Regulatory Role and Biomarker Potential of Gut Microbiota Metabolites in the Progression of Metabolic Dysfunction-Associated Steatotic Liver Disease to Hepatocellular Carcinoma

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Nonalcoholic fatty liver disease is the most prevalent chronic liver disease worldwide. It is now updated as metabolic dysfunction-associated steatotic liver disease (MASLD). The progression of MASLD to hepatocellular carcinoma (HCC) involves complex mechanisms, with the gut microbiota (GM) and its metabolites playing a pivotal role in this transformation through the "gut-liver axis." This review systematically summarizes the characteristics of GM dysbiosis in patients with MASLD and the regulatory mechanisms of its metabolites (e.g., short-chain fatty acids, secondary bile acids, trimethylamine N-oxide, and lipopolysaccharides) in the progression from MASLD to HCC. Short-chain fatty acids exert protective effects in the early stages by enhancing the intestinal barrier and modulating immune and metabolic responses. However, metabolic disturbances, such as the "paradoxical effect" of butyrate and the lipogenic effect of acetate, may promote the formation of a tumor microenvironment in the later stages. Secondary bile acids (e.g., deoxycholic acid) exacerbate liver fibrosis and carcinogenesis by activating inflammatory pathways (nuclear factor-κB and mitogen-activated protein kinase), inducing oxidative stress, and inhibiting foresaid X receptor signaling. Trimethylamine N-oxide directly drives HCC progression by activating the mitogen-activated protein kinase/nuclear factor-κB pathway, promoting epithelial-mesenchymal transition, and creating an immunosuppressive microenvironment. Lipopolysaccharide accelerates fibrosis and metabolic reprogramming through toll-like receptor 4-mediated chronic inflammation and hepatic stellate cell activation. This review highlights that the dynamic changes in GM metabolites are closely associated with MASLD-HCC progression. Specific monitoring of these metabolites may serve as potential biomarkers for early detection. Furthermore, gut-targeted therapies (e.g., fecal microbiota transplantation) have shown translational potential. Future studies are needed to further validate their clinical value and develop precise prevention and treatment strategies.

KEYWORDS: nonalcoholic fatty liver disease; metabolic dysfunction-associated steatotic liver disease; hepatocellular carcinoma; gut microbiota; gut-liver axis; short-chain fatty acids; secondary bile acids; trimethylamine N-oxide; lipopolysaccharides

ABBREVIATIONS: BAs, bile acids; FMT, fecal microbiota transplantation; GM, gut microbiota; HCC, hepatocellular carcinoma; LPS, lipopolysaccharides; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, nonalcoholic fatty liver disease; SCFAs, short-chain fatty acids; TMAO, trimethylamine N-oxide

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease worldwide, affecting approximately 30% of the adult population and more than half of obese individuals (1). NAFLD can progress to metabolic dysfunction-associated steatohepatitis (MASH), which may further develop into liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), posing significant threats to patient health and survival (2–4).

According to the 2023 international expert consensus, the term "nonalcoholic fatty liver disease (NAFLD)" has been replaced by "metabolic dysfunction-associated steatotic liver disease (MASLD)." The 2 are highly similar in clinical characteristics, diagnostic criteria, and prognosis. Studies have shown that 99% of NAFLD patients meet the diagnostic criteria for MASLD, and the predictive accuracy of noninvasive testing for both is almost the same. At the same time, there is no significant

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difference in mortality between patients with MASLD and NAFLD, suggesting that their long-term prognosis is similar. By comparing the NAFLD database with the data from the National Health and Nutrition Examination Survey, it is found that the diagnostic consistency between MASLD and NAFLD is extremely high, with a Cohen κ coefficient of 0.968 (95% confidence interval 0.962–0.973). This means that in most cases, NAFLD and MASLD can be used interchangeably, and research results based on NAFLD can be directly applied to MASLD (5). However, although the vast majority of NAFLD patients meet the MASLD criteria, 5.29% of NAFLD cases do not. Such patients usually lack at least 1 cardiovascular metabolic risk factor, and their unique clinical characteristics and prognosis need further research to clarify. In clinical practice, more individualized evaluation and management are required for this small number of patients.

HCC is the most common type of primary liver cancer, accounting for 80%–90% of all cases (6). HCC is the sixth most frequently diagnosed cancer globally and the third leading cause of cancer-related mortality in 2020, with an estimated 5-year survival rate of approximately 20%. With the widespread adoption of vaccination and advancements in modern medicine, the incidence of liver disease caused by hepatitis B virus and hepatitis C virus infections has been steadily declining. Consequently, MASLD is becoming an increasingly important driver of HCC development (7).

Increasing evidence suggests that dysbiosis or imbalance of the gut microbiota (GM) may disrupt normal liver homeostasis and has become a significant contributing factor in the pathogenesis and progression of various liver diseases (8). The gut-liver axis serves as a bridge for the interaction between the GM and the liver, which is regulated by a combination of dietary, genetic, and environmental factors. Gut-derived substances are directly transported to the liver through the portal vein, while the liver provides feedback regulation to the gut through the secretion of bile acids (BAs) and antibodies. The intestinal mucosa and vascular barriers function as key structural components of this interaction, acting as an "interactive platform" that restricts the systemic spread of microbes and toxins while facilitating the smooth entry of nutrients into circulation and their delivery to the liver (9). In patients with MASLD-HCC, enrichment of *Bacteroidetes* and *Ruminococcaceae* members has been observed, which correlates with systemic inflammatory and immune markers (10,11). The GM produces a variety of metabolites that can alter host metabolism, immune responses, and gene expression (12). Several studies have identified distinct GM signatures that differentiate HCC from non-HCC cases, highlighting their potential as biomarkers for early diagnosis (13).

This review will summarize the changes and roles of GM and their metabolites during the progression of MASLD and the transition to HCC in patients with MASLD. It will also explore the therapeutic potential of targeting GM to modulate the tumor microenvironment and immune responses as a strategy for preventing the progression of MASLD to HCC in the future (14).

RELATIONSHIP BETWEEN GM AND MASLD

The human gastrointestinal tract harbors hundreds of trillions of microorganisms, comprising more than 500 different genera and species, primarily including members of the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (15). Recent studies have highlighted the emerging role of the GM in the progression of MASLD. Although the exact pathogenesis of MASLD remains unclear, several key risk factors have been identified, including obesity, type 2 diabetes, hypercholesterolemia, insulin resistance (IR), and poor dietary habits (16,17). Furthermore, GM dysbiosis has been recognized as a critical factor in the onset and progression of MASLD (18,19). Several preclinical studies have shown that germ-free mice can develop MASLD after being colonized with microbiota from MASLD mice, indicating that GM directly contributes to MASLD development. Alterations in GM composition have also been observed in patients with MASLD (20).

Under normal conditions, a symbiotic relationship exists between GM and the host, maintaining intestinal microecological balance, promoting nutrient absorption and metabolism, and regulating immune function. In healthy mice, the composition of the GM is relatively stable, with beneficial bacteria (e.g., *Prevotellaceae_UCG-001* and unclassified members of *Lachnospiraceae*) occupying a certain proportion. These beneficial bacteria help maintain intestinal barrier function and regulate host metabolic balance. The normal GM is dominated by *Firmicutes* and *Bacteroidetes*, which account for more than 90% of the total microbiota (21). These microbial communities play a crucial role in maintaining gut homeostasis through (i) Metabolic function: GM can ferment dietary fibers to produce short-chain fatty acids (SCFAs), providing an energy source for the host. They also participate in the metabolism of endogenous substances such as BAs and vitamins. In addition, they are involved in choline metabolism, converting choline into trimethylamine, which is further converted into trimethylamine N-oxide (TMAO) in the liver. Choline is essential for the synthesis of phospholipids (e.g., phosphatidylcholine), and phospholipids, as important components of very low-density lipoprotein, are crucial for hepatic lipid export (21). Notably, GM dysbiosis may lead to abnormal choline metabolism, reducing the choline available to the host, which in turn affects hepatic lipid transport and results in lipid accumulation in hepatocytes (22,23). (ii) Immune defense: GM form a "biological barrier" by attaching to the surface of intestinal epithelial cells, secreting antimicrobial substances (e.g., bacteriocins), and lowering the local pH through microbial metabolites, thereby preventing the colonization of pathogenic bacteria (24,25).

A balanced GM maintains the integrity of the intestinal barrier, preventing gut bacteria and their metabolites from entering the bloodstream and protecting the liver from gut-derived endotoxins transported through the portal vein (26). This "gut-liver axis" plays a crucial role in maintaining hepatic homeostasis. When the GM is balanced, it effectively regulates BA metabolism, SCFA levels, and immune responses, thereby reducing inflammation and fat accumulation, which lowers the risk of developing MASLD.

In MASLD mouse models, the composition of the GM undergoes significant changes, characterized by a decrease in beneficial bacteria and an increase in the relative abundance of potentially harmful bacteria (e.g., norank_f__Desulfovibrionaceae) (27). This imbalance leads to increased production of endotoxins such as LPS, which can cross the damaged intestinal barrier and enter the bloodstream. LPS activates toll-like receptor 4 (TLR4) in the liver, triggering an inflammatory response. This process stimulates hepatic cells, including Kupffer cells, to secrete inflammatory mediators, contributing to immune injury and inflammation in the liver. Therefore, the ecological imbalance of the GM may affect the progression of MASLD through the gut-

liver axis (28,29). GM dysbiosis may also alter SCFA levels, which play a crucial role in regulating energy metabolism and immune responses. Changes in SCFA levels can impair hepatic metabolic function. Increased gut permeability due to a damaged intestinal barrier allows more bacteria and their metabolites to enter the circulation, further activating hepatic immune responses and causing hepatocyte injury and inflammation.

However, the specific changes exhibit heterogeneity: Some studies show an increased relative abundance of Firmicutes, with genera such as Dorea and Roseburia significantly enriched in the feces of patients with MASLD (29,30), while others observe a decrease in Firmicutes accompanied by elevated Bacteroidetes (31,32). These differences may be related to the dietary structure, obesity status, and disease stage of the study populations (33). Furthermore, the relative abundance of beneficial bacteria (e.g., Bifidobacteria and Lactobacilli) decreases, while that of potentially harmful bacteria (e.g., endotoxin-producing Escherichia coli and members of Proteobacteria) increases significantly (34,35). For instance, levels of Epsilonproteobacteria and Gammaproteobacteria (both belonging to Proteobacteria) in the gut of patients with MASH, including children, are significantly higher than those in healthy controls (36).

Dysregulated GM promotes MASLD progression through multiple mechanisms. Moreover, it exacerbates hepatic metabolic dysfunction by influencing BA metabolism, SCFA levels, and insulin sensitivity. The degree of GM dysbiosis correlates with the severity of MASLD; more severe dysbiosis is linked to greater hepatic inflammation and fibrosis. Notably, in MASLD patients with liver fibrosis, the abundance of gut Proteobacteria (e.g., *Escherichia coli*) is significantly increased, while the levels of some potentially beneficial genera (e.g., *Ruminococcus obeum*) are reduced. Predictive models (such as random forest models) constructed based on these microbial characteristics have shown high diagnostic accuracy for advanced fibrosis (up to 93.6%), indicating that GM features hold potential as noninvasive diagnostic biomarkers (37). In addition, GM composition may influence the response to MASLD treatments. Some gut-targeted therapies (e.g., probiotics, prebiotics, and fecal microbiota transplantation [FMT]) have shown potential in improving MASLD symptoms (38). For instance, specific probiotic strains (e.g., *Lactobacillus rhamnosus* GG) and prebiotics (e.g., fructooligosaccharides) have been demonstrated to improve liver injury indicators by restoring microbial balance and reducing circulating endotoxin levels (39,40). In animal models, FMT has even been proven effective in alleviating hepatic steatosis (41), highlighting the great potential of GM as a therapeutic target for MASLD.

ROLE OF GM METABOLITES IN THE PROGRESSION FROM MASLD TO HCC

The progression of MASLD remains incompletely understood, but it is known that a subset of individuals develop MASH, a more severe form of liver injury characterized by lobular inflammation and hepatocellular ballooning. MASH can progress to fibrosis through the activation of Kupffer cells and hepatic stellate cells (HSCs), establishing a vicious cycle of cell damage and death, which promotes compensatory proliferation and disease progression. GM dysbiosis in patients with MASLD leads to significant alterations in the metabolite profile, primarily involving SCFAs, secondary BAs (e.g., deoxycholic acid and DCA), TMAO, lipopolysaccharides (LPSs), and tryptophan metabolites (e.g., indole derivatives). Although direct evidence elucidating the specific mechanisms by which the Veillonella genus contributes to the progression from MASLD to HCC is currently lacking, its proinflammatory role in both MASLD and MASH may create a microenvironment conducive to HCC development. Chronic inflammation is a significant driver of HCC, and by promoting inflammatory responses and metabolic dysregulation, Veillonella likely accelerates HCC occurrence indirectly (42). Furthermore, metabolites produced by Veillonella (such as SCFAs) may further promote HCC development by influencing the hepatic microenvironment and immune responses (43). In some individuals, MASH eventually progresses to cirrhosis or HCC, which may account for liver-related mortality (44). The following sections will explore the roles of these metabolites in the progression from MASLD to HCC.

Short-chain fatty acids

Production of SCFAs and characteristic changes in patients with MASLD. GM produce SCFAs, including acetate, propionate, and butyrate, through the fermentation of dietary fibers, starch, and other carbohydrates. SCFAs exhibit protective anti-inflammatory effects in most cases by enhancing barrier integrity, regulating hepatic metabolism, and even exerting antitumor effects, thereby counteracting disease progression (44). In patients with MASLD, the fecal microbiota shows a significant reduction or absence of beneficial genera from *Ruminococcaceae*, *Lachnospiraceae*, and *Pasteurellaceae*, which are known SCFA producers (45). Studies have found that the levels of acetate and butyrate in the feces of patients with MASLD are significantly reduced, and these changes correlate with MASLD severity, being observed across mild, moderate, and severe MASLD cases (46,47). YYang et al reported that butyrate could distinguish mild MASLD from non-MASLD cases with an area under the curve of 0.83. Acetate had an area under the curve of 0.84 and 0.70 in distinguishing moderate and severe MASLD from non-MASLD, respectively (46).

Core mechanisms by which SCFAs improve MASLD (barrier integrity, metabolic regulation, anti-inflammatory, and immune modulation). SCFAs enhance the expression of tight junction proteins such as zonula occludens-1 (ZO-1) and occludin, thereby reducing intestinal permeability (48), limiting the translocation of endotoxins (e.g., LPS) into the bloodstream, and mitigating inflammatory responses (49). Acetate is the most abundant SCFA in both the intestinal lumen and systemic circulation and participates in cholesterol metabolism and lipogenesis (50). SCFAs activate G-protein-coupled receptors (GPR41 and GPR43), which are expressed on intestinal L cells and stimulate the secretion of glucagon-like peptide-1 (GLP-1) and peptide YY. GLP-1 is a key regulator of body weight and glucose metabolism. It enhances insulin secretion, improves insulin sensitivity, and promotes β-cell mass while inhibiting gastric emptying and appetite, thereby reducing lipid intake. Activation of G-protein-coupled receptor also promotes peptide YY release, which delays gastric emptying and increases satiety (51). In patients with MASLD, hepatic GLP-1 receptor expression is significantly downregulated. Supplementation with butyrate has been shown to enhance GLP-1 receptor expression in the liver of MASLD mice by inhibiting histone deacetylase-2 (HDAC-2), thereby improving energy metabolism and reducing lipid accumulation (52). GLP-1 is a gut-derived incretin hormone with hypoglycemic and anti-inflammatory effects, improving insulin

sensitivity and reducing IR, thereby exerting protective effects in MASLD (53,54). Studies have demonstrated that FMT significantly improved insulin sensitivity after 6 weeks, and this improvement was associated with an increase in butyrate-producing bacteria (55).

Regulation of lipid metabolism. SCFAs regulate hepatic lipid metabolism by activating AMP-activated protein kinase and inhibiting sterol regulatory element-binding protein-1c, thereby reducing hepatic lipogenesis and promoting fatty acid oxidation, which alleviates hepatic steatosis (56).

Anti-inflammatory effects. SCFAs reduce the production of inflammatory cytokines by inhibiting nuclear factor-κB (NF-κB) signaling. NF-κB is a transcription factor activated in various immune and inflammatory responses, promoting the expression of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). SCFA-mediated inhibition of NF-κB reduces TNF-α and IL-6 production, thereby decreasing hepatic inflammation. SCFAs also modulate immune cell function, further reducing inflammation and protecting the liver (57,58). They regulate the activity of neutrophils, macrophages, dendritic cells (DCs), and T cells. SCFAs are natural inhibitors of HDACs in T cells, modulating immune responses by suppressing T-cell activation (59). This immunomodulation reduces inflammation and protects hepatic cells (60,61). Certain compounds targeting GM, such as hawthorn polysaccharides, have been shown to increase SCFA levels in MASLD mouse models, thereby restoring metabolic homeostasis in the liver (27).

Dual role of SCFAs in MASLD progression to HCC (protective vs tumor-promoting mechanisms). SCFAs play a dual role in the transition from MASLD to HCC. On one hand, SCFAs regulate immune responses by modulating cytokine production, reducing proinflammatory cytokines, and increasing anti-inflammatory cytokines, thereby maintaining immune balance (62). However, chronic inflammation caused by dysbiosis and pathogenic microorganisms may induce a proinflammatory state that promotes liver injury, fibrosis, cirrhosis, and carcinogenesis, contributing to the progression from MASLD and MASH to HCC (63,64). SCFAs have also demonstrated antitumor properties, potentially inhibiting HCC cell growth through multiple mechanisms, including apoptosis induction, cell proliferation inhibition, and modulation of signaling pathways.

Butyrate. Butyrate exerts a protective effect in early-stage MASLD, but its role becomes complex as the disease progresses to MASH and eventually HCC, a phenomenon termed the "butyrate paradox."

Antitumor activity. Butyrate can inhibit HDAC activity, thereby enhancing the expression of tumor suppressor genes such as *p21*, leading to the suppression of HCC cell proliferation (65). Butyrate supplementation has been shown to inhibit HCC proliferation and metastasis by triggering calcium signaling pathways and increasing reactive oxygen species (ROS) production (66).

Tumor-promoting potential. In patients with MASLD-HCC, Behary et al (11) found significantly increased fecal levels of acetate and butyrate, along with elevated serum butyrate and propionate levels compared with MASLD-cirrhosis and non-MASLD controls, suggesting that these SCFAs might play a tumor-promoting role in the progression of HCC. The paradox lies in butyrate's concentration-dependent effects—at high concentrations, it inhibits HDAC and exerts an antitumor effect, while at lower concentrations, it may promote tumor progression by supporting cancer cell survival and metabolic adaptation (67).

Acetate. When the body consumes a large amount of fructose, the small intestine cannot fully absorb it. GM metabolize the unabsorbed fructose into SCFAs, including acetate, which is transported to the liver through the portal vein. In the liver, acetyl-CoA synthetase 2 (ACSS2) uses acetate to generate acetyl-CoA, providing the substrate for fatty acid synthesis and thereby promoting lipogenesis. Excessive lipid accumulation in the liver can lead to hepatic steatosis, an early hallmark of MASLD. Over time, this lipid accumulation increases the risk of progression to HCC (68). On the other hand, hypoxia is a common feature of the tumor microenvironment, where acetate and ACSS2 play critical roles in activating the hypoxia-inducible factor (HIF) pathway. Under hypoxic conditions, ACSS2 uses acetate to produce acetyl-CoA for the acetylation of HIF-2α, facilitating the formation of the CBP/HIF-2α complex, which enhances the expression of the erythropoietin gene (69). Tumor cells efficiently take up acetate and, in the cytoplasm, catalyze the conversion of acetate into acetyl-CoA through ACSS2. Compared with normal cells, cancer cells exhibit increased acetate uptake, and it has been found that ACSS2 is involved in the process of converting acetate into acetyl-CoA (70). The expression of ACSS2 is typically elevated in tumor cells, enabling them to survive and remain active under hypoxic or nutrient-deprived conditions. This may support tumor cell growth and survival under unfavorable conditions (71,72). Studies have shown that knocking down ACSS2 in hypoxic HCC cells reduces HIF-2α acetylation, thereby inhibiting tumor cell migration and invasion (68).

Conversely, acetate exhibits antitumor activity by regulating macrophage polarization and enhancing the activation of cytotoxic $CD8^+$ T cells. This effect is mediated through the modulation of fatty acid biosynthesis in tumor-associated macrophages, which enhances the effector function of $CD8^+$ T cells (73).

Propionate. As a primary SCFAs product of GM fermentation of dietary fiber, propionate exerts diverse regulatory roles in the progression of MASLD to HCC. Propionate improves intestinal barrier function, thereby reducing the influx of endotoxins such as LPS into the bloodstream and subsequently lowering the hepatic inflammatory burden. Studies demonstrate that propionate upregulates the expression of tight junction proteins (e.g., occludin and claudin-1), decreases intestinal permeability, and reduces portal vein LPS levels. This inhibits TLR4-mediated NF-κB pathway activation, diminishing the release of proinflammatory cytokines such as TNF-α and IL-6 (74). This anti-inflammatory effect can delay the progression from MASLD to MASH, reducing the risk of liver fibrosis and carcinogenesis. Furthermore, propionate remodels GM structure, increasing the abundance of beneficial bacteria such as *Akkermansia* and *Bifidobacterium*, while suppressing the overgrowth of proinflammatory bacteria (e.g., *Blautia*). This action helps mitigate microbiota dysbiosis-induced hepatic lipotoxicity at its source.

Propionate also modulates hepatic energy metabolism pathways to reduce abnormal lipid accumulation. On one hand, it activates AMP-activated protein kinase, inhibiting acetyl-CoA carboxylase activity. This suppresses *de novo* lipogenesis and

promotes mitochondrial β-oxidation, thereby lowering intrahepatic triglyceride levels. On the other hand, propionate inhibits hepatic glycogenolysis and gluconeogenesis, improving IR and reducing glucose-driven and fructose-driven hepatocyte proliferation signals. In HCC cell models, propionate downregulates the expression of oncogenes such as c-Myc and Cyclin D1, induces cell cycle arrest, and inhibits tumor cell clonogenic capacity (75).

Formate. The dual role of formate in MASLD to HCC progression: The role of formate in the transition from MASLD to HCC exhibits a stage-dependent duality. During the MASLD stage, studies indicate (76) that partially hydrolyzed guar gum, acting as a prebiotic, modulates the GM to promote the production of SCFAs, including formate, in the cecum. Formate can improve intestinal barrier function, thereby reducing the translocation of LPS into the portal vein. This subsequently inhibits the activation of endotoxin-induced TLR (TLR4/TLR9) signaling in the liver, lowers levels of inflammatory cytokines such as TNF-α and monocyte chemoattractant protein-1, and reduces the expression of fibrosis markers such as collagen 1α1 and α-smooth muscle actin, thereby delaying MASLD progression. Furthermore, formate may attenuate hepatic inflammatory injury by inhibiting neutrophil infiltration (evidenced by reduced hepatic myeloperoxidase activity), providing protection during the early stages of MASLD to HCC transition.

However, as the disease progresses to HCC, the role of formate shifts toward promoting tumor invasion and progression. Research has shown (77) that HCC patients with microvascular invasion exhibit significantly higher preoperative serum formate levels than those without microvascular invasion ($P = 0.023$), suggesting a potential association between formate and tumor cell invasiveness. This observation, coupled with findings of elevated urinary formate levels in patients with HCC, may reflect increased utilization of formate during the metabolic reprogramming of HCC cells or abnormal formate excretion due to gut-liver axis dysfunction. Extrapolating from studies on colorectal cancer, GM-derived formate can activate the aryl hydrocarbon receptor (AhR) signaling pathway, enhancing the self-renewal and invasive capabilities of cancer stem cells. This mechanism may also apply to HCC—that is, during the progression from MASLD to HCC, gut dysbiosis leads to excessive formate production, which promotes HCC cell stemness through the AhR signaling pathway, accelerating tumor metastasis (78).

Notably, the differential effects of formate in MASLD vs HCC may stem from context-dependent factors such as concentration and timing: during early MASLD, moderate formate levels exert anti-inflammatory and antifibrotic effects by protecting the gut-liver axis. By contrast, during the HCC stage, disruption of the intestinal barrier or aberrant tumor metabolism can lead to excessive formate exposure, which then activates pro-oncogenic signaling pathways to drive disease progression. Although partially hydrolyzed guar gum restores the beneficial effects of formate by remodeling the GM (e.g., increasing Bacteroides and Clostridium subcluster XIVa, reducing proinflammatory bacteria), dysbiosis in HCC may disrupt this balance, transforming formate into a tumor-promoting factor (76).

Secondary BAs

BAs, synthesized in the liver and metabolized by GM into secondary BAs, serve as crucial metabolic regulators. Through activation of BA receptors (including farnesoid X receptor [FXR], vitamin D receptor, and Takeda G protein-coupled receptor 5 [TGR5]) in the liver and intestine, secondary BAs precisely modulate lipid and glucose metabolism (79).

Emerging evidence indicates that MASLD development is closely associated with gut dysbiosis and altered serum BA profiles. Clinical studies demonstrate characteristic reductions in secondary BA levels coupled with significant alterations in BA-transforming microbiota in patients with MASLD (80,81). Notably, specific secondary BAs (e.g., lithocholic acid [LCA] and DCA) exhibit progressive elevation during MASLD progression (82). These BAs participate in MASLD pathogenesis by binding to tissue-specific receptors that regulate lipid and glucose metabolic signaling. Particularly, DCA levels show strong positive correlation with MASLD severity, including fibrosis staging (79). DCA derivatives such as glycodeoxycholate, 7-ketodeoxycholic acid, and dehydrocholic acid also increase progressively with disease advancement. The biosynthesis of DCA and its metabolites requires coordinated action of microbial bile salt hydrolase, BA-inducible operon, and 7α-hydroxysteroid dehydrogenase. Given that BAs disrupt bacterial membranes through detergent effects and alter intracellular macromolecular conformations, only BA-tolerant microbial species achieve intestinal dominance (83). Metagenomic analyses reveal increased abundance of Bacteroides and Lachnospiraceae (harboring DCA-synthesizing genes), alongside progressive depletion of DCA-sensitive Ruminococcaceae during disease progression (84). Mechanistically, DCA-induced oxidative stress activates key inflammatory pathways (NF-κB and mitogen-activated protein kinase [MAPK]), upregulating proinflammatory cytokines including TNF-α, IL-6, and IL-1β, thereby exacerbating hepatic inflammation (85,86). These inflammatory mediators not only directly damage hepatocytes but also promote fibrogenesis through HSC activation, potentially triggering hepatocellular carcinogenesis (87). At the metabolic level, DCA significantly impairs mitochondrial function: It reduces mitochondrial DNA content in muscle fibers through TGR5-dependent mechanisms, compromising structural integrity (88), while synergizing with cholic acid to suppress mitochondrial biogenesis pathways, leading to diminished mitochondrial mass (89). Such mitochondrial dysfunction ultimately hinders fatty acid β-oxidation, resulting in pathological intracellular lipid accumulation.

Emerging evidence demonstrates that hydrophobic BAs including LCA, DCA, and chenodeoxycholic acid serve as key promoters of HCC development (90,91). Preclinical studies suggest that although DCA and other FXR agonists show therapeutic potential in ameliorating hepatic steatosis, their mechanistic complexity warrants careful consideration. Notably, as an endogenous FXR antagonist, elevated DCA levels significantly suppress FXR activity. Given FXR's central role in maintaining BA homeostasis, lipid metabolic balance, and inflammatory responses, DCA-mediated FXR inhibition exacerbates MASLD progression (92,93). Mechanistically, FXR deficiency promotes hepatocarcinogenesis: FXR-null mice spontaneously develop liver tumors through Wnt/β-catenin pathway activation and oxidative stress accumulation (94,95). In addition, elevated DCA correlates with intestinal inflammatory microenvironment formation and metabolic dysregulation. Specifically, DCA activates myeloid cell FXR to induce type I interferon production, aggravating intestinal inflammation (96) while simultaneously suppressing FXR activity through negative feedback, ultimately

leading to BA dysregulation and carcinogenic microenvironment formation (67,97).

Secondary BAs (e.g., LCA and DCA) and their conjugated forms are identified as potent agonists for TGR5 (98). Pathologically elevated secondary BAs-induced TGR5 overactivation shows significant correlation with HCC progression. TGR5 exerts antitumor effects through dual mechanisms: attenuating hepatic inflammation through NF-κB and STAT3 pathway inhibition, and enhancing cAMP production through adenylate cyclase activation, thereby suppressing IκB kinase phosphorylation and nuclear factor P65 translocation (99). This systemic regulation of inflammatory signaling constitutes the core mechanism of TGR5-mediated cancer suppression.

Regarding oxidative stress, DCA induces ROS burst-mediated DNA double-strand breaks, directly compromising genomic stability. This genotoxic effect not only promotes tumorigenesis through p53 tumor suppressor inhibition but also exacerbates malignant transformation through redox imbalance (100). Epigenetic studies further reveal DCA's capacity to upregulate proto-oncogene c-Myc expression. As a master regulator of cell proliferation, metabolic reprogramming, and apoptosis resistance, c-Myc dysregulation plays pivotal roles in HCC progression (101).

Studies demonstrate that secondary BA LCA induces endoplasmic reticulum stress in $CD8^+$ T cells, upregulating transcription factors NUR77 and TOX to suppress effector molecule secretion (e.g., IFN-γ), thereby impairing antitumor immune responses. Primary BAs such as taurochenodeoxycholic acid promote immune escape by generating ROS that cause mitochondrial dysfunction and programmed cell death in T cells. Ursodeoxycholic acid (UDCA) restores T-cell cytotoxicity through multiple mechanisms: reducing LCA proportion, alleviating endoplasmic reticulum stress, and enhancing granzyme B expression, leading to significant suppression of hepatic tumor growth. In MASLD animal models, UDCA may delay disease progression through similar mechanisms. Future research should focus on delineating dynamic changes in BA profiles across MASLD stages and identifying critical therapeutic targets.

Preclinical and clinical evidence confirms that in SV40-driven murine HCC models, either genetic ablation of BA synthesis enzymes (e.g., BAAT) or UDCA supplementation effectively reduces tumor burden and enhances antitumor immunity. Human HCC tissue analyses reveal an inverse correlation between BAAT overexpression and $CD8^+$ T-cell infiltration, suggesting clinical relevance of secondary BA accumulation in immune suppression. These findings indicate that inhibiting BA synthesis may potentiate the efficacy of immune checkpoint therapies such as PD-1 inhibitors (Figure 1).

Glycodeoxycholic acid (GCDA) plays a critical and dynamic role in the progression of MASLD to HCC. Metabolomic analyses reveal significant variations in GCDA levels across disease stages: Hepatic GCDA is markedly downregulated in MASH (the severe stage of MASLD), potentially falling to as low as 9% of normal levels. Conversely, plasma GCDA levels increase on transition to HCC. This shift likely reflects metabolic reprogramming during the transition from hepatic inflammation and fibrosis (MASH) to malignant proliferation (HCC), suggesting GCDA may participate in regulating this process or serve as a potential mediator (102).

Mechanistically, GCDA upregulates the sphingosine-1-phosphate receptor 2 (S1PR2), triggering activation of the PI3K/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway. This significantly enhances the expression of key downstream effectors (p-AKT, p-mTOR, vascular endothelial growth factor, serum and glucocorticoid-inducible kinase 1, and protein kinase C alpha), molecules critically involved in malignant phenotypes such as cell proliferation, invasion, and migration, thereby promoting the MASLD-to-HCC transition. S1PR2 knockdown abolishes GCDA's ability to activate this pathway and its effectors, confirming GCDA's actions are strictly dependent on S1PR2-mediated signaling. In summary, fluctuations in GCDA levels during the MASLD-HCC continuum and its pro-oncogenic role through the S1PR2-PI3K/AKT/mTOR axis underscore its pivotal contribution to disease progression (103).

Trimethylamine N-oxide

TMAO is primarily generated through gut microbial metabolism of dietary nutrients such as choline and carnitine, which are abundant in red meat, egg yolks, and certain fish. GM convert these precursors into trimethylamine, subsequently oxidized by hepatic flavin monooxygenases to form TMAO (104). As a key modulator of IR, TMAO is closely associated with MASLD pathogenesis. Its mechanistic involvement includes activation of inflammatory pathways such as TLR signaling, promoting systemic inflammation and accelerating MASLD progression (105). Clinical evidence demonstrates that elevated circulating TMAO levels correlate with MASLD and MASH, while fecal TMAO content shows positive correlation with MASLD severity, indicating its critical role in disease progression (106).

Molecular studies reveal TMAO inhibits FXR-mediated BA signaling, leading to hepatic lipid accumulation (107). Concurrently, TMAO exacerbates hepatic inflammation through proinflammatory cytokine release and potentiates glucose metabolism dysregulation and IR, promoting MASLD development in high-fat diet mice (108). These findings collectively suggest TMAO accelerates MASLD progression and drives transition to end-stage liver diseases such as MASH through gut-liver axis regulation.

In vitro and *in vivo* experiments confirm TMAO significantly promotes proliferation and migration of HCC cells (HepG2 and Huh-7). Subcutaneous xenograft models demonstrate TMAO-treated groups exhibit larger tumor volumes and weights compared with controls. Immunohistochemical and Western blot analyses show upregulated expression of proliferation markers (Ki-67), migration-related proteins, and epithelial-mesenchymal transition (EMT) indicators (Vimentin, Snail, Slug) with concurrent E-cadherin downregulation in TMAO-treated tumors (108). Morphological observations reveal mesenchymal-like phenotypes in TMAO-treated HCC cells, characterized by elongated morphology and disrupted intercellular junctions (109,110).

Mechanistic investigations identify TMAO activates phosphorylation of MAPK pathway components (p38, c-Jun N-terminal kinase [JNK], extracellular signal-regulated kinase [ERK]). Western blot analyses demonstrate dose-dependent increases in p-p38, p-JNK, and p-ERK expression levels with TMAO concentration, indicating that MAPK pathway activation mediates TMAO's oncogenic effects (111,112).

Chronic inflammation is a key factor in the occurrence and development of liver cancer. In addition, TMAO activates NF-κB signaling and NLRP3 inflammasome, upregulating proinflammatory factors (TNF-α, IL-6), adhesion molecules, and

Bile Acid	Influence			Consequence
Deoxycholic Acid（DCA）	TGR5 →mitochondrial quality（-）→fatty acid oxidation（-）		fat accumulation	Promote HCC
	FXR	→Wnt/β-catenin signaling pathway →oxidative stress →FXR in myeloid cells→	Aggravate inflammation， Bile acid accumulation	Promote HCC
	induce reactive oxygen species		DNA double-strand breaks， Genomic instability（-），P53 （-），Cellular damage	Promote HCC
	c- Myc Gene expression (+)		Oncogene expression	Promote HCC
Lithocholic Acid（LCA）	NF-κB +MAPK→TNF-α， IL-6,IL-1β TGR5→NF-κB+STAT3 adenylate cyclase（+）→cAMP（+），P65 nuclear translocation→ NF-κB（-）		exacerbate inflammatory response inhibit inflammatory response	Promote HCC Inhibit HCC
	transcription factor（NUR77、TOX）（+） cD8⁺T ER stress inhibit cellular secretion（IFN-γ）		attenuate immune response	Promote HCC
Tauro chenodeoxycholic Acid(TCDCA)	Ros→T-cell mitochondrial dysfunction		T-cell death resistance	Promote HCC
Ursodeoxycholic Acid(UDCA)	Reduce the proportion of LCA、Alleviate endoplasmic reticulum stress、Enhance T-cell cytotoxic function (e.g. expression)			Inhibit HCC
Glycodeoxycholic acid (GCDA)	Upregulate the expression of S1PR2 receptor and activate the PI3K/AKT/mTOR signaling pathway			Promote HCC

Figure 1. Schematic diagram of the mechanism of secondary bile acids in the progression from MASLD to HCC. This figure summarizes the key mechanism of action of secondary bile acids in the transition from MASLD to HCC. HCC, hepatocellular carcinoma; MASLD, metabolic-associated steatotic liver disease.

chemokines while inducing oxidative stress, collectively driving MASLD-MASH-HCC transition (113–115). TNF-α is a proinflammatory cytokine associated with the occurrence and development of HCC. In fact, we found that TNF-α promotes the proliferation of Hepa1-6 mouse HCC cells in a dose-dependent manner, 50 ng/mL TNF-α synergizes with specific TMAO concentrations to enhance proliferation in Hepa1-6 murine HCC cells (116). Emerging research reveals TMAO promotes HCC progression through upregulation of stromal protein periostin (POSTN). POSTN is a stromal cell protein that is closely related to the invasiveness of HCC. In the inflammatory-induced HCC microenvironment, high expression of POSTN is associated with an immunosuppressive state of the tumor microenvironment. As a key tumor microenvironment regulator, POSTN activates an integrin-linked kinase/AKT/mTOR signaling axis, enhancing HCC cell proliferation, migration, and survival. Single-cell sequencing analyses demonstrate that TMAO-induced POSTN and HTRA3 overexpression correlates with increased neutrophil and

regulatory T-cell infiltration, suggesting TMAO fosters immunosuppressive microenvironments to facilitate HCC development (109,117,118).

LPS and chronic inflammation-driven carcinogenesis

LPS, a major component of Gram-negative bacterial cell walls, activates immune responses through TLR4. As a pivotal pattern recognition receptor, TLR4 initiates inflammatory cascades by detecting pathogen-associated molecular patterns. Gut dysbiosis compromises intestinal barrier function, facilitating endotoxin (e.g., LPS) translocation into circulation, which subsequently activates inflammatory pathways through TLR4 signaling and induces oxidative stress through nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase. Experimental studies demonstrate that exogenous LPS administration significantly promotes HCC development in mice (119). Gut-derived bacterial products accelerate MASH progression by activating TLR4 in various hepatic cells (including hepatocytes and Kupffer cells), triggering downstream cytokine cascades (120). Mechanistically, the LPS-TLR4 axis activates the NF-κB pathway, promoting proinflammatory cytokine production including TNF-α, IL-6, and IL-1β (121). Within a hepatic microenvironment, LPS binding to TLR4 on Kupffer cells induces TNF-α and IL-6 release, activating HSCs transdifferentiation into myofibroblasts. Activated myofibroblasts exacerbate oxidative stress through NADPH oxidase upregulation, elucidating how gut-liver axis disruption drives disease progression in MASLD (122).

HSCs reside in the perisinusoidal space between sinusoidal endothelial cells and hepatocytes, remaining quiescent and storing vitamin A under physiological conditions (123). When LPS activates these cells through TLR4, HSCs transform into myofibroblasts and begin to secrete a variety of cytokines and matrix components, including transforming growth factor β1 (TGF-β1). TGF-β1 is a potent profibrotic factor that promotes the deposition of extracellular matrix, particularly collagen synthesis, a process crucial for liver fibrosis. TGF-β1 not only promotes collagen deposition but also inhibits the expression of matrix metalloproteinases, which are responsible for matrix degradation. Therefore, oversecretion of TGF-β1 leads to the accumulation of extracellular matrix, resulting in fibrosis. In the liver, collagen deposition alters the liver's structure, thereby affecting its function. Under the influence of the LPS-TLR4 axis, the liver environment gradually shifts to a profibrotic microenvironment. The activation of HSCs and the secretion of TGF-β1 lead to collagen accumulation, which not only affects the liver's structure but also alters its hemodynamics, increasing intrahepatic pressure. The chronic high-pressure state can further activate HSCs, creating a vicious cycle that exacerbates liver fibrosis. Liver fibrosis is an important risk factor for HCC. As liver fibrosis progresses, the liver microenvironment also changes, providing a fertile ground for the development of liver cancer.

The LPS/TLR4 axis can also regulate the expression of liver glutaminase (GLS). On LPS stimulation, the NF-κB p65 subunit translocates to the nucleus and binds to the promoter region of the GLS gene, thereby promoting the transcription of GLS. This directly enhances the transcription of GLS mRNA. The increased level of GLS mRNA further leads to increased synthesis of GLS protein, thereby enhancing the activity of the GLS enzyme and consequently promoting hepatic ammonia accumulation (124). HCC cells exhibit glutamine addiction, relying on GLS-catalyzed conversion of glutamine to glutamate and α-ketoglutarate (α-KG). The increased activity of GLS promotes the metabolism of glutamine, generating glutamate and α-KG, α-KG entry into the tricarboxylic acid cycle provides bioenergy and biosynthetic precursors, with this metabolic reprogramming markedly promoting HCC proliferation and migration. Mechanistic investigations reveal elevated GLS activity enhances tumor invasiveness through EMT regulation. The increased activity of GLS is also closely related to the glycolytic pathway in HCC cells. The α-KG generated from glutamine metabolism can promote the expression of key glycolytic enzymes, increase lactate production, and thereby support the rapid proliferation of HCC cells (125). Notably, selective GLS inhibitor Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) effectively suppresses HCC cell growth and migration (Figure 2).

Metabolites of amino acid metabolism

Tryptophan metabolites. Tryptophan metabolites play a crucial bidirectional regulatory role in the transition from MASLD to HCC by modulating the GM-liver axis, the inflammation-fibrosis-carcinogenesis axis, and the immune microenvironment. Within its major metabolic pathways, the dominant kynurenine pathway (catalyzed by indoleamine 2,3-dioxygenase/tryptophan 2,3-dioxygenase) is activated in the chronic inflammatory environment of MASLD, leading to kynurenine accumulation. Kynurenine promotes HSC activation and liver fibrosis progression while also suppressing antitumor immunity (e.g., reducing T-cell infiltration) by activating the AhR, thereby fostering a protumorigenic immunosuppressive microenvironment. Conversely, indole pathway metabolites, which depend on GM (e.g., *Clostridium*, *Bacteroides*), such as indole-3-propionic acid (IPA), exhibit anticancer potential: IPA enhances gut barrier function and reduces endotoxin translocation by activating the pregnane X receptor. It also alleviates liver fibrosis and inflammation (e.g., by inhibiting the NF-κB pathway) through suppressing HSC activation and the TGF-β1 signaling pathway (126,127). In the serotonin-melatonin pathway, serotonin is primarily synthesized by gut enterochromaffin cells. In MASLD, serotonin may promote HSC proliferation and collagen deposition by activating hepatic serotonin receptors (e.g., HTR2A), accelerating fibrosis (128), thereby indirectly increasing HCC risk. Melatonin, further metabolized from serotonin, provides protection through its antioxidant and anti-inflammatory effects. Gut dysbiosis leading to reduced indole metabolites weakens the barrier function and exacerbates intrahepatic TLR4-NF-κB inflammatory cascades and DNA damage. In summary, tryptophan metabolic imbalance (e.g., elevated kynurenine/reduced IPA) is a key driver in the MASLD-to-HCC transition. Targeting this metabolic pathway (e.g., inhibiting IDO, supplementing indole-producing microbiota) holds promise as a novel strategy to prevent MASLD progression to HCC (129).

Tyrosine metabolites. Although the direct role of tyrosine metabolites in the progression from MASLD to HCC has not been exhaustively studied, existing evidence strongly suggests their potential involvement. Patients with MASLD commonly exhibit significant tyrosine metabolic dysregulation, with plasma levels positively correlating with the degree of hepatic steatosis. This abnormality is independent of obesity and IR, suggesting it may be a hallmark characteristic of MASLD (130). This dysregulation involves "gut-liver axis" modulation (where gut dysbiosis leads to tyrosine accumulation) and interactions with oxidative stress and

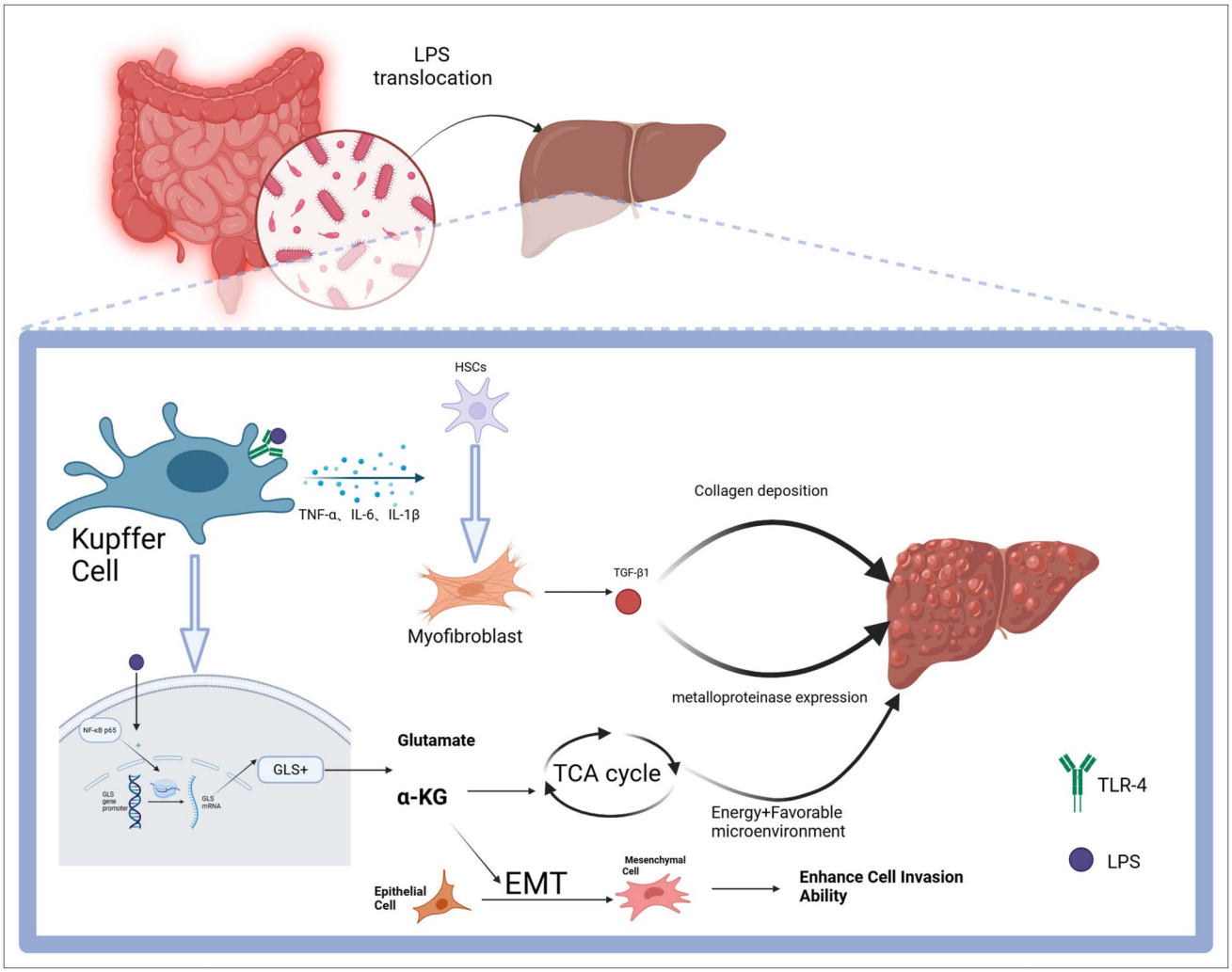


Figure 2. Mechanism diagram of LPS-regulating GLS expression through the TLR4/NF-κB signaling axis to promote HCC progression. This figure illustrates that LPS activates the NF-κB signaling pathway through TLR4, thereby upregulating the expression of hepatic GLS. GLS promotes glutamine metabolism to generate glutamate and α-KG; the latter enters the TCA cycle to provide energy and biosynthetic precursors, supporting the proliferation and migration of HCC cells. In addition, enhanced GLS activity also promotes tumor invasion through EMT. The figure also indicates that the GLS inhibitor BPTES can effectively inhibit the growth and migration of HCC cells. α-KG, α-ketoglutarate; EMT, epithelial-mesenchymal transition; GLS, glutaminase; HCC, hepatocellular carcinoma; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; TCA, tricarboxylic acid; TLR4, toll-like receptor 4.

inflammation (e.g., elevated tyrosine levels coincide with increased inflammatory cytokines and oxidative markers) (131). In the multistep progression from MASLD to HCC, tyrosine metabolites may accelerate malignant transformation through several mechanisms: (i) Exacerbating lipotoxicity: Its degradation product, acetyl-CoA, drives hepatic lipid synthesis and deposition, inducing hepatocyte damage and genetic mutations (130). (ii) Enhancing chronic inflammation and oxidative stress: Accumulation of intermediate products (e.g., homogentisic acid) can induce ROS generation and activate inflammatory pathways, leading to sustained liver injury and increased risk of DNA mutations (132). However, current research primarily focuses on the MASLD stage, with a lack of direct evidence linking tyrosine metabolites to HCC transformation. Specific molecular targets and microbiota regulatory mechanisms also remain unclear. Future studies are needed to validate their value as progression biomarkers in clinical cohorts and to elucidate their molecular mechanisms in depth.

Phenylalanine and branched-chain amino acid metabolites. Studies indicate that plasma levels of both phenylalanine and branched-chain amino acids significantly increase ($P < 0.001$) with the severity of MASLD (progressing from simple steatosis to MASH and cirrhosis), and their elevation, coupled with glutathione depletion, signals metabolic imbalance. Phenylalanine accumulation can induce oxidative stress (e.g., its metabolite phenylacetic acid damaging DNA) and exacerbate chronic inflammation and fibrosis through the "gut-liver axis." Branched-chain amino acids, on the other hand, promote IR (by activating the PI3K/Akt/mTOR pathway), thereby fostering abnormal hepatocyte proliferation. They simultaneously enhance lipid synthesis (e.g., increasing sterol regulatory element-binding protein-1c

expression) and fibrosis (through stellate cell activation), creating a predisposing microenvironment for HCC development (64).

FECAL MICROBIOTA TRANSPLANTATION

As an innovative method for regulating GM, FMT shows broad application prospects in the treatment of various diseases.

Dysbiosis of the GM is associated with HCC caused by multiple etiologies, including viral hepatitis, liver cirrhosis, and MASLD (133,134). FMT is a clinical technique used to modify the GM. By introducing a healthy microbial community, FMT helps restore microbial diversity in the recipient's gut and improve gut microbial metabolites. According to the new formulations approved by the US Food and Drug Administration (FDA), there are ingestible capsules and rectal suspensions. FMT was first approved for the treatment of recurrent *Clostridioides difficile* infection (135).

In the intestines of patients with HCC caused by viral hepatitis, a significant increase in SCFA-producing bacteria has been observed (136). By contrast, in the intestines of patients with cirrhosis-related HCC, a decrease in butyrate-producing bacteria and an increase in LPS-producing bacteria have been found (137). Another study showed that in HCC patients with both cirrhosis and MASLD, the GM is characterized by increased numbers of Bacteroides and Ruminococcus, and decreased numbers of Bifidobacteria (10). Imbalances in GM with different functions are associated with different etiologies, ultimately promoting the development of HCC.

In recent years, a growing body of research has indicated that the GM and its metabolites play a key role in regulating the host immune system, modulating antitumor immunity, and influencing the response to immunotherapy. However, to date, no clinical trials have explored the application of FMT in patients with HCC.

Effects on NAFLD or MASLD

Several trials have investigated the use of FMT in patients with hepatic steatosis (i.e., patients with NAFLD/MASLD). Patients who received a 6-week FMT course from lean healthy donors showed a trend toward improved hepatic insulin sensitivity and increased concentrations of butyrate-producing microbes. Butyrate can prevent the translocation of endotoxin bacterial particles and significantly regulate IR (55). Importantly, the gut-liver axis in patients with hepatic steatosis seems to be rebalanced through FMT. Impaired small intestinal permeability was significantly restored 6 weeks after allogeneic FMT (138).

The efficacy of FMT in treating liver diseases such as MASLD and alcoholic hepatitis has also been confirmed. Xue et al (139) conducted a randomized clinical trial on 75 patients with NAFLD/MASLD, dividing them into 2 groups: one group received FMT (3 enemas from allogeneic healthy donors through colonoscopy) and the other group received oral probiotics. A reexamination after 1 month showed that patients who received FMT had reduced hepatic fat accumulation, decreased blood lipid levels, and restored gut dysbiosis (confirmed by fecal 16S rRNA sequencing), and lean patients with NAFLD/MASLD responded better to FMT than obese patients. Gomez-Hurtado et al found that FMT from healthy donors to 47 patients diagnosed with MASLD resulted in significant improvement in their clinical symptoms. Similarly, in 2017, Philips et al published a compelling study demonstrating that patients with severe alcoholic hepatitis showed significant clinical and biochemical improvement after receiving FMT (140).

Effects on HCC

Emerging evidence suggests that the GM can influence the efficacy of immunotherapy for HCC. Specific microbial combinations are associated with improved responses to immune checkpoint inhibitors (such as anti-programmed death 1 [PD-1]/programmed death-ligand 1 [PD-L1] and anti-cytotoxic T-lymphocyte-associated protein 4 therapies). These findings have led to the hypothesis that modulating the GM through interventions such as probiotics, prebiotics, FMT, and dietary adjustments may enhance the efficacy of immunotherapy for HCC (141). Preclinical studies based on HCC animal models have yielded positive results (142–144). One study showed that transplanting fecal microbiota from wild-type mice into HCC mice enhanced their anticancer response (145). Another study using FMT revealed the role of cholesterol-regulated microbiota in the progression of MASLD-related HCC: Germ-free mice gavaged with fecal microbiota from mice on a high-fat, high-cholesterol diet developed hepatic lipid accumulation, inflammation, and cell proliferation, confirming that dietary cholesterol is an important driver of MASLD-HCC (142).

However, there is currently 1 study on the effect of FMT on immune cells in the TME of HCC mice. *Lactobacillus reuteri* is a well-known probiotic with positive effects on various diseases, such as constipation, MASLD, metabolic syndrome, acute diarrhea, allergic rhinitis, and asthma, and enhances immune checkpoint inhibitor [ICI]-based melanoma treatment (146,147). Recent studies have shown that the concentration of gut-derived *L. reuteri* and its metabolite (acetic acid) is associated with the development of HCC. Treating HCC mouse models (in which L. reuteri and acetic acid concentrations were significantly reduced) with antibiotics and supplementing with L. reuteri or feces from wild-type mice resulted in a reduction in tumor number and size. L. reuteri inhibits IL-17A-producing ILC3 cells in HCC by inhibiting HDAC activity and inducing acetylation of SRY-box transcription factor 13 (145). Other studies have shown that IL-17A can cause MASH and HCC, while IL-17A inhibitors can prevent MASH and HCC in high-risk patients (148).

Combination with ICIs. Strategies targeting the GM to enhance ICI efficacy have emerged as promising therapeutic approaches (149). Given the different microbial profiles associated with patients who respond well to ICIs, interest in integrating FMT into immunotherapy strategies is growing. Early studies combining FMT with immunotherapy have shown that FMT can influence T-cell and natural killer-cell pathways, thereby enhancing immune responses and promoting tumor suppression. A recent study focused on oral fecal capsules for FMT in patients receiving ICI treatment, showing that it can increase the levels of bacteria associated with improved anti-PD-1 treatment responses, enhance $CD8^+$ T cell activation, and reduce IL-8-expressing myeloid cells.

Li et al found in HCC mouse models that gut dysbiosis impairs antitumor immune responses through the cyclic GMP-AMP synthase-stimulator of interferon genes-type I interferon (cGAS-STING-IFN-I) pathway, and activation of this pathway can enhance the antitumor efficacy of PD-1 blockade in HCC, suggesting that the combination of FMT and ICIs is a promising therapeutic strategy for HCC. Bertrand et al found that

transplanting feces from tumor patients who responded to ICI treatment into tumor-bearing mice improved the efficacy of ICI treatment (150). ICI therapy activates the patient's own immune system to attack tumor cells but may also induce immune-related adverse events (irAEs), such as through inducing excessive immune activation and intestinal inflammation. However, Routy et al confirmed that incorporating FMT into anti-PD-1 therapy does not increase the incidence of irAEs; Spreafco et al tested microbial ecosystem therapeutic 4 as an alternative to FMT in combination with ICIs for advanced solid tumors and found no worsening of ICI-related irAEs (151). Renga et al found that direct intestinal delivery of indole-3-carboxaldehyde can activate the AhR/IL-22 pathway, maintaining epithelial barrier function and immune homeostasis during colitis. Indole-3-carboxaldehyde can also increase ZO-1 expression, reduce inflammation severity, and restore intestinal epithelial barrier function (152). Sun et al demonstrated that oral administration of RP 10 can enhance the expression levels of ZO-1 and mucin 2 in the colonic mucosa of mice with colitis. This effect can reduce the severity of inflammation and restore intestinal epithelial barrier function (153). In summary, the GM and their products can enhance the efficacy of chemotherapy and immunotherapy for gastrointestinal tumors while reducing treatment-induced adverse reactions.

Combination with radiotherapy. A prospective longitudinal study was conducted on patients with HCC receiving radiotherapy, collecting their fecal samples for 16S rRNA sequencing and using them for FMT in HCC mouse models. It was found that the gut microbiome regulates HCC sensitivity to radiotherapy through the cGAS-STING signaling in DCs, and bacterial-derived c-di-AMP in feces can regulate radiotherapy-induced cGAS-STING activation in DCs (149).

In anti-HCC metastasis

Significant GM dysbiosis has been observed in patients with intrahepatic metastatic HCC and animal models, characterized by reduced diversity and decreased abundance of core beneficial genera (such as butyrate-producing Anaerotruncus colihominis and Dysosmobacter welbionis). These genera can exert antitumor effects by regulating host immunity, metabolism, and inhibiting inflammation; their absence may promote excessive formation of neutrophil extracellular traps, thereby accelerating tumor angiogenesis (e.g., upregulated expression of vascular endothelial growth factor, CD31, and matrix metalloproteinase 9) and tissue necrosis, ultimately driving intrahepatic metastasis of liver cancer.

By constructing a humanized flora-associated HCC mouse model, studies found that FMT intervention from healthy donors can significantly reverse GM dysbiosis, restore the colonization of beneficial bacteria, such as Anaerotruncus and Dysosmobacter, and inhibit the progression of intrahepatic metastasis. Compared with no intervention, the FMT treatment group showed significantly reduced intrahepatic metastatic tumor burden, malignancy, and necrotic foci, and significantly improved the survival rate. Mechanistically, FMT repairs microbiota balance, inhibits neutrophil inflammatory activation and neutrophil extracellular traps formation, thereby reducing the expression of proangiogenic factors and improving the tumor microenvironment (154).

However, FMT also has some drawbacks, such as the risk of disease transmission from donor to recipient and variations in patient acceptance (155). Although FMT seems promising in patients with NAFLD/MASLD, important issues remain to be addressed. Safety concerns remain significant due to 2 cases of invasive infections involving extended-spectrum β-lactamase-producing Escherichia coli in immunocompromised patients. Therefore, according to European consensus and the FDA, inclusion and exclusion criteria should be stricter to avoid any adverse reactions (156,157).

CONCLUSION

This review systematically elucidates the regulatory network involving the GM and its metabolites in the progression from NAFLD/MASLD to HCC. Accumulating evidence indicates that patients with NAFLD/MASLD exhibit characteristic gut dysbiosis whose metabolites drive malignant transformation through mechanisms mediated by the gut-liver axis. Core metabolites, including SCFAs, secondary BAs, TMAO, and LPS, demonstrate dynamic bidirectional regulatory roles at different stages of NAFLD/MASLD-HCC progression.

In the early stages of NAFLD/MASLD, SCFAs exert protective effects by enhancing intestinal barrier integrity, modulating immune homeostasis, and suppressing inflammatory responses. However, their metabolic dysregulation (e.g., the concentration-dependent "paradoxical effect" of butyrate and the prolipogenic effect of acetate) may promote the formation of a tumor microenvironment in later disease stages. Secondary BAs, represented by DCA, significantly exacerbate liver fibrosis and carcinogenesis by activating the NF-κB/MAPK pathway, inducing oxidative stress, and suppressing FXR signaling. Conversely, targeting BA metabolism (e.g., supplementing UDCA) shows therapeutic potential. TMAO directly drives malignant phenotypes by activating the MAPK/NF-κB axis, promoting EMT, and shaping an immunosuppressive microenvironment. LPS lays the pathological foundation for HCC development through TLR4-mediated chronic inflammation, HSC activation, and metabolic reprogramming.

Current research suggests that the dynamic changes in gut microbial metabolites are closely associated with the clinical stages of NAFLD/MASLD-HCC, and specific metabolite profiles (e.g., decreased SCFAs, increased TMAO/DCA) hold potential as biomarkers. Based on the mechanisms outlined in this review, strategies targeting the GM and its metabolites—including the selection of specific probiotic strains, precision FMT, and the development of metabolic enzyme inhibitors—are considered a promising direction for future interventions in NAFLD/MASLD-HCC and possess theoretical potential for translation into novel therapeutics. However, it must be emphasized that the actual clinical efficacy and safety of these strategies for treating liver cancer are not yet established and require rigorous validation through large-scale, well-designed clinical trials. Although this review synthesizes compelling preclinical evidence, certain limitations warrant attention. First, most mechanistic insights are derived from animal models, which may not fully recapitulate the progression from human NAFLD/MASLD to HCC. Second, the heterogeneity of the GM across different populations (diet, genetics, and geographical factors) poses challenges for developing universal biomarkers or therapeutic regimens.

Future research directions should focus on systematically elucidating the dynamic changes and regulatory mechanisms of microbial metabolites across different disease stages (from NAFLD/MASLD and MASH to HCC), developing personalized intervention strategies, and evaluating the clinical efficacy and

long-term safety of prebiotics, probiotics, and FMT. Future multicenter clinical studies will be crucial for validating the diagnostic value of microbial metabolites and the efficacy of microbiota-modulating therapies. Integrating clinical data with the analysis of gut microbial metabolites will provide novel insights for the prevention and control of NAFLD/MASLD-HCC, ultimately reducing the incidence and mortality of liver cancer.

CONFLICTS OF INTEREST

Guarantor of the article: Zongyuan Che, MS.

Specific author contributions: Z.C.: made substantial contributions to conceptualization, writing of the original draft, and critical revision of the manuscript. W.X.: contributed equally to literature review, writing—review and editing, and participated in critical discussion of the study. X.Z.: participated in literature review and data collation/analysis. C.H.: assisted in writing the original draft and data collection. Y.T.: supervised the entire study, finalized the manuscript, and is responsible for all aspects of the work to ensure integrity and accuracy of the data and results.

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