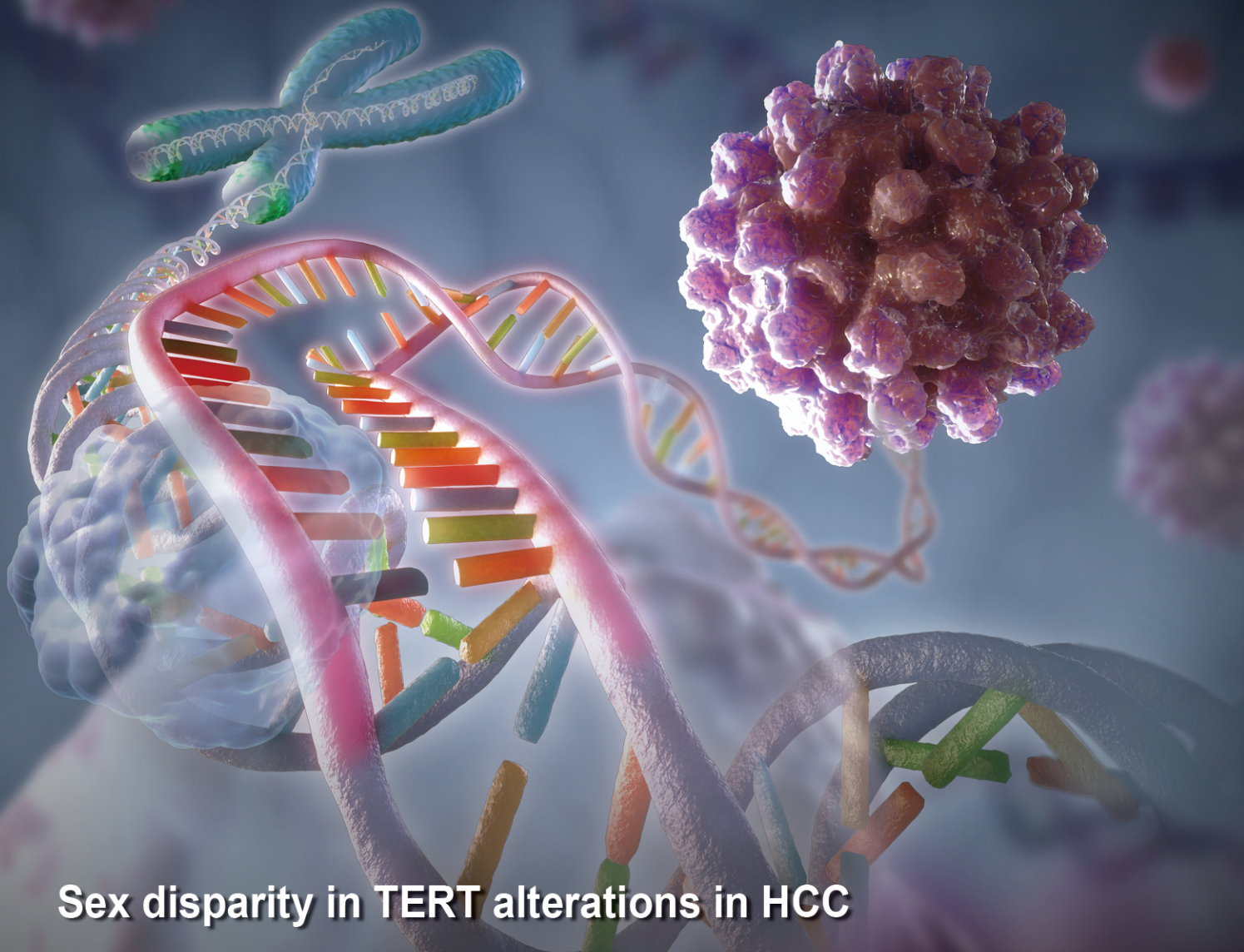


VOLUME 31 NUMBER 2 April 2025

pISSN 2287-2728
eISSN 2387-285X

CLINICAL and MOLECULAR HEPATOLOGY

The forum for latest knowledge of hepatobiliary diseases



Sex disparity in TERT alterations in HCC

Economic evaluation of hepatitis C elimination

GOLM1 promotes MASH-related gallstone formation

Molecular characterization of sarcomatoid HCC

Gut microbiota-mediated gut-liver axis: a breakthrough point for understanding and treating liver cancer

Chenyang Li^{1,2,3,*}, Chujun Cai^{4,5,*}, Chendong Wang^{1,2,3,*}, Xiaoping Chen^{1,2,3,6}, Bixiang Zhang^{1,2,3,6}, and Zhao Huang^{1,2,3}

¹Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology; ²Clinical Medical Research Center of Hepatic Surgery at Hubei Province; ³Hubei Key Laboratory of Hepato-Pancreatic-Biliary Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology; ⁴Department of Obstetrics and Gynecology, National Clinical Research Center for Obstetrics and Gynecology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology; ⁵Key Laboratory of Cancer Invasion and Metastasis (Ministry of Education), Hubei Key Laboratory of Tumor Invasion and Metastasis, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology; ⁶Key Laboratory of Organ Transplantation, Ministry of Education, Chinese Academy of Medical Sciences; NHC Key Laboratory of Organ Transplantation, Wuhan, China

The trillions of commensal microorganisms living in the gut lumen profoundly influence the physiology and pathophysiology of the liver through a unique gut-liver axis. Disruptions in the gut microbial communities, arising from environmental and genetic factors, can lead to altered microbial metabolism, impaired intestinal barrier and translocation of microbial components to the liver. These alterations collaboratively contribute to the pathogenesis of liver disease, and their continuous impact throughout the disease course plays a critical role in hepatocarcinogenesis. Persistent inflammatory responses, metabolic rearrangements and suppressed immunosurveillance induced by microbial products underlie the pro-carcinogenic mechanisms of gut microbiota. Meanwhile, intrahepatic microbiota derived from the gut also emerges as a novel player in the development and progression of liver cancer. In this review, we first discuss the causes of gut dysbiosis in liver disease, and then specify the pivotal role of gut microbiota in the malignant progression from chronic liver diseases to hepatobiliary cancers. We also delve into the cellular and molecular interactions between microbes and liver cancer microenvironment, aiming to decipher the underlying mechanism for the malignant transition processes. At last, we summarize the current progress in the clinical implications of gut microbiota for liver cancer, shedding light on microbiota-based strategies for liver cancer prevention, diagnosis and therapy. (*Clin Mol Hepatol* 2025;31:350-381)

Keywords: Gut microbiota; Liver cancer; Gut-liver axis; Intratumoral microbiota; Clinical translation

Corresponding author : Zhao Huang

Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan 430030, China
Tel: +86-27-83665293 34, Fax: +86-27-83663500-35, E-mail: huangzhao@tjh.tjmu.edu.cn
<https://orcid.org/0000-0002-1151-4942>

Bixiang Zhang

Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan 430030, China
Tel: +86-27-83665293 34, Fax: +86-27-83663500-35, E-mail: bixiangzhang@163.com
<https://orcid.org/0000-0002-1609-7260>

*These authors contribute equally.

Editor: Ki Tae Suk, Hallym University Medical Center, Korea

Received: Sep. 30, 2024 / Revised: Nov. 22, 2024 / Accepted: Dec. 6, 2024

INTRODUCTION

The concept of the 'gut-liver axis' refers to the reciprocal relationships between the gut and the liver, encompassing the anatomical proximity and functional interactions of these organs, as well as clinical relevance of pathogenesis. The gut microbiota represents a centerpiece in the bidirectional gut-liver communication – a healthy microbiome not only maintains homeostasis in the gut but also profoundly shapes hepatic physiology by delivering a myriad of metabolites through the portal vein.¹ The liver, in turn, modulates the microbiota by secreting bile acids and antimicrobial molecules directly into the intestine. Disruption of the balance on either side can set off a chain of healthy events in the gut-liver axis.

Hepatocarcinogenesis is a pathogenic process that typically evolves from chronic conditions. Cirrhosis, as a common end-stage of various chronic liver diseases (CLDs), represents the primary predisposing factor for the development of hepatocellular carcinoma (HCC).² Meanwhile, intrahepatic cholangiocarcinoma (iCCA) is closely linked to CLDs including viral hepatitis, alcohol consumption, and metabolic dysfunction-associated steatotic liver disease (MASLD).³ Primary sclerosing cholangitis (PSC), whose pathogenesis is known to be associated with intestinal disorders, is also a strong risk factor for CCA.³ It has been established that disturbance in gut microbial communities (dysbiosis), characterized by deterioration in microbial compositions and metabolic dysfunction, is associated with development and progression of liver diseases.^{4,5} Although mechanisms of pathogenesis vary among hepatic diseases, intestinal dysbiosis has been shown to promote hepatocarcinogenesis, which can be transmitted with fecal microbiota from CLD and HCC patients in preclinical models.⁶ Moreover, the microbial composition and function

also shift with HCC development, indicating certain pro-carcinogenic patterns of microbiome and metabolome.⁷ In this review, we focus on the role of gut dysbiosis and the resulting aberrant gut-liver axis in the progression from CLDs to liver cancer, elucidating the underlying mechanisms of cancer formation and highlighting clinical translation opportunities for liver cancer prevention, diagnosis and therapy.

DYSBIOSIS-MEDIATED ABERRANT GUT-LIVER AXIS IN LIVER DISEASES

Origin of gut dysbiosis in CLDs

Large population-based studies have shown that the composition of human gut microbiota varies among individuals and is predominantly influenced by environmental factors.⁸ Diet is considered a dominant determinant of inter-individual microbiome variation. Certain dietary patterns can modulate the composition and metabolism of gut microbiota, which in turn affect human health (Fig. 1A).⁹ Preclinical studies have demonstrated that a high fat diet (HFD) induced dysbiosis, which impacts the lipid metabolism in the liver and promotes the development of MASLD.¹⁰ An increase in LPS-producing bacteria, such as *Enterobacteriaceae* and *Desulfovibrionaceae* and a decrease in the production of short-chain fatty acid (SCFA)-producing bacteria including *Bacteroides*, *Ruminococcaceae* and *Lachnospiraceae* were commonly observed in HFD-induced MASLD mice.¹¹ Moreover, a western diet characterized by low-choline, high fat and high sugar leads to an increased abundance of *Blautia producta* and elevated level of its outcome metabolites 2-oleoylglycerol, thereby contributing to the path-

Abbreviations:

AhR, aryl hydrocarbon receptor; AIH, autoimmune hepatitis; AUC, area under the curve; BDL, bile duct ligation; CCA, cholangiocarcinoma; CCl₄, carbon tetrachloride; CDCA, chenodeoxycholic acid; CDHF, choline-deficient high-fat; CLDs, chronic liver diseases; CXCL16, C-X-C motif chemokine ligand 16; DCA, deoxycholic acid; DEN, diethylnitrosamine; DMBA, 7,12-dimethylbenz [a]anthracene; DSS, dextran sodium sulfate; EGFR, epithelial growth factor receptor; Elf4, E74-like ETS transcription factor 4; FASN, fatty acid synthase; FGF15, fibroblast growth factor 15; FXR, farnesoid X receptor; FMT, fecal microbiota transplantation; GVB, gut vascular barrier; HCC, hepatocellular carcinoma; HFD, high fat diet; HFHC, high fat and high cholesterol; HIF-1, hypoxia-inducible factor; HSCs, hepatic stellate cells; ICB, immune checkpoint blockade; ICD, inulin-containing diet; IFN γ , interferon- γ ; IL-22, interleukin-22; ILC3s, type 3 innate lymphoid cells; LCA, lithocholic acid; LPS, lipopolysaccharide; LSECs, liver sinusoidal endothelial cells; LTA, lipoteichoic acid; MASLD, metabolic dysfunction-associated steatotic liver disease; MDP, muramyl dipeptide; Mdr2, multidrug resistance protein 2; MDSCs, myeloid-derived suppressor cells; mTOR, mammalian target of rapamycin; MUP-uPA, major urinary protein-urokinase plasminogen activator; Myd88, myeloid differentiation primary-response gene 88; NEMO, NF- κ B essential modulator; NLRP6, NOD-like receptor family pyrin domain containing 6; NOD2, nucleotide-binding oligomerization domain 2; PAMPs, pathogen-associated molecular patterns; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PGE₂, prostaglandin E₂; PMN-MDSCs, polymorphonuclear myeloid-derived suppressor cells; PPI, pump inhibitors; PRR, pathogen recognition receptors; PSC, primary sclerosing cholangitis; PTGER4, prostaglandin E receptor 4; REG3G, regenerating islet-derived 3 gamma; RIP2, receptor-interacting-serine/threonine-protein kinase 2; SASP, senescence-associated secretory phenotype; SCFAs, short-chain fatty acids; SREBP1, sterol regulatory element-binding transcription factor 1; STZ, streptozotocin; TGR5, Takeda G-protein-coupled receptor 5; TLRs, Toll-like receptors; TNF, tumor necrosis factor; T_{reg}, regulatory T

ogenesis of metabolic dysfunction-associated steatohepatitis (MASH).¹² Others like high dietary fructose or cholesterol were also shown to alter the gut microbiota, whereby they were implicated in the development of MASLD and HCC.¹³⁻¹⁵ Alterations in several *Clostridium* species and disrupted bile acid metabolism were identified as key mediators in fructose-induced MASLD.¹³ In mice fed a high-fat/high-cholesterol diet, *Bifidobacterium* and *Bacteroides* were reduced, a finding also corroborated in hypercholesterolemia patients.¹⁵ Additionally, alcoholic consumption has a profound impact on gut microbiota, potentiating the pathogenesis of alcohol-associated liver diseases.¹⁶ Intestinal microbiota dysbiosis and bacterial overgrowth have been observed in both acute and chronic alcohol exposure.^{16,17} Loss of butyrate-producing bacteria belonging to *Lachnospiraceae*, *Ruminococcaceae*, and *Clostridiaceae* families, along with downregulation of butyrate synthesis pathways, has been

suggested as key pathogenic features of ethanol-induced dysbiosis.¹⁸ A recent study has corroborated that ethanol-induced gut microbiota alterations are a result of elevated acetate levels, rather than direct ethanol metabolism by gut microbiota.¹⁹ Collectively, despite the demonstration of diet's ability to remodel gut microbiota in preclinical animal models, the significance of this cause-and-effect relationship in human and the extent to which it contributes to the development and progression of liver disease remain to be investigated.

Antibiotics are known to have a dramatic impact on the gut microbiota communities due to their direct antibacterial activity. Long-term, low-dose penicillin exposure, particularly during early life, has been shown to exacerbate metabolic disturbances and hepatic steatosis in HFD-fed mice by inducing gut dysbiosis, characterized by a significant reduction in genera *Lactobacillus*, *Allobaculum*, *Rikenellaceae*,

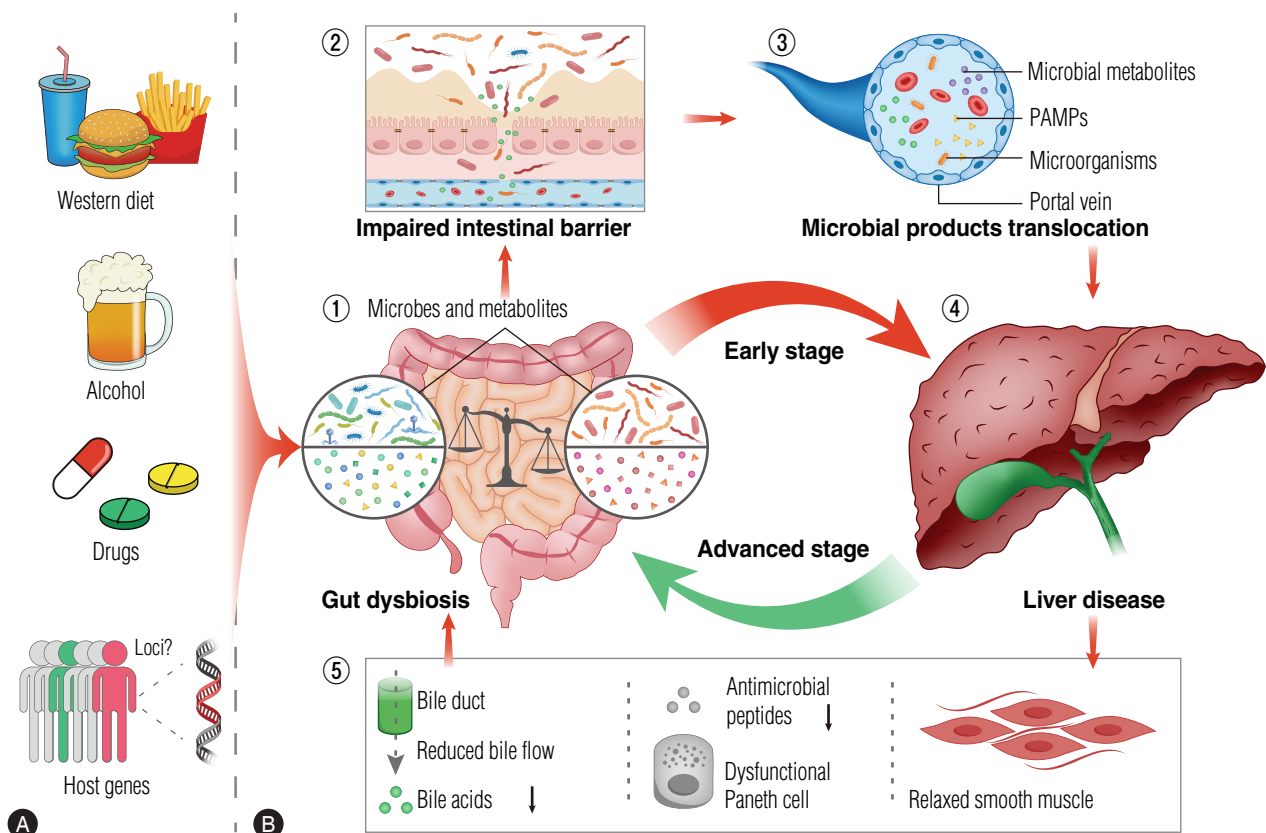


Figure 1. Origin and outcomes of gut dysbiosis in the gut-liver axis. (A) Environmental factors such as the Western diet, alcohol consumption, drugs, and genetic predispositions are primary contributors to gut dysbiosis in the context of liver diseases. (B) This dysbiosis disrupts microbial metabolism, leading to intestinal barrier dysfunction and subsequent translocation of microorganisms and microbial products to the liver. These events typically occur early in the pathogenic process.

and *Candidatus Arthromitus*.^{20,21} A human study also demonstrated that prior antibiotic use, including fluoroquinolones, cephalosporins, macrolides, penicillins and tetracyclines, was associated with an increased risk of future MASLD, with the highest risk linked to fluoroquinolones.²² Furthermore, antibiotic exposure around the time of treatment initiation was found to correlate with a worse prognosis in HCC patients receiving targeted or immune-based therapy, suggesting impaired treatment efficacy preceded by antibiotic-induced dysbiosis.²³ In addition to the effects of antibiotics, the long-term consumption of several non-antibiotic drugs like proton pump inhibitors (PPI), also influences the gut microbiome function and composition (Fig. 1A), consequently leading to hepatic inflammation in the liver.²⁴ Consumption of PPI can cause an oralization of the gut microbiome due to the reduction of gastric acidity.²⁴ It has been demonstrated that expansion of intestinal *Enterococcus faecalis* induced by PPI promoted progression of alcoholic liver disease (ALD) and MASLD in preclinical models.²⁵ Prospective cohort studies have also observed the positive correlation between use of PPI and readmission risk in patients with cirrhosis.²⁶

While host genetics are known to regulate the gut microbiome, as demonstrated in general population studies, there is currently a lack of research specifically investigating the influence of host genetics on gut microbiome in patients with liver diseases (Fig. 1A).^{27,28} Most of the present evidence comes from experimental mouse models. E74-like ETS transcription factor 4 (*Elf4*) was identified as a host protective gene that maintains the integrity of intestinal barrier and gut homeostasis. Ablation of *Elf4* in mice induced gut dysbiosis characterized by an increase in *Helicobacter* and *Escherichia* genera within the *Proteobacteria* phylum and exaggerated alcohol-induced hepatic steatosis and liver injury.²⁹ Another host gene, G protein-coupled receptor 35 (*Gpr35*), was also shown to modulate hepatic steatosis by regulating gut microbial ecology.³⁰ Expansion of pro-inflammatory bacteria from *Bacteroides* and *Ruminococcus* genera and reduction in probiotics such as *Akkermansia muciniphila* were observed in *Gpr35*^{KO} mice. Additionally, hepatocyte-specific deletion of myeloid differentiation primary-response gene 88 (*Myd88*) induced enrichment of specific bacterial genera including *Sutterella*, *Allobaculum*, *Ruminococcus*, and *Oscillospira* in mice fed with normal diet.³¹ This effect may be related to the alterations of hepatic bile acid metabolism induced by *Myd88* deficiency, suggesting the potential liver-

to-gut communication.³¹ In addition to protective genes, a gene encoding miRNA-21 was found to promote liver injury in cholestasis by modulating small intestinal *Lactobacillus* in mice.³²

Conversely, conditions such as liver cirrhosis-induced small bowel dysmotility, reduced bile acids and impaired intestinal immunity also contribute to gut dysbiosis, indicating a reciprocal effect in the advanced stages of liver disease.

Aberrant gut-liver communicating axis owing to dysbiosis

Among the intestinal microorganisms, the bacteria take the main responsibility for microbial metabolic processes, encompassing the transformation of bile acids, SCFAs via fermentation of indigestible carbohydrates, and the synthesis of essential amino acids.³³ These microbial metabolites serve as essential messengers and regulators of the gut-liver communicating axis, maintaining the gut homeostasis *in situ* and influencing the distal liver function after translocating to the liver through the portal vein.^{1,33} A direct consequence of disturbances in the microbial composition community is the alteration of microbiome-derived or microbiome-modified metabolites, as evidenced by fecal metabolomics studies in patients with various CLDs.³⁴⁻³⁸ Disruption of metabolic processes within the microbiome can impair the integrity of intestinal epithelial, immune and vascular barriers (Fig. 1B and Table 1), which is a prerequisite for microbiota-associated hepatic pathogenesis.³⁹ Unconjugated bile acids such as deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) have been shown to increase intestinal permeability via epithelial growth factor receptor activation,⁴⁰ while conjugated bile acids prevent the unconjugated bile acids-mediated intestinal and hepatic damage by forming micelles to sequester them.⁴¹ SCFAs like propionate and butyrate have been demonstrated to protect the integrity of intestinal mucus and epithelial barriers.⁴²⁻⁴⁴ Butyrate, in particular, enhances the tight junction of intestinal epithelial cells by promoting epithelial O₂ consumption and stabilizing hypoxia-inducible factor, a transcription factor that coordinates barrier protection.⁴³ Microbial tryptophan metabolites, especially indoles, which are reduced in patients with alcoholic hepatitis also play a crucial role in maintaining the intestinal immune and epithelial barrier by activating the aryl hydrocarbon receptor (AhR) signaling pathway.^{45,46} Indole-3-acetic

Table 1. Microbial metabolites involved in the gut-liver communicating axis during the pathogenesis of CLDs

Class	Metabolite	Effect	Mechanism	Pathway	Reference
Bile acids	Unconjugated bile acids	Impair gut epithelial barrier	Occludin dephosphorylation and tight junction rearrangement	Activation of EGFR/Src kinase pathway in intestinal epithelial cells	40
		Disrupt hepatic lipid metabolism	Suppress intestinal FXR activity	Inhibition of FXR-FGF15 signaling in enterocytes	48
	Conjugated bile acids	Protect gut epithelial barrier	Form micelles to sequester unconjugated bile acids	A signaling-independent and physicochemical way	41
	Secondary bile acids	Impair enterohepatic circulation and inhibit hepatic FXR activation by inducing ileitis	CD8 ⁺ T cell-mediated ileitis	Activation of TGR5/mTOR/oxidative phosphorylation signaling pathway in CD8 ⁺ T cells	49
SCFAs	Propionate	Protect gut mucus layer and epithelial barrier	-	-	42
	Butyrate	Alleviate alcohol-induced liver injury	Alleviate endoplasmic reticulum stress	Activation of major urinary protein 1 in hepatocytes	47
Tryptophan derivatives		Protect gut epithelial barrier	Enhance O ₂ consumption and stabilize HIF-1 by uncompetitively inhibiting HIF prolyl hydroxylases	-	43,44
	Indole	Alleviate hepatic steatosis and inflammation	Upregulate PFKFB3 expression and suppress pro-inflammatory activation in macrophage	Activation of AhR signaling pathway in macrophages	50
	Indole-3-acetic acid	Protect gut immune barrier	Upregulate intestinal IL-22 and REG3G expression	Activation of AhR signaling pathway in ILC3s	45,51
		Alleviate hepatic inflammation and cytokine-mediated lipogenesis	Reduce pro-inflammatory cytokines expression and migration of macrophages; downregulate FASN and SREBP-1c expression in hepatocytes	Activation of AhR signaling pathway in hepatocytes	52
	Indole-3-propionic acid	Protect gut epithelial barrier and alleviate hepatic inflammation	Upregulate expression of tight junction proteins and reduce production of pro-inflammatory cytokines in macrophages	-	46
	Indole-3-aldehyde	Trigger <i>Tet2</i> deficiency-associated AIH	Induce IFN γ -producing CD8 ⁺ T cell differentiation	Activation of AhR signaling pathway in CD8 ⁺ T cells	53
	Tryptamine	Alleviate hepatic inflammation	Reduce pro-inflammatory cytokines expression and migration of macrophages	-	52
Others	N,N,N-trimethyl-5-aminovaleric acid	Promote hepatic steatosis	Reduce carnitine synthesis by competitively inhibiting γ -butyrobetaine hydroxylase, and decrease fatty acid oxidation	-	54

AhR, aryl hydrocarbon receptor; AIH, autoimmune hepatitis; CLDs, chronic liver diseases; EGFR, epithelial growth factor receptor; FASN, fatty acid synthase; FGF15, fibroblast growth factor 15; FXR, farnesoid X receptor; HIF-1, hypoxia-inducible factor; IFN γ , interferon- γ ; IL-22, interleukin-22; ILC3s, type 3 innate lymphoid cells; mTOR, mammalian target of rapamycin; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; REG3G, regenerating islet-derived 3 gamma; SCFAs, short-chain fatty acids; SREBP1, sterol regulatory element-binding transcription factor 1; TGR5, Takeda G-protein-coupled receptor 5.

acid, for instance, can enhance the antibacterial function of the intestinal mucus layer by stimulating interleukin-22 (IL-22) production in type 3 innate lymphoid cells (ILC3s), thereby inducing the expression of antibacterial proteins such as regenerating islet-derived 3 gamma.⁴⁵ In addition to mucus layer and epithelial barrier, a diet induced-dysbiosis also leads to the disruption of gut vascular barrier (GVB) and the farnesoid X receptor (FXR) agonist obeticholic acid protects against the GVB damage by driving β -catenin activation in endothelial cells, although by which microbial metabolites this is mediated is still unascertained.³⁹

Beyond the intestine, specific microbial metabolites also directly influence hepatic lipid metabolism and inflammation through interacting with hepatocytes and macrophages after they are absorbed by the gut and transported to the liver (Table 1).⁴⁰⁻⁵⁴ Propionate can mitigate alcohol-induced liver injury by activating major urinary protein 1 and suppressing endoplasmic reticulum stress in hepatocytes.⁴⁷ Indoles and tryptamine inhibit proinflammatory activation and migration of macrophages through direct activation of the AhR signaling pathway. Additionally, N,N,N-trimethyl-5-aminovaleric acid, a microbial metabolite derived from trimethyllysine, has been shown to reduce carnitine synthesis and decrease fatty acid oxidation in hepatocytes, thereby promoting hepatic steatosis.

Gut barrier dysfunction facilitates the translocation of microorganisms and microbial products, such as lipopolysaccharide (LPS) and exotoxins, into the liver through the portal circulation, thereby triggering a continuous inflammatory response in the liver.⁵⁵ Human studies have demonstrated that patients with various liver diseases, including MASLD, ALD, cirrhosis, and PSC, present increased intestinal permeability and concomitant increase in endotoxin levels in both the serum and the liver.⁵ LPS were found to be more frequently present in the liver of patients with MASLD compared to those without MASLD, and these LPS particles predominantly localize in the portal tracts, suggesting an intestinal origin.⁵⁶ These microbial components can activate pathogen recognition receptors (PRR) such as Toll-like receptors (TLRs) on Kupffer cells in the liver, resulting in increased hepatic inflammation, hepatocyte damage and liver fibrosis.⁵⁷ Administration of dextran sodium sulfate, which is known to compromise the integrity of gut barrier, has been shown to facilitate hepatic tumorigenesis in both choline-deficient high-fat diet MASH mouse model⁵⁸ and

diethylnitrosamine (DEN)-induced HCC model.⁵⁹ Moreover, *E. faecalis*, that is abundant in human CLD and HCC feces, could increase gut permeability conferred by GelE, a metallopeptidase, and thereby promote the formation of HCC in collaboration with other bacteria in a TLR4-dependent manner.⁶

In addition to microbial products, the intrahepatic microbiota has recently received increasing attention, with respect to its physiologic roles and pathophysiologic contributions to CLDs.⁶⁰ The liver microbiome, which is suggested to originate from the gut, exhibits distinct characteristics from the gut microbiome, possibly as a result of the selective filtering by the gut-liver barrier.⁶¹ The bacterial signature within the liver has been identified in patients suffering from MASLD and showed diverse patterns associated with obesity.⁵⁶ Findings from the preclinical models have corroborated that translocated pathobionts such as *E. faecalis* on the premise of impaired gut barrier could exacerbate ALD via secretion of cytolysin that is toxic to hepatocytes, and/or activation of TLR2 on Kupffer cells.^{25,62}

It is extensively accepted that gut dysbiosis predisposes individuals to liver diseases. However, hepatic abnormalities can also have a reciprocal effect on the gut microbiome (Fig. 1), creating a cyclical relationship where it seems difficult to discern which factor comes first—much like the classic conundrum of the chicken and the egg. Indeed, diet or alcohol-induced gut dysbiosis can determine at least partially the individual's susceptibility to MASLD and ALD, which tends to be an early event in the pathogenic process.^{10,63,64} Nevertheless, as diseases progress to advanced stages like cirrhosis, they can in turn lead to changes in the microbiota composition due to reduced small bowel motility, abnormal bile acid levels and impaired intestinal immunity.⁶⁵ The biliary system serves as the central mediator of liver-to-gut communication by transporting bile, a solution rich in bile acids, immunoglobulin A (IgA) and other antimicrobial molecules, to the intestinal lumen. Liver cirrhosis is characterized by impaired bile transport and reduced bile acid levels in the gut, resulting from extensive destruction of liver tissue structure and hepatocyte dysfunction.⁶⁶ Bile acids, particularly unconjugated bile acids, can directly disrupt bacterial membranes due to their detergent properties.^{67,68} Notably, different gut microorganisms exhibit varying sensitivity to bile acid toxicity.⁶⁸ Gram-negative bacteria tend to be more resistant than Gram-positive bacteria, possibly due to their

differences in cell wall structure.⁶⁸ Some probiotic bacteria, such as *Lactobacillus* and *Bifidobacterium*, also show greater resistance, which may be linked to the activation of glycolysis.⁶⁸ In addition to their direct bactericidal effects, bile acids also stimulate the production of antimicrobial molecules such as angiogenin, inducible NO synthase and IL-18 in the intestine through activation of FXR.⁶⁹ These mechanisms may collaboratively contribute to the connection between disrupted bile acid metabolism and microbial dysbiosis in cirrhosis. Additionally, diminished production of antimicrobial peptides by Paneth cells, such as α -defensins 5 and 7, was found in experimental decompensated cirrhosis rats and contributed to intestinal bacterial overgrowth and translocation.⁷⁰ Others like IgA antibodies in the gut lumen were decreased in cirrhotic patients, suggesting a reduction of antimicrobial immunity.⁷¹

GUT DYSBIOSIS IN LIVER CARCINOGENESIS

Gut dysbiosis is suggested to preexist in a variety of precancerous liver diseases stemmed from diverse etiologies, including MASLD,^{34,72,73} ALD,^{64,74} viral hepatitis,^{36,75} and

autoimmune liver disease.^{37,76,77} In general, patients with CLDs exhibit a reduced gut microbial diversity, with a decrease in probiotics and overgrowth of detrimental bacteria that tend to be pathobionts.⁷⁸ Changes in the gut mycobiome and virome are also specified in several CLDs.⁷⁹⁻⁸² As we described above, this microbial dysbiosis leads to a chain of disturbances in the gut-liver axis, involving dysfunctional microbial metabolism, compromised intestinal barrier and translocation of microbial products, which make up the foundation for gut microbiota-associated hepatocarcinogenesis. In this part, we delve into the functional outcomes of gut dysbiosis and consequent aberrant gut-liver axis in liver cancer development, focusing on key microbes and metabolites identified through current clinical and experimental research.

Hepatocellular carcinoma

Clinical studies have delineated the dynamics of gut microbiota during the progression from CLDs to HCC, shedding light on potential anti-tumorigenic probiotics and protumorigenic pathobionts (Fig. 2 and Table 2).^{6,7,83-105} In MASLD patients with HCC, the stool microbiota exhibited an

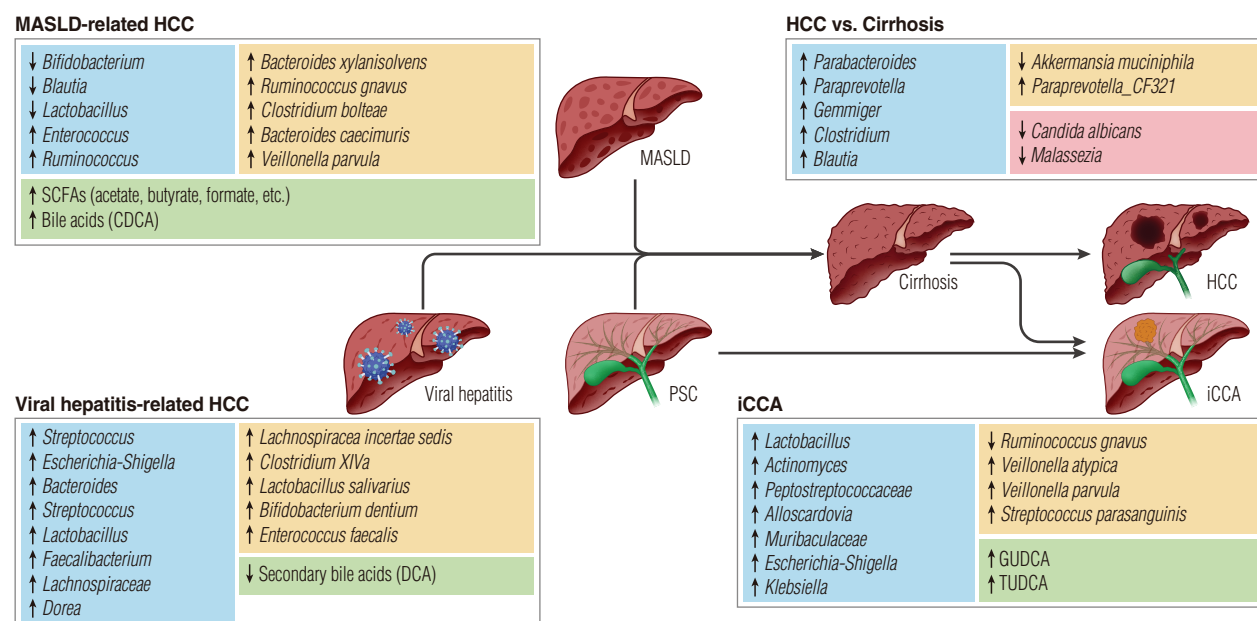


Figure 2. Gut microbiome evolves with liver carcinogenesis from different etiologies. Hepatocellular carcinoma developing from different etiologies exhibits diverse fecal microbiome profiles. Similarly, iCCA is associated with specific changes in gut bacteria and metabolites. Blue boxes represent bacterial genus, yellow boxes species, red boxes fungi and green boxes metabolites. CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GUDCA, glyoursodeoxycholic acid; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; MASLD, metabolic dysfunction-associated steatotic liver disease; PSC, primary sclerosing cholangitis; SCFAs, short-chain fatty acids; TUDCA, tauroursodeoxycholic acid.

increased abundance of SCFA-producing bacteria such as *Bacteroides caecimuris* and *Veillonella parvula*, and a corresponding functional enrichment in SCFA synthesis compared to those with MASLD-cirrhosis. In this study, the MASLD-HCC microbiota was confirmed to elicit an immunosuppressive phenotype in the peripheral blood from non-MASLD controls, which is specific to HCC rather than cirrhosis.⁷ In addition, an integrated analysis of gut microbiome and tumor transcriptome has revealed a correlation of intestinal microbiota dysbiosis with the tumor immune microenvironment and bile acids metabolism in patients with hepatitis B virus (HBV)-related HCC, indicating potential mechanistic relationships.⁸³ Another study has characterized the gut microbial profiles in patients with HCV-related HCC, revealing a notable rise in the abundance of *E. faecalis* and several species belonging to genera *Streptococcus* and *Lactobacillus*.⁶ This study further validated that the colonization of *E. faecalis*, linked to decreased intestinal concentrations of DCA, could promote the liver carcinogenesis in mice.⁶ Additionally, studies have also compared the differences in intestinal bacteria composition between viral and non-viral HCC, consistently uncovering an increase in *Faecalibacterium* in viral HCC.^{84,85} Numerous studies have examined the compositional shifts of gut microbiota during cirrhosis-associated hepatocarcinogenesis (Table 2). However, the taxonomic changes in gut microbiota were not aligned across these investigations, potentially due to the diverse etiologies, varying study designs, and differences in confounder controls. Notably, cirrhotic patients with HCC also exhibited a gut fungal dysbiosis characterized by an increase in opportunistic pathogenic fungi such as *Malassezia* sp. and *Candida albicans*.⁸⁶ Characterization of gut microbial profiles in prospectively recruited patients with cirrhosis has revealed a correlation between specific microbes and future HCC risk, such as *Alloprevotella*, whose reduction predicts a higher risk for developing HCC.⁸⁷ Intestinal permeability was also increased in patients with cirrhosis and HCC, as suggested by elevated levels of colonic tight junction protein zonulin-1 and microbial components LPS in the serum, as well as calprotectin, a surrogate marker of intestinal inflammation, in the feces.⁸⁸ Overall, these clinical studies substantiate the correlation between the aberrant gut-liver axis and the development of HCC, highlighting selective expansion or depletion of specific microorganisms during hepatocarci-

nogenesis.

Experimental studies have confirmed the casual relationship between pre-existing gut microbiota dysbiosis in CLDs and carcinogenesis within the liver. Transplantation of fecal microbiota from patients with HCV-related CLD or HCC, but not from healthy individuals, into microbiota-depleted mice could increase the intestinal permeability and promote tumorigenesis induced by DEN and carbon tetrachloride (CCl₄) administration.⁶ This carcinogenic effect was also verified in a streptozotocin and HFD-induced MASH mouse model.⁶ In addition, the pro-tumorigenic role of gut microbiota has been confirmed in other spontaneous HCC models, such as models induced by high fat and high cholesterol (HFHC) diet, combination of HFD with 7,12-dimethylbenz [a]anthracene (DMBA), and several genetically engineered mouse models (Table 3).^{15,57-59,106,109-114} The involvement of intestinal microbiota in hepatocarcinogenesis was initially observed in DEN and CCl₄-induced HCC mouse models, in which LPS derived from the intestinal microbiota collaborated with the chemical carcinogen-induced chronic liver injury to promote HCC formation by activating TLR4 pathway.⁵⁷ Gut sterilization efficiently prevents this malignant progression.⁵⁷ Furthermore, depletion of gut commensal microbiota using an antibiotic cocktail, consisting of vancomycin, neomycin and primaxin, also showed a protective effect on HCC development in MYC transgenic mice.¹⁰⁷ Mechanistically, gut microbiota was demonstrated to suppress liver antitumor immunity via modulating NKT cells accumulation through regulating primary-to-secondary bile acid conversion, in which *Clostridium* sp. were suggested to play key roles.¹⁰⁷ These results indicated that the gut microbiota, when considered as an integral unit, may constitutively shape the hepatic microenvironment and therefore amplify carcinogenic effects in combination with other carcinogens. Experimental studies also verified the hypothesis that the gut microbiota determines the susceptibility to HCC. It is demonstrated that a dysbiotic gut microbiota induced by an inulin-containing diet (ICD) predispose TLR5-deficient mice to HCC.¹⁰⁶ Moreover, supplementing this with a high fat diet, known for inducing dysbiosis, further exacerbated the ICD-induced formation of HCC. A dysregulated microbial metabolism of fermentable fiber, leading to the subsequent overproduction of SCFAs such as butyrate, was confirmed to mediate this process.¹⁰⁶ Vancomycin treatment prevented this carcinogenic effect by depleting the SCFAs and major

Table 2. Investigations into the gut microbiota composition and function in HCC and CCA patients

Etiologies	Study design and participants detail	Diversity	Taxonomic changes	Functional shifts	Reference
MASLD-related HCC					
MASLD-cirrhosis	- Cohort study of fecal microbiota and microbial metabolites in patients with MASLD - Patients with MASLD-HCC (n=32) - Patients with MASLD-cirrhosis (n=28) - Non-MASLD controls (n=30)	- HCC vs. controls - Decreased - HCC vs. cirrhosis - Not significant	- HCC vs. cirrhosis - Family: <i>Enterobacteriaceae</i> ↑ - Species: <i>Bacteroides caecimuris</i> ↑, <i>Veillonella parvula</i> ↑ - HCC vs. controls - Family: <i>Oscillospiraceae</i> ↓, <i>Erysipelotrichaceae</i> ↓ - Species: <i>Bacteroides xylanisolvens</i> ↑, <i>Ruminococcus gnavus</i> ↑, <i>Clostridium bolteae</i> ↑	The abundance of many bacterial genes involved in SCFA synthesis (<i>pycA</i> , <i>pta</i> , <i>ptb</i> , <i>frd</i> , <i>sucC</i>) was increased in MASLD-HCC patients, as well as elevated levels of SCFAs (acetate, butyrate and formate) in the faeces and serum.	7
MASLD-cirrhosis	- Cohort study of fecal microbiota in patients with MASLD-related cirrhosis - MASLD patients with cirrhosis and HCC (n=21) - MASLD patients with cirrhosis without HCC (n=20) - Healthy controls (n=20)	- HCC vs. cirrhosis - Not significant	- HCC vs. cirrhosis - Family: <i>Bacteroides</i> ↑, <i>Ruminococcaceae</i> ↑ - Genus: <i>Enterococcus</i> ↑, <i>Phascolarctobacterium</i> ↑, <i>Oscillospira</i> ↑, <i>Bifidobacterium</i> ↓, <i>Blautia</i> ↓	<i>Akkermansia</i> was positively correlated with fecal calprotectin. <i>Bacteroides</i> was associated with IL-8 and IL-13, activated circulating monocytes and MDSC.	88
MASLD	- Cohort study of fecal microbiota and serum bile acids in patients with MASH - Patients with MASH without cirrhosis (n=23) - Patients with MASH and cirrhosis (n=11) - Patients with MASH-HCC without cirrhosis (n=14) - Patients with MASH-HCC with cirrhosis (n=19)	- Non-cirrhotic HCC vs. HCC-cirrhosis - Increased	- Controls → MASH → HCC - Genus: <i>Bifidobacterium</i> ↑, <i>Lactobacillus</i> ↓ - MASH-HCC without cirrhosis vs. MASH-HCC with cirrhosis - Genus: <i>Ruminococcus</i> ↑	<i>Lactobacillus</i> was associated with serum bile acid levels.	89
Hepatitis virus-related HCC					
HBV	- Cohort study of fecal microbiota in patients with HBV infection and healthy controls - Patients with HBV-HCC (n=124) - Healthy controls (n=91) - Patients with HBV without cirrhosis (n=48) - Patients with HBV and cirrhosis (n=39)	Not mentioned	- HCC vs. controls - Phylum: <i>Proteobacteria</i> ↑ - Genus: <i>Streptococcus</i> ↑ - Between all groups <i>Streptococcus</i> and <i>Escherichia-Shigella</i> display an ascending trend as the disease progresses from HBV to HCC.	HCC vs. controls Increased amino acid metabolism	90

Table 2. Continued

Etiologies	Study design and participants detail	Microbial alterations and summary of results			Reference
		Diversity	Taxonomic changes	Functional shifts	
HBV	• Cohort study of fecal microbiota and host transcriptome in patients with HBV-related HCC • Patients with HBV-HCC (n=113) • Subgroup: Small HCC (n=36) vs. non-small HCC (n=77), non-cirrhotic HCC (n=22) vs. cirrhotic HCC (n=91) • Healthy controls (n=100)	• HCC vs. controls • Not significant • Small HCC vs. non-small HCC • Decreased	• HCC vs. controls • Genus: <i>Bacteroides</i> ↑ • Species: <i>Lachnospiraceae incertae sedis</i> ↑, <i>Clostridium XI</i> ↑ • Non-small HCC vs. small HCC • Genus: <i>Bacteroides</i> ↑, <i>Parabacteroides</i> ↑ • Species: <i>Lachnospiraceae incertae sedis</i> ↑, <i>Clostridium XI</i> ↑	The gut microbiota characterizing HBV-HCC was associated with tumor immune environment and bile acid metabolism.	83
	• Meta-analysis of public gut microbiome datasets for HBV-related liver diseases and a cohort study for validation • Meta-analysis: 139 controls, 133 chronic hepatitis B (CHB), 74 cirrhosis, 140 HCC • Validation cohort: 15 controls, 23 CHB, 20 cirrhosis, 22 HCC	• HCC vs. others • Decreased	• HCC vs. controls • Genus: <i>Lachnospiraceae_ND300</i> ↓, <i>Eubacterium ventriosum</i> ↓ • HCC vs. CHB • Genus: <i>Lachnospiraceae</i> ↓, <i>Dorea</i> ↓	-	91
HCV	• Analysis of fecal microbiota in patients with HCV-related chronic liver disease (HCV-CLD) • Patients with HCV without HCC (n=21) • Patients with HCV and HCC (n=23) • Healthy controls (n=24)	• HCC vs. controls • Increased • HCC vs. HCV-CLD • Increased	• HCC vs. controls • Species: 9 <i>Streptococcus</i> spp. ↑, 4 <i>Lactobacillus</i> spp. ↑, <i>Bifidobacterium dentium</i> ↑, <i>Enterococcus faecalis</i> ↑ • HCC vs. HCV-CLD • Species: 4 <i>Streptococcus</i> spp. ↑, <i>Lactobacillus salivarius</i> ↑, <i>Bifidobacterium pseudocatenulatum</i> ↑	HCC vs. HCV-CLD Increased amino acids metabolism and xenobiotics biodegradation	6
HCC developing from cirrhosis					
Cirrhosis	• Cohort study of fecal microbiota in cirrhotic patients with early HCC • Patients with HCC (n=150) • Patients with cirrhosis (n=40) • Healthy controls (n=131)	• HCC vs. cirrhosis • Increased	• HCC vs. cirrhosis • Phylum: <i>Actinobacteria</i> ↑ • Genus: <i>Gemmiger</i> ↑, <i>Parabacteroides</i> ↑, <i>Paraprevotella</i> ↑ • HCC vs. controls • Genus: <i>Verrucomicrobia</i> ↓, <i>Alistipes</i> ↓, <i>Ruminococcus</i> ↓, <i>Phascolarctobacterium</i> ↓, <i>Klebsiella</i> ↑, <i>Haemophilus</i> ↑	Butyrate-producing bacterial genera were decreased, while LPS-producing genera were increased.	92

Table 2. Continued

Etiologies	Study design and participants detail	Microbial alterations and summary of results			Reference
		Diversity	Taxonomic changes	Functional shifts	
Cirrhosis	• 2 Cohorts of fecal microbiota in male patients with cirrhosis: a prior HCC cohort and a future HCC cohort • Cirrhotic patients with prior HCC (n=38)/without prior HCC (n=38) • Cirrhotic patients with future HCC (n=33)/without future HCC (n=33)	• Not significant	• HCC vs. cirrhosis • Genus: <i>Clostridium sensu stricto</i> ↓, <i>Anaerotruncus</i> ↓, <i>Raoultella</i> ↑, <i>Haemophilus</i> ↑	Increased amino acid metabolism and toluene metabolism as well as decreased metabolism of urea cycle intermediates	93
Cirrhosis	• Cohort study of fecal fungi in cirrhotic patients • Patients with HCC and cirrhosis (n=34) • Patients with cirrhosis • Healthy controls (n=18)	• HCC vs. controls • Decreased • HCC-cirrhosis vs. cirrhosis • Not significant	• HCC vs. others • <i>Malassezia</i> ↑, <i>Malassezia</i> sp. ↑, <i>Candida</i> ↑, <i>Candida albicans</i> ↑	-	86
Cirrhosis	• Prospective cohort study of duodenal microbiota in patients with cirrhosis • Patients with cirrhosis (n=227) • Patients developing HCC during the follow-up period (n=14)	• Not significant	• HCC vs. cirrhosis • Family: <i>Bacillaceae</i> ↓, <i>Christensenellaceae</i> ↓, <i>Lactobacillaceae</i> ↓ • Genus: <i>Listeria</i> ↑, <i>Gemella</i> ↑, <i>Alloprevotella</i> ↑, <i>Anaerostipes</i> ↑	-	87
Cirrhosis	• Cohort study of fecal microbiota and diet in HCC patients • Patients with HCC and cirrhosis (n=30) • Patients with HCC without cirrhosis (n=38) • Healthy controls (n=27)	• HCC-cirrhosis vs. cirrhosis • Not significant • HCC vs. controls • Decreased	• HCC vs. controls • Butyrate-producing bacteria ↓ • HCC-cirrhosis vs. cirrhosis • Genus: <i>Clostridium</i> ↑ • Species: <i>Paraprevotella</i> CF321 ↑, <i>Akkermansia muciniphila</i> ↓	Consumption of artificial sweeteners was correlated with presence of <i>A. muciniphila</i> .	94
Cirrhosis	• Cohort study of fecal microbiota • Patients with HCC (n=75, 52 with cirrhosis and 23 without cirrhosis) • Patients with cirrhosis (n=24) • Healthy controls (n=20)	• HCC-cirrhosis vs. cirrhosis • Increased • Non-cirrhotic HCC vs. HCC- cirrhosis • Increased	• Non-cirrhotic HCC vs. others • Genus: <i>Intestinibacter</i> ↑, <i>Intestinimonas</i> ↑ • HCC-cirrhosis vs. others • Genus: <i>Blautia</i> ↑	-	95
Cirrhosis	• Cohort study of fecal microbiota in patients with cirrhosis • Cirrhotic patients with HCC (n=25) • Matched cirrhotic patients without HCC (n=25)	• HCC vs. non-HCC • Increased	• Family: <i>Bacteroidaceae</i> ↑, <i>Erysipelotrichaceae</i> ↑, <i>Prevotellaceae</i> ↓, <i>Leuconostocaceae</i> ↓ • Genus: <i>Fusobacterium</i> ↑, <i>Odoribacter</i> ↑, <i>Butyrivibrio</i> ↑, <i>Lachnospiraceae</i> ↓	Enrichment of NOD-like receptor pathways	96

Table 2. Continued

Etiologies	Study design and participants detail	Diversity	Taxonomic changes	Functional shifts	Reference
Heterogenous HCC					
Mixed	• Comparison of fecal microbiota between virus and non-virus-related HCC • Patients with HBV-HCC (n=35) • Patients with non-hepatitis virus related HCC (n=22) • Healthy controls (n=33)	• Higher in HBV-HCC	• Non-viral HCC vs. HBV-HCC • Genus: <i>Escherichia-Shigella</i> ↑, <i>Enterococcus</i> ↑, <i>Faecalibacterium</i> ↓, <i>Ruminococcus</i> ↓, <i>Ruminoclostridium</i> ↓	- Non-viral HCC vs. HBV-HCC - Reduced amino acid and glucose metabolism, high level of transport and secretion activity	84
Mixed	• Comparison of fecal microbiota between virus and non-virus-related HCC • Patients with virus-related HCC (n=33) • Patients with non-virus-related HCC (n=18) • Healthy controls (n=16)	• Higher in hepatitis virus-related HCC	• Viral HCC vs. others • Genus: <i>Faecalibacterium</i> ↑, <i>Agathobacter</i> ↑, <i>Coprococcus</i> ↑ • Non-viral HCC vs. others • Genus: <i>Bacteroides</i> ↑, <i>Streptococcus</i> ↑, <i>Ruminococcus gnavus</i> group ↑, <i>Parabacteroides</i> ↑, <i>Erysipelatoclostridium</i> ↑	- Non-viral HCC vs. viral HCC - Reduced short-chain fatty acid-producing bacteria and declined fecal butyrate level	85
General characterization of gut microbiome in HCC					
Mixed	• Cohort study of fecal microbiota and liver transcriptome • Patients with MASLD (n=21) • Patients with cirrhosis (n=27) • Patients with HCC (n=111)	• HCC vs. cirrhosis • Not significant • HCC vs. MASLD • Decreased	• HCC vs. MASLD • SCFAs-producing genera (<i>Blautia</i> and <i>Agathobacter</i>) ↓	Several host genes, such as MT1B, were associated with specific microbial genera when comparing HCC to cirrhosis and MASLD.	97
Mixed	• Cohort study of fecal microbiota in patients with primary liver cancer • Patients with HCC (n=143) • Healthy controls (n=40)	• Decreased	• Phylum: <i>Actinobacteria</i> ↓, <i>Firmicutes</i>, <i>Bacteroidetes</i> ↑ • Genus: <i>Faecalibacterium</i> ↓, <i>Lachnospiraceae</i> ↓, <i>Streptococcus</i> ↑, <i>Collinsella</i> ↑, <i>Akkermansia</i> ↑	-	98
Mixed	• Analysis of fecal microbiota • Patients with HCC (n=21) • Patients with CLD (n=11) • Healthy controls (n=9)	• Not mentioned	• HCC/CLD vs. controls • Phylum: <i>Firmicutes</i> ↓, <i>Proteobacteria</i> ↑ • Genus: <i>Blautia</i> ↓	-	99
Not mentioned	• Analysis of fecal microbiota in elderly patients with HCC • Patients with HCC (n=25) • Healthy controls (n=21)	• Decreased	• Genus: <i>Blautia</i> ↓, <i>Anaerostipes</i> ↓, <i>Fusicatenibacter</i> ↓, <i>Escherichia-Shigella</i> ↑, <i>Fusobacterium</i> ↑, <i>Megasphaera</i> ↑, <i>Veillonella</i> ↑	Reduced enrichment in metabolic process, such as amino acid metabolism	100
Not mentioned	• Analysis of fecal microbiota • Patients with HCC (n=21) • Healthy first-degree relatives (n=21)	• Not significant	• Genus: <i>Veillonella</i> ↑, <i>Lachnospiraceae</i> ↑, <i>Ruminococcaceae</i> UCG-014 ↑, <i>Peptostreptococcaceae</i> ↓, <i>Citrobacter</i> ↓, <i>Romboutsia</i> ↓	-	101

Table 2. Continued

Etiologies	Study design and participants detail	Diversity	Microbial alterations and summary of results		Reference
Intrahepatic CCA					
Not mentioned	• Cohort study of fecal microbiota in patients with ICC • Patients with ICC (n=28) • Patients with HCC (n=28) • Patients with cirrhosis (n=16) • Healthy controls (n=12)	• ICC vs. others • Increased	• ICC vs. others • Genus: <i>Lactobacillus</i> ↑, <i>Actinomyces</i> ↑, <i>Peptostreptococcaceae</i> ↑, <i>Alloscardovia</i> ↑	<i>Lactobacillus</i> and <i>Alloscardovia</i> were positively correlated with the plasma-stool ratio of tauroursodeoxycholic acid in ICC patients.	102
Not mentioned	• Cohort study of fecal microbiota in patients with primary liver cancer • Patients with ICC (n=19) • Patients with HCC (n=25) • Healthy controls (n=76)	• Not significant	• ICC vs. others • Species: <i>Veillonella atypica</i> ↑, <i>V. parvula</i> ↑, <i>Streptococcus parasanguinis</i> ↑, <i>Ruminococcus gnavus</i> ↓	The gut microbiota of patients with ICC displayed increased amino acid metabolism, nucleotide metabolism and glycolysis pathways.	103
Not mentioned	• Cohort study of fecal microbiota in patients with primary liver cancer • Patients with ICC (n=46) • Patients with HCC (n=143) • Healthy controls (n=40)	• ICC vs. controls • Not significant	• ICC vs. others • Phylum: <i>Firmicutes</i> ↓, <i>Bacteroidetes</i> ↑ • Genus: <i>Muribaculaceae</i> ↑, <i>Escherichia Shigella</i> ↑, <i>Klebsiella</i> ↑, <i>Megamonas</i> ↓	-	98
General characterization of gut microbiome in CCA					
Not mentioned	• Cohort study of fecal microbiota in patients with CCA • Patients with CCA (n=22) • Healthy controls (n=16)	• Not significant	• Phylum • <i>Firmicutes</i> ↓, <i>Actinobacteriota</i> ↓, <i>Proteobacteria</i> ↑, <i>Bacteroidetes</i> ↑ • Genus • <i>Bifidobacterium</i> ↓, <i>Klebsiella</i> ↑	-	104
Not mentioned	• Cohort study of fecal microbiota in patients with CCA • Patients with CCA (n=53) • Patients with cholelithiasis (n=47) • Healthy controls (n=40)	• CCA vs. controls • Not significant • CCA vs. cholelithiasis • Increased	• CCA vs. cholelithiasis • <i>Bacteroides</i> ↑, <i>Muribaculaceae</i> ↑, <i>Muribaculum</i> ↑, <i>Alistipes</i> ↑ • CCA vs. controls • <i>Faecalibacterium</i> ↓, <i>Burkholderia-Caballeronia-Paraburkholderia</i> ↓, <i>Ruminococcus</i> ↓	-	105

CCA, cholangiocarcinoma; CLDs, chronic liver diseases; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; IL-8, interleukin-8; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MDSCs, myeloid-derived suppressor cells; SCFAs, short-chain fatty acids; -, not available.

Table 3. Mouse models for HCC and CCA involving the gut microbiota

Models	Description	Liver disease	HCC/CCA development	Microbial alterations	Reference
HFHC diet	High fat and high cholesterol diet (HFHC, 43.7% fat, 36.6% carbohydrate, 19.7% protein, 0.203% cholesterol)	MASLD-HCC	14 mo	Gut dysbiosis and altered gut bacterial metabolites such as increased taurocholic acid and decreased 3-indolepropionic acid.	15
DEN	i.p. injection of DEN (40 mg/kg) weekly	Chemical carcinogens-induced HCC	14 wk	Gut dysbiosis characterized by decreased probiotics such as <i>Lactobacillus</i> and <i>Bifidobacterium</i> .	59
DEN+CCL ₄	i.p. injection of DEN (100 mg/kg) at ages 6-14 weeks followed by 6-12 biweekly i.p. injections of CCL ₄ 0.5 mL/kg in C3H mice	Chemical carcinogens-induced HCC	54 wk	Gut microbiota contributed hepatocarcinogenesis through LPS-induced TLR4 activation.	57
DEN+HFHC diet	Single injection i.p. DEN (25 mg/kg)+HFHC diet	MASLD-HCC	26 wk	Gut dysbiosis characterized by depletion of <i>Bifidobacterium pseudolongum</i> .	109
DEN+CDHF diet	Single injection i.p. DEN (25 mg/kg)+choline deficient and high fat diet (CDHF, 60 kcal% fat, no choline)	MASLD-HCC	28 wk	-	109
STAM	Single subcutaneous injection of 200 µg streptozotocin (STZ) at 4 days after birth+high fat diet at 4 weeks of age	MASH-HCC	16 wk	Gut dysbiosis characterized by reduction in <i>A. muciniphila</i> .	110
DSS+CDHF diet	Intermittent administration of 1% dextran sodium sulfate (DSS) in the drinking water+CDHF diet	MASH-HCC	12 wk	Gut dysbiosis	58
DMBA+HFD	Single application of 50 µl 0.5% DMBA (7,12-dimethylbenz [a]anthracene) in acetone+high fat diet (HFD, 60% fat, 20% protein, 20% carbohydrates)	Obesity-HCC	30 wk	Gut dysbiosis characterized by increased gram-positive bacteria such as <i>Clostridium</i> , which may result in an increase of DCA.	111
MUP-uPA mice+HFD	Overexpression of major urinary protein-urokinase plasminogen activator (MUP-uPA)+high fat diet	MASH-HCC	32 wk	Gut dysbiosis	112
Tlr5 KO+ICD	TLR5 deficient mice are fed with inulin-containing diet (ICD, 7.5% inulin and 2.5% cellulose)	Cholestatic HCC	6 mo	Gut dysbiosis characterized by increased fiber-fermenting bacteria and <i>proteobacteria</i> .	106
NEMO ^{Δhepa} /Nlrp6 ^{-/-} mice	Deletion of NF-κB essential modulator (NEMO) and NOD-like receptor family pyrin domain containing 6 (NLRP6)	MASH-HCC	52 wk	Gut dysbiosis characterized by reduction in <i>A. muciniphila</i> .	113
Hydrodynamic transfection+BDL	Hydrodynamic injection of plasmids encoding activated AKT and YAP+bile duct ligation (BDL)	PSC-CCA	3 wk	Gut dysbiosis	114
Hydrodynamic transfection+Mdr2 KO	Hydrodynamic injection of plasmids encoding activated AKT and YAP+deletion of multidrug resistance protein 2 (Mdr2)	PSC-CCA	3 wk	Gut dysbiosis	114

CCA, cholangiocarcinoma; CCL₄, carbon tetrachloride; CDHF, choline-deficient high-fat; DCA, deoxycholic acid; DEN, diethylnitrosamine; HCC, hepatocellular carcinoma; HFHC, high fat and high cholesterol; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; PSC, primary sclerosing cholangitis; -, not available.

secondary bile acid-producing bacteria.¹⁰⁸

As microbiome sequencing technologies advance, recent studies have been increasingly dedicated to precisely pinpointing specific microorganisms and metabolites that are crucial in either promoting or impeding liver carcinogenesis. The probiotics *A. muciniphila* and *Bifidobacterium pseudolongum*, whose reduction has been consistently observed in many human studies,^{36,77,88,95,115} were confirmed to protect against MASLD-HCC.^{109,110} *A. muciniphila* suppressed HCC formation in the STAM model by promoting the accumulation of NKT cells.¹¹⁰ Supplementation of *B. pseudolongum* restored a healthy gut microbiome composition, improved gut barrier function and prevented the development of HCC in a MASLD-HCC mouse model induced by DEN and HFHC diet.¹⁰⁹ Acetate generated from *B. pseudolongum* was identified as the functional mediator. It can not only restore gut homeostasis *in situ*, but also bind to GPR43 on hepatocytes via the gut-liver axis and inhibit the oncogenic IL-6/JAK1/STAT3 signaling pathway, thereby suppressing cell proliferation and inducing apoptosis.¹⁰⁹ Another SCFA, valeric acid generated by *Lactobacillus acidophilus*, also exhibits anti-tumorigenic effects through binding to GPR41/43 and inhibiting oncogenic Rho-GTPase signaling pathway.¹¹⁶ D-lactate, a microbiome small-molecule metabolite, has recently been identified to promote the transformation of M2 tumor-associated macrophages to M1 via inhibition of PI3K/Akt pathway and activation of NF- κ B pathway, and remodel immunosuppressive microenvironment for HCC.¹¹⁷ Additionally, gut microbiota-associated bile acids also profoundly affect the development of HCC. Primary bile acids such as CDCA and taurocholic acid facilitated NKT cells accumulation in the liver, while secondary bile acids lithocholic acid (LCA) or ω -muricholic acid showed an opposite effect, and therefore suppressed the hepatic antitumor immunity.¹⁰⁷ Moreover, increased taurocholic acid and depleted indole-3-propionic acid were found in excess dietary cholesterol-induced MASLD-HCC mouse model, functionally aggravating hepatic lipid accumulation and promoting hepatocyte proliferation.¹⁵ In a DMBA and HFD-induced obesity-associated HCC mice model, an increase in DCA resulted from gut dysbiosis provoked the senescence-associated secretory phenotype (SASP) in hepatic stellate cells (HSCs), which in turn secreted multiple tumor-promoting factors and eventuated in the promotion of HCC.¹¹¹

Cholangiocarcinoma

CCA is known to be associated with liver fluke infection and chronic biliary diseases such as choledocholithiasis and PSC.³ Liver cirrhosis, viral hepatitis, alcohol consumption and MASLD also represent strong risk factors for CCA, especially intrahepatic CCA. Studies on gut microbiota have provided new insights into the pathogenesis of CCA. Compositional alterations of gut microbiome have been observed in patients with CCA (Table 2). Changes of specific genera in CCA such as increase in *Muribaculacea*^{98,105} and *Klebsiella*,^{98,104} and decrease in *Ruminococcus*^{103,105} were concordantly seen in different studies. Examination of stool microbiota and serum bile acids in patients with intrahepatic CCA revealed a positive correlation between the genera *Lactobacillus* and *Alloscardovia* and the plasma-stool ratio of taurooursodeoxycholic acid, indicating potential interacting mechanisms.¹⁰² The gut leakiness preceded by microbial dysbiosis and subsequent translocation of microbial components and microorganisms was also shown to facilitate the development of CCA in a bile duct ligation-induced PSC mouse model.¹¹⁴ Although solid evidence of gut dysbiosis in CCA has been attained in clinical studies and the role of aberrant gut-liver axis in cholangiocarcinogenesis was also verified in preclinical models, the processes leading to gut dysbiosis, the distinctions among CCA subtypes, and the mechanisms underlying microbiota-associated malignant transformation of cholangiocytes still remain largely unknown and need to be further explored.

UNDERLYING MECHANISMS

Hepatocarcinogenesis typically arises from the interplay between chronic inflammation-induced persistent cell death and compensatory regeneration, which expedites oncogenic mutations in hepatic parenchymal cells.¹¹⁸ This oncogenic transformation in the liver is profoundly influenced by gut-liver interactions, where gut microbiota on the one hand constantly transmits tumor-promoting inflammatory signals through the leaky gut to the liver, and on the other hand modulates the metabolic and immunological environment through its metabolites (Fig. 3).

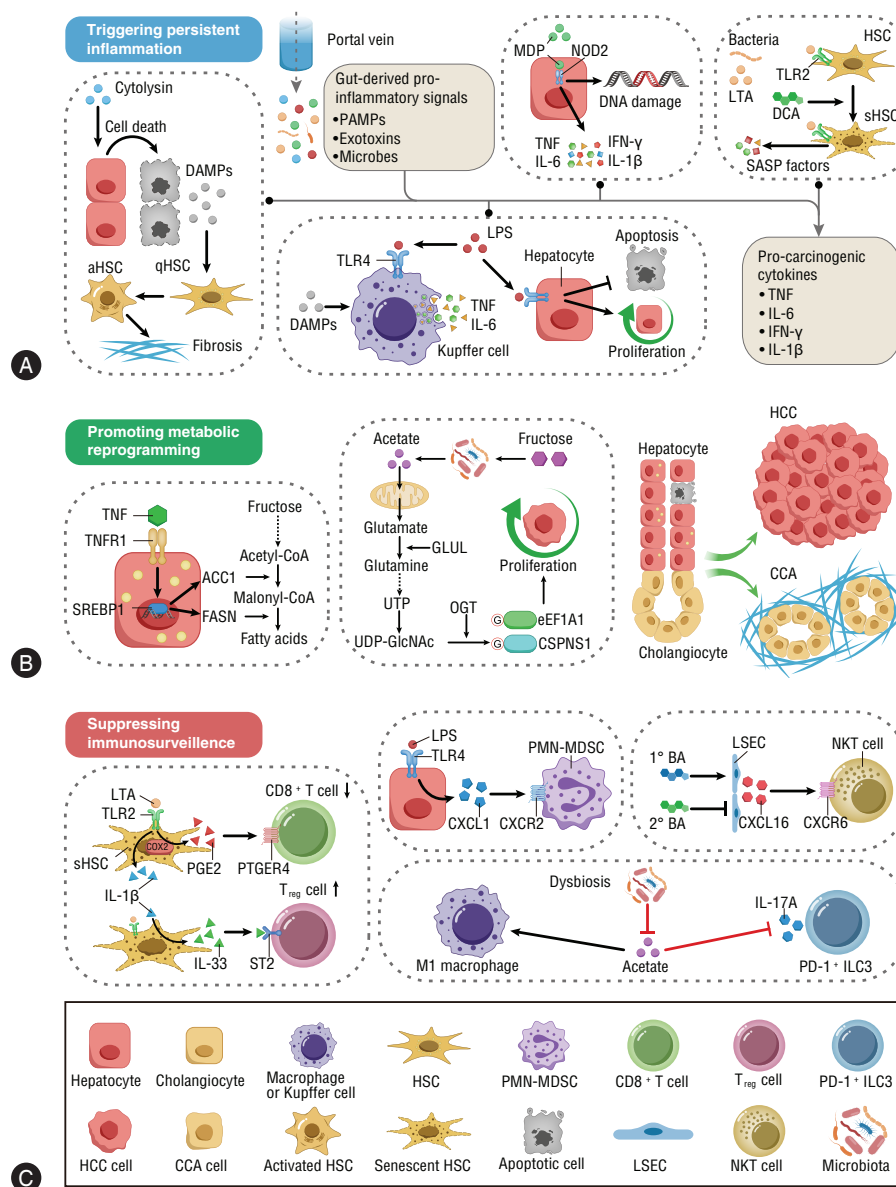


Figure 3. Underlying mechanisms by which aberrant gut-liver axis promotes hepatobiliary carcinogenesis. (A) As a result of gut leakiness, the liver is exposed to a large number of gut-derived inflammatory signals, including pathogen-associated molecular patterns (PAMPs), exotoxins and live microorganisms. Cytolysin directly causes hepatocyte death by inducing cell lysis and contributes to subsequent fibrosis. Lipopolysaccharide (LPS) stimulates Kupffer cells to produce pro-inflammatory cytokines and exerts pro-proliferative and anti-apoptotic effects on hepatocytes through Toll-like receptor 4 (TLR4). Muramyl dipeptide (MDP) induces DNA damage and secretion of inflammatory cytokines in hepatocytes by binding to nucleotide-binding oligomerization domain 2 (NOD2). Senescent hepatic stellate cells (HSCs) provoked by lipoteichoic acid (LTA) and deoxycholic acid (DCA) also exacerbate hepatic inflammation by releasing several senescence-associated secretory phenotype (SASP) factors. (B) Gut microbiota mediates the effects of high dietary fructose intake on pro-carcinogenic metabolic reprogramming. Tumor necrosis factor (TNF) upregulates the expression of key genes in lipid synthesis through the activation of TNF signaling, thereby promoting *de novo* lipogenesis. High fructose diet elevates the levels of microbiota-derived acetate, which promotes HCC cell proliferation by enhancing glutamine synthesis and O-GlcNAcylation of downstream proteins. (C) Gut dysbiosis induces an immunosuppressive microenvironment in the liver. Senescent HSCs suppress anti-tumor immunity by producing prostaglandin E2 (PGE2) and interleukin-33 (IL-33), which inhibit the expansion of cytotoxic CD8⁺ T cells and activate T_{reg} cells, respectively. Activation of LPS-TLR4 signaling in hepatocytes promote the recruitment of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs). Secondary bile acids inhibit hepatic NKT cells accumulation by downregulating the expression of C-X-C motif chemokine ligand 16 (CXCL16) in liver sinusoidal endothelial cells (LSECs). Gut dysbiosis also restrains immune activation and promotes immunosuppression by reducing acetate levels. CCA, cholangiocarcinoma; DAMPs, damage associated molecular patterns; HCC, hepatocellular carcinoma; IFN- γ , interferon- γ .

Triggering persistent inflammation

Under physiological conditions, the liver continuously receives moderate inflammatory stimuli from gut-derived microbial pathogens, achieving a balance between immune elimination and immunotolerance.¹¹⁹ However, prolonged exposure to pathogen-associated molecular patterns (PAMPs), microbial exotoxins, and live microorganisms as a result of a compromised intestinal barrier, disturbs this delicate immunological balance in the liver, leading to tissue damage and sustained inflammation that promotes carcinogenesis (Fig. 3A). Cytolysin, a microbial exotoxin secreted by *E. faecalis*, has been identified as a direct cause of hepatocyte death and liver injury during the progression of ALD.⁶² Furthermore, accumulation of PAMPs activates multiple pro-inflammatory signaling pathways via PRRs, resulting in the production of tumor-promoting cytokines and the malignant transformation of hepatic parenchymal cells. LPS, one of the most well-known PAMPs, was shown to activate TLR4 signaling in multiple hepatic cells, including Kupffer cells, HSCs, endothelial cells and hepatocytes.¹²⁰ It has been demonstrated that activation of LPS-TLR4 pathway in Kupffer cells enhanced the production of TNF and IL-6, which are known to be crucial mediators in hepatic carcinogenesis.^{121,122} Moreover, TLR4 activation also promotes hepatocytes proliferation by upregulating expression of epiregulin in HSCs and prevents cell apoptosis through NF- κ B signaling.⁵⁷ Another influential PAMP, LTA from Gram-positive bacteria, was shown to enhance the SASP of HSCs in collaboration with DCA-induced DNA damage through activation of TLR2.^{111,123} Consequentially, pro-inflammatory cytokines and chemokines such as IL-1 β , Gro α , and IL-6, termed SASP factors, were secreted by senescent HSCs and contributed to hepatocarcinogenesis.¹²³ In addition to TLR, nucleotide-binding oligomerization domain 2, an intracellular PRR that recognizes muramyl dipeptide from the gut bacteria, has also been demonstrated to promote hepatic tumorigenesis via receptor-interacting-serine/threonine-protein kinase 2-mediated pro-inflammatory response and nuclear autophagy-mediated DNA damage in hepatocytes.¹²⁴ Intriguingly, intestinal inflammatory cytokines owing to dysbiosis may also participate in the development of HCC. IL-25, generated from colonic epithelial tuft cells, was found to stimulate hepatic M2 macrophages polarization and secretion of CXCL10, which fostered the epithelial-to-mesen-

chymal transition of HCC cells.¹²⁵ Additionally, it is noteworthy that the intrahepatic colonization of bacteria such as *Stenotrophomonas maltophilia* also functions as an oncogenic factor by promoting hepatic fibrosis and senescent HSC-associated inflammation through activating TLR4.¹²⁶ However, the relationship between this process and bacterial translocation resulting from gut permeability was not investigated in this study.

Promoting metabolic reprogramming

Metabolic rearrangement is a critical feature during the development of liver cancers, particularly on a MASLD background, where metabolic dysfunction resulting from long-term dietary challenges leads to the sequential progression of steatosis, fibrosis, and ultimately HCC.^{127,128} Gut microbiota is involved in this process by promoting *de novo* lipogenesis and glutamine synthesis under stimulation of high dietary fructose (Fig. 3B).^{14,129} Excessive fructose intake causes gut dysbiosis and intestinal barrier deterioration in mice, resulting in the translocation of endotoxins such as LPS to the liver.¹²⁹ In addition to the aforementioned pro-inflammatory effects of LPS-TLR4 pathway, the production of TNF triggered by TLR4 signaling also induces *de novo* lipogenesis in hepatocytes.¹³⁰ Mechanistically, TNF signaling activates sterol regulatory element-binding transcription factor 1 (SREBP1), a key regulator of lipogenic genes, by upregulating the expression of caspase-2, which triggers proteolytic activation of SREBP1 by cleaving site 1 protease.¹³⁰ This *de novo* lipogenesis promoted by fructose-associated inflammation facilitated the progression from MASLD to MASH and the spontaneous development of HCC.¹²⁹ Furthermore, gut microbiota-mediated conversion from fructose to acetate has recently been demonstrated to promote HCC progression by influencing glutamine synthesis.¹⁴ Microbiota-derived acetate, as an additional carbon source, supports glutamine synthesis via the tricarboxylic acid cycle and subsequently upregulates uridine diphospho-N-acetylglucosamine levels through the hexosamine biosynthetic pathway, ultimately enhancing O-GlcNAcylation of downstream proteins and promoting HCC cell proliferation.¹⁴ Taking into consideration the intricate metabolic networks and numerous dysregulated metabolic pathways in liver cancers, investigations into the role of gut microbiota and microbial metabolites in reprogramming the tumor metabolic

microenvironment remain relatively limited. Future studies are anticipated to profile the relationship between the gut microbiota and the tumor metabolic microenvironment in the liver, as well as to unravel the underlying interactional mechanisms.

SUPPRESSING IMMUNOSURVEILLANCE

Another pivotal carcinogenic mechanism mediated by gut microbiota involves suppressing hepatic immunosurveillance that is primarily regulated by liver-infiltrating lymphocyte populations, including CD8⁺ T cells, NKT cells, regulatory T (T_{reg}) cells, macrophages and myeloid-derived suppressor cells (MDSCs) (Fig. 3C).¹¹⁸ As suggested in *Nlrp6*^{-/-} mice, characterized by intestinal dysbiosis and barrier impairment, dysbiotic microbiota can reshape the hepatic environment into an immunosuppressive state by inducing a TLR4-dependent expansion of monocytic MDSCs and reduction of CD8⁺ T cells.¹¹³ Moreover, aberrant gut-liver axis in PSC mice induced by bile duct ligation or knockout of *Mdr2* also causes hepatic accumulation of polymorphonuclear MDSCs, thereby promoting cholangiocarcinogenesis.¹¹⁴ Mechanistically, excessive exposure of hepatocytes to LPS induced the upregulation of CXCL1 expression in a TLR4-dependent manner, leading to the subsequent recruitment of CXCR2+ MDSCs.¹¹⁴ In addition, the aforementioned SASP phenotype of HSCs provoked by LTA and DCA also exerts suppressive effects on antitumor immunity.¹²³ Activation of LTA-TLR2 pathway upregulated COX2 expression and promoted COX2-mediated production of prostaglandin E2 (PGE2) in senescent HSCs. PGE2 bound to prostaglandin E receptor 4 (PTGER4) expressed on immune cells to attenuate antitumor immune responses. Blockade of PTGER4 effectively increased the number of CD103⁺ dendritic cells (DCs) while diminishing the level of T_{reg} cells and CD8⁺PD-1⁺ T cells.¹²³ Another SASP factor released from senescent HSCs, IL-33, has also been demonstrated to suppress antitumor immunity by activating T_{reg} cells through its receptor growth stimulation expressed gene 2.¹³¹ Furthermore, hepatic NKT cell accumulation was shown to be regulated by gut microbiota-mediated bile acid metabolism.¹⁰⁷ Primary bile acids could increase the accumulation of CXCR6⁺ NKT cells by regulating the expression of CXCL16 on liver sinusoidal endothelial cells, whereas secondary bile acids showed the opposite

effect. Therefore, depletion of secondary bile acids by antibiotics reversed NKT cells accumulation and antitumor immunosurveillance in the liver.¹⁰⁷ Notably, secondary bile acids, including ω -muricholic acid, 3 β -hydroxydeoxycholic acid, 3-oxoLCA and isoalloLCA, have been identified as significant promoters of T_{reg} expansion.¹³²⁻¹³⁴ 3 β -hydroxydeoxycholic acid enhances forkhead box protein P3 induction by suppressing the immunostimulatory properties of DCs as an antagonist of FXR.¹³² 3-oxoLCA inhibits T helper cell 17 differentiation by directly binding to retinoid-related orphan receptor- γ t, while isoalloLCA increased T_{reg} differentiation through the generation of mitochondrial reactive oxygen species.¹³³ Although this bile acid-mediated modulation of T_{reg} was first reported in inflammatory colitis,¹³⁴ the producers of these bile acids – such as *Proteobacteria* and *Bacteroidetes* – are also elevated in HCC patients, indicating a potential involvement of this regulation in microbe-mediated immunosuppression in liver cancer.^{98,99,135}

The gut commensal microbiota plays a fundamental role in governing the liver physiological immunity, not only maintaining the immune tolerance to innocuous antigens, but also enhancing the liver's immunogenic potential.^{55,61} The latter was supported by the dramatic reduction of hepatic immune cells following the elimination of gut and intrahepatic microbes through oral broad-spectrum antibiotic treatment with ampicillin, vancomycin, neomycin and metronidazole.⁶¹ As a consequence of disruptions in the gut-liver axis, this immune competence endowed by microbes seems to be disturbed by alterations in specific microbes and microbial metabolites, ultimately resulting in a weakened antitumor immunosurveillance. A recent study has demonstrated that SCFAs, particularly acetate generated by *Lactobacillus reuteri*, which were reduced in DEN and CCL-induced HCC mice, were able to inhibit tumor growth and enhance the effectiveness of anti-PD-1/PD-L1 therapy by reducing the production of immunosuppressive cytokines IL-17A in hepatic ILC3s and the infiltration of PD-1⁺ ILC3.¹³⁶ A decreased level of acetate and increased ILC3 infiltration were observed in HCC patients, suggesting a conserved mechanism.¹³⁶ Moreover, *Bacteroides thetaiotaomicron*-derived acetate was also found to modulate the polarization of M1 macrophages by increasing acetyl-CoA carboxylase 1-mediated fatty acid synthesis, thereby enhancing the antitumoral function of CD8⁺ T cells.¹³⁷ Correlation analysis showed that *B. thetaiotaomicron* was negatively associated

with HCC recurrence.¹³⁷

INTRAHEPATIC MICROBIOTA: GUT-DERIVED TUMOR COMPONENTS

In recent years, with the development of next-generation sequencing technology, intratumoral microbiota have been revealed as a novel component of the tumor microenvironment.¹³⁸ Intriguingly, besides tumors spatially adjacent to microorganism-inhabited mucosa, like colorectal, lung, and cervical cancers, intratumoral bacteria and fungi have also been discovered in organs once believed to be sterile, such as the breast and liver.^{139,140} Although these microbes exist in low biomass within the tumor microenvironment, they signi-

ficantly regulate the behavior of both tumor cells and tumor-infiltrating stroma cells, as evidenced by studies in breast, gastrointestinal and lung cancers.¹⁴¹⁻¹⁴³ The liver occupies a unique anatomical position, receiving blood directly from the portal vein which carries nutrients and antigens from the intestine. As we described above, the aberrant gut-liver axis in precancerous conditions exposes the liver to increased live microorganisms. Hence, it is postulated that the intrahepatic microbiota is derived from the gut and evolves alongside changes in the intestinal flora. This hypothesis was supported by a recent study, in which fecal microbial transplantation to germ-free mice recapitulated the liver microbiome of the naive specific pathogen-free mice.⁶¹ Moreover, this liver microbiome was found to vary with age, sex and feeding environment in mice.⁶¹ Perturbation of gut microbiome by di-

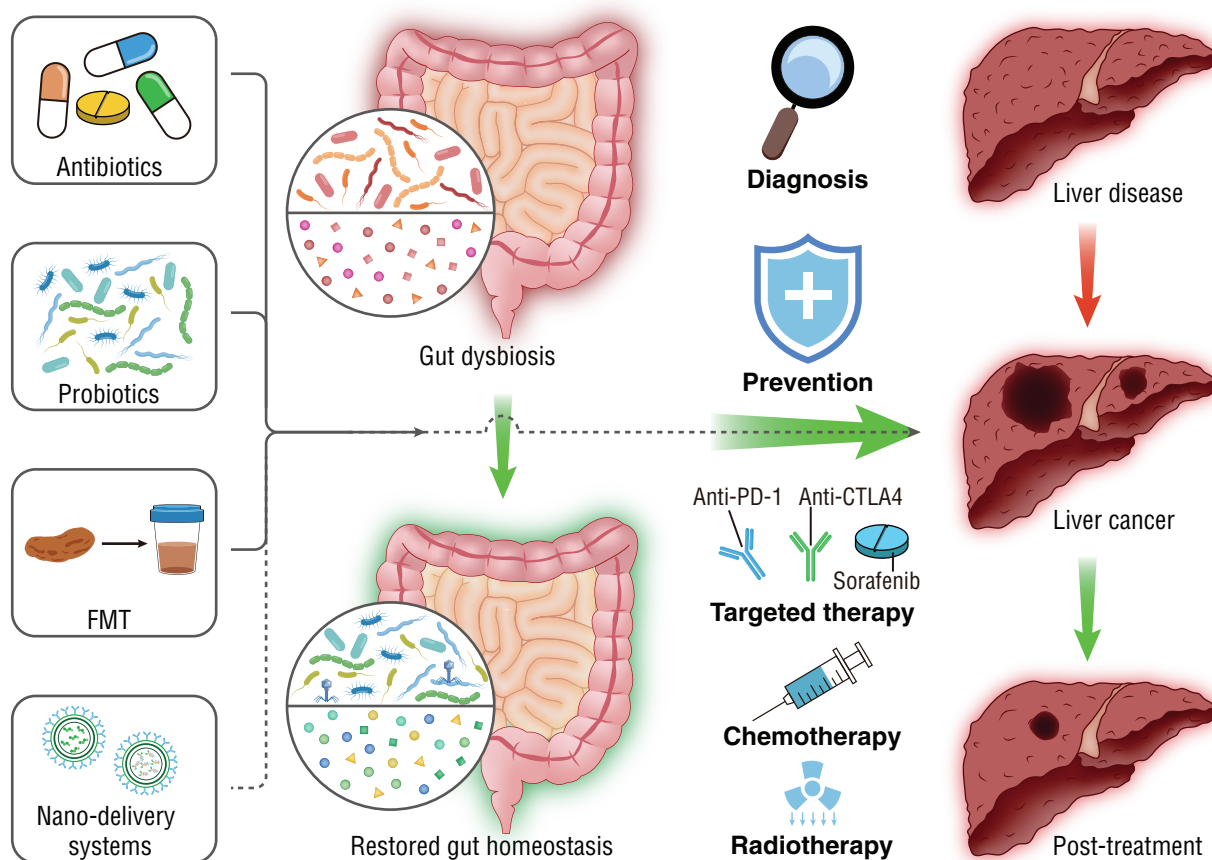


Figure 4. Clinical implications of gut microbiota in liver cancer prevention, diagnosis and therapy. The gut microbiome can serve as a non-invasive biomarker for the diagnosis of liver cancer. Gut microbiota-based therapeutic strategies, such as antibiotics, probiotics, and fecal microbiota transplantation (FMT), have the potential to prevent the malignant progression of liver cancer from benign liver diseases and enhance the efficacy of liver cancer treatments, including targeted therapy, chemotherapy, and radiotherapy, by restoring gut homeostasis. A novel approach, the nano-delivery system, can transport gut microbial metabolites to tumor sites, improving both efficacy and safety.

fferent combinations of oral antibiotics also reprogramed the hepatic bacterial communities.⁶¹

As the immunosuppressive and hypoxic microenvironment of tumors can influence microbial colonization, the intra-

Table 4. Clinical trials of microbiota-based strategies for liver cancer prevention and therapy

Interventions	Disease and number of participants (n)	Phase	Outcomes	Reference
Stool collection for testing the gut microbiome profiles and other detection of circulating micro-RNA, as well as metabolome in urine and plasma	Cirrhosis (750) Chronic hepatitis B (930) Chronic hepatitis C (20) MASLD/MASH (300)	Not applicable	Recruitment still ongoing	NCT05148572 NCT04965259
Probiotic cocktail (<i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i> , <i>Streptococcus faecalis</i> and <i>Bifidobacterium brevis</i>)	Cirrhosis (280)	Not applicable	Not yet recruiting	NCT03853928
Probiotic capsules (<i>Bifidobacterium</i> , <i>Lactobacillus</i> and <i>Enterococcus</i>) after hepatectomy	Hepatocellular carcinoma (180)	Not applicable	Bifidobacterium-rich probiotic treatment reduced the rates of delayed recovery, shortened hospital stays, and improved overall 1-year survival.	NCT04303286 NCT05178524174
Nivolumab, tadalafil and oral vancomycin	Hepatocellular carcinoma (6) Liver metastases (16)	II	The combination therapy demonstrated minimal efficacy in patients with refractory HCC.	NCT03785210
Carralizumab and apatinib mesylate only or plus probiotics tablets (<i>Bifidobacterium</i> , <i>Lactobacillus</i> and <i>Streptococcus thermophilus</i>)	Hepatocellular carcinoma (30)	I/II	Recruitment still ongoing	NCT05620004
Atezolizumab and bevacizumab combined with EXL01, a pharmacological preparation of <i>Faecalibacterium prausnitzii</i>	Hepatocellular carcinoma (34)	II	Not yet recruiting	NCT06551272
Oral enterobacterial capsules combined with immune checkpoint inhibitors and anti-angiogenesis targeted agents	Hepatocellular carcinoma progressed after treating with immune checkpoint inhibitors in combination with anti-angiogenesis targeted agents (30)	II	Not yet recruiting	NCT06563947
Standard of care immunotherapy (atezolizumab and bevacizumab) only or plus FMT via capsule	Hepatocellular carcinoma (48)	II	Not yet recruiting	NCT05690048
FMT combined with triple therapy consisting of transarterial chemoembolization, lenvatinib and sintilimab	Hepatocellular carcinoma progressed despite the triple therapy (15)	II	Not yet recruiting	NCT06643533
FMT combined with atezolizumab and bevacizumab	Hepatocellular carcinoma failed to achieve a complete or partial response to atezolizumab plus bevacizumab (12)	Ila	Recruitment still ongoing	NCT05750030

FMT, fecal microbiota transplantation; HCC, hepatocellular carcinoma.

tumoral microbiome of liver cancers may undergo further evolution in both overall abundance and spatial distribution during hepatocarcinogenesis.¹⁴⁴ Current studies have already profiled the intratumoral microbiome in HCC and CCA, and found its correlation with the clinical features and prognosis of patients.¹⁴⁵⁻¹⁴⁷ The intratumoral microbiome of HCC was suggested to be different from adjacent nontumor tissues and highly heterogeneous among individuals.^{145,148,149} Higher bacterial abundance in HBV-related HCC patients was associated with increased infiltration of M2 macrophages and MDSCs, indicating a potential role of these bacteria in modulating the immune microenvironment.^{149,150} In HCC mouse model, a specific correlation between several intratumoral bacteria and tumor metabolic distortion was detected, offering insights into the interacting mechanisms.¹⁵¹ In iCCA, *Paraburkholderia fungorum* was found to be significantly reduced in tumor tissues compared to paracancerous regions.¹⁴⁶ *In vitro* and *in vivo* experiments confirmed that *P. fungorum* exerted antitumor effects by inhibiting tumor cells proliferation and migration.¹⁴⁶ Abnormalities in alanine, aspartate and glutamate metabolism revealed by metabolomics and transcriptomics analysis of tumors may underlie the mechanisms.¹⁴⁶ Currently, the majority of the studies on the intratumoral microbiota in liver cancers primarily focus on correlation analysis of its signatures. Exploring the causal relationship between the source of these microbes and the tumor's immunological and metabolic microenvironment, as well as their biological effects on tumor development and progression, holds great promise for future research.

CLINICAL IMPLICATIONS FOR THE PREVENTION, DIAGNOSIS AND THERAPY OF LIVER CANCER

Given the encouraging results from numerous preclinical studies showing its efficacy in halting the progression of CLDs and preventing hepatobiliary carcinogenesis in murine models, targeting the gut microbiota-mediated gut-liver axis represents a promising translational opportunities for clinical prevention of liver cancers in addition to treating the primary liver diseases (Fig. 4). Gut microbiota also holds potential as a non-invasive biomarkers for early diagnosis and prognosis of liver cancer, with valuable gut microbiome-based signatures being gradually identified through multiple human

studies.^{92,98,152} Of note, recent studies have expanded their attention to applying gut microbiota for liver cancer therapy, particularly when combined with immunotherapy (Fig. 4), as gut microbiota has been demonstrated to modulate tumor immune microenvironment in experimental animals and clinically correlate with response to immune checkpoint blockade (ICB) therapy.^{153,154} A variety of microbiota-based therapeutic approaches such as antibiotics, probiotics and fecal microbiota transplantation (FMT) have been detailed elsewhere;^{120,155} therefore, here, we mainly discuss the current clinical translation status and recent advancements concerning the gut microbiota-liver axis in liver cancer prevention, diagnosis and therapy.

Targeting the gut microbiota for liver cancer prevention

On the basis of ample preclinical evidence and the emergence of innovative therapeutic strategies, significant advancements have been made in the clinical translation of manipulating the gut microbiota for treating liver diseases, particularly MASLD and cirrhosis.⁷⁸ Therapeutic approaches including probiotic supplementation, rifaximin, and FMT from healthy donors have shown success in ameliorating hepatic steatosis in MASLD and reducing complications and hospitalizations among cirrhotic patients, as demonstrated by numerous human clinical trials.⁷⁸ While direct clinical trials investigating the preventive effects of microbiota-based therapies on the development of HCC are yet to be completed (Table 4), improvements in microbial composition, intestinal permeability, and anti-inflammatory effects, all pivotal factors in the malignant progression of CLDs, have been observed in many clinical studies. For example, rifaximin, a non-absorbable broad-spectrum antibiotic, was shown to reduce circulating neutrophil TLR4 expression and plasma TNF- α levels in the RIFSYS trial.¹⁵⁶ Concurrently, it fostered an intestinal environment resistant to pathobionts and conducive to gut barrier repair, as indicated by elevated fecal levels of TNF- α and IL-17. Moreover, rifaximin suppressed oralization of the gut microbiome by reducing mucin-degrading species rich in sialidase, such as *Streptococcus* spp., *Veillonella atypica* and *V. parvula*.¹⁵⁶ In addition, probiotic supplementation has also exhibited moderate efficacy in gut-derived inflammation and intestinal permeability. Treatment with probiotic *Lactobacillus casei* Shirota reduced plasma

IL-1 β in alcoholic cirrhosis and IL-17A in non-alcoholic cirrhosis.¹⁵⁷ Another clinical trial revealed that a probiotic blend containing six *Lactobacillus* and *Bifidobacterium* species helped sustain the expression of CD8⁺ T cells and zonulin-1 in the duodenal mucosa of MASLD patients.¹⁵⁸ Surprisingly, a retrospective study that included 1,267 patients with HBV-related cirrhosis found that probiotic treatment was associated with a lower risk of HCC, exhibiting a dose-response pattern.¹⁵⁹ In addition to gut microbiota, targeting gut-liver barrier may also offer a promising approach to prevent HCC development. Non-selective β -blockers, utilized for preventing variceal bleeding and managing portal hypertension in cirrhotic patients, can additionally reduce intestinal transit time and mitigate gut bacterial overgrowth, intestinal permeability, and bacterial translocation.¹⁶⁰ A recent meta-analysis has revealed that nadolol and carvedilol could decrease the risk of HCC in cirrhotic patients by 26% and 38%, respectively.¹⁶¹ Others like bile acids, an important regulator of the intestinal barrier through activation of FXR, have been demonstrated to prevent the progression of MASLD and PSC in multiple clinical trials.^{78,162} Nevertheless, the preventive effect of FXR agonist on HCC has yet to be directly verified in human studies.

Monitoring the gut microbiota for liver cancer diagnosis and prognosis

The feasibility of using gut microbiome profiling as a diagnostic tool for early screening of HCC has been validated by a cross-regional population study, in which the prediction model consisting of 30 microbial markers achieved an area under the curve (AUC) of 80.64% between 75 early HCC and 105 non-HCC samples.⁹² Other studies have further strengthened this diagnostic potential in HCC and extended its application to CCA, demonstrating superior diagnostic performance of gut microbiota-based signatures compared to the classic markers alpha-fetoprotein and carbohydrate antigen 19-9.^{98,105,152} Fungal biomarkers such as increased abundance of *C. albicans* and depletion of *Saccharomyces cerevisiae* have also been associated with the progression from cirrhosis to HCC.¹⁶³

In addition to its diagnostic role, the gut microbiome also shows predictive potential for the progression and prognosis of HCC. Integrating *Veillonella* and *Streptococcus pneumoniae* with TNM stages and aspartate transaminase levels

achieved an AUC of 78.0% for predicting early recurrence of HCC.¹⁶⁴ Another study identified six microbial markers through integrated analysis of the microbiome and host transcriptome, demonstrating their potential to predict clinical outcomes in HCC patients, with an AUC of 81%.⁸³ A recent study has also validated the predictive ability of a specific gut microbiome signature, consisting of nine microbial markers, for microvascular invasion in HCC patients, achieving an AUC of 79.80%.¹⁶⁵ Nonetheless, the current diagnostic and prognosis models based on selected gut microbial markers are limited to HBV-related HCC and are restricted to the genus-level analysis of gut microbiome. Future studies involving HCC patients with diverse etiologies, along with more in-depth microbiome profiling, are essential for advancing this field.

Targeting the gut microbiota for liver cancer therapy

As we described above, specific microbes and their metabolites play an important role in modulating hepatic immunity, with some enhancing immune competence while others dampening immunosurveillance. An immunosuppressive tumor environment can thus be attributed to the disturbance of homeostatic gut microbiota. Multiple cohort studies have confirmed the association between the gut microbiome and the efficacy of immune checkpoint inhibitors, including tremelimumab,¹⁶⁶ durvalumab,¹⁶⁶ nivolumab,^{167,168} camrelizumab,¹⁵³ and pembrolizumab,¹⁶⁸ in HCC patients, identifying specific bacterial species and microbial metabolites that may be either beneficial or detrimental.^{154,169} Reported probiotics, including *A. muciniphila*,^{153,166,167} species of *Bifidobacterium*,^{153,166} *Ruminococcus*,^{153,169} and *Faecalibacterium*,^{154,168,169} were enriched in patients with superior treatment responses, while higher abundance of *Enterobacteriaceae*,^{166,168} *Bacteroides*^{153,167} was associated with poorer treatment outcomes. Additionally, a study investigating gut microbial metabolites revealed that the microbial metabolites ursodeoxycholic acid and ursolic acid were more abundant in patients with objective responses.¹⁶⁸ These studies highlight the potential of manipulating the gut microbiota to enhance the efficacy of liver cancer immunotherapy. Several phase I/II clinical trials have already been initiated to evaluate the combined efficacy of ICB therapy and microbiota-based treatments, including vancomycin, probiotics, and FMT (Table 4). While one

completed trial combining vancomycin with anti-PD-1 therapy showed no significant efficacy in 6 patients with refractory HCC (NCT03785210), the outcomes of other trials, involving larger cohorts, are still pending.

In addition to immunotherapy, the gut microbiome may also influence the efficacy of other liver cancer treatments, including the tyrosine kinase inhibitors, chemotherapy and radiotherapy.¹⁷⁰⁻¹⁷² A microbiota-derived protein, *Staphylococcal* superantigen-like protein 6, was found to sensitize HCC cells to sorafenib-induced apoptosis by blocking CD47 and downregulating PI3K/Akt-mediated glycolysis.¹⁷⁰ Moreover, the microbial metabolite butyrate can directly inhibit the proliferation and migration of HCC cells by disrupting intracellular calcium homeostasis, and synergistically improve the efficacy of sorafenib in preclinical models.¹⁷³ In HCC patients undergoing chemotherapy, depletion of intestinal anaerobic bacteria, such as *Blautia*, due to the overuse of antibiotics targeting anaerobes, was associated with poor prognosis.¹⁷¹ The gut microbiome has also been associated with the response to radiotherapy in HCC patients, with *Faecalibacterium* identified as being enriched in responders.¹⁷² Mechanistically, gut dysbiosis impaired radiotherapy-associated antitumor immune responses by suppressing stimulator of interferon genes (STING) signaling pathway.¹⁷² Cyclic-di-AMP, a bacterium-derived molecule that is elevated in the stool of responders, may enhance the efficacy of radiotherapy as a STING agonist.¹⁷²

It is worth mentioning that encouraging success has been made in promoting liver function recovery after hepatectomy in HCC patients using a probiotic bacteria cocktail containing *Bifidobacterium longum*.¹⁷⁴ These benefits may derive from diminished liver inflammation, reduced liver fibrosis and enhanced hepatocyte regeneration facilitated by several microbial metabolites such as 5-hydroxytryptamine, secondary bile acids and SCFAs, as indicated in mouse experiments.¹⁷⁴ Although this study concentrated solely on postoperative liver recovery of HCC patients, its implications in improving liver function hold pivotal extending potential in various liver diseases, signifying substantial progress in clinical translation of microbiota-based strategies.

Notably, in addition to antibiotics, probiotics and FMT, novel microbiota-based therapeutic strategies have been developed for liver cancer therapy in recent years. A recent study reported a nanoparticle/engineered bacteria-based triple-strategy delivery system for HCC therapy.¹⁷⁵ This

system utilized non-pathogenic *Escherichia coli* with a synchronized lysis circuit as both an immunostimulatory agent, and a source of hepatitis B surface antigen upon bacterial lysis.¹⁷⁵ Anchored with a nanoparticle loaded with doxorubicin and plasmid encoded human sulfatase 1, the delivery system effectively stimulated the anti-tumor immune responses and suppressed tumor growth.¹⁷⁵ Nano-delivery systems carrying gut microbial metabolites have also made significant advancements in HCC therapy. Based on the anti-tumor effects of butyrate and its synergistic action with sorafenib, glypican-3-targeted nanoparticles encapsulating butyrate and sorafenib were developed and shown to inhibit HCC progression.¹⁷³ Moreover, nanoparticles loaded separately with obeticholic acid, an FXR agonist, and 5 β -cholic acid 3, a G-protein-coupled bile acid receptor 1 antagonist, elicited robust anti-tumor immune responses against HCC.^{176,177} Collectively, the precise delivery of gut microbiota metabolites by nano-delivery systems amplifies their therapeutic efficacy, limits side effects, and holds promise as a future direction for HCC therapy.

IMPLICATIONS AND FUTURE DIRECTIONS

Disturbance of the intestinal ecosystem preceded by several pathogenic triggers such as improper diet, alcohol, drugs and genetics upsets the symbiotic balance between the gut microbiota and the host; here, we focus on the liver. The aberrant gut-to-liver axis underlies the pathogenesis and progression of liver cancer by shaping a pro-inflammatory and immunosuppressive environment, as elucidated in this review. However, these mechanisms seem insufficient to completely cover the intricate process of hepatobiliary carcinogenesis. Current conclusion is limited to the notion that alterations of the gut-liver axis promote, but do not determine, the onset of liver cancer.⁵⁷ This may be related to the heterogeneity of hepatocarcinogenesis and primary disease-specific properties. Hence, a crucial aspect of the success of cancer-preventive strategies targeting the gut-liver axis lies in the ability to precisely discriminate specific patients with higher susceptibility. Thorough determination of the microbial signature within a disease population and exact identification of key markers will be paramount in the future.

Intratumoral microbiota is a whole new area in the view of

tumor microenvironment. The enrichment of microorganisms within human tumors is thought to be related to the tumor specific immunosuppressive, hypoxic and nutritional microenvironment.¹⁷⁸ This is supported by the observed decrease in tumor-resident microbes following ICB therapy, particularly in responsive patients.¹⁷⁹ The presence of intratumoral microorganisms has been closely linked to cancer development and progression. For instance, specific intratumoral bacteria like *Peptostreptococcus anaerobius* and *P. stomatis* have been shown to promote the colonic tumorigenesis and worsen colorectal cancer progression.^{180,181} Additionally, targeting tumor-resident intracellular bacteria with cell-penetrating antibiotics, including doxycycline, clarithromycin and azithromycin, has also been found to effectively reduce the lung metastasis in breast cancer.¹⁴¹ As we mentioned above, various live gut-derived microorganisms have been detected in tumors of patients with primary liver cancer. However, their interactions with hepatic cells and the resulting functional outcomes remain unclear. Future investigations into these mechanisms are needed and could provide new strategies for liver cancer therapy.

Despite significant advances in understanding the role of gut microbiota in liver cancer, the clinical translation of gut microbiota-related products as a potential strategy for managing liver cancer still lags behind. Future population studies with larger sample sizes, more stringent confounding control, in-depth metagenomic profiling, and cross-regional sample validation are expected to accurately characterize gut microbial biomarkers for liver cancer. Risk assessment and patient stratification based on the gut microbiome will be essential for the success of clinical trials involving microbiota-targeted treatments. Innovative therapeutic approaches such as engineered bacteria and nano-delivery systems also hold promising potential for precision cancer therapy. Another important avenue that creates new opportunity for the clinical translation of microbiota-targeted therapies is the combination with ICB drugs. The significant impact of gut microbiota on the efficacy of immunotherapies across different cancer types has been increasingly established in recent years.¹⁸²⁻¹⁸⁵ Whole or unique live bacteria-based therapies such as probiotics and FMT have been initiated to test the efficacy of combination therapy in liver cancer (Table 4). Other treatments, such as prebiotics and postbiotics, also show promising potential in sensitizing ICI-based immunotherapy, as demonstrated in cutting-edge

research at a pan-cancer level, although a notable gap remains in liver cancer.¹⁸⁶⁻¹⁸⁸ Furthermore, the mechanisms underlying the combination of immunotherapy and gut microbiota are still largely unexplored. Therefore, in-depth mechanistic preclinical studies and multi-dimensional clinical research are urgently needed to better understand the microbiota-metabolite-immune axis in liver cancer.

Authors' contributions

Chenyang Li and Chujun Cai wrote and edited the manuscript. Chenyang Li and Chendong Wang generated the figures and tables. Xiaoping Chen revised the manuscript. Zhao Huang and Bixiang Zhang initiated the study and revised the manuscript. Zhao Huang obtained the funding. All authors have read and approved the final manuscript.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (82203809), Tongji Hospital (Huazhong University of Science and Technology) Foundation for Excellent Young Scientist (24-2KYC13057-15).

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

1. Pabst O, Hornef MW, Schaap FG, Cerovic V, Clavel T, Bruns T. Gut-liver axis: barriers and functional circuits. *Nat Rev Gastroenterol Hepatol* 2023;20:447-461.
2. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2021;7:6.
3. Brindley PJ, Bachini M, Ilyas SI, Khan SA, Loukas A, Sirica AE, et al. Cholangiocarcinoma. *Nat Rev Dis Primers* 2021;7:65.
4. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* 2021;19:55-71.
5. Chopyk DM, Grakoui A. Contribution of the intestinal microbiome and gut barrier to hepatic disorders. *Gastroenterology* 2020;159:849-863.
6. Iida N, Mizukoshi E, Yamashita T, Yutani M, Seishima J,

- Wang Z, et al. Chronic liver disease enables gut *Enterococcus faecalis* colonization to promote liver carcinogenesis. *Nat Cancer* 2021;2:1039-1054.
7. Behary J, Amorim N, Jiang XT, Raposo A, Gong L, McGovern E, et al. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat Commun* 2021;12:187.
8. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018;555:210-215.
9. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;334:105-108.
10. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* 2013;62:1787-1794.
11. Chen J, Xiao Y, Li D, Zhang S, Wu Y, Zhang Q, et al. New insights into the mechanisms of high-fat diet mediated gut microbiota in chronic diseases. *Imeta* 2023;2:e69.
12. Yang M, Qi X, Li N, Kaifi JT, Chen S, Wheeler AA, et al. Western diet contributes to the pathogenesis of non-alcoholic steatohepatitis in male mice via remodeling gut microbiota and increasing production of 2-oleoylglycerol. *Nat Commun* 2023;14:228.
13. Zhang D, Wang H, Liu A, Wang S, Xu C, Lan K, et al. The chronic consumption of dietary fructose promotes the gut *Clostridium* species imbalance and bile acid alterations in developing nonalcoholic fatty liver disease. *J Nutr Biochem* 2023;121:109434.
14. Zhou P, Chang WY, Gong DA, Xia J, Chen W, Huang LY, et al. High dietary fructose promotes hepatocellular carcinoma progression by enhancing O-GlcNAcylation via microbiota-derived acetate. *Cell Metab* 2023;35:1961-1975 e1966.
15. Zhang X, Coker OO, Chu ES, Fu K, Lau HCH, Wang YX, et al. Dietary cholesterol drives fatty liver-associated liver cancer by modulating gut microbiota and metabolites. *Gut* 2021;70:761-774.
16. M CCC, N LL, C MF, J LG, D A, C G, et al. Comparing the effects of acute alcohol consumption in germ-free and conventional mice: the role of the gut microbiota. *BMC Microbiol* 2014;14:240.
17. Keshavarzian A, Farhadi A, Forsyth CB, Rangan J, Jakate S, Shaikh M, et al. Evidence that chronic alcohol exposure promotes intestinal oxidative stress, intestinal hyperpermeability and endotoxemia prior to development of alcoholic steatohepatitis in rats. *J Hepatol* 2009;50:538-547.
18. Singhal R, Donde H, Ghare S, Stocke K, Zhang J, Vadhanam M, et al. Decrease in acetyl-CoA pathway utilizing butyrate-producing bacteria is a key pathogenic feature of alcohol-induced functional gut microbial dysbiosis and development of liver disease in mice. *Gut Microbes* 2021;13:1946367.
19. Martino C, Zaramela LS, Gao B, Embree M, Tarasova J, Parker SJ, et al. Acetate reprograms gut microbiota during alcohol consumption. *Nat Commun* 2022;13:4630.
20. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* 2014;158:705-721.
21. Mahana D, Trent CM, Kurtz ZD, Bokulich NA, Battaglia T, Chung J, et al. Antibiotic perturbation of the murine gut microbiome enhances the adiposity, insulin resistance, and liver disease associated with high-fat diet. *Genome Med* 2016;8:48.
22. Ebrahimi F, Simon TG, Hagström H, Sun J, Bergman D, Forss A, et al. Antibiotic use and development of nonalcoholic fatty liver disease: A population-based case-control study. *Liver Int* 2023;43:2186-2197.
23. Pinato DJ, Li X, Mishra-Kalyani P, D'Alessio A, Fulgenzi CAM, Scheiner B, et al. Association between antibiotics and adverse oncological outcomes in patients receiving targeted or immune-based therapy for hepatocellular carcinoma. *JHEP Rep* 2023;5:100747.
24. Weersma RK, Zhernakova A, Fu J. Interaction between drugs and the gut microbiome. *Gut* 2020;69:1510-1519.
25. Llorente C, Jepsen P, Inamine T, Wang L, Bluemel S, Wang HJ, et al. Gastric acid suppression promotes alcoholic liver disease by inducing overgrowth of intestinal *Enterococcus*. *Nat Commun* 2017;8:837.
26. Bajaj JS, Acharya C, Fagan A, White MB, Gavis E, Heuman DM, et al. Proton pump inhibitor initiation and withdrawal affects gut microbiota and readmission risk in cirrhosis. *Am J Gastroenterol* 2018;113:1177-1186.
27. Sanna S, Kurilshikov A, van der Graaf A, Fu J, Zhernakova A. Challenges and future directions for studying effects of host genetics on the gut microbiome. *Nat Genet* 2022;54:100-106.
28. Liu C, Sun G, Wang H, Shang G, Yan X, Zou X. Analysis of the intestinal flora in patients with primary liver cancer*. *Oncology and Translational Medicine* 2023;9:28-34.
29. Liu T, Yu H, Zhang Z, Xie Y, Yang L, You F. Intestinal ELF4

- deletion exacerbates alcoholic liver disease by disrupting gut homeostasis. *Int J Mol Sci* 2022;23.
30. Wu X, Chen S, Yan Q, Yu F, Shao H, Zheng X, et al. Gpr35 shapes gut microbial ecology to modulate hepatic steatosis. *Pharmacol Res* 2023;189:106690.
 31. Duparc T, Plovier H, Marrachelli VG, Van Hul M, Essaghir A, Ståhlman M, et al. Hepatocyte MyD88 affects bile acids, gut microbiota and metabolome contributing to regulate glucose and lipid metabolism. *Gut* 2017;66:620-632.
 32. Santos AA, Afonso MB, Ramiro RS, Pires D, Pimentel M, Castro RE, et al. Host miRNA-21 promotes liver dysfunction by targeting small intestinal *Lactobacillus* in mice. *Gut Microbes* 2020;12:1-18.
 33. Krautkramer KA, Fan J, Bäckhed F. Gut microbial metabolites as multi-kingdom intermediates. *Nat Rev Microbiol* 2021;19:77-94.
 34. Carr RM, Li Y, Chau L, Friedman ES, Lee JJ, Adorini L, et al. An integrated analysis of fecal microbiome and metabolomic features distinguish non-cirrhotic NASH from healthy control populations. *Hepatology* 2023;78:1843-1857.
 35. Oh TG, Kim SM, Caussy C, Fu T, Guo J, Bassirian S, et al. A Universal Gut-Microbiome-Derived Signature Predicts Cirrhosis. *Cell Metab* 2020;32:878-888.e876.
 36. Shen Y, Wu SD, Chen Y, Li XY, Zhu Q, Nakayama K, et al. Alterations in gut microbiome and metabolomics in chronic hepatitis B infection-associated liver disease and their impact on peripheral immune response. *Gut Microbes* 2023;15:2155018.
 37. Kummén M, Thingholm LB, Rühlemann MC, Holm K, Hansen SH, Moitinho-Silva L, et al. Altered gut microbial metabolism of essential nutrients in primary sclerosing cholangitis. *Gastroenterology* 2021;160:1784-1798.e1780.
 38. Lee G, You HJ, Bajaj JS, Joo SK, Yu J, Park S, et al. Distinct signatures of gut microbiome and metabolites associated with significant fibrosis in non-obese NAFLD. *Nat Commun* 2020;11:4982.
 39. Mouries J, Brescia P, Silvestri A, Spadoni I, Sorribas M, Wiest R, et al. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. *J Hepatol* 2019;71:1216-1228.
 40. Raimondi F, Santoro P, Barone MV, Pappacoda S, Barretta ML, Nanayakkara M, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G906-913.
 41. Li DK, Chaudhari SN, Lee Y, Sojoodi M, Adhikari AA, Zukerberg L, et al. Inhibition of microbial deconjugation of micellar bile acids protects against intestinal permeability and liver injury. *Sci Adv* 2022;8:eabo2794.
 42. Xu Q, Zhang R, Mu Y, Song Y, Hao N, Wei Y, et al. Propionate ameliorates alcohol-induced liver injury in mice via the gut-liver axis: focus on the improvement of intestinal permeability. *J Agric Food Chem* 2022;70:6084-6096.
 43. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, et al. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 2015;17:662-671.
 44. Wang RX, Henen MA, Lee JS, Vögeli B, Colgan SP. Microbiota-derived butyrate is an endogenous HIF prolyl hydroxylase inhibitor. *Gut Microbes* 2021;13:1938380.
 45. Hendriks T, Duan Y, Wang Y, Oh JH, Alexander LM, Huang W, et al. Bacteria engineered to produce IL-22 in intestine induce expression of REG3G to reduce ethanol-induced liver disease in mice. *Gut* 2019;68:1504-1515.
 46. Zhao ZH, Xin FZ, Xue Y, Hu Z, Han Y, Ma F, et al. Indole-3-propionic acid inhibits gut dysbiosis and endotoxin leakage to attenuate steatohepatitis in rats. *Exp Mol Med* 2019;51:1-14.
 47. Zhang H, Li C, Han L, Xiao Y, Bian J, Liu C, et al. MUP1 mediates urolithin A alleviation of chronic alcohol-related liver disease via gut-microbiota-liver axis. *Gut Microbes* 2024;16:2367342.
 48. Hartmann P, Hochrath K, Horvath A, Chen P, Seebauer CT, Llorente C, et al. Modulation of the intestinal bile acid/farnesoid X receptor/fibroblast growth factor 15 axis improves alcoholic liver disease in mice. *Hepatology* 2018;67:2150-2166.
 49. Zheng C, Wang L, Zou T, Lian S, Luo J, Lu Y, et al. Ileitis promotes MASLD progression via bile acid modulation and enhanced TGR5 signaling in ileal CD8(+) T cells. *J Hepatol* 2024;80:764-777.
 50. Ma L, Li H, Hu J, Zheng J, Zhou J, Botchlett R, et al. Indole alleviates diet-induced hepatic steatosis and inflammation in a manner involving myeloid cell 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3. *Hepatology* 2020;72:1191-1203.
 51. Kouno T, Zeng S, Wang Y, Duan Y, Lang S, Gao B, et al. Engineered bacteria producing aryl-hydrocarbon receptor agonists protect against ethanol-induced liver disease in mice. *Alcohol Clin Exp Res (Hoboken)* 2023;47:856-867.
 52. Krishnan S, Ding Y, Saedi N, Choi M, Sridharan GV, Sherr DH, et al. Gut microbiota-derived tryptophan metabolites modulate inflammatory response in hepatocytes and macro-

- phages. *Cell Rep* 2018;23:1099-1111.
53. Pandey SP, Bender MJ, McPherson AC, Phelps CM, Sanchez LM, Rana M, et al. Tet2 deficiency drives liver microbiome dysbiosis triggering Tc1 cell autoimmune hepatitis. *Cell Host Microbe* 2022;30:1003-1019.e1010.
54. Zhao M, Zhao L, Xiong X, He Y, Huang W, Liu Z, et al. TMA-VA, a Metabolite of intestinal microbes, is increased in plasma from patients with liver steatosis, inhibits γ -butyrobetaine hydroxylase, and exacerbates fatty liver in mice. *Gastroenterology* 2020;158:2266-2281.e2227.
55. Wang R, Tang R, Li B, Ma X, Schnabl B, Tilg H. Gut microbiome, liver immunology, and liver diseases. *Cell Mol Immunol* 2021;18:4-17.
56. Sookoian S, Salatino A, Castaño GO, Landa MS, Fijalkowky C, Garaycochea M, et al. Intrahepatic bacterial metataxonomic signature in non-alcoholic fatty liver disease. *Gut* 2020;69:1483-1491.
57. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012;21:504-516.
58. Achiwa K, Ishigami M, Ishizu Y, Kuzuya T, Honda T, Hayashi K, et al. DSS colitis promotes tumorigenesis and fibrogenesis in a choline-deficient high-fat diet-induced NASH mouse model. *Biochem Biophys Res Commun* 2016;470:15-21.
59. Zhang HL, Yu LX, Yang W, Tang L, Lin Y, Wu H, et al. Profound impact of gut homeostasis on chemically-induced pro-tumorigenic inflammation and hepatocarcinogenesis in rats. *J Hepatol* 2012;57:803-812.
60. Lang S, Schnabl B. Microbiota and fatty liver disease-the known, the unknown, and the future. *Cell Host Microbe* 2020;28:233-244.
61. Leinwand JC, Paul B, Chen R, Xu F, Sierra MA, Paluru MM, et al. Intrahepatic microbes govern liver immunity by programming NKT cells. *J Clin Invest* 2022;132.
62. Duan Y, Llorente C, Lang S, Brandl K, Chu H, Jiang L, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019;575:505-511.
63. Leung H, Long X, Ni Y, Qian L, Nychas E, Siliceo SL, et al. Risk assessment with gut microbiome and metabolite markers in NAFLD development. *Sci Transl Med* 2022;14:eabk0855.
64. Llopis M, Cassard AM, Wrzosek L, Boschhat L, Bruneau A, Ferrere G, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. *Gut* 2016;65:830-839.
65. Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J Hepatol* 2020;72:558-577.
66. Schwartz CC, Almond HR, Vlahcevic ZR, Swell L. Bile acid metabolism in cirrhosis. V. Determination of biliary lipid secretion rates in patients with advanced cirrhosis. *Gastroenterology* 1979;77:1177-1182.
67. Sannasiddappa TH, Lund PA, Clarke SR. In vitro antibacterial activity of unconjugated and conjugated bile salts on staphylococcus aureus. *Front Microbiol* 2017;8:1581.
68. Tian Y, Gui W, Koo I, Smith PB, Allman EL, Nichols RG, et al. The microbiome modulating activity of bile acids. *Gut Microbes* 2020;11:979-996.
69. Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A* 2006;103:3920-3925.
70. Teltschik Z, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, et al. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* 2012;55:1154-1163.
71. Pelletier G, Briantais MJ, Buffet C, Pillot J, Etienne JP. Serum and intestinal secretory IgA in alcoholic cirrhosis of the liver. *Gut* 1982;23:475-480.
72. Hoyle L, Fernández-Real JM, Federici M, Serino M, Abbott J, Charpentier J, et al. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* 2018;24:1070-1080.
73. Moran-Ramos S, Cerqueda-García D, López-Contreras B, Larrieta-Carrasco E, Villamil-Ramírez H, Molina-Cruz S, et al. A metagenomic study identifies a *Prevotella copri* enriched microbial profile associated with non-alcoholic steatohepatitis in subjects with obesity. *J Gastroenterol Hepatol* 2023;38:791-799.
74. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G966-978.
75. Yang X, Mai H, Zhou J, Li Z, Wang Q, Lan L, et al. Alterations of the gut microbiota associated with the occurrence and progression of viral hepatitis. *Front Cell Infect Microbiol* 2023;13:1119875.
76. Wei Y, Li Y, Yan L, Sun C, Miao Q, Wang Q, et al. Alterations of gut microbiome in autoimmune hepatitis. *Gut* 2020;69:569-577.

77. Liwinski T, Casar C, Ruehlmann MC, Bang C, Sebode M, Hohenester S, et al. A disease-specific decline of the relative abundance of *Bifidobacterium* in patients with autoimmune hepatitis. *Aliment Pharmacol Ther* 2020;51:1417-1428.
78. Hsu CL, Schnabl B. The gut-liver axis and gut microbiota in health and liver disease. *Nat Rev Microbiol* 2023;21:719-733.
79. Demir M, Lang S, Hartmann P, Duan Y, Martin A, Miyamoto Y, et al. The fecal mycobiome in non-alcoholic fatty liver disease. *J Hepatol* 2022;76:788-799.
80. Lang S, Demir M, Martin A, Jiang L, Zhang X, Duan Y, et al. Intestinal virome signature associated with severity of nonalcoholic fatty liver disease. *Gastroenterology* 2020;159:1839-1852.
81. Lang S, Duan Y, Liu J, Torralba MG, Kuelbs C, Ventura-Cots M, et al. Intestinal fungal dysbiosis and systemic immune response to fungi in patients with alcoholic hepatitis. *Hepatology* 2020;71:522-538.
82. Lemoine S, Kemgang A, Ben Belkacem K, Straube M, Jegou S, Corpechot C, et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. *Gut* 2020;69:92-102.
83. Huang H, Ren Z, Gao X, Hu X, Zhou Y, Jiang J, et al. Integrated analysis of microbiome and host transcriptome reveals correlations between gut microbiota and clinical outcomes in HBV-related hepatocellular carcinoma. *Genome Med* 2020;12:102.
84. Liu Q, Li F, Zhuang Y, Xu J, Wang J, Mao X, et al. Alteration in gut microbiota associated with hepatitis B and non-hepatitis virus related hepatocellular carcinoma. *Gut Pathog* 2019;11:1.
85. Jinato T, Anuntakun S, Sathawiwat N, Chuaypen N, Tangkijvanich P. Distinct alterations of gut microbiota between viral- and non-viral-related hepatocellular carcinoma. *Appl Microbiol Biotechnol* 2024;108:34.
86. Zhang L, Chen C, Chai D, Li C, Qiu Z, Kuang T, et al. Characterization of the intestinal fungal microbiome in patients with hepatocellular carcinoma. *J Transl Med* 2023;21:126.
87. Dong TS, Jacobs JP, Agopian V, Pisegna JR, Ayoub W, Durazo F, et al. Duodenal microbiome and serum metabolites predict hepatocellular carcinoma in a multicenter cohort of patients with cirrhosis. *Dig Dis Sci* 2022;67:3831-3841.
88. Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. *Hepatology* 2019;69:107-120.
89. Sydor S, Best J, Messerschmidt I, Manka P, Vilchez-Vargas R, Brodesser S, et al. Altered microbiota diversity and bile acid signaling in cirrhotic and noncirrhotic NASH-HCC. *Clin Transl Gastroenterol* 2020;11:e00131.
90. Yang J, He Q, Lu F, Chen K, Ni Z, Wang H, et al. A distinct microbiota signature precedes the clinical diagnosis of hepatocellular carcinoma. *Gut Microbes* 2023;15:2201159.
91. Li R, Yi X, Yang J, Zhu Z, Wang Y, Liu X, et al. Gut microbiome signatures in the progression of hepatitis B virus-induced liver disease. *Front Microbiol* 2022;13:916061.
92. Ren Z, Li A, Jiang J, Zhou L, Yu Z, Lu H, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut* 2019;68:1014-1023.
93. Albhaisi S, Shamsaddini A, Fagan A, McGeorge S, Sikaroodi M, Gavis E, et al. Gut microbial signature of hepatocellular cancer in men with cirrhosis. *Liver Transpl* 2021;27:629-640.
94. Lapidot Y, Amir A, Nosenko R, Uzan-Yulzari A, Veitsman E, Cohen-Ezra O, et al. Alterations in the gut microbiome in the progression of cirrhosis to hepatocellular carcinoma. *mSystems* 2020;5.
95. Zheng R, Wang G, Pang Z, Ran N, Gu Y, Guan X, et al. Liver cirrhosis contributes to the disorder of gut microbiota in patients with hepatocellular carcinoma. *Cancer Med* 2020;9:4232-4250.
96. Piñero F, Vazquez M, Baré P, Rohr C, Mendizabal M, Sciarra M, et al. A different gut microbiome linked to inflammation found in cirrhotic patients with and without hepatocellular carcinoma. *Ann Hepatol* 2019;18:480-487.
97. Effenberger M, Waschina S, Bronowski C, Sturm G, Tassello O, Sommer F, et al. A gut bacterial signature in blood and liver tissue characterizes cirrhosis and hepatocellular carcinoma. *Hepatology* 2023;77.
98. Deng T, Li J, He B, Chen B, Liu F, Chen Z, et al. Gut microbiome alteration as a diagnostic tool and associated with inflammatory response marker in primary liver cancer. *Hepatology* 2022;16:99-111.
99. Chen T, Ding R, Chen X, Lu Y, Shi J, Lü Y, et al. Firmicutes and *Blautia* in gut microbiota lessened in chronic liver diseases and hepatocellular carcinoma patients: a pilot study. *Bioengineered* 2021;12:8233-8246.
100. Zhang W, Xu X, Cai L, Cai X. Dysbiosis of the gut microbiome in elderly patients with hepatocellular carcinoma. *Sci Rep* 2023;13:7797.
101. Feng J, Wu Y, Dai P, Wang D, Liu L, Chai B. Gut microbial signatures of patients with primary hepatocellular carcinoma

- and their healthy first-degree relatives. *J Appl Microbiol* 2023;134.
102. Jia X, Lu S, Zeng Z, Liu Q, Dong Z, Chen Y, et al. Characterization of gut microbiota, bile acid metabolism, and cytokines in intrahepatic cholangiocarcinoma. *Hepatology* 2020;71:893-906.
103. Pomyen Y, Chaisaingmongkol J, Rabibhadana S, Pupacdi B, Sripan D, Chornkrathok C, et al. Gut dysbiosis in Thai intrahepatic cholangiocarcinoma and hepatocellular carcinoma. *Sci Rep* 2023;13:11406.
104. Zhang N, Zhu W, Zhang S, Liu T, Gong L, Wang Z, et al. A novel bifidobacterium/klebsiella ratio in characterization analysis of the gut and bile microbiota of CCA patients. *Microb Ecol* 2023;87:5.
105. Zhang T, Zhang S, Jin C, Lin Z, Deng T, Xie X, et al. A predictive model based on the gut microbiota improves the diagnostic effect in patients with cholangiocarcinoma. *Front Cell Infect Microbiol* 2021;11:751795.
106. Singh V, Yeoh BS, Chassaing B, Xiao X, Saha P, Aguilera Olvera R, et al. Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. *Cell* 2018;175:679-694.e622.
107. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* 2018;360.
108. Singh V, Yeoh BS, Abokor AA, Golonka RM, Tian Y, Patterson AD, et al. Vancomycin prevents fermentable fiber-induced liver cancer in mice with dysbiotic gut microbiota. *Gut Microbes* 2020;11:1077-1091.
109. Song Q, Zhang X, Liu W, Wei H, Liang W, Zhou Y, et al. Bifidobacterium pseudolongum-generated acetate suppresses non-alcoholic fatty liver disease-associated hepatocellular carcinoma. *J Hepatol* 2023;79:1352-1365.
110. Li T, Lin X, Shen B, Zhang W, Liu Y, Liu H, et al. Akkermansia muciniphila suppressing nonalcoholic steatohepatitis associated tumorigenesis through CXCR6(+) natural killer T cells. *Front Immunol* 2022;13:1047570.
111. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97-101.
112. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature* 2017;551:340-345.
113. Schneider KM, Mohs A, Gui W, Galvez EJC, Candels LS, Hoenicke L, et al. Imbalanced gut microbiota fuels hepatocellular carcinoma development by shaping the hepatic inflammatory microenvironment. *Nat Commun* 2022;13:3964.
114. Zhang Q, Ma C, Duan Y, Heinrich B, Rosato U, Diggs LP, et al. Gut microbiome directs hepatocytes to recruit MDSCs and promote cholangiocarcinoma. *Cancer Discov* 2021;11:1248-1267.
115. Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, et al. Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. *Gut* 2018;67:891-901.
116. Lau HC, Zhang X, Ji F, Lin Y, Liang W, Li Q, et al. Lactobacillus acidophilus suppresses non-alcoholic fatty liver disease-associated hepatocellular carcinoma through producing valeric acid. *EBioMedicine* 2024;100:104952.
117. Han S, Bao X, Zou Y, Wang L, Li Y, Yang L, et al. d-lactate modulates M2 tumor-associated macrophages and remodels immunosuppressive tumor microenvironment for hepatocellular carcinoma. *Sci Adv* 2023;9:eadg2697.
118. Li X, Ramadori P, Pfister D, Seehawer M, Zender L, Heikenwalder M. The immunological and metabolic landscape in primary and metastatic liver cancer. *Nat Rev Cancer* 2021;21:541-557.
119. Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. *Nat Immunol* 2018;19:222-232.
120. Yu LX, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol* 2017;14:527-539.
121. Yu LX, Yan HX, Liu Q, Yang W, Wu HP, Dong W, et al. Endotoxin accumulation prevents carcinogen-induced apoptosis and promotes liver tumorigenesis in rodents. *Hepatology* 2010;52:1322-1333.
122. Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010;140:197-208.
123. Loo TM, Kamachi F, Watanabe Y, Yoshimoto S, Kanda H, Arai Y, et al. Gut microbiota promotes obesity-associated liver cancer through PGE(2)-mediated suppression of antitumor immunity. *Cancer Discov* 2017;7:522-538.
124. Zhou Y, Hu L, Tang W, Li D, Ma L, Liu H, et al. Hepatic NOD2 promotes hepatocarcinogenesis via a RIP2-mediated proinflammatory response and a novel nuclear autophagy-mediated DNA damage mechanism. *J Hematol Oncol* 2021;14:9.

125. Li Q, Ma L, Shen S, Guo Y, Cao Q, Cai X, et al. Intestinal dysbacteriosis-induced IL-25 promotes development of HCC via alternative activation of macrophages in tumor microenvironment. *J Exp Clin Cancer Res* 2019;38:303.
126. Liu B, Zhou Z, Jin Y, Lu J, Feng D, Peng R, et al. Hepatic stellate cell activation and senescence induced by intra-hepatic microbiota disturbances drive progression of liver cirrhosis toward hepatocellular carcinoma. *J Immunother Cancer* 2022;10.
127. Satriano L, Lewinska M, Rodrigues PM, Banales JM, Andersen JB. Metabolic rearrangements in primary liver cancers: cause and consequences. *Nat Rev Gastroenterol Hepatol* 2019;16:748-766.
128. Tian Y, Yang B, Qiu W, Hao Y, Zhang Z, Yang B, et al. ER-residential Nogo-B accelerates NAFLD-associated HCC mediated by metabolic reprogramming of oxLDL lipophagy. *Nat Commun* 2019;10:3391.
129. Todoric J, Di Caro G, Reibe S, Henstridge DC, Green CR, Vrbancic A, et al. Fructose stimulated de novo lipogenesis is promoted by inflammation. *Nat Metab* 2020;2:1034-1045.
130. Kim JY, Garcia-Carbonell R, Yamachika S, Zhao P, Dhar D, Loomba R, et al. ER stress drives lipogenesis and steatohepatitis via caspase-2 activation of S1P. *Cell* 2018;175:133-145.e115.
131. Yamagishi R, Kamachi F, Nakamura M, Yamazaki S, Kamiya T, Takasugi M, et al. Gasdermin D-mediated release of IL-33 from senescent hepatic stellate cells promotes obesity-associated hepatocellular carcinoma. *Sci Immunol* 2022;7:eabl7209.
132. Campbell C, McKenney PT, Konstantinovskiy D, Isaeva OI, Schizas M, Verter J, et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* 2020;581:475-479.
133. Hang S, Paik D, Yao L, Kim E, Trinath J, Lu J, et al. Bile acid metabolites control T(H)17 and T(reg) cell differentiation. *Nature* 2019;576:143-148.
134. Song X, Sun X, Oh SF, Wu M, Zhang Y, Zheng W, et al. Microbial bile acid metabolites modulate gut RORγ(+) regulatory T cell homeostasis. *Nature* 2020;577:410-415.
135. Collins SL, Stine JG, Bisanz JE, Okafor CD, Patterson AD. Bile acids and the gut microbiota: metabolic interactions and impacts on disease. *Nat Rev Microbiol* 2023;21:236-247.
136. Hu C, Xu B, Wang X, Wan WH, Lu J, Kong D, et al. Gut microbiota-derived short-chain fatty acids regulate group 3 innate lymphoid cells in HCC. *Hepatology* 2023;77:48-64.
137. Ma H, Yang L, Liang Y, Liu F, Hu J, Zhang R, et al. B. thetaio-taomicron-derived acetic acid modulate immune microenvironment and tumor growth in hepatocellular carcinoma. *Gut Microbes* 2024;16:2297846.
138. Yang L, Li A, Wang Y, Zhang Y. Intratumoral microbiota: roles in cancer initiation, development and therapeutic efficacy. *Signal Transduct Target Ther* 2023;8:35.
139. Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* 2020;368:973-980.
140. Narunsky-Haziza L, Sepich-Poore GD, Livyatan I, Asraf O, Martino C, Nejman D, et al. Pan-cancer analyses reveal cancer-type-specific fungal ecologies and bacteriome interactions. *Cell* 2022;185:3789-3806.e3717.
141. Fu A, Yao B, Dong T, Chen Y, Yao J, Liu Y, et al. Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. *Cell* 2022;185:1356-1372.e1326.
142. Bertocchi A, Carloni S, Ravenda PS, Bertalot G, Spadoni I, Lo Cascio A, et al. Gut vascular barrier impairment leads to intestinal bacteria dissemination and colorectal cancer metastasis to liver. *Cancer Cell* 2021;39:708-724.e711.
143. Ma Y, Chen H, Li H, Zheng M, Zuo X, Wang W, et al. Intratumor microbiome-derived butyrate promotes lung cancer metastasis. *Cell Rep Med* 2024;5:101488.
144. Galeano Niño JL, Wu H, LaCourse KD, Kempchinsky AG, Baryames A, Barber B, et al. Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. *Nature* 2022;611:810-817.
145. Sun L, Ke X, Guan A, Jin B, Qu J, Wang Y, et al. Intratumoral microbiome can predict the prognosis of hepatocellular carcinoma after surgery. *Clin Transl Med* 2023;13:e1331.
146. Chai X, Wang J, Li H, Gao C, Li S, Wei C, et al. Intratumor microbiome features reveal antitumor potentials of intrahepatic cholangiocarcinoma. *Gut Microbes* 2023;15:2156255.
147. Sitthirak S, Suksawat M, Phetcharaburanin J, Wangwiatsin A, Klanrit P, Namwat N, et al. Chemotherapeutic resistant cholangiocarcinoma displayed distinct intratumoral microbial composition and metabolic profiles. *PeerJ* 2022;10:e13876.
148. He Y, Zhang Q, Yu X, Zhang S, Guo W. Overview of microbial profiles in human hepatocellular carcinoma and adjacent nontumor tissues. *J Transl Med* 2023;21:68.
149. Li S, Xia H, Wang Z, Zhang X, Song T, Li J, et al. Intratumoral microbial heterogeneity affected tumor immune microenvironment and determined clinical outcome of HBV-related HCC. *Hepatology* 2023;78:1079-1091.

150. Liu Y, Kim ES, Guo H. Hepatitis B virus-related hepatocellular carcinoma exhibits distinct intratumoral microbiota and immune microenvironment signatures. *J Med Virol* 2024;96:e29485.
151. Xue C, Gu X, Shi Q, Ma X, Jia J, Su Y, et al. The interaction between intratumoral bacteria and metabolic distortion in hepatocellular carcinoma. *J Transl Med* 2024;22:237.
152. Zhang H, Wu J, Liu Y, Zeng Y, Jiang Z, Yan H, et al. Identification reproducible microbiota biomarkers for the diagnosis of cirrhosis and hepatocellular carcinoma. *AMB Express* 2023;13:35.
153. Zheng Y, Wang T, Tu X, Huang Y, Zhang H, Tan D, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. *J Immunother Cancer* 2019;7:193.
154. Mao J, Wang D, Long J, Yang X, Lin J, Song Y, et al. Gut microbiome is associated with the clinical response to anti-PD-1 based immunotherapy in hepatobiliary cancers. *J Immunother Cancer* 2021;9.
155. Rajapakse J, Khatiwada S, Akon AC, Yu KL, Shen S, Zekry A. Unveiling the complex relationship between gut microbiota and liver cancer: opportunities for novel therapeutic interventions. *Gut Microbes* 2023;15:2240031.
156. Patel VC, Lee S, McPhail MJW, Da Silva K, Guilly S, Zamalloa A, et al. Rifaximin- α reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. *J Hepatol* 2022;76:332-342.
157. Macnaughtan J, Figorilli F, García-López E, Lu H, Jones H, Sawhney R, et al. A double-blind, randomized placebo-controlled trial of probiotic lactobacillus casei Shirota in stable cirrhotic patients. *Nutrients* 2020;12.
158. Mohamad Nor MH, Ayob N, Mokhtar NM, Raja Ali RA, Tan GC, Wong Z, et al. The effect of probiotics (MCP® BCMC® strains) on hepatic steatosis, small intestinal mucosal immune function, and intestinal barrier in patients with non-alcoholic fatty liver disease. *Nutrients* 2021;13.
159. Shi K, Zhang Q, Zhang Y, Bi Y, Zeng X, Wang X. Association between probiotic therapy and the risk of hepatocellular carcinoma in patients with hepatitis B-related cirrhosis. *Front Cell Infect Microbiol* 2022;12:1104399.
160. Madsen BS, Havelund T, Krag A. Targeting the gut-liver axis in cirrhosis: antibiotics and non-selective β -blockers. *Adv Ther* 2013;30:659-670.
161. He X, Zhao Z, Jiang X, Sun Y. Non-selective beta-blockers and the incidence of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Front Pharmacol* 2023;14:1216059.
162. Nevens F, Trauner M, Manns MP. Primary biliary cholangitis as a roadmap for the development of novel treatments for cholestatic liver diseases(†). *J Hepatol* 2023;78:430-441.
163. Jiang S, Xu L, Chen Y, Shu Z, Lv L, Zhao Y, et al. Longitudinal gut fungal alterations and potential fungal biomarkers for the progression of primary liver disease. *Sci China Life Sci* 2024;67:1183-1198.
164. Zheng C, Lu F, Chen B, Yang J, Yu H, Wang D, et al. Gut microbiome as a biomarker for predicting early recurrence of HBV-related hepatocellular carcinoma. *Cancer Sci* 2023;114:4717-4731.
165. Peng YC, Xu JX, You XM, Huang YY, Ma L, Li LQ, et al. Specific gut microbiome signature predicts hepatitis B virus-related hepatocellular carcinoma patients with microvascular invasion. *Ann Med* 2023;55:2283160.
166. Ponziani FR, De Luca A, Picca A, Marzetti E, Petito V, Del Chierico F, et al. Gut dysbiosis and fecal calprotectin predict response to immune checkpoint inhibitors in patients with hepatocellular carcinoma. *Hepatol Commun* 2022;6:1492-1501.
167. Chung MW, Kim MJ, Won EJ, Lee YJ, Yun YW, Cho SB, et al. Gut microbiome composition can predict the response to nivolumab in advanced hepatocellular carcinoma patients. *World J Gastroenterol* 2021;27:7340-7349.
168. Lee PC, Wu CJ, Hung YW, Lee CJ, Chi CT, Lee IC, et al. Gut microbiota and metabolites associate with outcomes of immune checkpoint inhibitor-treated unresectable hepatocellular carcinoma. *J Immunother Cancer* 2022;10.
169. Wu H, Zheng X, Pan T, Yang X, Chen X, Zhang B, et al. Dynamic microbiome and metabolome analyses reveal the interaction between gut microbiota and anti-PD-1 based immunotherapy in hepatocellular carcinoma. *Int J Cancer* 2022;151:1321-1334.
170. Zhang X, Wu L, Xu Y, Yu H, Chen Y, Zhao H, et al. Microbiota-derived SSL6 enhances the sensitivity of hepatocellular carcinoma to sorafenib by down-regulating glycolysis. *Cancer Lett* 2020;481:32-44.
171. Iida N, Mizukoshi E, Yamashita T, Terashima T, Arai K, Seishima J, et al. Overuse of antianaerobic drug is associated with poor postchemotherapy prognosis of patients with hepatocellular carcinoma. *Int J Cancer* 2019;145:2701-2711.
172. Li Z, Zhang Y, Hong W, Wang B, Chen Y, Yang P, et al. Gut

- microbiota modulate radiotherapy-associated antitumor immune responses against hepatocellular carcinoma Via STING signaling. *Gut Microbes* 2022;14:2119055.
173. Che Y, Chen G, Guo Q, Duan Y, Feng H, Xia Q. Gut microbial metabolite butyrate improves anticancer therapy by regulating intracellular calcium homeostasis. *Hepatology* 2023;78:88-102.
 174. Yu J, Zhu P, Shi L, Gao N, Li Y, Shu C, et al. *Bifidobacterium longum* promotes postoperative liver function recovery in patients with hepatocellular carcinoma. *Cell Host Microbe* 2024;32:131-144.e136.
 175. Yang M, Chen W, Gupta D, Mei C, Yang Y, Zhao B, et al. Nanoparticle/engineered bacteria based triple-strategy delivery system for enhanced hepatocellular carcinoma cancer therapy. *Int J Nanomedicine* 2024;19:3827-3846.
 176. Ji G, Si X, Dong S, Xu Y, Li M, Yang B, et al. Manipulating liver bile acid signaling by nanodelivery of bile acid receptor modulators for liver cancer immunotherapy. *Nano Lett* 2021;21:6781-6791.
 177. Ji G, Ma L, Yao H, Ma S, Si X, Wang Y, et al. Precise delivery of obeticholic acid via nanoapproach for triggering natural killer T cell-mediated liver cancer immunotherapy. *Acta Pharm Sin B* 2020;10:2171-2182.
 178. Cao Y, Xia H, Tan X, Shi C, Ma Y, Meng D, et al. Intratumoral microbiota: a new frontier in cancer development and therapy. *Signal Transduct Target Ther* 2024;9:15.
 179. Battaglia TW, Mimpfen IL, Traets JJH, van Hoeck A, Zevenijn LJ, Geurts BS, et al. A pan-cancer analysis of the microbiome in metastatic cancer. *Cell* 2024;187:2324-2335.e2319.
 180. Liu Y, Wong CC, Ding Y, Gao M, Wen J, Lau HC, et al. *Peptostreptococcus anaerobius* mediates anti-PD1 therapy resistance and exacerbates colorectal cancer via myeloid-derived suppressor cells in mice. *Nat Microbiol* 2024;9:1467-1482.
 181. Huang P, Ji F, Cheung AH, Fu K, Zhou Q, Ding X, et al. *Peptostreptococcus stomatis* promotes colonic tumorigenesis and receptor tyrosine kinase inhibitor resistance by activating ERBB2-MAPK. *Cell Host Microbe* 2024;32:1365-1379.e1310.
 182. Lu Y, Yuan X, Wang M, He Z, Li H, Wang J, et al. Gut microbiota influence immunotherapy responses: mechanisms and therapeutic strategies. *J Hematol Oncol* 2022;15:47.
 183. Jia D, Wang Q, Qi Y, Jiang Y, He J, Lin Y, et al. Microbial metabolite enhances immunotherapy efficacy by modulating T cell stemness in pan-cancer. *Cell* 2024;187:1651-1665.e1621.
 184. Zhang SL, Wang X, Cai QQ, Chen C, Zhang ZY, Xu YY, et al. Acarbose enhances the efficacy of immunotherapy against solid tumours by modulating the gut microbiota. *Nat Metab* 2024;6:1991-2009.
 185. Zhang Q, Zhao Q, Li T, Lu L, Wang F, Zhang H, et al. *Lactobacillus plantarum*-derived indole-3-lactic acid ameliorates colorectal tumorigenesis via epigenetic regulation of CD8(+) T cell immunity. *Cell Metab* 2023;35:943-960.e949.
 186. Huang J, Liu D, Wang Y, Liu L, Li J, Yuan J, et al. Ginseng polysaccharides alter the gut microbiota and kynurenine/tryptophan ratio, potentiating the antitumour effect of anti-programmed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy. *Gut* 2022;71:734-745.
 187. Ferrari V, Lo Cascio A, Melacarne A, Tanasković N, Mozzarelli AM, Tiraboschi L, et al. Sensitizing cancer cells to immune checkpoint inhibitors by microbiota-mediated upregulation of HLA class I. *Cancer Cell* 2023;41:1717-1730.e1714.
 188. Cho YS, Han K, Xu J, Moon JJ. Novel strategies for modulating the gut microbiome for cancer therapy. *Adv Drug Deliv Rev* 2024;210:115332.