



Mechanisms and therapeutic implications of interactions between the gut microbiome and host epigenetics in hepatocellular carcinoma

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Abstract

This narrative review summarizes current evidence on reciprocal interactions between the gut microbiome and host epigenetic regulation in hepatocellular carcinoma (HCC), with emphasis on mechanistic pathways and potential translational implications. Disruption of the intestinal barrier can increase hepatic exposure to microbial products and activate inflammatory signaling, including toll-like receptor 4 (TLR4)-nuclear factor kappa B (NF- κ B) and interleukin 6 (IL-6)-Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3) pathways, which may interact with epigenetic regulators. Microbiota-derived short-chain fatty acids can modulate histone deacetylase (HDAC) activity and acetylation-related processes, while microbiota-dependent bile acid remodeling influences hepatic metabolic and immune signaling through receptors such as farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5). Polyamine, one-carbon, and α -ketoglutarate-related metabolism provide plausible biochemical links between microbial metabolism and DNA or RNA modification, although direct causal evidence for these connections in HCC remains limited. HCC-specific studies also support roles for N6-methyladenosine (m6A) regulation in metabolic reprogramming and antitumor immunity, whereas evidence that host epigenetic regulation reshapes the gut microbiota derives mainly from non-HCC intestinal models. These findings highlight a heterogeneous evidence base in which selected microbiome-metabolite-host pathways are mechanistically supported, while others remain inferential. Future studies should integrate longitudinal microbiome and metabolite profiling with cell-resolved epigenomic, immune, and clinical data. These approaches may help identify reproducible biomarkers and mechanistically validated therapeutic targets. However, prospective and experimental validation remains necessary before microbiome- or epigenetics-based strategies can inform routine HCC management.



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INTRODUCTION

Primary liver cancer remains a major global cause of cancer-related mortality, and hepatocellular carcinoma (HCC) represents its predominant histological subtype. Global burden models from 2020 project increases in the incidence and mortality rates of primary liver cancer over the subsequent decades^[1]. Epidemiological analyses from 2022 quantify regional variations in the contribution of nine modifiable risk factors, categorizing them primarily into infectious factors [hepatitis B or C virus (HBV, HCV) infection or *Clonorchis sinensis*], metabolic factors [obesity, type 2 diabetes, and metabolic dysfunction-associated steatotic liver disease (MASLD)], and behavioral/toxic exposures (heavy alcohol consumption, smoking, and aflatoxin B1)^[2]. HCC formation rarely results from an isolated somatic mutation; it develops progressively within the altered architectural framework of chronic liver disease. This process involves persistent hepatic inflammation, continuous cycles of hepatocyte necrosis and regeneration, the deposition of a cirrhotic extracellular matrix, systemic metabolic anomalies, and the progressive attenuation of localized immune surveillance^[3].

The gut-liver axis provides a direct vascular connection through the portal circulation, allowing intestinal-derived molecules, including microbial metabolites and inflammatory mediators, to reach hepatic cells. The portal vein transports dietary nutrients, microbial metabolites, and microbe-associated molecular patterns (MAMPs) to the hepatic sinusoids, where the fenestrated endothelium and resident Kupffer cells process these inputs before systemic distribution. The enterohepatic circulation of bile acids represents an interconnected metabolic network that coordinates hepatic metabolism, intestinal epithelial function, and gut microbial activity. During chronic liver disease progression, disruption of intestinal barrier integrity increases permeability and facilitates the translocation of microbial components, including lipopolysaccharide (LPS), peptidoglycans, and unmethylated bacterial CpG DNA, into the hepatic immune microenvironment. Research correlates specific gut microbiota compositions, the enzymatic capacity for bile acid transformation, peripheral short-chain fatty acid concentrations, and subsequent clinical responses to immunotherapy^[4-8]. These data indicate that the gut microbiome participates in the pathogenesis of HCC, necessitating the characterization of specific host transcriptional and epigenetic responses to these microbial stimuli.

Epigenetic dysregulation operates alongside genetic mutations to drive HCC pathogenesis. Documented abnormalities include global DNA hypomethylation coupled with specific promoter hypermethylation, the reduction of 5-hydroxymethylcytosine (5hmC) marks, the abnormal genomic deposition of histone H3 lysine 27 trimethylation (H3K27me3), imbalances in global histone acetylation, the dysregulation of N6-methyladenosine (m6A) RNA modifications, and the structural remodeling of non-coding RNA regulatory networks^[9-13]. Aberrant promoter DNA methylation can transcriptionally silence tumor suppressor-related genes such as *RASSF1A*, *CDKN2A/p16*, *SOCS1*, and *GSTP1*^[12,14]. In addition, abnormalities in DNA methylation, histone modifications, RNA methylation, and non-coding RNAs may regulate cell-cycle proteins, major histocompatibility complex (MHC)-associated antigen processing and immune-recognition pathways, and immune checkpoint molecules such as programmed death-ligand 1 (PD-L1), thereby promoting tumor-cell proliferation and immune escape.

Epigenetic enzymes require specific intracellular metabolites for their catalytic functions. DNA methyltransferases (DNMTs) and RNA methyltransferase complexes (METTL3/METTL14) utilize S-adenosylmethionine (SAM) as the methyl group donor. The ten-eleven translocation (TET) family of dioxygenases requires divalent iron (Fe²⁺) and alpha-ketoglutarate (α -KG) for catalytic activity. The sirtuin (SIRT) family of histone deacetylases depends on nicotinamide adenine dinucleotide (NAD⁺), whereas histone acetyltransferases (HATs) use acetyl-CoA as the acetyl donor. Microbiota-derived short-chain fatty acids (SCFAs) modulate histone acetylation through several complementary mechanisms^[15-17]. These findings illustrate biochemical links between microbial metabolites and host epigenetic regulation.

This review focuses on intestine-derived inflammatory signals, SCFAs, bile acids, tryptophan metabolism, polyamine metabolism, and host epigenetic regulation, and further discusses their relationships with etiological heterogeneity, immunotherapy, and biomarker development in HCC [Figure 1]. The principal challenge in the field is not a lack of candidate pathways, but the scarcity of studies that simultaneously manipulate microbial composition, quantitatively measure key metabolites, and resolve epigenetic changes in specific intrahepatic cell populations within the same experimental system. The supporting evidence and biological context of each pathway are discussed in the relevant sections to distinguish well-established mechanisms from emerging or insufficiently validated models in HCC. Table 1 summarizes the experimental contexts and limitations of these pathways.

FUNCTIONAL SIGNIFICANCE OF GUT MICROBIOTA ALTERATIONS IN HCC

Microbiome profiling studies in patients with HCC have revealed changes in microbial diversity, altered abundance of specific bacterial taxa, together with changes in predicted or inferred microbial functional potential^[18,19]. However, these cross-sectional observations may be influenced by multiple confounding factors, including the underlying cause of liver disease, geographic differences, dietary patterns, cirrhosis severity, and recent medication exposure, including antibiotics. Characterizing specific functional pathways provides a stronger foundation for mechanistic biological linkage than taxonomy alone. Relevant functional pathways include the capacity for short-chain fatty acid biosynthesis, the enzymatic deconjugation and dehydroxylation of bile acids, LPS biosynthesis potential, and the metabolic processing of tryptophan and polyamines.

Several independent HCC cohorts have reported reductions in selected SCFA-producing or SCFA-associated bacterial taxa, although the affected taxa and the direction of these alterations vary according to disease etiology, stage, and comparator group^[18-20]. For example, reduced butyrate-producing genera have been reported in early HCC, while reductions in *Faecalibacterium* and *Bifidobacterium* have been observed in selected etiological subgroups of HCC^[21]. In mouse models of non-alcoholic fatty liver disease (NAFLD)-associated HCC, *Bifidobacterium pseudolongum*-derived acetate suppresses tumor development through G protein-coupled receptor 43 (GPR43) activation and subsequent inhibition of the interleukin 6 (IL-6)/Janus kinase 1 (JAK1)/signal transducer and activator of transcription 3 (STAT3) signaling pathway^[22]. This model provides direct mechanistic evidence by linking a defined bacterial strain, its specific metabolite, the disease context, and the corresponding host signaling pathway.

Bile acid metabolism constitutes an additional enterohepatic pathway associated with HCC pathophysiology. The gut microbiota regulates the conversion of primary bile acids into secondary bile acids through specific enzymatic processes, particularly bile salt hydrolase (BSH)-mediated deconjugation and 7 α -dehydroxylation. Microbiota-driven remodeling of bile acid composition subsequently influences host receptor signaling, including pathways mediated by farnesoid X receptor (FXR), Takeda G protein-coupled receptor 5 (TGR5), vitamin D receptor (VDR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR)^[23-27].

In parallel, enrichment of pro-inflammatory bacterial taxa and Gram-negative bacteria-associated functional pathways may increase systemic exposure to LPS. Preclinical HCC evidence further indicates that modulation of the gut microbiota and intestinal barrier can reduce LPS leakage and attenuate hepatic toll-like receptor 4 (TLR4)-nuclear factor kappa B (NF- κ B) signaling^[28]. These microbial alterations occur in the broader context of cirrhosis, bile acid dysregulation, intestinal barrier dysfunction, and immune disturbance, all of which may independently influence microbiome composition and HCC biology and therefore confound cross-sectional associations^[4,5,29,30].

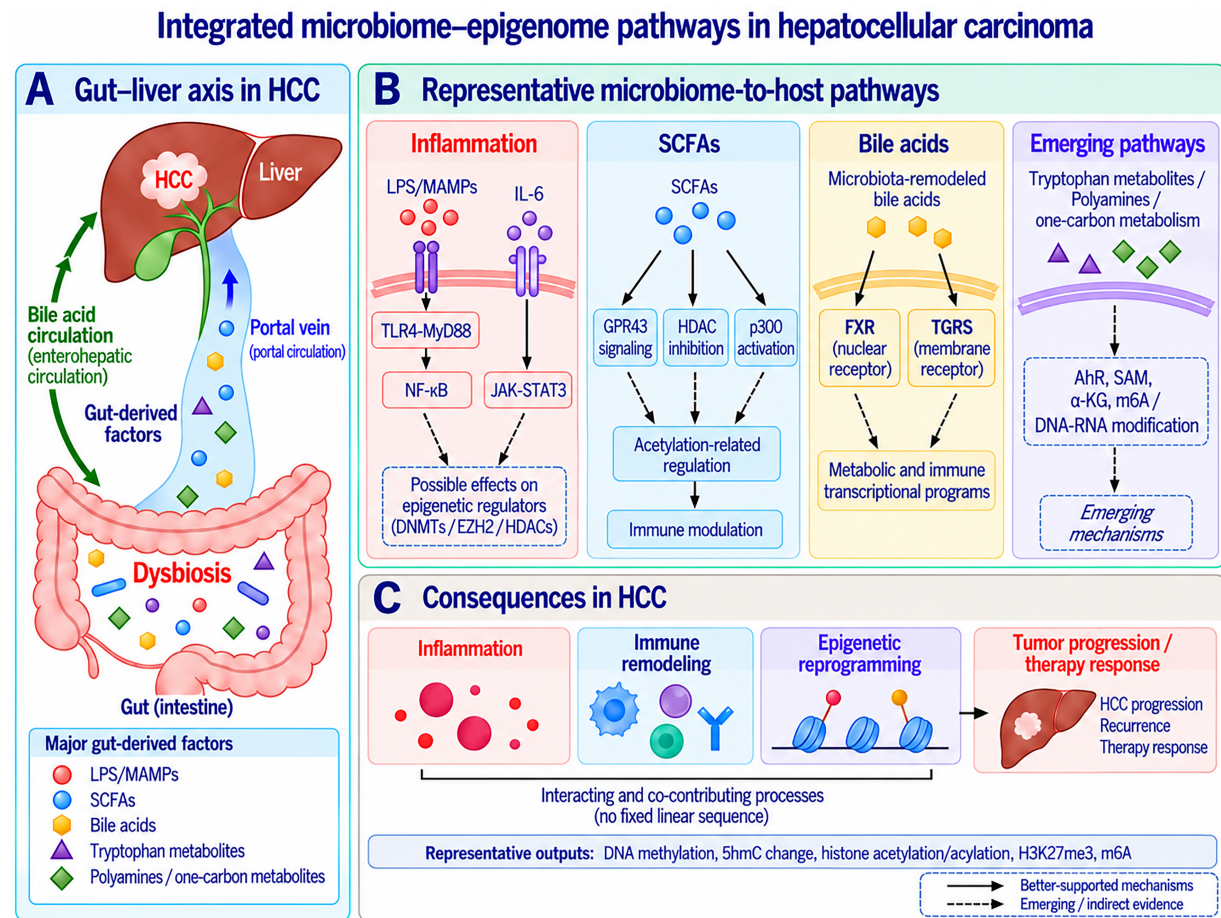


Figure 1. Integrated microbiome-epigenome pathways in HCC. (A) Gut dysbiosis and intestinal barrier dysfunction increase hepatic exposure to gut-derived factors through the portal circulation, including LPS/MAMPs, SCFAs, bile acids, tryptophan metabolites, and polyamine/one-carbon metabolites. Bile acids additionally participate in enterohepatic circulation; (B) Representative microbiome-to-host pathways include inflammatory signaling involving TLR4-MyD88-NF- κ B and IL-6-JAK-STAT3, SCFA-mediated GPR43 signaling and acetylation-related regulation, microbiota-dependent bile acid signaling through FXR and TGR5, and emerging pathways involving tryptophan, polyamine, and one-carbon metabolism. Potential links between inflammatory signaling and epigenetic regulators, as well as between microbial metabolism and DNA/RNA modification, remain incompletely established in HCC; (C) These pathways may contribute to inflammatory responses, immune remodeling, epigenetic reprogramming, tumor progression, and therapeutic response. Representative epigenetic outputs include DNA methylation, 5hmC changes, histone acetylation/acylation, H3K27me3, and m6A RNA modification. Solid arrows denote relatively better-supported mechanisms, whereas dashed arrows indicate emerging or indirect evidence. The pathways are presented schematically and do not imply a fixed linear or temporal sequence. AhR: Aryl hydrocarbon receptor; α -KG: alpha-ketoglutarate; 5hmC: 5-hydroxymethylcytosine; DNA: deoxyribonucleic acid; DNMTs: DNA methyltransferases; EZH2: enhancer of zeste homolog 2; FXR: farnesoid X receptor; GPR43: G protein-coupled receptor 43; H3K27me3: histone H3 lysine 27 trimethylation; HCC: hepatocellular carcinoma; HDAC: histone deacetylase; HDACs: histone deacetylases; IL-6: interleukin 6; JAK: Janus kinase; LPS: lipopolysaccharide; m6A: N6-methyladenosine; MAMPs: microbe-associated molecular patterns; MyD88: myeloid differentiation primary response 88; NF- κ B: nuclear factor kappa B; p300: histone acetyltransferase p300; RNA: ribonucleic acid; SAM: S-adenosylmethionine; SCFAs: short-chain fatty acids; TGR5: Takeda G protein-coupled receptor 5; TLR4: toll-like receptor 4; STAT3: signal transducer and activator of transcription 3.

INTESTINE-DERIVED INFLAMMATORY SIGNALS AND EPIGENETIC REGULATION

Following the structural compromise of the intestinal epithelial barrier, MAMPs enter the portal venous system. Microbial components such as LPS can translocate to the liver, where they engage pattern recognition receptors on various hepatic cell types, including Kupffer cells, liver sinusoidal endothelial cells, hepatic stellate cells, and hepatocytes^[4,5]. Activation of the TLR4-myeloid differentiation primary response 88 (MyD88)-NF- κ B axis together with the IL-6/JAK/STAT3 signaling pathway reshapes the expression of inflammatory cytokines, chemokines, and anti-apoptotic mediators in these intrahepatic cells. Increasing evidence indicates that these inflammatory signaling pathways interact with epigenetic regulators to influence gene expression programs.

Table 1. Representative microbiome-associated and host epigenetic pathways relevant to hepatocellular carcinoma

Principal pathway and epigenetic relevance	Cell type or experimental model	Evidence context and limitations	Potential therapeutic or translational relevance	References
TLR4-NF- κ B signaling; proposed links to DNMTs, EZH2 and HDACs	Hepatic cells in HCC models; complementary non-HCC tumor studies	HCC inflammatory-pathway evidence; direct gut-derived epigenetic coupling remains unproven	Barrier restoration and inflammatory-pathway studies; epigenetic targets require validation	[28,31-33]
GPR43 activation; suppression of IL-6/JAK1/STAT3 signaling. No direct epigenetic endpoint	Hepatocytes in NAFLD-associated HCC mouse models	HCC preclinical microbiome-metabolite mechanism	Defined strain-metabolite intervention; clinical efficacy remains unestablished	[22]
HDAC-associated Sox13 acetylation and reduced IL-17A; a non-histone mechanism	HCC mouse models, isolated hepatic ILC3s, and complementary transfected 293T-cell assays	HCC-related functional evidence with histone H3 acetylation and complementary Sox13 assays; locus-specific chromatin mediation remains unproven	Test acetate-ILC3 immunomodulation with HDAC, Sox13 and IL-17A readouts	[38]
HDAC inhibition; p300 auto-acylation; histone acetylation, methylation and butyrylation	Cultured cells; non-tumor mouse liver, intestine and adipose tissue	Biochemical and mainly non-HCC evidence; HCC chromatin effects require validation	Measure metabolite exposure and cell-specific histone marks in HCC	[15-17,41,42]
CXCL16-CXCR6 ⁺ NKT-cell recruitment and antitumor immunity. No direct epigenetic endpoint	Liver sinusoidal endothelial cells and NKT cells in mouse liver-tumor models	Preclinical microbiome-bile acid-immune mechanism; human correlative support	Investigate bile acid composition and NKT-cell recruitment as linked intervention readouts	[45]
Microbial butyrate induces CXCL14 and DC/CD8 ⁺ T-cell responses. No direct epigenetic endpoint	Hepatocyte-specific <i>Af6</i> deficiency in HCC models	HCC preclinical host-microbiota-metabolite-immune mechanism	Test butyrate-CXCL14 immunomodulation; distinguish it from direct bile acid effects	[46]
Nuclear FXR transcriptional coregulation; membrane TGR5-cAMP signaling	Hepatic and intestinal receptor studies, mainly outside HCC	Receptor mechanisms support plausibility; microbiota-driven HCC chromatin remodeling is unproven	Study receptor- and tissue-specific effects alongside quantitative bile acid profiles	[43,44]
TIE2-PI3K/AKT signaling and tumor promotion. No direct epigenetic endpoint	HCC cells; conventional and germ-free mouse models	HCC preclinical bacterial species-metabolite-receptor mechanism	Investigate bacterial, metabolite or receptor targeting in preclinical HCC	[56]
Ligand- and cell-dependent AhR immune regulation; proposed RNA-modification links	Intestinal immune and general tumor models; host kynurenine and microbial indoles	Mainly non-HCC mechanisms; a microbial metabolite-AhR-m6A axis in HCC is unproven	Separate host and microbial metabolite sources and test cell-specific effects	[54,55]
SAM/dcSAM partitioning; putative effects on methyl-donor availability	Biochemical and microbial metabolic studies; HCC ODC1 models	Biochemical rationale; microbial control of hepatic DNA/RNA methylation flux is unproven	Combine isotope tracing with metabolomic and epigenomic measurements	[50-53]
m6A-SCAP-cholesterol and m6A-TUG1-PD-L1/CD47 pathways	HCC cells and mouse models; downstream CD8 ⁺ T-cell and macrophage effects	HCC host mechanistic evidence; an upstream microbiome link was not established	Preclinical rationale for m6A-targeted treatment combined with checkpoint blockade	[48,49]
miR-122a/miR-200c-3p-occludin; PIM1-HDAC2; fecal miRNA-bacterial interactions	Intestinal epithelial, goblet-cell, bacterial and colitis models	Non-HCC experiments; barrier effects and direct bacterial regulation are distinct mechanisms	Test barrier/mucus regulation and host miRNA signaling in HCC-relevant models	[58-61]
Microbial, metabolite and inflammatory associations with outcome; no defined epigenetic target	Clinical HCC cohorts receiving ICI monotherapy or combination regimens	Observational HCC associations; findings and response definitions vary across cohorts	Candidate biomarkers require prospective external validation and control of clinical confounding	[67-72]

Evidence descriptions specify the experimental setting, microbiome linkage and directly demonstrated endpoints; they do not constitute a formal ranking of study quality. Preclinical antitumor effects do not establish clinical efficacy. *Af6*: *Afadin*; *AhR*: aryl hydrocarbon receptor; *AKT*: protein kinase B; *cAMP*: cyclic adenosine monophosphate; *CD8⁺*: cluster of differentiation 8-positive; *CXCL14*: C-X-C motif chemokine ligand 14; *CXCL16*: C-X-C motif chemokine ligand 16; *CXCR6*: C-X-C motif chemokine receptor 6; *dcSAM*: decarboxylated S-adenosylmethionine; *DC*: dendritic cell; *DNMT*: DNA methyltransferase; *DNA*: deoxyribonucleic acid; *EZH2*: enhancer of zeste homolog 2; *FXR*: farnesoid X receptor; *GPR43*: G protein-coupled receptor 43; *HCC*: hepatocellular carcinoma; *HDAC*: histone deacetylase; *ICI*: immune checkpoint inhibitor; *IL-6*: interleukin 6; *IL-17A*: interleukin 17A; *ILC3*: group 3 innate lymphoid cell; *JAK1*: Janus kinase 1; *m6A*: N6-methyladenosine; *miR*: microRNA; *miRNA*: microRNA; *NAFLD*: non-alcoholic fatty liver disease; *NF- κ B*: nuclear factor kappa B; *NKT*: natural killer T cell; *ODC1*: ornithine decarboxylase 1; *PD-L1*: programmed

death-ligand 1; PI3K: phosphoinositide 3-kinase; p300: histone acetyltransferase p300; PIM1: proviral integration site for Moloney murine leukemia virus 1; RNA: ribonucleic acid; SAM: S-adenosylmethionine; SCFA: short-chain fatty acid; Sox13: SRY-box transcription factor 13; STAT3: signal transducer and activator of transcription 3; TGR5: Takeda G protein-coupled receptor 5; TIE2: TEK receptor tyrosine kinase; TLR4: toll-like receptor 4; TUG1: taurine upregulated 1.

Mechanistic links between inflammatory transcription factors and epigenetic enzymes have been demonstrated in non-HCC tumor models. In particular, STAT3 acetylation at Lys685 promotes its interaction with DNMT1 and contributes to promoter methylation and transcriptional silencing of selected tumor-suppressor genes^[31]. Independently, dysregulation of DNMTs, enhancer of zeste homolog 2 (EZH2), and histone deacetylases (HDACs) is well documented in HCC^[9,32]. However, current evidence does not establish intestine-derived inflammatory signaling as the direct upstream cause of these epigenetic abnormalities in HCC^[30,33].

The abnormal hypermethylation of promoter CpG islands regulating genes such as RASSF1A, CDKN2A/p16, SOCS1, and GSTP1 is extensively characterized in HCC tissue arrays^[12,14]. EZH2 is the catalytic subunit of Polycomb Repressive Complex 2 (PRC2) and promotes local deposition of H3K27me3, thereby facilitating transcriptional silencing of selected differentiation-related genes, tumor-suppressor genes, and tumor-suppressive miRNA loci^[32]. In chronic liver disease, persistent exposure to intestine-derived LPS and downstream inflammatory cytokines such as IL-6 and TNF may maintain intrahepatic cells in prolonged states of inflammatory stress and regeneration and may thereby influence the expression or activity of epigenetic regulators such as DNMTs, EZH2, and HDACs. Ohtani reviewed how persistent senescence-associated secretory phenotype (SASP) programs can connect cellular stress, chronic inflammation, and cancer, providing a conceptual framework for long-lasting inflammatory remodeling of the tissue microenvironment^[34]. In murine models simulating obesity-driven HCC, Yoshimoto *et al.* demonstrate that specific gut microbial metabolites induce a SASP in hepatic stellate cells, which promotes hepatocarcinogenesis^[35]. Collectively, these findings indicate that intestine-derived inflammation and microbial metabolic signals can contribute to long-term remodeling of the premalignant microenvironment.

Alterations in TET enzyme function and the genomic distribution of 5hmC connect cellular oxidative stress, epigenetic remodeling, and immunomodulation. The TET dioxygenase family initiates active DNA demethylation by sequentially oxidizing 5-methylcytosine to 5hmC, 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC)^[36]. Lu *et al.* showed that oxidative stress within the HCC microenvironment activates TET2 in B cells. Aryl hydrocarbon receptor (AhR) recruits TET2 to specific genomic sites, facilitating hydroxymethylation at the Il10 locus and promoting IL-10⁺ regulatory B-cell generation; conversely, B-cell-specific Tet2 inactivation reduces this immunosuppressive population and enhances antitumor immunity^[37]. These studies identify distinct regulatory mechanisms of different evidentiary strength rather than a single established epigenetic cascade initiated by intestine-derived inflammation. Establishing relationships between intestinal-derived inflammatory signals, TET enzyme activity, 5hmC patterns, and the functional status of tissue-resident immune cells requires integrated quantitative analyses using intact liver specimens or comprehensive liquid biopsy approaches.

SCFAs AND HISTONE ACETYLATION/ACYLATION MODIFICATIONS

SCFAs, predominantly acetate, propionate, and butyrate, are synthesized through the anaerobic bacterial fermentation of dietary fiber in the intestinal lumen. The enzymatic mechanisms connecting SCFAs with host epigenetic regulation have been increasingly characterized. Waldecker *et al.* showed that butyrate inhibits HDAC activity in nuclear extracts from human colon carcinoma cells, whereas propionate exerts a

weaker inhibitory effect^[15]. Subsequently, Thomas and Denu reported that propionate and butyrate can be converted into their corresponding acyl-coenzyme A (acyl-CoA) species - activated metabolic intermediates formed from fatty acid-derived acyl groups and coenzyme A - and enhance the histone and protein acetyltransferase activity of p300 by promoting auto-acylation of its autoinhibitory loop^[16]. Therefore, the effects of SCFAs on histone modifications are likely mediated through multiple mechanisms, including HDAC inhibition, p300 activation, and alterations in intracellular acyl-CoA availability. The relative contribution of each mechanism may depend on the specific metabolite, its absolute concentration, exposure duration, and the metabolic state of the target cells.

Extrapolating the enzymatic interactions of SCFAs to HCC requires accounting for absolute molecular concentrations and tissue-specific metabolic rates. SCFA availability varies substantially across different biological compartments, including the colonic lumen, fecal samples, peripheral blood, and portal circulation, resulting in distinct exposure levels within the liver. Furthermore, the capacity to transport and metabolize SCFAs differs among functional hepatocytes, glycolytic tumor cells, resident Kupffer cells, hepatic stellate cells, and specific subsets of infiltrating lymphocytes. Donohoe *et al.* showed in a colorectal cancer model that the effect of butyrate depends on its intracellular metabolic fate. Normal colonocytes efficiently oxidized butyrate as an energy substrate, whereas the Warburg phenotype of cancerous colonocytes limited butyrate oxidation, allowing intracellular accumulation and HDAC inhibition, with consequent changes in histone acetylation and cell proliferation^[17]. The findings from colorectal cancer raise the testable possibility that SCFA effects in HCC may vary with the metabolic state of malignant and non-malignant intrahepatic cell populations.

In HCC-bearing mice, Hu *et al.* showed that microbiota-associated acetate inhibits HDAC activity, increases Sox13 K30 acetylation, reduces Sox13 mRNA expression and Sox13-dependent IL-17A transcription in hepatic group 3 innate lymphoid cell (ILC3s), and thereby enhances antitumor immunity^[38]. In non-HCC immune models, Arpaia *et al.* found that butyrate and propionate promote peripheral regulatory T cell (Treg) differentiation^[39]. In a non-tumor systemic bacterial infection model, Balmer *et al.* showed that stress-associated increases in circulating acetate expand the acetyl-CoA pool in memory CD8⁺ T cells and promote glyceraldehyde-3-phosphate dehydrogenase (GAPDH) acetylation, glycolysis, and rapid effector responses^[40]. Krautkramer *et al.* further demonstrated that microbial colonization and dietary composition alter histone acetylation and methylation in mouse liver, colon, and adipose tissue, and that SCFA supplementation partially reproduces the chromatin changes induced by colonization^[41]. Gates *et al.* identified microbiota-regulated H3K9 and H3K27 butyrylation in mouse intestinal epithelial cells and showed that histone butyrylation is responsive to tributyrin administration; H3K27 butyrylation was associated with active regulatory elements and higher gene expression^[42]. Together, these studies demonstrate that SCFAs can influence host cellular states through metabolic remodeling, HDAC-dependent regulation, protein acetylation, and histone modifications across several experimental systems. However, direct HCC-specific evidence is currently strongest for the acetate-HDAC-ILC3 pathway, whereas several histone-level mechanisms remain extrapolated from non-HCC tissues and models. HCC research necessitates the precise quantification of discrete chromatin marks - including histone H3 lysine 27 acetylation (H3K27ac), histone H3 lysine 9 acetylation (H3K9ac), histone H4 acetylation (H4ac), and site-specific histone butyrylation - within isolated intrahepatic cellular populations, and the correlation of these measurements with the functional genomic capacity of the microbiome and absolute concentrations of circulating metabolites.

BILE ACIDS, NUCLEAR RECEPTORS, AND CHROMATIN STATES

Primary bile acids are synthesized in the liver and subsequently transformed by gut microbial enzymes into secondary bile acids through intestinal deconjugation and dehydroxylation processes. The bile acid pathway simultaneously connects microbial metabolism, intrahepatic immune surveillance, and nuclear

receptor-dependent transcriptional regulation. In HCC models, animal studies provide relatively direct evidence that microbiota-driven bile acid remodeling influences intrahepatic immune-cell composition and tumor progression. However, the relationship between bile acid receptor signaling and chromatin regulation has been established mainly through investigations of FXR-responsive genes and associated coregulatory factors. Beyond their role in lipid absorption, bile acids also function as signaling molecules through the nuclear receptor FXR and the membrane G protein-coupled receptor TGR5. Selected bile acids and their derivatives can additionally engage VDR- and PXR-related signaling, while CAR contributes to bile acid detoxification and metabolic homeostasis^[23-27,43,44]. Through these receptor systems, bile acids regulate lipid and glucose metabolism, inflammatory cascades, drug detoxification, and cellular proliferation. In mice, Ma *et al.* reported that depletion of Gram-positive bacteria responsible for converting primary to secondary bile acids increased CXCL16 expression in liver sinusoidal endothelial cells, promoted accumulation of CXCR6⁺ NKT cells and IFN- γ production, and suppressed liver tumor growth; supplementation with secondary bile acids or colonization with bile acid-metabolizing bacteria reversed these effects. In non-tumor liver tissue from patients with primary liver cancer, CDCA levels correlated positively with CXCL16 expression, whereas GLCA levels correlated negatively, providing human correlative support for the microbiota-bile acid-CXCL16 axis^[45]. A recent study further showed that hepatocyte-specific *Af6* deficiency increases primary bile acid production, reshapes the gut microbiota, and elevates microbiota-derived butyrate. Butyrate, rather than bile acids directly, upregulated hepatic CXCL14, promoted recruitment of activated dendritic cells, and enhanced CD8⁺ T-cell cytotoxicity, thereby suppressing HCC progression^[46].

Bile acid signaling can influence host chromatin states through nuclear receptor-associated coregulatory mechanisms, with this relationship being best characterized for FXR in non-HCC experimental systems^[43]. Following ligand binding, FXR heterodimerizes with retinoid X receptor (RXR), binds specific genomic response elements, and recruits coactivator or corepressor complexes according to the target gene and cellular context, thereby influencing local histone modifications and transcriptional activity. Reported FXR-associated coregulators include the acetyltransferase p300 and other chromatin-modifying cofactors, whereas the participation of nuclear receptor corepressor (NcoR)/silencing mediator of retinoid and thyroid hormone receptor (SMRT)-type corepressor complexes in FXR regulation is less well defined^[43]. These interactions can alter histone acetylation, FXR post-translational modification, chromatin accessibility, and target-gene transcription. The activation of the membrane-bound receptor TGR5 engages the Gs-protein-coupled cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA)-cAMP response element-binding protein (CREB) signal transduction cascade, regulating the transcription of specific inflammatory and metabolic genes^[44]. Receptors such as VDR, PXR, and CAR coordinate the expression of gene networks involved in bile acid homeostasis, xenobiotic detoxification, and drug responses. Shifts in the physiological bile acid profile translate into altered transcriptional landscapes across hepatocytes and local immune populations via these transcription factors and coregulators.

FXR and TGR5 signaling is highly context- and tissue-dependent, whereas stage-specific effects in HCC remain incompletely defined. Within hepatocytes, FXR activation contributes to suppression of bile acid synthesis and maintenance of metabolic homeostasis. In the intestinal epithelium, FXR activation induces fibroblast growth factor 15 (FGF15) in mice or FGF19 in humans, which signals through hepatic FGFR4 to suppress bile acid synthesis and can influence hepatocyte proliferative signaling^[43,44]. Golonka *et al.* found that combined *Tlr5* and *Fxr* deficiency was associated with more severe spontaneous hepatocarcinogenesis, aggravated bile acid dysmetabolism, and marked gut microbial dysbiosis compared with *Fxr* deficiency alone^[47]. This model indicates that genetic interactions between innate immune signals that maintain microbial homeostasis and FXR-dependent bile acid homeostasis can jointly influence susceptibility to liver cancer.

TRYPTOPHAN, POLYAMINE, AND ONE-CARBON METABOLISM IN HCC EPIGENETIC AND IMMUNE REGULATION

The biochemical cofactor requirements of key epigenetic enzymes relevant to these pathways are well established: DNA and RNA methyltransferases use SAM, whereas TET enzymes and the m6A demethylases fat mass and obesity-associated protein (FTO) and alkB homolog 5 (ALKBH5) belong to the Fe²⁺/α-KG-dependent dioxygenase family^[10,11,36]. In HCC, direct experimental studies further demonstrate that m6A regulation can influence tumor metabolic reprogramming and antitumor immunity^[48,49]. Whether microbially derived metabolites directly remodel DNA or RNA methylation in HCC by altering these substrate pools remains a mechanistic connection that requires validation.

DNA and RNA methylation and demethylation reactions depend on defined metabolic substrates and cofactors. DNMTs and the m6A writer complex centered on METTL3/METTL14 use SAM as the methyl donor, whereas TET dioxygenases and the RNA demethylases FTO and ALKBH5 require α-KG, Fe²⁺, and molecular oxygen for catalysis. Accordingly, intracellular SAM/S-adenosylhomocysteine (SAH) balance and the availability of α-KG, Fe²⁺, and oxygen can constrain the catalytic potential of methylation and demethylation reactions^[10,11,36]. Krautkramer *et al.* demonstrated that diet and microbial colonization alter histone modification states across multiple host tissues and that SCFA supplementation can partially reproduce colonization-associated chromatin changes^[41]. This study provides general evidence that microbial metabolism can influence host epigenetic states, but it does not directly establish microbiota-dependent control of SAM- or α-KG-driven DNA/RNA methylation in HCC.

Polyamine metabolism is biochemically linked to one-carbon metabolism and SAM consumption. Polyamine cellular synthesis initiates with ornithine decarboxylase (ODC1) and proceeds via spermidine synthase and spermine synthase to produce spermidine and spermine^[50]. Each of these subsequent synthesis steps requires decarboxylated SAM to supply aminopropyl groups^[51]. In HCC, ODC1 is upregulated and can promote tumor-cell proliferation, migration, and invasion through AKT/GSK3β/β-catenin signaling and modulation of the acidic microenvironment^[52]. Endogenous polyamine synthesis converts a portion of SAM to decarboxylated SAM, which then supplies aminopropyl groups for spermidine and spermine synthesis. Polyamine metabolism can therefore affect the metabolic allocation between SAM and decarboxylated SAM. Gut microbial polyamines can contribute to host-accessible polyamine pools^[53]. Direct mechanistic evidence for the polyamine-SAM-methylation axis in HCC remains relatively limited compared with the established evidence supporting SCFA- and bile acid-associated pathways. Biochemically, increased flux from SAM into dcSAM provides a testable mechanism by which polyamine synthesis could alter methyl-donor allocation; whether this contributes materially to global or locus-specific DNA/RNA methylation changes in HCC has not been demonstrated. This hypothesis is therefore suitable for integrated isotope-tracing, metabolomic, and epigenomic analysis.

The evaluation of tryptophan metabolism requires the separation of host-intrinsic pathways from microbiota-driven enzymatic processes. Host cells direct tryptophan into the kynurenine pathway primarily through indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO2). Some gut microorganisms use unabsorbed tryptophan to generate indole and its derivatives, including indole-3-acetic acid, indole-3-propionic acid, and indole-3-aldehyde. Kynurenine and selected microbiota-derived indole metabolites can both modulate AhR signaling, but their biological effects depend on ligand structure, exposure concentration and duration, and target-cell type and may therefore be divergent or even opposing^[54,55]. A distinct microbial metabolite-TEK receptor tyrosine kinase (TIE2) pathway has been demonstrated in HCC models. Zhang *et al.* showed that *Catenibacterium mitsuokai* disrupts the intestinal barrier and translocates to the liver, where its secreted quinolinic acid binds to and activates TIE2 on HCC

cells, leading to downstream phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) activation and tumor promotion^[56]. Using conventional and germ-free mice together with bacterial, metabolite, and receptor-level interventions, the study provides direct HCC-specific causal evidence linking a defined bacterial species, quinolinic acid, TIE2, and PI3K/AKT signaling^[56].

The IDO1/TDO2-kynurenine-AhR pathway is a well-characterized immunoregulatory axis across multiple tumor types^[55]. HCC-specific evidence is more limited, although IDO1 upregulation has been shown to contribute to adaptive resistance to immune checkpoint blockade in experimental HCC models^[57]. In addition, m6A RNA modifications have emerged as potential regulators of the HCC immune microenvironment and therapeutic response^[48,49]. Pan *et al.* demonstrated that METTL3 promotes cholesterol biosynthesis through m6A-dependent enhancement of sterol regulatory element-binding protein cleavage-activating protein (SCAP) translation, resulting in cholesterol-mediated impairment of CD8⁺T-cell function; METTL3 inhibition enhanced the efficacy of anti-PD-1 therapy in NAFLD-HCC models^[49]. Xi *et al.* further showed that METTL3-mediated m6A modification increases taurine upregulated 1 (TUG1) expression, leading to reduced CD8⁺ T-cell effector function and impaired macrophage phagocytosis through regulation of PD-L1 and CD47, respectively^[48]. These findings indicate that metabolic reprogramming, AhR signaling, and m6A regulation may all participate in shaping HCC immunity; however, they do not yet directly demonstrate a unified microbial metabolite-AhR-RNA methylation axis. Future studies should combine microbiota and metabolite interventions, cell-specific manipulation of AhR or METTL3, and joint measurement of m6A and metabolic flux to define their interactions and causal order.

RECIPROCAL IMPACT OF HOST EPIGENETICS ON THE INTESTINAL MICROECOLOGY

Epigenetic and post-transcriptional regulation not only responds to microbial signals but can also indirectly shape the ecological niches available to the microbiota by altering the intestinal epithelial barrier, mucus secretion, and mucosal immune programs. Current mechanistic evidence for this reverse regulation derives mainly from intestinal epithelial, inflammatory bowel disease, and colitis-associated cancer models and provides a framework for understanding bidirectional host-microbiota interactions in chronic liver disease. To maintain immunological and barrier homeostasis, the host leverages DNA methylation and specific histone modifications to orchestrate the transcriptional programs of resident immune and epithelial cells, thereby resisting local dysbiosis. For example, TNF- α induces miR-122a in intestinal epithelial cells, and miR-122a directly binds the 3' untranslated region of occludin mRNA and promotes its degradation, thereby increasing intestinal tight-junction permeability^[58]. Separately, Rawat *et al.* showed that IL-1 β induces miR-200c-3p, which likewise targets occludin mRNA and increases intestinal permeability^[59]. Such post-transcriptional disruption of tight-junction integrity increases intestinal permeability and may alter ecological conditions relevant to microbial colonization. Host epigenetic mechanisms can influence microbial colonization, growth, and community structure through targeted histone regulation and cross-kingdom molecular communication. Histone regulation in the host can reshape the intestinal niche by changing goblet-cell abundance and the mucus layer. Yang *et al.* showed that intestinal epithelial PIM1 interacts with and phosphorylates HDAC2, reduces HDAC2 protein abundance, and decreases HDAC2 occupancy at selected Wnt-related promoters, thereby altering Wnt-associated transcription^[60]. PIM1 also affected Notch signaling, although the HDAC2 dependence of this effect was less clearly established. Increased epithelial PIM1 was accompanied by reduced goblet cells and MUC2 and aggravated experimental colitis and colitis-associated cancer; conversely, PIM1 deficiency increased goblet cells and MUC2 and was associated with higher relative abundances of *Roseburia*, *Alloprevotella*, and *Bifidobacterium*. This study therefore links the intestinal epithelial PIM1-HDAC2 axis and mucus-barrier remodeling with changes in microbial community structure, although the causal direction and contribution of the microbiota to the disease phenotype remain incompletely resolved^[60]. Host-derived miRNAs may also participate in cross-kingdom signaling. Liu *et al.* demonstrated that fecal miRNAs derived in part from intestinal epithelial cells,

including extracellular-vesicle-associated miRNAs, can enter specific bacterial species and regulate bacterial transcripts and growth through sequence-dependent interactions^[61,62]. However, because these effects depend highly on individual miRNAs and bacterial taxa, their broader significance within complex microbial ecosystems and chronic liver disease remains largely unresolved. Together, these studies indicate that host miRNAs, histone regulation, and epithelial secretory programs can reciprocally influence the gut microbiota by altering barrier integrity and microbial ecological niches. Extending these mechanisms to HCC will require simultaneous analysis of epithelial epigenetic states, barrier function, and microbiota dynamics in liver disease models and longitudinal patient cohorts.

INFLUENCE OF ETIOLOGICAL BACKGROUND ON MECHANISTIC INTERPRETATIONS

HCC is etiologically heterogeneous, and viral, alcohol-associated, and metabolic liver diseases create distinct inflammatory, metabolic, microbial, and epigenetic contexts in which HCC develops^[4,5,29,63-65]. HBV-associated HCC develops in the context of chronic viral persistence and may involve HBV DNA integration together with host epigenetic alterations, including aberrant DNA methylation^[63]. In HCV-associated HCC, specific transcriptional and epigenetic aberrations persist in the host cellular memory even following viral clearance^[64]. Alcohol-associated liver disease is frequently accompanied by increased intestinal permeability and endotoxin exposure, together with ethanol- and acetaldehyde-related oxidative and inflammatory stress; these processes may remain relevant in the background from which alcohol-associated HCC develops. MASLD/metabolic dysfunction-associated steatohepatitis (MASH)-associated HCC is connected with systemic obesity, insulin resistance, disruption of bile acid metabolism, and chronic low-grade inflammatory signaling^[29,65].

These distinct etiologies alter the interpretation of any microbiome-epigenetic interaction. SCFA-related pathways may warrant particular investigation in metabolically defined HCC populations, especially where diet-related dysbiosis or reduced microbial SCFA-producing capacity is documented. Similarly, bile acid receptor-directed strategies may be most appropriately evaluated in mechanistically stratified studies that document baseline bile acid composition and receptor-signaling states, rather than being assigned solely on the basis of HCC etiology. LPS translocation and TLR4 signaling may be particularly relevant in alcohol-associated liver disease and advanced cirrhosis with impaired intestinal barrier function, although their relative contribution to HCC biology is likely to vary among patients. Clinical cohorts should therefore systematically document underlying etiology, cirrhosis severity, antiviral treatment history, antibiotic and proton-pump-inhibitor exposure, dietary patterns, and systemic metabolic status so that major confounders can be assessed or adjusted for and cross-study comparability can be improved. More broadly, HCC prognosis is determined by multiple interacting factors, including tumor biology, tumor burden, liver functional reserve, underlying etiology, and treatment selection. Recent clinical commentary has therefore emphasized individualized, patient-centered risk stratification rather than reliance on any single molecular marker^[66]. These considerations are particularly important when evaluating whether microbiome or epigenetic features provide prognostic or predictive information beyond established clinical determinants.

MECHANISM-DRIVEN THERAPEUTIC IMPLICATIONS

The mechanisms outlined above provide potential therapeutic entry points for targeting the gut microbiome and associated epigenetic pathways; however, HCC etiology and measurable biological phenotypes should currently be used primarily to prioritize mechanistic hypotheses and stratify clinical studies rather than to select therapy directly. In HBV- or HCV-related HCC, virological control, persistent inflammation, and virus-induced host epigenetic abnormalities remain fundamental considerations^[63,64]. Alcohol-associated liver disease and advanced cirrhosis with marked barrier dysfunction may involve greater microbial-product translocation and endotoxin-related inflammation. In MASLD/MASH-associated HCC, direct preclinical

evidence supports selected metabolic pathways, including the acetate-GPR43-IL-6/JAK1/STAT3 axis^[22] and the METTL3-m6A-SCAP-cholesterol axis^[49], whereas other microbiome-metabolic pathways remain less completely validated.

For HCC characterized by prominent barrier dysfunction and endotoxin-related inflammation, restoration of intestinal barrier integrity and attenuation of LPS-TLR4-NF- κ B signaling represent mechanistically plausible intervention strategies that require clinical validation. These approaches may be particularly relevant for investigation in alcohol-associated liver disease or advanced cirrhosis with endotoxemia. In an HCC mouse model, microbiota-directed intervention improved intestinal barrier integrity, reduced LPS leakage, and attenuated hepatic TLR4/NF- κ B signaling^[28]. However, a pro-inflammatory microbial signature cannot currently be used to directly select anticancer therapy. Future clinical trials should incorporate barrier-function measures, circulating LPS and inflammatory cytokines, microbial functional modules, and immune-cell phenotypes as mechanistic endpoints to determine whether an intervention truly acts on the prespecified gut-liver inflammatory axis.

Future mechanistic and translational studies of SCFA-targeted interventions may reasonably prioritize MASLD/MASH-associated HCC or biologically defined populations with reduced microbial SCFA-producing capacity. Compared with broadly defined probiotics that lack functional specification, strains with defined metabolic capabilities, compositionally defined postbiotics, or standardized metabolite formulations are more suitable for establishing links among a strain, its metabolite, and host effects. Using two diet-induced MASLD-HCC mouse models, Song *et al.* demonstrated that *Bifidobacterium pseudolongum*-derived acetate mediates antitumor effects through hepatocyte GPR43 activation, attenuation of IL-6/JAK1/STAT3 signaling, and restoration of gut microbial composition and barrier integrity^[22]. This study provides preclinical support for etiology-matched strain-metabolite intervention, although clinical translation requires definition of dose, colonization stability, and target population. Related studies should separately quantify fecal and circulating acetate, propionate, and butyrate and evaluate their biological effects together with HDAC activity, p300-associated modifications, and immune-cell function.

The therapeutic goal of targeting bile acid pathways should not be simplified to increasing or decreasing total bile acid concentrations; instead, it should focus on restoring the balance among bile acid composition, microbial transformation capacity, and receptor signaling. Potential bile acid-directed approaches - including modulation of FXR or TGR5 signaling, alteration of enterohepatic bile acid circulation, and intestine-selective FXR regulation - have been investigated principally in metabolic and liver-disease contexts^[22,24-26,43]. Their therapeutic value in HCC remains to be established. Because individual primary and secondary bile acids differ in receptor affinity and immunological effects, such strategies are best studied in patients whose absolute baseline bile acid profiles, microbial bile salt hydrolase and 7 α -dehydroxylation capacities, and receptor signaling states are clearly characterized. MASLD/MASH-related HCC may warrant priority attention to the link between bile acid imbalance and metabolic inflammation, whereas viral- or alcohol-related HCC requires separation of the effects of the underlying etiology from those of cirrhosis, cholestasis, and impaired liver function on the bile acid profile. At present, these agents should be evaluated in HCC primarily through mechanistically defined, phenotype-stratified clinical studies.

Combining epigenetic agents with immune checkpoint inhibitors (ICIs) offers another strategy for linking host epigenetic states to antitumor immunity. DNMT, HDAC, EZH2, and BET inhibitors can modulate antigen processing and presentation, inflammatory signaling, chemokine production, and immune-cell recruitment; however, their effects vary substantially depending on the specific molecular target, cellular context, and disease background. Therefore, epigenetic therapies should not be considered a uniform class of nonspecific immune-sensitizing agents^[9]. In virus-related HCC, the relationships among virological status,

DNA methylation, and immune escape may represent a priority area. In MASLD/MASH-related HCC, metabolic-epitranscriptomic pathways have more direct preclinical support. Pan *et al.* demonstrated that METTL3 promotes SCAP translation through m6A modification, increases cholesterol biosynthesis, and impairs CD8⁺ T-cell function; genetic or pharmacological METTL3 inhibition enhanced the efficacy of anti-PD-1 therapy in NAFLD-HCC models^[49]. Xi *et al.* further showed that METTL3-dependent m6A modification influences CD8⁺ T-cell activity and macrophage phagocytic function through regulation of TUG1, PD-L1, and CD47^[48]. Collectively, these findings provide a rationale for exploring combined m6A-directed and immunotherapeutic strategies.

At present, the translational potential of gut microbiome profiling in HCC immunotherapy is being explored primarily for patient stratification and response prediction, but no microbiome feature has been validated for routine treatment selection. In the small longitudinal cohort reported by Zheng *et al.*, anti-PD-1 responders showed greater microbial richness and enrichment of taxa including *Akkermansia muciniphila* and Ruminococcaceae species^[67]. Ponziani *et al.* reported lower pretreatment fecal calprotectin and higher *Akkermansia* abundance in patients who achieved disease control^[68]. Lee *et al.* further identified pretreatment microbiota-bile acid associations with ICI outcome, including enrichment of selected secondary bile acids in objective responders and a microbiota signature associated with longer survival^[69]. In an atezolizumab-bevacizumab cohort, *Bacteroides stercoris* and *Parabacteroides merdae* were enriched in responders, although overall alpha and beta diversity did not differ significantly between response groups^[70]. Xin *et al.* identified specific taxa associated with progression-free survival (PFS) and overall survival (OS) despite no significant difference in alpha diversity between responders and nonresponders^[71]. Conversely, the prospective study by Shen *et al.* found no significant association of baseline alpha diversity, richness, or overall community composition with objective response and observed no major community-level change during the first 8 weeks of ICI therapy^[72]. These heterogeneous findings indicate that associations between the gut microbiome and ICI outcomes are sensitive to cohort composition, geography, diet, HCC etiology, liver function, concomitant medications, treatment regimen, sampling time, and sequencing methodology. No single bacterial species or microbial signature has yet been reproducibly validated across independent HCC cohorts for clinical decision-making.

Evidence from other solid malignancies further indicates that the gut microbiome can influence responses to PD-1/PD-L1 blockade. Studies across epithelial cancers and melanoma have linked antibiotic exposure, microbial diversity, and specific commensal taxa with variations in immunotherapy efficacy^[73,74]. In patients with anti-PD-1-refractory melanoma, responder-derived fecal microbiota transplantation (FMT) followed by anti-PD-1 rechallenge restored clinical benefit in a subset of individuals, providing initial clinical evidence that microbiome-based interventions may partially overcome treatment resistance^[75,76]. These results, however, cannot be directly extrapolated to HCC. Because many patients with HCC have underlying cirrhosis and may have portal hypertension, impaired barrier function, or cirrhosis-associated immune dysfunction, the safety profile of FMT in this population may differ from that in melanoma cohorts. These comorbidities raise potential concerns regarding microbial translocation and infection, but HCC-specific safety data remain limited. FMT should therefore remain experimental in HCC, with stringent donor and pathogen screening, stratification by liver function, and long-term safety monitoring. Compared with whole-community FMT, compositionally defined synthetic consortia or functionally traceable postbiotics may offer greater controllability.

Novel diagnostic, prognostic, and treatment-response models should integrate microbial function, metabolites, epigenetic features, and etiological context rather than relying on a single genus or methylation site. In addition to fecal and circulating biomarkers, intratumoral microbial signals may provide complementary information for HCC prognostic stratification. Jiang *et al.* combined 16S rRNA sequencing

with bacterial RNA *in situ* detection in HCC tumors and matched non-tumor tissues and found that intratumoral microbial composition was associated with survival after surgery, further supporting its potential as a tissue-level prognostic biomarker^[77]. However, microbial signatures identified to date remain heterogeneous across studies, highlighting the need for multicenter validation with harmonized workflows, particularly for low-biomass sample processing and analysis. Translationally promising models could integrate fecal metagenomic functional modules; absolute plasma concentrations of bile acids, SCFAs, and tryptophan metabolites; barrier and inflammatory indices; tissue or circulating DNA methylation features; and clinical markers such as alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II). Aberrant DNA methylation of RASSF1A, CDKN2A, GSTP1, and SOCS1 has been consistently reported in multiple HCC studies and supported by meta-analytic evidence^[12,14]. In contrast, microbiome-derived biomarkers remain supported largely by single-center observational cohorts, relatively small treatment-response cohorts, or computationally derived public datasets, with limited external validation. Treatment timing should also be recorded and incorporated into biomarker analyses as a potential clinical confounder. A systematic review and meta-analysis found no statistically significant pooled difference in OS or disease-free survival (DFS) between delayed and non-delayed HCC treatment groups; however, the delayed-treatment group had smaller tumors and differed in treatment allocation, illustrating how tumor biology and clinical selection can complicate interpretation of time-to-treatment effects^[78]. Accordingly, associations between microbial or epigenetic biomarkers and outcome should be adjusted for treatment timing together with tumor burden, liver function, and treatment modality. Therefore, integrated biomarker models should be evaluated in prospective, multicenter cohorts with prespecified documentation and statistical adjustment for etiology, liver function, tumor burden, treatment regimen, concomitant medications, and treatment timing, followed by independent external validation.

Overall, etiological context can help define research priorities for microbiome-epigenetic interventions. Virus-related HCC should be assessed with simultaneous consideration of virological control, persistent inflammation, and host epigenetic status. Alcohol-associated HCC and advanced cirrhosis characterized by intestinal barrier disruption may represent priority populations for investigating barrier restoration strategies, microbial product translocation, and TLR4-mediated inflammatory pathways. In contrast, MASLD/MASH-associated HCC may provide a particularly relevant context for studying SCFA metabolism, bile acid signaling, cholesterol homeostasis, and m6A-mediated regulation. In patients receiving ICIs, microbiota, metabolites, and intestinal inflammatory indices may serve as cross-etiology adjunctive stratification factors, but they cannot replace established clinical, liver-function, and tumor biomarkers before prospective and cross-cohort validation.

STUDY DESIGN AND EVIDENCE INTEGRATION

Many human microbiome studies in HCC rely on cross-sectional comparisons or relatively short-term pre- and post-treatment correlative analyses^[5]. Such designs are vulnerable to confounding by cirrhosis severity, dietary patterns, antibiotic and proton-pump-inhibitor exposure, tumor burden, underlying etiology, hepatic function, and treatment history^[4,29,30,65]. Prospective longitudinal studies beginning before HCC diagnosis are therefore needed to distinguish pre-existing microbial and metabolic features from changes caused by progressive liver disease, established cancer, or treatment. These studies should incorporate serial fecal and blood sampling, quantitative metabolite measurements, detailed exposure and medication data, and, when HCC subsequently develops, molecular and epigenomic profiling of tumor and adjacent liver tissue. Longitudinal sampling can help establish temporal ordering and reduce reverse-causation bias, but observational associations still require experimental validation to establish causal mechanisms. Candidate mechanisms can subsequently be tested using complementary approaches, including germ-free or microbiota-depleted models, defined bacterial colonization, controlled microbiota transfer, metabolite supplementation, and receptor- or enzyme-specific perturbation in appropriate HCC models^[22,35,45,56].

A major unresolved issue is the temporal sequence of microbiome, metabolic, and epigenetic changes before HCC diagnosis. Molecular pathological epidemiology (MPE) provides a framework for linking long-term environmental, dietary, metabolic, and microbial exposures to molecularly defined disease phenotypes rather than treating HCC as a biologically homogeneous endpoint^[79,80]. The prospective cohort incident-tumor biobank method (PCIBM) is particularly relevant because it links prospectively collected prediagnosis exposure and biomarker data with biospecimens from tumors that subsequently arise within the cohort^[81]. Applied to HCC, a PCIBM-type design could integrate serial prediagnosis stool and blood samples with incident tumor tissue, adjacent liver tissue, microbiome and metabolite profiles, tumor epigenomic and immune phenotypes, treatment information, and longitudinal clinical outcomes. Artificial intelligence and machine-learning methods may assist in integrating these complex longitudinal datasets, but they cannot reconstruct biological exposures that were never prospectively measured and therefore cannot substitute for prediagnosis biospecimen collection.

Longitudinal exposure assessment should also extend beyond signaling metabolites to microbial genotoxins. In non-HCC systems, *Morganella morganii*-derived indolamines cause host DNA damage and enhance colorectal tumorigenesis in gnotobiotic mice^[82]. Recent colorectal cancer genomic studies further illustrate the temporal dimension of microbial genotoxic exposure by showing that colibactin-associated mutational signatures can be imprinted early during tumor evolution^[83]. These findings should not be directly extrapolated to HCC, but they raise the testable question of whether long-term exposure to microbial genotoxins leaves identifiable mutational or other molecular footprints in incident liver tumors.

Beyond fecal and peripheral blood samples, the intratumoral microbiome has emerged as an additional important compartment for investigating microbiota-tumor interactions in liver cancer. Current studies suggest that microbial signals in liver tumors may originate from translocation from the intestine or biliary tract, hematogenous dissemination, adjacent non-tumor tissue, or other mucosal sites and may be associated with local inflammation, immune state, metabolic features, and clinical outcomes^[84]. The intratumoral microbiome should not, however, be considered equivalent to the fecal microbiome. Studies of the liver tumor microbiome should incorporate DNA extraction blanks, reagent controls, and batch-effect controls, while integrating *in situ* localization of bacterial DNA or RNA with microbial culture or transcriptional activity assays to differentiate true tissue-associated microbial signals from extracellular microbial nucleic acids and technical contamination.

The analytical resolution of the selected platforms constrains the level of mechanistic inference that can be made. Standard 16S rRNA gene amplicon sequencing at the genus level lacks the resolution required to distinguish the divergent metabolic capabilities that exist among distinct strains within the same bacterial genus^[29,30]. When strain-level functional inference is required, shotgun metagenomics, metatranscriptomics, high-resolution metabolomics, and strain-resolved genome reconstruction can improve characterization of microbial metabolic pathways. Multi-omics integration frameworks such as Data Integration Analysis for Biomarker discovery using Latent variable approaches (DIABLO) can identify correlated feature sets across omics layers that distinguish predefined clinical or biological phenotypes^[85]. Such statistical associations should not be interpreted as causal molecular networks without experimental validation. Spatial metabolomics and spatial epigenomic approaches may map metabolite distributions and chromatin states within the same tissue context; however, spatial co-localization alone does not establish causality.

CONCLUSION

The gut microbiome may influence HCC through interconnected inflammatory, metabolic, immune, and epigenetic pathways. Intestine-derived microbial products such as LPS activate inflammatory signaling, while microbiota-derived metabolites, including SCFAs and bile acids, can affect host transcriptional and

epigenetic regulation. Among these mechanisms, direct HCC-specific evidence is strongest for selected pathways involving microbial metabolites, immune regulation, and histone-related processes. In contrast, the extent to which microbiota-derived polyamines, tryptophan metabolites, or one-carbon intermediates directly alter DNA or RNA methylation in HCC remains to be established. Similarly, although inflammatory signaling can interact with DNMTs, EZH2, HDACs, and TET enzymes, direct evidence linking intestine-derived inflammation to stable epigenetic remodeling in HCC is still limited.

The relative importance of these pathways is likely to vary according to HCC etiology and clinical context. SCFA, bile acid, cholesterol, and m6A-related pathways may be particularly relevant for mechanistic studies in MASLD/MASH-associated HCC, whereas intestinal barrier dysfunction and LPS-related inflammation may warrant greater attention in alcohol-associated liver disease and advanced cirrhosis. Microbiome and epigenetic features may also contribute to biomarker development and immunotherapy stratification, but they are not yet sufficiently validated for routine treatment selection. Future studies should integrate longitudinal microbiome and metabolite profiling with tissue epigenomic, immune, and clinical data to distinguish association from causation and to determine whether microbiome-epigenetic interactions can provide reproducible biomarkers or therapeutic targets for HCC.

DECLARATIONS

Authors' contributions

Conceptualized the review, designed the review framework, refined the core arguments, and performed the final review of the manuscript: Xiong XH

Collected and reviewed the relevant literature, extracted key information, and drafted the initial manuscript: Zhou J

Contributed to the interpretation and synthesis of the literature, critically revised the manuscript for important intellectual content, and supervised its overall organization: Zhao RC

All authors reviewed and approved the final manuscript.

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

During the preparation of this manuscript, ChatGPT (version GPT-4o, released 2024-05-13) was used to assist with language editing and manuscript organization/refinement. In addition, ChatGPT (version GPT-4o, released 2024-05-13) was used to prepare and refine [Figure 1](#). The authors independently reviewed and approved the scientific interpretations, literature selection, reference verification, pathway classification, and final wording. The tool did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. All authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

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Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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