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Review Article

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# The Intratumor Microbiome: An Untapped Avenue for Translational Applications in Cancer Immunotherapeutics

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**Abstract**
The human body harbors distinct microbial communities at each body site. One microbial niche of particular interest within the human body is the tumor microenvironment. These intratumor microbes are linked to tumor initiation, progression, and metastasis through diverse mechanisms, including activation of oncogenic pathways and modulation of antitumor immunity. Recent studies have emphasized the role of intratumor microbes in influencing the response and outcome of cancer immunotherapeutics and vaccines. Further data suggest a crucial role of microbial metabolites in the metabolic rewiring of CD8+ T cells controlling antitumor immunity. This knowledge is vital to promote our understanding of the role of microbes in the tumor microenvironment and advance translational applications. In this review, we discuss factors that shape the structure of the intratumor microbiome, such as the translocation of gut microbes and the development of local microbial communities. This study highlights the remote control of gut microbes in the tumor microenvironment, disease progression, and therapy outcome. We detail interactions of intratumor microbes and their crosstalk with tumor and immune cells, such as tissue-resident and tumor-infiltrating T cells. We discuss open research questions in this field, including defining oncomicrobiotics, the subset of microbiota with biotherapeutic potential in inducing antitumor immunity. We highlight challenges and opportunities, emphasizing the future direction of developing next-generation engineered probiotics that can advance delivery, maximize the efficacy of cancer therapy, or even serve as a non-invasive technique to sense and detect tumor cells.

**Keywords:**
intratumor microbiome; tumor microenvironment; immunotherapeutics; engineered probiotics; chemotherapy resistance

## 1. Introduction

Tumor microenvironment (TME) plays a major role in shaping tumor behavior. Microbes residing inside the tumor cells, referred to as intratumor microbiome or oncobiomes, play a crucial role in tumor development, progression, response to therapeutics, prognosis, and clinical outcome [1–5]. Recent research has shown that these microbes may either translocate from the gut or oral cavity following dysbiosis or be local residents that thrive in the tumor microenvironment due to its immunosuppressive nature and the presence of a leaky vascular network within cancerous lesions [6,7]. Intratumor microbes can manipulate the anti-tumor immunity in multiple types of cancers, including colon, pancreas, prostate, breast, and lung [1–5] (Figure 1). Intratumor microbes can reprogram cytotoxic CD8+ T cells, among other immune cells, and affect their tumor infiltration rates. In addition, these microbes control the levels of proinflammatory cytokines. Multiple studies suggest that tumor-infiltrating tissue-resident

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memory T cells (TRM) are crucial to achieve the desired response in solid tumors [8–11] and especially in patients receiving PD-1 therapy [12]. Interestingly, memory responses by IFN–γ–secreting CD8+ and CD4+ T cells specific for *Bacteroides fragilis*, *Enterococcus hirae*, and *Akkermansia muciniphila* correlated with positive outcomes in cancer therapy [13–16]. Microbiome signatures, whether in the gut or within the tumor microenvironment (TME), are gaining attention as a major factor contributing to interpatient heterogeneity and influencing the response to immunotherapies such as anti-PD-1. For example, the abundance of *Collinsella aerofaciens*, *Bifidobacterium longum*, and *E. faecium* in the feces of patients is linked to a good response to anti-PD-1, and their fecal transplant to germ-free (GF) mice resulted in increased T cells and improved therapy outcome [4].

In this review, we showcase the recent advances in understanding the structure and function of the intratumor microbiome and its influence on the disease's progression and therapy outcome. This study highlights the potential application of this knowledge to enhance the efficacy of immunotherapeutics or to develop novel microbiome-based diagnostic biomarkers and therapeutics.

## 2. Intratumor Microbial Colonization

Various pathways facilitate microbiota access to the TME. Tumors originating in organs directly exposed to the external environment, such as in nasopharyngeal cancer, may carry bacteria from the local microbiome. In a cohort of 800 patients with nasopharyngeal cancer, higher intratumoral bacterial loads were associated with reduced survival rates. The analysis pinpointed the nasopharyngeal microbiota as the principal origin of intratumoral bacteria [17]. The disturbed epithelial or mucosal barrier in some tumors can promote the colonization of resident microbiota. For example, tumors with TP53 mutations, known to impede epithelial function, exhibit a distinct bacterial consortium, primarily featuring *Acidovorax temporans* in lung cancer [18].

Several factors enable the intracellular colonization of bacteria within the tumor cells. The hypoxic tumor microenvironment favors the survival of anaerobic and facultative anaerobic bacteria, with varying oxygen levels in different tissues contributing to differences in the residing bacteria [19]. Except for lung cancer, most cancers show a predominance of anaerobic bacteria. TME is an immunosuppressive region [20], which impairs immune-mediated clearance of bacteria. The disrupted vascular system is also a favorable condition for rapid bacterial entry and colonization in tumors [21]. Alongside this, tumor tissues with necrotic regions provide nutrients and molecules that support bacterial outgrowth [22].

A recent study showed that the diversity of the microbiome is linked to the biopsy site, emphasizing the influence of the surrounding environment, rather than the primary tumor type [23]. Further, bacteria possess the capability to disseminate from remote anatomical sites and establish colonization within tumor tissues via the bloodstream or other physical channels. For example, the breakdown of barriers caused by genetic lesions initiating colorectal cancer leads to the invasion of adenomas by microbiota and microbial products. These products, in turn, activate inflammation initiated by the tumor, fostering tumor growth [24]. Oral-originated microbiota, including four *Fusobacterium* spp., were found to be enriched in colorectal tumors [25].

## 3. The Impact of Gut Microbes on Intratumor Microbial Landscape

Growing evidence supports that gut microbes translocate to the tumor site, where they reside and shape the tumor microbiome landscape. These microbes, together with local microbial residents, can rewire the CD8+ T cells and guide them to promote or inhibit anti-tumor immunity and hence determine tumor growth and outcome, together with the host factors and tumor genetics [26–31]. Diversity of gut microbiota is linked to local and distant immune signatures that could be either favorable or unfavorable in tumor progression and metastasis [32]. Dysbiosis and leaky gut create a chronic inflammatory status conducive to tumor development and progression [3]. Gut microbes play a crucial role in the maturation of the immune system and controling the anti-tumor immunity. During early gut colonization, commensal microbial antigens are transported to the thymus by dendritic cells, promoting T cell expansion [33]. More insights have been gained from studies on germ-free mice that support the notion that microbial antigens control the development of T cells [34]. Early evidence of the microbiota's role in anti-tumor immunity emerged in 2007 with the discovery that commensals activate antigen-presenting cells via Toll-like receptor 4 (TLR4) [13]. Further, a study shows that TLR4 agonists modulate tumor necrosis factor (TNF) and initiate anti-tumor activity [14]. In addition, secreted microbial metabolites from the leaky gut can exert a remote control over the TME as detailed in the mechanisms below.

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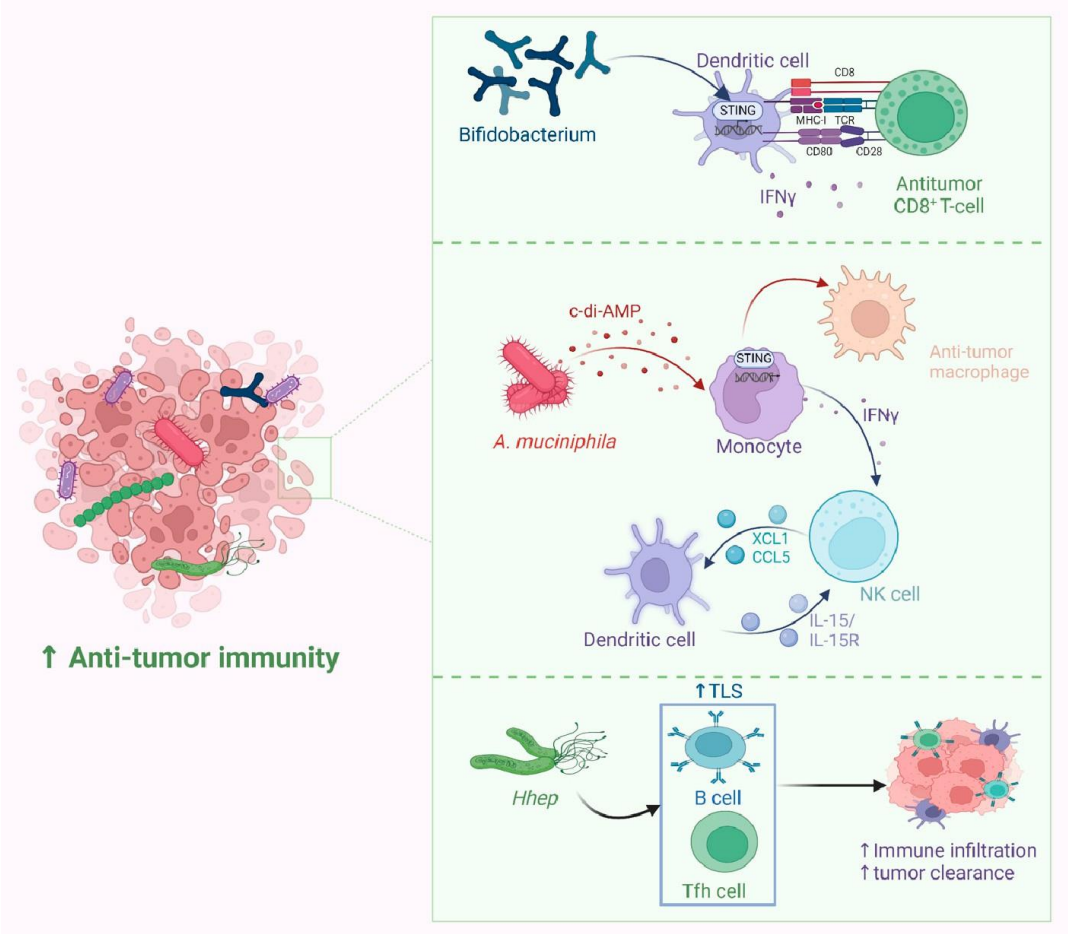



**Figure 1: Mechanisms of Antitumor Activity Mediated by Tumor-Resident Microbes.** Anti-tumorigenic microbes enhance the host's anti-tumor immunity, thereby improving the outcomes of immunotherapies. Intratumor microbiome, such as *Bifidobacterium,* accumulates within the tumor and enhances the response to anti-CD47 immunotherapy. Upon detection of *Bifidobacterium* by dendritic cells, the stimulator of interferon genes (STING) pathway is activated, increasing type I IFN signaling. Moreover, the activation of dendritic cells leads to the upregulation of antitumor $CD8^+$ T-cells. Similarly, *A. muciniphila* secretes a STING agonist, c-di-AMP, in monocytes. c-di-AMP contributes to the polarization of macrophages and triggers the intratumoral IFNγ-NK cell-DC axis through cytokines. Colonization of *Helicobacter hepaticus* (*Hhep*) in colorectal tumors induces Hhep-specific T follicular helper (Tfh) cells and supports the development of peritumoral tertiary lymphoid structures (TLSs), which boost immune infiltration and enhance anti-tumor immunity in the colon.

# 4. The Mechanistic Insights Underpinning the Role of Microbiota on Cancer Development, Progression, and Metastasis

## 4.1. Microbiota Mediate a State of Chronic Inflammation Leading to Tumor Initiation

Several studies suggest that dysbiosis in the gut contributes to oncogenesis, especially for cancers of the colon, liver, and pancreas. This effect is mediated by leaked microbial metabolites that modulate the host immune response [27, 35,36]. A leaky gut and increased circulating levels of lipopolysaccharide (LPS) that create a chronic state of inflammation are drivers for cancer development. For example, increased levels of circulating microbial-derived LPS in obesity and type 2 diabetes are associated with a higher risk of colorectal cancer [37]. Another line of evidence shows that elevated levels of secondary bile acids in mice are linked to overexpression of cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin pathways, which are linked to inflammation and cancer [38]. Other metabolites derived from *Clostridium* spp. and implicated in inflammation pathways are lithocholic acid and muricholic acid, which suppress chemokine (C-X-C motif) lig-

and 16 (CXCL16) in the liver, hindering the recruitment of natural killer T (NKT) cells, resulting in tumor progression and metastasis in mice [35]. Interestingly, the administration of oral antibiotics that deplete *Clostridium* increased the expression of CXCL16, resulting in the accumulation of NKT cells and achieving more control over tumor growth [35].

The chronic inflammatory response worsens tissue damage and consequent influx of infiltrating microbes/microbiyalmetabolites, resulting in excessive production of cytokines and chemokines, which might foster angiogenesis [39] (Figure 2). High levels of pro-inflammatory mediators were associated with the tumor microbiome [40]. A study on a genetically engineered mouse model found that the microbiota can induce inflammation and advance the progression of cancer by acting through the lung-resident γδ T cells. In this model, lung tumor growth was associated with an increase in total bacterial load and a decrease in bacterial diversity within the airway. Commensal bacteria increase Myd88-dependent IL-1β and IL-23 production from myeloid cells, stimulating the activation of Vγ6+Vδ1+ γδ T cells that produce IL-17 and other effector molecules, promoting inflammation and tumor cell proliferation. Moreover, neutralization of IL-17, a key effector molecule produced by γδ T cells, resulted in reduced neutrophil infiltration and tumor burden [41]. Another study reported that intratumor bacteria induced the production of IL-17, which promoted an influx of B cells and the development of tumors [42]. In an attempt to study the link between microbiome, inflammation, and cancer, Hoste et al. employed a mouse model of wound-induced skin cancer and studied the mechanism by which the skin microbiota contributes to inflammation and tumorigenesis. In the presence of skin microbes, the removal of various innate immune sensors, including TLR-5, TNF receptor (TNFR)-1/-2, and MYD-88, protects against tumorigenesis, with inflammation showing a correlation with tumor incidence. Notably, the administration of antibiotics hinders tumor formation, while flagellin injection induces tumors, both in a TLR-5 dependent manner [43].

## 4.2. Microbes Modulate Host Immunity Affecting Tumor Progression

Microbes can enhance the progression of tumors by modulating the activity of several pathways related to host immunity. One example is by altering the immune response towards the cancer cells. This leads to remodeling of the TME, inducing an immunosuppressive environment which makes the cancer cell unrecognizable or non-responding to the immune system. For example, intratumor *F. nucleatum* promotes tumor growth by mediating antitumor immunity, represented by suppression of tumor-infiltrating CD8+ T cells [44]. Mathiasen et al. showed that cytolethal distending toxin (CDT), produced by many pathogenic gram-negative bacterial species, can induce premature senescence in activated CD4 T cells [45]. This suggests that bacterial toxins reduce the anticancer response and promote the proliferation of cancer. Another mechanism for the modulation of the immune response is activating TLRs by the bacterial antigens. Activation of TLR can promote the activation of certain proliferation and angiogenesis responses, such as STAT3, NFkB, and ROS [46].

Other microbial metabolites that contribute to antitumor immunity are short-chain fatty acids (SCFAs) via their direct interaction with CD8+ T cells, which leads to improving their capacity to differentiate and exert antitumor activity [47–49]. A study found that the SCFA-producing *Ruminococcaceae* family is associated with an increase in T cell accumulation inside the tumor [50]. In support of this finding, another study showed that fecal transplantation from metformin-fed mice (that showed a higher abundance of *Ruminococcaceae)* resulted in an elevated level of SCFAs coupled with a suppression of tumor proliferation in a murine model [51]. Another study identified a positive correlation between the abundance of SCFAs-producing *Lachnoclostridium* genus (originally resides in the gut), inside the tumor, and the concentration of intratumor cytotoxic CD8+ T cells mediated by overexpression of chemokines C-C motif chemokine ligand 5 (CCL5), CXCL9, and CXCL10 [52].

## 4.3. Microbial Metabolites Can Induce DNA Damage and Initiate Cancer Development

Bacteria produce metabolites, proteins, and molecules that aid in directly damaging and altering the stability of the host genome, thus contributing to the development of mutations. For example, colibactin is a metabolite produced by *pks+ Escherichia coli;* this metabolite acts as a DNA alkylator and causes double-strand breaks as a consequence of DNA crosslinks [53,54]. Colibactin possesses a unique mutational pattern in organoids treated with genotoxic *pks+ E. coli*, similar to the mutation present in 5876 human cancer genomes [55]. *Bacteroides fragilis* could promote DNA damage by secreting *B. fragilis* toxin (BFT), although without a distinct mutational profile [56, 57]. Through cell culture and animal models, Goodwin et al. reported that BFT induces the expression of spermine oxidase (SMO), which is a polyamine catabolic enzyme, resulting in higher reactive oxygen species (ROS) and DNA damage. Furthermore, they showed that inhi-

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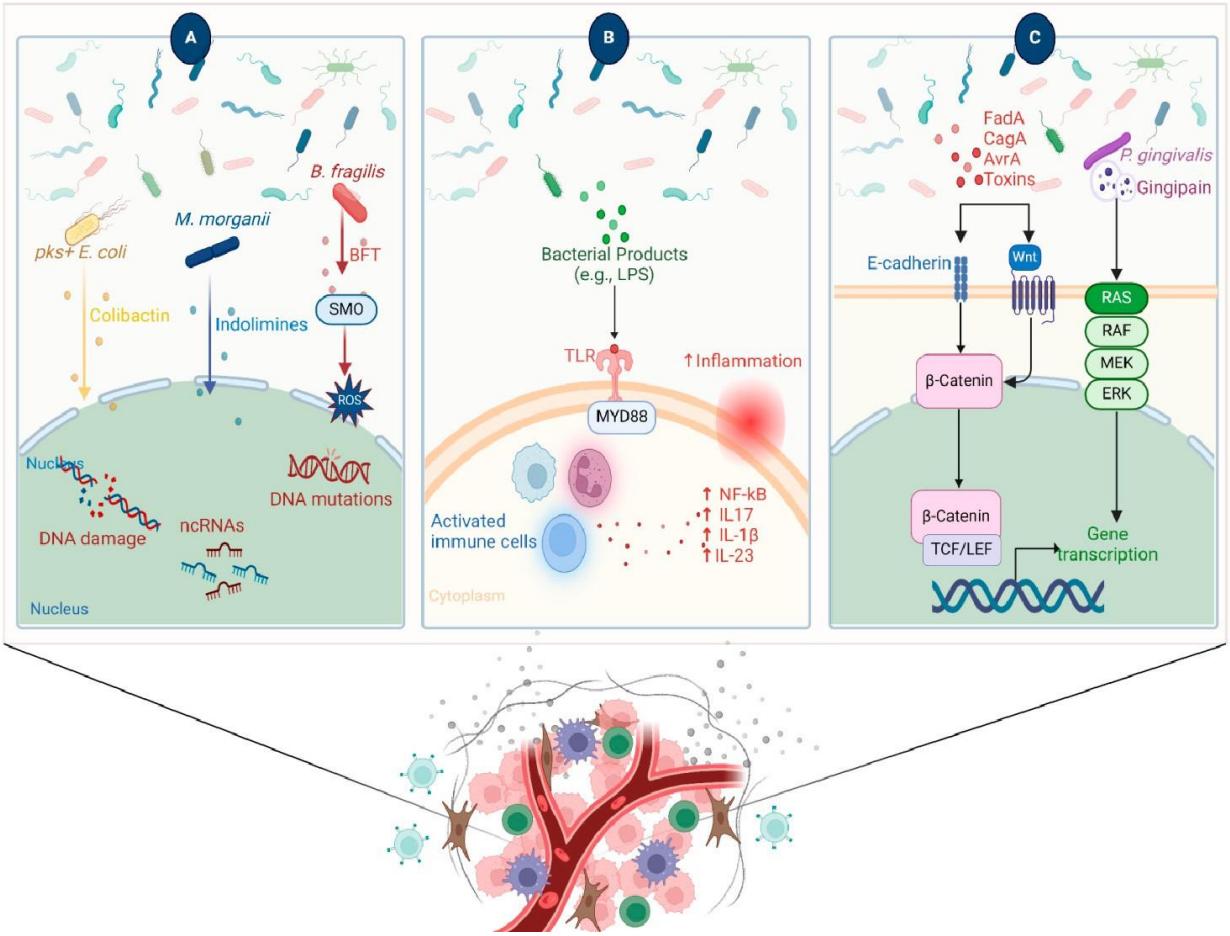



**Figure 2: Main Strategies Adopted by Microbiome to Develop Cancer.** (**A**) Microbial metabolites have a genotoxic effect that leads to cancer development. Polyketide synthase-expressing strain of *E. coli* (pks+ *E. coli*) and *M. morganii* secrete toxins such as colibactin and indolamines, respectively. These toxins directly induce DNA damage, cause mutations, or alter the levels of non-coding RNAs (ncRNAs) upon reaching the genetic material of the cell. Additionally, *B. fragilis* upregulates the expression of spermine oxidase (SMO), which increases the levels of reactive oxygen species (ROS) within the cell and further contributes to DNA impairment. (**B**) Sustained inflammation is a known risk for cancer. Microbiota-derived components and products, such as lipopolysaccharide, are recognized by pattern recognition receptors such as toll-like receptors (TLRs). This detection stimulates inflammatory pathways, activates various immune cells, and elevates the production of pro-inflammatory cytokines. (**C**) Microbiomes interfere with host pathways involved in carcinogenesis through the secretion of proteins and toxins. The activation of β-catenin signaling through E-cadherin or Wnt can modulate the transcription of genes responsible for oncogenesis, immunity, and inflammation. Moreover, *P. gingivalis* secretes protease virulence factors called gingipains, which activate mitogen-activated protein kinase (MAPK) signaling, also known as the Ras-Raf-MEK-ERK pathway. This cascade is involved in cell proliferation and survival.

bition of elevated SMO in *B. fragilis*-infected mice significantly reduces chronic intestinal inflammation and inhibits colon tumorigenesis [58]. Recently, a new genotoxic small molecule secreted by colorectal cancer-associated species, *Morganella morganii,* was discovered by Cao et al., named indolamines. These metabolites elicit DNA damage in intestinal epithelial cells (IECs) and are implicated in the development of colon tumors in gnotobiotic mouse models [59].

The microbiome can also disrupt the body's DNA damage response. During DNA replication, base-base mismatches and insertion-deletion loops can occur when the primer slips against the template strand during the synthesis of a new strand [60]. DNA mismatch repair (MMR) functions to correct these errors. The inactivation of DNA MMR, both genetically and epigenetically, has the potential to induce mutations in genes associated with cancer and subsequently contribute to the development of cancer [61]. MMR genes are found to be downregulated in response to *Helicobacter pylori* [62]. Interestingly, *H. pylori* infection induces expression of microRNAs (miRs), such as miR-150-5p, miR-155-5p, and miR-3163, which in turn modulate and target MMR genes, such as POLD3, MSH2, and MSH3, respectively [62].

### 4.4. Microbes Control Signaling Pathways Involved in Carcinogenesis

Several microbes secrete molecules that interact with host pathways involved in carcinogenesis. For example, *H. pylori* produces a protein called CagA, which modulates β-catenin to drive gastric cancer and prostate cancer. CagA-mediated β-catenin activation leads to up-regulation of genes involved in cellular proliferation, survival, migration, and angiogenesis [63–65]. *F. nucleatum* is a member of the oral microbiota and is associated with human cancers [66]. *F. nucleatum* expresses FadA, a bacterial cell surface adhesion component that binds host E-cadherin, leading to β-catenin activation [66]. Enterotoxigenic *B. fragilis*, which is enriched in some human colorectal cancers, can stimulate E-cadherin cleavage via Bft, leading to β-catenin activation [67]. *Salmonella typhi* strains secrete AvrA, which can activate epithelial β-catenin signaling and are associated with colonic cancers [68].

## 5. Defining the Oncobiomes and Microbial Signatures that Impact Therapy Outcome in Different Types of Tumors

A growing body of data suggests a distinctive effect of local tumor microbiota that is independent of gut microbes. For example, in gastric cancer, the abundance of *Methylobacterium* inside the tumor, independent of the concentration in the feces, was found to be negatively correlated with tumor-infiltrating CD8+ T cells, downregulation of transforming growth factor-β (TGF-β) [69]. In line with this finding, another study reported the presence of a unique microbial signature composed of *Acinetobacter*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas*, *Brevundimonas*, and *Ralstonia* residing inside the thyroid tumor [70,71]. Another interesting study revealed that the progression of lung cancer is associated with local intratumor residents, not gut-translocated microbes. This intratumor signature is characterized by the abundance of *Herbaspirillum* and *Sphingomonadaceae*, inducing proinflammatory mediators such *as* IL-1β, IL-17, and IL-23. In this study, intratracheal transplant of microbes from lung tumor into mice at the initial stage of tumor development accelerated tumor progression [41].

The full characterization of the microbiome structure in the TME remains challenging due to the low biomass of these communities [72,73]. A set of guidelines for the minimum standards for conducting microbiome studies with low microbial biomass has been suggested [74]. Currently, metagenomics and proteomics analyses are widely used to facilitate the detection and identification of bacteria, depending on their DNA or their metabolites from samples directly [75].

An in-depth investigation of the intratumor microbiomes of 1526 tumor tissues across seven cancer types revealed that all tumors contain detectable levels of bacterial metabolites and genetic material, while live bacterial cells were detected residing mostly intracellularly in both tumor and immune cells [76]. The study showed that each tumor type harbors unique and distinct microbial communities, with *F. nucleatum* being one of the most abundant species in breast and pancreatic tumors. Colon tumors showed a high abundance of *Firmicutes* and *Bacteroidetes*, while non-intestinal tumors were enriched in *Corynebacteriaceae* and *Micrococcaceae*. Another study found an association between survival rate and signature intratumor microbiota in pancreatic ductal adenocarcinoma (PDAC) enriched in *Bacillus clausii*, *Saccharopolyspora rectivirgula*, and *Streptomyces* [77]. Fecal transplant from survivors to mice with pancreatic cancer increased tumor infiltration of activated CD8+ T cells and augmented serum levels of IFN-γ and IL-2, which enhanced anti-tumor immunity, while fecal transplant from short-term survivors resulted in increased tumor infiltration of Treg cells and subsequently led to an immune suppression state [77].

There are several reports identifying the oncobiome structure and unique signature in each tumor type (Table 1).

**Table 1:** The structure of the microbiome of various cancer types and its impact on cancer behavior.

| Cancer Type | Study Design | Sample Size | Intratumor Microbes | Outcomes | Mechanism of Action | References |
|---|---|---|---|---|---|---|
| **lung cancer** | Meta transcriptomics pilot study | 49 | ↑*Brevundimonas diminuta*, ↑*Acinetobacter radioresistens* ↑*Enterobacter cloacae* ↑*Mycobacterium chelonae* ↑*Mycobacterium franklinii* ↑*Staphylococcus sp.* ↑*Bacillus megaterium* ↑*Pseudomonas aeruginosa* ↑*Rhodococcus erythropolis* | The development of cancer progression and metastasis leads to a poor prognosis. | Unknown mechanism of inducing carcinogenesis. | [78] |

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**Table 1:** *Cont.*

| Cancer Type | Study Design | Sample Size | Intratumor Microbes | Outcomes | Mechanism of Action | References |
|---|---|---|---|---|---|---|
| | Prospective observational study | 38 | ↑ *Gammaproteobacteria* | Lower response to anti-PD-L1 Reduced survival rate by worsening the recurrence-free survival (RFS) and overall survival (OS) rate. | By lowering programmed death-ligand 1 (PD-L1) expression on cancer cells. | [79] |
| **Breast cancer (BC)** | Cross-sectional study | 221 | ↓*Streptococcus*<br>↓*Propionibacterium*<br>↓*Anaerococcus*,<br>↓*Caulobacter*<br>↓*Streptococcus*<br>↑*Porphyromonas*<br>↑*Lacibacter*<br>↑*Ezakiella*,<br>↑*Fusobacterium* | Enhancing tumor suppression | - *Streptococcus* and *Propionibacterium* activate an anti-tumor response by activating T-cells. | [80] |
| | Cross-sectional study | 33 | ↑*Gluconacetobacte*<br>↑*Fusobacterium*<br>↑*Atopobium,*<br>↑*Lactobacillus*<br>↑*Hydrogenaphagar* | Stimulating tumor progression and metastasis. | Creating a proinflammatory environment and secreting virulence factors that induce carcinogenesis. | [81] |
| **Pancreatic cancer** | In vivo/in vitro study | 125 | ↑*Fusobacterium nucleatum* | Induction of pancreatic tumor growth and metastasis, leading to poor prognosis. | - Promoting the secretion of motif chemokine ligand 1(CXCL1), which will activate the autocrine signaling pathway.<br>- Modifying the tumor microenvironment (TME) by suppressing the activity of the infiltrating tumor CD8+ cells. | [44] |
| | Retrospective cohort study | 68 | +*Pseudoxanthomonas*<br>+*Streptomyces*<br>+*Saccharopolyspora*<br>+*Bacillus clausii* | Enhancing the therapy outcomes, as they were found to be more abundant in long-term survival patients. | Activating and recruiting CD8+ immune cells to the tumor cells. | [77] |
| **Liver cancer** | Retrospective analysis | 28 | ↓*Pseudomonadaceae*<br>↑*Rhizobiaceae*<br>↑*Agrobacterium* | - Peudomonadaceae: exerts anti-tumor effect and acts as an effective therapeutic agent.<br>- High abundance of Rhizobiaceae and *Agrobacterium* in the cancer cells may be associated with tumor progression. | Unknown mechanisms | [82] |
| | Retrospective analysis | 91 | ↑ *Proteobacteria*<br>↑*Actinobacteria,*<br>↓*Deinococcus thermus.*<br>↑*Akkermansia*<br>↑*Methylobacterium* | Proteobacteria & Actinobacteria: increase pathogenesis and tumor progression. *Akkermansia* and *Methylobacterium*: acting as effective predictors for better recurrence-free survival (RFS) and overall survival (OS). | Proteobacteria: It is involved in the pathogenicity of endotoxemia and inflammation. Actinobacteria: highly present in patients with poor prognosis. | [83] |
| **Cervical cancer** | Retrospective analysis | 72 | ↑ *Klebsiella*<br>+*Micromonospora*<br>+*Microbispora*<br>+*Methylobacter* | Induction of metastasis and tumor progression. | Increase the production of expression of HIF-mRNA in the epithelial cells, causing epithelial-mesenchymal transition. | [84] |

**Table 1:** *Cont.*

| Cancer Type | Study Design | Sample Size | Intratumor Microbes | Outcomes | Mechanism of Action | References |
|---|---|---|---|---|---|---|
| **Colorectal cancer (CRC)** | Multi-omics analysis | 372 | + *Clostridium*<br>+ *Flavonifractor*<br>+ *Parvimonas micra*<br>+ *Fusobacterium nucleatum*<br>+ *Alistipes*<br>+ *Oscillibacter*<br>+ *Akkermansia* | - *Clostridium, Fusobacterium nucleatum*: confer a more malignant phenotype to CRC cells and promote colorectal tumorigenesis and metastasis.<br>- *Akkermansia*: increase therapy response.<br>- *Parvimonas micra*: contribute to tumorigenesis.<br>- Odoribacter splanchnicus: protection against tumorigenesis.<br>Flavonifractor: negative correlation with survival time. | - *Akkermansia*: modulates the tumor microenvironment (TME) and activates immune cells like t-T-cells and natural killer (NK) cells.<br>- *Odoribacter splanchnicus*:- induce intestinal th17 cells development against CRC.<br>- *Clostridium* may affect tumor-infiltrating immune cells (TIICs), particularly mucosa-associated invariant T (MAIT) cells.<br>- *Fusobacterium nucleatum*: The abundance of tumor-infiltrating M2-like macrophages will be increased.<br>- *Parvimonas micra*: It promotes differentiation of CD4+ T cells to Th17, increases the oncogenic signaling pathway. | [85] |
| **Squamous cell carcinoma (SCC)** | Case-control study | 353 | ↑ *Staphylococcus aureus (S. aureus)* | Promoting tumor development and progression. | Induce chronic inflammation in the skin, leading to the production of tumor necrosis factor (TNF), which will activate nuclear factor-κB (NF-κB), a transcription factor. | [86] |
| **Brain tumor (glioma)** | multi-omics study | 50 | ↑*Fusobacterium nucleatum*<br>↑*Longibaculum*<br>↑*Intestinimonas*<br>↑ *Pasteurella*<br>↑*Limosilactobacillus*<br>↑ *Arthrobacter*. | Contribute to tumor progression and metastasis. | *F. nucleatum* increases N-acetylneuraminic acid and CCL2, CXCL1, CXCL2, and chemokine expression levels. | [21] |
| **Kidney cancer (KC)** | Case-control study | 24 | ↑ *Deinococcus*.<br>↑*Rhodoplanes*<br>↓*Cyanobacteria (class Chloroplast and the order Streptophyta)* | *Cyanobacteria* restrict metastasis and tumor growth. *Deinococcus* and *Rhodoplanes* cause cancer development. | *Cyanobacteria* produce bioactive substances that can induce cancer cells' apoptosis. | [87] |
| **Gastric tumor** | Mouse model And single-cell sequencing. | 53 | ↑ *Methylobacterium* | Causing tumor progression and poor prognosis | - Reduction in CD8+ and Tissue-resident memory cells (TRM).<br>- Reduction in the level of TGF-beta in tumor microenvironment (TME), which will inhibit the production of CD103 TRM cells, leading to the tumor's escape from the immune system. | [69] |
| **prostate tumor** | Cross-sectional study | 16 | ↑ *Staphylococcus spp.*<br>↑ *Propionibacterium spp.* | -Increase in tumor invasiveness and progression. | -*Propionibacterium* spp. Able to make biofilms and adhere to the components of the extracellular matrix. | [88] |

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**Table 1:** *Cont.*

| Cancer Type | Study Design | Sample Size | Intratumor Microbes | Outcomes | Mechanism of Action | References |
|---|---|---|---|---|---|---|
| **Bladder cancer** | Observational study | 400 | *+E. coli*, *+butyrate-producing bacterium SM4/1* *+species of Oscillatoria* | Epithelial–mesenchymal transition (EMT) genes are involved in the progression and metastasis of tumors. | - important correlations between the abundance of those bacteria and 30 epithelial–mesenchymal transition (EMT) genes in bladder cancer. | [89] |
| **Ovarian cancer** | Cross-sectional study | 50 | *↑Ratio of Proteobacteria/Firmicutes* *↑Acinetobacter lwoffii* *↓Lactococcus piscium* | High Ratio of *Proteobacteria/Firmicutes* and *Acinetobacter lwoffii* associated with tumor progression and metastasis. *Lactococcus piscium* can act as a marker for tumorigenesis absence. | - Activation of the inflammation-related pathways was observed in tumor tissue samples. - *Acinetobacter lwoffii* causes persistent infection and escapes the host immune system. *Lactococcus piscium* acts as a microbial biomarker to distinguish between benign and malignant tissue. | [90] |

↑ denotes more abundance in the tumor cells compared to healthy tissue; ↓ denotes less abundance in the tumor cells compared to healthy one; + denotes being detected in the tumor samples; HIF: Hypoxia-inducible factors/CD4 cells: Clusters of differentiation 4 cells/CXCL 1,2: C-X-C motif chemokine ligand 1,2./ccl2: chemokine (C-C motif) ligand 2. /TGF-β: Transforming growth factor-β./TRM: resident memory cells.

## 5.1. Breast Cancer

The first study highlighting the potential pathological significance of the oncobiome in breast cancer (BC) dates back to 1971 [91]. BC exhibits a high abundance of the intratumoral microbiome [76] with significant enrichment in *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. On average, a total of 16.4 distinct bacterial species could be detected within each sample. In contrast, it was observed that the average number of bacterial species present in all other types of tumors was found to be less than nine. BC samples were enriched in *F. nucleatum* in addition to other genera such as *Corynebacterium* US_1715, *Lactobacillus iners*, and *Streptococcus infantis*. Moreover, they investigated that different breast cancer subtypes show a distinct microbiome that is very distinct from the microbiome in adjacent normal tissue and the microbiome between cancer and normal cells [76,80]. Other studies showed that *Enterobacteriaceae* and *Staphylococcus* are more abundant in BC patients compared to healthy subjects. Bacterial isolates from these BC subjects, including *E. coli* and *S. epidermidis*, were shown to elevate the levels of phosphorylated H2AX (gamma-H2AX) in treated HeLa cells, indicating DNA damage [92].

In a study that compared the microbiome in breast skin and BC tissue under aseptic conditions, cancer tissue showed greater species richness and distinct composition. In addition, they observed demonstrable differences in the microbiome between benign and malignant tissues. *Fusobacterium*, *Atopobium*, *Hydrogenophaga*, *Gluconacetobacter*, and *Lactobacillus* were significantly higher in women with malignant cancer. Interestingly, some metabolic pathways were predicted to be severely suppressed in malignant cancer patients, such as glycosyltransferases, methionine and cysteine metabolism, fatty acid biosynthesis, and C5-branched dibasic acid metabolism [81]. Another study showed that the advancement of malignancy is associated with a reduction in the relative abundance of *Bacteroidaceae* and an increase in the *Agrococcus* genus, suggesting a correlation between the abundance of certain microbiota within the breast and the invasiveness of the cancer [93]. A recent study utilized the PathoChip array to reveal distinct microbiome signatures for breast cancer subtypes. The study revealed that Estrogen receptor positive (ER+) BC is the most diverse, while Triple Negative (TN) BC showed the lowest oncobiome diversity [94]. Another study revealed that TN tumors exhibited an increase in seven genera, six of which were depleted in ER+ tumors [80]. Main mechanisms summarizing the impact of breast and gut microbiomes on BC are illustrated in Figure 3.

## 5.2. Pancreatic Cancer

Multiple studies reported similarities in the microbiome between the duodenum and pancreatic tissues [95], suggesting a possible translocation of the microbiome from the gut into the pancreas (Figure 4). A study revealed that bacterial DNA was detectable in 76% of PDAC samples, compared to 15% of control samples. Deep sequencing analysis identified *Enterobacteriaceae* and *Pseudomon-*

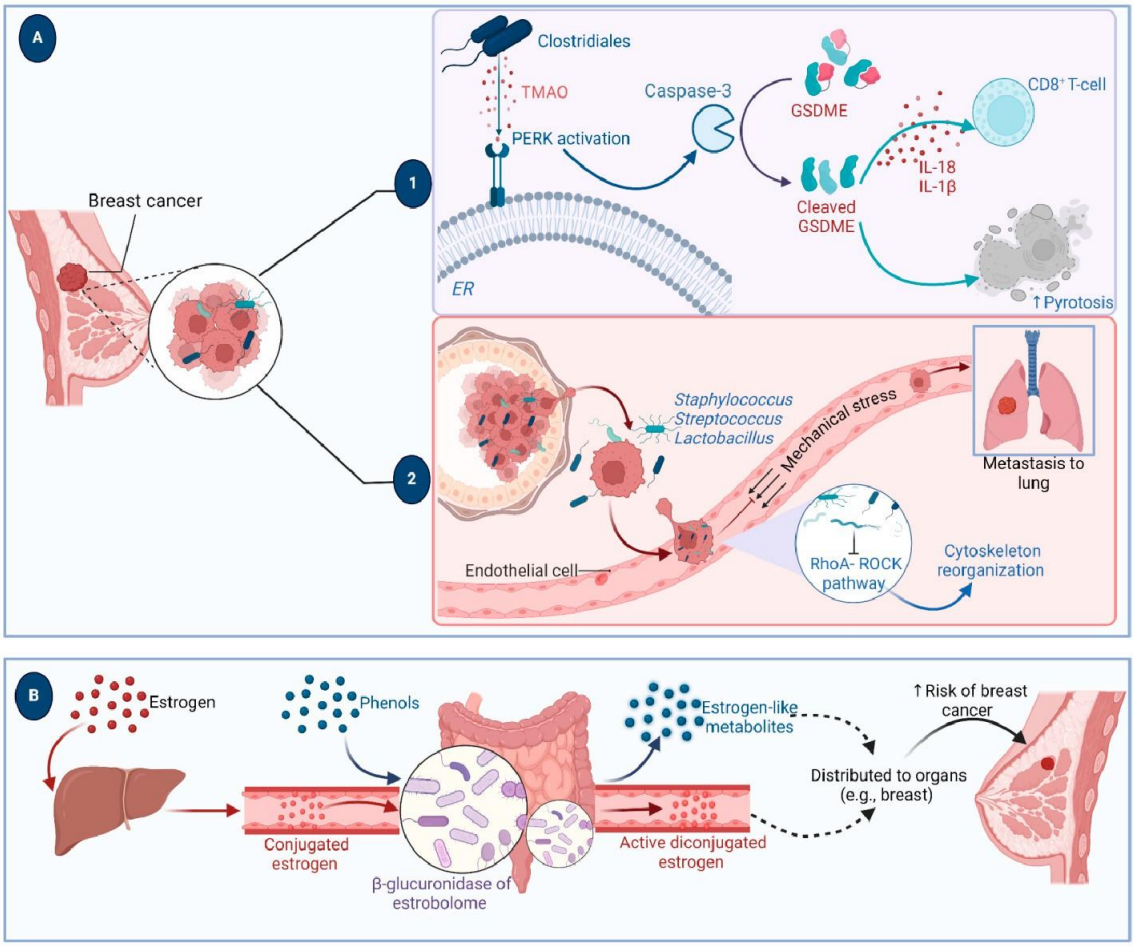



**Figure 3: Mechanistic insights on the role of intratumor and gut microbiome in breast cancer.** (**A**) A distinct microbiome has been found within the breast cancer samples. These species can initiate an anti-tumor activity or, in contrast, promote metastasis and growth of the tumor. (1) *Clostridiales-related* genera secrete trimethylamine N-oxide (TMAO). This metabolite activates the endoplasmic reticulum stress kinase PERK, which activates caspase 3, which mediates the cleavage of GSDME. Cleaved GSDME mediates anti-tumor immunity by initiating pyroptosis and activating $CD8^+$ T-cells through secreting cytokines such as IL-18 and IL-1β. (2) To the contrary, other intratumor microbiota, such as *Staphylococcus, Streptococcus,* and *Lactobacillus,* promote metastasis by augmenting resistance to fluid shear stress through reorganizing the actin cytoskeleton. This occurs through inhibiting the RhoA-ROCK pathway, the main cascade responsible for cellular cytoskeleton dynamics. (**B**) The gut microbiome plays a crucial role in terms of breast cancer. After the conjugation of blood circulating estrogen by the liver, the gut 'estrobolome' reactivates the conjugated estrogen via microbial β-glucuronidase. Moreover, the gut microbiome produces estrogen-like compounds from dietary phenols. These events contribute to a disturbance in estrogen hormone levels in the body and lead to a higher risk of breast cancer.

*adaceae* families as the most abundant families in PDAC. Interestingly, further research showed that members of *Enterobacteriaceae* express cytidine deaminase, which can deactivate the anticancer drug, gemcitabine [96]. On the other hand, other bacterial taxa are associated with long-term survival, such as *Saccharopolyspora, Pseudoxanthomonas, B. clausus,* and *Streptomyces* [77].

Several studies have identified some oral microbiomes, such as *F. nucleatum*, *P. gingivalis*, *Tannerella denticola*, and *Tannerella forsythia*, that cause infections such as periodontal diseases, as risk factors for developing pancreatic cancer [97–100]. This suggests a translocation of oral bacteria or diffusion of their metabolites to the pancreas. Mitsuhash et al. detected *Fusobacterium* species, originally resident in the mouth, in 8.8% of pancreatic cancer specimens [101]. Other studies supported the claim of colonization of *F. nucleatum* in pancreatic tumor tissue. Furthermore, DNA from *F. nucleatum* was detected in 15.5% of pancreatic tumors. Mechanistically, *F. nucleatum* stimulates the secretion of some CXC cytokine groups, such as CXCL1 and IL-8, further confirmed by increased expression of mRNA coded for CXCL1 and IL-8. Both types of cytokines bind to CXCR2 to promote

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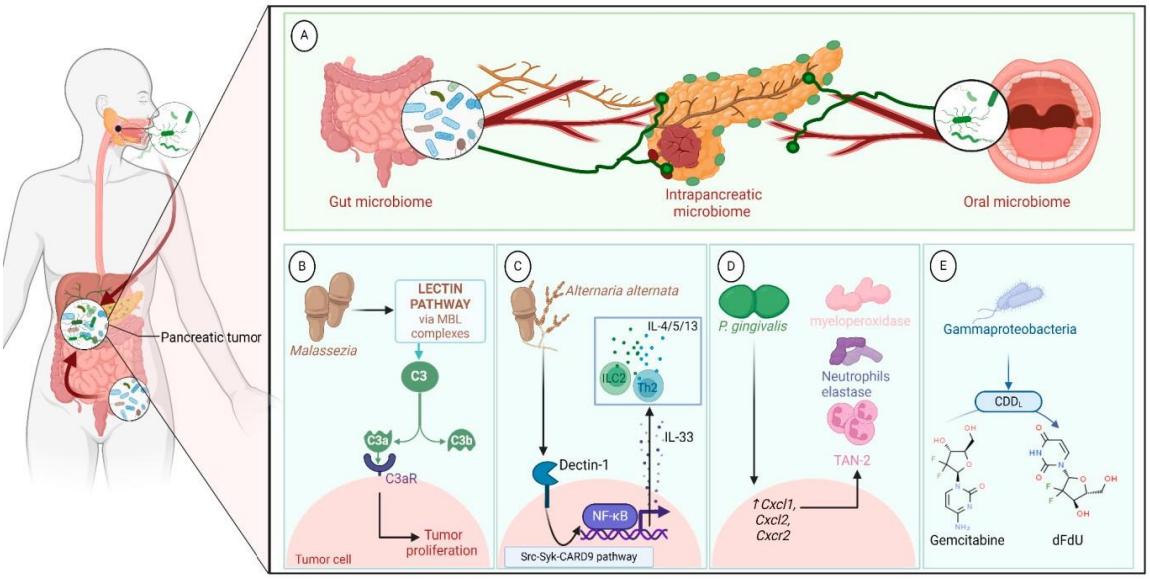



**Figure 4: Microbiome Translocation to the Pancreas and Its Role in Pancreatic Tumor.** (**A**) The pancreatic tumor microenvironment has a high abundance of gut and oral microbiomes. Due to the structural connection between the pancreas and the gastrointestinal tract, the microbiome can migrate to the pancreas, passing through the pancreatic duct or via blood or lymphatic vessels. Likewise, the oral microbiome can also colonize the pancreas in similar ways. (B-E) Intratumor microbiome plays different roles in proliferating pancreatic cancer and mediates resistance to therapy. (**B**) Fungi species such as *Malassezia* activate complement 3 (C3) cascades by binding to the mannose-binding lectin (MBL) through their cell wall glycans. C3 activation yields a complement factor (C3a) that activates the C3a receptor (C3aR), promoting cancer cell proliferation and supporting epithelial-to-mesenchymal transition. (**C**) Fungal components of *Malassezia* and *Alternaria alternata* activate the pattern recognition receptor, dectin-1. Consequently, the Src-Syk-CARD9-NFkB pathway is triggered and enhances the secretion of IL-33 from the pancreatic cancer cells that activate $T_H2$ and ILC2 and promote tumor progression. (**D**) *P. gingivalis* induces the secretion of neutrophil chemokines (CXCL1 and CXCL2) in the tumor microenvironment, supporting the accumulation of tumor-associated neutrophil 2 (TAN2) and its proteases, including neutrophil elastase (NE) and myeloperoxidase, which contribute to higher pancreatic tumorigenesis via an unknown mechanism. (**E**) Gammaproteobacteria are linked to chemotherapeutic drug resistance. The bacterial enzyme cytidine deaminase ($CDD_L$) metabolizes gemcitabine to its inactive form, 2',2'-difluorodeoxyuridine (dFdU).

cell migration by inducing autocrine signaling, leading to a poor prognosis [44].

An interesting study showed that while pancreatic cancer samples are enriched in *A. ebreus* and *Acinetobacter baumannii* compared to healthy subjects, this enrichment is consistently higher in males as compared to females. This indicates that microbiota can adopt different pathways in cancer progression according to gender or smoking status [102].

## 5.3. Colorectal Cancer

Colorectal cancer (CRC) is the second leading cause of cancer-related death after lung cancer, and metastasis is the leading cause of mortality among CRC patients [103]. Several reports support that the *Fusobacterium* genus is strongly associated with CRC [104–106], Figure 5. Interestingly, the abundance of *F. nucleatum* increases with advances in cancer stage [107]. Mechanistically, *F. nucleatum* induces inflammation and triggers the expression of oncogenic responses through its unique membrane protein FadA. FadA binds to E-cadherin, leading to E-cadherin phosphorylation and internalization of E-cadherin, subsequently activating β-catenin signaling, which triggers the overexpression of oncogenes. FadA genes were overexpressed in colon cancer tissues by 10–100 times compared to tissues from normal individuals [66].

Other studies reported the association between intratumoral *F. nucleatum* and specific tumor behavior, such as high-level microsatellite instability (MSI) [108], metastasis [109], treatment resistance [110] and poor survival.

A study showed that *F. nucleatum* promotes the metastasis of CRC by activation of the ALPK1/NF-κB/ICAM1 pathway. Mechanistically, *F. nucleatum* stimulates Alpha kinase 1 (ALPK1) receptor, which in turn activates the NF-κB, leading to the upregulation of intracellular adhesion molecule 1 (ICAM-1). ICM1 is a cell membrane glycoprotein engaged in cell-cell communication and assists in metastasis by promoting the adhesion of CRC cells to endothelial cells [109]. Kong et al. postulated the ability of *F. nucleatum* to initiate TLR4 signaling, thereby inducing the upregulation

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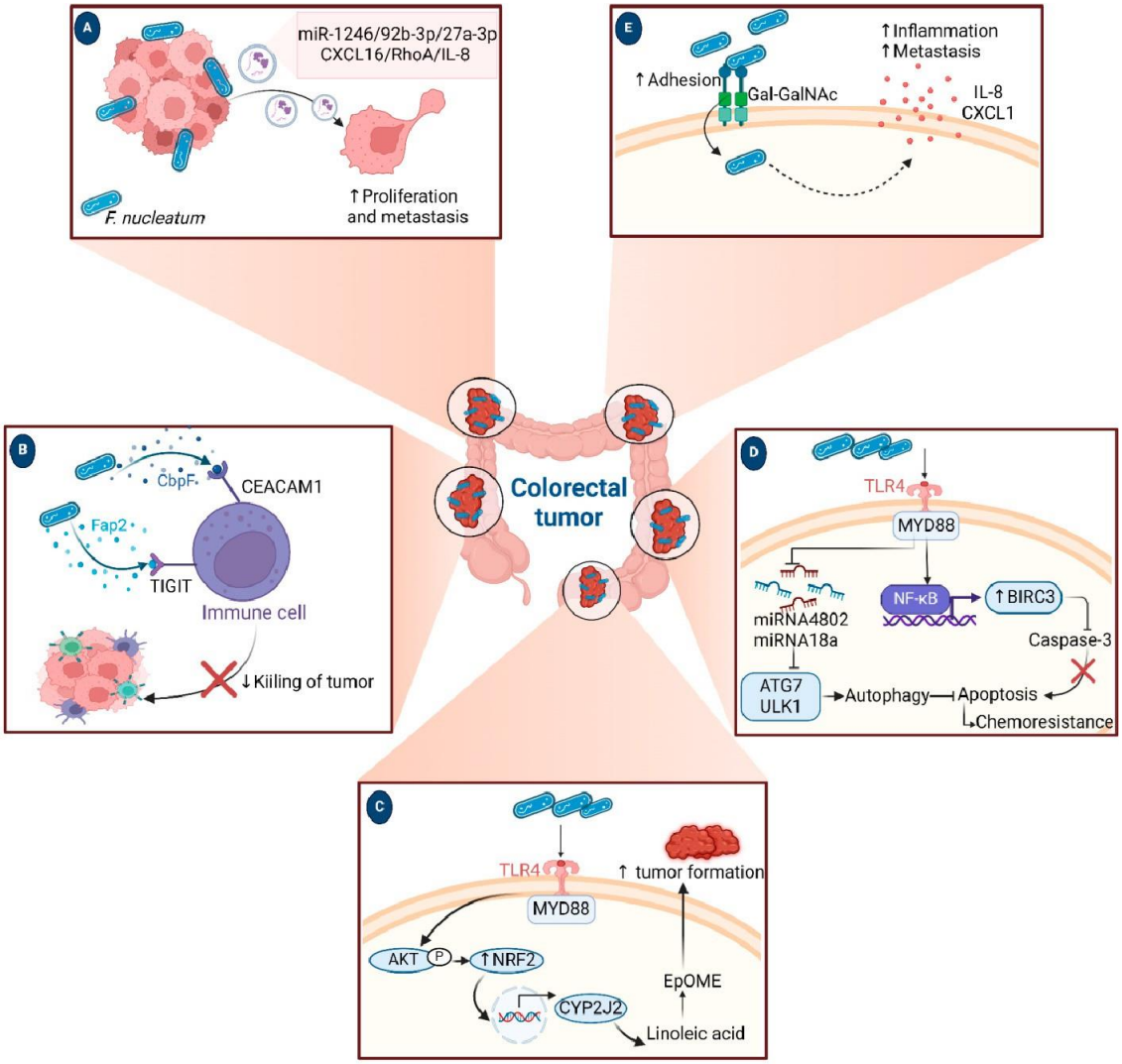



**Figure 5: Role of *F. nucleatum* in Colorectal Cancer (CRC).** (**A**) The presence of *F. nucleatum* in CRC stimulates the secretion of oncogenic exosomes carrying miR-1246/92b-3p/27a-3p and CXCL16/RhoA/IL-8 to other uninfected cells, which promote cell migration ability and metastasis. (**B**) *F. nucleatum* modulates the immune response against cancer cells by secreting trimeric autotransporter adhesin CbpF and Fap2. These proteins bind to inhibitory receptors, such as CEACAM1 and TIGIT, on the surface of immune cells, thereby inhibiting their cytotoxic activity against cancer. (**C**) *F. nucleatum* supports tumorigenesis and metastasis of CRC through TLR4/AKT/NRF2 signaling pathway, which upregulates cytochrome P2J2 (CYP2J2) that converts linoleic acid to 12,13-epoxyoctadecenoic acid (12,13-EpOME). This promotes CRC formation by transforming normal epithelial cells into cancer cells and upregulating the epithelial-mesenchymal transition. (**D**) *F. nucleatum* contributes to chemotherapy failure by activating autophagy pathways and inhibiting apoptosis. This is mediated through TLR4/MYD88 signaling and suppressing miR-18a* and miR-4802, increasing autophagy signaling elements such as ATG7 and ULK1. Moreover, *F. nucleatum* upregulates Baculoviral IAP repeat containing 3 (BIRC3) that encodes for apoptosis inhibition by inhibiting the caspase-3 cascade. (**E**) The over-expressed Gal/GalNAc on the tumor cell surface facilitates the adhesion of Fap2 lectin and elevates IL-8 and CXCL1 secretion from tumor cells. These cytokines act as metastatic signals and inducers for inflammation.

of CYP2J2 expression within cells. Subsequently, this increased expression facilitates the catalysis of linoleic acid, resulting in the production of a larger quantity of the 12,13-epoxyoctadecenoic acid (12,13-EpOME) metabolite. This metabolite contributes to the initiation of epithelial-mesenchymal transformation (EMT), a process closely associated with the development and progression of colorectal cancer [111]. Studying the role of *F. nucleatum* in CRC cell lines and mice models reveals that 50 miRNAs increased significantly, and 52 miRNAs were significantly negatively regulated. miR21 was the most up-regulated and contributes to carcinogenesis through stimulation of the TLR4-Myd88-NFkB pathway [112]. Another study reported similar findings on the role of

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*F. nucleatum* in the TLR4-Myd88-NFkB pathway. They showed that *F. nucleatum* can cause selective loss of miR-18a and miR-4802, which activate cancer autophagy and consequently promote chemoresistance in patients with colorectal cancer [110].

Another bacterium implicated in CRC is *E. coli*. A study showed that the detection of *E. coli* within colorectal biopsies is 20% in the mucosa of healthy individuals compared to 55% in CRC patients [113]. Some strains of *E. coli* might contribute to CRC by producing the genotoxin colibactin [55]. *Campylobacter* is another genotoxin-producing bacterium that is enriched among CRC patients [114]. Similar to *E. coli*, *Campylobacter* is associated with host DNA double-strand breaks [114]. CRC patients with a high abundance of *Campylobacter* show a mutational signature and genetic alterations such as *HRAS, TSC2, AR, FGFR3,* and *AKT1* [115].

Metatranscriptomic analysis revealed other dominant gram-negative anaerobic bacteria among 65 cohorts. *Leptotrichia* and *Campylobacter* spp. are enriched in CRC. This signature composition (*F. nucleatum, Leptotrichia, and Campylobacter*) has been linked to the overexpression of certain genes in the CRC host, such as IL-8 and cathepsin Z. [116]. Other studies reveal a microbial signature characterized by a higher abundance of the *Coriobacteridae* subclass (*Slackia* and *Collinsella*), together with a lower abundance of *Enterobacteriaceae (Kluyvera, Citrobacter, Serratia, Cronobacter, Shigella,* and *Salmonella* spp.) in CRC [117].

## 5.4. Gastric Cancer

Gastric cancer (GC) is the fifth most prevalent malignant cancer and is ranked as the fourth leading cause of cancer-related mortality [118].

Studies revealed that the GC microbiota has lower microbial diversity with enrichment in *Oceanobacter*, *Syntrophomonas* and *Methylobacterium* genera [69]. Furthermore, *Methylobacterium* levels are inversely correlated with $CD8^+$ TRM and TGFβ in TME. [69]. However, the mechanism by which *Methylobacterium* suppresses TGFβ is not understood. Besides, a higher abundance of *Propionibacterium acnes*, primarily found within the skin, was found in stage III of GC tissues than in stages I and II. *P. acnes* stimulates the M2 polarization of macrophages through TLR4/PI3K/Akt signaling, leading to overexpression of IL-10 [119]. *H. pylori* (HP) infection is among the major risk factors for GC [120,121] Approximately 70% of GC patients were diagnosed as HP+, while the eradication of HP could be a preventive measure for GC [122,123]. However, HP shows a decreased relative abundance inside gastric tumor tissues compared to normal tissues [122,124] suggesting that HP might have a role in driving chronic inflammation, enabling GC initiation, but not as an intratumor resident. HP infection activates NF-κB in bile duct carcinoma cells, thereby increasing expression of VEGF, a major angiogenic factor. Additionally, VEGF may elevate nuclear expression of E2F, which increases proliferation in bile duct carcinoma [125].

## 5.5. Lung Cancer

Lung cancer (LC) is the leading cause of cancer deaths despite the huge advances in detection methods and treatment availability. Pulmonary infection and dysbiosis of the lungs are linked to many respiratory disorders, including LC, mainly via triggering a state of chronic inflammation [126]. This occurs by stimulating Myd88-dependent IL-1β and IL-23 production from myeloid cells, consequently leading to the activation of lung-resident γδ T cells producing IL-17 and other effector molecules that promote inflammation and stimulate tumor cell proliferation [41]. However, due to ethical considerations, obtaining lung biopsy samples from healthy human subjects is not applicable. Therefore, the majority of studies used bronchoalveolar lavage (BAL) [127], sputum [128], or bronchoscopic brushing [129] to study lung microbiota (Figure 6). In one investigation involving BAL fluid in LC patients, a notable rise in abundance was observed in two phyla, namely *Saccharibacteria* (TM7) and *Firmicutes*, as well as four genera, *Selenomonas*, *Atopobium*, *Megasphaera*, and *Veillonella*. [127]. Another study linked the higher level of chromosomal aberrations in LC patients with a higher sputum abundance of *Lachnoanaerobaculum, Bacteroides, Mycoplasma, Porphyromonas,* and *Fusobacterium* in their sputum [128].

To investigate if LC microbiome composition differs according to the type of sample, a study conducted by Bingula et al. characterized the lung microbiota from three different lung tissues (tumor tissue, peritumoral tissue, and non-malignant tissue) and compared it with BAL (obtained directly on an excised lobe) and saliva samples. The microbiome in the oral and lung shows differences in diversity and taxonomy. Lung tissue samples were predominantly with *Proteobacteria*. While saliva and BAL samples show a high abundance of *Firmicutes*. However, the dominant class among saliva was *Bacilli*, whereas *Clostridia* was the dominant class among BAL samples [130]. In the same way, Patnaik et al. identified variations in the microbiome between tissue, BAL, and saliva samples [131]. This indicates the importance of sample sources to analyze lung microbiota, and it is essential to note that BAL fluid, sputum, or saliva may not precisely represent the lung microbiota due to the potential

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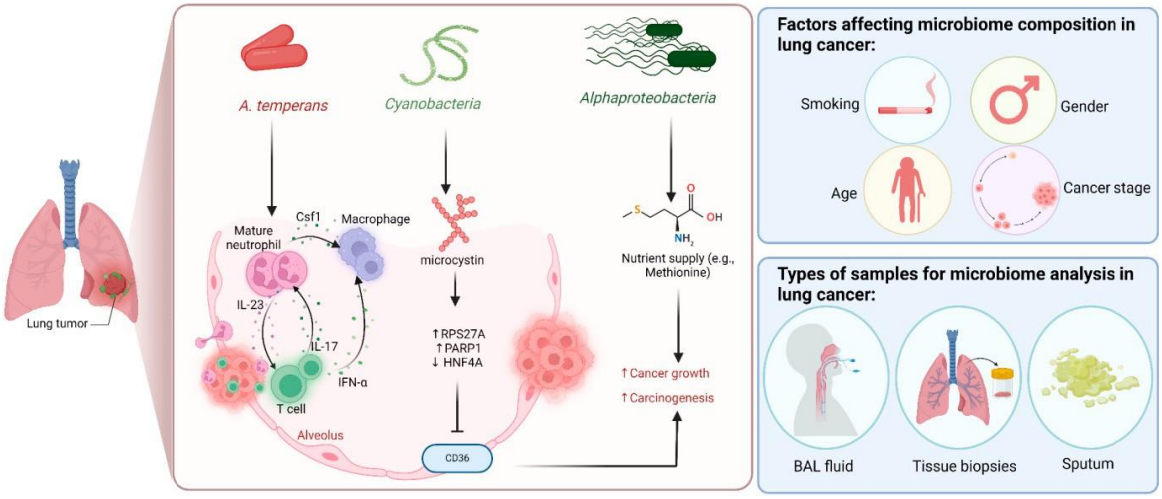



**Figure 6: Roles of Intratumor Microbiome in Cancer Progression, Influencing Factors, and Sample Types for Microbiome Analysis.** High abundance of *A. temperans* within the tumor microenvironment contributes to the promotion of lung adenocarcinoma. This microbe influences the development and maturation of neutrophils and promotes the secretion of cytokines. IL-23 and Csf1 secreted from the mature neutrophils stimulate the differentiation of monocytes and activate $CD4^+$ T cells, polarizing them to an IL-$17A^+$ phenotype, leading to a pro-inflammatory tumor microenvironment facilitating tumor growth. Through the production of the toxin, microcystin, the phylum *Cyanobacteria* increases the expression of ribosomal protein S27A (RPS27A) and procyclic acidic repetitive protein 1 (PARP1), combined with reducing the expression of HNF4A, which enhances inflammation by inhibiting CD36. *Alphaproteobacteria* are another microorganism commonly detected in lung cancers. This class of bacteria supplies crucial nutrients, such as methionine, to the cancer cells, which supports the proliferation of the latter. Several factors have been linked to the diversity of microbiome species found within the lung tumor cells. High levels of microbiomes that degrade chemicals found in cigarettes are enriched in tumor samples obtained from smoking patients. Furthermore, the gender and age of the patient, together with the stage of lung cancer, are other factors that affect the composition of the intratumor microbiome within lung cancer. To analyze the tumor-associated microbiome, different sample types have been widely used. While bronchoalveolar lavage (BAL) fluid is commonly employed, the use of biopsies from the cancer region or saliva samples has also been reported to detect lung cancer-related microbes.

contamination of the upper respiratory tract or oral microbiota.

A recent study investigated the association between intratumoral microbiome in non-small cell lung cancer (NSCLC) patients without lung infection and various factors such as malignancy, response to first-line treatment, and survival. *Serratia marcescens*- and *Enterobacter cloacae*-rich tumors were more likely to metastasize to the brain and mediastinal lymph nodes, respectively. Furthermore, *Haemophilus parainfluenzae* was negatively correlated with response to the first-line treatment for stage IV lung cancer; consequently, it was related to poor progression-free survival (PFS) while *S. haemolyticus* was linked to longer PFS [132]. *Gammaproteobacteria* were linked to low PD-L1 expression and poor response to checkpoint-based immunotherapy, translating into poor survival [79]. Additionally, the association of six bacterial biomarkers (*Clostridioides, Shewanella, Succinimonas, Acidovorax, Dickeya,* and *Leuconostoc*) with survival in patients with lung cancer indicated their potential to identify recurrence or metastasis [133]. By applying RNA-seq to investigate the metatranscriptome of human lung cancer, Chang and colleagues identified nine enriched bacteria in lung cancer. These nine species were correlated with a low overall survival among patients with LC. Moreover, the presence of two bacterial species, *Mycobacteroides franklinii* and *B. megaterium*, was associated with high levels of CD4+ T cells and Th2 cells, respectively. This suggests that these two bacteria can play an important role in the carcinogenesis process of LC [78].

Lung microbiome is also associated with the prognosis of lung cancer. Microbial composition differences were noted according to the cancer stage. The advanced-stage lung cancer group is enriched with the genera *Staphylococcus*, *Burkholderia*, *Caballeronia*, *Paraburkholderia*, and *Peptoniphilus* [134].

Recent studies have revealed significant variations in the microbiota based on histopathological types of lung cancer. For example, differential abundances were observed within the NSCLC subtypes. The abundance is significantly higher in adenocarcinoma (ADC) compared to squamous cell carcinoma (SCC). *Cyanobacteria* can produce a toxin called microcystin, which increases the expression of Poly [ADP-ribose] polymerase 1. Through the CD36 receptor, Poly [ADP-ribose] polymerase 1 can activate inflammatory pathways, thereby contributing to

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inflammation-associated lung carcinogenesis [135]. Similarly, in another study, microbiome profiles in BALF showed higher microbial diversity in SCC compared to the microbiota in ADC, in which *Acinetobacter*, *Brevundimonas*, and *Propionibacterium* were more enriched in ADC. In contrast, *Enterobacter* was more enriched in SCC [136].

Among smokers, colonization of bacteria that degrade cigarette smoke metabolites, such as nicotine, phenolic compounds, toluene, and anthranilate, is higher compared to non-smokers and lung cancer patients [76, 137]. Furthermore, the abundance of *Adinovorax temperans* was higher in smoker LC patients compared to non-smoker LC patients. Smoking, together with TP53 mutation, was linked to impairment in epithelial function, which may facilitate the invasion of carcinogenesis bacteria such as *A. temperans* [18]. On the contrary, *Acidovorax* was more abundant among non-smokers in a Chinese study conducted recently. However, enrichment of polycyclic aromatic hydrocarbon-degrading bacteria such as *Massilia* and *Sphingobacterium* was observed. Both studies reported the link between TP53 mutations, smoking, and the presence of the oncobiome [138].

## 5.6. Brain Cancer

Less data is available regarding the role or abundance of the microbiome in brain tumors. Recently, a study differentiated between microbial community composition in glioma tissues versus adjacent normal brain tissues by utilizing transcriptome sequencing and metabolomics, supported by an animal model, bacterial RNA and LPS were found within glioma tissues. Six genera were found to be significantly enriched in glioma tissues compared to their adjacent normal brain tissues, including *Fusobacterium*, *Longibaculum*, *Intestinimonas*, *Pasteurella*, *Limosilactobacillus*, and *Arthrobacter*. Moreover, results from animal studies revealed that *F. nucleatum* promoted glioma growth by increasing the levels of N-acetylneuraminic acid and the expression levels of CCL2, CXCL1, and CXCL2. Several significantly abnormal metabolic pathways were found in glioma samples, such as several amino acid metabolisms, nitrogen metabolism, and aminoacyl-tRNA biosynthesis [21].

## 5.7. Liver Cancer

Liver cancer is the sixth most diagnosed cancer and is the third leading cause of death among cancer-related mortality. An estimated 1.3 million people will die from liver cancer in 2040 by an increase of 56.4% compared to 2020. Hepatocellular carcinoma (HCC) represents 80% of primary liver cancer cases [139].

The alteration of normal gut microbiota increases the permeability of the gut, which leads to liver exposure to many microbial products [140]. For example, LPS-producing genera increased in early HCC patients compared to normal subjects. LPS binds to TLR4, which directly promotes HCC [141,142]. This suggests the gut microbiome as a target to prevent HCC [143]. A higher abundance of *Actinobacteria* was observed in HCC tissues, whereas *Deinococcus-Thermus* was significantly enriched in normal tissues. Additionally, *Methylobacterium* and *Akkermansia* emerged as significant prognostic markers for both overall survival (OS) and recurrence-free survival (RFS) [83]. Song et al. developed a microbiome-related score (MRS) model. This model identifies a 27-microbe prognostic signature of microbial abundances related to OS and disease-specific survival (DSS) in patients with HCC. The MRS model can predict prognosis, particularly 1-, 3-, and 5-year OS and DSS rates of HCC patients. Among the 27 microbes, some genera such as *Ornithinimicrobium*, *Caldimonas*, *Holophaga*, and *Rheinheimera* are associated with decreased overall response (OR), while others such as *Robinsoniella*, *Snodgrassella*, *Amycolatopsis*, *Alicyclobacillus*, and *Tetragenococcus* are linked to increased overall survival (OS) in HCC patients [144].

## 5.8. Cervical Cancer

A study linked the presence of *L. iners* in cervical tumors to treatment resistance and decreased patient survival. The Lactobacilli genus in general utilizes carbohydrates and uses lactate dehydrogenase (LDH) to produce lactate as the final product of fermentation [145]. However, *L. iners* does not express the D-LDH gene, and only L-lactate enantiomers are produced. Interestingly, L-lactate production increases after exposure of cells to metabolic stress such as ionization radiation. Lactate can provide energy to the tumor cells and contribute to communication between tumor cells and surrounding cells. Furthermore, lactate can activate certain signaling pathways that contribute to treatment resistance, such as hypoxia-inducible factor 1 (HIF-1) transcription targets and ROS-induced cellular signaling [146].

## 5.9. Skin Cancer

The main phyla of normal skin tissue are *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* [147], with the most represented genera being *Corynebacteria*, *Propionibacteria*, and *Staphylococci* [148]. Kullander et al. reported that the higher prevalence of *S. aureus* is associated with skin SCC, but not basal cell carcinoma, compared to healthy skin by analyzing tumor biopsies and swab samples. However, whether *S. aureus* influences

carcinogenesis or if SCC has an increased susceptibility to S. aureus colonization still needs more investigation [86]. Furthermore, *S. aureus* overabundance was also significantly linked to increased human beta defensin-2 (hBD-2) expression in SCC samples (Figure 7). The challenge of SCC cells directly with hBD-2 promoted keratinocyte tumor cell proliferation [149]. Some studies suggest that skin damage promotes the opportunity for *S. aureus* to infect the skin and secrete its virulence factor, regulated by the staphylococcal accessory regulator (SarA) protein. These virulence proteins induce chronic inflammation, consequently leading to skin cancer development [150]. On the other hand, in cell culture experiments, Nakatsuji et al. identify a skin commensal microbe, *S. epidermidis*, that can produce 6-N-hydroxyaminopurine (6-HAP). This molecule works as a DNA polymerase inhibitor that blocks the proliferation of tumor cells. Moreover, treating mice models with 6–HAP–producing *S. epidermidis* reduced the incidence of UV-triggered tumors compared to control mice. Consequently, these results suggest the role of skin commensals in protection against skin cancer [151]. Furthermore, a mouse study showed that the growth of melanoma cells was inhibited upon intratumoral administration of the commensal *P. acnes*. The proposed mechanism was through the induction of Th1-type cytokines such as IL-12, TNF-α, and IFN-γ. Moreover, they found that the induction of IFN-γ promotes cytotoxic effects by activating CD8+ T cells, NK cells, and B cells and elevates chemokines, including CXCL9 (MIG) and CXCL10 (IP-10) that suppress vascular proliferation [152].

## 5.10. Genitourinary Cancers

### 5.10.1. Prostate Cancer

Analysis of prostate tumor specimens from 242 patients revealed that microbes were more abundant in tumor samples than in normal samples [153]. Findings from another study suggest that 70% of bacterial genera detected in prostate tumor samples were gram-negative bacteria, in which *Proteobacteria* were the most abundant, followed by *Firmicutes*, *Actinobacteria*, and *Bacteroides*. Additionally, DNA from *H. pylori*, specifically the sequences of the cagA gene, was detected in specific host chromosomes in prostate tumor cells. The cagA gene encodes for the immune-dominant cagA virulence factor [64]. Moreover, *P. acnes* infection was positively associated with chronic inflammation of the prostate. Consequent to *P. acnes* infection, the body activates transcription factors NF-κB and STAT3 that induce plasminogen-matrix metalloproteinase and COX2-prostaglandin pathways activation, leading to chronic inflammation. Prolonged exposure to *P. acnes* not only affects host cell proliferation but also induces cellular transformation [154].

### 5.10.2. Ovarian Cancer

Tissue samples from ovarian cancer showed different bacterial, fungal, viral, and parasitic characteristics in comparison with normal samples [155]. Evidence suggests a significant decrease in both the total number and diversity of bacterial communities in ovarian cancer tissues compared to those in normal distal fallopian tube tissues. Moreover, inflammation-associated signaling pathways, such as cytokine–cytokine receptor interaction, NF-κB signaling, and chemokine signaling, were significantly activated in ovarian cancer tissues [90].

### 5.10.3. Bladder Cancer

Comparing microbiome composition in urine and tumor tissue in bladder cancer patients revealed similarity in phyla levels, where both sample types showed *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, and *Bacteroidetes* as the most abundant phyla. However, in terms of genera, urine samples were enriched in *Lactobacillus*, *Staphylococcus*, *Streptococcus*, and *Corynebacterium*. Whereas, *Akkermansia*, *Bacteroides*, *Klebsiella*, *Enterobacter*, and *Clostridium sensu stricto* are abundant in tissue samples [156]. Another study showed that genes of EMT, including TWIST1, E-cadherin, SNAI2, SNAI3, and vimentin, are associated with the presence of butyrate-producing bacteria [89].

### 5.10.4. Kidney Cancer

The kidney microbiome is originally translated from the gut, circulatory system, or ascended from the lower urinary tract [87]. It has been observed in a study that species diversity was decreased in renal cell carcinoma (RCC). In addition, a noted reduction in *Streptophyta* was observed in tumor tissue compared to healthy. Of note, 9 KEGG pathways were significantly different between the two groups. For example, membrane transport, transcription, and cell growth and death pathways were abundant in tumor tissues, whereas the other 6 pathways, such as energy, cofactors, and vitamins metabolism, and cell motility, were abundant in normal tissues [87].

# 6. The Impact of Intratumor Microbes on Cancer Therapeutics

Several studies revealed the significant role of the intratumor microbiome in influencing the response to cancer therapy and, in particular, immunotherapeutics (Table 2).

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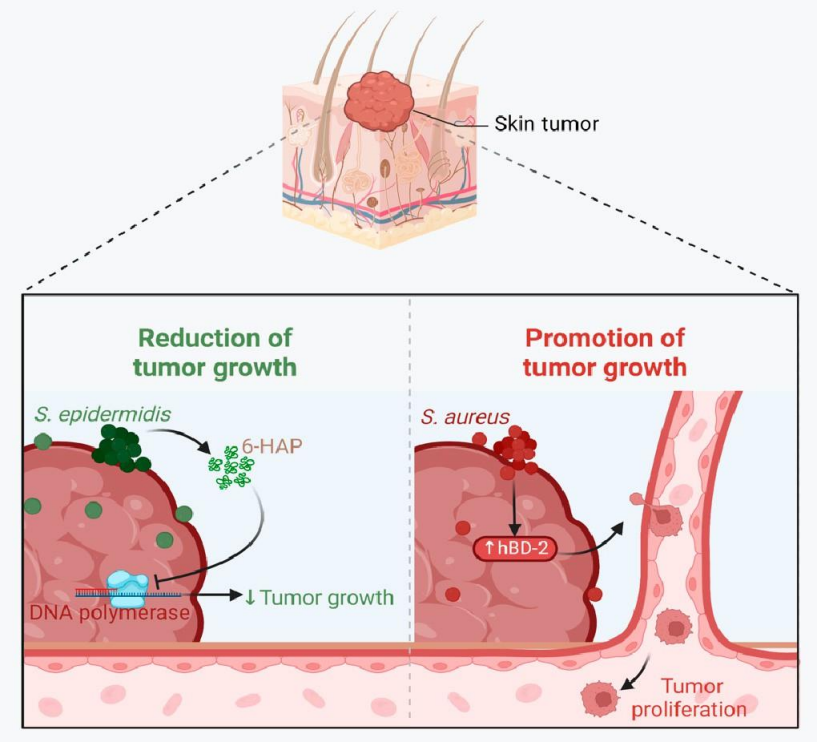



**Figure 7: The Dual Role of *Staphylococcus* Bacteria in Skin Cancer.** *S. epidermidis* produces 6-N-hydroxyaminopurine (6-HAP), a substance that inhibits skin tumor proliferation by interfering with DNA polymerase activity, thereby slowing the growth of cancer cells while remaining safe for normal skin cells. In contrast, the presence of *S. aureus* in the skin tumor microenvironment promotes tumor proliferation by stimulating host cells to overproduce human β-defensin-2 (hBD-2).

For example, the efficacy of various chemotherapeutic drugs, such as gemcitabine, fludarabine, and cladribine, could be attenuated or enhanced by bacteria commonly present in tumor tissues. This influence is, in part, mediated by bacterial modification of the chemical structure of drugs. For instance, intratumor *Gammaproteobacteria*, expressing the bacterial enzyme cytidine deaminase, have been linked to gemcitabine resistance in cancers, including colon and pancreatic cancer. Conversely, microbiota-derived tryptophan metabolite indole-3-acetic acid has shown promise in enhancing chemotherapeutic effects in pancreatic cancer by modulating ROS accumulation and downregulating autophagy (https://doi.org/10.1002/mco2.376). In colorectal cancer, *F. nucleatum* has been implicated in activating pathways, like TLR4, to enhance autophagy in cancer cells, leading to chemoresistance [157]. In prostate cancer, the intratumor LPS-activated NF–κB–IL6–STAT3 axis has been associated with increased proliferation and chemoresistance (https://doi.org/10.1016/j.cell.2017.07.008). It comes as no surprise that the significant impact of intratumor microbiota on the efficacy of immunotherapeutics, such as checkpoint inhibitors, is observed, given the crucial interaction of these microbes with the immune system. For example, a study showed that some defined taxa can improve anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), increase the accumulation of IFN-γ-producing CD8+ T cells, and improve the efficacy of anti-PD-1 therapy [158]. These taxa include *Bacteroides*, *Ruminococcaceae*, *Parabacteroides*, and *Alistipes*. Further studies showed that fecal transplant enriched with SCFAs producers increased tumor infiltration of CD8+ T cells and improved the outcome of anti-PD-1 immunotherapy in melanoma patients [159,160]. A recent study showed that a higher abundance of *Ruminococcus*, *Bacteroides*, and *Faecalibacterium* is associated with increased responses to CAR-T cell therapy in B-cell malignancies [161]. Another study reported two oncomicrobiotics named *E. hirae* and *Barnesiella intestinihominis, to* enhance the recruitment of IFN-γ-producing γδ T cells and CD8+ effector tumor-infiltrating lymphocytes while reducing Treg cells and γδ T17 inside tumor cells, leading to improved outcome of cyclophosphamide therapy [14].

**Table 2:** The impact of Gut microbiome on the efficacy of Immune checkpoint inhibitors therapy.

| Type of Cancer | Study Size | Type of Immuno-Therapy | Sample | Outcomes | References |
|---|---|---|---|---|---|
| **Melanoma** | 25 | Anti-PDI-1 or anti PDI-1/Anti-CTLA-4) | Feces | ↑*E. biforme, Ruminococcus gnavus, E. coli, Streptococcus salivarius*, and *Phascolarctobacterium succinatutens*, in respondent patients.<br>↑*B. longum, Prevotella copri, Coprococcus sp, Eggerthella*, and *Eubacterium ramulus* in non-respondent patients.<br>↑*Streptococcus parasanguinis* carriers → longer Overall Survival.<br>↑*B. massiliensis* → higher in Progression-free survival.<br>↑*Peptostreptococcaceae* carriers → shorter overall survival and progression-free survival rate. | [162] |
| **Advanced thoracic carcinoma** | 42 | PD-1 blockade | Feces | ↑*Enterobacteriaceae, Carnobacteriaceae, Akkermansiaceae, Enterococcaceae*, and *Clostridiales* in the respondent group correlated with a longer Progression-free survival rate. | [163] |
| **Advanced-stage GI Carcinoma** | 74 | Anti-PDI-1, or Anti PD-1/Anti-CTLA-4 | Feces | ↑*Ruminococcaceae, Lachnospiraceae, and Prevotellaceae* in Respondent individuals.<br>↓*Bacteroidaceae* in Respondent individuals.<br>*Prevotella/Bacteroides* ratio decreased in the respondent individuals.<br>Producing short-chain fatty acid (*Lactobacillus, Streptococcus, and Eubacterium*) → positively correlated with anti-PD-1/PD-L1 response. | [164] |
| **Hepatocellular carcinoma** | 8 | PD-1 blockade | Feces | ↑*Proteobacteria* abundance in non-respondents during therapy. | [165] |
| **Non-Small Cell Lung Cancer** | 11 | PD-1 blockade | Feces | ↑*A. muciniphila, B. longum, Faecalibacterium prausnitzii* in Respondents.<br>↑*Staphylococcus aureus, Veillonella, Propionibacterium acnes, Peptostreptococcus, Sutterella, Dialister, and Ruminococcus bromii* in non-respondent patients.<br>↑*Streptococcus, Lactobacillus, Enterobacteriaceae, Prevotella, Bacteroides plebeius, Oscillospira*, and *Rikenellaceae* are present in cancer patients compared to healthy control participants. | [166] |
| **Melanoma** | 112 | Anti-CTLA-4, Anti-PDI-1 | Feces and oral samples | ↑variety of alpha and relative abundance of *Ruminococcaceae* bacteria in respondents. | [2] |

Up arrow: means increase; Down arrow: Means decrease.

## 7. Development of Microbiome-Based Cancer Therapeutics and Diagnostic Biomarkers

Probiotics have been widely employed to confer health benefits [167] by restoring the healthy microbiome structure and its associated beneficial functions [168,169]. Multiple studies show the beneficial effect of using specific microbes as an adjuvant with chemotherapeutics (Figure 8). For example, co-administration of *Eudoraea* spp. anti-PD-1 resulted in a better outcome of immunotherapy through the activation of CD8+ T cells and cytolytic T cells in the mouse model [170]. Combining *Bifidobacterium* with anti-PD-L1 therapy reduced tumor expansion by enhancing the activity of dendritic cells and increasing the intratumor accumulation of CD8+ T cells [171]. A study conducted on the CRC mice model fed on nano-sized *L. plantarum* showed a reduction in the number of tumor lesions compared to the control. These changes were attributed to the induction of cell cycle arrest and apoptosis, the suppression of inflammation, and increased IgA secretion [172]. Another study showed that the probiotic VSL#3, which is composed of *Bifidobacterium* and *Lactobacillus* species, reduced the proliferating cell nuclear antigen labeling index, TNFα, IL-1β, IL-6 production, and COX-2 expression, and increased IL-10 levels in colon tissue [173]. Emerging data suggest that the intratumor microbiome signature could be used as a diagnostic biomarker [174]. Although being technically challenging due to the difficult-to-access sampling sites, low microbial biomass, and the high chance of contamination [174]. For example, a study examining the microbiota associated with esophageal SCC revealed that patients have a reduced microbial diversity characterized by lower abundances of *Bacteroidetes*, *Fusobacteria*, and *Spirochaetes*. Interestingly, the authors claim that this mi-

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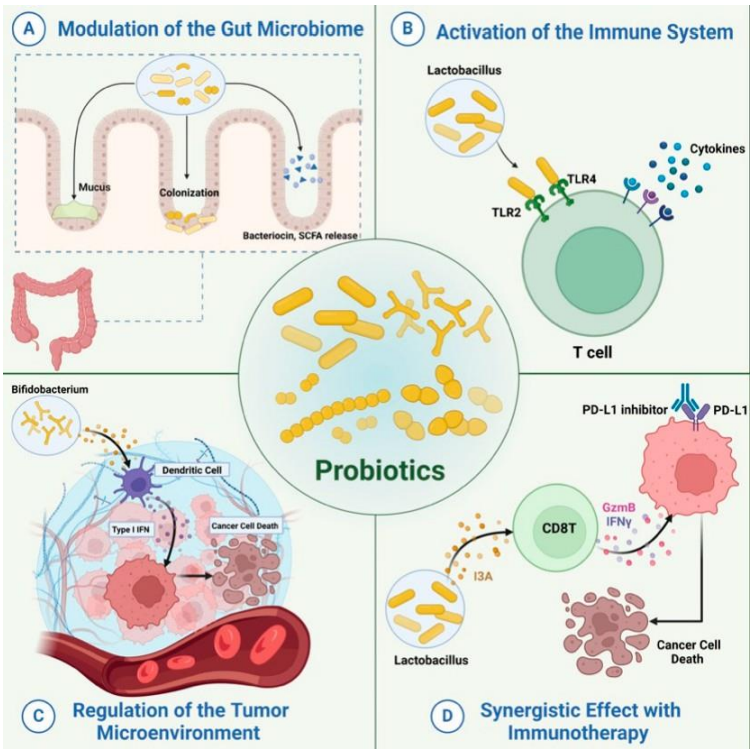



**Figure 8: Impact of probiotics on cancer therapy**. (**A**) Probiotics modify gut microbiome composition and diversity. Additionally, it helps reestablish the gut microbiome balance disturbed in cancer patients. (**B**) Probiotics can activate different types of immune cells and stimulate the production of cytokines and chemokines. (**C**) Probiotics can affect the tumor microenvironment by interacting with the gut-tumor axis and reconfiguring the metabolic and immunological landscape of TME to suppress tumor progression. (**D**) Probiotics enhance the response and durability of tumor immunotherapies in various cancers.

crobial shift could effectively distinguish between patients and healthy controls. Furthermore, this dysbiosis affected the metabolic profile with a change in nitrate reductase level [175]. Another study suggests that *P. somerae* can be used as a biomarker for endometrial cancer. *P. somerae* upregulates *the* hypoxia-inducible factor pathway, a hallmark of endometrial cancer (10). Another study suggested that oral microbiota, such as *P. gingivalis* and *Aggregatibacter actinomycetemcomitans*, can be used to predict the possibility of developing pancreatic cancer [176].

Routy et al. reported the ability of *A. muciniphila* to modulate the link between immunotherapy and treatment response. Administration of *A. muciniphila* after the initiation of fecal microbiota transplantation using feces from non-responding mice has restored the responsiveness to PD-1 blockade, showing a promising interleukin-12-dependent mechanism [5]. Another study involving preclinical oral probiotics in mice with bladder cancer and melanoma showed that administering Bifidobacterium will enhance the tumor control significantly when combined with PD-L1 blockade [171]. Le Noci et al. reported that *L. rhamnosus* could enhance the immunosuppression reversal and inhibitory effect of lung tumor implantation, while also further reducing the number of metastases when alternating with antibiotics. Together, they show that the microbiota of the local environment seems to play key roles in the immune response and its implication in lung cancer [177]. Another study investigated the influence of intratumor microbiota on CD47-based cancer immunotherapy in colon cancer. Shi et al. administered Bifidobacterium to colon cancer patients, and found that it has been colonized and accumulated inside tumor sites, resulting in the augmentation of local anti-CD47 treatment via a STING-dependent route [178]. Moreover, Iida et al. showed that administering *Alistipes shahii* via oral gavage was sufficient for bringing back the immunotherapeutic response against colon tumors in mouse models, which had been treated previously with antibiotics [3].

## 8. Engineered Probiotics, an Emerging Trend in the Development of Cancer Biomarkers and Therapeutics

The design and development of engineered or programmed probiotics for treating a range of human con-

ditions, from inflammatory bowel diseases to cancer, is gaining momentum [168]. This interest is fueled by the advancement in gene editing technology, including third-generation Clustered Regularly Spaced Short Palindromic Repeats (CRISPR)/CRISPR-associated Protein (CRISPR-Cas) system [179]. Engineered probiotics are modified microorganisms that can deliver a more controlled outcome compared to conventional probiotics, with unpredictable interactions within the host context [180]. The application of engineered probiotics in cancer therapy includes their use as; (1) adjuvant therapy to enhance the efficacy of immunotherapeutics, (2) heterologous host to express anticancer drugs, (3) vectors to ensure the precise delivery of anti-tumor drugs, and (4) a non-invasive technique to sense and detect tumor cells. Examples of bacteria highly utilized in engineered probiotics include *E. coli*, *Bifidobacterium*, and *S. typhimurium*. These microbes are anaerobes that can easily survive, effectively colonize, and carry anticancer proteins, drugs, and compounds to the intratumor anaerobic environment. *E. coli* Nissle 1917 (EcN) is one of the most utilized strains in the field of engineered probiotics, due to its well-known tolerability in humans and high safety margin, in addition to being easily genetically manipulated [181,182]. Various studies have been carried out in this regard, utilizing in vitro cell lines, in vivo models, and clinical trials, to prove the efficacy and safety of such living biotherapeutic products (LBP) [183]. Data indicates that metabolic modulation of the intratumor environment via engineered probiotics can act synergistically with other immunotherapy agents to achieve durable and potent eradication of cancer [184]. Examples of the use of engineered probiotics in cancer therapy or diagnosis are detailed (Figure 9).

Many reports highlight the promise and efficacy of engineered probiotics in provoking anti-tumor activity and enhancing the activity of cancer therapy. For example, an engineered strain of *S. typhimurium* expressing IL-15/Flagellin B (FlaB) proteins causes tumor regression in animal models of metastatic colon tumors [185]. FlaB is a protein used as an adjuvant in vaccines due to its strong ability to activate the innate immune response, primarily by enhancing the recruitment of immune cells [186,187]. IL-15 has immunostimulatory action mainly by promoting maturation, development, and activation of NK, NKT, and CD8+ cells, and increasing proliferation of the specialized CD8+ T memory cells [188,189]. Engineered *S. typhimurium* induces both the innate and adaptive immune response, suppressing tumor growth in mice and enhancing the development of immune memory toward the tumor cells. Besides that, the combination of engineered *S. typhimurium* producing IL15/FlaB and PD-L1 blockade treatment revealed an improved efficacy of this synergistic combination, including in metastatic cancers [185]. Another engineered probiotic strain, EcN, was developed to constantly convert the tumor byproduct ammonia to L-arginine, a key element in provoking the immune response, mainly through increasing the proliferation of T cells. The use of this engineered probiotic increased the number of tumor-infiltrating T cells, resulting in the suppression of tumor growth in the MC38 tumor model when combined with PD-L1 antibodies. Additionally, mice injected with the EcN-engineered strain were found to form T cell memory specifically against MC38 tumors, which yields long-term protection [190]. Engineered *B. longum* (BL) was developed to express tumstatin, a potent angiogenesis inhibitor. Tumstatin-transformed BL exerted significant anti-tumor activity, supported by a reduction in the volume, weight, growth, and microvessel density of the tumors. Also, the intratumorally expressed tumstatin generated apoptosis and stimulated the immune response toward tumor cells. The implication of the Tum-BL system is expected to gain momentum when thinking about new approaches to treat solid tumors [191]. Interestingly, some engineered probiotics have reached clinical trials, such as SYNB1891, an engineered EcN developed by Synlogic (NCT04167137) [192]. SYNB1891 activates antigen-presenting cells, triggering innate immunity in addition to stimulation of the interferon pathway through the production of di-AMP.

Multiple studies showed the efficacy of engineered probiotics in the targeted delivery of therapeutics inside the tumor cells. For example, *E. coli* SLIC was engineered to deliver checkpoint inhibitors such as PD-L1 and CTLA-4 antagonists in the form of nanobodies and control their release inside the tumor cells utilizing a stabilized lysing release system that was optimized based on computational and experimental studies. Data shows that a single intravenous or intratumor injection of such a system resulted in a higher therapeutic response compared to antibodies, resulting in restriction of tumor growth in mice. The authors suggested that this activity is mediated by a systemic stimulation of the immune response, as suggested by the increased number of T cells [193]. Another study employed tumor tropism to enable guiding the bacteria to tumor cells. An example is EcN, an engineered *E. coli* Nissle 1917, which is designed to deliver tumor suppressors such as tumor suppressor p53 and the angiogenic inhibitor TUM-5 to the tumor sites. Use of this engineered strain resulted in restricted tumor growth in mice [194]. Data shows that EcN can accumulate inside the hypoxic tumor microenvironment in nude mice. Blue light is employed to control the expression of specific TNFα in the genetically engineered EcN (EcN@EL222-TNFα). Such strain was modified to be sensitive to the applied blue light

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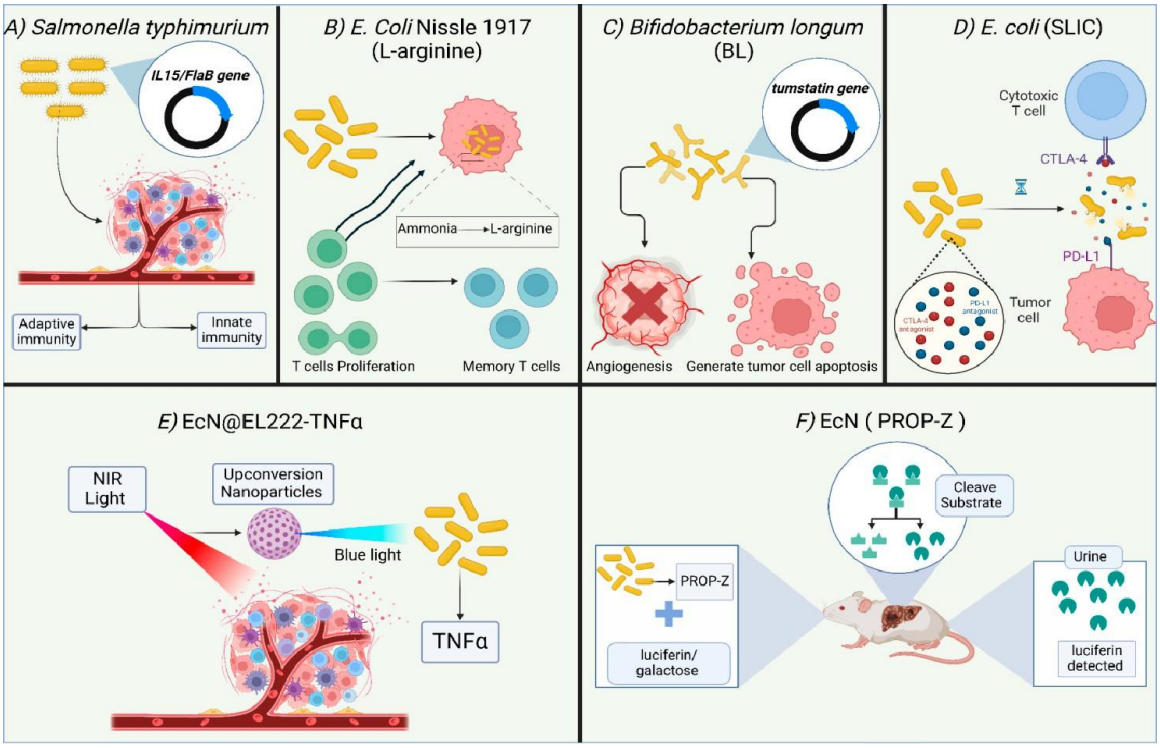



**Figure 9:** Illustration of the mechanisms underpinning some examples of Engineered Probiotics for cancer treatment and diagnosis. (**A**) Engineered strain of *Salmonella typhimurium* expressing Interleukin-15 (IL15)/Flagellin B (FlaB) proteins resulting in activation of innate and adaptive immune systems to suppress tumor cells, (**B**) Engineered strain of EcN that converts cancer cells byproduct ammonia to L-arginine which provokes the proliferation of T cells and formation of memory cells, (**C**) Engineered strain of *Bifidobacterium longum* (BL) expressing tumstatin to inhibit angiogenesis and generate apoptosis in cancer cells, (**D**) Engineered strain of EcN integrated into the optimized platform "SLIC" enabling the controlled lyse of the bacteria in the tumor cells to deliver checkpoints inhibitors in form of nanoparticles, (**E**) Engineered strain of EcN that respond to the blue light with the subsequently injected upconversion nanoparticles which converts the exogenous NIR to blue light shed to stimulate the secretion of tumor necrotic factor (TNFα) by EcN, (**F**) Engineered strain of EcN expressing PROP-Z that serves as diagnostic biomarker for hepatic metastasis detection in urine.

and accordingly produces TNFα in tumor tissues. Specialized nanoparticles were subsequently injected following the delivery of engineered blue-light sensitive *E. coli* strain, to accomplish the key role, which is the conversion of near-infrared light (NIR) that originates from a laser light applied exogenously, to a local blue light, resulting in a direct illumination endogenously toward the specific EL222 in the *E. coli* strain, stimulating it to produce TNFα. As long as laser light is shed from outside, the engineered probiotic will continue to produce necrosis factor from inside, and once removed, the whole process of TNFα expression will stop. The results exhibit a considerable efficacy of NIR light-responsive *E. Coli* strain to inhibit the tumor growth both in vitro against stage IV human breast cancer cell lines, and in vivo using mouse models. This provided a valuable approach for the precise regulation of intratumor drug delivery [195].

Another emerging application of engineered probiotics is the sensing and diagnosis of tumors. The TME is attractive to anaerobic microbes such as *E. coli* and *Clostridium* EcN named PROP-Z, which was designed to selectively detect liver metastasis in mice. PROP-Z is engineered to co-express luciferase and β-galactosidase and thus can generate luminescent and colorimetric signals [196]. Oral treatment of PROP-Z coupled with intraperitoneal injection of D-luciferin resulted in a detectable tumor-specific signal in the urine that is proportional to the size of the tumor in a murine model of liver metastasis. A further modification that included the introduction of the gene *dlp7* from *B. subtilis* and a toxin-antitoxin system has further enhanced the efficacy and stability of the construct. Oral administration of PROP-Z and a combined luciferin/galactose molecule, named LuGal, resulted in the release of luciferin by the action of β-galactosidase. luciferin is then detected in the urine. Interestingly, this programmed strain was not able to colonize healthy tissues [197].

## 9. Conclusion and Future Perspectives

Multiple studies suggest a strong correlation between intratumor microbiota and tumor infiltration of immune cells such as cytotoxic CD8+T cells and Treg cells, exerting either a negative or positive effect on anti-tumor immunity, and implicating tumor progression and clinical outcome [198]. In spite of the paramount significance of intratumor microbiota and its implication in translational application, we still lack a comprehensive understanding of the microbiota-immunity-tumor cells interactions and signals within the TME. Key unanswered questions include how to elucidate the mechanisms underpinning the crosstalk between gut and local tumor microbiota, the impact of intratumor microbes on cancer metastasis, detailed characterization of intratumor microbial communities, large-scale cohorts of clinical studies to determine the impact of intratumor microbes on response to therapeutics and their applications, and the development of microbiome-based diagnostic biomarkers and live therapeutics to enhance activity of cancer therapy. An interesting area of research in this field is the modulation of therapy outcomes through a controlled diet that affects the structure of gut microbes. For example, in mouse models of adenocarcinoma, oral administration of the polysaccharide dietary fiber inulin increased the effectiveness of anti-PD-1 therapy [199]. A trending research in this field is the design and development of engineered probiotics concurrent with the advances and development of next-generation gene editing tools [200,201]. Engineered probiotics could revolutionize cancer diagnosis and treatment protocols, particularly in the targeted delivery of anticancer drugs, provoking anti-tumor immunity, or sensing metastatic tumor cells in a non-invasive manner.

## Abbreviations

| | |
|---|---|
| 12,13-EpOME | 12,13-epoxyoctadecenoic acid |
| 6-HAP | 6-N hydroxyaminopurine |
| ADC | Adenocarcinoma |
| ALPK1 | Alpha-protein kinase 1 |
| BAL | Bronchoalveolar lavage |
| BC | Breast cancer |
| BFT | Bacteroides fragilis Toxin |
| BIRC3 | Baculoviral IAP repeat containing 3 |
| C3 Receptor | Complement 3 receptor |
| CagA | Cytotoxin-associated gene A |
| CAR-T cell | Chimeric antigen receptor T cell |
| ccl2 | Chemokine (C-C motif) ligand 2 |
| CD8+ T cells | Cytotoxic T lymphocytes |
| CDDL | Cytidine deaminase |
| c-di-AMP | Cyclic di-adenosine Monophosphate Monophosphate |
| CDT | Cytolethal distending toxin |
| CEACAM1 | Carcinoembryonic antigen-related cell adhesion molecule 1 |
| COX-2 | Cyclooxygenase-2 |
| CRC | Colorectal cancer |
| CRISPR | Clustered Regularly Interspaced Short Palindromic Repeats |
| Csf1 | Colony-stimulating factor 1 |
| CTLA-4 | Cytotoxic T-Lymphocyte-Associated Protein 4 |
| CXCL 1,2 | C-X-C motif chemokine ligand 1,2. |
| DC | Dendritic cells |
| dFdU | Difluorodeoxyuridine |
| DSS | Disease-specific survival |
| EMT | Epithelial-mesenchymal transition |
| ER+ | Estrogen receptor positive |
| GC | Gastric cancer |
| GF | Germ free |
| GSDM E | Gasdermin E |
| hBD-2 | Human beta defensin-2 |
| HCC | Hepatocellular carcinoma |
| Hhep | Helicobacter hepaticus |
| HIF | Hypoxia-inducible factors |
| HIF-1 | Hypoxia-inducible factor 1 |
| HNF | Hepatocyte nuclear factor |
| HP | H. pylori |
| ICAM1 | Intercellular adhesion molecule 1 |
| IECs | Intestinal epithelial cells |
| IFN | Interferon |
| IgA | Immunoglobulin A |
| IL-1β | Cytokine interleukin-1β |
| IL-23 | Interleukin 23 |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| LC | Lung cancer |
| LDH | Lactate dehydrogenase |
| LPS | Lipopolysaccharide |
| MAPK | Mitogen-activated protein kinase |
| MBL | Mannose-binding lectin |
| miRs | MicroRNAs |
| MMR | Mismatch repair |
| MRS | Microbiome-related score |
| MSI | Microsatellite instability |
| MYD protein | Myeloid differentiation primary response protein |
| ncRNAs | Non-coding RNAs |
| NE | Neutrophil elastase |
| NFkB | Nuclear factor kappa B |
| NIR | Near-infrared light |
| NK Cells | Natural killer cell |

| | |
|---|---|
| NSCLC | Non-small cell lung cancer |
| OS | Overall survival |
| PARP1 | Procyclic acidic repetitive protein 1 |
| PD-1 | Programmed Cell Death Protein 1 |
| PDAC | Pancreatic ductal adenocarcinoma |
| PD-L1 | Programmed Cell Death Ligand 1 |
| PFS | Progression-free survival |
| pks+ E.coli | Polyketide synthetase-positive Escherichia coli |
| RCC | Renal cell carcinoma |
| RFS | Recurrence-free survival |
| RhoA- ROCK pathway | Rho-Associated Protein Kinase |
| ROS | Reactive oxygen species |
| RPS27A | Ribosomal protein S27A |
| SarA | Staphylococcal accessory regulator A |
| SCC | Squamous cell carcinoma |
| SCFAs | Short-chain fatty acids |
| SMO | Spermine oxidase |
| SNAI | Snail family transcriptional repressor-1 |
| STAT3 | Signal transducer and activator of transcription 3 |
| STING | Stimulator of interferon genes |
| TAN2 | Tumor-associated neutrophil 2 |
| Tfh | T follicular helper |
| TGF-β | Transforming growth factor-β |
| TH cells | T helper cells |
| TIGIT | T cell immunoreceptor with immunoglobulin and ITIM domain |
| TLR4 | Toll-like receptor 4 |
| TLSs | tertiary lymphoid structures |
| TMAO | Trimethylamine N-oxide |
| TME | Tumor microenvironment |
| TNBC | Triple Negative breast cancer |
| TNF | Tumor necrosis factor |
| TRM | Tissue-resident memory T cells |
| VEGF | Vascular endothelial growth factor |
| γδ T cells | Gamma delta T cells |

## Author Contributions

W.K.M. conceptualized the study and review structure, developed tables, figures, and collected and analyzed data. A.A.A. curated data of the microbiome of cancer types, developed figures, and tables. R.W.A.A. collected data related to the application of microbiome therapeutics and managed reference citations. R.A.S. organized and developed tables. N.A.R. collected data on engineered probiotics and developed figures. S.M. collected introductory data on TME. R.G. and TAI developed the review structure and analyzed the literature. All authors wrote, edited, and approved the final version of the manuscript.

## Availability of Data and Materials

This is a review article and does not contain any original data. All sources of information are cited in the reference list.

## Consent for Publication

All authors have read and approved the final manuscript and consent to its publication. There are no conflicts of interest to declare.

## Conflicts of Interest

The authors declare no conflicts of interest.

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