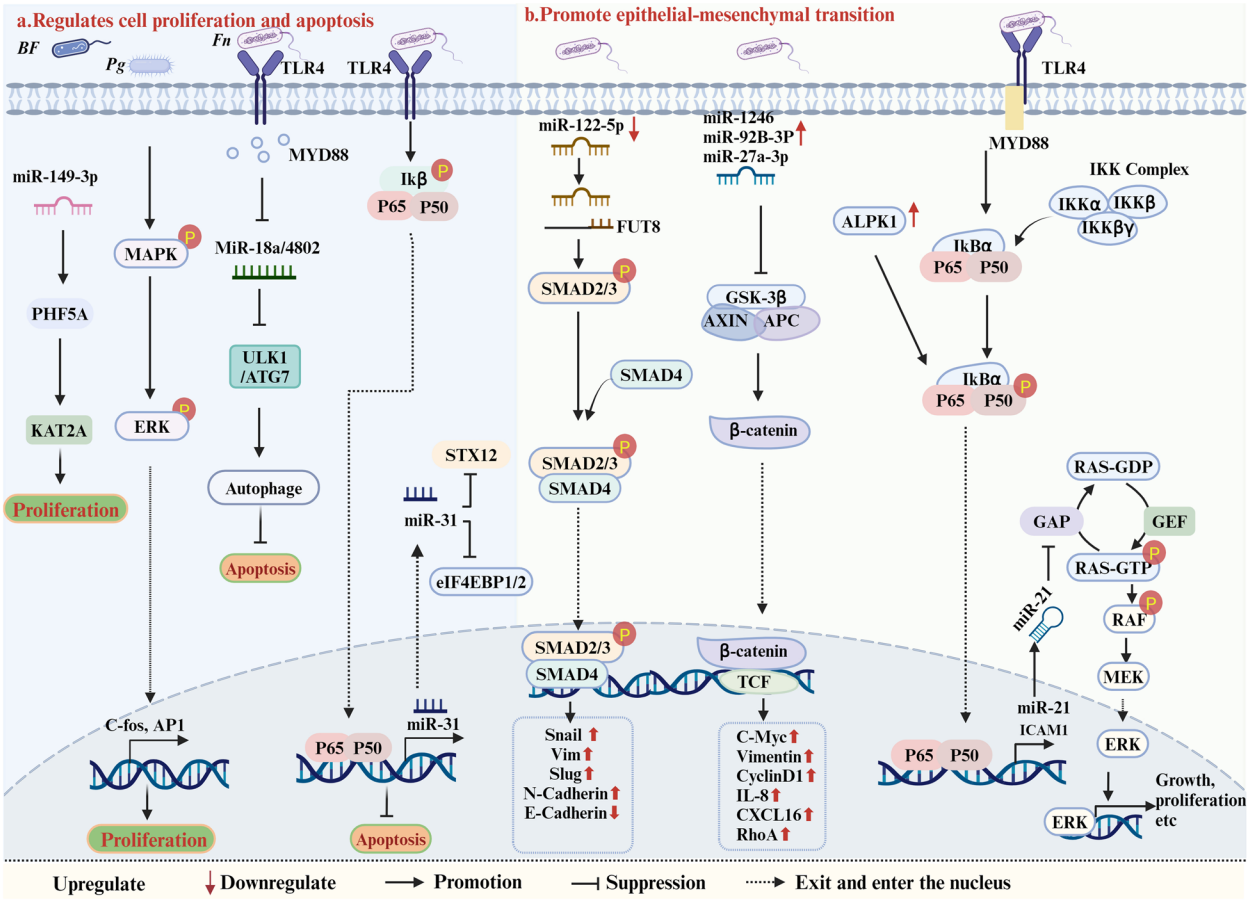

Fig. 3 Effect of intratumoral microorganisms on tumor cells. **a** Intratumoral microorganisms regulate tumor cell proliferation and apoptosis; **b** Intratumoral microorganisms promote tumor epithelial-to-mesenchymal transition. Graphics created using BioRender.com

and invasive capabilities, playing a pivotal role in CRC metastasis. Intratumoral microbes have been identified as promoters of CRC metastasis by mediating EMT (Fig. 3b). Exosomes released by microbe-infected cells participate in various aspects of tumor formation and invasion [76]. For instance, the tumor suppressor miR-122-5p is upregulated in the serum of CRC patients but downregulated in paired tumor tissues. Ex vivo and in vivo experiments have validated that *Fn* enhances CRC metastasis by facilitating the exocytosis of miR-122-5p, which subsequently activates the FUT8/TGF-β1/Smads axis and induces EMT [77]. *Fn* also prompts tumor cells to release exosomes rich in miR-1246/92b-3p/27a-3p and CXCL16/RhoA/IL-8. These exosomes, transmitted from *Fn*-infected to *Fn*-uninfected tumor cells, promote cell migration capacity, thereby fostering CRC liver metastasis [78]. Concurrently, intratumoral microorganisms metastasize to the liver along with colon cancer cells [44]. *Fn* additionally enhances CRC cell adhesion to endothelial cells by triggering the ALPK1/NF-κB/ICAM1 axis, thus facilitating extravasation and metastasis [79]. Moreover, it activates the TLR4/MYD88 signaling pathway, upregulating miR21 expression and consequently reducing the level of RAS GTPase-activating protein 1 (RASA1), thereby promoting CRC cell proliferation and migration [80]. Studies have also shown that *Fn* synergistically increases the invasiveness and EMT characteristics of CRC cells treated with dextran sulfate sodium (DSS) [81].

Metabolic reprogramming

The metabolic reprogramming is a crucial biological trait in tumor development, providing the energy and materials essential for tumor growth. Glycolysis is the main metabolic mode of tumor cells to meet their energy and macromolecule requirements. Increased glycolytic dependence also promotes tumor resistance to radiotherapy as well as triggers competition for nutrients between tumor cells and tumor-infiltrating cells. Zheng et al. [82] discovered that *Fn* stimulates glycolysis in cancer cells by inducing ANGPTL4 expression in CRC cells. Then, the increased ANGPTL4

levels promote *Fn* colonization by upregulating GLUT1 expression and glucose uptake. *Fn* also enhances the transcription of long non-coding RNA ENO1-IT1 by increasing the binding efficiency of transcription factor SP1 to the promoter region of lncRNA ENO1-IT1. The increased ENO1-IT1 acts as a guide for KAT7 histone acetyltransferase, promoting ENO1 histone modification and consequently enhancing tumor cell glycolysis [83]. Moreover, *Fn* activates the TLR4/Keap1/NRF2 pathway to elevate the expression of the tumor cell metabolic enzyme CYP2J2 and its product 12,13-EpOME, promoting fatty acid metabolism [84]. Tsoi et al. [52] found that *Peptostreptococcus anaerobius (P. anaerobius)* could induce reactive oxygen species (ROS) via TLR2 or TLR4 and activate the expression of Sterol-regulatory element binding protein 2 (SREBP2) to promote cholesterol synthesis, thereby promoting the proliferation of tumor cells. mRNAs related to the regulation of lipid metabolism are overexpressed in *pks*$^{+}$*E.coli*-enriched tumor tissues, and imbalance of lipid metabolism also promotes CRC progression [34]. Redox reactions are present in a wide range of metabolic processes and are key to energy conversion. *Lactobacillus reuteri* and its metabolite rotenone, which is down-regulated in mouse and human CRC tissues, rotenone alters redox homeostasis and reduces proliferation and survival of colon cancer cells [85]. The influence of intratumoral microbes on the metabolic reprogramming of tumor cells is bidirectional. In CRC, the downregulation of the Farnesoid X receptor (FXR) disrupts bile acid (BA) metabolism, leading to alterations in the bile acids profile, which positively regulates secretory immunoglobulin A (sIgA) secretion, and the dual regulation of BAs and sIgA enhances the adhesion and biofilm formation of *ETBF*, consequently promoting colorectal tumorigenesis [86] (Fig. 4).

Another aspect, intratumoral microorganisms influence the metabolism of anticancer drugs, diminishing their effectiveness. For instance, *Fn* induces chemoresistance to 5-fluorouracil (5-FU) in CRC cells by upregulating baculovirus inhibitor of apoptosis (IAP) 3 (BIRC3) expression through the Toll-like receptor 4 (TLR4)/NF-κB pathway [87]. Additionally, *Fn* activates autophagy and reduces CRC cell response to 5-FU chemotherapy by targeting TLR4 and myeloid differentiation primary response 88 (MYD88), resulting in a selective loss of miRNA expression [69]. Moreover, in colon cancer, intratumoral *Gammaproteobacteria* promote tumor cell

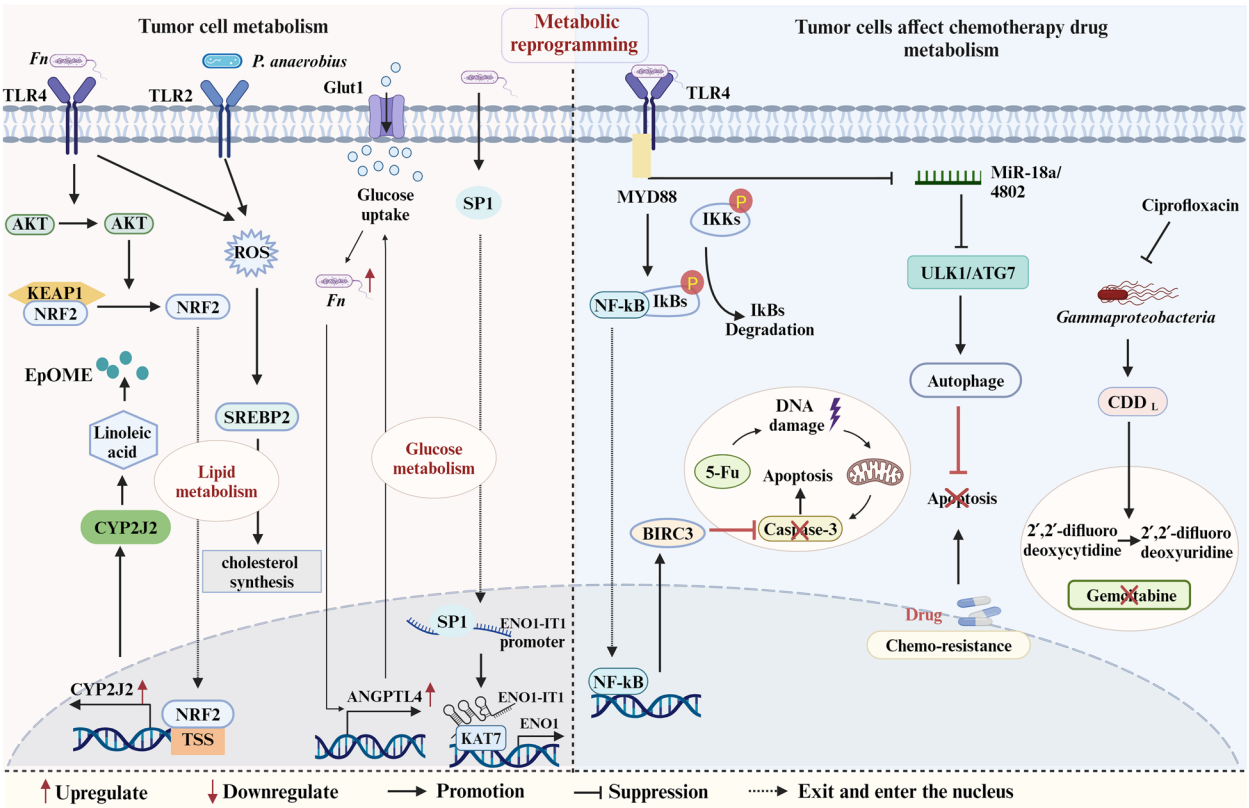

Fig. 4 Effect of intratumoral microorganisms on metabolic reprogramming of tumor cells. Graphics created using BioRender.com

resistance to gemcitabine through the synthesis of bacterial cytidine deaminase (CDD_L) [88] (Fig. 4).

Effect of intratumoral microbiota on the tumor microenvironment

Inflammatory microenvironment

The microenvironment of precancerous colonic polyps exhibits enrichment with non-enterotoxin-producing *Bacteroides fragilis* (*NTBF*), with significant upregulation of lipopolysaccharide (LPS) biosynthesis genes in *NTBF* strains. This *NTBF*-enriched milieu activates TLR4, instigating localized inflammation that promotes polyp growth and colonization by additional "passenger" bacteria such as *ETBF*, *pks*$^+$*E.coli* and *Fn*, which potentially contribute to CRC progression [89]. Additionally, *pks*$^+$*E.coli* enhances inflammation by secreting virulence factors [90]. Intratumoral microorganisms play a dual role in inflammation in tumors, both promoting tumor development and mediating inflammation. For instance, *Fn* directly targets human CRC stem cells by activating CEACAM-1-dependent protein tyrosine phosphorylation signaling, leading to increased expression of CXCL1, CXCL8, and NF-kB, thereby eliciting pro-inflammatory and oncogenic responses [91]. *Streptococcus gallolyticus* can selectively colonize tumor cells and promote chronic inflammation and angiogenesis, thereby promoting carcinogenesis [92]. The *P. anaerobius* surface protein PCWBR2 can also upregulate the expression of a large number of inflammation-associated genes through direct interaction with colonic epithelial cells or tumor cells via integrin α2/β1 [93]. In addition, *Helicobacter pylori*, a microorganism closely associated with the development of gastric cancer, as well as *Streptococcus gallolyticus* induce pro-inflammatory and oncogenic responses in the colon [84, 86].

Immunity Microenvironment

Numerous studies have shown that intratumoural microbes play a key role in remodeling the tumor immune microenvironment. Multiple lines of evidence show that the higher the intratumoural microbial diversity, the fewer tumor-infiltrating lymphocytes there are in the tumor microenvironment [94–96]. In addition, the regulation of the tumor immune microenvironment by intratumoural microorganisms exhibits a two-fold character that enhances or promotes anti-tumor immunity and pro-tumor immunity (Fig. 5).

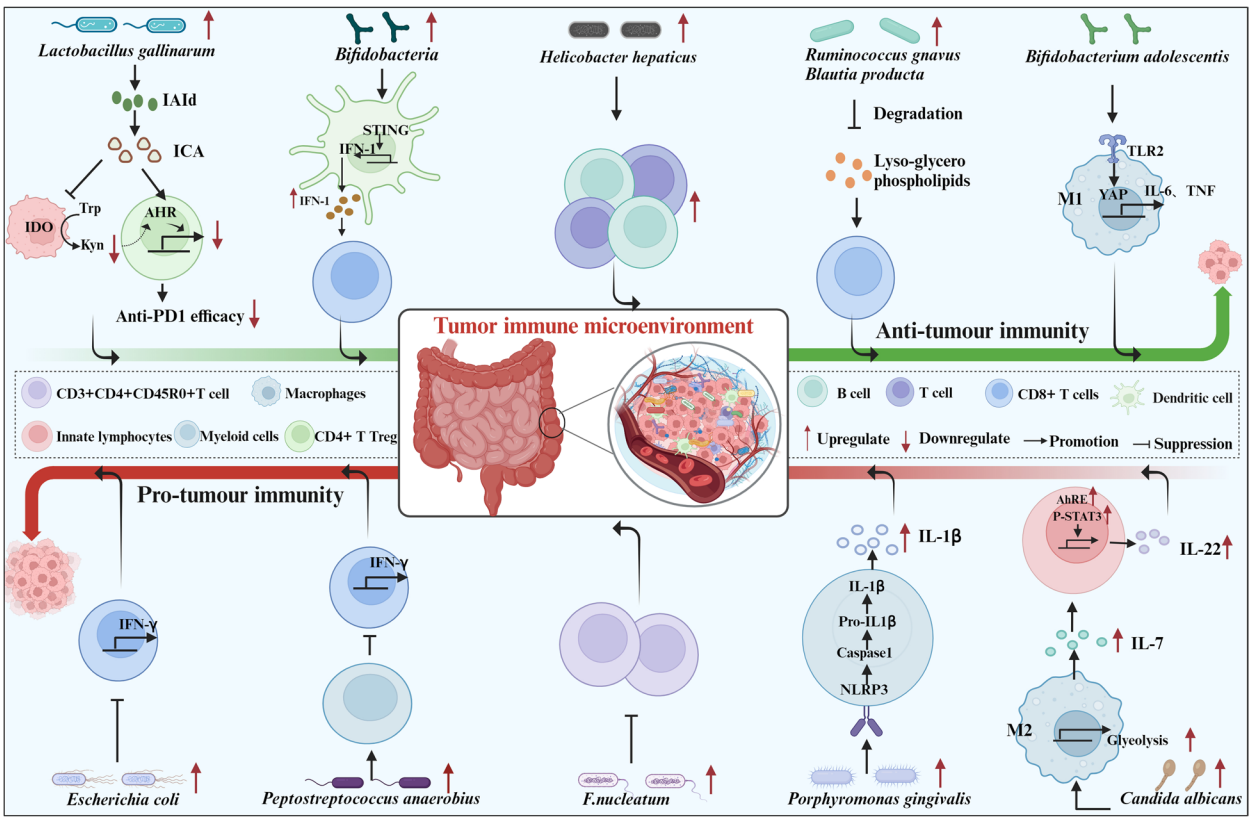

Fig. 5 The impact of intratumoral microorganisms on the tumor microenvironment. Graphics created using BioRender.com

Borowsky et al. [97] observed a negative correlation between the amount of *Fn* DNA in CRC tissues and the presence of CD3 + CD4 + CD45RO + cells (memory Th cells) in the tumor stroma, suggesting that *Fn* suppresses antitumor immunity, as evidenced by two prospective cohort studies. Another oral source bacterium, *P. anaerobius*, promotes tumor growth by inducing CXCL1 secretion, increasing the number of MDSC within the tumor and reducing IFN-γ + CD8 + T cells [98]. Reduced infiltration of IFNγ + CD8 + T cells was also found in *pks*$^{+}$*E. coli*-enriched tumor tissues [34]. Wang et al. [50] found that *Porphyromonas gingivalis* recruits bone marrow-derived immune cells, induces NLRP3, caspase-1, IL1β and pro-IL1β expression, and alters the tumor immune environment to promote CRC development. *Candida albicans*, another microorganism found in the tumor immune microenvironment, triggers glycolysis and IL-7 secretion in macrophages, leading to IL7-induced IL-22 secretion by acting on AhRE and STAT3 in innate lymphocytes, thus fostering CRC development [26].

In contrast to the inhibitory effect of intratumor microorganisms on antitumor immunity, some microorganisms promote antitumor immunity. Zhang et al. [55] demonstrated that tissue-resident Trichosporonaceae bacteria *Ruminococcus gnavus* (*Rg*) and *Blautia producta* (*Bp*) degrade lysoglycerophospholipids, thereby attenuating their inhibition of CD8 + T cell activity and preserving the immune surveillance function of CD8 + T cells, which delays CRC progression. Indole-3-carboxylic acid, a metabolite of the probiotic *Lactobacillus gallinarum*, also inhibits CD4 + Treg differentiation and enhances CD8 + T cell function through modulation of the IDO1/Kyn/AHR axis and improves anti-PD1 efficacy in CRC [99]. Overacre-Delgoffe et al. [100] confirmed that *Helicobacter hepaticus* (*Hhep*) reduces the number and size of tumors in a mouse model of colitis-associated colon cancer, increases tumor-infiltrating T cells and B cells recruitment, induces more Tfh-cells, and activates tertiary lymphoid structures to promote antitumor immunity. Additionally, *Bifidobacteria* can enhance dendritic cell IFN-β expression via the STING signaling pathway, stimulating adaptive immune responses and enhancing the antitumor effect of anti-CD47 antibodies [101]. *Bifidobacterium adolescentis*, a specific strain belonging to the *Bifidobacterium*, also induces Decorin + macrophage infiltration into tumor tissue and inhibits CRC [102].

The potential of intratumoral microbiota in clinical application of CRC

Diagnostic value of intratumoral microbiota

Owing to the unique distribution characteristics of the intratumoral microbiota in CRC, these microbial communities exhibit significant predictive capabilities. They are adept at distinguishing cancerous tissues from normal ones and hold promise as biomarkers for the classification of molecular subtypes and the staging of CRC. For instance, OCS1 enriched with oral pathogens such as *Fusobacteria*, exhibiting high CRC grade, positive MSI-H, CpG island methylation phenotypes, and commonly containing BRAF V600E and FBXM7 mutations, mostly located on the right side of the colon. Additionally, OCS2 enriched with *Firmicutes* and *Bacteroidetes*, and OCS3 enriched with *Escherichia* and *Shigella*, associated with chromosomal instability and occurring on the left side of the colon [10]. Furthermore, *Fn* was found to account for 5.9% of adenoma tissue, escalating to 81.8% as CRC progressed to later stages [29]. It also has been observed that the ratio of *Candida* to *Yeast* is generally low in early-stage CRC but significantly increases in stage IV tumors [26]. Fecal and intratumoral microbiotas provide a dual approach to CRC diagnostics. Testing of fecal microbiota is well-suited for non-invasive, broad population screenings, whereas intratumoral microbiota analysis offers personalized insights, crucial for precision medicine [103]. Overall, identifying the microbial composition and alterations in pathological tissues can be used as an adjunct approach to the diagnosis of CRC.

Therapeutic effects of intratumoral microbiota

Chemotherapy

Intratumoral microorganisms can effectively alter the activity of CRC chemotherapy drugs. *Fn* induces chemoresistance to 5-fluorouracil (5-FU) in CRC cells by upregulating baculovirus inhibitor of apoptosis (IAP) 3 (BIRC3) expression through the TLR4/NF-κB pathway [87]. Additionally, *Fn* activates autophagy and reduces CRC cell response to 5-FU chemotherapy by targeting TLR4 and MYD88, resulting in a selective loss of miRNA expression [69]. Intriguingly, 5-FU exhibits potent inhibitory effects on *Fn*, while *E. coli* can modify 5-FU, thereby reducing its toxicity to sensitive *Fn* and intestinal epithelial cells [104]. Moreover, after injecting *E. coli* into CT26 subcutaneous transplanted tumors, it was found that *E. coli* can reduce the anti-tumor activity of gemcitabine; it can also activate CB1954 cytotoxicity and significantly increase the median survival time of mice [105]. In colon cancer, intratumoral *Gammaproteobacteria* promote tumor cell resistance to gemcitabine through the synthesis of bacterial CDD_L, and combining the antibiotic ciprofloxacin with gemcitabine can effectively neutralize the impact of *Gammaproteobacteria* [88]. CRC intratumoral microorganisms can inhibit the activity of multi-types of chemotherapy drugs. Different bacteria may have synergistic effects, and the combination of chemotherapy drugs and antibiotics can eliminate the inhibitory effect

of some bacteria on the activity of chemotherapy drugs. The diagnosis of CRC will be facilitated by monitoring differences in intratumoural microbial abundance and composition.

Immunotherapy

In addition to chemotherapy, intratumoral microorganisms also have potential impact on CRC immunotherapy [106]. The use of immune checkpoint inhibitors is an important therapeutic strategy in anti-tumor therapy. *Clostridium* is enriched in CRC patients who are insensitive to immune checkpoint blockade (ICB). Further research have shown that intratumoral microorganisms may affect ICB therapy by mediating tumor infiltrating immune cell (TIIC), especially mucosal-associated invariant T cells [96]. *P. Anaerobius* creates an immunosuppressive tumor microenvironment through a dual pathway, inducing CXCL1 secretion and recruiting MDSCs through the integrin α2 β1-NF-κB signaling pathway, and directly activating MDSCs through the secretion of lytC22, thereby weakening T cell-mediated anti-tumor immune response [98]. Furthermore, *Bifidobacterium* accumulated in the CRC tumor microenvironment ultimately promoted CD47-based immunotherapy by stimulating interferon gene transcription and increasing dendritic cell crosstalk in an interferon-dependent mode [101]. Thus, intratumoural microorganisms have both advantages and disadvantages for CRC immunotherapy.

Targeted therapy

Most individuals with advanced metastatic CRC are treated with a combination of chemotherapy and molecularly targeted therapies. For instance, cetuximab has been shown to extend the overall survival of patients with wild-type *KRAS* gene CRC, yet a significant number of patients do not derive benefits from this treatment [107]. Investigations have revealed that *P. stomatis* can adhere to CRC cells by binding to the integrin α6/β4 receptor through its surface protein, fructose-1,6-diphosphate aldolase, subsequently activating the Erb-B2 receptor tyrosine kinase 2 (ERBB2) and the downstream MEK-ERK-p90 signaling pathway. This activation, driven by *P. stomatis*, can override the blockade of epidermal growth factor receptor inhibitors such as cetuximab and erlotinib, resulting in drug resistance in KRAS mutant CRC [16]. Concurrently, studies have demonstrated that *P. stomatis* can also diminish the effectiveness of BRAF inhibitors like vemurafenib in BRAF V600E mutant CRC xenografts.

Other therapies

The treatment of CRC by designing nanomedicines to target intratumoral microorganisms, live bacterial therapy using bacteria as drug carriers, or oral probiotics as auxiliary treatment of CRC are all research hotspots in the field of CRC treatment in recent years. Short-chain fatty acid butyrate can downregulate the expression of adhesion-related outer membrane proteins, thereby inhibiting Fn activity in CRC tissues. Chen et al. [74]. encapsulated sodium butyrate in liposomes to make tablets, which significantly inhibited *Fn* and attenuated chemoresistance by intravenous injection. Intratumoral *Fn* promotes CRC progression and leads to chemoresistance. Although great efforts have been made to overcome *Fn*-induced chemoresistance by co-delivering antibacterial and chemotherapeutic drugs, improving drug loading capacity and achieving controlled release of drugs Still challenging. Yan et al. [72]. designed a new type of nanoparticle by incorporating a positively charged polymer with *Fn* inhibitory ability and a negatively charged oxaliplatin prodrug, which can improve the drug loading capacity and achieve controlled release of the drug. Research have also been conducted to enhance the antitumor activity of anti-programmed cell death 1 (PD-1) immunotherapy by oral administration of the commensal bacterium Lactobacillus rhamnosus GG (LGG) to mice with subcutaneous transplanted tumors of CRC to increase tumor-infiltrating dendritic cells and T cells [108]. A research team has also established a microbiota targeted drug delivery system Oxa@HMI Hydrogel, this system shows high efficiency in CRC targeting and colorectal retention, and excellent anti-tumor effect [109].

Prognostic potential of intratumoral microbiota

Intratumoral microorganisms have a certain predictive effect on CRC tumor metastasis and patient survival. Microbiome analysis of biopsy samples from different parts of primary CRC patients showed that *Acinetobacter*, *Burkholderia*, *Corynebacterium*, *Cutibacterium*, *Flavobacterium*, *Pelomonas*, *Rheinheimera*, *Sphingobium*, *Staphylococcus* and *Streptococcus* were the 10 most common bacterial genera in metastatic CRC tissues [95]. Xu et al. [43] obtained two clear clusters after clustering 533 CRC tissue samples. Cluster 1 has a higher relative abundance of *Proteobacteria* and *Bacteroidetes*, and cluster 2 has a higher relative abundance of *Firmicutes* and *Actinobacteria*, and the *Firmicutes/Bacteroidetes* ratio is significantly higher than in cluster 1. Finally, it was found that some pathogenic bacteria enriched in cluster 1 can promote the development of colorectal cancer, resulting in lower patient survival rates. In contrast, the abundance of some probiotic and anti-cancer development genera

increased significantly in cluster 2. Research have also shown that patients with *Fn*-enriched right-sided colon cancer have shorter progression-free survival [35].

Currently, the field of intratumoral microbiota research is in its nascent phase, with limitations in the scope and depth of investigation. The majority of studies concentrate on particular microbial species, falling short of a holistic and systematic comprehension. The intricacy of the intratumoral microbiota composition is compounded by the absence of standardized protocols for sampling, processing, and analysis, leading to challenges in comparing and replicating findings across studies. This variability hampers the generalizability of research outcomes and their translation into clinical practice. Future endeavors necessitate profound exploration across various strata, encompassing foundational theories, methodological approaches, and clinical trial designs.

Current status and challenges in the study of intratumoral microbiota and colorectal *cancer*

Current status

In this review, we discussed the origins of CRC intratumoral microorganisms and the methods used for their detection. We described the microbial composition in different segments of the colorectum and discussed variations in CRC microbial composition across different molecular phenotypes and disease stages. We summarized how intratumoral microorganisms influence CRC progression by impacting epithelial cells, tumor cells and the tumor immune microenvironment, as well as the potential application of intratumoral microorganisms in the clinical of CRC. Based on these findings, our investigation reveals a decrease in α-diversity and an increase in β-diversity of CRC intratumoral microbiota [9], and compared with fecal microorganisms, the low biomass of CRC intratumoral microorganisms, which are relatively less affected by diet and environment, can be used together with fecal microorganisms as a target for CRC diagnosis and treatment [103]. Moreover, various intratumoral microorganisms play distinct roles in CRC progression. Some act as probiotics, inhibiting tumor progression, while others exert major functional effects at different stages of CRC progression. For instance, *ETBF* and *pks*$^{+}$*E.coli* are mainly associated with early inflammation or DNA damage in CRC, while *Fn* predominantly affects advanced CRC, promoting an immunosuppressive microenvironment and tumor cell metastasis.

Cutting-edge technologies are driving advancements in intratumoral microbiology research. In constructing ex vivo and in vivo models, such as mouse intratumoral multipoint injections and organoids, researchers have tools to investigate the interaction between specific microorganisms and tumors [50, 55]. Particularly, organoid-microbe co-cultures offer a valuable platform for directly studying microbial effects on the tumor microenvironment, providing insights into their complex pathophysiological relationship [110, 111]. Furthermore, traditional antibiotic cocktails and intravenous antibiotic injections are important experimental tools for studying microbial functions [112]. At the detection level, microscopy, immunology, and multi-omics techniques are combining forces to advance intratumor microbial detection. Innovations such as the 5R 16S rDNA sequencing technology, SAHMI process, and INVADE seq methods demonstrate this integration. Therapeutically, there is a growing focus on targeting intratumoral microorganisms for CRC treatment. Strategies include designing nanomedicines to specifically target these microorganisms and exploring live bacterial therapies utilizing bacteria as drug carriers. These approaches have emerged as research hotspots in CRC detection and treatment over the past few years [72–75, 113]. By employing nano-targeted drugs that selectively act on intratumoral microorganisms, the potential adverse effects on the intestinal flora can be effectively minimized.

Challenges

Despite recent breakthroughs in intratumoral microbiology research, several limitations remain. Firstly, the low content of intratumoral microorganisms poses a challenge, requiring the exclusion of host DNA and other environmental microorganisms to enhance detection accuracy. Secondly, current research models, such as organoid-microbe co-cultures, may not fully replicate the in vivo environment, highlighting the need for more appropriate in vitro models to explore the intricate mechanisms of tumor-microbe interactions. Thirdly, the dynamic changes in composition and abundance of intratumoral microbiota throughout the CRC process necessitate consideration of factors like sampling time and site when designing experiments. Fourthly, intratumoral microorganisms mainly exist in tumor cells or immune cells, but most studies do not distinguish between intracellular and extracellular microorganisms. Whether the mechanism of action of intracellular and extracellular microorganisms is the same requires more research to further reveal. Lastly, while the dual effects of intratumoral microorganisms on CRC are the mechanisms underlying their impact on antitumor therapy remain poorly understood, impeding the clinical application of microbe-related therapeutic strategies in oncology. Thus, extensive validation through additional preclinical models and clinical trials is imperative.

Abbreviations

12，13-EpOME	12,13-epoxyoctadecenoic acid
ATG7	Autophagy-related protein 7

AXIN2	Axin Inhibition Protein 2
BF	*Bacteroides fragilis*
BIRC3	Baculoviral IAP Repeat Containing 3
Bp	*Blautia producta*
CDDL	Cytidine deaminase
cmDNA	Circulating microbial DNA
CMSs	Consensus molecular subtypes
C-Myc	Myelocytomatosis oncogene
CRC	Colorectal cancer
CYP2J2	Cytochrome P450 2J2
DSS	Dextran sulfate sodium
E. coli	*Escherichia coli*
eIF4EBP1/2	Eukaryotic initiation factor 4F-binding protein 1/2
ERBB2	Erb-B2 receptor tyrosine kinase 2
ENO1-IT1	Enolase1-intronic transcript 1
ERK	Extracellular signal regulated kinase
ETBF	*Enterotoxigenic Bacteroides fragilis*
Fa	*Fusobacterium animalis*
Fap	fibroblast activation protein
Fn	*Fusobacterium nucleatum*
Gal-GalNAc	Galactose-N-acetylgalactosamine
Glut1	Glucose Transporter 1
Hhep	*Helicobacter hepaticus*
HVM	High-variable microbe
ICAM1	Intercellular Cell Adhesion Molecule-1
ILC3	Group 3 Innate Lymphoid Cells
IHC	Immunohistochemistry
KAT2A	Lysine acetyltransferase 2A
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
MyD88	Myeloid differentiation factor88
OCS	Oncomicrobial community subtypes
P. anaerobius	*Peptostreptococcus anaerobius*
P. stomatis	*: Peptostreptococcus stomatis*
Pg	*Porphyromonas gingivalis*
PHF5A	PHD-finger domain protein 5A
RAF	Rapidly accelerated fibrosarcoma
Rg	*Ruminococcus gnavus*
RhoA	Ras homolog family member A
STX12	Syntaxin-12
TCF	T-cell factor
TLR4	Toll-Like Receptor4
ULK1	UNC-51-like kinase 1

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Authors' contributions
L.G. designed the review protocol, conducted the search, drafted the manuscript, and prepared all Figures and tables. J.M.W. screened potentially eligible studies, extracted and analyzed data, and updated reference lists. X.C. and F.M.Y. were responsible for the funding acquisition. X.K.L. contributed to the design of the review protocol, arbitrating potentially eligible studies, and interpreting results. Y.F.J. and X.K.L. provided feedback on the report. All authors approved the final version of the manuscript.

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Data sharing is not applicable to this article as no datasets were generated or analyzed in the study.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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