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REVIEW

Interactions between microbiota and innate immunity in tumor microenvironment: Novel insights into cancer progression and immunotherapy

Zhaokai Zhou[#], Yingying Lv[#], Anning Zuo[#], Xudong Zhu, Yudi Xu, Lulu Zuo, Hui Xu, Shutong Liu, Yuyuan Zhang, Siyuan Weng, Yuhao Ba, Peng Luo, Quan Cheng, Jiaxin Xu, Xing Zhou, Shuang Chen, Boxuan Li, Chuhan Zhang, Yukang Chen, Jinhai Deng, Xiulin Jiang, Zaoqu Liu, Ran Xu[*], Xinwei Han[*]

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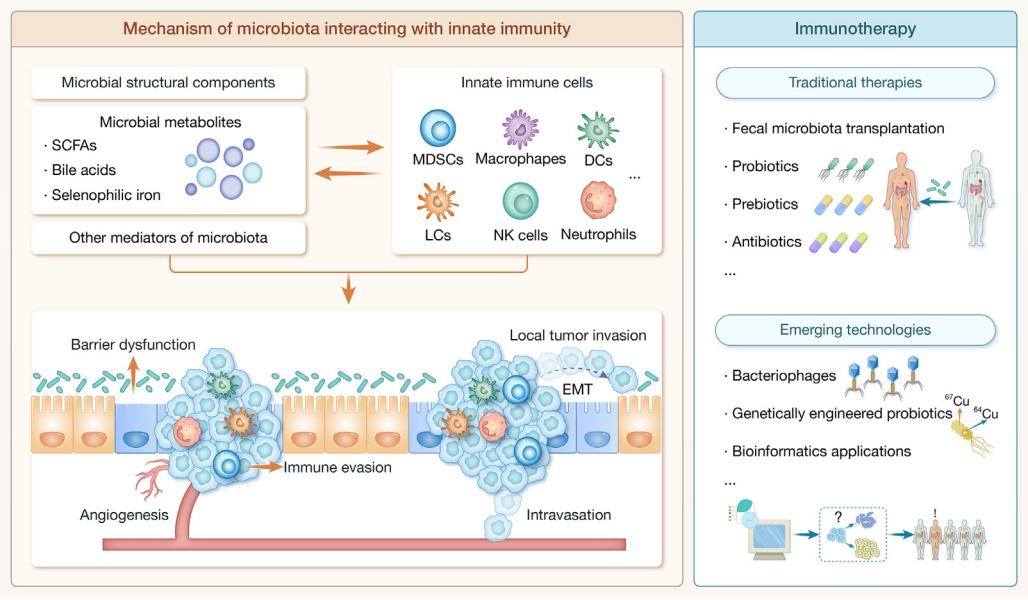
GRAPHICAL ABSTRACT



HIGHLIGHTS

- **Microbiota shapes immune system development.**
- **Microbial components bidirectionally regulate the tumor microenvironment.**
- **Innate immunity–microbiota crosstalk impacts cancer initiation and progression.**
- **Bioinformatics opens novel avenues for precision cancer microbiota-immunotherapy.**

REVIEW

Interactions between microbiota and innate immunity in tumor microenvironment: Novel insights into cancer progression and immunotherapy

Zhaokai Zhou[1,2,#], Yingying Lv[1,3,4,#], Anning Zuo[1,#], Xudong Zhu[5], Yudi Xu[6], Lulu Zuo[7], Hui Xu[1], Shutong Liu[1], Yuyuan Zhang[1], Siyuan Weng[1], Yuhao Ba[1], Peng Luo[8], Quan Cheng[9], Jiaxin Xu[1,10], Xing Zhou[11], Shuang Chen[7], Boxuan Li[1], Chuhan Zhang[12], Yukang Chen[1], Jinhai Deng[13], Xiulin Jiang[14], Zaoqu Liu[1,15,16,17], Ran Xu[2,*], Xinwei Han[1,15,16,*]

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ABSTRACT

The human microbiota constitutes a complex and dynamic community that interacts with host innate immunity at various anatomical sites, influencing both physiological and pathological states. In individuals with a genetic predisposition, disruptions to the "innate immunity–microbiota" axis appear to rewire immune responses within the tumor microenvironment (TME), thereby driving cancer pathogenesis. Emerging evidence highlights the critical role of these reciprocal interactions in maintaining immune homeostasis, promoting immune tolerance, and modulating immune recognition and polarization within the TME. However, a comprehensive understanding of these mechanisms remains elusive. Here, we summarize the intricate crosstalk between the microbiota and innate immunity in both healthy and cancerous states, focusing on the modulation of immune recognition and polarization during tumor progression. We also highlight recent advances and challenges in leveraging the microbiota for cancer immunotherapy, covering innovations in bacteriophages, genetically engineered probiotics, and bioinformatics applications. Moreover, we propose potential approaches to enhance therapeutic efficacy by targeting innate immunity–microbiota interactions. Further mechanistic insights into these interactions may pave the way for innovative microbiota-based cancer immunotherapy.

KEYWORDS innate immunity; microbiota; polarization; tumor microenvironment; immunotherapy; bioinformatics

INTRODUCTION

The human microbiota forms a complex symbiotic community residing on mucosal and skin surfaces. It is estimated that the human body harbors approximately 3 trillion bacteria [1], exhibiting remarkable structural diversity and spatial distribution across barrier sites, including the skin, intestines, oral cavity, respiratory tract, and vagina [2–6]. In healthy individuals, these dynamic yet substantially stable commensal microbial communities act as a microbial barrier, interacting with the innate immune system to facilitate its maturation and function. However, when the microbial microenvironmental homeostasis is disrupted, the resulting dysbiosis could contribute to cancer progression.

The history between microbiota and cancer is inextricably intertwined (Figure 1) and dates back to Imhotep's early use of infections to treat tumors [7]. As crude immunotherapy gained traction, researchers began to explore potential connections between microbiota and cancer [8,9]. The discovery of intratumoral microbial communities represented a significant breakthrough, while the identification of *Helicobacter pylori* and its link to stomach cancer turned the spotlight on the role of microbiota in carcinogenesis [10]. More recently, the publication of 11 oncogenic microorganisms and the elucidation of pro-carcinogenic molecular mechanisms have heightened awareness of the crucial interplay between innate immunity and microbiota in tumor recognition and immune polarization [11,12].

Advances in high-throughput sequencing technologies have significantly expanded our understanding of the molecular mechanisms underlying microbiota–innate immunity interactions and their implications for cancer diagnosis and therapy. Developments in metagenomic sequencing have enabled the development of microbial species-level co-abundance networks, while the establishment of quantitative polymerase chain reaction (qPCR) scores using a panel of 21 bacterial probes offers a dynamic diagnosis tool to guide microbiota-driven cancer immunotherapies [13]. Preclinical studies have demonstrated the efficacy of microbiota-targeted approaches in enhancing tumor immunotherapy. In pancreatic cancer mouse models, the microbial metabolite trimethylamine N-oxide (TMAO) synergizes with immune checkpoint blockade by reprogramming tumor-associated macrophages (TAMs) and dendritic cells (DCs) toward immunostimulatory phenotypes, thereby boosting anti-tumor immunity [14]. Microbiota transplantation from wild-type (WT) mice or *Lactobacillus reuteri* enhances acetate production and reduces interleukin-17A (IL-17A)-producing group 3 innate lymphoid cell (ILC3) infiltration in a mouse mode. This acetate-driven immunomodulation, when combined with immune checkpoint inhibitors, synergistically enhances anti-tumor immunity, presenting promising avenues for the clinical management of hepatocellular carcinoma (HCC) [15]. In rectal cancer, combining the microbial metabolite methylglyoxal with radiotherapy and immunotherapy (iRT) enhances anti-tumor immunity *via* immunogenic cell death, which induces intratumoral infiltration of natural killer (NK) cells and $CD8^+CD69^+$ T lymphocytes compared to iRT alone [16]. A recent study has identified a dual immunomodulatory role of the intratumoral microbiota in gastric cancer. *Fusobacterium nucleatum* facilitates tumor-associated neutrophil (TAN) recruitment and their polarization toward the regenerative

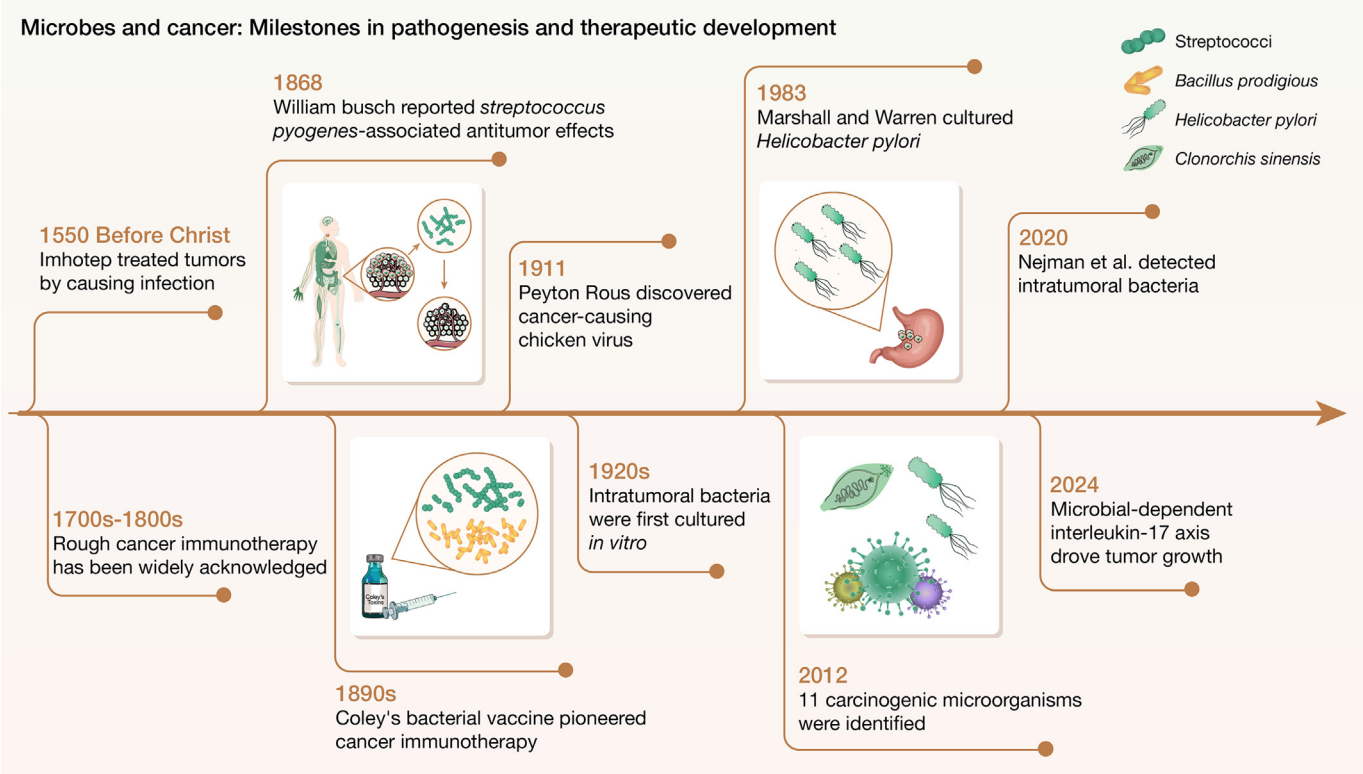

Figure 1. Microbes and cancer: Milestones in pathogenesis and therapeutic development
A historical chronology of the association between human microbiota and cancer is retrospectively summarised along with ten key research milestones.

phenotype. This microbiota–neutrophil axis induces programmed cell death-ligand 1 (PD-L1) overexpression on TANs, thus suppressing $CD8^+$ T cell cytotoxicity to promote immune evasion and improve anti-PD-L1 antibody therapeutic responsiveness [17]. In a melanoma clinical trial, vancomycin pretreatment induced dysbiosis and immune hyperactivation, resulting in incomplete recovery from immune checkpoint blockade therapy compared to the placebo group. However, patients pretreated with vancomycin followed by SER-401, an orally delivered Firmicutes-enriched spore formulation, and anti-programmed cell death 1 (PD-1) therapy, exhibited fewer adverse events (AEs) than the placebo group. A further retrospective evaluation of 114 cases demonstrated that elevated Ruminococcaceae levels correlated with improved therapeutic response and survival [18].

Despite significant advances, comprehensive and systematic discussions of the reciprocal interplay between innate immunity and microbiota in regulating immune recognition and polarization within TME and its latest applications in cancer immunotherapy are still lacking. Herein, we highlight how innate immunity and microbiota jointly modulate immune homeostasis in healthy individuals and immune recognition and polarization in cancer patients. Furthermore, we summarize the latest advancements and dilemmas in this domain and propose potential solutions to provide novel perspectives for establishing rational microbiota-based cancer immunotherapy strategies.

INTERACTIONS BETWEEN MICROBIOTA AND INNATE IMMUNITY IN HEALTHY HUMANS

The mechanisms of interaction between innate immunity and microbiota are highly complex. The host's normal flora functions as a microbial barrier, contributing to the composition of innate immunity and modulating the development of innate immune cells. Conversely, the innate immune system selects normal microbiota and establishes immune tolerance. Stable and balanced interactions between innate immunity and microbiota are fundamental to maintaining health.

Microbial Barrier in Innate Immunity

The microbial barrier consists of normal flora residing on the skin and mucosal surface. Specifically, most bacteria colonizing the skin belong to three phyla, Actinobacteria, Firmicutes, and Proteobacteria, whereas Firmicutes and Bacteroidetes predominate within the mucosal surface [19,20]. Most of these could mediate colonization resistance to pathogens by competing for nutrients and secreting bactericidal and bacteriostatic substances. Certain strains of skin commensal bacteria, such as *Staphylococcus hominis*, produce antimicrobial peptides as antibiotics against *Staphylococcus aureus*, the dominant skin pathogen, while synergizing with host keratinocyte-derived antimicrobial peptide LL-37 [21]. Similarly, *Staphylococcus epidermidis* could achieve this synergistic effect by phenol-soluble modulin gamma (PSMγ) and phenol-soluble modulin delta (PSMδ), which mediate colonization resistance against opportunistic skin pathogens, including methicillin-resistant *S. aureus* (MRSA) and *Streptococcus pyogenes* (Figure 2A) [22]. *Candida albicans* or *S. aureus* skin infection facilitated Langerhans cells to secrete large amounts of the T helper 17 (Th17) cells differentiating cytokines IL-6, IL-1β, and IL-23, thus regulating innate immunity [23]. Additionally, DC, an integral component of innate immunity, could modulate innate immunity and activate adaptive immune responses against pathogens by recognizing diverse microbial structural components and microbial metabolites (Figure 2A). Single-cell transcriptome analysis reveals that Batf3-dependent type I conventional dendritic cell (Batf3-cDC1) uniquely upregulates the expression of the *IL12b* and *Stat4* genes, which are essential for Th1 polarization during cryptococcal infection, whereas other DC subsets do not exhibit this transcriptional signature [24]. Type II conventional dendritic cell (cDC2) and double negative DC (DN DC) promote Th2 cell differentiation by responding to thymic stromal-derived lymphopoietin (TSLP), thereby regulating adaptive immune response [25,26]. In the intestine, DCs regulate innate immunity by stimulating NK cells to secrete transforming growth factor beta (TGF-β) and ILC3 cells to secrete IL-22. They also participate in adaptive immune response *via* secreting IL-12 in synergy with macrophages to regulate Th1 cells (Figure 2B) [27–29]. Moreover, several bacteria such as *Escherichia coli* and *Bifidobacterium*, which colonize the human gastrointestinal tract, convert L-tryptophan to indole [30,31]. Indole subsequently strengthens the barrier function of intestinal epithelial cells and enhances resistance to pathogen colonization by increasing tight junction expression and mucin production [32]. Johansson et al. demonstrated an intimate connection between gut microbiota and mucus layer formation in germ-free mice models. The findings revealed that comparisons between germ-free and conventionally raised rodents showed clear differences in mucus layer thicknesses, penetrability, and host response protein alterations during colonization [33].

Recent studies have demonstrated the potential of the innate immunity–microbiota dialogue to remodel barrier function and improve cancer therapy efficacy. CPX-351, a combination of cytarabine and doxorubicin, exhibits superior efficacy in treating acute myeloid leukemia. This efficacy is closely associated with promoting colonization resistance and remodeling barrier function *via* microbiota [34]. Similarly, ectonucleotide pyrophosphatase/ phosphodiesterase family member 2 (ENPP2) inhibitor suppresses colorectal cancer (CRC) proliferation by attenuating M2 tumor-associated macrophage polarization and enhancing intestinal barrier function [35]. Further understanding the microbial barrier mechanisms in innate immunity will provide novel perspectives on microbiota-based precision cancer therapy.

Innate Immunity and Microbiota Interactions in Human Health

Microbiota-driven modulation of immune system development

Microbiota is essential in immune cell development, including proliferation, activation, and cell differentiation (Figure 2C). Aryl hydrocarbon receptor (AHR) ligands, which are metabolites generated by maternal commensal microbiota, promote ILC3 proliferation [36–38], which induces neutrophil recruitment through producing IL-17 [39]. Furthermore, maternal retinoic acid facilitates lymphoid tissue inducer (LTi) cell maturation and controls ILC3s [40]. Recent evidence has confirmed that the elevated levels of bile acids and microbiota-derived

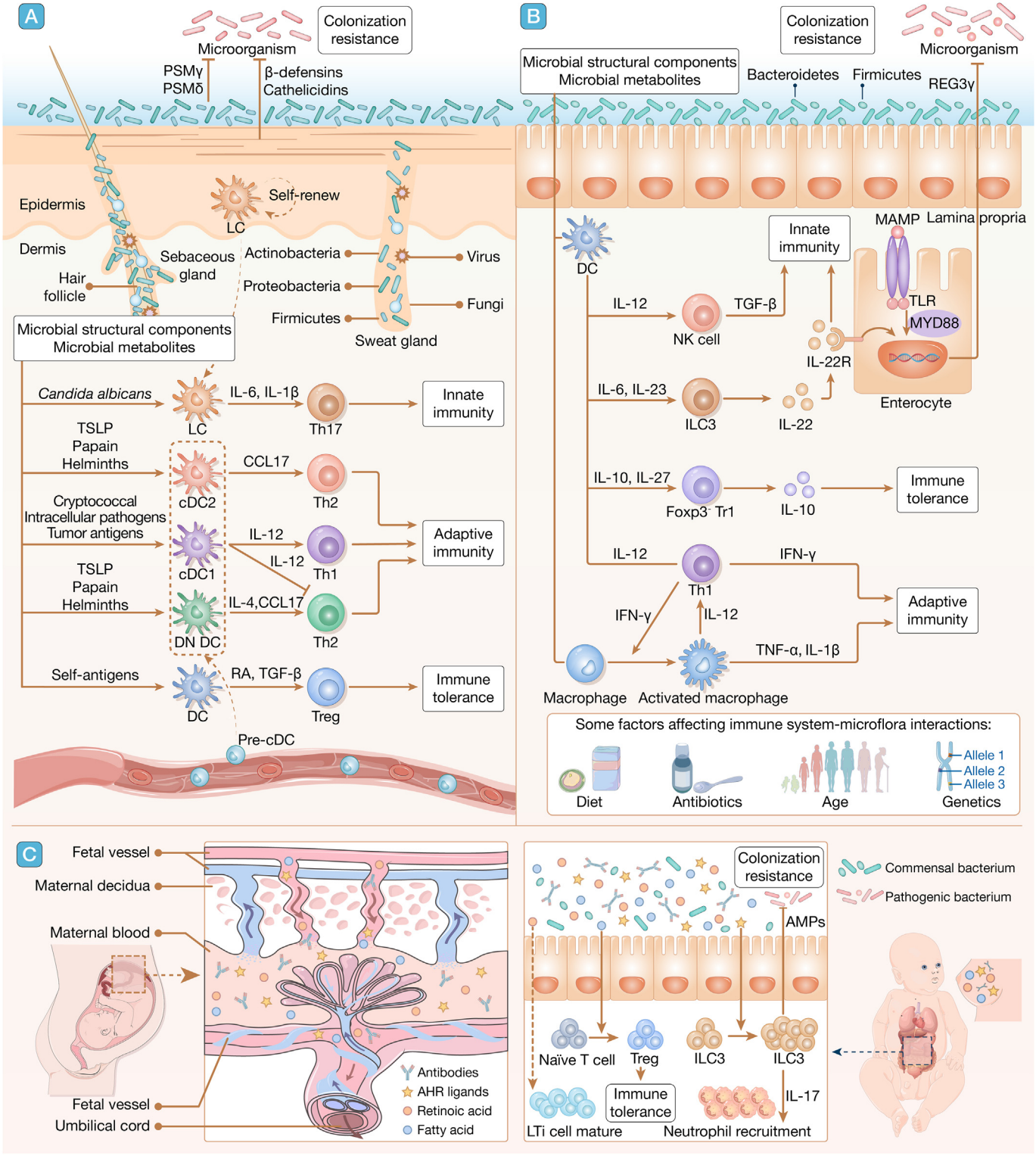

Figure 2. Microbiota and cross-talk with innate immunity in healthy humans

(A) Distinct classes of DCs modulate immune response by recognizing microbial structural components and microbial metabolites while establishing immune tolerance and colonization resistance in the skin. (B) Innate immune cells produce cytokines in response to microbiota, thus maintaining intestinal homeostasis. Some factors, such as diet, antibiotics, age, and genetics could affect immune system–microbiota interactions. (C) Microbiota influences immune system development through the placental barrier and breast milk. Abbreviations: PSMγ, phenol-soluble modulin gamma; PSMδ, phenol-soluble modulin delta; TSLP, thymic stromal-derived lymphopoietin; LC, Langerhans cell; DC, dendritic cell; cDC1, type I conventional dendritic cell; DN DC, double negative dendritic cell; IL-6, interleukin-6; CCL17, C-C motif chemokine ligand 17; RA, retinoic acid; TGF-β, transforming growth factor beta; Reg3γ, regenerating islet-derived 3 gamma; MAMP, microbe-associated molecular patterns; TLR, toll-like receptor; MYD88, myeloid differentiation factor 88; NK cell, natural killer cell; ILC3, group 3 innate lymphoid cell; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; AHR, aryl hydrocarbon receptor; LTi cell, lymphoid tissue inducer cell.

metabolites promote eosinophilia and activate ILC2s [41]. Additionally, antigen presentation by retinoic acid-related orphan receptor gamma T (RORγt) cells on or within the mesenteric lymph nodes (MLNs) proves that microbiota induces regulatory T cell (Treg) differentiation without affecting Th17 cell differentiation [42]. Moreover, studies in germ-free animals have shown that lacking gut microbiota can lead to severe immunodeficiency [43]. However, resident microbiota are not always beneficial. A recent study found that resident microbiota increased the levels of $CCR5^+CD4^+$ T cells, a type of human immunodeficiency virus (HIV) target cell, and promoted the development of acquired immunodeficiency syndrome (AIDS) [44]. HIV/AIDS-associated immunodeficiency predisposes individuals to opportunistic infections such as histoplasmosis, which exacerbate immune dysfunction and potentiate oncogenesis [45].

Immune system selection of normal flora and establishment of immune tolerance

The innate immune system can distinguish between pathogenic bacteria and normal flora, thereby mounting distinct responses. Neutrophils, the most abundant component of innate immune cells, play a significant role in maintaining microbial-innate immune homeostasis. On the one hand, they can defend against invading pathogenic bacteria through phagocytosis, reactive oxygen species (ROS) formation, and granule degranulation. On the other hand, excessive immune responses triggered by neutrophils can also damage healthy tissues, highlighting the importance of maintaining a balanced immune response [46]. In contrast, resident microbiota could promote their colonization by modulating the immune response. For instance, *Bifidobacterium breve* induces IL-10 secretion from $Foxp3^-$ type 1 regulatory T (Tr1) cells by stimulating DC production of IL-10 and IL-27 *via* the toll-like receptor 2 (TLR2)/myeloid differentiation factor 88 (MyD88) pathway, thereby establishing immune tolerance (Figure 2B) [47]. Additionally, immune cytokines modulate intestinal chemical barriers, including antimicrobial peptides (AMPs) and the regenerating islet-derived 3 (Reg3) protein family, which subtly isolate commensal microbiota from lamina propria, preventing unnecessary conflict, and thus maintaining the symbiotic relationship in the intestine [48]. Variations in nutrients, oxygen concentration, and pH across different intestinal regions create a heterogeneous microenvironment that selects for gut microbes suited for colonization [49]. Moreover, several other factors could affect gut microbes shaping, such as mode of delivery, diet, and antibiotics [50]. In skin microbial communities, the selective effects of sex hormones on microbiota are particularly noteworthy. During puberty, sexual maturation drives sebaceous glands to secrete lipid-rich sebum, selecting for microbiota species that metabolize these nutrients, such as lipophilic *Cutibacterium acnes* and *Malassezia* spp. on the skin surface [51]. Meanwhile, the hypoxic environment of pilosebaceous facilitates anaerobic bacteria colonization, such as the facultative anaerobe *C. acnes* [19]. Through normal flora selection and immune tolerance establishment, the host maintains dynamic stability of microbial communities and immune homeostasis, which are key protective factors in human health.

MECHANISMS OF MICROBIOTA INTERACTING WITH INNATE IMMUNITY IN TIME

Microbiota plays a complicated role in TME by interacting directly with cancer cells and by regulating the development and function of immune cells. In turn, innate immune cells recognize microbiota and regulate its composition and distribution, thus regulating tumor growth, proliferation, and metastasis (Figure 3). The complex synergistic and antagonistic interactions between different microbial communities and the immune system remain unclear, and additional studies are required.

Molecular Mechanisms Underlying Microbiota-mediated Regulation of Innate Immunity

Microbial structural components

Microbial structural components may modulate the development and function of the innate immune system through direct interactions with target innate immune cells, thereby affecting tumor progression. In response to lipopolysaccharide (LPS), microglia, the unique and essential resident macrophages in the retina, exhibit enhanced innate immune response and promote retinal tissue destruction in retinoblastoma. As proof, elevated expression of mitogen-activated protein kinase (MAPK) signaling pathway, as well as IL-6 and tumor necrosis factor-alpha (TNF-α) at both the messenger RNA (mRNA) and protein levels, were found in LPS-induced retinoblastoma 1-deficient microglia compared to WT microglia [52]. Further experiments are required to assess the potential for improving retinoblastoma outcomes by modulating the microbiota to inhibit microglia development. Besides, LPS enhanced IL-17 production *via* the TLR4/MyD88 pathway, thus enhancing the amount of granulocyte colony-stimulating factors (G-CSF). This process could drive granulocyte mobilization and tumor infiltration metastasis, especially in breast cancer models [39,53–56]. Conversely, microbiota could facilitate tumor cell killing by providing tumor cross-antigens to $CD8^+$ T cells. The antigenic epitope SVYRYYGL (SVY) of *B. breve* highly resembles the tumor antigen SIYRYYGL (SIY), while tape measuring protein 1 (TMP1) of *Enterococcus hirae* is analogous to PSMβ4 on tumor cells [57,58]. This cross-protective effect has also been demonstrated with viruses [59]. However, microbiota-induced cross-reactivity of over-activated T cells may also cause damage to healthy cells [60]. In addition to interacting with immune cells, microbiota could also affect cancer progression by binding directly to E-cadherins on cancer cells. *Fusobacterium* adhesin A (FadA) from *F. nucleatum* binds to E-cadherin and activates β-catenin signaling, contributing to elevated expression of oncogenes and inflammatory genes, loss of cellular polarity, and promotion of CRC cell growth [61]. Equivalently, *H. pylori* cytotoxin-associated gene A (CagA) induces aberrant expression of the intestinal-differentiation marker, goblet-cell mucin-2, by binding to E-cadherin, deregulating the β-catenin signal, and causing intestinal-type gastric adenocarcinoma [62]. Further exploration of the microbial contributions to the development and functional activation of immune cells is crucial for enhancing the efficacy of cancer immunotherapy, rather than facilitating cancer cell growth.

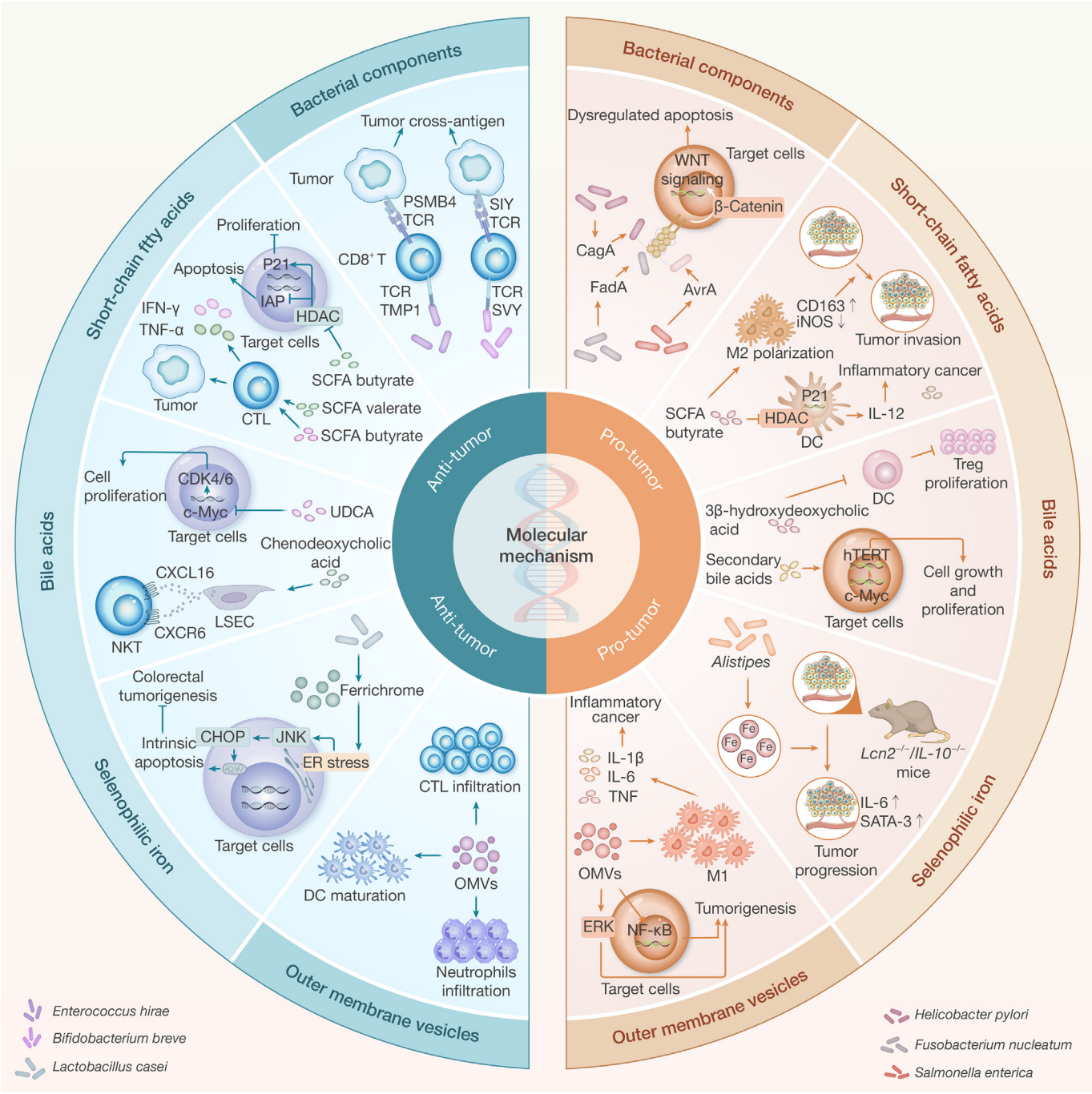

Figure 3. Molecular mechanisms underlying the bidirectional regulation of bacterial components and metabolites in cancer progression

Microbiota influence the proliferation, activity, and recruitment of immune cells, affecting apoptosis, growth, or metastasis of cancer cells. Microbiota could also act directly on cancer cells through metabolites such as SCFAs, bile acids, and selenophilic iron. The combined effect ultimately leads to a complex bi-directional regulation of tumor development by microbiota. Abbreviations: PSMB4, phenol-soluble modulin beta type 4; TCR, T cell receptor; SIY, SIYRYYGL; TMP1, tape measuring protein 1; SVY, SVYRYYGL; IAP, inhibitor of apoptosis protein; HDAC, histone deacetylase; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; CTL, cytotoxic T lymphocyte; SCFA, short-chain fatty acid; UDCA, ursodeoxycholic acid; LSEC, liver sinusoidal endothelial cells; CXCL16, C-X-C motif chemokine ligand 16; CXCR6, C-X-C motif chemokine receptor 6; c-Myc, cellular-myelocytomatosis oncogene; CDK4/6, cyclin-dependent kinase 4/6; NKT cell, natural killer T cell; JNK, c-Jun N-terminal kinase; CHOP, JNK-C/EBP homologous protein; ER, endoplasmic reticulum; DC, dendritic cell; OMVs, outer membrane vesicles; M1, type-1 macrophage; M2, type-2 macrophage; CagA, cytotoxin-associated gene A; FadA, *Fusobacterium* adhesin A; AvrA, *Salmonella enterica* virulence factor; iNOS, inducible nitric oxide synthase; hTERT, human telomerase reverse transcriptase; Lcn2, Lipocalin-2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ERK, extracellular signal-regulated kinase.

Microbial metabolites

Microbiota influences cancer initiation and progression by indirectly interacting with innate immune cells through secreted metabolites (Table 1). Microbial metabolites have been demonstrated as an intimate nexus with cancer, mainly including short-chain fatty acids (SCFAs), bile acids, and selenophilic iron [63]. These metabolites could reach distant tumor sites, traveling through the blood circulation, and thus have a double-edged sword effect on immune response in TME.

SCFAs. The impact of SCFAs on the direction of innate immune response regulation and cancer progression varies across subgroups and application contexts. Butyrate-mediated suppression of histone deacetylase activity induces upregulation of IL-12p19 mRNA in DCs, ultimately promoting inflammation-associated microenvironment development [64]. Butyrate-induced increased C-C motif chemokine ligand 20 (CCL20) recruits macrophages, polarizes them toward tumor-promoting M2 phenotypes, and enhances tumor invasion [65]. Additionally, butyrate can interact directly with cancer cells to promote cancer progression. Firmicutes-derived butyrate promotes colon epithelial cell hyperproliferation, thereby facilitating CRC in $APC^{min/+}MSH^{-/-}$ mice [66]. Interestingly, exogenous butyrate reverses M1 polarization of pro-inflammatory macrophages, suppresses activation of the NLRP3/Caspase-1 pathway, and alleviates intestinal inflammation, thereby inhibiting tumor growth [67]. These differences indicate that the biological effects of butyrate may depend on its specific source and application context. SCFAs may inhibit tumor progression by promoting immune cell removal of tumor cells and tumor cell apoptosis. SCFAs, especially valerate and butyrate, induce cytotoxic T lymphocytes (CTLs) to secrete interferon-gamma (IFN-γ) and TNF-α, and promote anti-tumor effects [68]. Additionally, SCFAs such as propionic acid produced by *Akkermansia muciniphila* upregulate the cell cycle inhibitor p21 and downregulate the inhibitor of apoptosis protein (IAP), thereby suppressing malignant cell multiplication and triggering apoptosis [69]. Modulating the species of microbiota to alter the proportion of SCFA subtypes may contribute to the effectiveness of anti-tumor immunity, and the feasibility has been demonstrated. Compared to germ-free HCC mice, the *L. reuteri* group with higher levels of acetate shows a reduction in the number and relative size of liver tumors [15].

Bile acids. Bacteria-derived bile acids modulate cancer cell growth by governing the proliferation and chemotaxis of innate immune cells. In contrast to the reductions in neutrophils and monocytes observed in germ-free mice, the microbiota in conventional mice regulates granulocyte monocyte progenitors (GMPs) proportions and promotes their differentiation and maturation [70]. In mice treated with deoxycholic acid, the expression of cell proliferation markers and the G2M gene *Mki67* in GMPs significantly increased, suggesting that microbial metabolite-mediated enhancement of myelopoiesis may become a promising direction for the treatment of hematological tumors [71]. Chenodeoxycholic acid upregulates C-X-C motif chemokine ligand 16 (CXCL16) level on liver sinusoidal endothelial cells (LSEC) and C-X-C motif chemokine receptor 6 positive ($CXCR6^{+}$) hepatic natural killer T (NKT) cells aggregation, thus inhibiting liver tumor growth [72]. Conversely, 3β-hydroxydeoxycholic acid attenuates the immunostimulatory properties of DCs, thereby facilitating Treg proliferation and enhancing immune escape of tumor cells [73]. Moreover, bile acids could interact directly with cancer cells, with different types regulating cancer cell proliferation variably. Acidified bile acids promote tumor progression by directly inducing cellular-myelocytomatosis oncogene (c-Myc) transcription and increasing human telomerase reverse transcriptase (hTERT) expression [74]. On the contrary, it was found that ursodeoxycholic acid (UDCA), a secondary bile acid generated by *Ruminococcus gnavus*, could restrain cancer cell proliferation by repressing *c-Myc* and cyclin-dependent kinase 4/6 (*CDK4/6*) [75,76]. Taking beneficial bile acids or modifying microbiota through direct administration may help impede cancer progression.

Selenophilic iron. Beyond SCFAs and bile acids, microbial metabolite selenophilic iron bidirectionally regulates tumorigenesis. In the Lipocalin-2 $(Lcn2)^{-/-}/IL\text{-}10^{-/-}$ double-deficient mouse model, facultative pathogenic *Alistipes* spp. utilize enterobactin as an iron source and produce selenophilic iron, driving colitis and tumorigenesis [77]. In contrast, ferrichrome produced by *Lactobacillus casei* activates endoplasmic reticulum (ER) stress, resulting in c-Jun N-terminal kinase (JNK)-C/EBP homologous protein (CHOP)-mediated intrinsic apoptosis and inhibiting colorectal tumorigenesis [78].

Other mediators of microbiota

Microbiota-derived outer membrane vesicles (OMVs) contain a variety of cargos, such as LPS, genetic material, virulence factors, and enzymes, which are released into the somatic circulation, thus participating in bacterial delivery system composition. *F. nucleatum* OMVs predominantly carrying the outer membrane protein of *F. nucleatum* (FomA) and FadA could act as activators of TLR, causing phosphorylation and activation of extracellular signal-regulated kinase (ERK), cAMP response element binding protein (CREB), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). These events stimulate the secretion of IL-8 and TNF-α, contributing to inflammation and CRC progression [79,80]. CagA and VacA of *H. pylori* OMVs could facilitate the generation of TNF, IL-6, and IL-1β by macrophages, inducing inflammation and potentially contributing to cancer development [81]. Additionally, the parallel decrease in VacA and LPS in *H. pylori*-OMVs under iron-deficient conditions suggests that imbalances in iron metabolism might affect OMVs content production. Nevertheless, accumulating studies illuminate that OMVs present potential anti-tumor properties. OMV-displaying tumor antigen peptides have been shown to intensely activate the immune system, promoting the maturation of DCs and the infiltration of neutrophils and CTLs [82]. Moreover, *E. coli* OMVs demonstrate anti-tumor synergism with anti-PD-1 antibody immunotherapy, inducing tumor cell growth inhibition [83]. Therefore, the application of OMVs in drug delivery and tumor therapy is anticipated to hold significant scientific and clinical promise.

Table 1. Mechanisms of microbial metabolite–innate immune cell interactions in tumor development

Microbial metabolites	Innate immune cells	Mechanisms	Outcomes	References
Short-chain fatty acids (SCFAs)				
Acetate	ILC3s	Inhibited histone deacetylase activity, increasing the acetylation of Sox13 at site K30, and decreasing the expression of Sox13	Reduced the production of IL-17A in hepatic ILC3s	[15]
Acetate	Neutrophils	Improved Th17 responses in an FFAR2-dependent manner and thus triggered chemotaxis of human neutrophils.	Drived a pro-tumorigenic intestinal microenvironment	[246,247]
Butyrate	Macrophages	Induced cancer cell autophagy and released higher levels of chemokine CCL20 that promote macrophage infiltration and polarization towards M2-type macrophages	Strengthened prostate cancer cell invasion	[65]
Bile acids				
Secondary bile acids				
3β-hydroxydeoxycholic acid	Dendritic cells	3β-hydroxydeoxycholic acid exhibited weakened immunostimulatory properties when acting on DCs, thus upregulating the number of Tregs	Promoted immune escape	[73]
Isolithocholic acid	Neutrophils	Isolithocholic acid inhibited retinoic acid receptor-related orphan nuclear receptor-γt, a key Th17-cell-promoting transcription factor	Suppressed Th17 cell differentiation and chemotaxis of neutrophils	[247,248]
Primary bile acids				
Chenodeoxycholic acid	NKT cells	Chenodeoxycholic acid upregulated chemokine CXCL16 level on liver sinusoidal endothelial cells and the accumulation of $CXCR6^+$ hepatic NKT cells	Inhibited liver tumor growth	[72]
Others				
Anacardic acid	Macrophages	Induced phosphorylation of ERK1/2, JNK, P38 kinase and NF-κB in macrophages	Promoted macrophages activation	[249]

(*Continued on next page*)

Table 1. *Continued*

Microbial metabolites	Innate immune cells	Mechanisms	Outcomes	References
c-di-AMP	Monocytes	Stimulator of interferon genes agonists induced intratumoral monocytes to produce IFN-I	Regulated macrophage polarization and natural killer cell-DC crosstalk	[143]
ATP	Neutrophils	Activated $CD70^{high}CD11c^{low}$ cells in the lamina propria to induce IL-6 and IL-23 production as well as TGF-β activation	Promoted Th17 cell differentiation and chemotaxis of neutrophils	[247,250]
trimethylamine-N-oxide	Macrophages	Induced macrophages to M1-type macrophage polarization	Improved anti-tumor immune responses	[14]
Indole-3-lactic acid	Dendritic cells	Accelerated IL12a production in dendritic cells by enhancing H3K27ac binding at the enhancer regions of IL12a	Primed $CD8^+$ T cell immunity against tumor growth	[194]

Abbreviations: ATP, adenosine 5′-triphosphate; c-di-AMP, cyclic di-adenosine monophosphate; CCL20, C-C motif chemokine ligand 20; CXCL16, C-X-C motif chemokine ligand 16; CXCR6, C-X-C motif chemokine receptor 6; DC, dendritic cell; ERK, extracellular signal-regulated kinase; FFAR2, free fatty acid receptor 2; H3K27ac, histone H3 lysine 27 acetylation; IFN-I, type I interferon; IL-6, interleukin-6; ILC3s, group 3 innate lymphoid cells; JNK, c-Jun N-terminal kinase; M1, type-1 macrophage; M2, type-2 macrophage; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NKT cells, natural killer T cells; Sox13, sex-determining region Y-box transcription factor 13; TGF-β, transforming growth factor beta; Th17, T helper 17 cells; Treg, regulatory T cell.

Genetic and Epigenetic Modifications of Innate Immunity–Microbiota Interactions

The interplay between the host innate immune system and the microbiota plays a crucial role in tumorigenesis and progression, with genetic polymorphisms and epigenetics modulating this crosstalk in the TME.

Microbiota can influence the expression levels of *TLR* genes in innate immune cells through transcriptional regulation, thereby reshaping host immune responses. A recent study demonstrated that stimulation of DCs with phospholipids derived from *Lactobacillus johnsonii* N6.2 selectively upregulated *TLR2* gene expression while downregulating *TLR3* and *TLR4* expression, as quantified by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) [84]. Similarly, differential regulation of *TLR* genes was observed during HPV infection. It was shown that HPV-induced type I interferon (IFN-I) was positively correlated with *TLR4* and *TLR9* and negatively correlated with *TLR3* [85]. Collectively, these studies underscore the complex transcriptional regulatory networks of *TLR* gene expression in innate immunity–microbiota interactions, where dysregulated expression patterns could critically impact anti-pathogen immune efficacy.

Epigenetic modifications, including DNA methylation, histone modification, and non-coding RNA regulation, can alter gene expression without altering the DNA sequence and play a crucial role in regulating the interaction between innate immunity and the microbiota [86]. Bacterial indole-3-propionic acid suppresses macrophage IL-1β generation by activating methionine metabolism and promoting DNA methylation of ubiquitin-specific peptidase 16, which in turn promotes TLR4 ubiquitination and inhibits NF-κB, showing its potential as a microbial-derived anti-inflammatory agent [87]. Interestingly, butyrate can act as a histone deacetylase inhibitor (HDACi), increasing IL-12p19 mRNA expression in DCs, thereby promoting an inflammatory microenvironment [64]. As well, it was found that *L. reuteri* could activate DCs through triggering the cells' histone H3 lysine 27 acetylation (H3K27Ac) of active gene transcription [88]. Microbiota could modulate the expression patterns of host miRNA, a type of non-coding RNA in innate immune cells. As proof, commensal gut bacteria suppress miR-107 levels in DCs and macrophages *via* TLR ligand-mediated interactions through MyD88 and NF-κB dependent signaling mechanisms, thereby reducing IL-23p19 expression in DCs [89]. Emerging studies reveal that microbiota-driven lactate metabolism critically regulates cancer progression through post-translational lactylation modifications. In CRC, *E. coli*-generated lactate enhances cancer cell metastasis by inducing lactylation of retinoic acid-inducible gene 1 (*RIG-I*), a mechanism that drives immunosuppressive M2 macrophage polarization within the TME [90]. Lysine lactylation modifications in cancer cells are shown to correlate with aggressive tumor phenotypes and adverse clinical outcomes [91]. However, the molecular mechanisms underlying microbiota regulation of lactylation levels in cancer cells remain unclear and need to be further explored.

In conclusion, the role of genetic and epigenetic factors in regulating innate immune–microbiota interactions in TME is complex and multifaceted, and further studies are needed.

Innate Immunity Response to Microbiota

The capacity to discriminate between pathogens and commensal microbiota is a fundamental feature of the innate immune system. When the innate immune system recognizes commensal microbiota, it downregulates the immune response and establishes immune tolerance. In contrast, upon detecting pathogens, it activates rapidly, regulates the composition and distribution of microbiota, and clears out undesirable species, thereby eliminating potential harm. For instance, macrophages facilitate dysbiosis through *Stat1*-induced inducible nitric oxide synthase (iNOS) generation. In support of this, researchers purposely fed LysM-*Stat1*$^{-/-}$ mice, whose macrophages are incapable of responding to IFN-γ *via Stat1* and have insufficient iNOS production compared with WT mice. Consequently, the relative abundance of Enterobacteriaceae was significantly increased in WT mice ($P < 0.05$), whereas it remained relatively unchanged in LysM-*Stat1*$^{-/-}$ mice and was about 12-fold lower than that in WT mice [92]. Pro-IL1β produced by mononuclear phagocytes is unresponsive to commensals but can be converted to an active state by pathogenic bacteria such as *Salmonella* or *Pseudomonas* in the gut [93]. Subsequently, neutrophils are recruited and produce large amounts of ROS, inhibiting microbial translocation and overgrowth [94]. In infants with immature adaptive immunity, brain macrophages expressing TLR5 are critical in protecting against pathogenic infections [95]. In mice with colon tumors, neutrophil depletion enhanced tumor growth and invasiveness, correlating with higher bacterial levels and IL17-driven inflammation. Antibiotics or IL-17 neutralization reduced tumor invasiveness, highlighting the role of neutrophils in restricting tumor-associated microbiota and limiting tumor progression [96]. Due to the complexity of the causal relationship between innate immunity and microbiota, as well as the difficulty of identifying microbial transfer sites, specific mechanisms of microbial composition and distribution modulated by innate immunity haven't yet been fully investigated, and further exploration is needed.

Microbiota and Innate Immune Homeostasis

Microbiota interactions with the innate immune system are critical for immune homeostasis and dysregulation. As a member of the human innate lymphocyte family, mucosal-associated invariant T (MAIT) cells are activated by antigens combined with the MHC class I-related protein 1 (MR1) [97]. The parallel absence of IgA secretion and MAIT cells in germ-free mice suggests that derivatives of the microbiota riboflavin biosynthesis pathway may promote MAIT cell production and activation *via* inducing IgA secretion and MR1 expression, favoring the homeostasis and integrity of the meningeal barrier [98–100]. Meanwhile, MAIT cells modulate the intestinal immune system by secreting inhibitory lymphokines to sense bacterial activity [99]. *A. muciniphila* threonyl-tRNA synthetase (AmTARS) promotes the production of anti-inflammatory IL-10 and the central inflammatory mediator NF-κB *via* interacting with TLR2 and activating MAPK and phosphatidylinositol 3-kinase (PI3K)/AKT signaling. In this manner, AmTARS restores IL-10-positive macrophages and induces M2 macrophage polarization, functioning as a monitor and regulator of immune homeostasis [101]. Conversely, fimbrial adhesin H (FimH) variants of adherent invasive *E. coli* (AIEC) regulate the secretion of pro-inflammatory cytokines IL-8 and CCL20 and promote the recruitment of DCs and macrophages, contributing to immune dysregulation and inflammation-related CRC [102,103]. Hence, restoration of innate immune homeostasis through microbiota control may contribute to cancer regression.

Cross-talk in Signaling Pathways within Bacterial Phyla

The synergism and antagonism within microbiota are highly complicated and remain incompletely elucidated. Competitive microbial–microbial interactions constitute both direct mechanisms, including nutrient and space competition, contact-dependent inhibition, inhibitory metabolites, and indirect mechanisms that interact through the mucus layer, oxygen gradients, and microbiota-mediated immune responses [49].

Microbiota could stimulate Wnt/β-catenin pathway by adhesin-induced target cell recognition, thus promoting dysregulated apoptosis and cancer development. Upon binding of *H. pylori* outer membrane adhesin HopQ to carcinoembryonic antigen-associated cell adhesion molecule (CEACAM) on epithelial cells, the *H. pylori* virulence factor CagA translocates into epithelial cells *via* the type 4 secretion system (T4SS) and triggers the Wnt/β-catenin pathway. This leads to pro-inflammatory cytokine production, cell proliferation activation, and tumor formation [104–106]. Similarly, *Salmonella enterica* virulence factor AvrA enters the cell *via S. enterica* T3SS and activates Wnt/β-catenin pathway, driving tumorigenesis [107]. Furthermore, *F. nucleatum* stimulates Wnt/β-catenin *via Fusobacterium* autotransporter protein 2 (Fap2) adhesin, generating a pro-tumorigenic effect [108].

Multiple factors, including microbiota, can influence the macrophage polarization direction, immune response, and cancer progression. Pro-inflammatory cytokines TNF-α, IL-1, and IL-12 induce the differentiation of undifferentiated macrophages (M0) into M1 macrophages. Conversely, macrophage colony-stimulating factors, or IL-4 and IL-10 trigger M2 differentiation [109–111]. Microbiota regulates macrophage polarization direction by inducing host cytokine production. Specifically, *F. nucleatum*, *H. pylori*, and *C. albicans* could activate host cells to produce TNF-α and drive M0 polarization towards M1 [79,81,112]. Reciprocally, *A. muciniphila*, *F. nucleatum*, and *Faecalibacterium* promote M2 polarization through the induction of IL-10 production in immune cells [61,101,113]. Acetate from *Bacteroides thetaiotaomicron* drives M1 polarization, promotes cytotoxic $CD8^+$ T cell function, and significantly inhibits tumor cell proliferation in HCC mice [114]. Interestingly, microbial metabolite butyrate promotes M1 polarization and activates NLRP3/Caspase-1 but favors tumor growth [67]. Bacterial-derived butyrate promotes lung cancer metastasis by increasing H19 expression in tumor cells and inducing M2 macrophage polarization [115]. These contrasting effects may be attributed to the distinct downstream signaling pathways activated by diverse microbial metabolites in varying contexts. Nevertheless, this dichotomous classification could not

fully reflect conditions *in vivo* due to the continuous changes in macrophage activation states [116]. Therefore, a more nuanced classification system is needed.

INTERACTIONS BETWEEN INNATE IMMUNITY AND MICROBIOTA IN CANCER INITIATION AND PROGRESSION

Multiple obstacles need to be overcome for cancer cell generation, local invasion, and metastatic growth. Innate immunity–microbiota interactions primarily affect several aspects of TME (Table 2), such as immune escape, barrier function disruption, chronic inflammatory transformation, and angiogenesis during cancer initiation, as well as local invasion, intravasation, and pre-metastatic niche formation during the tumor metastatic growth phase (Figure 4).

Innate Immunity and Cross-talk with Microbiota in Cancer Initiation

Promote immune evasion

In the normal tissue microenvironment, immune cells could identify and eradicate cells exhibiting heterologous antigens and exert potent selective pressures on tumor cells, thus constraining cancer outgrowth [117]. Intriguingly, emerging evidence suggests that microbiota may be involved in this process, whereas dysbiosis suppresses immune cell activity and promotes the formation of immune-suppressive TME in local tissues. Gram-negative bacteria/LPS have been shown to promote intratumoral myeloid-derived suppressor cells (MDSCs) infiltration through a TLR4/CXCL1/CXCR2-dependent signaling pathway [118]. MDSCs are immunosuppressive cells that suppress anti-tumor immunity by expressing TGF-β and decreasing the activity of immune cells [119]. Mechanistically, TGF-β induces forkhead box protein P3 (Foxp3) expression and generates immunosuppressive Tregs. These cells could suppress the function of T cells and DCs through TLR8 ligands/MyD88/TNF receptor-associated factor 6 (TRAF6) molecules, thus contributing to tumor cell escape from immune surveillance [120,121]. However, during early tumorigenesis, TGF-β potentiates cell cycle arrest and apoptosis *via* ataxia telangiectasia mutated protein (ATM) and p53, thus exerting an anti-tumor effect [122]. The role of TGF-β in tumorigenesis varies depending on the context and phase of tumor development [123]. Zhou et al. found that bacterial abundance was increased in right-sided adenomas, characterized by elevated Proteobacteria and reduced Fusobacteria [124]. Bacterial LPS-induced S100A11 expression impaired anti-tumor immunity with MDSC recruitment and cytotoxic $CD8^+$ T cells reduction. Subsequently, targeting the S100A11-receptor for advanced glycation end product (RAGE) axis with Azeliragon, a RAGE antagonist, has been shown to enhance the efficacy of anti-PD-1 therapy in colon cancer [124]. Frustratingly, the feasibility of inhibiting MDSC recruitment to enhance anti-tumor immunity by improving dysbiosis remains unverified, and further experiments are warranted. Multiple pathobionts, including *F. nucleatum* and *Klebsiella pneumoniae*, could modulate tumor-derived cytokine activity, inducing the infiltration of TAMs of the M2 subtype, which in turn favors tumor cell proliferation and invasion [125,126]. Mechanistically, *F. nucleatum* promotes M2 macrophage polarization by activating the TLR4/NF-κB/S100A9 cascade signaling pathway [125]; *K. pneumoniae* polarizes macrophages to an M2 phenotype through IKK-NF-κB inhibition and STAT6-KLF4-IL-10 activation [126]. Moreover, microbiota exerts differential effects on the transcriptional profiles of M1/M2 polarized macrophages [127]. Thus, modulating microbiota to switch macrophage polarization direction may represent a promising target for cancer immunotherapy.

Barrier dysfunction and impaired innate immune–microbiota crosstalk

Imbalances in the "microbiota-innate immunity" axis lead to altered microbial community and barrier dysfunction of the skin and multiple mucosal surfaces, facilitating tumorigenesis and tumor progression.

Alterations in the skin microbiota, predominantly characterized by increases in some skin microbiota strains, such as *S. aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* may promote chronic self-maintaining inflammation and carcinogenesis [2]. Specifically, *S. aureus*-expressed PSMα triggers keratinocyte MyD88 signaling and induces IL-1α and IL-36α generated by keratinocytes, promoting IL-17-mediated self-sustained inflammation and thus generating impaired skin barrier, proliferation, and migration of skin cancer cells [128]. Dietary or genetic obesity-induced gut microbiota changes are manifested by an increase in obesity-associated gram-positive bacteria such as *Clostridium* cluster XI and XIVa, leading to elevated bacterial metabolite deoxycholic acid (DCA). DCA could facilitate DNA damage and HCC development in mice after exposure to chemical carcinogens by provoking senescence-associated secretory phenotype (SASP) in hepatic stellate cells and secreting inflammatory and tumor-promoting factors [2]. Using 16S rRNA sequencing to compare the oral microbiota in DNA isolated from head and neck squamous cell carcinoma (HNSCC) tissues and healthy sites, the results suggest that high *F. nucleatum* and low-loading *Streptococcus* appear to be promising markers for predicting HNSCC initiation [3,129]. Research from a transcriptomic perspective found that the transcriptome signature relevant to the etiology of lung cancer was correlated with increased lower respiratory tract microbiota. Concretely, oral bacteria (*Streptococcus* and *Veillonella*) dominated the lower respiratory tract in lung cancer patients compared to healthy individuals, and these bacteria were shown to upregulate ERK and PI3K signaling pathways *in vitro* [4]. Additionally, a decreased prevalence of lung cancer was demonstrated in germ-free mice [130]. Microtrauma during human papillomavirus (HPV) infection disrupts the vaginal epithelial barrier and local immune microenvironment, leading to large numbers of abnormal flora proliferation [131]. The disruption results in an imbalance in the vaginal microecosystem, which promotes HPV protein expression and increases the adhesion, invasion, and colonization of abnormal flora. This process exacerbates the local micro-ecosystem imbalance, creating a vicious cycle that consequently accelerates the cervical precancerous lesions progression [5,132].

In the local micro-ecosystem, specific niches select microbial communities that differ greatly based on their particular locations. As mentioned earlier, alterations in the flora of various mucous membranes, including the skin, intestines, mouth, respiratory tract, and vagina, contribute to dysbiosis and ecosystem

Table 2. Microbiota and innate immune cells on tumor immunity

Cancer types	Microbiota	Immune cells	Mechanism of action	Outcomes	References
Pro-tumor effects					
Colorectal cancer	*Fusobacterium nucleatum*	Macrophages	Activated TLR4/NF-κB/S100A9 cascade	Facilitated M2 macrophage polarization	[125]
Colorectal cancer	*F. nucleatum*	Macrophages	Promoted miR-1322/CCL20 axis	Facilitated M2 macrophage polarization	[251]
Colorectal cancer	*Klebsiella pneumoniae*	Macrophages	Inhibited IKK-NF-κB and activated STAT6-KLF4-IL-10	Facilitated M2 macrophage polarization	[126]
Colorectal cancer	Malnutrition-induced gut microbiota dysbiosis	Macrophages	Recruited macrophages to activate mucosal immunity in CRC	Regulated macrophages and accelerated CRC progression.	[252]
Colorectal cancer	*Proteus mirabilis*, *Parabacteroides distasonis*	Kupffer cells	Negatively affected the proliferation of Kupffer cells	Promoted CRC liver metastasis	[253]
Colorectal cancer	*F. nucleatum*	MDSCs, NK cells	Recruited MDSCs into the liver and reduced NK cell infiltration	Potentially promoted CRC liver metastasis	[254]
Colorectal cancer	*Peptostreptococcus anaerobius*	MDSCs, neutrophils TAMs	Caused an expansion of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and granulocytic tumor-associated neutrophils	Potentially worsened cancer progression	[138]
Cholangiocarcinoma	Gram-negative commensal bacteria	MDSC	Controlled accumulation of hepatic MDSC through a TLR4/CXCL1/CXCR2 dependent mechanism	Derived the formation of immunosuppressive TME	[118]
Pancreatic ductal adenocarcinoma	Commensal microbiota from *Rag1*$^{-/-}$ mice	NK cells	Inhibited the cytotoxicity and migration of NK cells	Promoted PDAC tumor progression	[255]
Breast cancer	*Mycobacterium*	Tie2^{+}/VEGF^{hi} macrophages	Promoted Tie2^{+}/VEGF^{hi} macrophages mobilization to tumors *via* Ang2/Tie2 signaling	Led to endothelial cell-cell junctions disruption and facilitated intravasation	[183,184]

(*Continued on next page*)

Table 2. *Continued*

Cancer types	Microbiota	Immune cells	Mechanism of action	Outcomes	References
Colon cancer	*Candida albicans*	Macrophages	Triggered glycolysis and IL-7 secretion which IL-7 induced IL-22 production in ILC3s	Promoted tumorigenesis	[256]
Anti-tumor effects					
Solid tumor	*Akkermansia muciniphila*, *Enterococcus hirae*	Dendritic cells	Induced dendritic cells to secrete IL-12	Suppressed tumor angiogenesis	[160]
Breast cancer, hepatocellular carcinoma	*Escherichia coli* strain Nissle 1917	Dendritic cells	Enhanced dendritic cell activation	Mitigated the immunosuppressive TME	[257]
Melanoma	Commensal microbiota from *Rnf5*$^{-/-}$ mice	Dendritic cells	Recruited and activated dendritic cells	Controlled melanoma growth	[258]
Melanoma	Commensal microbiota	NK cells	Promoted intestinal NK cell migration from the gut to the tumor-bearing bones	Restrained melanoma bone growth	[259]
Human papilloma virus-induced cancer	*Lactobacillus casei* BL23	NK cells	Enhanced the local recruitment of NK cells and cytotoxic activity	Reduced the number of tumor cells	[260]

Abbreviations: Ang2, angiopoietin-2; CCL20, C-C motif chemokine ligand 20; CXCL1, C-X-C motif chemokine ligand 1; CXCR2, C-X-C motif chemokine receptor 2; CRC, colorectal cancer; IKK, inhibitor of NF-κB kinase; ILC3s, group 3 innate lymphoid cells; KLF4, Krüppel-like factor 4; LPS, lipopolysaccharide; M1, type-1 macrophage; M2, type-2 macrophage; MDSC, myeloid-derived suppressor cells; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK cells, natural killer cells; PDAC, pancreatic ductal adenocarcinoma; STAT6, signal transducer and activator of transcription 6; TAM, tumor-associated macrophage; TLR4, toll-like receptor 4; TME, tumor microenvironment; VEGF, vascular endothelial growth factor.

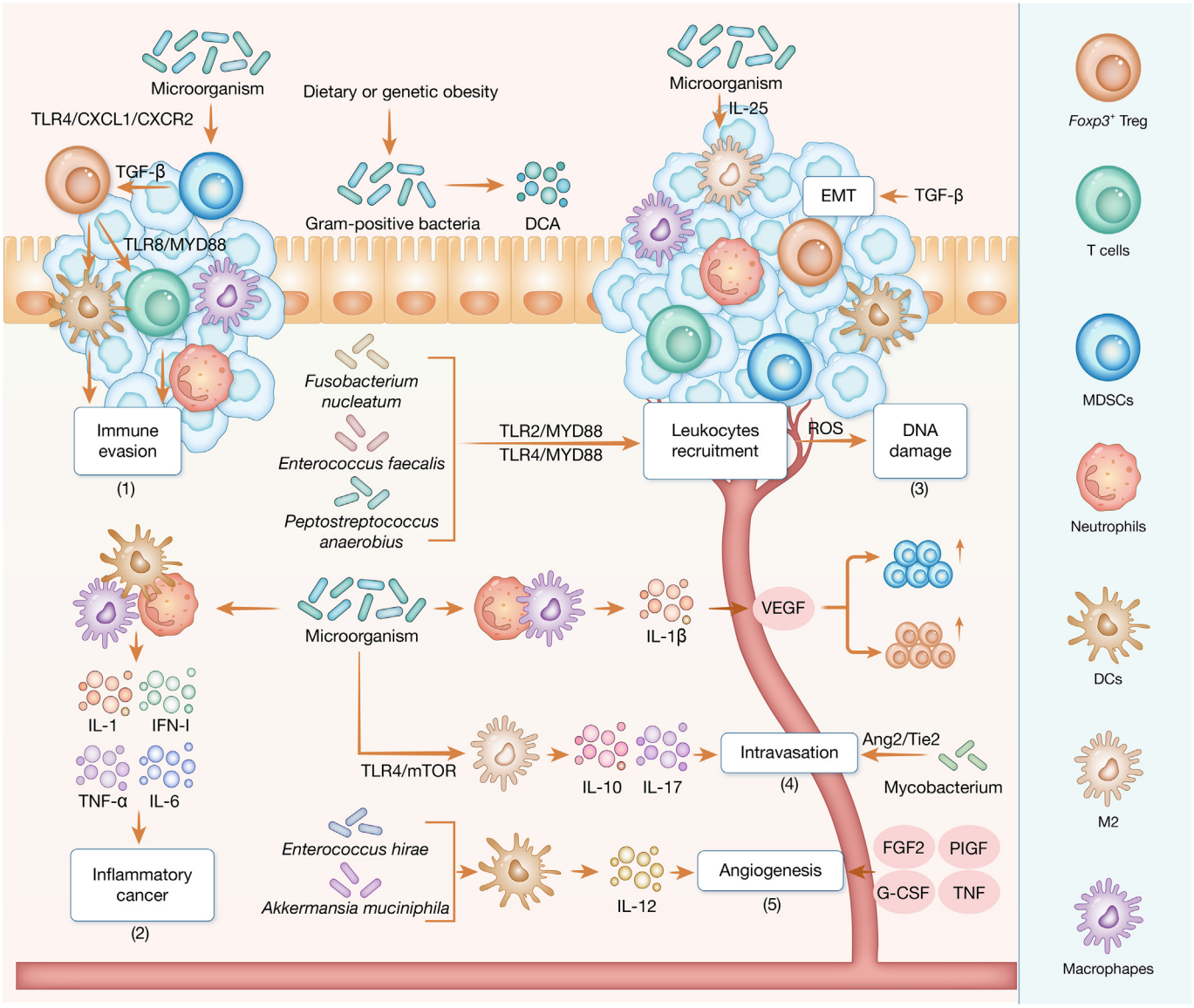

Figure 4. Regulation of the microenvironment during tumor initiation and metastatic spread by microbial and innate immune interactions

(1) Microorganisms promote MDSC infiltration *via* TLR4/CXCL1/CXCR2 pathway and *Foxp3*$^+$ Treg cell generation, which suppress T cell and dendritic cell functions through TLR8/MyD88, thereby facilitating tumor immune escape. (2) Innate immune cells detect PAMPs/DAMPs *via* TLRs on their cell membranes, which triggers the upregulation of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and IFN-I. (3) *Fusobacterium nucleatum*, *Peptostreptococcus anaerobius*, and *Enterococcus faecalis* activate the TLR2/4 signaling pathway through their adaptor protein MyD88, resulting in leukocyte recruitment and DNA damage, thus promoting cancer initiation. (4) Microorganisms promote M2 polarization of TAMs through TLR4/mTOR pathway. Subsequently, M2 TAMs stimulate NF-κB pathway by secreting IL-10 and IL-17, thereby facilitating tumor metastasis. (5) *Akkermansia muciniphila* and *Enterococcus hirae* trigger DCs to generate IL-12 and suppress tumor angiogenesis. Other molecules promoting angiogenesis, such as FGF2, PIGF, TNF, and G-CSF, have been identified in tumors. Abbreviations: TLR4, toll-like receptor 4; CXCL1, C-X-C motif chemokine ligand 1; CXCR2, C-X-C motif chemokine receptor 2; Foxp3, forkhead box protein P3; MYD88, myeloid differentiation factor 88; PAMP, pathogen-associated molecular patterns; DAMP, damage-associated molecular patterns; IL-6, interleukin-6; DCA, deoxycholic acid; IFN-I, interferon type I; TNF-α, tumor necrosis factor-alpha; TGF-β, transforming growth factor beta; EMT, epithelial–mesenchymal transition; VEGF, vascular endothelial growth factor; mTOR, mammalian target of rapamycin; Ang2, angiopoietin-2; FGF2, fibroblast growth factor; PIGF, placental growth factor; G-CSF, granulocyte colony-stimulating factors.

disruption. This disruption compromises the mucosal barrier, facilitating cancer initiation and progression. There are numerous unresolved complex mechanisms and multifactorial challenges, and further animal experiments and clinical trials are needed to elucidate the interaction mechanisms between cancer and microbiota at the biological barrier level and the potential for antitumor therapy.

Transformative potential of chronic inflammation

While genetic damage establishes the molecular foundation for carcinogenesis initiation, some types of chronic inflammation may act as the sustaining force that drives cancer progression [133]. Microbiota could further amplify this process by activating the inflammatory response through infecting innate immune cells, including macrophages, monocytes, neutrophils, and

DCs. These cells detect pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) *via* TLR on their cell membranes, leading to enhanced expression of proinflammatory cytokines such as TNF-α, IL-1, IL-6, IFN-I [134–136]. For instance, *F. nucleatum*, *Peptostreptococcus anaerobius*, and *Enterococcus faecalis* activate TLR2/4 signaling through their adaptor MyD88, resulting in NF-κB activation and the expression of downstream pro-inflammatory cytokines and adhesion molecules, which leads to leukocyte recruitment to inflammation sites [137–139]. ROS released by recruited neutrophils drives DNA damage and genomic instability, thus triggering oncogenic alterations and promoting cancer initiation [140]. What's more, bacterial metabolites may be involved in the inflammatory response. In people consuming high-fat diets, the bile acids production, such as tyrosocholic acid (TyrChol) by *Clostridium* spp. is increased, which may contribute to inflammation-derived tumorigenesis in Crohn's disease [141]. Tremendous studies have suggested that the activated bacterial-induced inflammatory cells and cytokines found in tumors favor gene alterations, immunosuppression, tumor formation, and progression [133]. Therapeutic measures targeting the interaction between microbiota and inflammatory tumors have reduced the incidence of some cancers. Mouse models of colitis-associated CRC imply that chronic inflammation and dysbiosis, particularly genotoxin-producing *E. coli* strains, contribute to CRC development. Furthermore, restricting Enterobacteriaceae bloom by oral administration of sodium tungstate suppresses intestinal inflammation and decreases colonic tumor incidence [142].

Beyond most inflammatory TME, there exists TME with an immunosuppressive state, where the enhancement of host immune response may contribute to cancer regression. For instance, c-di-AMP, a microbial-derived stimulator of interferon genes (STING) agonist, ameliorates immunosuppressive TME by inducing IFN-I generated from intratumoral monocytes and polarizing M0 to anti-tumor M1. Increased IFN-I promotes NK cell infiltration and secretion of CCL5 and XCL1, thereby recruiting DCs, which in turn activate NK cells *via* IL-15/IL15-R [143].

Angiogenesis: A promoter of cancer progression

Angiogenesis, referring to the generation of new blood vessels, acts critically in tumorigenesis by supplying tumors with oxygen and nutrients. Conversely, failure of angiogenesis may lead to tumor dormancy and delayed clinical manifestation of cancer [144,145]. In different cancer types, such as breast cancer, kidney renal clear cell carcinoma, and lung adenocarcinoma, elevated levels of vascular endothelial growth factor (VEGF) in patients are intimately associated with poor disease prognosis. Anti-VEGF treatments have been shown to suppress cancer progression without severe toxicities, highlighting the critical role of angiogenesis in tumorigenesis [146–150]. Other molecules promoting angiogenesis, such as basic fibroblast growth factor (FGF2) and placental growth factor (PIGF), TNF, and G-CSF, have been identified in tumors [151]. Macrophages and neutrophils secrete IL-1β in response to LPS and promote VEGF generated by endothelial cells (ECs), providing an inflammatory microenvironment for tumor formation [152]. Microbiota could affect angiogenesis by prompting innate immune cells to secrete ROS *via* the TLR pathway. This occurs directly on the ECs themselves or by inducing hypoxia-inducible factor 1α, a major regulator of the hypoxic response, which drives the VEGF expression [140,153,154]. *H. pylori* infection triggers ECs to release inflammatory chemokine IL-8, an angiogenesis-inducing factor [155,156]. In gastric cancer, upregulated IL-8 activated the VEGF receptor through the CXCR2 pathway, which was validated by IL-8 overexpression and knockdown experiments using gastric cancer cells [157,158]. Tumor-induced angiogenesis could facilitate immune evasion and tumor metastasis [159,160]. VEGF impacts immune response *via* suppressing DC maturation and increasing the number of both Tregs and MDSCs within the tumor [161–163]. Reciprocally, vascular normalization attenuates immunosuppression by polarizing TAMs to a pro-inflammatory M1 phenotype [159]. Excitingly, some microbiota, such as *A. muciniphila* and *E. hirae*, have been shown to trigger DC to generate IL-12 and suppress tumor angiogenesis [164]. Yet the intricate mechanisms of microbiota and tumor angiogenesis in distinct TME, as well as how cancer cells migrate into the circulation along generated blood vessels, are not fully understood [160]. This gap in knowledge provides an essential direction for future studies.

Interplay between Innate Immunity and Microbiota Drives Tumor Metastasis

Local tumor invasion

Metastasis is one of the major hallmarks of cancer, and it begins when cancer cells detach from each other and intrude into the surrounding stroma from the primary tumor, a process known as tumor invasion [165]. In the context of tumor-innate factors and the TME, cancer cells migrate in three modes (single-cell migration, multicellular streaming, and collective migration), often accompanied by mesenchymal trait acquisition to achieve local invasion [166–168]. The main key factor promoting invasion is the epithelial–mesenchymal transition (EMT), a process referred to as the transition of cancer cells from an epithelial phenotype to a mesenchymal state, thus promoting stem-like characteristics and infiltration [169]. Microbes induce EMT through direct interactions with cancer cells, thereby promoting tumor progression. *F. nucleatum* drives CRC invasion through adhesin FadA-mediated binding to E-cadherin and activating β-catenin signaling [61]. In oral squamous cell carcinoma, *F. nucleatum* exerts similar pro-tumor effects by upregulating partial EMT-associated gene expression in cancer cells [170]. *Enterococcus* isolated from mice with oropharyngeal candidiasis degrades epithelial E-adhesin *via* the secretion of the metalloproteinase GelE and increases oral epithelial barrier permeability, thereby enhancing the risk of invasive infection and death [171]. Moreover, microbes promote EMT in cancer cells through indirect modulation of the inflammatory response. Dysbiosis of intestinal microbiota activates macrophages to produce IL-6 and TNF-α and promotes EMT in CRC [172]. Intratumoral *Lachnoclostridium* and *Sutterella* enhance the inflammatory response in bladder tumors, promoting EMT. This is evidenced by cohorts with the highest expression of EMT-associated genes showing the highest enrichment of macrophages and $CD4^+$ T cells [173]. Equivalently, dysbiosis-derived IL-25 induces M2 phenotype infiltration and suppresses EMT-

related protein E-cadherin expression in HCC cells, thereby promoting tumor progression [174]. Another study observed a similar outcome that treatment of mice with Banxia Xiexin Decoction improved bacterial dysbiosis, upregulated E-cadherin expression, reduced M2 macrophage infiltration, and delayed the progression of colitis to cancer [175]. These findings suggest that modulating microbiota during early cancer invasion may represent a promising therapeutic target. Excitingly, recent studies have found that some microbial metabolites can exert anti-tumor effects by inhibiting EMT in cancer cells. The bacterial metabolite lithocholic acid suppresses β-catenin signaling and epithelial transformation in breast cancer cells, thereby exerting antitumor effects [176]. The microbial metabolite urolithin A (UroA) and its structural analog UAS03, when combined with 5-fluorouracil (5FU), significantly upregulate E-cadherin expression and suppress CRC cell migration compared with 5FU monotherapy [177].

Intravasation

After acquiring a migratory phenotype and invading the surrounding tissue of the primary tumor, the next step in tumor metastasis is the intravasation of malignant cells into the circulatory or lymphatic systems, where adhesion to ECs and intravasation of tumor cells are critical steps [178,179]. By targeting the pattern recognition receptor ALPK 1, *F. nucleatum* stimulates the NF-κB pathway and upregulates the expression of intercellular adhesion molecule-1 (ICAM1), a member of the immunoglobulin superfamily. This enhances CRC cell adhesion to ECs, ultimately fostering CRC metastasis [180]. ICAM1 expression levels are positively correlated with tumor progression and metastasis [181]. Similarly, LPS-induced metastasis-related secretory protein cathepsin K (CTSK) could bind to TLR4 to foster the M2 polarization of TAMs *via* an mTOR-dependent pathway. Subsequently, M2 TAMs stimulate the NF-κB pathway by secreting cytokines, including IL-10 and IL-17, promoting tumor metastasis [182]. *Mycobacterium* promotes $Tie2^{+}$/$VEGF^{hi}$ macrophages mobilization to tumors *via* angiopoietin-2 (Ang2)/Tie2 signaling pathway, which leads to the endothelial cell-cell junction disruption and facilitates intravasation in a contact-dependent manner with Ecs [183,184]. Bacteria enhance the expression of TGF-β on immunosuppressed cells *via* TLR4/CXCL1/CXCR2, which in turn upregulates the production of angiopoietin-like 4 by tumor cells. This process increases pulmonary capillary permeability and ultimately promotes the transendothelial metastasis of tumor cells to achieve lung metastasis of breast tumors [118,185]. Following vascular intravasation, tumor cells encounter profound hemodynamic shear stress, which induces apoptosis through the caspase-dependent signaling pathway [186]. Intriguingly, intratumoral microbiota, including *Staphylococcus xylosus*, *Lactobacillus animalis*, and *Streptococcus cuniculi*, enhance cancer cell survival under fluid shear by dynamically remodeling the actin cytoskeletal structures [187]. The probiotic's capacity to suppress cancer cell intravasation remains undefined. Current evidence focuses on the role of probiotics in angiogenesis inhibition rather than targeting intravasation. Besides, lymphatic vessels are structurally different from blood vessels and thus may require different intravasation patterns. The underlying mechanisms of these are not fully understood and require further exploration [188].

Formation of premetastatic ecological niche

Interestingly, the effect of the microbiota on cancer extends beyond the local TME but triggers a cascade of events that generates a microenvironment favorable for cancer cell growth in distant organs even before the primary tumor undergoes metastatic spread. The microbiota-mediated systemic inflammation and immunosuppression provide premetastatic niches for metastatic spread of cancer cells. *F. nucleatum* establishes tumor-specific colonization in breast cancer *via* Fap2 adhesin-mediated recognition of Gal-GalNAc (galactose-β1,3-N-Acetylgalactosamine) glycans on malignant epithelia, thereby facilitating primary tumor progression and metastatic dissemination to lung parenchyma [189]. In addition, microbial barrier disruption is an accomplice in cancer cell metastasis. Capsaicin-mediated changes in microbial structure increase intestinal barrier permeability, thereby disrupting the intestinal vascular barrier to promote bacterial translocation to the liver, whereas translocated increased bile-acid metabolizing bacteria recruit hepatic NKT cells to form a pre-metastatic ecological niche [190]. Excitingly, a recent study has shown that using glycyrrhizic acid to modulate microbiota and decrease M1 macrophage proportion prevents the formation of pre-metastatic ecological niches enhanced by a high-fat diet [191]. Therefore, targeting the microbiota to inhibit the formation of pre-metastatic niches may provide novel insights into preventing cancer metastasis.

Microbial Modulation of Innate-adaptive Immune Crosstalk in Carcinogenesis

The tightly integrated network of innate and adaptive immunity allows microbiota to reshape adaptive responses while modulating innate immunity. Therefore, dissecting the contribution of the microbiota in carcinogenesis requires acknowledging the interdependence of this immune circuitry.

The microbiota modulates innate-adaptive immune cascades through structural components and metabolites. Compared to conventional pancreatic ductal adenocarcinoma (PDAC) mouse models, germ-free conditions or antibiotic treatment delayed PDAC progression, whereas microbiota transplantation from PDAC mice accelerated tumorigenesis. These findings underscore the critical involvement of microbiota in promoting cancer progression. Mechanistically, microbiota promote the polarization of M1 macrophages *via* TLR activation and thus suppress Th1 differentiation of $CD4^{+}$ T cells and $CD8^{+}$ T cell activation [192]. Emerging evidence indicates that diverse microbial metabolites exhibit anti-tumor effects by triggering innate-adaptive cascades. SCFAs attenuate CRC progression by interacting with free fatty acid receptor 2 (FFAR2) on DCs, inhibiting DC activation and IL-27 production, and restricting $CD8^{+}$ T cell depletion [193]. Similarly, *Lactobacillus plantarum*-derived indole-3-lactic acid inhibits tumorigenesis *via* promoting IL-12a production in DCs and enhancing $CD8^{+}$ T cell function [194]. The gut microbial metabolite butyrate activates G protein-coupled receptor 109a (GPR109a) expressed on macrophages and DCs, driving Treg differentiation and IL-10-secreting $CD4^{+}$ T cell production, which in turn prevents immune overactivation and ameliorates

inflammation-driven colon carcinogenesis [195]. These findings suggest that microbial metabolites exert an anti-tumor effect by promoting the maintenance of immune homeostasis. Specifically, microbial metabolites enhance tumor cell clearance by stimulating adaptive immunity to prevent immune evasion, while inhibiting adaptive immune cell hyperactivation that contributes to inflammation-associated carcinogenesis. Conversely, the microbiota can exert tumor-promoting effects. In PDAC mouse models, *Lactobacillus*-derived indole promotes the production of immunosuppressive macrophages *via* AHR, which in turn suppresses IFN-γ expression in $CD8^+$ T cells and anti-tumor immunity [196].

The microbiota can interact directly with adaptive immune cells, thereby influencing cancer development and progression. Isoallolithocholic acid potentiates Treg differentiation by increasing the production of mitochondrial ROS and FoxP3 expression [197]. SCFAs amplify the immunosuppressive capacity of Tregs through *Ffar2*-dependent upregulation of G protein-coupled receptor 43 (GPR43) on Tregs [198]. Inosine produced by *L. reuteri* attenuates the differentiation of Th1 and Th2 cells by binding to adenosine 2A receptor (A_2AR) on the surface of T cells [199]. However, in the presence of exogenous IFN-γ, inosine promotes Th1 differentiation through A_2AR-dependent signaling and enhances anti-tumor immunity in various cancer types, such as CRC, melanoma, and bladder cancer [200].

Emerging evidence indicates that intratumoral microbiota regulate cancer progression *via* immunomodulation. In HCC, intratumoral hepatitis C virus (HCV) recruits Treg cells to cancer-infiltrating tissues by upregulating the expression of Treg chemokine receptors while amplifying their immunosuppressive capacity, thereby fostering immune evasion [201]. In contrast, *Lachnoclostridium* regulates the production of chemokines, including CXCL9, CXCL10, and CCL5, and thus impacts $CD8^+$ T cell infiltration in cutaneous melanoma [202]. Intratumoral-resident viruses, such as hepatitis B virus (HBV), human papillomavirus, and Merkel cell polyomavirus can modulate $CD8^+$ T cell infiltration in a similar manner [203–205]. While these studies highlight the interplay between microbiota and immunity in the course of tumor progression, the precise mechanisms of immunomodulation remain to be further investigated.

MICROBIOTA MODULATES CANCER IMMUNOTHERAPY RESPONSES

Understanding the molecular mechanisms by which microbiota interact with innate immune cells and their impact on various periods of TME development is essential for cancer immunotherapy through microbiota modulation. It is further supported by recent discoveries in microbial-based anti-tumor therapy mechanisms [206–208]. Studies have shown that microbiota improves the efficacy of cancer immunotherapy, mitigates immunotherapy-related AEs, and reduces graft *versus* host disease (GVHD) undergoing hematopoietic cell transplantation (HCT). Related clinical trials on microbiota modulation in cancer immunotherapy are listed in Table 3. Currently, the main microbiota-based cancer immunotherapies include fecal microbiota transplantation (FMT), probiotics, prebiotics and antibiotics, bacteriophage therapy, and genetically engineered probiotics (Figure 5).

Fecal Microbiota Transplantation (FMT)

FMT, a microbial treatment by transferring feces from healthy humans to patients, has been shown to enhance the efficacy and reduce side effects of cancer immunotherapy. By transplanting commensal microbiota from anti-CD47 immunotherapy-responsive mice into non-responsive mice, researchers found that *Bifidobacterium* aggregated in TME, effectively stimulated STING signaling, and increased cross-priming of DCs, thus enhancing the anti-tumor effects of $CD8^+$ T cells [209]. Similarly, *Bacteroides fragilis* stimulates CD11b DCs to secrete IL-12, increasing Th1 responses in anti-cancer immunotherapy with cytotoxic T lymphocyte-associated antigen 4 (CTLA4) blockers [210]. Clinical studies exploring microbiota-mediated enhancement of immunotherapy have been conducted for various cancers, such as HCC, prostate cancer, renal cell carcinoma, gastrointestinal cancer, and melanoma (Table 3). Furthermore, FMT efficiently reduces immunotherapy-related AEs such as diarrhea/colitis. In a clinical study involving 12 cancer patients with immune-mediated colitis who were treated with FMT from healthy donors, it was found that 10 patients experienced symptomatic improvement, and an increase in the abundance of *Collinsella* and Bifidobacteria was found in stool samples from complete responders [211]. This clinical remission was verified in another clinical study [212]. Furthermore, microbiota potentially prevents GVHD in patients with hematologic malignancy undergoing HCT, and relevant clinical studies are ongoing (Table 3). Although accumulating clinical trials have demonstrated the efficiency of FMT in improving cancer immunotherapy, its long-term safety remains a concern. It was reported that two patients developed β-lactamase *E. coli* bacteremia after undergoing FMT, and one of them died, suggesting potential infection risks in FMT [213]. Therefore, it is worth learning from these initial trials for further optimization and conducting further clinical studies to ensure its safety and efficacy.

Probiotics

Probiotics such as *Bifidobacterium*, *A. muciniphila*, and *Faecalibacterium prausnitzii* have been shown to facilitate the efficacy of cancer immunotherapy [214–216]. As exogenous bacteria, probiotics can be recognized by innate immune cells, thereby activating immune response and modulating cell polarization, which collectively facilitate tumor cell clearance. For instance, *Bifidobacterium* increases DC function and promotes $CD8^+$ T cell infiltration in TME and tumor cell reduction, thus facilitating anti-PD-L1 efficacy [214]. A clinical trial has demonstrated that *B. fragilis* ZY-312 induces M1 polarization and upregulates gene expression of *IL12* and *IL1β* as well as CD80 and CD86 expression on cells, enhancing innate immunity [217]. The OMVs derived from *A. muciniphila* could recruit macrophages and polarize them to the M1 phenotype [215]. Additionally, bacterial PAMPs/DAMPs act as cancer therapeutic vaccine adjuvants to enhance anti-tumor immunity by binding to TLR on innate immune cells [218]. Conversely, probiotics reduce the side effects of immunotherapy by down-regulating the immune response and promoting inflammation reduction. In B16 tumor-bearing rats, *F. prausnitzii* mitigated immune checkpoint inhibitor (ICI)-mediated colitis by suppressing innate immune cell infiltration, such as macrophages, DCs, and neutrophils [216]. However,

Table 3. Clinical trials on microbiota modulation in cancer immunotherapy

NCT numbers	Cancer types	Phase	*n*	Interventions	Primary Outcomes
FMT					
Improve efficacy of cancer immunotherapy					
NCT05286294	Solid tumor	II	20	FMT + immunotherapy	Safety, ORR
NCT04264975	Solid tumor	NA	60	FMT + immunotherapy	ORR
NCT05273255	Solid tumor	NA	30	FMT + immunotherapy	Change in the intestinal microbiome community
NCT05750030	Hepatocellular carcinoma	II	12	FMT + atezolizumab + bevacizumab	Safety, efficacy
NCT05690048	Hepatocellular carcinoma	II	48	FMT + ICI *versus* Placebo FMT + ICI	Immunotherapy-related AEs, differential tumoral $CD8^+$ T cell infiltration
NCT06206707	Melanoma/Kidney cancer	NA	20	FMT + ICI *versus* Placebo FMT + ICI	Clinical remission of immune-mediated diarrhea
NCT04577729	Melanoma	NA	5	Allogenic FMT *versus* Autologous FMT	PFS
NCT04521075	Melanoma, MSI-H, dMMR, or NSCLC	I, II	42	FMT + anti-PD-1	Immunotherapy-related AEs, ORR
NCT05251389	Melanoma	I, II	24	ICI non-responding donor FMT *versus* ICI responding donor FMT	Efficacy
NCT03772899	Melanoma	I	20	FMT + immunotherapy	Safety
NCT03353402	Melanoma	I	40	FMT + immunotherapy	Immunotherapy-related AEs, proper implant engraftment
NCT04988841	Melanoma	II	60	FMT + ipilimumab + nivolumab *versus* Placebo + ipilimumab + nivolumab	Safety
NCT03341143	Melanoma	II	18	FMT + pembrolizumab	ORR
NCT05008861	NSCLC	I	20	FMT + anti-PD-1	Immunotherapy-related AEs, anti-PD-1/PD-L1-related AEs
NCT05502913	Lung cancer	II	80	FMT + SoC *versus* SoC	PFS
NCT04924374	Lung cancer	NA	20	FMT + anti-PD-1 *versus* Anti-PD-1	Safety
NCT05279677	Colorectal cancer	II	30	FMT + sintilimab + fruquintinib	ORR
NCT04729322	Colorectal cancer	II	15	FMT + pembrolizumab *versus* FMT + nivolumab	ORR

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Table 3. *Continued*

NCT numbers	Cancer types	Phase	*n*	Interventions	Primary Outcomes
NCT04130763	Gastrointestinal cancer	I	10	FMT + anti-PD-1	Immunotherapy-related AEs, ORR, rate of abnormal vital signs and laboratory test results
NCT04758507	Renal cell carcinoma	I, II	50	Donor FMT *versus* Placebo FMT	PFS
NCT04116775	Prostate cancer	II	32	FMT + pembrolizumab + enzalutamide	Anticancer effect of fecal microbiota transplant from responders to pembrolizumab to non-responders
Prevent immunotherapy-related AEs					
NCT04883762	Solid tumor	I	4	FMT + immunotherapy	Immunotherapy-related AEs
NCT06218602	Lymphoma	II	40	FMT + chemotherapy + CAR-T therapy *versus* Chemotherapy + CAR-T therapy	Immunotherapy-related AEs
NCT04163289	Renal cell carcinoma	I	20	FMT + SoC with nivolumab and ipilimumab	Occurrence of immune-related colitis associated with ipilimumab/ nivolumab treatment
NCT04038619	Genitourinary cancer	I	40	FMT + loperamide	Immunotherapy-related AEs
NCT04038619	Genitourinary cancer	I	40	FMT + loperamide	Immunotherapy-related AEs, clinical response/remission of immune-related diarrhea/colitis
NCT03819296	Melanoma or genitourinary cancer	I, II	800	FMT + SoC with prednisone, infliximab, or vedolizumab	Immunotherapy-related AEs, difference in stool microbiome pattern
Prevent GVHD undergoing HCT					
NCT06026371	Hematopoietic and lymphatic system Neoplasm	II	138	FMT *versus* Placebo	Grade III-IV acute GVHD
NCT06355583	Leukaemia	II	50	EBX-102 *versus* Placebo	Change in gut microbiota diversity
NCT05067595	Hematologic malignancy	I	72	Upper FMT *versus* Lower FMT *versus* Upper FMT + fiber supplementation *versus* Lower FMT + fiber supplementation	Bacterial composition of stool, bacterial genes in stool, immunotherapy-related AEs
NCT04935684	Hematologic malignancy	II	150	Allogeneic FMT *versus* No treatment	Graft-*versus*-host disease and relapse-free survival
NCT04139577	Hematologic malignancy	I	10	FMT	Proportion of patients able to swallow no less than 40 capsules

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Table 3. *Continued*

NCT numbers	Cancer types	Phase	*n*	Interventions	Primary Outcomes
NCT04269850	Hematologic malignancy	I, II	20	FMT + ruxolitinib + steroids	Overall survival
NCT03812705	Hematopoietic and lymphatic system Neoplasm	II	30	FMT	ORR
NCT04769895	Hematologic malignancy	III	75	MaaT013	ORR
Probiotics					
Improve efficacy of cancer immunotherapy					
NCT05939791	Acute lymphoblastic leukemia	NA	29	*Lactobacillus casei*: CNCMI-1518 + physical exercise	The shift of the gut microbiome
NCT03829111	Renal cell carcinoma	I	30	*Clostridium butyricum*: CBM588 + nivolumab/ipilimumab *versus* Nivolumab/Ipilimumab	Change in *Bifidobacterium* composition of stool
NCT03775850	Colorectal carcinoma, triple-negative breast cancer and checkpoint inhibitor relapsed tumors	I	69	*Bifidobacterium*: EDP1503 + pembrolizumab	Immunotherapy-related AEs, ORR
NCT01895530	Colorectal cancer	NA	33	*Saccharomyces boulardii*: probiotic *versus* No intervention	Difference in gene expression of cytokines, postoperative complications
NCT03817125	Melanoma	I	14	Defined bacterial consortia: SER-401 + nivolumab *versus* Placebo + nivolumab	Immunotherapy-related AEs
NCT03686202	Solid tumor	II, III	65	*Bifidobacterium*: MET-4 + SoC *versus* SoC	Immunotherapy-related AEs, immunotherapy-responsiveness associated species/MET-4
NCT05079503	Rectal cancer	NA	40	*Lactococcus lactis*: GEN-001 + total neoadjuvant therapy	Change of gut microbiome, immune modulation
NCT04208958	Colorectal cancer gastric/ Gastroesophageal junction adenocarcinoma, or melanoma	I, II	56	11 commensal bacterial: VE800 + nivolumab + vancomycin oral capsule	Immunotherapy-related AEs, ORR
NCT03595683	Melanoma	II	8	*Bifidobacterium*: EDP1503 + pembrolizumab + anti-PD1 naïve *versus* EDP1503 + pembrolizumab + anti-PD1 refractory	ORR

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Table 3. *Continued*

NCT numbers	Cancer types	Phase	*n*	Interventions	Primary Outcomes
NCT03637803	Renal cell carcinoma, bladder cancer, melanoma, or NSCLC	I, II	63	*Enterococcus gallinarum*: MRx0518 + pembrolizumab	Immunotherapy-related AEs, clinical benefit
Prevent immunotherapy-related AEs					
NCT02169388	Colorectal cancer	I	30	*Clostridium butyricum*: probiotic *versus* Placebo	Immunotherapy-related AEs, short-chain fatty acids, composition of microorganisms in stool
NCT01790035	Gastrointestinal cancer	I	23	LGG (containing 10^{10} viable bacteria) *versus* Placebo *versus* No intervention	Safety, efficacy
NCT06039644	Breast cancer	NA	100	*Lactobacillus paracasei/ Lactobacillus plantarum/ Lactobacillus reuteri*: probiotic *versus* Placebo	Immunotherapy-related AEs
NCT03704727	Gastrointestinal cancer	NA	5	*Lactobacilli* + *Bifidum* + *Streptococcus thermophilus*: probiotic treatment	Intestinal permeability
NCT02771470	Lung cancer	I	41	*Clostridium butyricum*: probiotic *versus* Placebo	Composition of microorganisms in stool
NCT00197873	Colorectal cancer	NA	84	*Lactophilus*: probiotic *versus* Placebo	Effect on the treatment-related grade 2 to 4 diarrhoea
NCT04699721	NSCLC	I	40	*Bifidobacterium trifidum*: probiotic + nivolumab + paclitaxel + carboplatin AUC5	Safety, efficacy
NCT03705442	Colorectal cancer	II	76	OMNi-BiOTiC® 10AAD: probiotic + loperamide *versus* Placebo + loperamide	Incidence of grade III/IV diarrhoea
Prevent GVHD undergoing HCT					
NCT00946283	Hematologic cancer or myelodysplastic syndrome	NA	30	*Lactobacillus GG*: probiotic treatment	Safety
NCT02144701	Hematologic malignancy	NA	33	*Lactobacillus GG*: probiotic *versus* Placebo	Rate of GVHD
NCT03922035	Hematopoietic and lymphatic system Neoplasm	I	36	*Clostridium butyricum* CBM588: probiotic treatment	Immunotherapy-related AEs, feasibility of CBM588
Genetically engineered microbiota					

(*Continued on next page*)

Table 3. *Continued*

NCT numbers	Cancer types	Phase	*n*	Interventions	Primary Outcomes
NCT04025307	Solid tumor	I	5	*Bifidobacterium longum*: bacTRL-IL-12	Immunotherapy-related AEs
NCT04857697	Breast or lung cancer	I	6	Probiotic + biospecimen collection	Length and adherence of probiotics; percentage of $CD8^+$, $CD4^+$, and T-reg cells; cytokine counts
NCT04167137	Lymphoma, solid tumor	I	70	*Escherichia coli*: SYNB1891 *versus* SYNB1891+ atezolizumab	Incidence of dose-limiting toxicities
NCT03358511	Breast cancer	NA	7	Primal Defense ULTRA: probiotic treatment	Mean number of $CD8^+$ lymphocytes
Prebiotics					
NCT06250335	Melanoma	II	60	Prebiotic food-enriched diet + ipilimumab + nivolumab	Immunotherapy-related AEs
NCT05303493	Melanoma, NSCLC	I	45	Camu camu capsules + ICI	Immunotherapy-related AEs
NCT05821751	Head and neck squamous cell carcinoma	NA	40	Inulin + pembrolizumab *versus* Inulin + nivolumab	Diversity in the gut microbiota
NCT05135351	Lymphoma, multiple Myeloma	NA	30	Resistant starch *versus* Maltodextrin	Percentage of subjects who adhere to > 70% of scheduled doses of the intervention
Vaccines					
NCT05350501	Colorectal Cancer	II	1	EO2040 + nivolumab	Response to treatment at 6 months
NCT04187404	Adrenocortical carcinoma, pheochromocytoma, or paraganglioma	I, II	70	EO2401 + nivolumab	Vaccines-related AEs
NCT02718430	Colorectal cancer	I	6	VXM01	Vaccines-related AEs
NCT03821272	Head and neck cancer	I, II	21	PepCan *versus* Placebo	Vaccines-related AEs
NCT02625857	Prostatic neoplasms	I	26	JNJ-64041809	Incidence of dose-limiting-toxicity, antigen-specific T-cell response, Vaccines-related AEs
NCT03421236	Non-muscle-invasive Bladder Cancer	I	25	Intravesical administration of Ty21a	Vaccines-related AEs
NCT04116658	Glioblastoma	I, II	100	EO2041 + nivolumab + bevacizumab	Vaccines-related AEs
Antibiotics					

(*Continued on next page*)

Table 3. *Continued*

NCT numbers	Cancer types	Phase	*n*	Interventions	Primary Outcomes
NCT02366884	Solid tumor	II	250	Anti-bacterial agents *versus* Anti-fungal agents *versus* Anti-protozoal agents *versus* Anti-bacterial + anti-fungal + anti-protozoal agents	ORR
NCT04281667	Colorectal cancer	IV	604	Oral antibiotics + mechanical bowel preparation *versus* Placebo + mechanical bowel preparation	Comprehensive complication index
NCT02809729	Breast cancer	NA	124	Cefazolin *versus* Placebo	Surgical site infection in oncologic breast surgery
NCT05777603	NSCLC	I	20	Aerosolized aztreonam + aerosolized vancomycin + pembrolizumab	Dose limiting toxicities

Abbreviations: AEs, adverse events; dMMR, mismatch-repair deficient; FMT, fecal microbiota transplant; GVHD, graft versus host disease; HCT, hematopoietic cell transplantation; ICI, immune checkpoint inhibitor; MET, microbial ecosystem therapeutics; MSI-H, microsatellite instability-high; n, number of patients; NA, not applicable; NSCLC, non-small cell lung cancer; ORR, objective response rate; PD-1, programmed cell death 1; PD-L1, programmed cell death-ligand 1; PFS, progression-free survival; SoC, standard of care.

in some immunocompromised cancer patients, probiotics may be converted into opportunistic pathogens and fail to benefit patients [219]. Therefore, exploring precision probiotics tailored to individual clinical characteristics as adjuvants for cancer immunotherapy may be a promising direction.

Prebiotics and Antibiotics

Prebiotics, substrates that are selectively utilized by host microbiota for health benefits, can enhance cancer immunotherapy efficacy by promoting certain microbiota growth. For instance, prebiotics, such as fructans and galactans, may act by enriching *Lactobacillus* and/or *Bifidobacterium* [220]. Mechanistically, a high-fiber diet promotes microbial metabolite c-di-AMP production, which induces IFN-I generation by intratumoral monocytes. This process facilitates NK cell infiltration and DC recruitment in TME, and the recruited DCs can activate NK cells *via* IL-15/IL15-R [143]. An observational study of 128 cancer patients undergoing immunotherapy found that higher levels of prebiotic dietary fiber were significantly associated with better outcomes and lower rates of AEs, showing great potential for prebiotics in cancer immunotherapy [221].

Antibiotics can inhibit or clear cancer-promoting microbiota, thereby strengthening the anti-tumor immune response. Vancomycin-mediated intestinal microbiota enhances IL-12 production by CD8α^+ DCs, thereby maintaining the anti-tumor efficacy of effector T cells [222]. Compared to narrow-spectrum antibiotics, broad-spectrum antibiotics are more likely to disrupt the host microbial balance, induce dysbiosis, and promote cancer progression, thus limiting their clinical use. In melanoma patients undergoing immunotherapy, broad-spectrum antibiotics reduced the probiotic *Bifidobacterium* or *Akkermansia* population, weakening therapeutic effects [223].

As such, precisely regulating the microbial balance in the host through probiotics or antibiotics presents a significant challenge. Future strategies should focus on developing targeted interventions that selectively eliminate harmful microbiota while preserving beneficial components of the microbiota to optimize cancer immunotherapy outcomes.

Bacteriophages

Bacteriophage therapy has emerged as a promising vehicle for drug delivery in cancer immunotherapy and targeted therapies, attributed to its antigenicity, high specificity, and low toxicity. Mechanistically, bacteriophages activate the immune system and promote cancer cell destruction while effectively reducing harm to healthy cells. Engineered bacteriophage T7 carrying granulocyte-macrophage CSF (GM-CSF) effectively inhibited the growth of B16F10 melanoma cells. In treated groups, all mice survived until the end of the experiment, compared to a 40% survival rate in the untreated group. Mechanistically, bacteriophage T7 recruited anti-tumor immune cells such as DCs, macrophages, and CD8$^+$ T cells, as confirmed by immunohistochemical analysis of tumor tissues [224]. Similar results were observed in another animal study investigating CRC treatment with M13 bacteriophage therapy [225]. Additionally, bacteriophage therapy offers a targeted and efficient approach to addressing bacterial infections. High-fat diets have been shown

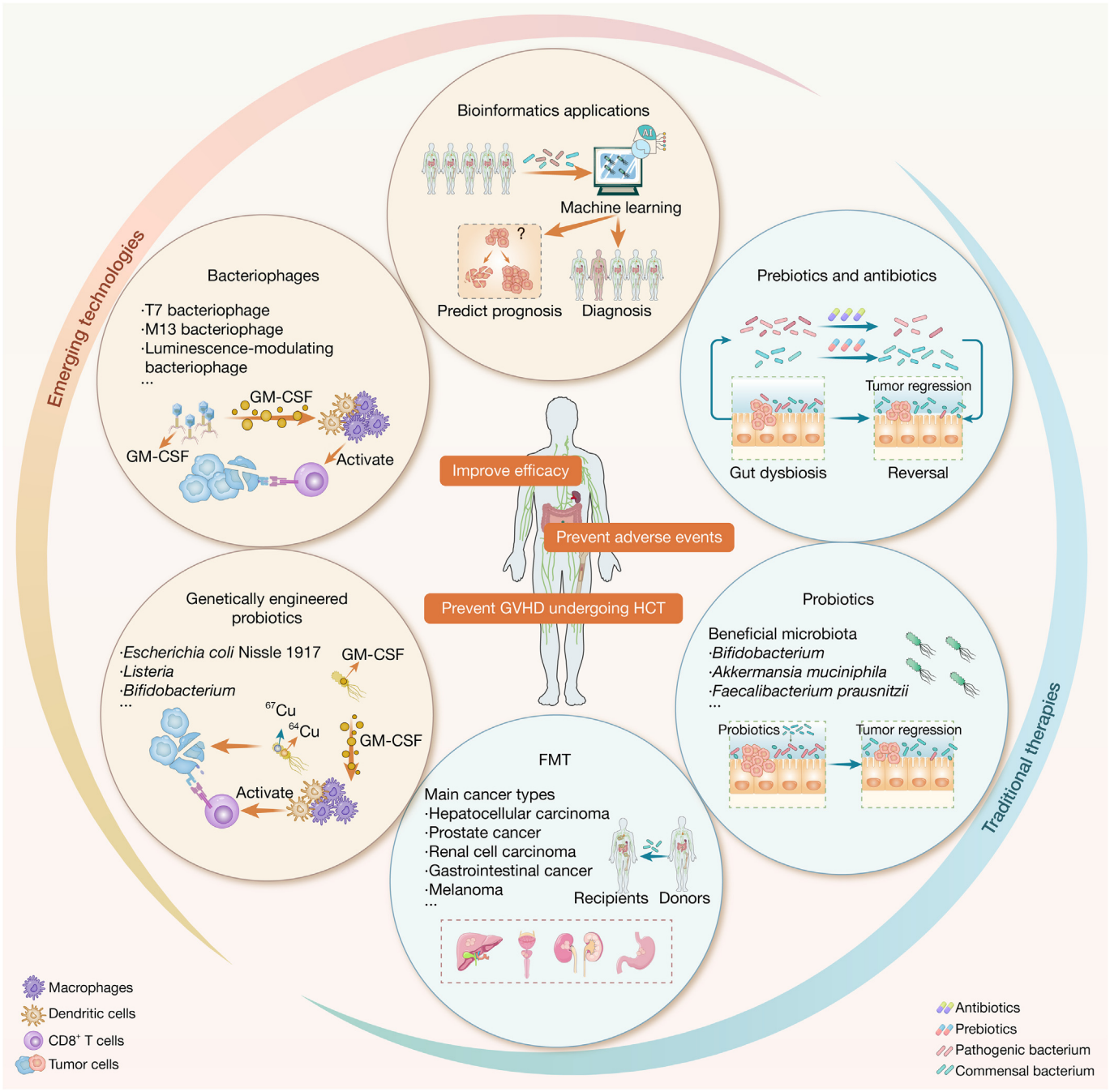

Figure 5. Microbiota in cancer immunotherapy
Microbiota-based cancer immunotherapies mainly include traditional therapies, including FMT, probiotics, prebiotics and antibiotics, and emerging technologies, including bacteriophage therapy, genetically engineered probiotics, and artificial intelligence. Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; FMT, fecal microbiota transplantation; GVHD, graft *versus* host disease; HCT, hematopoietic cell transplantation.

to increase the abundance of Firmicutes and Proteobacteria, which activate NF-κB and promote cancer progression [226,227]. However, transplantation of bacteriophage-rich fecal virus-like particles reduced high-fat diet-induced intestinal dysbiosis in mice, an effect similarly observed in human studies [228,229]. Furthermore, the utility of phages as biomarkers in cancer diagnosis has been demonstrated, achieving 80% sensitivity and 75% specificity in detecting grade 4 and 5 prostate cancer using luminescence-modulating phages [230]. Compared with natural bacteriophages, engineered bacteriophages are more susceptible to inactivation by the complement system, posing challenges for phage-induced therapies [231].

Genetically Engineered Probiotics

Genetically engineered probiotics represent a novel class of microbiota created by gene editing existing probiotics and can be employed as adjuvants for cancer immunotherapy to enhance anti-tumor responses. Currently, genetically engineered

attenuated, auxiliary, and inducible microbiota such as *E. coli*, *Bifidobacterium*, and *Listeria* have been modified and demonstrated anti-tumor responses in preclinical models *via* intravenous, intratumor, and oral administration routes [232]. Gurbatri et al. found that engineered probiotics yielded GM-CSF, which strengthened the antigen-presenting effect of innate immune cells, recruited T cells to TME, and ultimately enhanced immunotherapy efficacy in mice with poor immunogenicity [233]. In addition, genetically engineered probiotics can also be used as carriers of radiopharmaceuticals to better target tumor tissue independent of tumor epitope and receptor phenotype, thereby reducing damage to normal tissue. It was confirmed by the remarkable efficacy of *E. coli* Nissle 1917 (EcN) carrying ^{64}Cu and ^{67}Cu in the treatment of mice with solid tumors [234]. Genetically engineered bacteria have made significant progress in CRC screening and treatment. In a clinical trial, EcN was modified to selectively colonize tumor tissue, with PCR analysis revealing a higher concentration of EcN-specific amplicons in tumors *versus* normal tissues in treated patients [235]. Producing salicylate detectable in urine, EcN offered a non-invasive route for CRC detection. Additionally, EcN secreted GM-CSF and nanobodies targeting PD-L1 and CTLA-4 at the tumor site, reducing tumor burden by approximately 50%. CRC growth inhibition and melanoma metastasis blockage were achieved by modifying tumor antigens on engineered bacterial-derived OMV surfaces, showing great potential for personalized tumor vaccines [82]. Current attempts at microbiota-based vaccines have achieved success in preventing *Mycobacterium tuberculosis* infection [236].

CONCLUDING REMARKS AND PERSPECTIVES

Microbiota modulate the development and function of the innate immune system through bacterial components, microbial metabolites, and other mediators. In turn, innate immunity specifically recognizes microbiota, establishing immune tolerance or clearing pathogens. This interaction impacts several aspects of cancer initiation and progression. Although the potential of microbiota to enhance cancer immunotherapy is well-recognized, considerable clinical and translational challenges must be overcome to fully leverage its therapeutic benefits. Most findings are derived from laboratory studies, but when these exogenous microbiota enter the human gut, they interact with complex resident microbial communities, potentially impacting immunotherapy efficacy. Utilizing humanized mouse models to better simulate human microbiota and conducting larger, rigorously designed clinical trials offers a prospective path forward [13].

The reproducibility and generalizability of microbiota-based cancer immunotherapy remain limited due to variability in studied populations, methods, or statistics [237]. The advancement of bioinformatics applications in medicine presents a promising avenue for addressing these challenges [238–240]. Bioinformatics, particularly machine learning, has emerged as a powerful data analysis tool to identify microbiota and metabolites with critical roles and elucidate their potential molecular mechanisms, thereby enhancing result generalizability. This approach presents a new frontier in microbiota identification, cancer diagnosis, and prognosis prediction (Figure 5). Machine learning models leveraging gut microbiota data have shown substantial promise in diagnosing HCC (NCT04339725) [241]. Villani et al. employed iterative Random Forest models to analyze gut microbiota differences between patients with metastatic and non-metastatic pancreatic cancer, subsequently developing predictive models to estimate the impact of microbial population abundances on cancer metastasis [242]. This predictive model offers guidance for prognostic assessment in pancreatic cancer and a potential approach to enhance outcomes through modulating gut microbiota. Random Forest machine learning models derived from Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation-associated gut microbiota enable the investigation of molecular interactions between microbiota and tumor-infiltrating immune cells influencing tumor progression, underscoring the potential for microbiota-based cancer immunotherapy [243]. However, current microbiota-based machine-learning models are often constrained by small, ethnically homogeneous samples and lack extensive validation. Access to large, multi-ethnic datasets from open databases may further improve model accuracy and stability. There is also a lack of in-depth research on integrating machine learning predictive models with cancer treatment strategies.

AEs associated with microbial therapy constrain the application of microbiota in cancer immunotherapy, and emerging technologies offer potential solutions to address these limitations. These AEs include FMT-induced infections, opportunistic infections from probiotics in immunocompromised patients, and dysbiosis caused by broad-spectrum antibiotics [213,219,223]. Infection risk may be reduced by integrating bioinformatics applications to refine FMT donor screening protocols and enable the timely detection of potential pathogens. The personalized nature of synthetic biology approaches, such as genetically engineered probiotics and phage therapies, enables them to minimize adverse reactions associated with diverse microbial strains.

Hence, future efforts should prioritize the advancement of emerging technologies and their integration with traditional approaches to developing more precise and personalized microbiota-mediated cancer immunotherapies. Furthermore, standardization of microbiological methodologies and advances in serological testing represent critical research avenues for enhancing experimental reproducibility [244,245]. However, how can we reconcile the differences between animal models and the dynamic, heterogeneous human microbiota to ensure translatability? Could dynamic, real-time microbiome monitoring systems be developed to inform the adjustment of cancer treatment regimens and predict prognosis? How to balance microbial intervention efficacy with host immune tolerance to avoid paradoxical hyperprogression? By addressing these open questions, it is anticipated to offer novel insights for developing microbiota-based cancer immunotherapy strategies.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

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