Review

Current understanding of the intratumoral microbiome in various tumors

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https://doi.org/10.1016/j.xcrm.2022.100884

It is estimated that in the future, the number of new cancer cases worldwide will exceed the 19.3 million recorded in 2020, and the number of deaths will exceed 10 million. Cancer remains the leading cause of human mortality and lagging socioeconomic development. Intratumoral microbes have been revealed to exist in many cancer types, including pancreatic, colorectal, liver, esophageal, breast, and lung cancers. Intratumoral microorganisms affect not only the host immune system, but also the effectiveness of tumor chemotherapy. This review concentrates on the characteristics and roles of intratumoral microbes in various tumors. In addition, the potential of therapies targeting intratumoral microbes, as well as the main challenges currently delaying these therapies, are explored. Furthermore, we briefly summarize existing technical methods used to characterize intratumoral microbes. We hope to provide ideas for exploring intratumoral microbes as potential biomarkers and targets for tumor diagnosis, treatment, and prognostication.

INTRODUCTION

Cancer is a major health problem worldwide.[1] According to the World Health Organization, cancer will become the leading or second-leading cause of death in patients younger than 70 in most countries.[2] Among cancers, lung cancer remains the leading cause of cancer deaths, with approximately 350 people dying from lung cancer every day. The incidence of breast cancer (BC) in women is expected to surpass that of lung cancer, and BC is expected to become the most commonly diagnosed cancer in women.[3] In addition, the diagnosis and treatment of cancer patients have been delayed to varying degrees because of the impact of the COVID-19 pandemic; furthermore, the incidence of cancer is expected to decrease in the short term, but the mortality rate in the later stage could increase due to untimely treatment.[4] The study of cancer has continued for many years, and as a result, more means to diagnose, treat, and prevent cancer have been revealed. For example, human papillomavirus (HPV) vaccination can significantly reduce the incidence of gynecological tumors in women, such as cervical cancer (CC).[5] Early screening and eradication of *Helicobacter pylori* have contributed to reducing the incidence of gastric cancer (GC) and improving the efficacy of early treatment and patient prognosis.[6] In conclusion, the exploration of cancer and cancer therapies is an ongoing process, and a great deal of effort remains necessary.

The human microbiome is a general term that includes all microorganisms in and on the human body, e.g., those on the mucosal surfaces of the gut and skin.[7] The genomes of these microorganisms are called microbial genomes. The number of genes in the microbial genomes of these organisms is more than 150 times that of the human genome.[8] Therefore, the micro-

biome is believed to have its own genome.[9] Microorganisms are able to coexist with host cells at multiple body sites, and these factors interact to influence the physiological and pathological processes of the host.[10] Currently, the gut microbiome is the best known and the most studied. The normal intestinal flora is of great significance for the maintenance of intestinal mucosal integrity. The functions of gut microbes mainly include nutrient and drug metabolism, vitamin synthesis, immune regulation, and gastrointestinal structure maintenance.[11,12] Microbial imbalances contribute to the development and progression of many human diseases, including cancer. For example, Ma et al. found that gut microbes regulated the level of the chemokine CXCL16 in liver sinusoidal endothelial cells (LSECs) via bile acids, thereby controlling the accumulation of CXCR6^{+} hepatic natural killer T (NKT) cells.[13] Furthermore, these microbes exert antitumor immunity in the liver. Recently, the discovery of the intratumoral microbiome has piqued the interest of researchers. Studies have found that there are a large number of microorganisms in tumor tissue, and some of these microorganisms are involved in tumor initiation and development.

In fact, bacteria were found in tumor tissue more than 100 years ago. However, due to the lack of technical means to exclude the possibility of contamination and the very low microbial content in tumors, the presence of these bacteria was not widely recognized and explored. In 2020, Nejman et al. published a research paper on the intratumoral microbiome in *Science*.[14] They employed more than 1,500 tumor samples and adjacent normal samples to study the relationships between seven cancers (breast, lung, ovarian, pancreatic, melanoma, bone, and brain) and bacteria. The researchers found that most tumors and their adjacent normal tissues contained

different types of bacteria, mostly in cancer cells and immune cells. This research has attracted widespread attention, and intratumoral bacteria have become a popular topic in modern research. Organs and tissues, traditionally considered sterile, are increasingly being found to contain diverse microbial populations.[15–17] Therefore, this review mainly focuses on summarizing the characteristics and roles of intratumoral microorganisms in different tumors and possible therapeutic options related to intratumoral microorganisms, and it briefly summarizes the existing technical means for detecting intratumoral microorganisms. The aim of this work is to provide guidance for the exploration of intratumoral microbes as potential biomarkers in cancer diagnosis, treatment, and prognostication.

METHODS USED TO CHARACTERIZE THE INTRATUMORAL MICROBIOME

Next-generation sequencing methods achieve comprehensive and detailed analysis of all microorganisms without the need for culture. Researchers have applied high-throughput sequencing technology to analyze and generate a large number of microbiome datasets to facilitate subsequent research. Common approaches for microbiome research are based on deep sequencing of universal marker gene amplicons, such as 16S rRNA genes in prokaryotes, 18S rRNA genes, and internal transcribed spacers (ITSs) in eukaryotes, or whole metagenome-based shotgun sequencing (WMS).[18–20] Apart from sequencing, methods such as immuno-histochemistry (IHC), fluorescence *in situ* hybridization (FISH), D-alanine-based methods, and tissue culture can be used to explore the presence and abundance of bacteria within the tumor. A recent study also described QIIME 2 as a reproducible, interactive, and scalable microbiome data analysis system.[21] It is an excellent tool for analyzing microbiome sequencing data, as are MaAsLin and Microbiome Analyst. Overall, the development of various technologies has made it possible to deeply explore the microbiomes of tumors.

16S rRNA gene sequencing
16S rRNA gene sequencing is currently one of the most commonly used amplicon sequencing methods. 16S rRNA is a conserved region with hypervariable sequences. Normally, hypervariable regions and conserved regions are alternately arranged, and a 16S rRNA fragment contains 9 hypervariable regions: V1–V9. Among them, the V4 region has good specificity and rich relevant data and is thus the best choice for bacterial diversity analysis and annotation.[22] Notably, Nejman et al. performed multiplex PCR amplification and sequencing of five regions on the 16S rRNA gene.[14] This method showed improved coverage and resolution of bacterial species detection compared with the traditionally used V4 or V3–V4 amplification. Generally, 16S rRNA is amplified by PCR, purified, and sequenced; finally, the sequences are clustered into operational taxonomic units (OTUs) based on similarity and are compared with existing database entries based on the representative sequences in each OTU.

WMS
Metagenomics is studied based on WMS, and many related terms are not discussed here. WMS mainly refers to the use of non-targeted sequencing methods to capture the DNA of all microorganisms present in environmental samples.[23] The object of metagenomics research is to determine the total DNA content in a specific environment, not the total DNA in a specific microorganism or its cells, and WMS does not require isolation, culture, or purification of microorganisms.[24] Compared with 16S rRNA gene sequencing, WMS is more precise, expensive, and complex and provides more extensive results.

IHC
The principle of IHC is the specific binding of antigen and antibody. In brief, the general procedure of this method is to first fix the sample and then coincubate it with a blocking solution. Subsequently, the sample is coincubated with primary antibody and secondary antibody followed by color development, and the sample is ultimately analyzed by microscopy. IHC is a qualitative and quantitative technique that provides location-specific data. Researchers commonly use antibodies against bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA) to detect Gram-negative and Gram-positive bacteria in samples.[25]

FISH
FISH can be used to detect bacterial DNA or RNA in tissues using probes against the bacterial 16S rRNA gene labeled with fluorescent dyes. FISH takes advantage of the principle of nucleic acid complementary base pairing. A nucleic acid probe directly labeled with fluorescein is mixed with the target DNA or RNA, and the two form a hybrid. Kostic et al. explored the enrichment of *Fusobacterium* in colorectal cancer (CRC) by FISH.[26]

D-alanine-based methods
Researchers typically label live bacteria *in situ* with fluorescently labeled D-alanine. Almost all bacteria have alanine arginase activity, which helps to generate D-alanine, thereby producing peptidoglycan, an important cell wall component.[27] The presence of living, metabolically active bacteria in tumors can be confirmed by culturing fresh tumor tissue *in vitro* and adding fluorescently labeled D-alanine.[28]

CHARACTERIZATION OF THE INTRATUMORAL MICROBIOTA IN DIFFERENT TUMORS

Growing evidence has confirmed the presence of microorganisms, mainly bacteria, in tumor tissues (Figures 1 and 2). Bacteria are mainly found in immune cells and tumor cells.[14] The presence of bacteria within the tumor can have diagnostic value. There is also evidence that intratumoral bacteria can be associated with patient prognosis, as reported in liver and pancreatic cancer. Tables 1 and 2 provide a detailed summary of the characteristics and potential biological functions of intratumoral microbes in different tumors. Table 1 highlights the same and different intratumoral microbial types reported by different groups within the same tumor type. Table 2 mainly provides some ideas for the subsequent treatment and prevention of tumors by revealing the possible effects of intratumoral microorganisms on the occurrence and development of tumors.
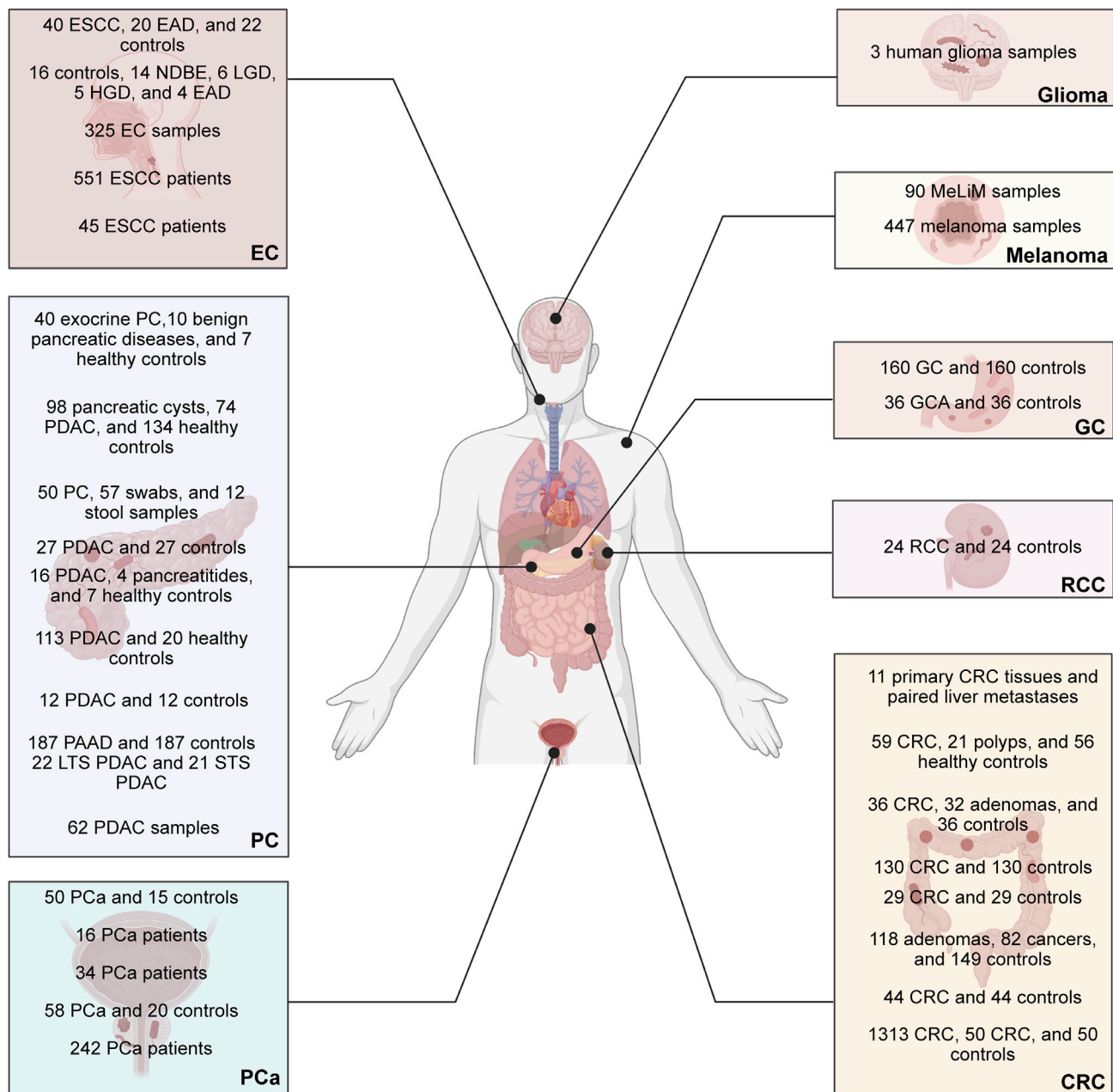
Figure 1. Intratumoral microbes are present in esophageal cancer (EC), pancreatic cancer (PC), prostate cancer (PCa), glioma, melanoma, gastric cancer (GC), renal cell cancer (RCC), and colorectal cancer (CRC)
Each box represents a cancer type. Number of clinical samples used by different study groups to study microorganisms in tumors is presented in the box.

Pancreatic cancer

Pancreatic cancer (PC) is a malignant tumor with a very high mortality rate.[89] Pancreatic ductal adenocarcinoma (PDAC) is the most common type of PC, accounting for more than 85% of total cases.[90] PDAC is inflammation-driven cancer characterized by a prominent fibroinflammatory microenvironment.[91] Recently, many studies have revealed the role of intratumoral microbes in the development of PC and their effects on PC treatment. Currently, there is no clear consensus on the role of

microbes in pancreatic tissue and the normal surrounding tissues of patients with PC. It is worth conducting more research to explore this issue in greater depth.

As early as 2006, Nilsson et al. found *H. pylori* DNA in the pancreas of 75% of PC patients.[29] Since then, an increasing number of studies have revealed that the pancreas is not a sterile organ. Researchers have found that the human pancreas has its own microbiota. Interestingly, some studies have reported that the bacterial composition of PC is different from that of the normal
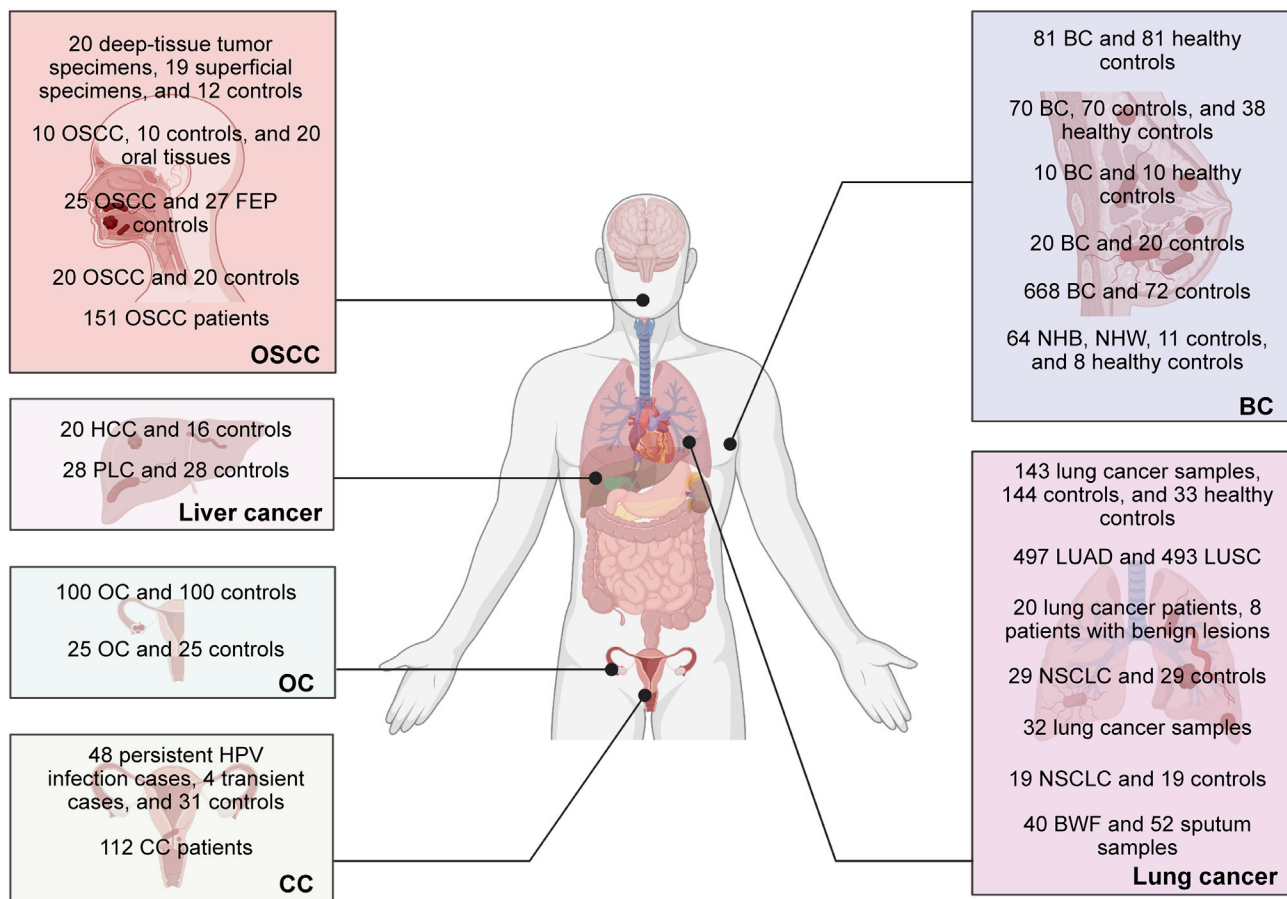
Figure 2. Intratumoral microbes were present in oral squamous cell carcinoma (OSCC), liver cancer, ovarian cancer (OC), cervical cancer (CC), breast cancer (BC), and lung cancer
Each box represents a cancer type. Number of clinical samples used by different study groups to study microorganisms in tumors is presented in the box.

pancreas.[31,30] However, other studies have found that the composition of the bacterial community in the pancreas is not distinct between normal and disease states.[32,33] This lack of difference could have been caused by the different sources, quantities, and qualities of tissue samples; the technical means; and the analysis methods used in the studies. Del Castillo et al. also found that the relative abundance of bacterial groups in the pancreas at the genus level varied significantly from person to person.[31] By contrast, in the same individuals, the bacterial composition in the pancreas was very similar to that in the duodenum. In exploring the possible impacts of intratumoral microbes on tumors, many studies have revealed the potential effects of bacteria on PC treatment outcomes, prognosis, and survival.[15,34–37] Gemcitabine is a common chemotherapeutic used in the treatment of PC patients. Research has indicated that bacteria in PDAC might modulate tumor sensitivity to gemcitabine. Bacteria break down gemcitabine into an inactive form via a specific isoform of cytidine deaminase.[15,92] Pushalkar et al. performed 16S rRNA gene sequencing on 12 PDAC patients.[34] The results showed that *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were the most abundant and prevalent groups of microorganisms. Further study found that ablation of the microbiome prevented the progression

of pancreatic disease. Mechanistically, the microbiome generated a tolerant immune program through differential activation of selected Toll-like receptors (TLRs) in monocytes. In addition to revealing the presence of a large number of bacteria in the pancreas, researchers have recently reported the discovery of fungi within tumors. The fungal community of PDAC tumors in both mice and humans is significantly enriched for *Malassezia*. Ligation of mannose-binding lectin (MBL), which binds to fungal glycan to activate the complement cascade, is required for the oncogenic progression of PDAC (Figure 3).[38]

Colorectal cancer
CRC is the third most common cancer worldwide and the second leading cause of cancer deaths in the United States. Intestinal flora imbalances play a significant role in the progression of CRC. In recent years, attention has been paid to the microbial communities within tumors. Currently, the mechanism of *Fusobacterium nucleatum* in CRC is relatively well understood.[93] Researchers have revealed that the effects of *F. nucleatum* mainly include inhibition of immunity, modulation of virulence, regulation of micro RNA (miRNA) and long noncoding RNA (LncRNA), and modulation of metabolism.[94,95]

Table 1. The characterization of the intratumoral microbiota in various cancers

Cancer types	Research groups	Number of clinical samples	Methods	Associated microbiota (same)	Associated microbiota (different)
Pancreatic cancer (PC)	Nilsson et al.[29]	40 exocrine PC, 14 neuroendocrine cancer, 8 multiple endocrine neoplasia, 5 chronic pancreatitis, 10 benign pancreatic disease, and 7 normal pancreas samples	PCR	–	*Helicobacter pylori*
PC	Kohi et al.[30]	134 normal pancreas control individual, 98 pancreatic cyst patient, and 74 PDAC patient samples	16S rRNA and 18S rRNA gene sequencing	–	Enterococcaceae, Lactobacillaceae, Bifidobacteriaceae *Nakaseomyces,* and *Skeletocutis*
PC	Del Castillo et al.[31]	50 PC patient samples, 189 tissue, 12 stool, and 57 swab samples	16S rRNA gene sequencing	–	*Fusobacterium*, Porphyromonas and *Prevotella*
PC	Nalluri et al.[32]	27 pairs of PDAC samples and adjacent normal samples	PCR and 16S rRNA gene sequencing	*Staphylococcus,* Enterobacteriaceae, Pseudomonadaceae	Ruminococcaceae and Bacillaceae
PC	Thomas et al.[33]	7 normal, 4 pancreatitis, and 16 PDAC patient samples	16S rRNA gene sequencing	*Staphylococcus,* Enterobacteriaceae	*Corynebacterium, Escherichia, Propionibacterium,* and *Streptococcus*
PC	Geller et al.[15]	113 PDAC samples and 20 normal samples	PCR and 16S rRNA gene sequencing	*Gammaproteobacteria,* Enterobacteriaceae, Pseudomonadaceae	–
PC	Pushalkar et al.[34]	12 pairs of PDAC samples and adjacent normal samples	PCR and 16S rRNA gene sequencing	*Proteobacteria,* Pseudomonadaceae	*Bacteroidetes, Firmicutes, Actinobacteria,* and *Elizabethkingia*
PC	Chakladar et al.[35]	187 pairs of PAAD samples and adjacent normal samples	Whole-transcriptome RNA sequencing	*Proteobacteria, Gammaproteobacteria,* Pseudomonadaceae	*Citrobacter freundii* and *Shigella sonnei*
PC	Riquelme et al.[36]	22 LTS PDAC patient and 21 STS PDAC patient samples	16S rRNA gene sequencing	–	*Saccharopolyspora, Pseudoxanthomonas, Streptomyces,* and *Bacillus clausii*
PC	Guo et al.[37]	62 PDAC samples	Metagenomic sequencing and FISH	Pseudomonadaceae	*Acinetobacter* and *Sphingopyxis*
PC	Aykut et al.[38]	–	18S rRNA gene sequencing and FISH	–	*Malassezia*
Colorectal cancer (CRC)	Bullman et al.[39]	11 primary CRC tissues and paired liver metastases	PCR, 16S rRNA gene sequencing, and WGS	*Fusobacterium, F. nucleatum*	*Fusobacterium necrophorum, Bacteroides fragilis, Bacteroides thetaiotaomicron, Prevotella intermedia,* and *Selenomonas sputigena*

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Table 1. *Continued*

Cancer types	Research groups	Number of clinical samples	Methods	Associated microbiota (same)	Associated microbiota (different)
CRC	Flemer et al.[40]	59 CRC patient samples, 21 samples from individuals with polyps, and 56 samples from healthy controls	PCR and 16S rRNA gene sequencing	*Firmicutes*	*Bacteroidetes* cluster 2, *Prevotella* cluster, *Pathogen* cluster, and *Firmicutes* cluster 2
CRC	Liu et al.[41]	36 CRC tissues, 32 adenoma tissues, and corresponding adjacent normal tissues	16S rRNA gene sequencing	*Fusobacterium, Firmicutes*	*Proteobacteria*
CRC	Warren et al.[42]	130 CRC and matched normal control tissues	Metatranscriptomic analysis	*Fusobacterium, Campylobacter*	*Leptotrichia*
CRC	Okuda et al.[43]	29 pairs of CRC and adjacent normal tissues	16S rRNA gene sequencing	*Fusobacterium, Campylobacter*	*Peptostreptococcus*
CRC	Yamamoto et al.[44]	118 adenomas, 82 cancers, and 149 matched adjacent normal tissues	PCR	*F. nucleatum*	–
CRC	Burns et al.[45]	44 pairs of CRC tissues and adjacent normal tissues	PCR and 16S rRNA gene sequencing	*Fusobacterium*	*Providencia*
CRC	Kosumi et al.[46]	1313 CRC patient samples, 50 pairs of CRC tissues and adjacent normal tissues	PCR and 16S rRNA gene sequencing	–	*Bifidobacteria*
Esophageal cancer (EC)	Wang et al.[47]	40 ESCC, 20 EAD, and 22 adjacent normal samples	TCMA, TCGA	*Firmicutes, Proteobacteria*	*Firmicutes* levels were significantly increased, while *Proteobacteria* levels were decreased in tumor samples.
EC	Yamamura et al.[48]	325 EC samples	PCR	*F. nucleatum*	–
EC	Liu et al.[49]	45 ESCC patient samples	16S rRNA gene sequencing	*Proteobacteria, Firmicutes*	*Bacteroidetes, Spirochaetes, Streptococcus, Prevotella,* and *Treponema*
EC	Yamamura et al.[50]	551 ESCC patient samples	PCR	*F. nucleatum*	–
EC	Snider et al.[51]	16 control, 14 NDBE, 6 LGD, 5 HGD, and 4 EAD samples	16S rRNA gene sequencing	*Proteobacteria, Firmicutes*	*Proteobacteria,* Enterobacteriaceae, and *Akkermansia muciniphila* were increased, while *Firmicutes* and *Veillonella* were reduced.
Oral squamous cell carcinoma (OSCC)	Hooper et al.[52]	20 deep-tissue tumor specimens, 19 corresponding superficial specimens, and 12 nontumorous control tissues	16S rRNA gene sequencing	–	*Exiguobacterium oxidotolerans, Prevotella melaninogenica, Staphylococcus aureus, Veillonella parvula*

Table 1. *Continued*

Cancer types	Research groups	Number of clinical samples	Methods	Associated microbiota (same)	Associated microbiota (different)
OSCC	Pushalkar et al.[53]	10 pairs of OSCC tissues and adjacent normal tissues, and 20 oral tissue samples	16S rRNA gene sequencing	–	*Streptococcus intermedius* was present in all samples. *Streptococcus sp. oral taxon 058*, *Peptostreptococcus stomatis*, *Streptococcus salivarius*, *Streptococcus gordonii*, *Gemella haemolysans*, *Gemella morbillorum*, *Johnsonella ignava*, and *Streptococcus parasanguinis I* were highly abundant in OSCC samples.
OSCC	Perera et al.[54]	25 OSCC patient and 27 FEP control samples	16S rRNA gene sequencing	*P. aeruginosa*	*F. nucleatum subsp. polymorphum*, *Streptococcus dysgalactiae*, *Citrobacter koseri*, and *P. aeruginosa* were significantly enriched in OSCC, while *Streptococcus mitis* and *Staphylococcus epidermidis* were abundant in FEP.
OSCC	Al-Hebshi et al.[55]	20 pairs of OSCC tissues and adjacent normal tissues	16S rRNA gene sequencing	*F. nucleatum, P. aeruginosa*	–
OSCC	Neuzillet et al.[56]	151 OSCC patient samples	PCR and 16S rRNA gene sequencing	*F. nucleatum*	–
OSCC	Nakashima et al.[57]	–	PCR	–	*Clostridium perfringens*
Lung cancer	Greathouse et al.[58]	33 control, 143 cancer, and 144 adjacent normal tissues	16S rRNA gene sequencing and FISH	*Firmicutes, Proteobacteria*	*Proteobacteria* levels were increased, while *Firmicutes* levels were decreased.
Lung cancer	Wong et al.[59]	497 LUAD and 493 LUSC patients	TCGA	–	*Escherichia coli* str. K-12 substr. W3110, *Pseudomonas putida* str. KT2440
Lung cancer	Lee et al.[60]	20 lung cancer patients and 8 patients with benign lesions	16S rRNA gene sequencing	–	*Veillonella*, *Megasphaera*, *Atopobium*, and *Selenomonas*
Lung cancer	Dumont-Leblond et al.[61]	29 pairs of NSCLC tissues and adjacent normal tissues	16S rRNA gene sequencing	–	Intestinal bacteria, potentially pathogenic or inflammatory bacteria
Lung cancer	Apostolou et al.[62]	32 lung cancer samples	PCR	–	*Mycoplasma, Staphylococcus epidermidis, Streptococcus mitis, Bacillus* strains, *Chlamydia, Candida, Listeria,* and *Haemophilus influenza*
Lung cancer	Peters et al.[63]	19 pairs of NSCLC tissues and adjacent normal tissues	16S rRNA gene sequencing	–	In normal tissues, greater abundance of the Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae families was associated with reduced RFS or DFS, whereas the Koribacteraceae family was associated with increased RFS and DFS.

Table 1. *Continued*

Cancer types	Research groups	Number of clinical samples	Methods	Associated microbiota (same)	Associated microbiota (different)
Lung cancer	Huang et al.[64]	40 BWF samples and 52 sputum samples from lung cancer patients	16S rRNA gene sequencing	*Firmicutes, Proteobacteria*	The most predominant phyla in the BWF samples were *Firmicutes* and *Proteobacteria*, and the most common genus was *Prevotella*. In sputum samples, *Firmicutes* was the predominant phylum, while *Streptococcus* was the most common genus.
Breast cancer (BC)	Urbaniak et al.[65]	Breast tissues from 81 women with and without cancer	16S rRNA gene sequencing	*Firmicutes, Proteobacteria*	–
BC	Urbaniak et al.[66]	70 pairs of BC tissues and adjacent normal tissues and 38 healthy control samples	16S rRNA gene sequencing	–	*Staphylococcus,* Enterobacteriaceae*, Bacillus, Staphylococcus epidermidis* and *Escherichia coli*
BC	Klann et al.[67]	10 BC patients and 10 healthy controls	PCR and 16S rRNA gene sequencing	*Firmicutes, Proteobacteria, Actinobacteria*	*Bacteroidetes*
BC	Xuan et al.[68]	20 pairs of BC tissues and adjacent normal tissues	PCR and 16S rRNA gene sequencing	–	*Methylobacterium radiotolerans* was abundant in tumor tissues, while *Sphingomonas yanoikuyae* was abundant in paired normal tissues.
BC	Thompson et al.[69]	668 breast tumor tissues and 72 noncancerous adjacent tissues	TCGA and 16S rRNA gene sequencing	*Firmicutes, Proteobacteria, Actinobacteria*	–
BC	Smith et al.[70]	64 breast tumor samples from NHB and NHW women, 11 adjacent normal tissues, and 8 healthy control samples	16S rRNA gene sequencing	*Proteobacteria*	–
BC	Fu et al.[71]	Murine spontaneous breast-tumor model and human samples	PCR, 16S rRNA gene sequencing, and FISH	*Firmicutes*	–
Prostate cancer (PCa)	Banerjee et al.[72]	50 PCa samples and 15 control samples	Array-based metagenomic and capture-sequencing	–	Viruses, bacteria, fungi, and parasites
PCa	Cavarretta et al.[73]	16 PCa patient samples	Massive ultradeep pyrosequencing	–	*Actinobacteria, Firmicutes,* and *Proteobacteria*
PCa	Cohen et al.[74]	34 PCa patient samples	16S rRNA gene sequencing	*Propionibacterium acnes*	–
PCa	Fassi Fehri et al.[75]	58 cancerous prostate tissues and 20 healthy prostate tissues	ISIF and PCR	*Propionibacterium acnes*	–

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Table 1. *Continued*

Cancer types	Research groups	Number of clinical samples	Methods	Associated microbiota (same)	Associated microbiota (different)
PCa	Ma et al.[76]	242 PCa patient samples	TCGA	–	*Listeria monocytogenes, Methylobacterium radiotolerans JCM 2831, Xanthomonas albilineans GPE PC73,* and *Bradyrhizobium japonicum*
Ovarian cancer (OC)	Al-Shabanah et al.[77]	100 pairs of OC tissues and adjacent normal tissues	PCR	–	HPV
OC	Zhou et al.[78]	25 OC tissues and 25 normal distal fallopian tube tissues	16S rRNA gene sequencing	–	*Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria,* and *Bacteria_unclassified*
Cervical cancer (CC)	Huang et al.[79]	112 CC patient samples	PCR	–	*F. nucleatum*
CC	Liu et al.[80]	48 persistent HPV infection samples, 4 transient infection samples, and 31 negative control samples	16S rRNA gene sequencing	–	Decreased levels of probiotics, including *Shuttleworthia, Prevotella, Lactobacillus,* and *Sneathia,* and increased levels of pathogenic bacteria, including *Dispar, Streptococcus,* and *Faecalibacterium prausnitzii*
Melanoma	Mrázek et al.[81]	90 samples from the MeLiM pig model	16S rRNA gene sequencing	–	*Fusobacterium* and *Trueperella*
Melanoma	Zhu et al.[82]	447 melanoma patient samples	TCGA	–	*Lachnoclostridium, Gelidibacter, Flammeovirga,* and *Acinetobacter*
Glioma	Zhao et al.[83]	3 human glioma samples	16S rRNA gene sequencing	–	Bacterial lipopolysaccharide
Gastric cancer (GC)	Yu et al.[84]	160 pairs of GC tissues and adjacent normal tissues	16S rRNA gene sequencing	*Firmicutes, Bacteroidetes, Proteobacteria*	*H. pylori*
GC	Shao et al.[85]	36 pairs of GCA tissues and adjacent normal tissues	16S rRNA gene sequencing	*Firmicutes, Bacteroidetes, Proteobacteria*	–
Renal cell carcinoma (RCC)	Wang et al.[86]	24 pairs of RCC tissues and adjacent normal tissues	16S rRNA gene sequencing	–	*Proteobacteria, Firmicutes, Bacteroidetes,* and *Actinobacteria, Chloroplast, Streptophyta*
Liver cancer	Huang et al.[87]	20 HCC samples and 16 control samples	16S rRNA gene sequencing and PCR	–	*H. pylori*
Liver cancer	Qu et al.[88]	28 pairs of PLC tissues and adjacent normal tissues	16S rRNA gene sequencing and PCR	–	*Proteobacteria, Actinobacteria, Bacteroidetes,* and *Firmicutes*

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; PAAD, pancreatic adenocarcinoma; LTS, long-term survival; STS, short-term survival; WGS, whole-genome sequencing; ESCC, esophageal squamous cell carcinoma; EAD, esophageal adenocarcinoma; TCMA, The Cancer Microbiome Atlas; TCGA, The Cancer Genome Atlas; NDBE, Barrett's esophagus without dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; FEP, fibroepithelial polyp; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; RFS, recurrence-free; DFS, disease-free survival; BWF, bronchial washing fluid; EMT, epithelial-to-mesenchymal transition; NHB, non-Hispanic Black; NHW, non-Hispanic White; MeLiM, melanoma-bearing Libechov minipig; GCA, gastric cardia adenocarcinoma; HCC, hepatocellular carcinoma; PLC, primary liver cancer.

Table 2. The function and implications of the intratumoral microbiota in various cancers

Cancer types	Associated microbiota	Main findings	Implications	Reference
PC	*Helicobacter pylori*	*H. pylori* was detected in PC patient tumors.	–	Nilsson et al.[29]
PC	Enterococcaceae, Lactobacillaceae, Bifidobacteriaceae, *Nakaseomyces,* and *Skeletocutis*	The duodenal fluid microbiome profile was altered in samples from patients with PDAC compared with samples from patients with pancreatic cysts and normal pancreas samples.	PC risk and survival after PC diagnosis are affected.	Kohi et al.[30]
PC	*Fusobacterium*, Porphyromonas and *Prevotella*	The microbial composition between PC and noncancer samples was different.	Identifying distinct bacterial taxa could shed new light on bacteria associated with etiology.	Del Castillo et al.[31]
PC	*Staphylococcus,* Enterobacteriaceae, Pseudomonadaceae, Ruminococcaceae and Bacillaceae	Intratumor bacterial colonization was more likely in patients who had pancreatic head tumors, had undergone preoperative biliary drainage with stent placement, had undergone the Whipple procedure, and had undergone neoadjuvant treatment with a combination of gemcitabine and paclitaxel.	–	Nalluri et al.[32]
PC	*Staphylococcus,* Enterobacteriaceae, *Corynebacterium, Escherichia, Propionibacterium,* and *Streptococcus*	The human pancreas microbiota composition was not different between normal and disease states.	–	Thomas et al.[33]
PC	*Gammaproteobacteria,* Enterobacteriaceae, Pseudomonadaceae	Intratumor bacterial colonization was more likely in patients who had undergone surgical modification of the pancreatic duct. Enterobacteriaceae were found to express CDD_L.	Resistance to chemotherapy is mediated.	Geller et al.[15]
PC	*Proteobacteria,* Pseudomonadaceae, *Bacteroidetes*, *Firmicutes*, *Actinobacteria,* and *Elizabethkingia*	The PC microbiome promoted tumorigenesis by inducing innate and adaptive immune suppression.	PC progression is promoted.	Pushalkar et al.[34]
PC	*Proteobacteria, Gammaproteobacteria,* Pseudomonadaceae, *Citrobacter freundii* and *Shigella sonnei*	The PC microbiome was linked to carcinogenesis and worse prognosis in men and smokers.	Prognosis might be affected.	Chakladar et al.[35]
PC	*Saccharopolyspora, Pseudoxanthomonas, Streptomyces,* and *Bacillus clausii*	The tumor microbial communities were significantly different between LTS and STS. The tumor microbiome affected the immune responses that promoted T cell activation.	Human PDAC survival could be predicted.	Riquelme et al.[36]
PC	Pseudomonadaceae, *Acinetobacter* and *Sphingopyxis*	Distinct microbial communities in basal-like tumors played an inflammation-inducing role and promoted pancreatic carcinogenesis.	Some bacteria are highly associated with carcinogenesis.	Guo et al.[37]
PC	*Malassezia*	The fungal flora promoted pancreatic tumorigenesis through MBL activation.	PC progression is promoted.	Aykut et al.[38]

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Table 2. *Continued*

Cancer types	Associated microbiota	Main findings	Implications	Reference
CRC	*Fusobacterium, F. nucleatum, Fusobacterium necrophorum, Bacteroides fragilis, Bacteroides thetaiotaomicron, Prevotella intermedia,* and *Selenomonas sputigena*	*Fusobacterium* co-occurred with other Gram-negative anaerobic bacteria in primary and matched liver metastases. Treatment of *Fusobacterium*-containing PDX models with metronidazole inhibited tumor growth.	Antimicrobial intervention is a potential treatment for patients with *Fusobacterium*-associated CRC	Bullman et al.[39]
CRC	*Bacteroidetes* cluster 2, *Prevotella* cluster, *Pathogen* cluster, and *Firmicutes* cluster 2	Alterations in the composition of the microbiota were not limited to cancer tissues and differed between distal and proximal cancer tissues.	–	Flemer et al.[40]
CRC	*Fusobacterium, Firmicutes, Proteobacteria*	The microbiota communities of CRC and precancerous adenoma were heterogeneous. This diversity could be associated with differences in the adenoma-cancer sequence and alterations of genes correlated with CRC.	–	Liu et al.[41]
CRC	*Fusobacterium, Campylobacter, Leptotrichia*	Anaerobic bacteria coexisted in CRC. Microbial characteristics were associated with the overexpression of host genes, including the gene encoding IL-8.	–	Warren et al.[42]
CRC	*Fusobacterium, Campylobacter, Peptostreptococcus*	*Fusobacterium* was related to *ATM* and *PIK3CA* alterations and alterations in genes encoding cell cycle proteins. Patients with high *Campylobacter* levels were more likely to have mutational signature 3, representing failure of double-strand DNA break repair	Substances released by bacterial infection cause DNA damage, which in turn can drive CRC progression.	Okuda et al.[43]
CRC	*F. nucleatum*	The level of *F. nucleatum* in tumors increased with pathological stage and was highest in stage III/IV samples.	–	Yamamoto et al.[44]
CRC	*Fusobacterium, Providencia*	Virulence-related bacterial genes were highly enriched in the CRC tumor microenvironment.	It is hinted that the microbiome could drive CRC progression.	Burns et al.[45]
CRC	*Bifidobacteria*	There was a strong association between intratumoral *Bifidobacteria* and signet ring cells.	–	Kosumi et al.[46]
EC	*Firmicutes* levels were significantly increased, while *Proteobacteria* levels were decreased in tumor samples.	The characteristics of the intratumoral microbiota were related to the subtype, stage, and prognosis of EC.	–	Wang et al.[47]
EC	*F. nucleatum*	*F. nucleatum* was associated with the survival of EC patients. *F. nucleatum* activated CCL20 to enhance the invasiveness of EC.	Prognosis is affected.	Yamamura et al.[48]

(*Continued on next page*)

Table 2. *Continued*

Cancer types	Associated microbiota	Main findings	Implications	Reference
EC	*Proteobacteria, Firmicutes, Bacteroidetes, Spirochaetes, Streptococcus, Prevotella,* and *Treponema*	The combined abundance of *Streptococcus* and *Prevotella* was suggested to be a prognostic biomarker for ESCC patients.	Action as a prognostic biomarker is demonstrated.	Liu et al.[49]
EC	*F. nucleatum*	*F. nucleatum* was linked to ESCC patients' adverse reaction to neoadjuvant chemotherapy.	Treatment response to neoadjuvant chemotherapy in ESCC could be predicted.	Yamamura et al.[50]
EC	*Proteobacteria,* Enterobacteriaceae*,* and *Akkermansia muciniphila* were increased, while *Firmicutes* and *Veillonella* were reduced.	The intratumoral microbiome changed dramatically during progression of Barrett's esophagus to EAD.	–	Snider et al.[51]
OSCC	*Exiguobacterium oxidotolerans, Prevotella melaninogenica, Staphylococcus aureus, Veillonella parvula*	Multiple bacterial groups were isolated from OSCC tissues.OSCC tissues differed in microbial composition from adjacent normal tissues.	–	Hooper et al.[52]
OSCC	*Streptococcus intermedius* was present in all samples. *Streptococcus sp. oral taxon 058*, *Peptostreptococcus stomatis*, *Streptococcus salivarius*, *Streptococcus gordonii*, *Gemella haemolysans*, *Gemella morbillorum*, *Johnsonella ignava*, and *Streptococcus parasanguinis I* were highly abundant in OSCC samples.	Bacterial colonization was different between tumor and nontumor tissues in OSCC patients.	–	Pushalkar et al.[53]
OSCC	*F. nucleatum subsp. polymorphum*, *Streptococcus dysgalactiae*, *Citrobacter koseri*, and *P. aeruginosa* were significantly enriched in OSCC, while *Streptococcus mitis* and *Staphylococcus epidermidis* were abundant in FEP.	Enrichment of the inflammatory bacterial population was found in OSCC.	Intratumoral microbes are pro-inflammatory.	Perera et al.[54]
OSCC	*F. nucleatum, P. aeruginosa*	For the first time, *F. nucleatum* and *P. aeruginosa* were found to be related to OSCC development.	–	Al-Hebshi et al.[55]
OSCC	*F. nucleatum*	*F. nucleatum*-related OSCC had a specific immune microenvironment and was more common in older patients without alcoholic disease.	Relationship shown to a favorable prognosis.	Neuzillet et al.[56]
OSCC	*Clostridium perfringens*	*Clostridium perfringens* enterotoxin induced claudin-4 to activate YAP, thereby promoting progression of OSCC.	Tumor progression is promoted.	Nakashima et al.[57]
Lung cancer	*Proteobacteria* levels were increased, while *Firmicutes* levels were decreased.	Tumors harboring TP53 mutations had distinct bacterial communities that were also relatively abundant in smoking-related tumors.	–	Greathouse et al.[58]

Table 2. *Continued*

Cancer types	Associated microbiota	Main findings	Implications	Reference
Lung cancer	*Escherichia coli* str. K-12 substr. W3110, *Pseudomonas putida* str. KT2440	Age- and sex-related differentially enriched microbes were associated with genomic alterations and immune dysregulation.	–	Wong et al.[59]
Lung cancer	*Veillonella*, *Megasphaera*, *Atopobium*, and *Selenomonas*	Bacterial communities differed between lung cancer patients and individuals with benign lesions.	The environment for lung cancer patients might be changed.	Lee et al.[60]
Lung cancer	intestinal bacteria, potentially pathogenic or inflammatory bacteria	Lung cancer tissues showed enrichment of intestinal and pro-inflammatory bacteria.	–	Dumont-Leblond et al.[61]
Lung cancer	*Mycoplasma, Staphylococcus epidermidis, Streptococcus mitis, Bacillus* strains, *Chlamydia, Candida, Listeria,* and *Haemophilus influenza*	Multiple pathogens were identified in tissue samples surgically extracted from lung cancer patients.	–	Apostolou et al.[62]
Lung cancer	In normal tissues, greater abundance of the Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae families was associated with reduced RFS or DFS, whereas the Koribacteraceae family was associated with increased RFS and DFS.	The diversity and overall composition of the microbiota in tumor tissue were not associated with RFS or DFS.	Survival is affected.	Peters et al.[63]
Lung cancer	The most predominant phyla in the BWF samples were *Firmicutes* and *Proteobacteria*, and the most common genus was *Prevotella*. In sputum samples, *Firmicutes* was the predominant phylum, while *Streptococcus* was the most common genus.	BWF samples could reflect the microbiome of lung cancer tissues better than sputum samples.	–	Huang et al.[64]
BC	*Firmicutes, Proteobacteria*	Breast tissue was not sterile, and a diverse microflora was present.	–	Urbaniak et al. (2014)
BC	*Staphylococcus,* Enterobacteriaceae, *Bacillus*, *Staphylococcus epidermidis* and *Escherichia coli*	The bacterial profiles in adjacent normal tissues from BC patients differed from those in breast tissues of healthy women. DNA damage-causing bacteria were present in higher abundance in BC patients.	These bacteria play a role in modulating risk of BC development.	Urbaniak et al. (2016)
BC	*Firmicutes, Proteobacteria, Actinobacteria,* and *Bacteroidetes*	There were differences in microbial composition and relative abundance between breast tumor tissues and normal tissues. Microbial composition varied significantly between women and between the left and right breasts of each woman.	–	Klann et al.[67]

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Table 2. *Continued*

Cancer types	Associated microbiota	Main findings	Implications	Reference
BC	*Methylobacterium radiotolerans* was abundant in tumor tissues, while *Sphingomonas yanoikuyae* was abundant in paired normal tissues.	Microbial DNA was present in the breast, and microbial dysregulation was associated with BC. Bacteria and their components could affect the local immune microenvironment in BC patients.	The local immune microenvironment was influenced.	Xuan et al.[68]
BC	*Firmicutes, Proteobacteria, Actinobacteria*	*Haemophilus influenza* was associated with proliferation pathway-related genes. *Listeria spp.* were linked to gene expression profiles associated with EMT.	–	Thompson et al.[69]
BC	*Proteobacteria*	The microbial community in breast tissue varied by race, stage, and breast tumor subtype.	–	Smith et al.[70]
BC	*Firmicutes*	The intratumoral microbiota promoted BC cell metastasis and colonization of the lung.	BC metastasis and colonization are promoted.	Fu et al.[71]
PCa	viruses, bacteria, fungi, and parasites	A diverse microbiome was observed in PCa samples compared with benign lesions. In some cases, viral (HPV18, KSHV) and bacterial (*H. pylori*) sequences led to perturbation of gene expression, in turn affecting the development of cancer.	Tumorigenesis is influenced at the genetic level.	Banerjee et al.[72]
PCa	*Actinobacteria, Firmicutes,* and *Proteobacteria*	The prostate contained a large number of bacteria, the distribution of which depended on the nature of the tissue.	–	Cavarretta et al.[73]
PCa	*Propionibacterium acnes*	For the first time, *P. acnes* was discovered and isolated from PCa tissue. *P. acnes* was positively correlated with prostate inflammation.	The bacterium was positively correlated with prostate inflammation.	Cohen et al.[74]
PCa	*Propionibacterium acnes*	*P. acnes* infection promoted the occurrence and development of PCa.	PCa progress is promoted.	Fassi Fehri et al.[75]
PCa	*Listeria monocytogenes, Methylobacterium radiotolerans JCM 2831, Xanthomonas albilineans GPE PC73,* and *Bradyrhizobium japonicum*	Microbes were more abundant in PCa samples than in normal samples. Some of these microbes were cancer promoting, and unexpectedly, most of the microbes were found to exert antitumor effects in PCa.	There is a double effect on the tumor.	Ma et al.[76]
OC	HPV	High-risk HPV types (HPV-16, 18, 45) were closely associated with advanced stage in OC.	–	Al-Shabanah et al.[77]

Table 2. *Continued*

Cancer types	Associated microbiota	Main findings	Implications	Reference
OC	*Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria,* and *Bacteria_unclassified*	The diversity and richness of bacterial communities were reduced in OC tissues compared with normal distal fallopian tube tissues. Microbiota dysbiosis was found to control OC initiation or progression by suppressing host inflammatory and immune responses.	–	Zhou et al.[78]
CC	*F. nucleatum*	The level of *F. nucleatum* was higher in CC tissues. High levels of *F. nucleatum* were associated with poorer overall survival and PFS in CC patients. *F. nucleatum* could be a potential diagnostic and prognostic biomarker for CC.	The bacterium provides potential CC diagnostic and prognostic biomarkers.	Huang et al.[79]
CC	decreased levels of probiotics including *Shuttleworthia*, *Prevotella*, *Lactobacillus*, and *Sneathia;* and increased levels of pathogenic bacteria, including *Dispar*, *Streptococcus*, and *Faecalibacterium prausnitzii*	The composition of the vaginal microbiota in HPV-infected cervical cancer patients varied with the duration of infection.	–	Liu et al.[80]
Melanoma	*Fusobacterium* and *Trueperella*	There were significant differences in bacterial composition and diversity between skin and melanoma microbiomes.	–	Mrázek et al.[81]
Melanoma	*Lachnoclostridium, Gelidibacter, Flammeovirga,* and *Acinetobacter*	The intestinal microbiota within tumors modulated chemokine (CXCL9, CXCL10 and CCL5) levels and influenced CD8$^+$ T cell infiltration. High levels of *Lachnoclostridium* significantly reduced the risk of death in melanoma patients.	The prognosis of patients receiving immunotherapy is affected.	Zhu et al.[82]
Glioma	Bacterial lipopolysaccharide	Researchers developed a 3D quantitative *in situ* microbiota imaging strategy that enabled direct, contamination-free visualization of bacteria within gliomas. Bacterial components in human glioma samples were irregular and sparsely distributed, mainly located near the nuclear membrane or in the intercellular space.	–	Zhao et al.[83]
GC	*Firmicutes, Bacteroidetes, Proteobacteria,* and *H. pylori*	*H. pylori* was the most enriched member of the gastric microbiota in samples from China and Mexico. The relative abundance of *H. pylori* was higher in nonmalignant tissues than in malignant tissues.	–	Yu et al.[84]

Table 2. *Continued*

Cancer types	Associated microbiota	Main findings	Implications	Reference
GC	*Firmicutes, Bacteroidetes, Proteobacteria*	Compared with the relative levels in nontumor tissues, there was decreased *Helicobacter* abundance and greater α diversity in GCA tumor tissues.	–	Shao et al.[85]
RCC	*Proteobacteria, Firmicutes, Bacteroidetes,* and *Actinobacteria Chloroplast, Streptophyta*	Species diversity was reduced in RCC tissues, and the bacterial community composition was significantly different from that in normal tissues. Compared with normal tissue, 25 taxa were increased, and 47 taxa were decreased in RCC tissue. The presence of *Chloroplast* and *Streptophyta* significantly distinguished normal tissue from RCC tissue.	–	Wang et al.[86]
Liver cancer	*H. pylori*	*H. pylori* was present in HCC tissues	The bacterium could be associated with carcinogenic processes in the liver.	Huang et al.[87]
Liver cancer	*Proteobacteria, Actinobacteria, Bacteroidetes,* and *Firmicutes*	Bacteria with antitumor effects, such as *Pseudomonas*, were reduced in PLC tissues, and their levels linearly correlated with the prognosis of PLC patients.	–	Qu et al.[88]

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; PAAD, pancreatic adenocarcinoma; LTS, long-term survival; STS, short-term survival; WGS, whole-genome sequencing; ESCC, esophageal squamous cell carcinoma; EAD, esophageal adenocarcinoma; TCMA, The Cancer Microbiome Atlas; TCGA, The Cancer Genome Atlas; NDBE, Barrett's esophagus without dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; FEP, fibroepithelial polyp; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; RFS, recurrence-free; DFS, disease-free survival; BWF, bronchial washing fluid; EMT, epithelial-to-mesenchymal transition; NHB, non-Hispanic Black; NHW, non-Hispanic White; MeLiM, melanoma-bearing Libechov minipig; GCA, gastric cardia adenocarcinoma; HCC, hepatocellular carcinoma; PLC, primary liver cancer.
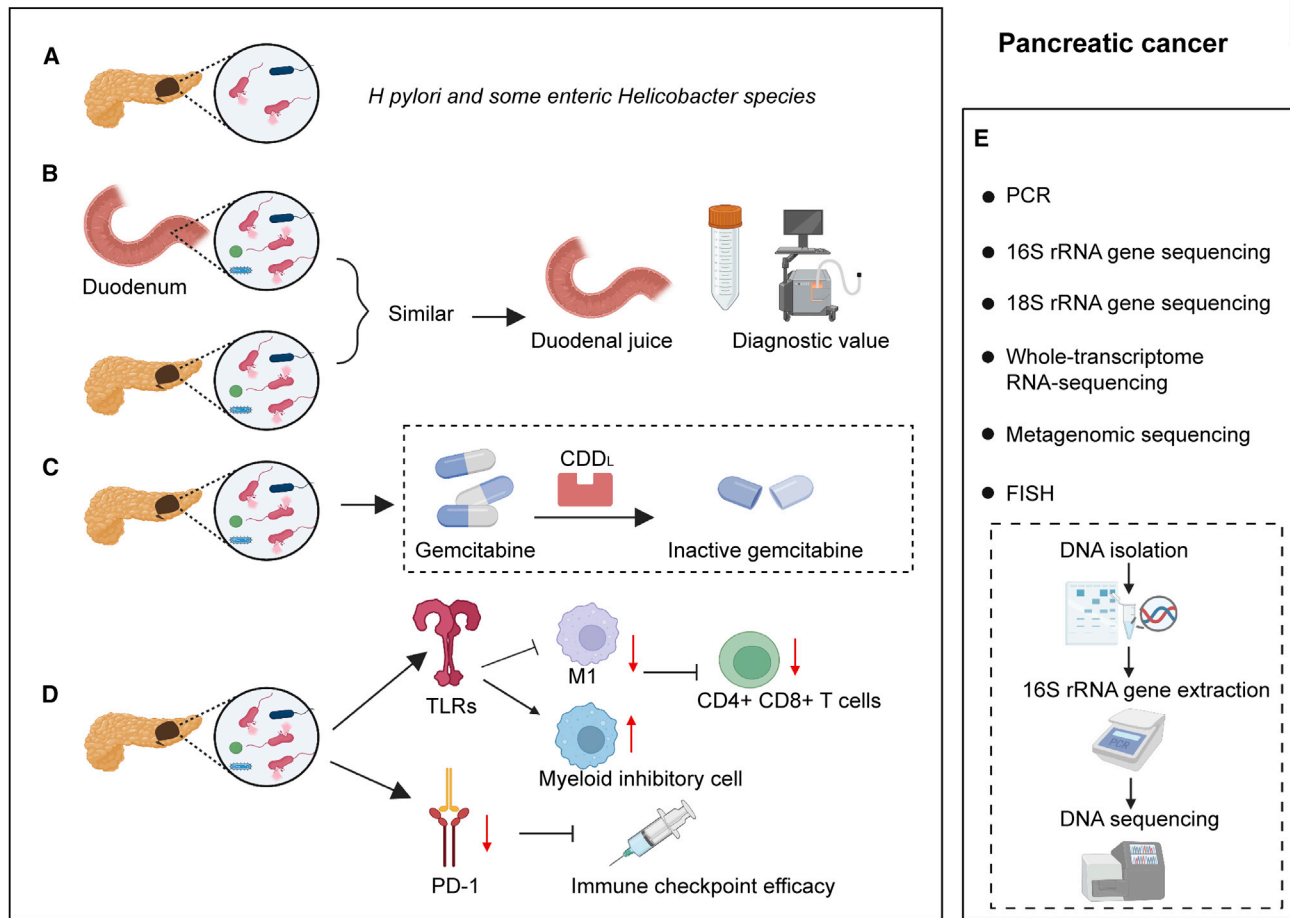
Figure 3. Characterization of the intratumoral microbiota in pancreatic cancer (PC)
(A) Gastric *H. pylori* and low levels of intestinal *H. pylori* were detected in tissue from PC patients but not in tissue from controls.
(B) Researchers believe that most intratumoral bacteria in PDAC patients result from retrograde migration from the duodenum, and the microbial composition of the two is similar.
(C) PDAC contains bacteria that modulate tumor sensitivity to gemcitabine.
(D) The microbiome promotes PC tumorigenesis by inducing innate and adaptive immune suppression.
(E) Methods commonly used by researchers to study microbes in PC tumors.

Compared with adjacent normal tissues, human CRC tissues show enrichment of *Fusobacterium* species.[42,96,97] *Fusobacterium* functions as a carcinogenic factor in the progression of CRC and has emerged as an influential bacterium associated with colorectal carcinogenesis. A series of studies have confirmed these observations. *F. nucleatum* was associated with different immune responses in tumors with microsatellite instability.[98] Its abundance was correlated with the CDKN2A methylation level and macrophage infiltration.[99] Bullman et al. found co-occurrence of *Fusobacterium* with other Gram-negative anaerobic bacteria in primary and matched liver metastases.[39] The researchers revealed consistency in the microbial community composition between paired primary and metastatic tumors. Furthermore, they reported that the treatment of *Fusobacterium*-containing patient-derived xenograft (PDX) models with the antibiotic metronidazole inhibited tumor proliferation and growth. Recently, Zhang et al. revealed an

F. nucleatum/ALPK1/NF-κB/ICAM1 axis in CRC.[100] The researchers found that the abundance of *F. nucleatum* in tumor tissues of CRC patients was positively correlated with ALPK1 and ICAM1 expression levels. *F. nucleatum* induced ALPK1 to activate the nuclear factor κB (NF-κB) pathway, leading to the upregulation of ICAM1. Ultimately, these factors promote the adhesion of CRC cells to endothelial cells and promote extravasation and metastasis. Selective targeting of *Fusobacterium* appears to be a potential therapeutic approach. Yamamoto et al. revealed the varied distribution of *F. nucleatum* in the development of CRC.[44] The presence of *F. nucleatum* was highest in stage III/IV disease. In addition, researchers have suggested that *F. nucleatum* could be used as a biomarker because of its association with CRC cell proliferation. In 2015, researchers reported another genus with a pathogenic role in CRC, *Providencia*. In addition, researchers found that virulence-related bacterial genes were highly enriched in
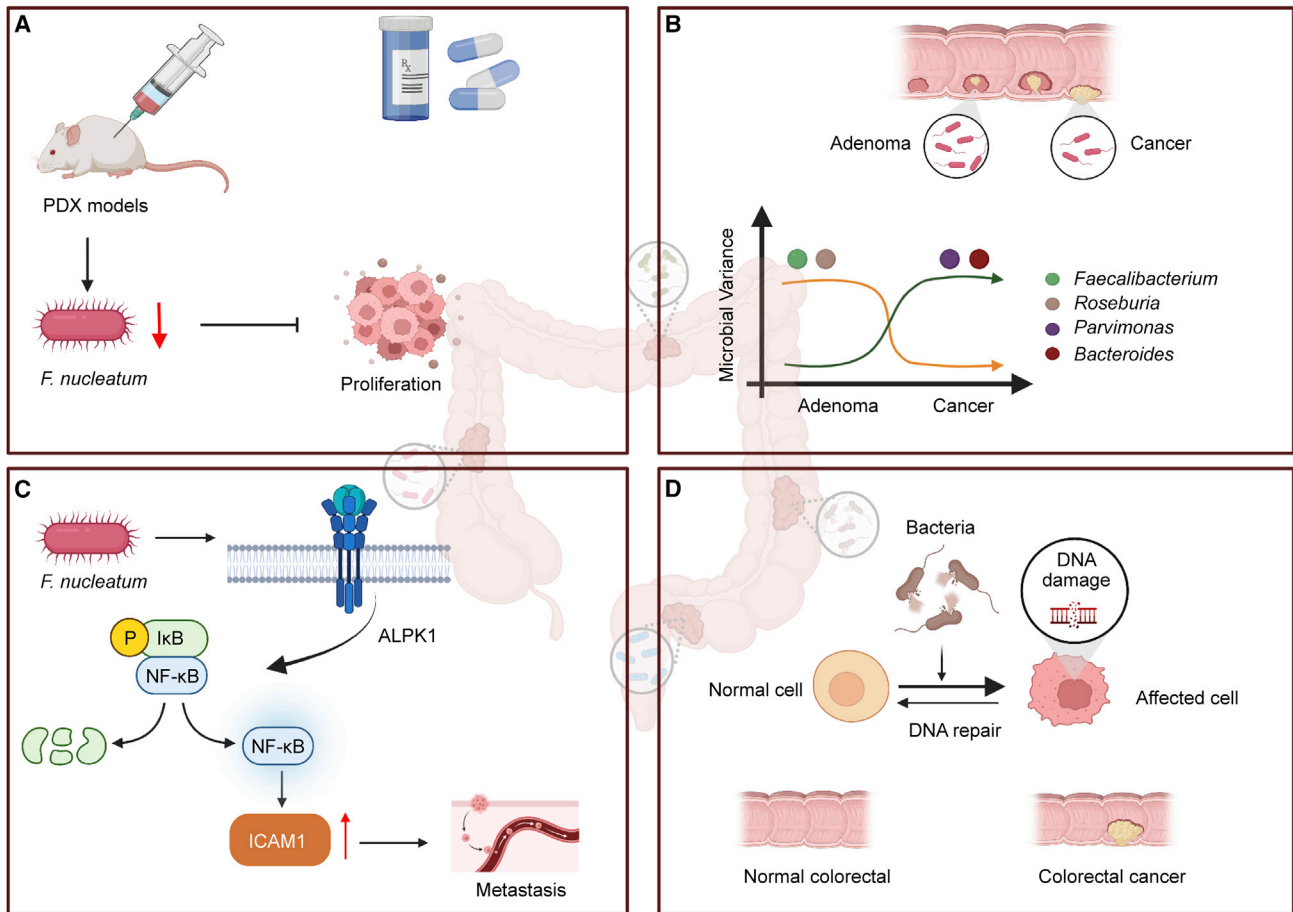
Figure 4. Characterization of the intratumoral microbiota in colorectal cancer (CRC)
(A) Treatment of *Fusobacterium*-containing PDX models with metronidazole inhibited tumor growth.
(B) Changes in individual microbial abundance within tumors vary along the adenoma-cancer sequence. The abundance of intratumoral variants of *Bacteroides* and *Parvimonas* increases from adenoma to carcinoma. The abundance of intratumoral variants of *Faecalibacterium* and *Roseburia* decreases from adenoma to carcinoma.
(C) *F. nucleatum* induced ALPK1 to activate the NF-κB pathway, leading to the upregulation of ICAM1.
(D) The development of CRC could be caused in part by DNA damage caused by substances released by bacterial infection.

the CRC tumor microenvironment.[45] Kosumi et al. found *Bifidobacteria* species in the tumors of 393 of 1,313 CRC patients.[46] There was a strong association between the presence of intratumoral *Bifidobacteria* species and signet ring cells. Signet ring-like cells form when cells are filled with a large amount of mucus that squeezes the nucleus to one side of the cell, giving it a ring-like appearance. Other interesting findings were that changes in the microbiota composition were not limited to cancer tissues and differed between distal and proximal cancers. *Prevotella* and *Firmicutes* (found in cluster 2) were more abundant in proximal cancers, whereas *Bacteroidetes* (found in cluster 2) and various pathogens (found in several clusters) were more abundant only in distal cancers.[40,101] Liu et al. reported microflora heterogeneity in CRC tumors.[41] The composition of the microbiota in biopsies from the same site was similar but significantly different between biopsies from different sites. These findings could explain the different results revealed by different research groups. As such, 2–3 biopsy

samples from each tumor should be collected whenever possible. In addition, the development of CRC might be caused in part by DNA damage induced by substances released by a bacterial infection (Figure 4).[43]

Esophageal cancer
Researchers have analyzed the microbial characteristics of esophageal cancer (EC) tissues based on two common databases: The Cancer Microbiome Atlas (TCMA) and The Cancer Genome Atlas (TCGA). The abundance of *Firmicutes* has been found to be significantly increased, while that of *Proteobacteria* has been found to be significantly decreased in tumor samples. In addition, studies have revealed that some microorganisms are closely related to the subtype, stage, and prognosis of EC. For example, a high abundance of *Proteobacteria*, *Negativicutes*, and *Lactobacillus* was linked to a favorable prognosis. The opposite was true for *Clostridiales* and *Fusobacteriales*.[47] Several studies have also revealed the relationships between

intratumoral microbes and the prognosis of EC. Yamamura et al. reported that *F. nucleatum* can be used as a prognostic biomarker due to its association with shorter survival in EC patients.[48] Liu et al. suggested that the combined abundance of *Streptococcus* and *Prevotella* could predict prognosis in esophageal squamous cell carcinoma (ESCC) patients.[49] The identification of similar biomarkers could be helpful for clinical prognostic assessment. In addition, researchers observed that *F. nucleatum* was linked to adverse reactions to neoadjuvant chemotherapy in ESCC patients.[50] Interestingly, the intratumoral microbiome was found to change dramatically during the progression of Barrett's esophagus to esophageal adenocarcinoma (EAD).[51] This finding is of great significance and could guide the clinical prediction of cancer risk based on microbial changes before the development of EAD.

Oral squamous cell carcinoma

In 2006, Hooper et al. first found that there were multiple bacterial groups in oral squamous cell carcinoma (OSCC) tissues.[52] The microbial composition was different between cancer and adjacent normal tissues. Subsequent studies confirmed these findings.[102,53] In addition to focusing on the intratumoral microbial composition, researchers have also explored the presence of functional bacteria in OSCC. They found that inflammatory bacteria were enriched in OSCC tissue. Although the results of studies of microbial composition have varied, they suggest that microbial communities composed of different species might be functionally similar.[54] Specifically, Al-Hebshi et al. were the first to discover that *F. nucleatum* and *Pseudomonas aeruginosa*, which are characteristic of inflammatory flora, were associated with OSCC development.[55] However, puzzlingly, another study revealed that *F. nucleatum* was associated with better outcomes in OSCC patients. Specifically, the *F. nucleatum* burden was negatively correlated with M2-type macrophages, insensitivity to pro-inflammatory signaling, and low TLR4 signaling, and its presence ultimately led to favorable clinical outcomes.[56] Both studies were limited by issues such as a small sample size, and the conclusions of the studies must be further confirmed. In addition, studies have revealed the potential utility of *Clostridium perfringens* enterotoxin (CPE) as a therapeutic target in OSCC.[57] On the one hand, CPE enhances the proliferation, migration, and invasion ability of OSCC cells by inducing CLDN4 nuclear translocation. On the other hand, by inhibiting the phosphorylation of YAP1, CPE promotes YAP1 expression, thereby promoting tumor progression. Inhibition of CPE could be a treatment strategy for OSCC.

Lung cancer

Although the respiratory tract is exposed to the outside world, the trachea and lungs of healthy individuals are traditionally considered sterile. In 2010, Hilty et al. described the microbiota in the airways of asthmatic patients.[103] Subsequently, reports of bacteria in lung cancer tissue began to appear. Researchers found that, in lung squamous cell carcinoma (LUSC), *Acidovorax* abundance differed between smokers and nonsmokers, with the former having a higher abundance. In addition, *Acidovorax* was more common in patients with TP53-mutated squamous cell carcinoma.[58] Another interesting study reported age- and sex-

related differences in microbiome composition in lung cancer. In addition to smoking, age and sex are important factors affecting the pathogenesis of lung cancer.[104] The high incidence of lung cancer occurs in patients between the ages of 55 and 65 years old, although recently, the incidence of lung cancer in younger patients has increased. Lung cancer incidence and mortality are higher in men than in women. In LUSC, *Pseudomonas putida* str. KT2440 is more common in young male patients and is associated with immune infiltration. In lung adenocarcinoma (LUAD), *Escherichia coli* str. K-12 substr. W3110 is related to survival and genomic alterations in older female and male patients.[59] Many studies have reported that bacterial communities differ between lung cancer patients and individuals with benign lesions.[61,60] Relationships between the human lung tissue microbiota and epidemiological and clinical features have also been revealed.[105] In addition, Lee et al. suggested that the *Veillonella* and *Megasphaera* genera could be used as biomarkers to predict lung cancer.[60] The researchers found multiple species of microflora in surgically resected lung cancer tissues, with *Mycoplasma* found in nearly all samples. They suggested that chronic infection plays an important role in the development of lung cancer.[62] Peters et al. conducted a study of the relationship between the lung microbiota and lung cancer prognosis.[63] They reported that the diversity and composition of the normal lung tissue microbiome were associated with recurrence-free survival (RFS) and disease-free survival (DFS). However, the microbial composition in tumor tissue was independent of RFS and DFS.

In conclusion, the lung microbiota might alter the lung cancer environment in patients and could play a significant role in the development of lung cancer. It is worth mentioning that the samples currently used to study the intratumoral microbiome of lung cancer patients have mainly been surgically resected tumor tissue, bronchial washing fluid (BWF), bronchial brushing tissue, and sputum samples. Although studies using surgically resected tumor tissues would be ideal, researchers have often used BWF and sputum samples instead. Huang et al. explored differences between BWF and sputum samples.[64] The results suggested that BWF samples might better reflect the microbiome of lung cancer tissue. Regardless of the metastatic status of lung cancer, differences between squamous cell carcinoma and adenocarcinoma have been identified. In different histological types of lung cancer, the genera related to distant metastasis were also different.

Breast cancer

As early as 2014, researchers revealed the potential of microorganisms in BC diagnosis and treatment strategies. They found that total bacterial counts were reduced in BC compared with matched normal tissue. Furthermore, bacterial DNA load was inversely associated with disease state. In addition, the expression levels of antibacterial response genes in tumors were lower than those in normal control tissues.[68] Thompson et al.[69] revealed a link between the microbiota and host gene expression in BC in a large-sample-size study. Some bacteria in the microbiota appeared to be closely linked to genetic profiles related to processes such as epithelial-mesenchymal transition (EMT) and proliferation. Smith et al.[70] studied the microbiome of breast tissue in non-Hispanic Black and non-Hispanic White patients.

They found that the microbial community in breast tissue varied by race, stage, and breast tumor subtype. Recent research has also found that the intratumoral microbiota promotes the metastasis and colonization of BC cells. Mechanistically, intratumoral bacteria enhance resistance to fluid shear stress by reorganizing the actin cytoskeleton, thereby promoting host cell survival and metastasis.[71] In conclusion, intratumoral microorganisms are widely present in BC tissues, and these microorganisms might participate in the occurrence, development, and prognosis of cancer. Antibiotic therapy that precisely targets microorganisms seems to have potential, but the use of such therapy in the clinic must be evaluated.

16S rRNA gene sequencing is one of the most commonly used methods to examine the microbial composition within tumors. Costantini et al.[106] reported that the V3 region of the 16S rRNA gene contains the most microbial information in breast tissue. They argued that the V4 and V6 + V7 regions were the most informative regions describing the gut microbiota, but using the same regions to describe the breast tissue microbiome might underestimate the total population analyzed. They explored the mammary tissue microbiota in various hypervariable regions (V2, V3, V4, V6+7, V8, and V9) of the 16S rRNA gene. The V3 region was ultimately found to be the most informative region in the breast tissue microbiome, accounting for 45% of all reads. They found that the results of core needle biopsy (CNB) and surgical excision biopsy (SEB) were significantly similar, while CNB is less invasive and more useful as a diagnostic modality. Interestingly, their study also revealed that the microbial composition of tumor tissue and adjacent normal tissue was similar. This finding seems to contradict some previous studies.[68] Klann et al.[67] suggested that this conflicting result could be due to inconsistencies in sample collection methods between studies. Normal control tissue was obtained from women who never had cancer in some studies, while in others, tissue adjacent to cancer in the same woman was used as normal control tissue.

Prostate cancer

In 2005, researchers isolated and cultured *Propionibacterium acnes* from prostate cancer (PCa) tissue for the first time.[74] Several previous studies have revealed the presence of microbes in PCa tissue, primarily the gut microbiota.[72,73] Researchers have also found that *P. acnes* is positively associated with prostate inflammation, which could be responsible for the subsequent development of cancer. Fehri et al.[75] confirmed previous findings and further revealed a possible mechanism by which *P. acnes* promotes cancer. After *P. acnes* infection, the transcription factors NF-κB and STAT3 are activated in the body, and the COX2-prostaglandin and plasminogen-matrix metalloproteinase pathways are also activated. The host exhibits a strong inflammatory response. Prolonged exposure to *P. acnes* affects host cell proliferation and causes cellular transformation. Ma et al.[76] conducted a large-sample-size study. They analyzed sequencing data from 242 PCa patients to gain insights into the distribution and potential roles of microbes present in PCa. The researchers found that microbes were more abundant in PCa samples than in normal samples. Some of these microbes were found to be cancer promoting, and unexpectedly, most of the microbes were found to

exert antitumor effects in PCa. Much research is needed in the future to further validate these findings.

Others

Studies of intratumoral microbes in ovarian cancer (OC),[77,78] CC,[79,80] melanoma,[81,82] glioma,[83] GC,[84,85] renal cell carcinoma (RCC),[86] and liver cancer[87,88] have also been reported (see Table 1 for details). For example, Al-Shabanah et al.[77] observed a high percentage of HPV+ cells in OC (42%), while only 8% of cells in normal adjacent tissues were HPV+. HPV-16 was the predominant genotype in malignant tissues, followed by HPV-18 and HPV-45. In addition, viruses easily integrate into the host genome, resulting in gene inactivation and chromosomal instability that can promote cancer progression. A study of liver cancer and intratumoral microbes was recently reported. The researchers used 16S rRNA MiSeq to characterize the tumor microbiome of primary liver cancer (PLC) patients for the first time. They found differences in microbial populations between cancerous tissue and matched adjacent nontumorous tissue, between different histopathological subtypes, and between PLC patients with different prognoses.[88] In conclusion, biomarkers reflecting the intratumoral microbiota could be noninvasive tools for cancer diagnosis and might also be useful in cancer treatment and prognostication.

THERAPEUTIC POTENTIAL OF THE INTRATUMORAL MICROBIOTA IN TUMORS

Traditional cancer treatments are mostly unable to accurately differentiate between malignant and healthy tissue, have a relatively broad scope of action, lack tumor specificity, and carry a high risk of recurrence. Furthermore, the unintended killing of healthy tissue results in side effects of varying severity. Researchers have tried for a long time to find ideal biomarkers for cancer diagnosis and treatment. Recent human microbiome studies have shown that microbes play important roles in carcinogenesis, therapeutic response, and drug resistance. Gut microbes can exert oncogenic or tumor-suppressive effects through a variety of mechanisms. Modulating the gut microbiota could be useful for cancer prevention and/or treatment. Recently, attention has shifted to the study of microbes within tumors. Although this research is mostly in the preliminary stage, many studies have begun to reveal the diagnostic value and therapeutic potential of intratumoral microbes. These microbes are closely related to immunity and can modulate the host immune system. With continuous advances in bioengineering technology, an increasing number of engineered bacteria have been designed and applied as cancer treatments. In conclusion, intratumoral microbes play a substantial role in cancer diagnosis and treatment.

Function as biomarkers in cancer

As a component of tumors, intratumoral microbes have the potential as novel diagnostic or prognostic markers. Poore et al.[17] revealed unique microbial signatures by whole-genome and whole-transcriptome sequencing of 18,116 samples from 33 types of cancer. Stringent filtering criteria removed 92.3% of the total sequence data, but the researchers still identified

intratumoral microbial plasma signatures that were predictive of cancer type. The study revealed the potential clinical utility of detecting markers of the intratumoral microbiome in blood and using them as a diagnostic tool. In addition, researchers have continued to report the prognostic value of intratumoral microbes in tumors.[50,107] For example, Yamamura et al.[50] found that in ESCC, high expression levels of *C. nucleatum* in tumors predicted poor patient outcomes. The intratumoral microbiome was also able to predict the prognoses of papillary thyroid carcinoma subtypes.[107] In conclusion, identifying potential biomarkers and applying them in the clinic are some of the main directions of future research.

Effects on the host immune system

In recent years, immunotherapy has played a vital role in the treatment of cancer. Immune-based anticancer therapy mainly inhibits cancer by enhancing the patient's autoimmune function or attacking cancer cells displaying foreign antigens with immune substances. Currently, monoclonal antibodies against programmed death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) are widely used in clinical practice. Studies have found that intratumoral microbes might mediate and influence the role of immune effector cells in the tumor microenvironment. The presence of intratumoral microorganisms can increase or decrease tumor immunogenicity and promote or inhibit antitumor immunity.[108] An in-depth understanding of the impact of intratumoral microbes on the cancer immune system is of great value. There is currently sufficient and extensive relevant research in PC. Pushalkar et al.[34] reported that the PDAC microbiome exerts oncogenic effects by affecting the host's immune system. The PDAC microbiome generates a tolerant immune program by activating TLRs, suppressing the activation of $CD4^+$ T cells and differentiated $CD8^+$ T helper 1 (Th1) cells, and inhibiting M1 macrophage differentiation. Elimination of PDAC microbes remodels the tumor microenvironment, in turn enhancing immunotherapy and potentially aiding the treatment of PDAC. The results of another study seemed to contradict the above findings. Based on 16S rRNA gene sequencing, researchers explored the microbial composition and function of the microbiota in PDAC patients. They found that the PDAC microbiota composition affects host immune responses and disease progression. They revealed that the three genera—*Saccharopolyspora*, *Pseudoxanthomonas*, *and Streptomyces*— in PDAC could promote antitumor immune responses by recruiting and activating $CD8^+$ T cells.[36] In head and neck squamous cell carcinoma, the pattern of immune cell infiltration in HPV+ tumor samples differs from that in non-virus-associated samples. HPV+ tumors are enriched in infiltrating $CD8^+$ T cells, myeloid dendritic cells (DCs), and pro-inflammatory chemokines.[109,110] In CRC, investigators reported that the Fap2 protein of *F. nucleatum* inhibits immune cell activity through TIGIT, protecting tumor cells from attack by the host immune system.[111] Studies in GC have also been performed. Panda et al.[112] reported benefits from treatment with the anti-PD-L1 antibody avelumab in a patient with metastatic GC. They found that the tumor tissues of Epstein-Barr virus (EBV)+ GC patients had higher lymphocyte infiltration and

expression of genes related to immune checkpoint pathways, suggesting that intratumoral microbes could be involved in avelumab treatment efficacy. In conclusion, the impact of intratumoral microbes on the immune status of the host tumor microenvironment is obvious and deserves further in-depth exploration.

Effects on chemotherapy efficacy

Intratumoral microbes also influence the efficacy of cancer chemotherapy. Geller et al.[15] reported the potential role of intratumoral bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. The researchers collected 15 fresh PDAC tissues and cultured them. They then found that 93% of the bacteria made CRC cells resistant to gemcitabine because the bacteria converted gemcitabine into an inactive form via the bacterial enzyme cytidine deaminase (CDD). Based on these findings, the researchers suggested that PDACs contain bacteria that could modulate tumor sensitivity to gemcitabine. In another ESCC study, the researchers found that higher levels of *C. nucleatum* were associated with adverse responses to neoadjuvant chemotherapy. Antibiotic therapy targeting the bacteria appeared to improve chemotherapy outcomes in patients.[50] In addition, commensal bacteria have also been reported to control the therapeutic response to oxaliplatin by regulating reactive oxygen species (ROS) production.[113]

Application of engineered bacteria

With the continuous development of nanomaterials and bioengineering technologies, researchers have modified natural bacteria to meet special needs, producing engineered bacteria. These bacteria are microorganisms that have been modified by modern bioengineering technology to have multiple functions, high efficiency, and strong adaptability. The main effects and advantages of treatment with engineered bacteria include the following: first, such treatments can directly induce the apoptosis or autophagy of cancer cells. Second, these treatments can induce an antitumor immune response.[114] Third, engineered bacteria have the ability to target and modulate tumor metabolic processes during tumor development. Fourth, engineered bacteria can be used as an excellent drug delivery system to precisely deliver drugs to tumor lesions.[115] Fifth, such strategies can be employed to generate *in situ* synthesis of specific therapeutic drugs. For example, Canale et al.[116] bioengineered a strain of *E. coli Nissle 1917*. The bacteria were able to colonize tumors and convert nitrogen-hydrogen compounds in tumors into L-arginine, which is a key determinant of effective antitumor T cell responses. Therefore, the engineered bacterium exerts its antitumor effect in a T cell-dependent, L-arginine-mediated manner. Similarly, Chowdhury et al.[117] designed a nonpathogenic strain of *E. coli*. The engineered bacteria were able to synthesize CD47 nanobodies and self-destructed *in vivo* to spill the nanobodies, which adhered to the CD47 protein of cancer cells, decreasing their evasion of the immune response and thus rendering them more vulnerable to immune system attack. In conclusion, the development and application of engineered bacteria are important for advances in the field of cancer therapy.

Oncolytic viruses

Oncolytic viruses are a class of replication-competent tumor cell-killing viruses.[118] They were discovered in a patient with CC who experienced a decrease in tumor size after being infected with the rabies virus. This phenomenon has attracted great attention from researchers. The effect of oncolytic virus therapy mainly depends on direct oncolysis, but the treatment also indirectly enhances host antitumor immunity.[119] The most studied oncolytic viruses include adenovirus and type I herpes simplex virus (HSV). Liu et al.[120] reported that JX-594 is a promising oncolytic virus that exerts anticancer effects in hepatocellular carcinoma (HCC). JX-594 has anti-hepatitis B virus (HBV) activity, and targeting of JX-594 can effectively inhibit tumor progression and angiogenesis in HCC patients. Intratumoral injection of JX-594 into primary or metastatic liver tumors showed therapeutic effects. In addition, oncolytic virus therapy has achieved very good results in melanoma, which responds well not only to talimogene laherparepvec (formerly OncoVEX), but also to vaccinia virus (VV) therapy.[121] Oncolytic viruses have also shown synergistic effects in killing tumor cells. A study found that VV synergistically enhances the antitumor activity of vesicular stomatitis virus (VSV), which mainly depends on the active gene product VV B18R.[122] Interestingly, Cronin et al.[123] reported an effect of bacteria on oncolytic virus therapy for cancer. _E. coli_ produces B18R, which greatly enhances VSVΔ51 infection _in vitro_ and _in vivo_, resulting in tumor growth inhibition, in turn improving mouse survival. Oncolytic virotherapy is now routinely used to treat cancer patients who do not respond to or achieve durable responses from immune checkpoint inhibitors. In conclusion, countless oncolytic virus studies and review articles have been published, and this article provides only a brief overview.

FUTURE PERSPECTIVES AND CHALLENGES

Challenges in the detection of intratumoral microbes

Characterizing the intratumoral microbiota is challenging because tumor samples are known to have low microbial biomass. Additionally, tumor samples have a very high host-to-bacterial DNA ratio, which can lead to biased amplicon-based sequencing results. In addition, because there are many steps in the process of clinical sample collection, ensuring the quality and quantity of tumor samples is also difficult. Ensuring that samples are not contaminated during the testing process and determining how to completely eliminate the influence of microbial contamination from the environment are important goals worth considering.

Challenges in determining the role of intratumoral microbes

Current research has not yet fully clarified whether specific changes in the microbes of tumors are the cause or the result of the development of cancer or whether these changes are unrelated to the cancer (for example, the result of a simple infection). The relationship between intratumoral microbes and host tumors is complex and unclear, and more research is needed to understand the mechanism.

Challenges in the therapeutic application of intratumoral microbes

As mentioned above, engineered microorganisms have been used in cancer treatment. However, their safety is an issue that cannot be ignored.[124] Further testing in animal models and humans is needed.

Other potential challenges

There remain major gaps in the clinical study of the cancer microbiome, and relevant clinical interventions must still be designed. The translation of scientific research into clinical applications is difficult, and there have been several contradictory conclusions between different studies. The consistency between the results of different studies has been poor, and although some differences in microbial composition between tumors and normal tissues have been identified, some of these differences were not significant.[125]

Future outlook

Researchers still have a long way to go to address these challenges and to identify potential undiscovered problems. In the foreseeable future, in-depth characterization of distinct intratumoral microbiomes could enable the manipulation of these bacterial communities to achieve cancer patient therapy. In addition to addressing these potential challenges, it is also very important to dig deeper into the molecular mechanisms of the role of microorganisms in tumors and to find new therapeutic targets and biomarkers. Clearly, the contribution of microbes to cancer biology is likely to take center stage in cancer research over the next decade.

Conclusion

In conclusion, this review mainly summarized the role of intratumoral microbes in different tumor types. The therapeutic potential of intratumoral microbes in tumors was also explored. In addition, a brief summary of methods commonly used to characterize intratumoral microbes was provided. Finally, the current challenges and future research directions in this field were presented. We hope to have provided some ideas for further innovation in the field of cancer microorganisms.

AUTHOR CONTRIBUTIONS

L.L. and J.L. designed the work and reviewed and edited the manuscript; C.X., Q.C., and Q.Z. participated in the original draft preparation; X.Y. helped to review the manuscript; and Y.S. and Z.B. collected the references. All authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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