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REVIEW

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Gut microbiota in hepatocellular carcinoma immunotherapy: immune microenvironment remodeling and gut microbiota modification

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ABSTRACT

Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, with limited treatment options at advanced stages. The gut microbiota, a diverse community of microorganisms residing in the gastrointestinal tract, plays a pivotal role in regulating immune responses through the gut-liver axis. Emerging evidence underscores its impact on HCC progression and the efficacy of immunotherapy. This review explores the intricate interactions between gut microbiota and the immune system in HCC, with a focus on key immune cells and pathways involved in tumor immunity. Additionally, it highlights strategies for modulating the gut microbiota – such as fecal microbiota transplantation, dietary interventions, and probiotics – as potential approaches to enhancing immunotherapy outcomes. A deeper understanding of these mechanisms could pave the way for novel therapeutic strategies aimed at improving patient prognosis.

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and one of the leading causes of cancer-related deaths worldwide.[1] Each year, HCC accounts for over 800,000 new cases and more than 700,000 deaths, with a poor prognosis reflected in a five-year survival rate of less than 20%.[2] Despite significant advancements in therapeutic strategies – including surgical resection, liver transplantation, and targeted therapies – prognosis remains dismal, particularly in advanced stages of the disease.[3] The emergence of immunotherapy, particularly immune checkpoint inhibitors, has introduced a promising avenue for HCC treatment. However, response rates remain inconsistent, with many patients failing to derive significant benefit from these therapies.[4]

Recent research has increasingly emphasized the pivotal role of gut microbiota in shaping the host immune system and influencing disease outcomes, including cancer.[5,6] The gut microbiota, a vast community of microorganisms – including bacteria, viruses, fungi, and archaea – plays a crucial role in maintaining physiological homeostasis, regulating immune function, metabolism, and inflammation.[7] Through the gut-liver axis, the gut microbiota exerts a profound influence on liver function and immune responses, making it a key player in the pathogenesis and progression of HCC.[8] This axis, which describes the bidirectional relationship between the gut and liver mediated by portal circulation, facilitates the direct impact of gut-derived microbial products – such as lipopolysaccharides (LPS), short-chain fatty acids (SCFAs), and bile acids (BAs) – on the liver's immune microenvironment.[9] Under physiological conditions, the gut microbiota helps maintain immune tolerance and prevents excessive inflammation. However, gut microbiota dysbiosis – an imbalance in microbial composition – can lead to chronic liver inflammation, creating a microenvironment conducive to tumorigenesis.[10]

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In the context of HCC, gut microbiota dysbiosis has been implicated in immune dysregulation, affecting both innate and adaptive immune responses.[11] For instance, certain gut microbes promote the expansion of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), both of which contribute to immune suppression and hinder anti-tumor immunity.[12] Conversely, a balanced gut microbiota can enhance immune responses by promoting the activation of cytotoxic T lymphocytes and natural killer (NK) cells – immune cells essential for targeting and eliminating cancer cells.[13] Furthermore, emerging evidence suggests that the gut microbiota can influence the efficacy of immunotherapy in HCC. Specific microbial compositions have been associated with improved responses to immune checkpoint inhibitors, such as anti-PD-1/PD-L1 and anti-CTLA-4 therapies.[14] These findings have led to the hypothesis that modulating the gut microbiota – through interventions such as probiotics, prebiotics, fecal microbiota transplantation (FMT), and dietary modifications – could enhance immunotherapy outcomes in HCC.[15]

Given the gut microbiota's central role in immune regulation and its potential to influence cancer treatment responses, further exploration of these interactions in HCC is imperative. This review aims to elucidate how the gut microbiota modulates the immune microenvironment in HCC, impacts immunotherapy efficacy, and how microbiota-targeted strategies could be leveraged to improve treatment outcomes. By deepening our understanding of these mechanisms, we may unlock novel therapeutic avenues to better manage HCC and enhance patient prognosis.

2. Regulation of immune response by gut microbiota in HCC

The gut microbiota plays a crucial role in shaping the immune landscape within the liver, influencing both innate and adaptive immune responses. Various microbial components, such as LPS, flagellin, and peptidoglycan, along with luminal metabolites derived from the gut microbiota, including SCFAs and bile acids, can enter the portal circulation. These molecules directly activate hepatocytes and modulate the hepatic immune system, thereby affecting liver function and immune regulation.[16] A deeper understanding of these interactions is essential for uncovering how the gut microbiota influences immune responses in HCC, potentially opening new avenues for therapeutic intervention (Table 1) (Figure 1).

2.1. B cells: activation enhances antibody-mediated tumor immunity in HCC

B cells play a crucial role in maintaining intestinal homeostasis. By producing immunoglobulins and cytokines, they help sustain a stable, non-inflammatory interaction between the host and its microbiota.[17] For instance, germ-free (GF) mice exhibit lower levels of immunoglobulin A (IgA), a key antibody secreted by B cells, along with impaired B-cell activity. Additionally, colonization of the intestine by *Escherichia coli (E. coli)*, *Bifidobacterium*, and *segmented filamentous bacteria (SFB)* has been shown to promote B cell maturation and enhance specific IgA antibody production.[18,19] Notably, in liver metastasis models, the gut microbiota regulates tumor growth by modulating the B cell immune response.[20] Similarly, the intestinal immune response – particularly the B cell-derived molecule IgA – is essential for stabilizing the gut microbiota. Research suggests that neutral sphingomyelinase can regulate IgA secretion by intestinal B cells, thereby influencing gut microbiota homeostasis and contributing to the development of metabolic dysfunction-associated steatohepatitis (MASH).[21]

The regulation of B cells by the gut microbiota and its byproducts is influenced by multiple factors, including IgA, various immune cells, chemokines, cytokines, and B cells themselves.[22] Specifically, immune cells such as intestinal epithelial cells (IECs), dendritic cells (DCs), T cells, and eosinophils can stimulate the production of B-cell activating factors. In conjunction with cytokines, these immune cells support the differentiation and survival of IgA-producing plasma cells.[23–25] Moreover, microbial metabolites such as SCFAs contribute to activating B-cell receptors (BCRs), inhibiting histone deacetylases (HDACs), and increasing adenosine triphosphate (ATP) levels.[26,27] The gut microbiota also drives the production of IL-1β and IL-6, promoting the

Table 1. Regulation of immune response by gut microbiota in HCC.

Immune Cell Type	Immune Cell Location	Primary Role	Key Mechanism	Ref.
B cell	Liver, gut	Regulate gut homeostasis and promote anti-tumor immunity	*E. coli*, *bifidobacteria*, and SFB promote the secretion of IgA	18,19
B cell	Liver, gut	Regulate gut homeostasis and tumor immunity	SCFAs activate B cells by inhibiting HDACs	26,27
B cell	Liver, gut	Regulate gut homeostasis and tumor immunity	Gut microbiota promote the differentiation of naïve B cells into Bregs	28
Macrophage	Liver	Inhibit progression of MASH to HCC	*Akkermansia muciniphila* reduces macrophage infiltration and decreases M2 macrophage levels	32
Macrophage	Liver	Enhance anti-tumor effects	*Bacteroides thetaiotaomicron*-derived acetate promotes M1 polarization by upregulating ACC1 through histone acetylation	35
Macrophage	Liver	Enhance immune evasion	Gut microbiota-derived bile acids promote M2-like TAMs	42
DC	Liver	Enhance anti-tumor immunity	EcN stimulates cDC activation	47
DC	Liver?	May strengthen anti-tumor immunity	OMVs from *Bacteroides thetaiotaomicron* trigger DCs to produce IL-10, supporting immune regulation	48
NK Cell	Liver	Improve hepatic immunity and restore function	*Bacteroides uniformis* and *Bifidobacterium bifidum* increase the number and function of liver NK cells by reducing inhibitory molecules	57
CD8+ T Cell	Liver	Strengthen anti-tumor immunity	Acetate derived from *Bacteroides thetaiotaomicron* enhances cytotoxic CD8 + T cell function	35
CD8+ T Cell	Liver, gut	Enhance immune response	Stigmasterol increases gut microbiota diversity, elevating CD8+ T cell levels and activity in both gut and tumor tissues.	67
CD8+ T Cell	Liver	Promote HCC progression	Gut dysbiosis and microbial metabolites suppress cytotoxic CD8+ T cell expansion in MASLD-related HCC	71
CD8+ T Cell	TME	Enhance IFN-γ and granzyme B production	SCFA butyrate suppresses HDACs in CTLs and Tc17 cells, boosting IFN-γ and granzyme B production	72
CD8+ T Cell	TME	Differentiate into memory T cells	SCFA butyrate facilitates the differentiation of activated CD8+ T cells into memory T cells	73
CD4+ T Cell	Liver	Diminished in chronic liver disease	Bacterial extracts from patients with HBV-related liver disease reduce the number of Th1 cells	79
CD4+ T Cell	Liver	Reduce tumor burden	Prohep probiotic mixture reduces Th17 levels in HCC mouse models, reshaping the gut microbiota and fostering beneficial bacteria	91
CD4+ T Cell	Liver	Prevent MASLD progression	*Akkermansia muciniphila* and *Bifidobacterium bifidum* reduce Th17 levels, preventing MASLD development	92
Treg	Liver	Enhance anti-tumor immunity	Stigmasterol alters gut microbiota diversity, increasing beneficial bacteria that reduce Tregs in tumors	67
Treg	Liver?	Enhance immune regulation	*B. fragilis* produces OMVs containing capsular PSA, increasing IL-10 expression in Tregs and activating TLR2 signaling	101,102

ACC1: Acetyl-CoA carboxylase 1; Bregs: Regulatory B cells; CTLs: Cytotoxic T lymphocytes; cDCs: Conventional dendritic cells; E. coli: Escherichia coli; HDACs: Histone deacetylases; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; IL-10: Interleukin-10; MASLD: Metabolic dysfunction-associated steatotic liver disease; MASH: Metabolic dysfunction-associated steatohepatitis; NK: Natural killer; OMVs: Outer membrane vesicles; PSA: Polysaccharide A; SCFAs: Short-chain fatty acids; SFB: segmented filamentous bacteria; TAMs: Tumor-associated macrophages; TLR2: Toll-like receptor 2; TME: Tumor microenvironment; Th1: T helper 1; Tregs: Regulatory T cells.

transformation of naïve B cells into regulatory B cells (Bregs).[28]

2.2. Macrophages: balancing M1/M2 polarization to regulate inflammation and tumor progression

Macrophages are primarily recognized as the body's frontline defense against pathogens. However, they also play a crucial role in interacting with commensal bacteria.[29] In the liver, macrophages are key players in immune defense, with gut microbiota influencing their polarization toward either a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype. For example, *Bacteroides fragilis* enhances the phagocytic function of macrophages by inducing M1 polarization.[30] Conversely, *Echinococcus multilocularis* secretes serpin, which promotes the expansion of M2 macrophages while simultaneously reducing M1 macrophages and increasing anti-inflammatory cytokine levels.[31] Additionally, a previous study demonstrated that treatment with *Akkermansia muciniphila* inhibited the progression from MASH to HCC, coinciding with a reduction in macrophage infiltration. This suggests that gut microbiota may hinder HCC progression by lowering M2 macrophage levels.[32] Moreover, another study found that the *Bacteroides uniformis* strain alleviates MASH by promoting *Akkermansia muciniphila* growth through the production of 3-succinyl bile acid (3-sucCA) in the colon,[33] providing theoretical support for the combined application of these strains.

Importantly, several studies have investigated the effects of microbial products on macrophages.

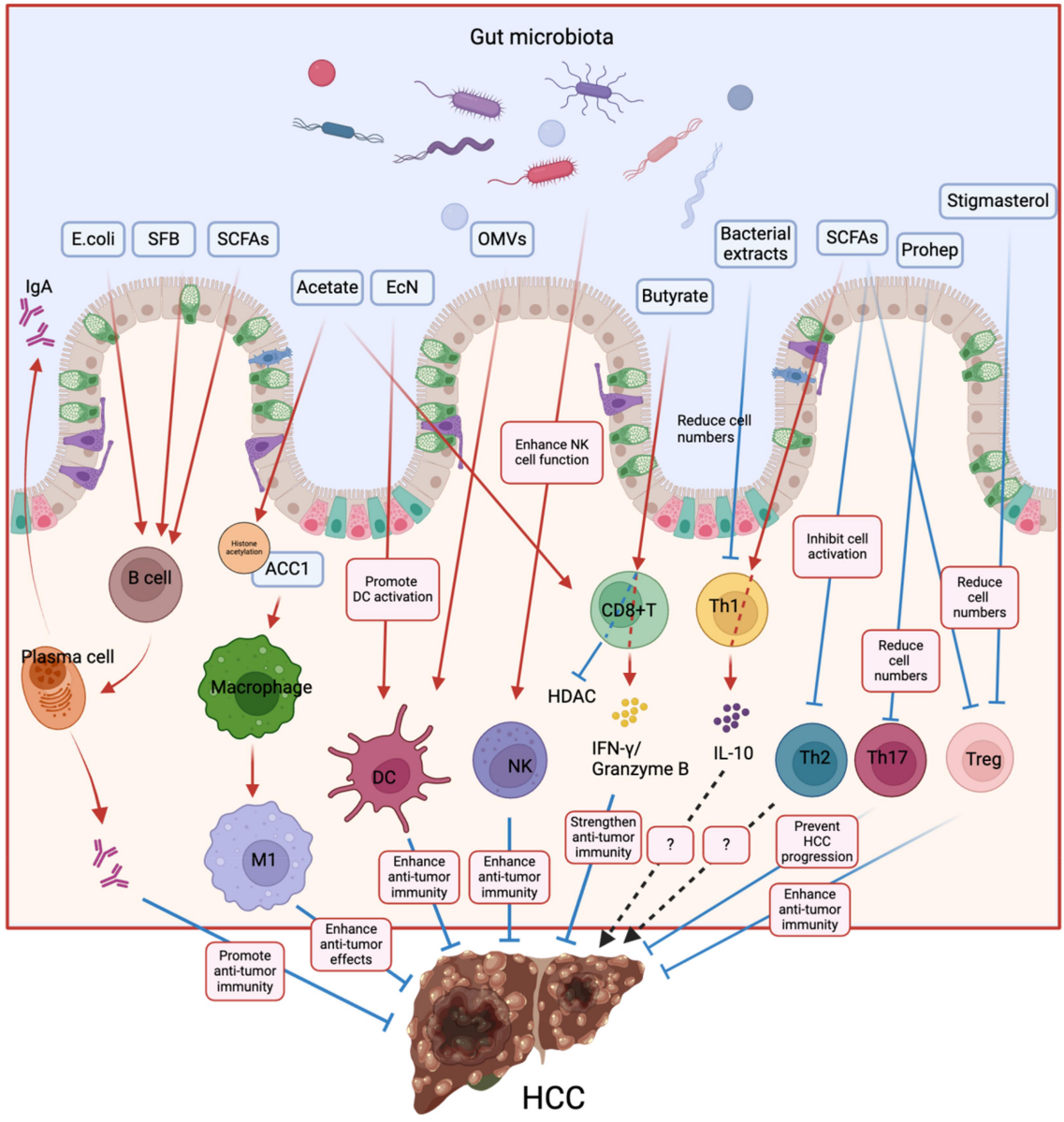

Figure 1. Regulation of immune response by gut microbiota in HCC. This complex microbial community interacts with various immune cells including B cells, macrophages, DCs, NK cells, CD8+T cells, CD4+T cells and Tregs directly affecting their function and, consequently, the progression of HCC (created in https://BioRender.com).

For instance, n-butyrate has been shown to aid colon macrophages in tolerating commensal bacteria by inhibiting the release of NO, IL-6, and IL-12.[34] In HCC fecal samples, *Bacteroides* exhibits the most significant difference between recurrence and non-recurrence groups. Further studies indicate that acetate derived from *Bacteroides thetaiotaomicron* promotes ACC1 transcription by inducing histone acetylation modifications in its promoter region. This enhances fatty acid biosynthesis, thereby promoting M1 macrophage polarization and exerting anti-tumor effects.[35] Additionally, butyrate has been linked to enhanced antimicrobial activity through changes in macrophage metabolism and increased LC3-associated antimicrobial clearance.[36] Meanwhile, TMAO-driven polarization of inflammatory macrophages intensifies Th1 and Th17 responses by altering the microenvironment, exacerbating inflammation-related diseases.[37]

SCFAs derived from the gut microbiota promote tumor cell TLR3-triggered autophagy, leading to increased secretion of the chemokine CCL20. This, in turn, reprograms the tumor

microenvironment (TME) by recruiting more macrophages and polarizing them toward the M2 phenotype, thereby contributing to tumor progression.[38] Future research should focus on determining whether gut microbiota-derived SCFAs drive M2 macrophage polarization within the HCC microenvironment, thereby promoting tumor progression. Specifically, this investigation could explore the involvement of SCFA receptors, such as GPR41 and GPR43,[39] and the activation of key signaling pathways like NF-κB and STAT3.[40] Furthermore, research could examine the role of epigenetic modifications, including histone acetylation, in facilitating these processes.[41] In parallel, gut microbiota-derived bile acids have been shown to enhance the polarization of M2-like tumor-associated macrophages (TAMs), thereby shaping the tumor immune microenvironment to promote immune evasion and drive HCC development and progression.[42]

2.3. DCs: enhancing antigen presentation to boost tumor-specific immunity

DCs are among the most powerful and versatile antigen-presenting cells (APCs) in the immune system, capable of initiating adaptive immune responses and strengthening innate immunity.[43] DCs are generally classified into two main types: plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs).[44,45]

Research suggests that cDCs may not achieve full activation due to insufficient interferon-I (IFN-I) signaling. Notably, the gut microbiota, which plays a critical role in regulating IFN-I production by pDCs, also influences the basal activation state of cDCs.[46] Interestingly, in HCC tumor models, *E. coli* strain Nissle 1917 (EcN), a widely used probiotic, has been shown to stimulate DC activation, leading to robust anti-tumor immune responses.[47] This indicates that alterations in gut microbiota composition may enhance DC-mediated anti-tumor immunity against HCC. Another example of this gut-immune system communication involves outer membrane vesicles (OMVs) from *Bacteroides thetaiotaomicron*, which are essential in triggering DCs to produce the regulatory cytokine IL-10.[48] Additionally, Bessman and colleagues discovered that in the colon, hepcidin, produced by cDCs in response to microbial signals, plays a crucial role in maintaining intestinal homeostasis.[49] Future research in HCC should explore how gut microbiota-derived factors, such as OMVs and microbial metabolites, influence DC function and the TME. Identifying specific microbial signals that regulate DC activity could reveal novel therapeutic targets for enhancing anti-tumor immunity.

2.4. NK cells: activating cytotoxic pathways to suppress tumor growth

NK cells are a vital component of the innate immune system, comprising approximately 15% of all lymphocytes.[50] Evidence suggests that specific NK cells in the gut, which contribute to mucosal defense, are regulated by the commensal microbiota through the expression of the transcription factor RORγt and the cytokine IL-22.[51] Multiple studies, including four clinical trials, have demonstrated that synbiotics or probiotics can improve gut microbiota composition, enhance NK cell activity, and increase levels of associated cytokines.[52–55] For example, Qiu et al.[56] found that the probiotic *Lactobacillus plantarum* significantly boosts IL-22 mRNA and protein expression in NK cells, thereby helping to protect the intestinal epithelial barrier from damage.

Additionally, fecal sample analysis has shown that *Bacteroides uniformis* and *Bifidobacterium bifidum*, which are abundant in healthy individuals, are significantly depleted in patients with MASH. These bacteria not only improve hepatic pathology and metabolic conditions but also increase the number of NK cells in the liver and restore their function. Transcriptomic and proteomic analyses suggest that these beneficial species may restore liver NK cell function by reducing inhibitory molecules within NK cells.[57] This finding indicates that the gut microbiota may help suppress liver cancer development by increasing the number and functionality of hepatic NK cells.

Notably, multiple factors – including diet, bile acids, gender, age, and antibiotic use – affect the structure and composition of the gut microbiota. Among these, a Western diet is a major risk factor for the progression of metabolic dysfunction-associated steatotic liver disease (MASLD) to

MASH, cirrhosis, and eventually HCC.[58] Studies have shown that this dietary pattern significantly alters the enterohepatic circulation (EHC) of bile acids.[59,60] Research by Ma et al. further revealed that gut microbiota-mediated conversion of primary bile acids to secondary bile acids significantly elevates secondary bile acid levels, such as deoxycholic acid (DCA). DCA suppresses CXCL16 expression in liver sinusoidal endothelial cells (LSECs), thereby reducing the accumulation of CXCR6+ hepatic natural killer T (NKT) cells and promoting HCC progression.[61] Additionally, DCA has been shown to exacerbate HCC by modulating the senescence-associated secretory phenotype (SASP) in hepatic stellate cells, fostering a tumor-promoting microenvironment.[62] Moreover, disruptions in EHC can lead to elevated levels of primary conjugated bile acids, such as TCA. TCA activates sphingosine-1-phosphate receptor 2 (S1PR2), which has been implicated in promoting pancreatic cancer metastasis.[63] Importantly, studies have demonstrated that S1PR2 facilitates MASLD-HCC progression via the PI3K/AKT/mTOR pathway.[64] Collectively, these findings suggest that gut microbiota-mediated EHC homeostasis influences the immune microenvironment through bile acid alterations, thereby regulating HCC progression.

2.5. CD8+T cells: activation strengthens cytotoxicity against tumor cells

T cells play a critical role in coordinating immune responses and eliminating damaged cells, with CD4+ and CD8+ T cells fulfilling distinct functions. CD8+ T cells, in particular, are essential for combating infections and cancer by secreting interferon-gamma (IFN-γ) and the protease granzyme B, both of which contribute to the destruction of infected or malignant cells.[65] Cytotoxic T lymphocytes (CTLs), a subset of CD8+ T cells, can be activated by the gut microbiota and its metabolites. Once activated, they exert direct cytotoxic effects and engage with other immune cells, particularly within the TME.[66] In the context of HCC, acetate derived from *Bacteroides thetaiotaomicron* has been shown to enhance the function of cytotoxic CD8+ T cells, thereby strengthening anti-tumor immunity.[35] Similarly, a recent study demonstrated that stigmasterol alters the α and β diversity of the gut microbiota in HCC mouse models, significantly increasing the abundance of *Lactobacillus johnsonii*, *Lactobacillus murinus*, and *Lactobacillus reuteri*. This shift in microbial composition led to elevated levels of CD8+ T cells in both the colon and tumor tissues, thereby enhancing the immune response within the host TME.[67]

Conversely, microbial imbalances can exacerbate chronic inflammation and increase tumor susceptibility, ultimately impairing CD8+ T cell activity and, in some cases, driving their exhaustion.[68–70] Notably, research has shown that gut dysbiosis is a hallmark of patients with liver cirrhosis, irrespective of HCC presence. In MASLD-related HCC, gut microbiota-derived metabolites have been implicated in suppressing the expansion of cytotoxic CD8+ T cells.[71] Further studies indicate that the gut microbiota modulates T cell immune responses through SCFAs.[71] Butyrate, for instance, has been shown to negatively regulate HDACs in CTLs and Tc17 cells, thereby enhancing IFN-γ and granzyme B production.[72] Additionally, butyrate has been found to facilitate the differentiation of activated CD8+ T cells into memory T cells.[73] Targeting the intricate interplay between CD8+ T cells and the gut microbiota presents significant therapeutic potential for future research, offering new avenues for enhancing anti-tumor immunity in HCC.

2.6. CD4+T cells: shaping immune responses through Th1, Th2, and Th17 pathways

CD4+ T cells play a critical role in coordinating both humoral and cellular immunity by promoting immune cell activation in a cytokine-dependent manner.[74,75] Different T cell subsets exhibit distinct roles in protective immunity and responses to gut microbiota, primarily due to their varying production of signature cytokines.[76] Th1 cells secrete cytokines such as IFN-γ, IL-2, and TNF-α, while Th2 cells are characterized by the production of IL-4, IL-5, and IL-13. Th17 cells, which are abundant in the gastrointestinal tract, regulate gut microbiota and are primarily defined by their secretion of IL-17A, IL-17F, and IL-22.[77] The functions of Th1 and Th2 cells are influenced by gut microbiota-

derived metabolites.[78] For example, bacterial extracts from patients with HBV-related chronic liver disease have been shown to reduce Th1 cell numbers.[79] Additionally, SCFAs have been linked to a reduced capacity to trigger a Th2 cell immune response.[80] Moreover, SCFAs can enhance microbe antigen-specific IL-10 production in Th1 cells via GPR43, while also promoting the expansion of the Th1 transcription factor T-bet.[81] Interestingly, cancer patients exhibit reduced plasma tryptophan (Trp) levels, which are associated with elevated Th1-type immune activation markers.[82]

A potential link between Th17 cells and gut microbiota has been observed in various diseases. Specific alterations in the intestinal mucosa-associated microbiota correlate with an increased presence of intestinal Th17 cells and a higher disease burden.[83] Preclinical studies further reinforce this association, demonstrating that an increased population of pathogenic colonic Th17 cells may contribute to HCC formation.[84] However, a direct causal relationship has yet to be established. Given their high plasticity, Th17 cells have been proposed as key drivers of intestinal immune disorders.[85,86] Research indicates that gut microbiota and their metabolites influence Th17 cell activation. In the absence of gut microbiota, microbial metabolites can restore the compromised plasticity of Th17 cells.[87,88] For example, SFB is known to induce homeostatic Th17 cells in the intestines.[89] Atarashi and colleagues demonstrated that SFB adhesion to IECs is critical for Th17 cell induction and antigen presentation to pro-Th17 DCs.[90] Additionally, Prohep, a novel probiotic mixture, has been shown to significantly reduce Th17 cell levels in an HCC mouse model, thereby inhibiting tumor growth. Shotgun metagenomic sequencing analysis of fecal samples further revealed intricate interactions between gut microbial metabolites and HCC progression. Probiotics reshaped the gut microbiota by promoting the growth of beneficial bacteria such as *Prevotella* and *Oscillibacter*, known producers of anti-inflammatory metabolites. This shift in microbial composition led to a reduction in Th17 polarization.[91] Similarly, *Akkermansia muciniphila* and *Bifidobacterium bifidum* have been found to decrease Th17 cell levels, thereby preventing the development of MASLD. These findings suggest that gut microbiota-mediated regulation of Th17 cells may influence HCC progression by modulating MASLD development.[92] Furthermore, various gut-derived metabolites, such as bile acids and SCFAs, contribute to Th17 cell function and differentiation.[93,94] Lastly, dietary factors have also been shown to exert complex effects on Th17 cells.[95]

2.7. Tregs: suppression of Tregs to counteract tumor immune escape

Tregs, derived from naïve CD4+ T cells, are indispensable for maintaining immune tolerance and homeostasis. They are defined by the expression of the transcription factor Foxp3 in the nucleus, along with CD25 and CTLA-4 on their surface, which collectively regulate their immunosuppressive function.[96] Importantly, these molecules are influenced by signals from the gut microbiota.[97,98] Recent studies have shown that stigmasterol alters the α and β diversity of the gut microbiota in fecal samples from an HCC mouse model, significantly increasing the abundance of *Lactobacillus johnsonii*, *Lactobacillus murinus*, and *Lactobacillus reuteri*. This shift in gut microbiota composition led to a reduction in tumor-infiltrating Tregs and enhanced the anti-tumor immune response in HCC.[67] Additionally, TGF-β, a key physiological driver of Foxp3 transcription and crucial for Treg development, can be induced by *Clostridia strains*.[99,100] Moreover, *Bacteroides fragilis* has been found to produce OMVs containing capsular PSA, which increases IL-10 expression in Tregs and activates TLR2 signaling in both T cells and DCs.[101,102] SCFAs also play a role in regulating the number and functionality of Tregs.[103,104] Specifically, butyrate promotes histone H3 acetylation at the Foxp3 gene locus, while propionate functions by inhibiting HDACs.[105,106]

Furthermore, interactions between the central nervous system and the gut microbiota contribute to intestinal immune homeostasis. For example, IL-6 produced by enteric neurons regulates the number and phenotype of microbe-responsive

Tregs.[97] Similarly, another study demonstrated that the liver-brain-gut neural arc sustains the Treg cell niche, thereby preserving intestinal immune balance.[107] These findings suggest that targeting the liver-brain-gut autonomic feedback system to modulate immune cells may represent a promising strategy for HCC treatment.

3. Impact of gut microbiota on immunotherapy outcomes in HCC

The field of cancer immunotherapy has made remarkable strides over the past few decades. Among these advancements, various immunotherapeutic strategies have been developed to restore immune function by targeting the inhibitory mechanisms that tumor cells exploit to evade immune surveillance. In recent years, groundbreaking research has increasingly highlighted the intricate relationship between the gut microbiota and cancer immunotherapy. This review provides an overview of the latest discoveries in this area, organized according to different types of immunotherapy (Table 2).

3.1. PD-1/PD-L1

PD-1 is a co-inhibitory receptor expressed on tumor-infiltrating lymphocytes (TILs).[108] Within the TME, PD-1 interacts with PD-L1, leading to the suppression of CTL-mediated cytolysis and inhibition of Fas-induced apoptosis, thereby allowing tumor cells to evade immune surveillance and continue proliferating.[109,110] PD-1/PD-L1 inhibitors, such as nivolumab, pembrolizumab, and atezolizumab, have demonstrated efficacy in clinical trials by enhancing immune responses against cancer cells.[111,112]

Notably, groundbreaking studies have linked PD-1/PD-L1 blockade with the gut microbiome. Preclinical research has explored several key mechanisms underlying this connection, including: (1) changes in gut microbiota composition due to immune checkpoint inhibitors (ICIs), (2) the influence of gut bacteria on intestinal immune cells, (3) microbiota-induced metabolic shifts affecting the host immune response, and (4) the role of the gut microbiome in recruiting immune cells to the TME. Sivan et al.[113] were among the first to highlight this crosstalk, demonstrating that *Bifidobacterium* enhances dendritic cell function and promotes the activation and accumulation of CD8+ T cells in the TME. Similarly, Routy et al.[114] conducted a clinical study using metagenomic analysis of patient fecal samples, revealing that certain microbes, such as *Akkermansia muciniphila* and *Enterococcus hirae*, are associated with improved responses to PD-1/PD-L1 blockade. Further animal experiments suggested that this antitumor effect was restored via an IL-12-dependent mechanism, leading to increased recruitment of CCR9+CXCR3+CD4+ T cells into the TME. Conversely, other studies have found that while *Prevotella* and *A. muciniphila* enhanced the effectiveness of PD-

Table 2. Impact of gut microbiota on immunotherapy outcomes in HCC.

Bacteria/Metabolites	Impacted Therapy	Prognosis	Potential Mechanism	Ref.
Lachnospiraceae bacterium-GAM79	PD-1/PD-L1 inhibitors	Significantly longer PFS and OS	May enhance antitumor immune response by modulating the immune microenvironment	118
Alistipes sp. Marseille-P5997	PD-1/PD-L1 inhibitors	Significantly longer PFS and OS	May regulate immune responses via gut microbiota-related metabolic shifts	118
Veillonellaceae	PD-1/PD-L1 inhibitors	Poorer PFS and OS	May be associated with immune suppression	118
Firmicutes	CTLA-4 inhibitors	Longer PFS and OS	Enhances Th1-dependent antitumor immune response by increasing ICOS expression on CD4+ T cells and sCD25 levels	136
Butyrate	CTLA-4 inhibitors	Inhibits therapeutic efficacy	Inhibits DC maturation and CD28 signaling pathway, reducing T cell activation	137
Firmicutes and Bacteroides	CTLA-4 inhibitors	Enhanced immunosuppressive response	Disruption of beneficial bacteria, leading to enhanced immunosuppressive effects of CTLA-4 inhibition	138
Butyrate	GPC3-specific CAR-T cells	Enhanced CAR-T cell efficacy, inhibited HCC growth	Enhances CAR-T cell activity through butyrate, promoting antitumor immune response	148
Butyrate nanoparticle drug	GPC3-specific CAR-T cells	Significantly boosted T cell immunity, inhibited HCC growth	Butyrate-encapsulated nanoparticles conjugated with GPC3 antibody enhance T cell immune response against HCC	149

CAR-T cells: Chimeric antigen receptor T cells; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; DC: Dendritic cell; GPC3: Glypican-3; HCC: Hepatocellular carcinoma; ICOS: Inducible costimulator; OS: Overall survival; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; PFS: Progression-free survival; sCD25: Soluble CD25.

1/PD-L1 inhibitors, *Bacteroides* was associated with reduced efficacy, potentially through its impact on glycerophospholipid metabolism and the regulation of IFN-γ and IL-2 expression in the TME.[115] In a mouse model of HCC, 16S rRNA sequencing of fecal samples revealed that anti-PD-1 therapy altered gut microbiota composition, which may have contributed to immune microenvironment remodeling in HCC.[116]

Subsequent clinical studies have reinforced the link between gut microbiota composition and ICI efficacy, expanding these insights beyond preclinical models. Zheng et al. examined the dynamic changes in gut microbiota composition and characteristics in fecal samples from HCC patients undergoing anti-PD-1 immunotherapy following prior treatment with sorafenib. Their findings revealed that responders exhibited greater microbial diversity and a higher gene count in their fecal microbiota compared to non-responders, highlighting the potential of gut microbiota as a therapeutic target.[117] Furthermore, in a study involving 65 patients with advanced hepatobiliary cancer undergoing anti-PD-1 therapy, those with higher abundances of *Lachnospiraceae bacterium-GAM79* and *Alistipes sp. Marseille-P5997* exhibited significantly longer progression-free survival (PFS) and overall survival (OS) compared to patients with lower levels of these bacteria. In contrast, patients with higher abundances of *Veillonellaceae* had poorer PFS and OS.[118] These findings suggest that different gut microbial species may have distinct roles in shaping the efficacy of immunotherapy in HCC, highlighting the urgent need to further investigate the underlying mechanisms.

Additionally, the study suggested that the antitumor functions of certain bacteria may be linked to SCFA production and bile acid metabolism,[118] providing a promising avenue for future research into the molecular mechanisms by which beneficial gut microbiota enhance PD-1 therapy efficacy. While several studies have associated favorable gut microbiota profiles with improved ICI responses in HCC patients,[119–121] a more recent study did not identify a positive correlation,[122] potentially due to differences in microbiota composition across patient cohorts. Future research should focus on identifying gut microbial species that enhance anti-PD-1 therapy efficacy and elucidating the molecular mechanisms involved to guide microbiota-based therapeutic strategies for HCC.

3.2. CTLA-4

CTLA-4 is a key inhibitory receptor on T cells, with its expression significantly increasing upon T-cell activation.[123–125] Given its critical role in immune regulation, CTLA-4 inhibitors such as ipilimumab and tremelimumab are designed to enhance antitumor immune responses by counteracting CTLA-4's potent immunosuppressive effects.[126,127] Notably, CTLA-4 inhibitors have already been applied in the treatment of HCC. Tremelimumab was initially evaluated as monotherapy in a phase II clinical trial for patients with HCV-related cirrhosis and secondary HCC, yielding an objective response rate (ORR) of 17.6%. The trial reported a time to progression of 6.48 months, along with a favorable safety profile.[128] Subsequently, another clinical study investigated the combination of tremelimumab with locoregional treatment, demonstrating an improved ORR of 26.3%, potentially due to the accumulation of intratumoral CD8+ T cells in treated patients.[129]

Mechanistically, blocking CTLA-4 influences the Th1 subset of CD4+ T cells, which are characterized by the expression of inducible costimulator (ICOS).[130,131] In addition, both effector T cells and Tregs are primary targets of CTLA-4 inhibition.[132,133] Research has revealed that specific gut microbiota species may enhance the clinical efficacy of anti-CTLA-4 immunotherapy. Initially, alterations in the gut microbiome were thought to trigger IL-12-dependent Th1 immune responses, thereby promoting antitumor effects.[134,135] For instance, Chaput et al.[136] conducted a prospective clinical study demonstrating that patients with a higher abundance of *Firmicutes* experienced longer PFS and OS, which correlated with increased ICOS expression on CD4+ T cells and elevated sCD25 levels. Conversely, recent findings suggest that certain microbial metabolites, such as butyrate, may negatively impact CTLA-4 inhibition. Specifically, systemic butyrate has been shown to suppress ipilimumab-induced DC

maturation and inhibit the CD28 signaling pathway, potentially reducing treatment efficacy.[137] Additionally, Feng et al. conducted a clinical study evaluating the relationship between gut microbiota alterations, clinical parameters, and peripheral immune responses in patients with HBV-HCC. Their results indicated that in HBV-HCC patients, the depletion of beneficial gut microbiota – primarily *Firmicutes* and *Bacteroides* —was associated with an enhanced CTLA-4-mediated immunosuppressive response.[138] However, whether changes in gut microbiota directly affect the efficacy of CTLA-4 inhibitors remains to be fully elucidated. To address this gap, future research should focus on developing in vivo HCC models to explore the therapeutic potential of combining microbiota transplantation with CTLA-4 inhibitors for HCC treatment.

3.3. Adoptive cell transfer (ACT)

ICI function by reactivating preexisting tumor-specific T cells.[139] However, for cancers that are not highly immunogenic, ACT offers a promising alternative.[140] ACT can be performed through two primary approaches: (1) extracting TILs from the TME or (2) modifying peripheral blood T cells with chimeric antigen receptors (CAR). In both methods, T cells are engineered in vitro before being reinfused into the patient.[141,142] Given the challenges associated with ACT, strategies that modify the immune microenvironment – such as altering the gut microbiota – have gained attention as potential ways to enhance treatment efficacy. These approaches aim to optimize immune cell function, counteract tumor-induced immune suppression, and improve the effectiveness of T-cell therapies.[143]

An early preclinical study by Paulos et al. revealed that microbial translocation enhances ACT efficacy and self-tumor-specific CD8+ T cell function in melanoma via the TLR4 signaling pathway. Activation of this pathway stimulates DCs and promotes the release of pro-inflammatory cytokines in the gut, ultimately improving ACT outcomes.[144] Similarly, another study on cervical cancer demonstrated that vancomycin treatment increases systemic CD8α+ DCs, which sustain adoptively transferred antitumor T cells through an IL-12-dependent mechanism.[145] Similarly, another study on cervical cancer demonstrated that vancomycin treatment increases systemic CD8α+ DCs, which sustain adoptively transferred antitumor T cells through an IL-12-dependent mechanism

In recent years, growing evidence has highlighted the potential of CAR-T therapy, particularly Glypican-3 (GPC3)-specific CAR-T cells, in effectively suppressing tumor growth in in vivo HCC models.[146,147] For instance, a recent study found that mice transplanted with fecal microbiota from gastric cancer patients developed more tumors during gastric cancer induction and exhibited lower butyrate levels. Further in vivo and in vitro investigations demonstrated that the gut microbiota could enhance CAR-Claudin 18.2+ CD8+ T cell activity through butyrate.[148] Notably, a nanodrug engineered by covalently conjugating butyrate-encapsulated nanoparticles with an antibody targeting GPC3 —a HCC-specific antigen – significantly enhanced T-cell immunity against HCC, thereby suppressing tumor growth in vivo.[149] These findings suggest a plausible hypothesis that gut microbiota, through its butyrate derivatives, could improve the therapeutic efficacy of GPC3-specific CAR-T cells in HCC treatment.

4. Gut microbiota modifcations in response to HCC immunotherapy

In recent years, research has increasingly demonstrated that the gut microbiota and its metabolites play a pivotal role in regulating the host immune system, modulating antitumor immunity, and influencing responses to immunotherapy. As a result, strategies targeting the gut microbiota to enhance ICI efficacy have emerged as promising therapeutic approaches. This section reviews both preclinical and clinical trials aimed at improving ICI treatment outcomes through gut microbiota modulation. The primary strategies explored in these studies include FMT, dietary interventions, probiotics, prebiotics, and the development of engineered microbial products (Figure 2).

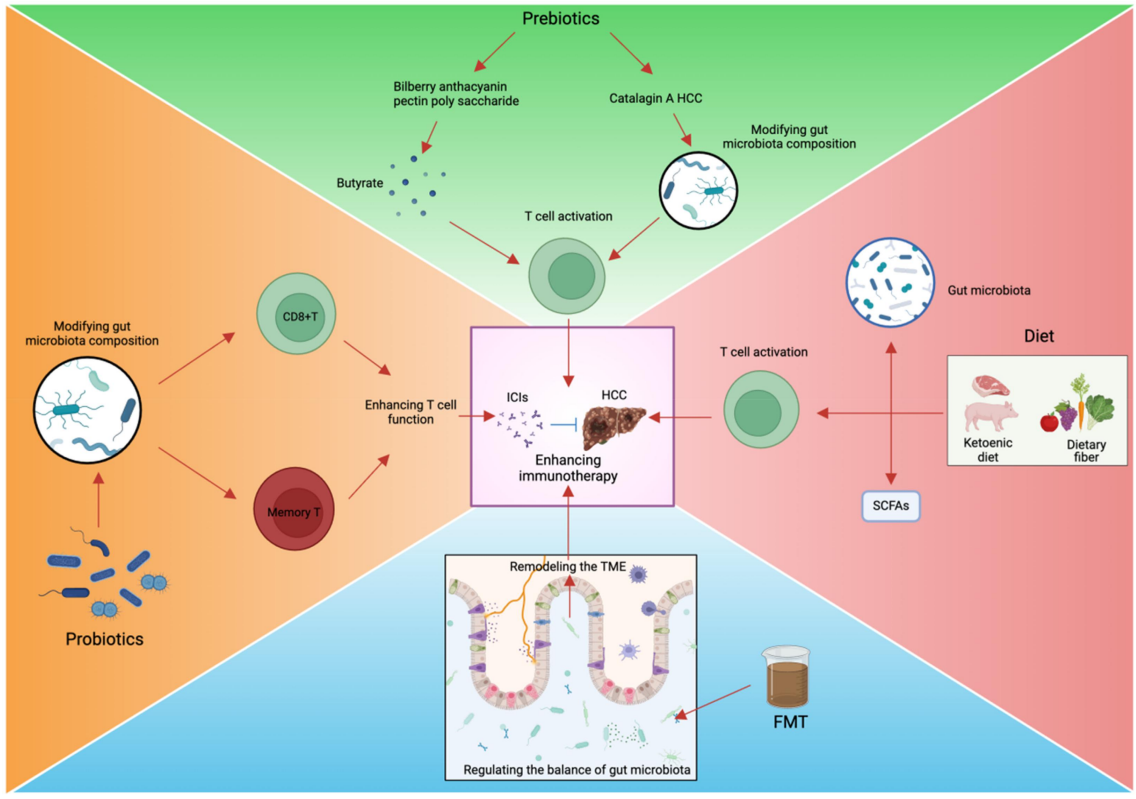

Figure 2. Gut microbiota modifications in response to HCC immunotherapy. The primary methods employed in these studies include fecal FMT, dietary regulation, probiotics, prebiotics, and the development of engineered microbial products (created in https://BioRender.com).

4.1. FMT

FMT is a well-established clinical technique for modifying gut microbiota.[150] By introducing healthy microbial communities, FMT helps restore microbial diversity in recipients' intestines.[151] The FDA currently endorses FMT for the treatment of recurrent *Clostridium difficile* infections.[152] Given the distinct microbial profiles associated with patients who respond favorably to ICIs, there is growing interest in integrating FMT into immunotherapy strategies. Early studies combining FMT with immunotherapy have demonstrated that FMT can influence T-cell and NK cell pathways, thereby enhancing immune responses and promoting tumor suppression.[114,153]

Recent research has focused on the use of oral stool capsules for FMT in patients receiving ICI treatment. The results have been promising, showing increased levels of bacteria linked to improved responses to anti-PD-1 therapy, enhanced CD8+ T cell activation, and reduced IL-8-expressing myeloid cells.[154–156] Additionally, Li et al. collected fecal samples from HCC patients and healthy donors and performed FMT in an HCC mouse model. Their study revealed that gut dysbiosis impairs antitumor immune responses by inhibiting antigen presentation and suppressing effector T cell function via the cGAS-STING-IFN-I pathway.[157] Notably, activation of the cGAS-STING pathway has been shown to enhance the antitumor efficacy of PD-1 blockade in HCC.[158] Collectively, these findings suggest that combining FMT with ICIs represents a promising therapeutic strategy for HCC, offering new opportunities to improve immunotherapy outcomes. In terms of safety, data from Routy et al. confirmed that incorporating FMT into anti-PD-1 therapy does not increase the incidence of immune-related adverse events (irAEs).[156] Similarly, Spreafco et al. tested a microbial consortium called Microbial Ecosystem Therapeutic 4 as an alternative to FMT in combination with ICIs for patients with advanced solid tumors, reporting no exacerbation of ICI-related irAEs.[159] Given these encouraging findings, large-scale clinical trials are warranted to further evaluate

the feasibility of FMT for enhancing ICI efficacy in HCC patients and to elucidate the underlying mechanisms.

Additionally, radiotherapy (RT) remains a crucial treatment option for patients with unresectable HCC. A prospective longitudinal study involving 24 hCC patients undergoing RT collected fecal samples for 16S rRNA sequencing, which were subsequently used for FMT in HCC mouse models. The study revealed that the gut microbiome regulates RT sensitivity in HCC through cGAS-STING signaling in DCs, with bacterial-derived c-di-AMP in the feces modulating RT-induced cGAS-STING activation in DCs.[157] Despite its potential, FMT also carries notable risks.[160] The full transfer of gut microbiota may disrupt the recipient's existing microbial balance, potentially leading to infectious diseases.[161] Future studies should prioritize establishing comprehensive safety profiles and optimizing treatment protocols to maximize therapeutic efficacy while minimizing potential risks.

4.2. Dietary regulation

Recent findings have underscored the profound influence of diet on gut microbiota composition and function.[162] Numerous studies have demonstrated that dietary modifications can directly shape the gut microbiome. For instance, the typical Western diet – high in fats and carbohydrates but low in fiber – has been linked to gut dysbiosis. This imbalance is characterized by an increased abundance of microbial populations such as *Firmicutes*, *Proteobacteria*, *Mollicutes*, *Bacteroides spp*., *Alistipes spp*., *Bilophila spp*., *Enterobacteriaceae*, *Escherichia*, *Klebsiella*, and *Shigella*, while beneficial bacteria such as *Bacteroidetes*, *Prevotella*, *Lactobacillus spp*., *Roseburia spp*., *Eubacterium rectale*, *Bifidobacterium*, and *Enterococcus* decline. Consequently, this dysbiosis leads to elevated BA secretion and reduced production of SCFAs.[163–165] In contrast, low-fat, high-fiber diets have been shown to promote beneficial shifts in the gut microbiota, increasing *Prevotella* and *Bacteroides* while reducing *Firmicutes* levels.[166] These findings suggest that dietary regulation of gut microbiota may serve as a promising strategy for enhancing cancer treatment efficacy.[167–169]

A clinical study further reinforced this concept, investigating the impact of the food-gut axis on ICI responses and revealing a positive correlation between high-fiber diets and improved anticancer immunotherapy outcomes. Specifically, patients on a high-fiber diet exhibited elevated expression of genes related to T-cell activation and the interferon response, likely induced by SCFAs produced by fiber-fermenting bacteria.[170] Interestingly, while mice fed fermentable fiber containing inulin exhibited an increase in liver CD8+ T cell levels, fermentation-induced gut microbiota dysbiosis led to bile acid accumulation, which in turn contributed to HCC development.[171] Thus, a high-fiber diet may enhance the efficacy of HCC immunotherapy by modulating immune responses. However, ensuring a stable gut microbiota environment is essential to prevent potential adverse effects, such as dysbiosis-induced bile acid accumulation, which may promote hepatocarcinogenesis. Future clinical trials are necessary to determine whether a high-fiber diet influences the efficacy of PD-1/PD-L1 immunotherapy in HCC and to identify the specific microbial taxa and metabolites involved.

Similarly, the ketogenic diet – characterized by high fat, low protein, and low carbohydrate intake – is well known for its ability to counteract tumor-induced immunosuppression mediated by lactate and influence tumor cell metabolism.[172] Ferrere et al.[173] investigated the potential benefits of combining a ketogenic diet with immunotherapy in a mouse model, finding that ketone body supplementation restored therapeutic responses when ICIs alone were insufficient to suppress tumor growth. Additionally, fecal sample analysis revealed that the ketogenic diet reshaped the gut microbiota, leading to the expansion of CXCR3+ T cells and the inhibition of IFNγ-driven PD-L1 expression on myeloid cells. Furthermore, the ketogenic diet has been shown to suppress HCC tumor growth by upregulating HMGCS2 protein expression.[174] Notably, HMGCS2 overexpression inhibits CXCL12 expression by suppressing HDAC1-dependent KLF5 expression, thereby alleviating the immunosuppressive TME. This mechanism enhances NK and cytotoxic T cell infiltration and improves the efficacy of anti-PD-1 therapy in colorectal cancer.[175] These findings provide a strong theoretical foundation for further

investigating how the ketogenic diet may enhance PD-1 antibody treatment efficacy for HCC by modulating immune homeostasis.

Additionally, the regional compartmentalization of intestinal immunity has been extensively studied and widely recognized for decades.[176,177] Consequently, some therapeutic interventions may overlook the spatial specificity of immune responses along different regions of the small and large intestines. Research has shown that IL-17- and IL-22-producing T cells are predominantly found in the small intestine, whereas IgA-producing B cells and a large population of Tregs are primarily localized in the colon.[178] These findings suggest that immune regional specializations may influence HCC through distinct mechanisms.[16,179,180] Furthermore, regional specialization extends to gut microbiota composition, with significant differences observed between the proximal gut and feces.[181] These variations in immune activation highlight the importance of considering spatial differences in microbial and immune functions when designing dietary interventions and microbiota-targeted therapies to optimize treatment precision and efficacy.

4.3. Probiotics

Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer health benefits to the host".[182] They have been widely used for the prevention and treatment of various diseases.[6,183] More recently, studies suggest that combining probiotics with PD-1 inhibitors and antiangiogenic agents can effectively improve clinical outcomes in patients with HCC.[184]

The use of single probiotic strains has shown promising therapeutic potential when combined with cancer immunotherapy. For instance, supplementation with *Bifidobacterium* has been found to significantly enhance the efficacy of PD-1 inhibitors.[113,185] Interestingly, *Bifidobacterium pseudolongum* supplementation was shown to restore a healthy fecal microbiome composition in a MASLD-HCC mouse model, significantly inhibiting tumor proliferation. This finding suggests a potential role for probiotics in enhancing the anti-HCC efficacy of ICIs. Additionally, research has demonstrated that *Lactobacillus* species can improve the effectiveness of PD-1 inhibitors by increasing the abundance of beneficial bacteria and reshaping the functional gut metagenome.[186] In the context of HCC, *Lactobacillus acidophilus* treatment was found to enrich symbiotic probiotics in fecal samples and significantly suppress MASLD-HCC progression.[187] In the context of HCC, *Lactobacillus acidophilus* treatment was found to enrich symbiotic probiotics in fecal samples and significantly suppress MASLD-HCC progression. Furthermore, while PD-1 inhibitors have shown remarkable efficacy in cancer treatment, they are also associated with irAEs, such as colitis and hepatitis. Notably, *Lactobacillus rhamnosus* has been shown to modulate inflammatory pathways in the gut and liver in HCC murine models, suggesting its potential as a therapeutic strategy to manage these irAEs while improving treatment outcomes.[188] Collectively, these findings further support the combined use of probiotics and PD-1 inhibitors in HCC therapy.

Compared to single probiotic strains, bacterial consortia may better replicate the diverse functional properties of the gut microbiota. For example, Tanoue et al.[189] tested an 11-strain commensal bacterial consortium in tumor-bearing mice and found that the improvement in ICI efficacy was dependent on CD103+ dendritic cells and major histocompatibility complex class IA cells. Similarly, another study demonstrated that a combination of four *Clostridiales* species exerted antitumor effects by activating CD8+ T cells and increasing tumor immunogenicity.[190] However, other studies suggest that multi-strain probiotics may have limitations. Suez et al.[191] reported that an 11-strain probiotic cocktail delayed the reconstitution of the gut mucosal microbiota, highlighting the need for personalized probiotic strategies that support mucosal integrity while allowing natural recolonization of the host microbiota following antibiotic treatment.

Despite growing interest in probiotic-based immunotherapy, debate remains regarding the efficacy of commercially available probiotics marketed as dietary supplements.[192] Challenges include a lack of standardization in strain

composition, dosage, and quality control. Additionally, clinical outcomes for probiotic formulations in immunotherapy have varied widely, with some studies reporting limited or no benefits.[170] These challenges underscore the need for further research to standardize probiotic formulations and elucidate their mechanisms of action, ensuring consistent and reliable therapeutic outcomes.

4.4. Prebiotics

A prebiotic is defined as a substrate that selectively benefits host microorganisms, thereby promoting health.[193] Emerging research suggests that prebiotics play a crucial role in immune modulation, gut barrier integrity, and metabolic function.[194]

Notably, prebiotics may enhance the immunomodulatory effects of ICIs by influencing SCFA levels. Studies have shown that natural prebiotics – such as bilberry anthocyanins, pectin, inulin (a plant polysaccharide), and ginseng polysaccharides – can positively impact anti-PD-1 therapy. These compounds enhance the production of beneficial SCFAs, which, in turn, stimulate systemic memory T-cell responses and promote T-cell infiltration and activation within the TME.[195] Similarly, artificial prebiotics like AHCC® (a standardized extract from *Lentinula edodes* mycelia) and castalagin have been found to improve ICI efficacy by modifying gut microbiota composition and enhancing T-cell functions within the TME (fecal samples).[196] Given the intricate connection between gut microbiota and liver health, further research should explore how various prebiotics influence the gut-liver axis and modulate immune responses in HCC. Additionally, clinical trials could evaluate the impact of specific prebiotic interventions combined with ICI therapies on patient outcomes, including tumor response rates and overall survival.

5. Challenges and future perspectives

The investigation of gut microbiota as a key regulator of immune responses in HCC has unveiled promising new avenues for research and therapy. However, several critical challenges must be addressed to fully harness the potential of gut microbiota modulation in clinical practice.

5.1. Complexity and variability of gut microbiota

One of the primary challenges stems from the inherent complexity and variability of the gut microbiota. Each individual harbors a unique microbial community influenced by factors such as genetics, diet, environment, and lifestyle. This variability makes it difficult to develop universal therapeutic strategies for microbiota modulation. Moreover, the gut microbiota is highly dynamic, continuously evolving over time and in response to external stimuli. This further complicates the standardization of interventions such as probiotics, prebiotics, and FMT, posing a significant hurdle in ensuring consistent and reproducible therapeutic outcomes.[197] Developing robust microbial biomarkers through metagenomic and metabolomic analyses could enable better patient stratification and response prediction. Additionally, computational models integrating multi-omics data could be developed to optimize individualized microbiota-targeted interventions.

5.2. Methodological challenges in clinical studies

Additionally, clinical studies on gut microbiota are often limited by inconsistencies in sampling methods, including variations in stool collection, storage, and processing protocols. These discrepancies can introduce biases and reduce the reproducibility of results.[198] Moreover, the absence of standardized microbiota sequencing protocols further complicates cross-study comparisons, making it difficult to draw definitive conclusions about microbiota-targeted therapies and their clinical applications. Establishing uniform methodologies will be essential to advancing research in this field. Establishing standardized clinical protocols, including harmonized sample collection, processing, and sequencing methodologies, is critical to ensuring data consistency across studies. Additionally, leveraging real-world data and AI-driven predictive models may provide valuable insights into the translational potential of microbiota-based therapies.

5.3. Need for personalized therapeutic approaches

Given this variability, there is a growing need for personalized therapeutic approaches. Precision medicine, which tailors treatments based on an individual's genetic and microbiota profiles, holds significant promise in this context. However, implementing such strategies requires extensive research to identify reliable biomarkers that can predict patient responses to microbiota-targeted therapies. For instance, microbial diversity indices could serve as potential biomarkers to assess the overall health and stability of the gut microbiota. Notably, HCC patients with higher microbial diversity have been associated with better outcomes following ICI treatment.[120] Additionally, specific microbial taxa have shown promise as predictive markers. A skewed *Firmicutes/Bacteroidetes* ratio and a low *Prevotella/Bacteroides* ratio have been linked to poor responses to ICIs, whereas the presence of *Akkermansia* species correlates with favorable treatment outcomes.[120] Furthermore, integrating microbiota profiling into routine clinical practice necessitates the development of cost-effective and reliable diagnostic tools. Standardizing sampling methodologies and sequencing protocols will be critical to ensuring the accuracy and comparability of microbiota data across clinical settings. Establishing clinical guidelines for these personalized approaches will be essential for their successful implementation and adoption in oncology.

5.4. Limited understanding of underlying mechanisms

Despite the growing evidence linking gut microbiota to immune modulation in HCC, the underlying mechanistic pathways remain incompletely understood. Further investigation is needed to elucidate the causal relationships between specific microbial taxa, their metabolites, and immune regulation in HCC. Functional validation studies using preclinical models will be essential to identify key microbial-derived metabolites that influence tumor progression and therapy response. Single-cell sequencing and spatial transcriptomics could provide deeper insights into how microbiota interact with the tumor immune microenvironment.

5.5. Safety concerns in microbiota modulation

Additionally, gut microbiota modulation carries potential risks and side effects that require careful evaluation. Interventions such as FMT, while promising, can lead to adverse outcomes if not properly controlled, including the transmission of harmful pathogens or unintended disruptions in microbial balance. Furthermore, the long-term safety of probiotics and prebiotics remains uncertain, particularly in the context of HCC, where an altered immune landscape may influence their effects in unpredictable ways. Rigorous clinical studies are needed to assess the safety, efficacy, and potential risks of these microbiota-targeted therapies before their widespread adoption in clinical practice.Therefore, stringent donor screening and rigorous quality control measures are essential to minimize the risks associated with FMT. Additionally, developing engineered probiotic strains with optimized immunomod ulatory properties could enhance therapeutic precision while reducing unintended consequences.[199,200] To ensure the safe and effective application of these strategies in clinical practice, comprehensive clinical trials are required to evaluate their safety, efficacy, and long-term impact.

5.6. Integration with existing HCC therapies

Integrating gut microbiota modulation with existing HCC therapies presents a significant challenge due to the complex interactions between gut microbiota and conventional treatments, such as chemotherapy, targeted therapy, and immunotherapy. These interactions can lead to both synergistic and antagonistic effects, influencing treatment outcomes in unpredictable ways. Future research should focus on identifying optimal treatment sequences and combination strategies to maximize the synergy between microbiota-targeted interventions and conventional therapies. Microbial-derived metabolites could serve as novel immunomodulatory agents, enhancing the efficacy of immune checkpoint inhibitors and other HCC treatments. Additionally, optimizing the timing and sequencing of microbiota-targeted therapies in conjunction with standard treatments will be crucial for improving therapeutic efficacy while

minimizing potential toxicities. Establishing a deeper understanding of these intricate relationships will pave the way for more effective, personalized treatment approaches for HCC.

Looking ahead, research should further validate the clinical applicability of gut microbiota modulation in HCC through large-scale, multicenter studies. Identifying effective microbial biomarkers, optimizing patient stratification, and assessing treatment responses will be crucial for advancing microbiota-based interventions. Additionally, refining targeted microbiota modulation strategies will enhance their specificity and controllability. Investigating the impact of diet and lifestyle on gut microbiota may also provide new avenues for HCC prevention and adjunctive therapy. To ensure the reliability and reproducibility of findings, efforts should focus on standardizing methodologies, including sample collection, processing, and sequencing protocols. Ultimately, interdisciplinary collaboration remains essential, integrating expertise from microbiology, immunology, oncology, and bioinformatics to accelerate the clinical translation of microbiota research and develop more precise and effective therapeutic strategies for HCC patients.

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Authors' contributions

MYH, QSJ and HYH wrote the manuscript. XQW and LW created the figures. XQW and LW conceived the final approval of the version to be submitted. All authors read and approved the final manuscript.

Consent for publication

All of the authors are aware of and agree to the content of the paper and their being listed as a coauthor of the paper.

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