Review

Interplay between Gut Microbiome, Metabolites, and Tumor Immunity: Mechanisms and Clinical Translation

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Abstract: Gut microbiota-derived metabolites critically modulate the efficacy of tumor immunotherapy, particularly immune checkpoint inhibitors, by orchestrating key immunological mechanisms within the tumor immune microenvironment. Through multifaceted pathways, they dynamically regulate both innate and adaptive immunity or reshape tumor immunogenicity, thereby maintaining the delicate equilibrium between antitumor immune activation and suppression. Moreover, some microbial metabolites (e.g., butyrate, polyamines, succinic acid) have paradoxical or dual functions depending on the context (e.g., cancer type). A comprehensive understanding of the complex interplay among microbiota, metabolism, and immunity is important for clarifying individual variations in immunotherapeutic outcomes and may help inform strategies to overcome resistance. Current microbiota-based therapies, including probiotics, genetically engineered bacteria, and fecal microbiota transplantation, as well as interventions targeting metabolic pathways, are emerging as promising strategies to enhance immunotherapy by modulating host metabolic processes. However, several major challenges hinder clinical translation, including the bidirectional effects and concentration-dependent activity of metabolites, issues in delivery efficiency, and significant inter-individual heterogeneity. This review aims to systematically summarize the main mechanisms by which gut microbial metabolites regulate antitumor immunity and to explore the current landscape, strategies, and obstacles in their clinical application. Overall, it may provide a theoretical framework and practical perspectives for the future development of personalized tumor immunotherapies based on microbiome and metabolic interventions.

Keywords: gut microbial metabolites; immunity; cancer immunotherapy; immune microenvironment; metabolic interventions

1. Introduction

Tumor immunotherapy offers a revolutionary treatment approach by harnessing the patient's immune system to achieve powerful and durable antitumor responses, distinct from traditional modalities like surgery, chemotherapy, radiotherapy, and targeted therapy [1]. Strategies such as immune checkpoint inhibitors (ICIs) and CAR-T cell therapy have demonstrated remarkable success. However, their effectiveness is limited by significant challenges, including variable patient response rates and common adverse events. These limitations may stem from inherent complexities in the tumor-immune microenvironment [2]. ICIs, such as inhibitors targeting PD-1/PD-L1 and CTLA-4, restore T cell-mediated antitumor immunity by blocking coinhibitory signals. These agents have demonstrated remarkable clinical efficacy in malignancies such as melanoma and non-small cell lung cancer, fundamentally transforming the landscape of cancer therapy [3]. However, the therapeutic response to ICIs exhibits marked tumor heterogeneity, with objective response rates remaining below 40%. Additionally, challenges such as acquired resistance and immune-related adverse events continue to significantly hinder their broader clinical application [4]. Improving the antitumor immune response and enhancing the sensitivity of tumor immunotherapy is very important for clinical practice.

The human gut microbiota constitutes a highly heterogeneous ecosystem, containing at least 38 trillion microbial cells [5] and the approximately 3.3 million microbial genes they encode. It has been referred to as the "second genome" of humans [6,7]. This system plays a central role in host health by regulating energy metabolism, mediating immune cell differentiation, and maintaining mucosal barrier homeostasis [8]. The gut microbiota can influence drug toxicity and modulate therapeutic efficacy, either attenuating or enhancing it, through various mechanisms including metabolite production, microbiota-host interactions, immune signal modulation, and biotransformation of drug molecules. As a result, it has emerged as a key biological variable in determining clinical drug responses [9].

In recent years, extensive clinical and preclinical studies have confirmed a close association between gut microbiota (especially its metabolites) and the therapeutic response to ICIs [10–13]. Specifically, microbial metabolites affect ICIs treatment outcomes by modulating three central pathways: innate immunity, adaptive immunity, and tumor immunogenicity. Short-chain fatty acids (SCFAs) produced by gut microbiota fermenting dietary fiber inhibit histone deacetylase (HDAC), relieve gene silencing, up-regulate tumor PD-L1 expression, and promote T cell infiltration, thereby amplifying the antitumor response of ICIs. Clinical studies have shown that higher concentrations of SCFAs are associated with longer progression-free survival for patients, and the concentration of fecal short-chain fatty acids may be related to the efficacy of PD-1 inhibitors [14]. *Lactobacillus reuteri* and its metabolite indole-3-acetaldehyde (I3A) enhanced antitumor immunity by activating the AhR signaling pathway of $CD8^+$ T cells and significantly improved the efficacy of ICIs [15]. Another clinical study revealed that in patients with advanced melanoma who were resistant to PD-1 treatment and received fecal microbiota transplantation (FMT) combined with anti-PD-1 immunotherapy, significant changes occurred in the gut microbiota-related metabolites (such as serum bile acids, hippuric acid, etc.) of these patients. Moreover, these changes in metabolites were associated with the clinical benefits of FMT combined with anti-PD-1 treatment for the patients, and they participated in the regulatory process of the treatment response [16]. Gut microbiota-derived metabolites may influence tumor development and cancer treatment through multiple immunological and metabolic pathways, and have significant potential in enhancing cancer immunotherapy.

This review aims to systematically summarize and critically discuss the key molecular mechanisms by which gut microbiota metabolites regulate antitumor immune responses, with an emphasis on the interplay among metabolites, immune cells, and the tumor microenvironment (TME). Additionally, the clinical translational potential of dietary interventions, probiotics, engineered bacteria, and FMT is evaluated, thereby providing a foundation for further research in tumor immunotherapy. Ultimately, this review integrates insights back into clinical practice to establish a basis for the development of novel immunotherapy combinations.

2. Classification and Source of Gut Microbiota Metabolites

Based on their dietary origins and metabolic pathways, gut microbial metabolites can be classified into several major categories: SCFAs, bile acids, tryptophan metabolites, trimethylamine *N*-oxide (TMAO), polyamines, nucleotide derivatives, and succinic acid [17]. SCFAs (including acetate, propionate, and butyrate) are the primary fermentation products of dietary fiber by gut microbiota. Their concentrations in the gut are influenced by microbial composition, the host's intake of dietary fiber, and intestinal transit time [18], whereas their production efficiency is jointly regulated by intestinal pH, microbial diversity, and host metabolic state [19,20]. Soluble fibers such as inulin and pectin have been shown to significantly increase SCFAs levels in the intestine [21]. Gut bacteria rich in bile salt hydrolase (BSH) can catalyze the deconjugation of primary bile acids synthesized in the liver, leading to the formation of secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid (LCA). These secondary bile acids can be further diversified through microbial-mediated dehydroxylation, epimerization, and oxidation [22]. Bile acids exert immunomodulatory and anti-inflammatory effects by activating nuclear receptors such as the pregnane X receptor (PXR), farnesoid X receptor (FXR), and TGR5. Tryptophan metabolites are produced by intestinal strains expressing tryptophanase, which metabolize tryptophan into various indole derivatives such as indole-3-acetic acid, indole-3-propionic acid, and indole-3-aldehyde. These compounds can function as agonists for the aryl hydrocarbon receptor (AhR) and PXR, contributing to host immune regulation [23]. The production of these metabolites is critically dependent on dietary tryptophan intake and the composition of the gut microbiota [24]. TMAO, an important gut microbial secondary metabolite generated from choline via microbial choline trimethylamine lyase (CutC/D)-mediated conversion to trimethylamine (TMA), followed by hepatic oxidation through flavin-containing monooxygenase 3 (FMO3), has been implicated in modulating tumor immunity [25]. Polyamines such as putrescine and spermidine are synthesized through the coordinated activity of both host and microbial pathways. Gut microbes catalyze the decarboxylation of amino acids using enzymes such as ornithine decarboxylase, arginine decarboxylase, and spermidine synthase to generate various polyamines [26].

Additionally, microbial enzymes such as purine nucleoside phosphorylase and adenylate cyclase mediate the biosynthesis of nucleotide derivatives like inosine and cyclic adenosine monophosphate (c-di-AMP). The abundance of these metabolites is directly influenced by dietary nucleotide intake [27]. Succinic acid is a key intermediate in the energy metabolism of anaerobic bacteria such as *Fusobacterium nucleatum* and is produced via the succinate dehydrogenase (SDH)-catalyzed conversion of fumarate [28].

3. Immune Regulatory Mechanisms of Metabolites of Gut Microbiota

Studies have demonstrated that gut microbiota-derived metabolites serve as critical mediators of immune regulation between the microbiota and the host. Therefore, it is essential to investigate the specific mechanisms through which these metabolites participate in antitumor immune modulation. In the following sections, we will explore the intricate roles of gut microbial metabolites in tumor immune regulation, focusing on their effects on innate immunity, adaptive immunity, and tumor immunogenicity (Figure 1 and Table 1).

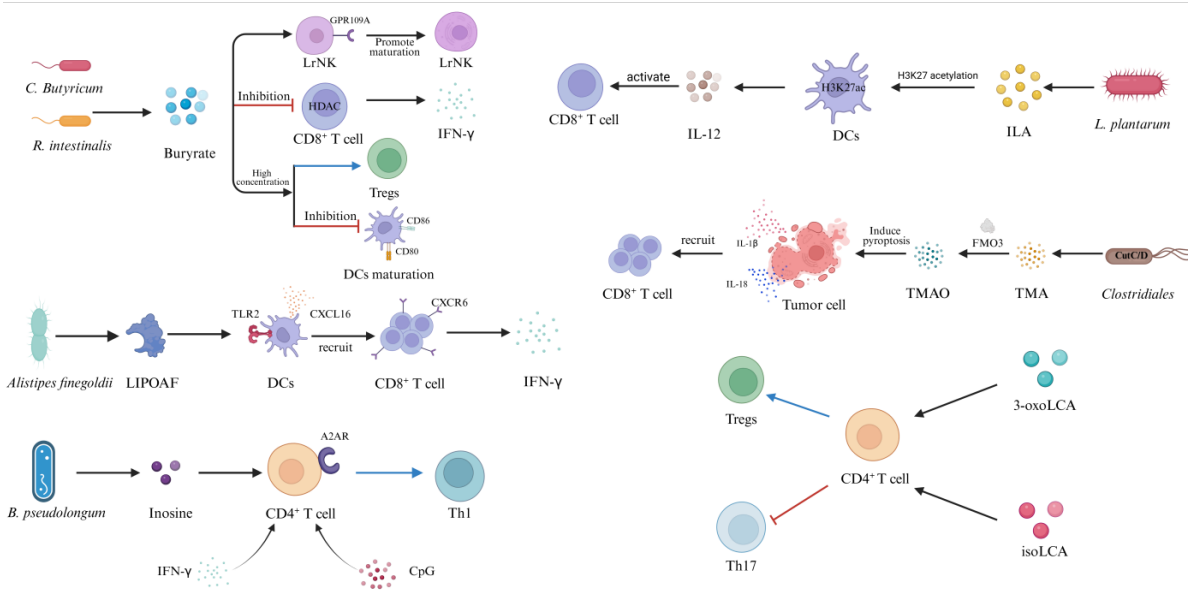

Figure 1. Microbiota-derived metabolites regulate antitumor immunity and immunotherapy response. Butyrate, produced by *C. butyricum* and *R. intestinalis*, promotes the maturation of LrNK cells through GPR109A and enhances their antitumor activity. It also increases IFN-γ secretion by $CD8^+$ T cells via HDAC inhibition. However, high concentrations of butyrate promote Treg differentiation, reduce CD80/CD86 expression on DCs, and inhibit DC maturation. LIPOAF secreted by *Alistipes finegoldii* binds TLR2 on DCs and induces CXCL16 secretion, which recruits $CXCR6^+$ cytotoxic $CD8^+$ T cells and enhances IFN-γ expression. Inosine produced by *B. pseudolongum* binds A2AR on $CD4^+$ T cells and, together with IFN-γ and CpG, promotes Th1 differentiation. ILA generated by *L. plantarum* enhances H3K27ac at the IL-12 enhancer in DCs and increases IL-12 secretion, thereby activating $CD8^+$ T-cell antitumor responses. *Clostridiales*-derived TMA is converted into TMAO by FMO3. TMAO induces GSDME-mediated pyroptosis in tumor cells through the PERK–eIF2α pathway and promotes the release of IL-1β and IL-18, thereby enhancing $CD8^+$ T-cell antitumor immunity. The LCA derivatives 3-oxoLCA and isoLCA regulate $CD4^+$ T-cell differentiation by promoting Treg cells and inhibiting Th17 cells.

Table 1. Gut microbiota-derived metabolites, immune targets, mechanisms, and cancer relevance.

Immune Cell Types	Metabolites	Related Species	Mechanism	Therapeutic Significance	Disease/Cancer Type	Reference
DCs	ILA	*L. plantarum* L168	Promotes IL-12 transcription, activates $CD8^+$ T cells	Enhance tumor immunity and inhibit tumor growth	CRC, Melanoma	[29]
	SCFAs	Dietary fiber fermenting bacteria (e.g., *Bacteroides* spp.)	Activation of FFAR2/HDAC enhances antigen presentation	Modulates antigen presentation	CRC, IBD	[30,31]
Macrophages	Propionate	*Prevotella* spp.	Inhibits M1 polarization	Alleviates inflammation but suppresses antitumor	IBD, CRC	[32]

Table 1. *Cont.*

Immune Cell Types	Metabolites	Related Species	Mechanism	Therapeutic Significance	Disease/Cancer Type	Reference
	Succinate	*F. nucleatum*	Promotes M2 polarization via HIF-1α	Promotes tumor angiogenesis	CRC	[28,33]
	Serine	Host-microbiota co-metabolism	Promotes M1, inhibits M2	Enhances antitumor immunity	Pancreatic cancer, CRC	[34]
NK cells	c-di-AMP	*E. coli* Nissle1917	Activates STING pathway	Enhances immunotherapy efficacy	Melanoma, solid tumors	[35,36]
	Serine	*E. recta* + *le*	Enhances NK activity	Improves immune response	Melanoma	[37]
	isoLCA	*Bacteroides ovatus*	Suppresses NK function	Tumor immune evasion	CRC	[38]
	Butyrate	*R. intestinalis* et al	Activates GPR109A, enhances LrNK function	Prevents liver cancer	Hepatocellular carcinoma	[39]
$CD8^+$ T cells	Butyrate	*C. butyricum*	HDAC inhibition, boosts effector function	Reverses T cell exhaustion	Melanoma	[40,41]
	TMAO	Choline metabolizing bacteria (e.g., *Proteobacteria*)	Promotes pyroptosis, IL-1β release	Enhances antitumor immunity	CRC	[42]
	DCA	*Bacteroides* spp.	Suppresses Ca^{2+}-NFAT2	Weakens antitumor immunity	CRC, Liver cancer	[43]
	IPA, I3A, IAA	*L. johnsonii, C. sporogenes, L. reuteri*	Activates AhR, boosts Tpex/Teff	Improves PD-1 response	Melanoma, CRC	[15,44,45]
$CD4^+$ T cells	DCA, LCA	*Clostridium* spp.	Modulates Treg/Th17	Promotes tolerance, cancer progression	CRC	[46,47]
	Kynurenine	*Lactobacillus* spp.	Activates AhR, induces Treg	Immunosuppression	Melanoma	[48,49]
	Inosine	*B. pseudolongum*	Regulates A2AR axis	Improves T cell function	Melanoma, CRC	[50,51]

3.1. Innate Immunity

3.1.1. Dendritic Cells (DCs)

DCs play a key role in the mammalian immune system. As the principal antigen-presenting cells of the innate immune system, DCs serve as a critical bridge between innate and adaptive immunity. Their functional status plays a pivotal role in determining the efficacy of tumor immunotherapy. By recognizing tumor-associated antigens and migrating to lymph nodes, DCs present these antigens to T cells, thereby initiating a specific antitumor immune response [52]. However, within the TME, DCs frequently exhibit upregulated expression of PD-L1, which contributes to an immunosuppressive milieu [53]. Different metabolites of gut microbiota can directly or indirectly affect the antitumor immune response by acting on DCs through different mechanisms.

The *Lactobacillus plantarum* L168 strain and its metabolite indole-3-lactic acid (ILA) significantly enhance antitumor immunity through an epigenetic mechanism. In DCs, ILA promotes histone H3K27 acetylation (H3K27ac) at the enhancer regions of the IL12a gene, thereby increasing IL12a production and promoting $CD8^+$ T-cell priming. Unlike short-chain fatty acids such as butyrate, this process does not rely on HDAC inhibition but instead involves the recruitment of pioneer transcription factors such as PU.1, which reshape enhancer–promoter interactions. In addition, ILA alters chromatin accessibility in $CD8^+$ T cells and transcriptionally inhibits Saa3 expression, thereby enhancing the function of tumor-infiltrating $CD8^+$ T cells. In the colorectal cancer (CRC) model, ILA promoted the proliferation and effector function of tumor-infiltrating $CD8^+$ T cells, accompanied by increased IFN-γ and granzyme B (GzmB) expression and reduced tumor growth [29].

SCFAs, as the core products of gut microbiota to utilize dietary fiber, directly regulate the immunophenotype of DCs by activating free fatty acid receptor 2 (FFAR2). In CRC mouse models, FFAR2 deficiency resulted in DC hyperactivation (increased percentage of $CD80^+$) and increased apoptosis, along with enhanced IL-27 secretion. IL-27 directly drives the exhaustion of $CD8^+$ T cells by inducing the expression of inhibitory receptors such as CD39 and PD-1. In contrast, exogenous FFAR2 agonist inhibited the activation of the NF-κB pathway in DCs, down-regulated IL-27 expression, and reduced the proportion of $CD39^+PD\text{-}1^+$ exhausted $CD8^+$ T cells in the tumor, ultimately reducing colon tumor burden. This mechanism was validated in clinical samples: IL-27 mRNA expression was significantly increased in tumor tissues of patients with CRC, and anti-IL-27 treatment inhibited tumor progression in a mouse model, highlighting the therapeutic potential of SCFA-FFAR2-IL-27 axis as a target [54]. Meanwhile, SCFAs profoundly influence the antigen-presenting function of DCs by regulating their maturation

and morphological plasticity. Studies have demonstrated that SCFAs, such as butyrate and propionate, enhance the activity of Rho family GTPases (Cdc42/Rac1) by inhibiting HDAC and activating the Src family kinase (SFK)/phosphatidylinositol 3-kinase (PI3K) signaling pathway. This activation drives actin polymerization, ultimately promoting dendrite extension in DCs. These morphological alterations significantly improve the capacity of DCs to capture both soluble antigens (e.g., ovalbumin) and particulate antigens (such as *Staphylococcus aureus*, and enhance the efficiency of major histocompatibility complex class II (MHC-II) molecules in presenting antigenic peptides, thereby promoting T cell activation [30]. Notably, this process is independent of SCFAs receptors (GPR41/43/109a) and is instead driven by HDAC inhibition–mediated chromatin remodeling. However, high concentrations of SCFAs (particularly butyrate), may impair the antitumor efficacy of CTLA-4 blockade by inducing regulatory T cells (Tregs) differentiation and suppressing the expression of DCs co-stimulatory molecules (CD80/CD86), suggesting a dual or context-dependent role in immune modulation [31].

3.1.2. Macrophages

Macrophages serve as key immune regulators within the TME, and their polarization status (M1 vs. M2) plays a pivotal role in determining the efficacy of immunotherapy. Metabolites derived from the gut microbiota have been shown to influence antitumor immune responses by regulating macrophage polarization into M1/M2 phenotypes. M1-polarized macrophages support antitumor immunity by secreting interleukin-12 (IL-12) and reactive oxygen species (ROS), whereas M2-polarized macrophages contribute to tumor progression by impairing T cell function through the production of interleukin-10 (IL-10) and arginase-1 (Arg-1) [55,56].

The MAPK family (ERK, JNK, p38) plays a dual role in promoting and suppressing tumors by regulating cell cycle, transcription factor activity, and signal cross-talk, while it mainly acts as a pro-inflammatory mediator in inflammation. Dysregulation of its homeostasis is a key mechanism in tumorigenesis and inflammatory diseases [57]. Supplementation with propionate has been shown to alleviate intestinal inflammation in the DSS-induced colitis mouse model by inhibiting the MAPK signaling pathway, thereby reducing both M1 macrophage polarization and the release of pro-inflammatory cytokines such as TNF-α and IL-1β [32].

Serine metabolism significantly influences macrophage polarization through epigenetic regulation involving S-adenosylmethionine (SAM)-dependent histone H3K27 trimethylation (H3K27me3). Serine deprivation or inhibition of the serine biosynthetic enzyme phosphoglycerate dehydrogenase (PHGDH) enhances IFN-γ-induced M1 macrophage polarization and promotes the expression of pro-inflammatory cytokines (e.g., IL-6, IL-1β) via the IGF1–p38–JAK/STAT1 axis, while simultaneously suppressing IL-4-induced M2 polarization [34].

The cGAS–IFN-β axis acts as a double-edged sword in cancer: the acute IFN-β response in early tumors inhibits tumor growth, while the chronic inflammatory microenvironment in late tumors changes the IFN-β signal into a pro-tumor effect [58]. Succinate, a key intermediate in the tricarboxylic acid (TCA) cycle, promotes M2 macrophage polarization by stabilizing hypoxia-inducible factor-1α (HIF-1α) [33], and concurrently suppresses antitumor immune responses mediated by the cGAS–IFN-β signaling pathway [28].In addition, succinate can exacerbate tumor angiogenesis by promoting vascular endothelial growth factor (VEGF) expression through SUCNR1-mediated ERK1/2 and STAT3 pathways and by driving M2 macrophage polarization, thereby reshaping the tumor microenvironment [59].

TMAO can activate the type I interferon (IFN) signaling pathway (significantly up-regulate key regulators such as IRF7, IFN-β, STING1 and STAT1) to drive the transformation of macrophages to an immunostimulatory phenotype (manifested as increased expression of MHCII/CD86 and decreased expression of Arg1) and enhance their antigen presentation ability. In this process, TMAO-treated macrophages reversed the inhibition of T cell proliferation by the TME and significantly increased the proportion of IFN-γ^{+}TNF-α^{+} effector subsets and the expression of activation markers CD44/Ki67 in $CD8^{+}$ T cells. Ultimately, this synergizes with immune checkpoint blockade therapy to inhibit pancreatic cancer progression [60].

Deficiency of ornithine decarboxylase (ODC) leads to reduced synthesis of putrescine, thereby aggravating arginine depletion. Exogenous supplementation of putrescine enhances histone H3K9 di- and tri-methylation, which upregulates the expression of MER Tyrosine-Protein Kinase (MerTK), ultimately improving the capacity of macrophages to clear apoptotic cells and promoting the resolution of inflammation [61].

3.1.3. Natural Killer Cells (NK Cells) and Neutrophils

NK cells and neutrophils, as central components of the innate immune system, play dual roles in tumor immunotherapy. NK cells contribute to antitumor immunity by directly killing tumor cells and secreting pro-inflammatory cytokines such as IFN-γ to activate adaptive immune responses; however, their function is often suppressed within the TME [62,63]. Neutrophils exhibit considerable heterogeneity, capable of promoting tumor-

associated inflammation—through the release of ROS and matrix metalloproteinases (MMPs)—as well as supporting antitumor immunity via antigen presentation and immune activation [64,65]. Gut microbiota-derived metabolites have emerged as critical modulators of immunotherapy efficacy by influencing NK cell metabolic pathways and regulating neutrophil functional states.

C-di-AMP induces the secretion of IFN-β and enhances the cytotoxic activity of NK cells and $CD8^+$ T cells by activating the STING–IRF3 signaling pathway. In the BALB/c mouse models of triple-negative breast cancer, B-cell lymphoma, and melanoma, delivery of c-di-AMP via engineered *Escherichia coli Nissle* 1917 strains (CIBT4523) activates STING-dependent immune responses within the tumor microenvironment, significantly increasing the proportions of $GzmB^+$ and $CD69^+$ tumor-infiltrating NK cells, while also promoting the effector function of $CD8^+$ T cells. This engineered strain further enhances antitumor immunity and establishes durable immune memory by inducing the production of Th1-type cytokines, such as IL-12 and IFN-γ [35] Additionally, c-di-AMP works synergistically with radiotherapy-induced tumor cell double-stranded DNA (dsDNA) to activate the cGAS–STING pathway, thereby boosting dendritic cell antigen presentation and facilitating robust $CD8^+$ T cell-mediated antitumor immune responses [36].

Eubacterium rectale relieved microenvironment-mediated inhibition of NK cells by depleting L-serine in the environment, thereby activating the FOS/FOSL2 signaling pathway to enhance NK cell function. *E. rectale* culture supernatant significantly enhanced the expression of killer molecules (PFN1/2, CCL2, CCL3) and cytotoxicity of NK cells. The mechanism was due to the reduction of L-serine concentration in the microenvironment by the serine catabolase expressed by *E. rectale*. Treatment with serine synthesis inhibitor NCT503 could mimic this effect and significantly increase the percentage of $GzmB^+$ and IFN-γ^+ in tumor-infiltrating NK cells, as well as NK cell activity. At the molecular level, L-serine inhibited NK function by inhibiting the expression of the key transcription factors FOS/FOSL2 in the MAPK pathway, and FOS knockdown attenuated the activation of NK cells induced by *E. rectale*. Analysis of clinical data further revealed that low expression of serine metabolic enzymes (PHGDH, phosphoserine phosphatase, serine hydroxymethyltransferase 1/2) was significantly associated with high NK cell activity, immunotherapy responsiveness, and prolonged patient survival [37]

Spironolactone (SPI), a structural analog of isoLCA, competitively binds to the bile acid receptor GPBAR1 and reverses the suppressive effect of isoLCA on NK-cell cytotoxicity by restoring CREB1 phosphorylation. In combination with anti-PD-1 monoclonal antibody, SPI significantly enhanced NK-cell infiltration and activation, as indicated by increased proportions of $CD44^+Ki67^+$ and IFN-γ^+TNF-α^+ cells, and synergistically inhibited tumor growth while prolonging survival in a liver cancer model. Clinical cohort analysis showed that increased serum isoLCA levels in HCC patients were significantly correlated with decreased fecal B. ovatus abundance and aldo-keto reductase family 1 member D1 (AKR1D1) expression, and isoLCA levels were positively correlated with poor prognosis in these patients. These findings suggest that targeting the isoLCA–GPBAR1–p-CREB1 axis may enhance the efficacy of immune checkpoint inhibitors [38].

Butyrate enhances the mitochondrial oxidative phosphorylation activity of liver-resident NK cells (LrNK) by activating the GPR109A receptor to promote the secretion of IL-18 by Kupffer cells and hepatocytes, thereby driving their functional maturation and enhancing their antitumor ability. Early antibiotic exposure leads to intestinal flora disorder and reduction of butyrate, which will continue to impair LrNK maturation and accelerate the progression of liver cancer, while butyrate or *Clostridium butyricum* supplementation can restore IL-18 signaling and LrNK function [39]. For neutrophils, butyric acid can significantly inhibit the production of proinflammatory cytokines (IL-6, TNF-α, IL-8), chemokines (CCL3/4), and calprotectin (S100A8/A9) by neutrophils from IBD patients by inhibiting HDAC. It also reduced the release of ROS, the level of myeloperoxidase (MPO), and the formation of neutrophil extracellular traps (NETs), and inhibited the migration ability of NETs. In the DSS-induced colitis model, oral butyrate reduced neutrophil infiltration and NETs formation in the colon, and alleviated mucosal inflammation [66].

3.1.4. Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs are key immunosuppressive components within the TME, weakening antitumor immune responses and promoting resistance to immunotherapy through multiple mechanisms. MDSCs suppress $CD8^+$ T cell function and promote the expansion of Tregs by secreting arginase (e.g., Arg1), ROS, nitric oxide (NO), and immunosuppressive cytokines such as IL-10 and TGF-β, as well as by expressing PD-L1 [67]. Clinical studies have demonstrated a negative correlation between MDSC abundance and the efficacy of ICIs. In hepatocellular carcinoma (HCC), therapies targeting the hypoxia-inducible factor (HIF) pathway reduce MDSC levels—including tumor-associated macrophages and monocytic MDSCs, thereby enhancing tumor clearance in response

to anti-PD-1 therapy [68]. In metastatic melanoma, patients with a baseline peripheral blood Lin-CD14$^+$ HLA-DR-/low MDSC frequency of less than 5.1% had significantly improved survival with ipilimumab (anti-CTLA-4) [69]

Butyric acid enhances the immunosuppressive function of MDSCs through epigenetic and metabolic reprogramming, playing a critical protective role in primary biliary cholangitis (PBC). In patients who do not respond to UDCA therapy, fecal levels of butyric acid are significantly reduced and positively correlated with impaired immunosuppressive function of circulating MDSCs, such as decreased expression of inducible nitric oxide synthase (iNOS) and ROS. Mechanistically, butyrate inhibits HDAC3 activity, thereby enhancing histone H3K27ac and selectively activating peroxisome proliferator-activated receptor delta (PPARD) along with key downstream genes in the fatty acid β-oxidation (FAO) pathway, including CPT1A and ACADVL. FAO-driven mitochondrial oxidative phosphorylation (OXPHOS) supplies energy for MDSCs, supporting their expansion and promoting the expression of immunosuppressive molecules such as arginase 1 and ROS. The regulatory effects of butyrate on MDSCs can be completely abolished by the CPT1A inhibitor etomoxir or by genetic knockout. In animal models, oral administration of butyrate or adoptive transfer of butyrate-treated MDSCs significantly ameliorated 2OA-BSA-induced cholangitis in mice, an effect dependent on MDSC FAO activity. Notably, MDSCs from UDCA non-responders exhibit impaired FAO gene expression and mitochondrial function, both of which can be restored by butyrate treatment, thereby recovering their immunosuppressive capacity [70].

3.2. Adaptive Immunity

3.2.1. CD8$^+$ T Cells

As the core effector cells of antitumor immunity, CD8$^+$ T cells directly mediate the killing of tumor cells by specifically recognizing antigen peptides presented by MHC-I molecules on the surface of tumor cells through the T cell receptor (TCR). However, the antigen-MHC-I complex present on the surface of tumor cells in the TME repeatedly activates CD8$^+$ T cells through TCR, drives the calcium-dependent NFAT signaling pathway to sustain activation, and induces high expression of TOX transcription factors, which jointly reshape chromatin openness and mediate irreversible epigenetic reprogramming. Meanwhile, the PD-L1/PD-1 immune checkpoint pathway inhibits T cell activation. Ido1-mediated tryptophan depletion hinders T cell proliferation. TGF-β secreted by Tregs inhibits effector function, and multiple immunosuppressive mechanisms jointly drive CD8$^+$ T cells into an exhausted state, leading to immunotherapy resistance [71,72].

Gut microbiota-derived metabolites have been shown to reverse T cell exhaustion and enhance the efficacy of ICIs by modulating T cell metabolic reprogramming and epigenetic modifications. Notably, butyrate enhances the antitumor activity of CD8$^+$ T cells through inhibition of HDACs. Treatment with butyrate significantly upregulates the expression of the transcription factor ID2 and promotes IL-12–dependent signaling, thereby increasing the secretion of IFN-γ and GzmB by CD8$^+$ T cells [40]. In CRC models, the butyrate-producing bacterium *Ruminococcus intestinalis* activates CD8$^+$ T cells via the TLR5/NF-κB pathway and enhances the efficacy of anti-PD-1 therapy in tumors with microsatellite stability (MSS) or low microsatellite instability (MSI-low) [73]. Additionally, butyrate alleviates T-cell exhaustion by increasing H3K27 acetylation at the T-box transcription factor 21 (Tbx21) promoter through HDAC3/8 inhibition, leading to transcriptional suppression of PD-1 expression [41]. Butyrate has also been shown to impair dendritic cell development and reduce its capacity to activate T cells by inhibiting HDAC3 [74]. However, under conditions of TLR activation, high concentrations of butyrate can induce a proinflammatory response via HDAC inhibition through activation of the NLRP3 inflammasome [75]. Acetate is absorbed into tumor cells by monocarboxylic acid transporter 1 (MCT1) and converted to acetyl-CoA by acetyl-COAsynthetase 2 (ACSS2), which activates the acetylation of c-Myc protein Lys148 mediated by dihydrolipoamide S-acetyltransferase (DLAT). Acetylated c-Myc recruits ubiquitin-specific peptidase 10 (USP10) for deubiquitination, thereby stabilizing c-Myc protein and promoting its transcriptional activity, and ultimately up-regulating PD-L1 expression. Elevated PD-L1 significantly inhibited CD8$^+$ T cell activation, as indicated by decreased IL-2 and IFN-γ secretion and reduced tumor invasion. Dietary acetic acid supplementation promoted the increase of TH2 cells and MDSCs in TME, while reducing the infiltration of cytotoxic CD8 T cells and the expression of GzmB. Knockout of MCT1 or c-Myc Lys148 acetylation-deficient mutation (K148R) reversed acetate-induced CD8$^+$ T cell suppression, whereas c-Myc acetylation-mimic mutation (K148Q) exacerbated immune escape. Analysis of clinical samples showed a significant inverse correlation between c-Myc Lys148 acetylation and CD8$^+$ T-cell infiltration [76].

TMAO induces GSDME-mediated pyroptosis in tumor cells by activating the PERK-eIF2α signaling pathway, leading to the release of proinflammatory cytokines such as IL-1β and IL-18, which in turn enhances the antitumor immune activity of CD8$^+$ T cells [42]. However, excessive accumulation of TMAO can trigger epithelial-mesenchymal transition (EMT) and promote tumor progression through activation of the MAPK

pathway. Moreover, elevated TMAO levels have been linked to increased risks of metabolic diseases, including cardiovascular disease and type 2 diabetes [77]. Therefore, its clinical application requires careful evaluation of dosing and administration strategies.

Indole derivatives, metabolites of gut microbes, can regulate T cell function through a variety of mechanisms. Indole-3-propionic acid (IPA) enhances the antitumor effect of immune checkpoint blockade through epigenetic regulation of $CD8^+$ T cell stemness. The specific mechanisms are: *Lactobacillus johnsonii* cooperates with Clostridium sporogenes to metabolize tryptophan to produce IPA. IPA promoted the differentiation and maintenance of stem cell-like exhausted precursor T cells (Tpex) by increasing histone H3K27ac in the transcription factor Tcf7 super-enhancer region in $CD8^+$ T cells, thereby enhancing the clonal expansion of TCR and the generation of effector T cells (Teff). Finally, the response of anti-PD-1 immunotherapy in multiple cancers (melanoma, breast cancer, CRC, etc.) is improved [44]; The oral probiotic *L. reuteri* could colonize melanoma and activate AhR signaling pathway of $CD8^+$ T cells in the tumor microenvironment by metabolize tryptophan to produce I3A. I3A promotes the differentiation of $CD8^+$ T cells into IFN-γ-secreting Tc1 effector cells through AhR-dependent phosphorylation of CREB and enhances antitumor activity. I3A alone can inhibit tumor growth, and its combination with anti-PD-L1 can significantly improve the efficacy of immune checkpoint inhibitors, which can be further amplified by a high-tryptophan diet. Clinical data show that elevated baseline serum I3A levels are significantly associated with immunotherapy response rate and prolonged survival in patients with advanced melanoma [15]. Acarbose, an oral antidiabetic drug, significantly increased the level of the tryptophan metabolite indoleacetic acid (IAA) by remodeling the gut microbiota, including increasing *Bifidobacterium* and lactic acid bacteria, thereby promoting CXCL10 expression in the tumor microenvironment, enhancing $CD8^+$ T-cell infiltration through the CXCL10–CXCR3 axis, and synergistically improving the efficacy of anti-PD-1 immunotherapy. This mechanism was validated in both colon cancer and melanoma models. Gut microbiota dependence was confirmed by fecal microbiota transplantation and antibiotic clearance assays, and supplementation with the acarbose-enriched bacterial species B. infantis independently enhanced immunotherapy efficacy [45].

DCA impairs the antitumor activity of $CD8^+$ T cells by inhibiting their calcium signaling pathway (Ca^{2+}-NFAT2) [43,78]. DCA, a secondary bile acid generated by the gut microbiota through the metabolism of primary bile acids, weakens antitumor immune responses by enhancing PMCA-mediated calcium efflux and suppressing the calcium-dependent NFAT2 signaling pathway in $CD8^+$ T cells [43].

The abundance of the gut microbe *A. finegoldii* is associated with improved efficacy of ICIs (such as anti-PD-1 antibodies) in treating solid tumors. The mechanism involves a lipoprotein named LIPOAF, secreted by *A. finegoldii*, which migrates via the bloodstream to the TME. There, it binds to Toll-like receptor 2 (TLR2) on the surface of conventional dendritic cells (cDCs), activating the downstream NF-κB signaling pathway. This activation promotes the secretion of the chemokine CXCL16 by cDCs. CXCL16, in turn, recruits cytotoxic $CD8^+$ T cells expressing the CXCR6 receptor into the tumor core, enhancing their activation and killer function, ultimately synergizing with immunotherapy to effectively inhibit tumor growth [79].

3.2.2. $CD4^+$ T Cell Subsets

$CD4^+$ T cells regulate the balance between activation and suppression of antitumor immunity by differentiating into various subsets, including Th1, Th17, and Treg cells.

Secondary bile acids, such as DCA and LCA, are produced by gut microbiota through the metabolism of primary bile acids. These metabolites influence $CD4^+$ T cell differentiation via nuclear receptors (e.g., FXR, VDR, RORγt) and membrane receptors (e.g., GPBAR1). Studies have demonstrated that bile acids promote Treg differentiation by modulating FXR signaling in DCs, inhibiting the NF-κB pathway, reducing proinflammatory cytokines such as TNF-α and IL-6, while enhancing IL-10 secretion [46,47]. 3-oxoLCA suppresses Th17 polarization by antagonizing RORγt, thereby indirectly promoting immune tolerance [47]. LCA derivatives, including 3-oxoLCA and isoLCA, inhibit Th17 differentiation through binding to RORγt [80,81], whereas LCA and its derivatives (such as isoalloLCA) enhance Treg function via VDR signaling [81], collectively alleviating autoimmune diseases.

However, in CRC, excessive DCA impairs effector T cell function and facilitates tumor progression by inhibiting the Ca^{2+}-NFAT2 signaling pathway in $CD8^+$ T cells [43]. These findings suggest that the immunoregulatory effects of microbial metabolites are disease-context dependent.

Kynurenine, a tryptophan derivative produced by indoleamine 2,3-dioxygenase 1 (IDO1) catalysis, has been demonstrated to promote Treg differentiation via activation of the AhR signaling pathway, thereby inhibiting $CD8^+$ T cell infiltration and suppressing antitumor immunity [82]. Tryptophan depletion induces overexpression of AhR and

enhances intracellular kynurenine uptake via a GCN2/LAT1-dependent mechanism, which synergistically amplifies AhR pathway activation, promotes regulatory T cell differentiation, and exacerbates immunosuppression [48]. Clinical data indicate that tumors with high IDO1 expression exhibit increased AhR pathway activity, which correlates significantly with resistance to PD-1 inhibitors [49]. Tryptophan deficiency may further amplify AhR signaling and promote Treg differentiation by upregulating AhR expression and increasing kynurenine uptake. Specifically, tryptophan depletion elevates AhR expression independently of GCN2, while kynurenine uptake mediated through the GCN2–LAT1 axis heightens AhR pathway sensitivity, thereby promoting regulatory T-cell induction and immune suppression [48]. AhR inhibitors can reverse kynurenine-driven Treg expansion and, when combined with PD-1 inhibitors, enhance antitumor efficacy [49] In addition, IDO1 inhibitors reduced CXCL10 secretion by inhibiting the Kyn–AhR pathway, whereas CXCL10 agonists synergistically enhanced the efficacy of IDO1 inhibitors [83].

Inosine modulates immune cell function by activating the adenosine A2A receptor (A2AR). It can suppress inflammatory responses, reduce apoptosis, and enhance Treg activity by upregulating the CD39/CD73-A2AR signaling axis, thereby restoring Th17/Th1 immune balance [50]. Moreover, inosine in combination with probiotics,such as *Akkermansia muciniphila*, can restore intestinal barrier integrity and modulate gut microbiota by increasing beneficial bacteria (e.g., *A. muciniphila*, Lactobacillus) and reducing harmful species (e.g., *Oscillibacter*), thereby alleviating alcohol-induced liver injury [84]. In an alcoholic liver disease model, co-administration of inosine and *A. muciniphila* significantly elevated the proportion of Treg cells and inhibited Th17/Th1 differentiation via upregulation of the CD39/CD73-A2AR axis—an effect that could be abrogated by A2AR antagonists [84]. At the metabolic level, inosine can serve as an alternative carbon source to glucose. It is catabolized by purine nucleoside phosphorylase (PNP) into ribose, supporting ATP production and biosynthesis in $CD8^+$ T cells, thereby alleviating the metabolic constraints of the TME [85]. A phase II clinical trial demonstrated that the combination of inosine and PD-1 inhibitors significantly prolonged progression-free survival in patients with advanced solid tumors [86]. Inosine regulates the differentiation of $CD4^+$ T cells into the Th1 subset via the adenosine A2AR signaling pathway, and this effect is strictly co-stimulation dependent. Upon co-stimulation with IFN-γ or a TLR agonist such as CpG, inosine binds to A2AR on T cells, activating the cAMP-PKA signaling axis, which leads to phosphorylation of the transcription factor CREB and a marked upregulation of IL-12 receptor β2 subunit (IL12Rβ2) expression. This promotes the differentiation of T-bet^+IFN-γ^+Th1 cells. The process can be completely inhibited by an A2AR antagonist or a PKA inhibitor, whereas a cAMP analog (db-cAMP) can substitute for inosine's function, confirming the specificity of this signaling pathway [87,88]. Moreover, the adoptive transfer of A2AR-deficient T cells to tumor-bearing mice significantly reduced the inosine-mediated intratumoral IFN-γ^+CD4^+ T cell expansion and the antitumor immune response [51].

3.3. Regulation of Tumor Immunogenicity

Tumor immunogenicity is determined by both the antigenicity and adjuvanticity of tumor cells. Immunogenic cell death (ICD) promotes antigen presentation and initiates antitumor T cell responses by activating pattern recognition receptors (e.g., TLR4, CD91) on DCs through the release of damage-associated molecular patterns (DAMPs), such as surface-exposed calreticulin (CALR), high mobility group box 1 (HMGB1), and adenosine triphosphate (ATP). This process enhances the efficacy of immunotherapy [89]. Tumor cells can evade immune detection by suppressing neoantigen expression via epigenetic regulation (such as DNA methylation) or metabolic reprogramming. Metabolites derived from gut microbiota remodel tumor immunogenicity by modulating antigen processing, immune checkpoint expression, and tumor angiogenesis. Inosine significantly improves the effectiveness of immune checkpoint blockade therapy (combined anti-PD-1/anti-CTLA-4 treatment) in melanoma and breast cancer models by inhibiting the ubiquitin-activating enzyme UBA6, upregulating MHC-I molecule expression and antigen-processing-related genes in tumor cells, thereby enhancing antigen presentation capacity [90].

4. Clinical Translation Strategies

Metabolites of gut microbiota have shown great potential in basic research to reshape the tumor immune microenvironment by regulating innate immunity, adaptive immunity and tumor immunogenicity. However, there are still multiple bottlenecks to be solved from mechanism research to clinical application, such as bidirectional effects of metabolites (such as butyrate concentration dependent regulation of Treg differentiation), individual differences in the host (such as heterogeneity of bacterial composition and metabolic characteristics), and delivery efficiency (such as insufficient cross-barrier targeting ability). This chapter systematically discusses the strategies of probiotics, prebiotics, engineered bacteria and fecal microbiota transplantation, aiming to break the translation

barriers between basic research and clinical practice, and provide an innovative path for precision immunotherapy based on the intestinal flora metabolic network (Figure 2).

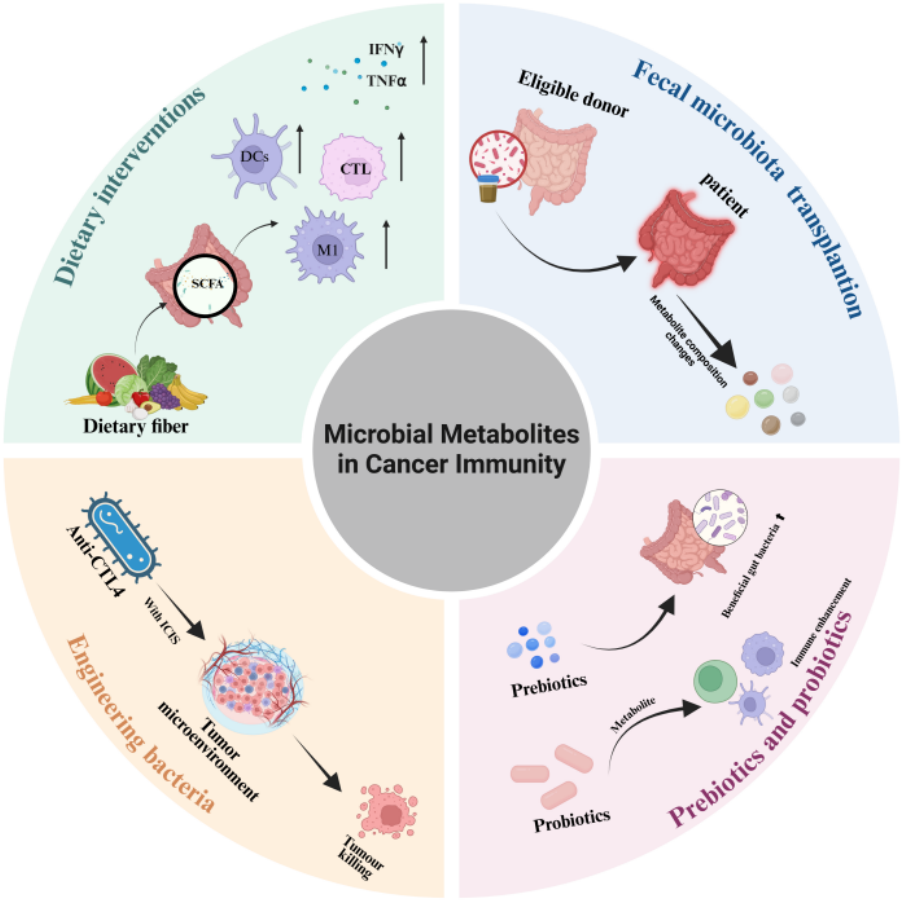

Figure 2. Intervention strategies through which microbial metabolites modulate cancer immunity. This figure summarizes four major intervention strategies targeting the microbiota–metabolite–immunity axis in cancer. Dietary interventions increase microbial fermentation of dietary fiber and promote SCFAs production, thereby enhancing antitumor immune responses through immune cells such as DCs, CTLs, and M1 macrophages, as well as cytokines including IFN-γ and TNF-α. Fecal microbiota transplantation (FMT) transfers microbiota from eligible donors to patients, thereby reshaping the gut microbial ecosystem and altering metabolite profiles. Engineered bacteria are designed to target the tumor microenvironment and deliver therapeutic molecules, thereby enhancing antitumor effects. Prebiotics and probiotics modulate gut microbial composition and metabolism, contributing to beneficial immune regulation. Together, these strategies highlight the therapeutic potential of microbial metabolites in cancer immunity.

4.1. Probiotics and Prebiotics

Probiotics and prebiotics, as key regulators of the gut microbiota, play vital roles in maintaining intestinal health and immune homeostasis. Probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts, while prebiotics are food components, typically dietary fibers or certain phytochemicals that promote the growth and activity of probiotics [91,92]. Research has demonstrated that probiotics can modulate immune responses and alleviate intestinal inflammation [93], whereas prebiotics enhance the intestinal barrier, improve microbial diversity, and reduce inflammation [94]. Overall, comprehensive modulation of the gut microbiota may influence host health through these mechanisms.

4.1.1. Probiotics

Lactobacillus rhamnosus GG is one of the most extensively studied probiotics and has demonstrated significant potential in enhancing immune system function. LGG enhances antitumor immunity by promoting IFN-γ secretion and $CD8^+$ T cell responses through TLR2-dependent activation of DCs [95]. Studies have shown that oral administration of LGG can boost host resistance to infections via the RIG-I signaling pathway and help combat

pathogens such as herpes simplex virus type 2 (HSV-2) by increasing IFN-I secretion [96]. Additionally, LGG promotes IL-10 production in monocytes by activating the STING/TBK1/NF-κB pathway, thereby ameliorating intestinal inflammation and remodeling gut microbial metabolism. This, in turn, enhances antitumor immune responses and synergizes with anti-PD-1 immunotherapy [97,98]. These findings suggest that LGG not only exerts immunomodulatory effects but can also be combined with immunotherapy to improve its efficacy.

Among the new generation of probiotics, *A. muciniphila* and its metabolites optimize immunotherapy responses through multiple mechanisms. Extracellular vesicles secreted by *A. muciniphila* (Akk-EVs) induce M1 polarization of macrophages, enhance GzmB and IFN-γ expression in $CD8^+$ T cells, and inhibit prostate cancer progression [99] Its outer membrane protein Amuc_1100 suppresses lung cancer immune evasion by inhibiting the JAK-STAT signaling pathway, reducing PD-L1 expression in tumor cells, and promoting $CD8^+$ T cell infiltration [100]. Clinical studies have also reported a positive correlation between *A. muciniphila* abundance and immune checkpoint inhibitor response rates [101]. Notably, structural variation (SV) regions within *A. muciniphila*, such as the gene encoding the YD repeat protein, show a negative correlation with ICI response, whereas regions containing glycosyltransferase genes correlate positively with response [102]. This suggests that specific SVs may serve as predictive biomarkers for ICI efficacy. Future research targeting these SV-related genes—via probiotic engineering or metabolic interventions—could optimize immunotherapy responses, potentially sensitizing patients who are initially non-responsive.

Oral administration of *Clostridium butyricum* MIYAIRI 588 (CBM588) enriches intestinal *Ruminococcaceae*, and activates the colonic IDO-1/IL-10 signaling axis to promote $ROR\gamma t^+$ Treg cell accumulation, while reducing infiltration of this immunosuppressive T-cell subset in tumor-draining lymph nodes, thereby alleviating local immunosuppression and enhancing $CD8^+$ T-cell function. In a murine lung cancer model, combining this bacterium with a PD-1 inhibitor reduced tumor volume threefold, and blocking IL-10 signaling restored IFN-γ secretion capacity in $CD8^+$ T cells, confirming that CBM588 overcomes PD-1 inhibitor resistance by remodeling the microbiota-immune microenvironment [103].In a clinical trial (NCT03829111), combining CBM588 with dual immune checkpoint inhibitors (nivolumab + ipilimumab) significantly prolonged progression-free survival (median PFS: 12.7 vs. 2.5 months) and improved objective response rates (ORR: 58% vs. 20%) in metastatic renal cell carcinoma (mRCC) patients without increasing toxicity [104]. Although it did not significantly increase *Bifidobacterium* abundance (primary endpoint unmet), responders showed significant *Bifidobacterium* enrichment alongside upregulated metabolic pathways (e.g., butyrate synthesis) and immune factors (e.g., CCL2, CXCL9) [104]. In another trial (NCT05122546), combining CBM588 with targeted and immune checkpoint inhibitors (cabozantinib + nivolumab) significantly improved ORR (74% vs. 20%) and 6-month PFS rates (84% vs. 60%) in mRCC patients [105]. These randomized trials demonstrate that CBM588 enhances immunotherapy efficacy in mRCC by modulating gut microbiota composition (e.g., enriching *Ruminococcaceae*) and metabolic functions (e.g., vitamin K2 pathway).

4.1.2. Prebiotics

Certain dietary fibers serve as prebiotics and play a vital role in maintaining intestinal health and regulating immunity. Dietary fibers exert significant immunomodulatory effects by being fermented by gut microbiota to produce SCFAs [106]. SCFAs, such as butyrate, enhance intestinal barrier integrity by upregulating tight junction protein expression, suppress the release of pro-inflammatory cytokines to alleviate intestinal inflammation, and promote Treg differentiation while inhibiting excessive immune responses via activation of GPR41/GPR43 receptors. Additionally, SCFAs boost $CD8^+$ T cell activity, thereby facilitating antitumor immunity [107]. Therefore, dietary fiber intake is not only essential for gut health but also holds promising potential for combating tumors and inflammatory diseases by increasing SCFA synthesis and modulating host immune responses.

The prospects for applying probiotics and prebiotics in immunotherapy are highly promising. Nevertheless, despite their substantial potential, challenges remain regarding standardization, dose optimization, and personalized treatment approaches. Future research focused on integrating probiotics and prebiotics into cancer immunotherapy through precise dosing and individualized therapeutic regimens represents an important avenue for further exploration.

4.2. Engineered Bacteria

Engineered bacteria, such as EcN 1917, have emerged as valuable tools in tumor immunotherapy through genetic engineering. EcN can modulate the immunosuppressive tumor microenvironment by secreting immunoreactive molecules (e.g., anti-PD-L1 antibodies) or metabolites (such as indole derivatives). For instance, genetically engineered EcN 1917 can express PD-L1 antagonists (e.g., antibodies) that block the PD-1/PD-L1

signaling axis between tumor cells and T cells, thereby reversing T cell exhaustion and enhancing antitumor immune responses [108]. EcN 1917 can also be integrated with nanomaterials for multifunctional delivery platforms. An example is indocyanine green (ICG)-loaded metal-organic framework (MOF)-modified engineered bacteria (ENZC), which, under near-infrared light activation, release anti-PD-L1 and anti-CD9 nanoantibodies. By targeting tumor-derived exosomes (TDEs) and immune checkpoints, this system enhances local T cell activation and promotes macrophage M1 polarization, effectively inhibiting tumor metastasis [109]. Furthermore, genetically modified EcN 1917 can achieve tumor-specific colonization and continuously convert ammonia metabolism into L-arginine, significantly elevating the local arginine concentration within tumors. This metabolic intervention enhanced $CD8^+$ T cell infiltration, reduced the proportion of Tregs, and had a synergistic effect with PD-L1 blockade, resulting in a significant complete response rate in a CRC model [110].

Clostridium butyricum was engineered (L-Trp CB) to overexpress tryptophan synthase, enabling the simultaneous intratumoral release of tryptophan and butyrate. Butyrate markedly reduced IDO activity by inhibiting the STAT1/HDAC pathway, thereby blocking the tryptophan-kynurenine metabolic axis. However, a high concentration of tryptophan directly acts as a "metabolic signal" to activate the mTORC1 pathway of $CD8^+$ T cells, promote glycolysis and oxidative phosphorylation metabolic reprogramming, and significantly increase the proportion of effector T cells. The synergistic effect of this dual pathway significantly delayed tumor growth in melanoma and breast cancer models, and combined with PD-L1 antibody resulted in the complete elimination of some tumors [111].

4.3. FMT

FMT significantly enhances the efficacy of ICI by reshaping the intestinal flora microecology. Clinical trials have shown that healthy donor FMT combined with anti-PD-1 therapy can significantly improve the objective response rate of patients with advanced melanoma, which is associated with the expansion of $ICOS^+CD8^+$ T cells and $CD38^+$ MAIT cells in the peripheral blood of the patients. The polyamine biosynthesis pathway was activated and the plasma histidine level was increased in responders after FMT, which may promote antitumor immunity by enhancing the mitochondrial function of $CD8^+$ T cells [112]. Mechanistically, FMT facilitates intestinal barrier repair and alleviates graft-versus-host disease by restoring microbial diversity, such as enrichment of genera like *Faecalibacterium,* and modulating immune cell balance, including increased Treg populations and decreased Th17 cells [113]. Furthermore, FMT can reverse dysbiosis caused by chemotherapy or antibiotics, restoring sensitivity to immune checkpoint blockade by increasing SCFA-producing bacteria and boosting $CD8^+$ T cell infiltration within the tumor microenvironment [114]. Results from clinical trial NCT03341143 demonstrate that responder-derived FMT combined with anti-PD-1 therapy overcomes immunotherapy resistance in melanoma patients: Among 15 anti-PD-1-refractory advanced melanoma patients, a single colonoscopic FMT infusion plus pembrolizumab yielded clinical benefit in 6 patients (40%), including 3 objective responses and 3 cases of durable stable disease(>12 months). This intervention significantly reduced resistance-associated serum factors (e.g., IL-8), remodeled gut microbiota (enriching beneficial taxa like *Ruminococcaceae* and *Bifidobacterium*), and reprogrammed the tumor microenvironment via $CD8^+$ T-cell activation ($CD56^+$ subset expansion) and myeloid suppression (reduced $IL\text{-}8^+$ cells). Multi-omics analyses confirmed synergistic microbiota-metabolite-immune axis interactions, such as the inverse correlation between *F. prausnitzii* and IL-8 [16].

In fact, the U.S. FDA has approved FMT for treating recurrent *Clostridioides difficile* infection in clinical practice. However, FMT should still be used with caution, as studies indicate it may lead to long-term, unintended negative health consequences for recipients. During the restoration of gut dysbiosis, FMT introduces anaerobic microbiota from the colon into the oxygen-rich small intestine environment. This anatomical mismatch causes abnormal colonization of anaerobic bacteria in the small intestine. Such region-specific mismatch persistently alters host metabolism and immune function: in murine models, jejunal microbiota transplantation (JMT) enhances lipid metabolism pathways, whereas FMT activates immune pathways and drives intestinal tissue gene expression toward colon-like features (e.g., SATB2 upregulation). These changes coincide with altered bile acid profiles and disrupted energy homeostasis. Human data reveal increased anaerobic bacteria in the duodenum post-FMT, alongside colon-characteristic gene expression in small intestinal tissue. Studies demonstrate that non-specific, fecal-based microbiota transplantation may trigger off-target effects, necessitating the development of region-specific precision therapies (e.g., combined small and large intestinal microbiota transplantation) [115].

However, the clinical application of FMT is limited by the lack of a standardized system. First, there is a lack of uniform criteria for donor screening. Studies have found that existing screening algorithms cannot quantify the functional characteristics of the microbiota, such as the ability to synthesize SCFAs, leading to significant heterogeneity in efficacy [112]. Secondly, the difference in the preparation process affects the activity of bacterial

flora. Different cryopreservation media can significantly vary the survival rate of bacterial flora, and rapid recovery is essential to maintain activity [112]. In addition, there is no consensus on the route of administration and dose. Meta-analysis showed that the colonization rate of colonoscopic instillation was significantly higher than that of oral capsules, but the invasive procedure increased the risk of infection. However, the immunomodulatory threshold for a single dose remains unclear [114]. Therefore, there is an urgent need to establish an international FMT quality control platform combined with multi-omics technology to dynamically monitor the function of flora.

5. Challenges and Prospects

The regulatory potential of gut microbiota-derived metabolites in tumor immunotherapy has been widely acknowledged; however, their clinical translation continues to face several significant challenges. One major limitation is the bidirectional effects and concentration-dependent nature of these metabolites, which complicates their precise therapeutic application. For instance, TMAO enhances antitumor immunity through the type I interferon pathway, but excessive accumulation may lead to an increased risk of cardiovascular disease, renal failure, and diabetes [60]. Moreover, the functional impact of metabolites is modulated by host genetic background, dietary patterns, and gut microbial composition (e.g., enterotypes), leading to marked interindividual variability in therapeutic outcomes [116].

In addition, the spatiotemporal heterogeneity and limited delivery efficiency of metabolic interventions remain major challenges. Metabolites derived from the gut microbiota must traverse multiple biological barriers—such as the intestinal mucosa—for effective targeted delivery. This process is complicated by host–microbe interactions, often resulting in poor bioavailability, subtherapeutic local concentrations, or off-target effects [117]. For instance, although oral administration of sodium butyrate can elevate systemic butyrate levels, its delivery efficiency to TME is constrained due to rapid metabolism at absorption sites, such as the small intestine, and the need to penetrate the intestinal mucosal barrier [40].

More importantly, the integration of multi-omics data and the development of individualized strategies are key to overcoming clinical bottlenecks. Although the combined application of metabolomics and single-cell technologies has revealed the intricate complexity of the microbiota–metabolism–immunity axis, data integration still suffers from a lack of standardization, outdated analytical algorithms, and high sample heterogeneity [116,118]. For instance, the microbiota–metabolite profiles of early-onset CRC (EO-CRC) and late-onset CRC (LO-CRC) differ significantly, necessitating the development of subtype-specific predictive models [118]. Moving forward, there is an urgent need to establish cross-omics databases and integrated analysis platforms that combine gut microbiota profiling, metabolic biomarkers, and host immune characteristics to advance the personalization of therapeutic strategies [117].

6. Conclusions

Metabolites of gut microbiota profoundly affect the efficacy of ICIs by reshaping the tumor immune microenvironment at multiple levels. These metabolites systematically regulate the function of innate immune cells and adaptive immune cells, enhance the immunogenicity of tumor cells, and jointly optimize the antitumor immune response. It is worth noting that gut-derived microbiota metabolites not only act locally on gut-associated lymphoid tissues but also enter the circulation system to exert systemic immunomodulatory effects and synergistic effects with ICIs. Therefore, strategies targeting the regulation of gut microbiota and its metabolic network, including probiotics, engineered bacteria, and FMT, have shown promise for clinical translation in synergy with ICIs. However, the key bottlenecks such as the bidirectional and concentration-dependent effects of metabolites, significant individual host heterogeneity, and delivery efficiency remain the core challenges of clinical translation. Future research needs to further analyze the interaction mechanism of the microbiota–metabolism–immunity axis, accurately identify immunostimulant and inhibitory metabolic pathways and strains, and combine multi-omics technology and individualized strategies to overcome existing obstacles and ultimately promote the development of precision therapy for tumor immunity based on microbiome and metabolism.

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