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Review Article

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Beyond Tumor Borders: Intratumoral Microbiome Effects on Tumor Behavior and Therapeutic Responses

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ABSTRACT

The human body contains a diverse array of microorganisms, which exert a significant impact on various physiological processes, including immunity, and can significantly influence susceptibility to various diseases such as cancer. Recent advancements in metagenomic sequencing have uncovered the role of intratumoral microbiome, which covertly altered the development of cancer, the growth of tumors, and the response to existing treatments through multiple mechanisms. These mechanisms involve mainly DNA damage induction, oncogenic signaling pathway activation, and the host's immune response modulation. To explore novel therapeutic options and effectively target and regulate the intratumoral microbiome, a comprehensive understanding of these processes is indispensable. Here, we will explore various potential actions of the intratumoral microbiome concerning the initiation and progression of tumors. We will examine its impact on responses to chemotherapy, radiotherapy, and immunotherapy. Additionally, we will discuss the current state of knowledge regarding the use of genetically modified bacteria as a promising treatment option for cancer.

Keywords: Microbiome; Immune response; DNA damage; Tumorigenesis; Immunotherapy; Genetic engineering

INTRODUCTION

Cancer is a multifaceted, complicated disease with high intra- and intertumoral heterogeneity (1). This heterogeneity emanates from a range of factors, including genetic mutations, epigenetic changes, and tumor microenvironment (TME) (1). This heterogeneity might lead to treatment resistance, ranging from insusceptibility to cytotoxicity to immunological treatments such as immunotherapy. TME exerts an important role in the process of cancer progression and treatment response, orchestrating alterations in the expression of key genes, cellular signaling cascades, and the composition of the microbiome (2). Despite genetic and environmental factors, various studies highlight the critical role of the microbiome in cancer development and advancement. Notably, these microorganisms possess the ability to regulate

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Abbreviations

AML, acute myeloid leukemia; BC, breast cancer; Bft, Bacteroides fragilis toxin; CagA, cytotoxin-associated gene A; CDD, cytidine deaminase; CDT, cytolethal distending toxin; CLL, chronic lymphocytic leukemia; ClyA, cytolysin A; CRC, colorectal cancer; CRT, chemoradiotherapy; DCA, deoxycholic acid; EBV, Epstein-Barr virus; EMT, epithelial-mesenchymal transition; EV, extracellular vesicle; FFAR, free fatty acid receptor; FMT, fecal microbiota transplantation; Fn, Fusobacterium nucleatum; HERV, human endogenous retroviruses; Hp, Helicobacter pylori; HTLV-1, human T-lymphotropic virus type 1; ILC, innate lymphoid cells; KSHV, Kaposi's sarcoma-associated herpesvirus; LCA, lithocholic acid; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; RT, radiotherapy; S. typhi, Salmonella typhi; SCFA, short-chain fatty acid; TLS, tertiary lymphoid structures; TMAO, trimethylamine N-oxide; TME, Tumor microenvironment; TRM, tissue-resident memory; VacA, vacuolar toxin A.

Author Contributions

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a variety of essential physiological functions, including inflammation and immunological responses—2 processes that are linked to cancer progression (3).

Several studies have highlighted the importance of the microbiome as a cancer diagnostic and prognostic factor. Recently, the strong association between the TME and the cancer etiology has been improved, suggesting the existence of specific strains of bacteria within tumor tissues (4). In an extensive metagenomic analysis of numerous solid tumors, Nejman et al. (4) revealed the presence of intracellular bacteria within both immune and cancer cells that constitute the TME. Interestingly, microbiome with distinct features was detected within each tumor subtype. Observations indicate that all of the conditions are met in tumors to sustain a high bacterial predominance (**Fig. 1**) (5). Notably, the intrinsic properties of the TME offer the ideal niches for diverse bacterial species, encompassing strict anaerobes (e.g., Clostridia spp.), facultative anaerobes (e.g., Clostridia spp.), and anaerobic bacteria (e.g., *Salmonella* spp. and lactic acid bifidobacteria) within tumors (6). The significant amounts of nutrients, especially purines, produced by necrotic tumor cells have a profound influence on the viability and relative abundance of bacterial communities (7). In addition, bacterial chemotaxis towards elevated chemoattractant substances found in necrotic regions (e.g., galactose, serine, aspartate, citrate, and ribose) increases the number of bacteria in tumors (8). Furthermore, the increased abundance of blood vessels, mostly due to neo-angiogenesis, surrounding most tumor tissues, along with the immunosuppressive environment they promote, provide an ideal habitat for the colonization of circulating bacteria (9).

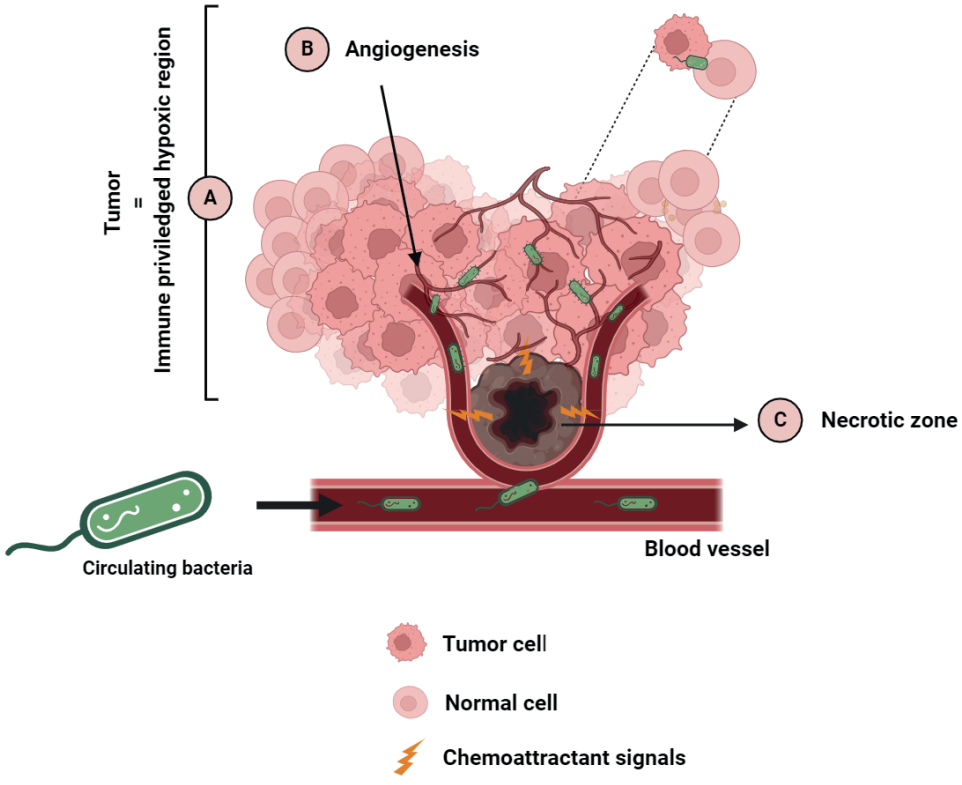

Figure 1. Diagram depicting bacterial invasion process in tumor tissue. (A) Tumor hypoxia promotes the proliferation of anaerobic bacteria, which thrive in low oxygen levels within the TME. (B) Necrotic zones within tumors provide abundant nutrition and emit chemoattractant signals, allowing bacterial infiltration and proliferation. (C) Tumor angiogenesis facilitates the development of aberrant blood vessels, providing pathways for bacteria to infiltrate tumor tissues via circulation or the surrounding environment.

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Dysbiosis, characterized by alterations in microbial community composition within tissues and blood, has emerged as a promising biomarker for various diseases, notably in oncology. In an investigative study involving saliva samples collected from glioma patients, the results showed substantial alterations in the oral microbiome profile between high-grade glioma cases and healthy controls (10). The oral Superphylum patescibacteria was decreasing with the advancement of glioma malignancy, pointing towards its potential utility as a diagnostic and prognostic factor for glioma malignancy (10). Likewise, the presence of Leptotrichia revealed a reverse association with glioma aggressiveness (10). These findings align with previous research, which has identified an association between higher incidence of Leptotrichia and lower risk of pancreatic cancer (11).

Based on extracellular vesicles (EVs) produced by bacteria, Jeong et al. (12) reported the complex dynamics of bacterial communities in pancreatic cancer tissue, depending on tumor stages. In comparison to normal pancreatic tissue, an elevated prevalence of Tepidimonas bacteria was detected. Leuconostoc and Sutterella, on the other hand, presented reduced levels in tumor pancreatic tissues. Yet, patients with additional lymph node metastases showed much higher levels of Comamonas and Turicibacter. Interestingly, with regard to tumor recurrence, *Streptococcus* and Akkermansia, both renowned for their anti-tumor properties, were found to be reduced in tumor tissues (12). These data emphasize the complex microbial role associated with the dynamics of tumor development and progression.

Further investigations into the complex interaction between gut microbiome and murine glioma models confirmed convincing associations. Analyses found an increase of Firmicutes, including Clostridia *Clostridiales*, *Clostridiales* Lachnospiraceae, and Oscillospira, in the gut microbiome of mice with gliomas, along with a concurrent decrease in Bacteroidia. This gastrointestinal dysbiosis inhibits Foxp3 expression within the brains of these mice, promoting glioma growth and malignancy (13).

The diagnostic utility of the cancer microbiome has been demonstrated through the analysis of a diverse array of materials, including live bacteria in tissues or their fragments encapsulated in EVs in blood and biopsies. Emerging evidence underlines the promise of microbiome analysis as a beneficial complement to conventional diagnostic modalities, enabling a more profound insight into cancer diagnosis and accurate monitoring of cancer progression (14). Numerous studies have shown that the intratumoral microbiome may have major effects on tumor development and treatment response, providing both positive and negative outcomes through a variety of complicated processes (15). More investigations in this emerging topic are needed to gain deeper knowledge of the microbiome's complicated function in carcinogenesis and tumor development (6).

In this context, we have compiled a number of studies, which illustrate the intratumoral microbiome effect on tumor initiation, development and progression, as well as on responses to chemotherapy, radiotherapy (RT) and immunotherapy. Furthermore, we aim to discuss the state of the art of the newly developed cancer therapies from genetically engineered bacteria.

INTRATUMORAL MICROBIOME IN TUMOR INITIATION AND PROGRESSION

Various factors have been systematically identified as primary causes of cancer, with current

research focusing on infectious agents (6). Among these factors, we will target the impact of the intratumoral microbiome on tumor development and clinical efficacy. Indeed, the link between tumor microbiome and cancer development has been relatively documented and 3 major processes have emerged as potential modes of action: 1) promoting carcinogenesis through DNA alteration, 2) regulating oncogenes and oncogenic pathways, and 3) modulating the host's immune response (16).

Intratumoral microbiome and DNA alterations

Bacteria have acquired the ability to synthesize substances that damage DNA, disturb the cell cycle, and induce genetic instability (**Fig. 2A**) (16). The release of these substances might

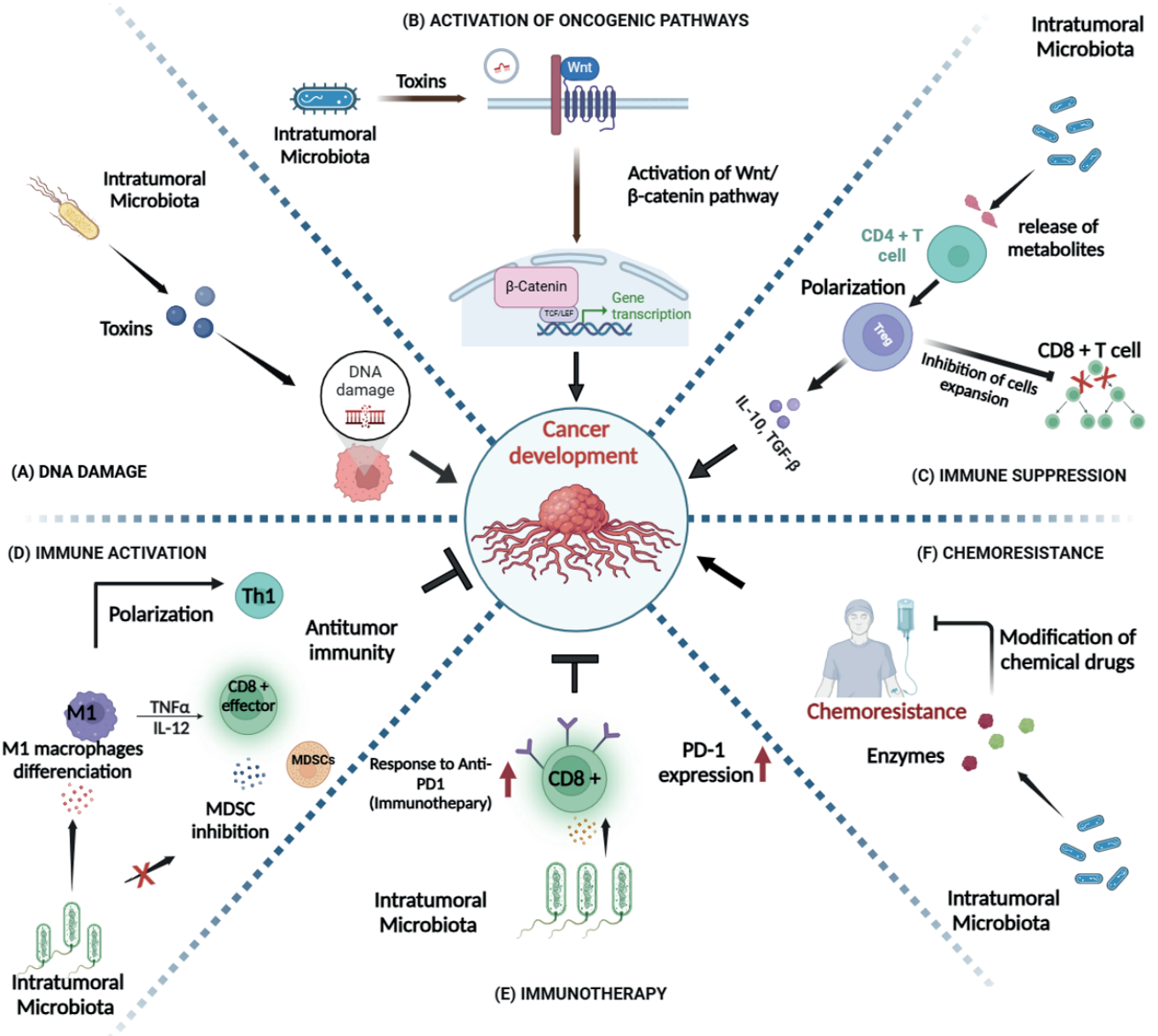

Figure 2. The role of the intratumoral microbiome in cancer. Several mechanisms have been suggested to elucidate the impact of intratumoral microbiome on cancer development: (A) Direct contribution to DNA damage and increased mutagenesis. (B) Activation of oncogenes and oncogenic signaling pathways. (C) Reducing antitumor immune responses and promoting cancer progression. (D, E) Enhancing antitumor immunity and immunotherapy efficacy by stimulating the recruitment of immune cells and modulating the expression of immune checkpoints. (F) Altering the bioactivity of chemotherapy drugs leading to chemoresistance.

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compromise host DNA integrity and raise the risk of oncogenic alterations in the colonized tissue by disrupting the physiological barriers that allow contact with epithelial and immune cells (17). Microbial toxins such as colibactin, cytolethal distending toxin (CDT), and Bacteroides fragilis toxin (Bft) all cause direct DNA damage, which triggers mutations (18). A significant proportion of *Escherichia coli* isolates from group B2 harbor genomic islands associated with the production of colibactin, a toxin that can induce double-strand DNA breaks, potentially contributing to genomic instability and promoting the development of colon cancer (19). CDT is produced by gram-negative bacteria belonging to both ε and γ classes of the Proteobacteria phylum (20). As a heterogeneous multimeric protein, CDT is composed of 3 subunits (CdtA, CdtB, and CdtC), with CdtB serving as the principal functional unit responsible for DNA damage (21). The activity of CdtB is dose-dependent and its effect gradually progresses from inducing single-strand DNA breaks to promoting double-strand DNA breaks (22). An aberrant DNA damage response may result in genomic instability and contribute to tumor initiation (23).

Additionally, *Streptococcus anginosus* and *Porphyromonas gingivalis* have been shown to transform ethanol into acetaldehyde, leading to the creation of DNA adducts or the inhibition of DNA repair enzymes, potentially causing DNA damage and oral carcinogenesis (24). Recent investigations revealed that bacteria such as Enteropathogenic *E. coli* and Enterohemorrhagic *E. coli*, may interact with intestinal epithelial cells via their type 3 secretion system and release genotoxin-UshA, which destructs the DNA of intestinal epithelial cells, leading to colon carcinogenesis (25). Microcystin toxin generated by *Cyanobacteria* has been detected in non-small cell lung cancer (NSCLC) patients (26). In an extensive in silico investigation, microcystin has been associated with reduced levels of CD36, a molecule related to advanced disease stages, poor prognostic outcomes, decreased survival rates, and elevated expression of PARP1, a crucial contributor to genome stability, which was confirmed in both cell lines and tumor tissue samples (26). These findings pinpoint that this toxin could have a substantial role in the inflammatory processes driving lung tumorigenesis.

Along with bacterial toxins that directly interact with host DNA, bacteria can produce ROS, which have been linked to oxidative DNA damage and potential carcinogenesis (27). The oral cavity is home to a wide range of species from the *Bifidobacterium*, *Lactobacillus* and *Streptococcus* genera. These species have the potential to produce hydrogen peroxide, which increases the possibility of DNA damage and promotes oral cancer (28). According to reports, the Bft raised ROS levels in intestinal epithelial cells, inducing oxidation and DNA damage, ultimately leading to malignant cell transformation (29). Furthermore, *Enterococcus faecalis* has been shown to produce extracellular superoxide. Accordingly, an increase in superoxide levels causes macrophages to produce 4-hydroxy-2-nonenal, a chemical molecule known for its capacity to promote genomic instability (30). Deoxycholic acid (DCA), a secondary bile acid derived from the gut microbiome, has been experimentally shown to cause DNA damage and accelerate carcinogenesis in mouse colorectal cancer (CRC) models. Also, this substance increases the amounts of ROS and causes mitochondrial oxidative stress (31). High ROS levels have the potential to overwhelm the host DNA repair system, leading to DNA damage and oncogenic mutations.

Intratumoral microbiome and its impact on oncogenic pathways

In addition to direct DNA damage, various microorganisms harbor proteins that activate host carcinogenesis-related pathways (**Fig. 2B**). Among these, the Wnt/β-catenin pathway is a crucial carcinogenic signaling cascade in cancer that regulates cell stemness, polarity,

and proliferation (32). *Helicobacter pylori* (Hp) secretes the cytotoxin-associated gene A (CagA) protein, which is naturally transported into the host cell cytoplasm, activating the β-catenin pathway and contributing to gastric cancer progression (33). CagA-mediated activation of β-catenin potentially generates a cascade effect characterized by the upregulation of genes essential for cell proliferation, survival, motility, angiogenesis, and other key carcinogenic processes (33). Furthermore, Fusobacterium adhesin A, a cell surface adhesion component generated by *Fusobacterium nucleatum* (Fn), interacts with the host's E-cadherin, and activates the β-catenin signaling. This activation can selectively alter the immunological, inflammatory, and cancer responses, thus contributing to CRC (34). Similarly, *Salmonella* generates AvrA, an enteric bacterial protein, which modifies host cell eukaryotic signaling pathways (35). It enhances β-catenin signaling by decreasing its ubiquitination, increasing phosphorylation, and raising its accumulation in the nucleus, thereby activating the expression of various transcription factors, such as TCF and NF-κB, and oncogenes, such as Myc and cyclin (35).

Furthermore, the Bft produced by Enterotoxigenic Bacteroides fragilis stimulates E-cadherin cleavage, which in turn enhances β-catenin activation. Bft promotes quick cleavage of E-cadherin in 2 steps. First of all, biologically active Bft induces detachment of the E-cadherin ectodomain and activates the host cell γ-secretase, which cleaves the intracellular fragment of E-cadherin (36). Following this, the proteolysis of E-cadherin enhances TCF-dependent β-catenin nuclear signaling, consequently enhancing the transcription and translation of the proto-oncogene c-Myc, promoting the expression of inflammatory genes, and eventually inducing the development of colon cancers (37).

Intratumoral microbiome and immune modulation

The impact of bacteria on the host immune system has been widely studied, dating back to the first characterization of intratumoral microbiome within the human body (19). Notably, the specific role of intratumor microorganisms in regulating the host immune system remains unclear. However, it is noteworthy that these prokaryotic organisms can either positively or negatively affect the host immunological responses (27). On the one hand, they can promote cancer development by creating an environment, which can lead to immune cell suppression. On the other hand, they can inhibit cancer cell proliferation and expansion by boosting the anti-tumor immunity (27).

The beneficial effect of the microbiome on antitumor immune responses is mainly due to innate immune mechanisms. It has been shown that intratumoral microbiome may evoke identification by immune cells through their pathogen-associated molecular patterns, triggering an anti-tumor immune response and activating immune cells such as macrophages and NK cells (38). Additionally, antigenic mimicry is a phenomenon based on similarities between specific antigens generated for instance by bacteria and a subset of tumor antigens, stimulating immune cells that identify the shared antigens. Accordingly, the immune response activated against microbial antigens can also target tumor cells that express similar antigens (39). Furthermore, intratumoral microbiome is crucial for the formation and maturation of tertiary lymphoid structures (TLS), which facilitate lymphocyte infiltration and activation in tumors, hence providing a permissive microenvironment for anti-tumor responses. Especially intratumoral Hp can stimulate T follicular helper cells and B lymphocytes in tumors, supporting TLS formation and suppressing colon cancer progression (40).

Also, a salt-rich diet may induce an enrichment of intratumoral *Bifidobacterium* in the melanoma microenvironment, enhancing NK cell activity and promoting melanoma

regression by the high production of hippurate, a metabolic by-product (41). Moreover, investigations indicate that the intratumoral microbiome may enhance the production of pro-inflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNF-α. This effect is particularly exemplified in primary human monocytes infected with Kaposi's sarcoma-associated herpesvirus (KSHV) (42).

Moreover, several studies have revealed the presence of a distinct microbiome in the liver, contradicting the traditional notion of the organ's sterility. Significant changes in hepatic immune cell populations and adaptive immunity were observed when targeting specific bacterial species, especially Bacteroidetes (43). Bacteroidetes-derived glycosphingolipids activate CCL5 signaling, resulting in hepatic leukocyte expansion and activation. These findings highlight the crucial role of the microbiome in regulating hepatic immunity and the presence of a novel microbial-glycosphingolipid-NKT-CCL5 axis inside the liver (43). Likewise, *Clostridiales*-produced trimethylamine N-oxide (TMAO) have been suggested to trigger the PERK-mediated endoplasmic reticulum stress response, resulting in tumor cell pyroptosis, boosted $CD8^+$ T-cell-mediated antitumor immunity, and improved immunotherapy effectiveness in triple-negative breast cancer (BC) (44).

Concurrently, the importance of outer membrane vesicles generated by bacteria such as *E. coli* should be mentioned, since they have been shown to improve IFN-γ responses and stop tumor development (45). This is mainly due to the suppression of angiogenesis within tumor tissue, induction of Tregs apoptosis, and activation of pro-inflammatory M1 macrophages, as perfectly demonstrated in a mouse colon adenocarcinoma model (45).

In addition to their immunostimulatory effect, specific microbiome species have a parallel and sometimes overlapping immunosuppressive effect in the TME at many levels. Microbiome-derived metabolites such as acetate and butyrate have been shown to activate Tregs and increase the levels of immunosuppressive cytokines such as IL-10 and aldehyde dehydrogenase 1, which promote tumor development (46). Additionally, the intratumoral microbiome frequently triggers tolerogenic programming through the engagement of pattern recognition receptors, resulting in reduced proportions of tumor-infiltrating lymphocytes, including $CD8^+$ T cells and elevated $CD4^+CD25^+$ $FOXP3^+$Tregs, as observed in pancreatic, lung, and CRCs (**Fig. 2C**) (47).

Using a 16S rRNA sequencing technique, distinct microbial profiles have been identified correlating with various stages of oral squamous cell carcinoma (OSCC). For example, *Capnocytophaga* and *Fusobacterium* were associated with the advanced and early stages of cancer, respectively. Although the most abundant bacteria in the TME show no association, or a negative correlation, with effector cells, this suggests that an immunosuppressive environment may be fostered to accelerate the development of cancer (48).

Studies have highlighted that the microbiome abundance within tumors may reshape the local anti-inflammatory TME, promoting tumor growth. It has been demonstrated that intratumoral microbiome stimulates the production of IL-17, which in turn promotes B cell infiltration into the intricate environment of tumor tissues (49). This finely orchestrated response appears as a significant contributor to the development of colon cancer. Moreover, Alam et al. (50) reported that the mycobiome in pancreatic ductal adenocarcinoma (PDAC) tissue, specifically *Malassezia* genus, increased tumor released IL-33 levels, which attracted Th2 cells and innate lymphoid cells (ILC)-2 to the TME, promoting tumor growth. The eradication

of the intratumoral mycobiome with antifungal therapy led to a lesser infiltration of type 2 immune cells, such as ILC2 and Th2, along with reduced tumor size and improved survival rates (50).

Previous research has shown that intratumoral *Staphylococcus aureus*, Nevskia ramose, HCV and HBV, promote immunosuppression via Tregs in the TME, resulting in prostate and liver cancer growth. Each of these microbiotas is linked to immune-related gene dysregulation, notably lysophosphatidylcholine acyltransferase 2, TLR3, and TGF-β2 (51). In mice treated with antibiotics, a decrease in bacterial levels was strongly associated with fewer Tregs and increased activation of T and NK cells, resulting in significant suppression of melanoma and lung metastases (52). Further research uncovered that *Aspergillus sydowii* in lung tumors causes macrophages to release IL-1β via the β-glucan/Dectin-1/CARD9 pathway, driving the proliferation and expansion of myeloid-derived suppressor cells (MDSCs), PD-1$^+$ T cell accumulation and suppression of cytotoxic T cell function (53).

In parallel, Fn fatty acid-binding protein 2 might directly impair anti-tumor immunity by binding to TIGIT, a receptor with immunoglobulin and ITIM domains expressed on T cells, and carcinoembryonic antigen cell adhesion molecule 1 receptors, expressed on human NK cells and other lymphocytes, thus inhibiting antitumor immune cell function in CRC (54,55). Conclusively, the intratumoral microbiome improves global tumor immunological tolerance by increasing the expression of M2 tumor-associated macrophages while decreasing the expression of the MHC class 1 protein. This allows tumor cells to escape CTL activity (56).

Although growing evidence supports dynamic interactions between the intratumoral microbiome and immunological populations, there remains a gap in mechanistic studies explaining how these bacteria influence immune characteristics. More research is needed to determine the role of the microbiome in boosting or inhibiting immune responses in the TME.

Intratumoral microbiome in blood tumors

Besides the above mentioned main human solid tumors, intratumoral microbiome have also been identified in blood cancer. However, the specific mechanisms whereby these microorganisms impact these cancers remain insufficiently understood. To fully understand the TME in blood cancer, it is crucial to consider the microbiome of the bone marrow and lymph nodes. Yet, while the gut microbiome has been well studied, intratumoral microbiome of these specific niches remains largely unexplored.

Recent research has highlighted a significant association between imbalances in the gut microbiota “dysbiosis” and the emergence of leukemia, in particular acute myeloid leukemia (AML) (57). The development of leukemia has been shown to be associated with dysbiosis, which can compromise the immune response and reduce the body’s ability to fight malignant cells (58). The reduction in microbial diversity and subsequent damage to the intestinal barrier in AML promote the development of the disease while causing increased leakage of LPS into the bloodstream and considerably reducing beneficial metabolites such as butyrate (57). Probiotics, butyrate supplementation, and fecal microbiota transplantation (FMT) could potentially restore the balance, thereby delaying the progression of leukemia and paving the way for new therapeutic options (58).

On the other hand, research has revealed associations between specific microbial species and the blood system’s leukemia cells. Epstein-Barr virus (EBV) has been identified in the blood

system's tumor cells, including Burkitt's lymphoma, a severe cancer of the immune B cells. Once invading these cells, EBV can enter a latent state that prevents detection by the immune system. Nevertheless, in some circumstances, particularly in immunosuppressed patients, the virus induces aberrant B cell proliferation. This proliferation is frequently associated with a chromosomal translocation within chromosomes 8 and 14, which triggers the MYC-C gene, a potent stimulator of cancer cell progression (59). Moreover, human T-lymphotropic virus type 1 (HTLV-1), the retrovirus that causes adult T cell leukemia, was found to suppress DNA repair pathways via the HTLV-1 Tax protein, resulting in genomic instability and the accumulation of carcinogenic mutations (60). Tax protein hijacks DNA repair signaling pathways, including RNF8 and UBC13, and activates the NF-κB along with other signaling pathways, consequently facilitating genomic instability essential for tumor cell proliferation (60). A further investigation explored the impact of human endogenous retroviruses (HERVs) in chronic lymphocytic leukemia (CLL) patients, with a focus on the expression of specific viral genes, and discovered that, in comparison to healthy controls, CLL patients overexpress the HERV-K np9 gene (61). The increased transcriptional activity of the np9 gene revealed its possible implication in the carcinogenic process, potentially acting as an oncogene that supports the emergence and progression of CLL (61). Nevertheless, more research is needed to fully understand the relationship between intratumoral microbiome and these cancer types.

IMPACT OF MICROBIOME-DERIVED PRODUCTS ON TUMOR DEVELOPMENT

Metabolites generated from the microbiome play critical roles in host-microbe interactions, exerting substantial effects on host processes such as metabolic pathways and immune responses. Also, they have shown a significant impact on both tumor progression and treatment efficacy.

Short-chain fatty acids (SCFAs)

SCFAs are the products of anaerobic bacteria, primarily consisting of acetate, butyrate, and propionate, demonstrating tumor-suppressive characteristics in various cancer types and decreasing intestinal inflammation. In addition, clinical investigations demonstrate that high levels of SCFAs are associated with favorable and effective responses to immunotherapy (62). On the other hand, eradicating bacteria that produce butyrate could contribute to elevated systemic inflammation and the development of tumors (63). Butyrate binds to G protein-coupled receptors 109a on dendritic cells and macrophages to influence the proliferation of $CD4^+$ T cells, eventually leading to the downexpression of anti-apoptotic proteins, Bcl-2 and Bcl-xl, and overexpression of the death receptor pathway (46). Besides, free fatty acid receptor 2 (FFAR2) is another SCFA receptor, whose absence enhances $CD8^+$ T cell exhaustion and raises tumor bacterial burden, consequently impairing the anti-tumor immune response (64).

Epigenetic alteration is another anti-tumor mechanism of SCFAs within the TME. Propionate has been demonstrated to inhibit histone deacetylase activity, thereby reducing the production of the inflammatory cytokines IL-17 and IL-22 (65). Additionally, inhibition of histone deacetylase 8 by butyrate and propionate improves the host's antitumor response by boosting the gene expression of interferon and granzyme B (66). Despite their significant anticancer properties, SCFAs may also contribute to cancer development under particular circumstances. High SCFA levels can reduce IFN-γ production by $CD4^+$ and $CD8^+$ T cells, eventually leading to the exhaustion of effector T cells (67). Butyrate stimulates the secretion

of anti-inflammatory cytokines and activates caspase-3/7, inducing apoptosis in activated T cells (68). Likewise, in the presence of antigen-presenting cells, butyrate prevents the proliferation of lymphocytes and Th1 cells. SCFAs in PDAC function by binding to FFAR2/FFAR3, which are widely expressed on tumor cells. This interaction increases the expression of G-protein coupled receptors, which promote tumor growth and spread, by stimulating several oncogenic pathways, including AKT, ERK, mTOR, and STAT3 (69).

Bile acids

Commensal bacteria were found to convert the host-derived substances, such as bile acids, into physiologically active compounds (70). Specifically, lithocholic acid (LCA), is a secondary bile acid transformed by *Clostridioides difficile*, that plays an intricate and contradictory role in carcinogenesis (70). On the one hand, LCA was shown to improve anticancer immunity and reduce oxidative stress by suppressing the epithelial-mesenchymal transition (EMT) and VEGF, thereby reducing the metastatic and proliferative capacity of BC (71). On the other hand, LCA may act as an endogenous cancer stimulator, particularly in the gastrointestinal tract. It generates ROS, which destruct the epithelial layer, causing cell growth, oxidative DNA damage, and inflammatory responses (72). Furthermore, LCA promotes cancer stemness via modulating muscarinic 3 receptors and Wnt/β-catenin signaling pathways, stimulating CRC progression as a result (73). In addition, LCA treatment stimulates the expression of matrix metalloproteinase (MMP) genes such as MMP-1, MMP-2, and MMP-7, activates the urokinase plasminogen activator receptor, and enhances tumor invasion and metastasis, thus supporting a carcinogenic epithelial phenotype (74). It also increases IL-8 production, which activates the ERK1/2/MAPK signaling pathway driving CRC angiogenesis and growth (75).

Additionally, *C. difficile* produces a 7-dehydroxylase enzyme that converts bile acids to DCA, which is involved in several types of cancer. In gallbladder cancer, DCA has been shown to decrease tumor development by reducing cell proliferation and to be associated with worse survival outcomes (76). Otherwise, it has been revealed that DCA may induce cyclooxygenase-2 activation and prostaglandin production, which leads to inflammation, DNA damage and fibrosis, promoting the growth of aggressive cells in colorectal, ovarian and pancreatic cancers (74).

LPS

LPS are crucial elements of gram-negative bacteria's outer membrane. LPS may bind to TLR4 and TLR2 on immune cells, which then recruit MyD88 or TRIF adaptor molecules. The latter may stimulate inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) through the MAPK and NF-κB pathways, promoting cancer cell progression (77). LPS has emerged as a significant contributor to tumor development and metastasis through different mechanisms. In PDAC-and BC, LPS increased cell invasiveness and aggressiveness by triggering the TLR/MyD88/NF-κB signaling pathway (69,78). In addition, LPS induces EMT in intrahepatic biliary epithelial cells, resulting in increased production of TGF-β1 and Smad2/3, supporting its function in chronic inflammation and fibrosis (79). LPS significantly contribute to the progression of OSCC by promoting inflammatory factors like IL-6 and VEGF leading to rapid STAT3 activation, potentially contributing to cancer progression (80). While in CRC, LPS have been demonstrated to significantly influence cell metastasis by enhancing glycolysis through the NF-κB/Snail/HK3 signaling axis or by activating SDF-1α/CXCR4 signaling (81). Finally, LPS have been shown to significantly decrease the epithelial marker E-cadherin and increase the interstitial markers N-cadherin and vimentin, supporting EMT (82).

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Other metabolites

Inosine, specially metabolized by Bifidobacteria, is another important microbial metabolite that boosts the effectiveness of immunotherapy. Actually, in a mouse model of melanoma, a combination of inosine supplementation with anti-PD1 antibody treatment led to impeded tumor progression and improved the patient's survival (83). Also, inosine serves as an alternate carbon source for $CD8^+$ T cells during glucose restriction and increases tumor immunogenicity by blocking the ubiquitin-activating enzyme UBA6 in tumor cells, an enzyme involved in cancer progression and metastasis (84). Together with dendritic cells, inosine concurrently increases T cell differentiation into Th1 cells, consequently enhancing the immune system's ability to initiate a strong and efficient anti-tumor response (83).

Moreover, bacteria can produce indoles through amino acid metabolic pathways that interact with aryl hydrocarbon receptors in macrophages, raising the expression of MHC II, CD40, PD-L1, and the amounts of $CD8^+$ T cells, thus leading to an overall reduction of PDAC invasion (85). Furthermore, it has been shown that TMAO, a bacterial compound formed from dietary L-carnitine and phosphatidylcholine, upregulated inflammatory factors, prevented the expression of immunosuppressive molecules, and supported the stimulation of tumors-infiltrating immune cells, thereby facilitating the creation of a strong anti-tumor immune response within the TME (86).

The influence of metabolites on tumor development is complicated and depends on numerous parameters, including cancer type, disease stage, and metabolite concentration. Further investigation is required to better elucidate the underlying mechanisms.

INTRATUMORAL MICROBIOME AND ITS INFLUENCE ON RESPONSE TO CHEMOTHERAPY AND IMMUNOTHERAPY

Tumor chemoresistance has been associated with intratumoral microbiome, which enzymatically produce substances such as cytidine deaminase (CDD) and purine nucleoside phosphorylase, rendering chemotherapeutic treatments ineffective (87). It has long been recognized that bacteria play the function of biotransformers, modifying organic chemical substrates with the help of their endogenous enzymes, supporting their impact on the therapeutic response to chemotherapeutic drugs (**Fig. 2F**) (6). Many studies have confirmed the negative impact of *E. coli* on gemcitabine, a cytotoxic antimetabolite often used in chemotherapy (88). In line with these findings, Geller et al. (89) showed that the bacterial CDD, produced by gammaproteobacterial strains frequently found in PDAC, metabolizes gemcitabine (2′,2′-difluorodeoxycytidine) into its inactive form (2′,2′-difluorodeoxyuridine), thereby affecting its efficacy and facilitating the emergence of drug-resistant pancreatic cancer.

Lehouritis et al. (90) investigated the effect of the tumor microbiome on the chemosensitivity of cancer cells, using a variety of bacterial species, including *E. coli* and *Listeria welshimeri*, as well as various cancer cell lines such as Lewis lung, mammary, and colorectal carcinoma. Their research showed that 10 of the 30 drugs evaluated *in vitro* had their efficacy reduced by particular bacterial strains. Significantly, these same microorganisms have improved the efficiency of 6 other drugs, offering a favorable therapeutic outcome. From this perspective, the identification of each tumor's microbiome composition before initiating therapies represents a potential step toward the development of a more personalized chemotherapeutic strategy (6). For example, manipulating the tumor microbiome holds promise in selectively

targeting microorganisms, like *Gammaproteobacteria*, that hinder chemotherapy effectiveness through targeted antibiotic treatments. Conversely, therapeutic results might be considerably improved by introducing, prior to treatment, bacteria that improve chemotherapy effectiveness in the TME (6).

Significant strides have been made in cancer treatment through recent studies that integrate chemotherapeutic drugs with the microbiome (91). For instance, cultivating the microbiome *in vitro* alongside specific chemotherapy agents like doxorubicin, gemcitabine, and vidarabine has resulted in significantly reduced levels of toxicity. Consequently, co-cultivating these drugs with the intratumoral microbiome *in vitro* holds promise for attenuating chemotherapy-induced toxicity and averting cancer resistance (91). However, additional research is essential to assess the efficacy of this approach in human cancer treatments.

Immunotherapy has advanced into a clinically validated therapeutic strategy for a range of cancers, following years of irrelevant results. One prominent avenue of research within the field of immunotherapy focuses on immune checkpoints consisting of a complex network of molecules involved in co-inhibition and co-stimulation of the global immune response, thus providing both effective and protective immunity (92,93). The clinical use of the immune checkpoint inhibitors has profoundly changed the landscape of cancer immunotherapy (92). Recent advances in various immunotherapeutic treatments, particularly focusing on monoclonal antibody inhibition of CTLA-4 and PD-1, have accelerated the emergence of this treatment approach (94). However, the effectiveness of immune checkpoint inhibitors varies substantially among patients. As a result, rapid exploration and testing of novel immunotherapeutic targets and agents currently aim to enhance the efficacy of immunotherapy across diverse types of cancers (92, 95- 99).

Recent investigations have demonstrated the undeniable significance of the intratumoral and intestinal microbiome in immunotherapy efficacy. The precise composition of these microbiomes has, however, emerged as a crucial variable that might either enhance or impair the immune system's potential against tumors (15). In pancreatic cancer, intratumoral bacteria have been reported to increase tumor immunogenicity. Several reports suggest that this microbiome has an impact on the TME, essentially by enhancing M1 macrophage differentiation and reducing MDSC. This dynamic process results in the augmentation of $CD8^+$ T cell activation, Th1 differentiation, PD-1 up-regulation and ultimately, an improved response to immunotherapy (91) (**Fig. 2D and E**).

Moreover, RNA-sequencing data from clinical trials involving Avelumab, an anti-PD1 monoclonal antibody used in treating EBV^+ gastric cancer, along with in vitro findings from primary human monocytes infected with KSHV, substantiate the assertion that intratumoral microbiomes contribute to enhanced expression of critical immunological checkpoints, notably PD-L1, which increase T cell sensitivity to immune checkpoint inhibitors and therefore improving immunotherapy efficacy (42,100). Furthermore, *Bifidobacterium* enhances the anti-CD47 immunotherapy by accumulating within tumors. Mechanistically, Bifidobacteria mainly improve dendritic cell crosstalk by stimulating interferon genes and in an interferon-dependent manner, thus promoting CD47-based immunotherapy (101). According to Anker et al. (102), CP1, a prostate-specific microbe, exhibits features of local immunostimulatory therapy, improved survival and decreased tumor burden in models of MYC- and PTEN-mutant prostate cancer and can be used to reprogram the "cold" tumor immune microenvironment to become more responsive to anti-PD-1 immunotherapy, thus

improving therapeutic outcomes. Moreover, Bacteroides fragilis have been shown to increase the effects of ipilimumab, a monoclonal antibody that targets CTLA-4. by increasing Th1 immune responses, thereby improving the efficiency of CTLA-4 blockade (103).

On the other side, the combination of microbiome with immune checkpoint inhibitors can improve the efficiency of cancer immunotherapy. *Lactobacillus*, a healthy intestinal bacterium, interacts synergistically with anti-PD-L1 antibodies forming a powerful combination that significantly suppresses tumors (104). In metastatic melanoma patients, a combined therapy of Talimogene Laherparepvec, an oncolytic herpes virus, along with Pembrolizumab, a PD-1 checkpoint inhibitor, demonstrated a significant objective response rate of 62% (105).

Another exploration about optimal indicators of cancer immunotherapy response has been conducted on the intratumoral microbiome. Specifically, the study examined how the intratumoral microbiome influences the prediction of response to neoadjuvant chemoimmunotherapy in patients diagnosed with resectable esophageal squamous cell carcinoma (106). The study revealed that longer disease-free survival and treatment efficiency were correlated with specific microbiome signatures, most notably the presence of *Streptococcus*. Interestingly, mouse model experiments indicated that transplanting faecal bacteria or colonization with *Streptococcus* from responders increased tumor-infiltrating $CD8^+$ T cells and improved anti-PD-1 response (106). These findings highlight the intratumoral microbiome's potential as a predictor of cancer immunotherapy efficacy.

Taking together, the positive impact that intratumoral microbiome could have in improving immunotherapy provides a potential solution to the ongoing challenge encountered in this field. Nonetheless, further research is needed to explore the full spectrum of functions that the intratumoral microbiome might have in the context of immunotherapy.

On the other hand, substantial data indicates that gut microbiome can also significantly affect immunotherapy outcomes, either by enhancing or suppressing local and systemic anti-tumor immune systems (3). Routy et al. (107) revealed a significant correlation between gut microbiome and anti-PD-1/PD-L1 immunotherapy effectiveness in patients with NSCLC, renal cell carcinoma, or urothelial carcinoma. The direct full metagenomic shotgun sequencing indicated a greater abundance of *Akkermansia muciniphila* in the fecal samples of the responder group compared to the non-responder. Furthermore, oral treatment of *A. muciniphila* and responders' FMT (responders-FMT) improved the efficacy of anti-PD-1 therapy in a mouse model (107). It is noteworthy that tumors from responders-FMT mice exhibited higher amounts of $CCR9^+CXCR3^+CD4^+$ T cells in the TME, leading to the improved efficacy of anti-PD-1 treatment in an IL-12-dependent manner (107), and highlighting the potential to predict and modulate anti-PD-1 immunotherapy responses through the gut microbiome (107). Concurrently, combining 16S rRNA sequences and metagenomic shotgun sequencing identified abundant 8 microbial species (e.g., *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*) in fecal samples, linked to improved anti-PD-1 efficacity in patients with metastatic melanoma (108). In contrast, *Ruminococcus obeum* and *Roseburia intestinalis* were found to be more prevalent in non-responder samples. These 8 beneficial microbiome species improve the efficacy of anti-PD-1 treatment by increasing $CD8^+$ T cells while decreasing the amount of $FOXP3^+$ cells in the TME (108).

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EXPLORING THE ROLE OF THE INTRATUMORAL MICROBIOME IN RT

RT uses radiation to target and destroy the DNA structure of malignant cells, slowing their development and promoting remission. Similar to chemotherapy, RT's effectiveness is partially based on its capacity to induce immunogenic cell death (109). Despite extensive research on the relationship between chemotherapy resistance and tumor microbiome, investigations into the effects of radiation remain mostly unexplored. A recent study revealed that *E. coli* K-12 colonizes necrotic areas of tumors in mice after being injected (110). This latter offers exciting possibilities for cancer treatment, combining engineered bacteria with radiation therapy for improved outcomes. When *E. coli* strain K-12 was engineered to produce cytolysin A (ClyA), a reduction in tumor growth was observed followed by a tendency for regrowth (110). However, in the mouse CT26 colon cancer model, tumor size dramatically reduced and tumors were completely eliminated when ClyA-producing *E. coli* was combined with radiation treatment. Additionally, this combined therapy suppressed metastatic tumor growth and prolonged survival in mice (110). Similarly, *Salmonella* has been employed as a carrier within melanoma cells for treatments including oral cytokine gene therapy. Yoon and colleagues' found (111) that attenuated *Salmonella typhimurium* (S. typhi) combined with gamma radiation raised ROS levels and activated phosphorylated H2A.X variant histone, which in turn activated caspase-3 and BCL2 in tumor cells promoting apoptosis.

In contrast, our current understanding of radiation therapy's impact on the human microbiome originates from gut-focused studies. Radiation therapy alters the microbiome's composition, crucial for regulating immune responses and potentially compromising the efficacy of RT (112). Radiation-induced changes in the microbiome arise from 2 primary inflammatory pathways (113). First, radiation induces tissue oxidation and inflammation, disrupting the local microenvironment and promoting dysbiosis, which disturbs immune function. Second, radiation affects epithelial cells, causing DNA and RNA damage, cell death, ulceration, bacterial translocation, and colonization, further exacerbating the inflammatory response (114). Several studies have reported alterations in microbiome composition following radiation exposure in various cancer types (115). El Alam et al. (116) detected significant changes in gut microbiome composition following pelvic chemoradiotherapy (CRT), characterized by an increase in Proteobacteria and a decrease in Clostridiale. Similarly, Oliva et al. (117) found that CRT reduced species richness and induced changes in gut-associated taxa in oropharyngeal samples from individuals with HPV^+ oropharyngeal squamous cell carcinoma. A randomized clinical trial by Jiang et al. (118) showed that probiotic administration during radiation treatment for patients with nasopharyngeal carcinoma undergoing concurrent CRT improved host immunity and reduced CRT-induced oral mucositis by modulating the gut microbiome. Moreover, Dong et al. (119) reported that oral microbiome composition affects the efficacy of RT in CRC and liver metastases, with distinct oral bacterial profiles in mousses at different stages of CRC. Notably, oral Fn migration to tumor sites contributes to RT resistance, although this effect can be mitigated by antibiotics such as Metronidazole (119). Collectively, these findings shed light on the pathogenic impact of the oral microbiome on radiation efficacy and underscore the importance of the oral-gut microbiome axis (119).

ADVANCES IN ENGINEERED BACTERIA FOR ENHANCED CANCER THERAPY

In order to address the current challenges of conventional cancer therapies, several strains of bacteria have been genetically modified (e.g., to minimize their virulence) and reassigned as therapeutic agents or local biosynthetic systems (120). Bacterial-mediated cancer immunotherapies have emerged as new approaches to induce tumor regression by perturbing cell metabolism, promoting apoptosis, delivering therapeutic agents, and stimulating the anti-tumor immune response (120). Historically, *Salmonella* and *Clostridium* species have always been recognized as the most effective anticancer microorganisms. However, recent research into tumor-targeting methods has identified attenuated S. typhi as a highly significant candidate, distinguished by its adaptability and specificity for cancer applications (121). S. typhi is known to preferentially invade mitotic cells with greater mobility, flexibility to genetic modification, and capability to proliferate in both aerobic and anaerobic environments (122). In contrast to *Clostridium*, which is strictly anaerobic and can form spores, enabling survival but not growth in oxygen-deprived conditions (123). Notably, VNP20009, a genetically modified strain of S. typhi, has demonstrated persistent efficacy against a variety of experimental cancer models and has even shown promise in targeting metastatic lesions (123). Once reaching the tumor site, S. typhi contribute to tumor destruction through processes such us nutritional competition and intrinsic toxicity resulting from bacterial proliferation and toxin production (124).

Moreover, genetically engineered bacteria may boost the antitumor activity by stimulating the immune system responses. An engineered strain of *E. coli* Nissle 1917 was modified to target mousse tumors and convert the excess metabolic waste product, ammonia, into L-arginine in the TME, which improved T cell activation and enhanced the effectiveness of immunotherapy (125). Likewise, in a mouse model of BC, galactosylceramide-harboured-Listeria proved to promote the NKT cell activation and further decrease metastasis (126). Furthermore, researchers engineered an attenuated S. typhi strain that produces Vibrio trauma flagellum B in the mouse's tumor tissue, leading to an increase of M1-like macrophages and a decrease of M2-like macrophages via TLR4 signaling pathways (127).

Overall, bacteria emerge as potential candidates for tumor targeting due to their natural characteristics, including their affinity for colonizing hypoxic regions and their active migration deep within solid tumors. In the near future, modified bacteria are anticipated to revolutionize cancer therapy and reshape the landscape of cancer treatment (123).

ANTI-CANCER EFFECT OF GENETICALLY ENGINEERED BACTERIA: MECHANISMS AND APPLICATIONS

Apoptosis and autophagy

Autophagy and apoptosis constitute essential catabolic mechanisms for maintaining organismal balance (128). Autophagy generally helps cells to survive by degrading and recycling damaged molecules. Apoptosis is the major pathway of programmed cell death, and the 2 processes are linked by distinct molecular interactions that allow synchronized control (128). Autophagy and apoptosis serve as tumor suppression processes; autophagy destroys oncogenic chemicals, thus preventing cancer growth, while apoptosis destroys malignant cells.

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Over the past century, numerous bacterial species have confirmed their capability to selectively accumulate within tumor tissues and be employed as anti-cancer agents (129). Growing evidence has revealed that genetically engineered bacteria may attack tumor cells either directly or indirectly by engaging different mechanisms, including apoptosis and autophagy (**Fig. 3**) (129). Various *Salmonella* species, including VNP20009, YB1, and SL7207 have been demonstrated to promote cancer cell apoptosis, subsequently reducing tumor development (130). For example, engineered S. typhi, notably VNP20009, has been shown to boost caspase-3 activity and elevate BAX protein expression, resulting in the initiation of apoptosis within cancer cells both *in vivo* and *in vitro*, therefore, inhibiting the progression of PDAC (130). In murine breast carcinoma and CT-26 colon cancer cells, S. typhi was effectively modified to release murine FasL, a pro-apoptotic cytokine, and showed a decrease in tumor development (131). In another study, S. typhi was genetically modified to produce TNF-related apoptosis-inducing ligand, inhibiting tumor development in mice with melanoma (132).

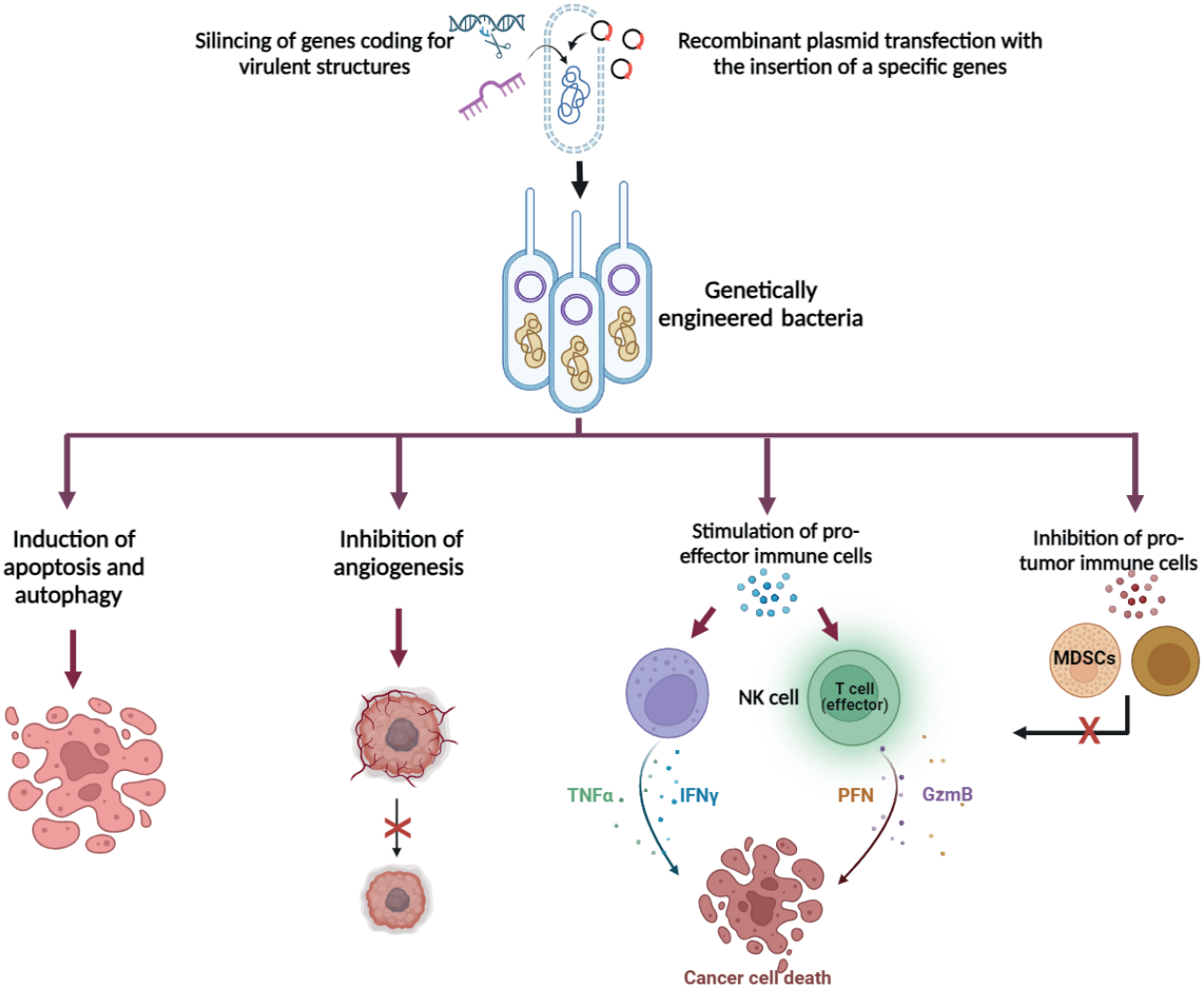

Figure 3. Genetically engineered bacteria can eliminate cancer cells either directly or indirectly through a variety of mechanisms. Within tumors, attenuated bacteria accumulate and have been identified to directly induce apoptosis and autophagy in tumor cells, as well as to inhibit tumor angiogenesis. Attenuated bacteria can also enhance the host immune response against tumors by stimulating and activating CTLs and NK cells, reducing the number of infiltrating Treg cells, and negating suppressive capabilities of MDSCs.

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Genetically modified S. typhi can also enhance autophagy, a conserved intracellular catabolic degradation process aimed at removing intracellular cargo (133). Lee et al. (134) found that modified S. typhi downregulated the AKT/mTOR pathway, resulting in autophagy and control of melanoma development in a mouse model in a dose-and time-dependent manner. Nevertheless, other studies have shown opposite results, claiming that genetically modified S. typhi-induced autophagy acts as a pro-tumoral mechanism, allowing tumor cells to survive and grow instead of contributing to their elimination (130).

Other bacterial genera have the capability to induce tumor cell death through various mechanisms (133). *E. coli* Nissle 1917 was *in vitro* found to induce apoptosis by increasing the expression of pro-apoptotic proteins, PTEN and BAX, while decreasing the expression of anti-apoptotic proteins, AKT1 and BCL-XL. This successfully inhibited the proliferation, invasion and growth of colon cancer (135). Listeria monocytogenes exhibit direct cytotoxicity toward tumor cells by inducing NADPH oxidase activity and increasing intracellular calcium levels; each of these contributes to ROS production promoting DNA damage and genetic instability. *C. novyi*-NT, an attenuated strain of *Clostridium novyi*, generates a variety of exotoxins, including phospholipases, haemolysins and lipases, which may disrupt cell membranes and immediately damage tumor cells (136). Alternatively, *P. gingivalis* infection has been demonstrated to increase autophagy, inhibit tumor cell development by triggering G1 cell cycle arrest, and promote apoptosis in host oral cancer cells (129). Vacuolar toxin A (VacA) has been identified as a significant pathogenic element in Hp and has been implicated in autophagy and gastric cancer. Besides, short-term exposure to VacA can enhance autophagy levels in gastric mucosal cells, thereby preventing tumor development (129). In mouse models, attenuated strains of *Salmonella choleraesuis* carry genes encoding anti-angiogenic factors such as endostatin and thrombospondin-1 and have shown anti-tumor activity in numerous mouse models by preventing the formation of new blood vessels (**Fig. 3**) (133).

Immunosuppressive shift

The immunosuppressive environment triggered by tumors, characterized by the infiltration of MDSCs and Treg cells, constitutes a significant obstacle to the effective development of anticancer treatment strategies (137). The selective use of genetically modified bacteria exerts a transformative effect on this immunosuppressive microenvironment, leading to immunogenic modifications in the host's innate and adaptive immune responses illustrated by inhibition of pro-tumoral immune cells and activation of anti-tumor effector immune cells as described in **Fig. 3**.

A pertinent example of this paradigm may be observed in the use of genetically modified *Salmonella YB1*. This strain has shown the ability to reduce metastatic spread across a wide range of cancer types. In this context, early *Salmonella* infection might result in the release of IFN-γ by NK cells, which would then enhance the accumulation, activation, and cytotoxicity of NK cells, leading to an anti-metastatic effect (138).

Recent research suggests that Listeria and *E. coli* may also prevent the progression of cancer by controlling the host's anticancer defense (133). Chandra et al. (139) reported the interesting capability of a highly attenuated strain of Listeria Monocytogenes to infiltrate MDSCs and then reprogram their immunosuppressive phenotype into an immune-stimulating one. This transformation involves the production of IL-12, followed by a significant increase in the $CD8^+$ T cells and NK cells-mediated antitumor response. In a parallel context, findings from a BC mouse model highlight the significant influence of *E. coli* colonization of the TME.

This colonization induces an exceptional redistribution of tumor-associated macrophages, which promotes a significant increase in TNF-α levels and enhanced production of MMP-9 (140).

ROLE OF CANCER CELL SECRETED EXOSOMES

Another possible way by which intratumoral microbiome influences tumor development and metastasis is the production of exosomes by infected cancer cells (133). These exosomes, which range from 50 to 100 nanometers, constitute external membranous vesicles enriched with distinct nucleic acids and protein cargos (141). They have been widely noticed as important agents within the advanced communication network that enhances tumor progression. The principal roles of exosomes within the TME range from promoting primary cancer development and inducing angiogenesis to activating stromal fibroblasts, shaping the cancer extracellular matrix, creating a premetastatic niche, and suppressing the host's immune response (142). Numerous investigations have suggested that the intratumoral microbiome may enhance the production of exosomes, thereby promoting tumor progression and spread. An illustrative instance of this phenomenon is currently provided by the facultative intracellular CRC-associated Fn bacteria (143). Notably, it has been reported that CRC cells infected with Fn release exosomes that are enriched with miR-1246, miR-92b-3p, miR-27a-3p, as well as CXCL16, Ras homolog family member A, and IL-8. These molecules actively contribute to tumor cell migration through interaction with glycogen synthase kinase-3 (a protein involved in cancer cell proliferation) triggering the Wnt/β-catenin pathway (143). Consistently, recent data suggest that metastasis may be initiated through paracrine exosome signaling rather than the direct influence of bacteria present within the TME (144). This has been observed in the context of lung cancer, where miRNAs and proteins are delivered to non-malignant cells via exosomes originating from infected tumor cells, thereby assuming a crucial role in driving lung cancer metastasis (142). Generally, exosomes generated by infected tumor cells have strong immunosuppressive characteristics, affecting the production of inflammatory proteins and regulating the proliferation of Treg cells, facilitating tumor development (144).

CONCLUSION AND PERSPECTIVES

The investigation of intratumoral microbiome has greatly improved our understanding of the cancer cell dynamic and its intricate interaction with the immune system. Recently, research has revealed that the intratumoral microbiome strongly impacts the TME, with both pro-carcinogenic and potentially therapeutic effects. This dual role highlights the potential of microbiome as biomarkers for cancer diagnosis and prognosis, as well as potential therapeutic targets. Current research faces challenges due to the dual impact of microbiome-driven molecules and pathways. Future studies should focus on identifying specific molecules/pathways, which could enhance anti-cancer responses while minimizing side effects.

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