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The role of gut microbiota in liver disease development and treatment★

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Abstract

Liver cancer is the sixth most common cancer worldwide, and the third most common cause of cancer-related death. Hepatocellular carcinoma (HCC), which accounts for more than 90% of primary liver cancers, is an important public health problem. In addition to cirrhosis caused by hepatitis B viral (HBV) or hepatitis C viral (HCV) infection, non-alcoholic fatty liver disease (NAFLD) is becoming a major risk factor for liver cancer because of the prevalence of obesity. Non-alcoholic steatohepatitis (NASH) will likely become the leading indication for liver transplantation in the future. It is well recognized that gut microbiota is a key environmental factor in the pathogenesis of liver disease and cancer. The interplay between gut microbiota and liver disease has been investigated in animal and clinical studies. In this article, we summarize the roles of gut microbiota in the development of liver disease as well as gut microbiota-targeted therapies.

Keywords

Microorganism; Hepatocellular carcinoma (HCC); Non-alcoholic fatty liver disease (NAFLD); Non-alcoholic steatohepatitis (NASH); Cirrhosis; Probiotics; Prebiotics; Synbiotics

1. Introduction

There are about 100 trillion (10^{14}) microorganisms and approximately 2000 different bacterial species in the human digestive tract.¹ The gut microbiota colonizes immediately after birth and plays an essential role in keeping the host healthy by assisting digestion, producing vitamins, generating bile acids, and modulating local and systemic immunity.^{2–5} Many factors, including diet, age, medication, illness, stress, and lifestyle, influence the gut microbiota community structure, which has an impact on disease development.⁶ It is

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Authors' contributions

L. Wang drafted the manuscript. Y.-J. Y. Wan edited and approved the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

important to note that genetic factors only contribute to 5–15% of most cancers. About 80% of cancers are caused by the environment or lifestyle.⁷ Emerging evidence reveals that the gut microbiota is a major environmental and etiological factor for liver disease development.^{8–12} In this review, we summarize publications on the topics of gut microbiota in liver disease development, as well as treatment, focusing on non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC).

We performed a literature search in PubMed for papers published within the past 10 years, using the keywords: microorganism, microbiota, bacteria, liver, liver disease, HCC, hepatocellular carcinogenesis, NAFLD, NASH, cirrhosis, probiotics, prebiotics, synbiotics, and their combinations.

2. Role of gut microbiota in liver diseases

2.1. Gut microbiota and NAFLD as well as NASH

NAFLD is a global public health problem because of the prevalence of obesity.¹³ NAFLD is a spectrum of chronic liver diseases, including simple steatosis, NASH, advanced fibrosis, cirrhosis, and HCC.⁶ Dysbiosis refers to unfavorable alteration of the microbiota. It is commonly characterized with a decreased ratio of autochthonous to nonautochthonous taxa. Increasing evidence indicates that gut dysbiosis has an important role in the development of NASH via regulation of inflammation, insulin resistance, bile acids, and choline metabolism.^{4,14–16}

Data generated from human studies have established the relationship between gut microbiota and NAFLD (Table 1).^{14,17–24} In 2013, Mouzaki *et al.*¹⁷ reported that NASH patients have significantly lower levels of Bacteroidetes compared to healthy individuals. Shen *et al.*¹⁸ showed similar findings. However, Wang *et al.*¹⁹ found that non-obese patients with NAFLD have significantly higher levels of Bacteroidetes and lower abundance of Firmicutes in addition to reduced diversity. In these non-obese patients with NAFLD, the depletion of Firmicutes included Lachnospiraceae, Ruminococcaceae, and Lactobacillaceae, which generated short-chain fatty acids (SCFAs).¹⁹ Additionally, a different Bacteroidetes abundance pattern in adolescents was revealed in a study conducted by Stanislawski *et al.*²⁰ The abundance of *Bacteroides* showed a U-shaped pattern based on hepatic fat; both low and high abundances were associated with elevated hepatic fat, while a moderate level was associated with reduced hepatic fat.²⁰ In addition, *Bacteroides* is associated with a high-fat diet (HFD).²⁵ However, certain species of *Bacteroides* have protective roles in obesity.²⁶

Stanislawski *et al.*²⁰ also found that *Bilophila*, *Paraprevotella*, *Suturella*, and *RF32* have a positive relationship with hepatic fat, while *Oscillospira* and *Varibaculum* correlate negatively. The positive association between hepatic fat and *Bilophila* is accompanied by reduced *Oscillospira*. These results suggest that *Bilophila* might contribute to fatty liver, while *Oscillospira* might counteract its effects.²¹ The abundance of *Bilophila wadsworthia* increases in response to a western diet or HFD.^{27,28} *Bilophila wadsworthia* is also associated with T helper 1 (Th1)-mediated intestinal inflammation. *Oscillospira* is reduced in pediatric NAFLD and NASH.^{21,22} Reduced *Oscillospira* accompanied by increased 2-butanone has

been identified as a gut microbiota signature of NAFLD onset. Increases in *Ruminococcus* and *Dorea* have been identified as gut microbiota signatures of NAFLD and NASH progression.²¹ *Oscillospira* is generally linked to leanness and health.²³ *Bilophila*, *Oscillospira*, and *Bacteroides* are associated with diets high in animal products.^{29–31} In addition, increased levels of *Lactobacillus* and selected members of the Firmicutes (Lachnospiraceae; genera, *Dorea*, *Robinsoniella*, and *Roseburia*) have been observed in NAFLD patients.²⁴ NAFLD patients and healthy subjects have a distinct intestinal microbiota community structure.

Further evidence shows that gut dysbiosis and altered metabolic function are linked with the severity of NAFLD. A study by Boursier *et al.*¹⁴ demonstrated that *Bacteroides* and *Ruminococcus* are associated with NASH and the severity of fibrosis. Patients with NASH and fibrosis severity F 2 have higher abundance of *Bacteroides* and lower abundance of *Prevotella* compared to those without NASH. Patients with F 2 fibrosis have higher abundance of *Bacteroides* and *Ruminococcus* and lower abundance of *Prevotella* compared with those with F0/F1 fibrosis.¹⁴ Patients with mild/moderate NAFLD have a higher abundance of Firmicutes, while patients with advanced fibrosis NAFLD have a higher abundance of Proteobacteria. Patients with advanced fibrosis have lower abundance of *Ruminococcus obeum* CAG: 39, *Ruminococcus obeum*, and *Eubacterium rectale* compared to those with mild/moderate NAFLD.³²

Small intestinal bacterial overgrowth (SIBO) is defined as bacterial culture >10⁵ CFU/ml in upper jejunal aspirate.^{33,34} SIBO has a direct relationship with the severity of liver disease. Many patients with chronic liver disease have dysbiosis with SIBO.^{3,35} SIBO in patients with NAFLD/NASH has an estimated prevalence of 39–85%.^{36–41} As a consequence of reduced intestinal motility and decreased bile acid production, SIBO has a role in NAFLD progression.⁴² Miele *et al.*³⁷ have reported that SIBO is implicated in increased intestinal permeability and development of fatty liver. SIBO increases lipopolysaccharide (LPS) secretion and inflammation. Hepatic expression of Toll-like receptor 4 (TLR4), together with release of interleukin-8 (IL-8) induced by SIBO, promotes inflammation.⁴ SIBO increases endogenous ethanol and intestinal permeability, favoring LPS production and increased inflammation via TLR4 signaling.^{43,44} SIBO is considered as an independent risk factor for the severity of NAFLD and is essential for NAFLD to progress into NASH, followed by development of cirrhosis.^{15,19,38,45,46}

Enteric dysbiosis or intestinal inflammation induced by HFD and dextran sulfate sodium significantly promotes liver fibrosis in mice with NASH.⁴⁷ The inflammasome-mediated dysbiosis, including increased Prevotellaceae and Porphyromonadaceae families as well as the TM7 taxa, promote NAFLD progression in mouse models.⁶ Apart from providing bacterial byproducts and increasing intestinal permeability, the gut microbiota might also inhibit small intestinal secretion of fasting-induced adipocyte factor, resulting in increased hepatic triglyceride deposition.⁴⁸ Antibiotic treatment or surgical removal of the bypassed section of the intestine can reverse SIBO and steatohepatitis.^{36,42} SIBO might be an important target for using antibiotics in treating NAFLD as well as NASH.^{49,50}

Patients with liver cirrhosis and liver or colon cancer have reduced bile acid receptor farnesoid X receptor (FXR).^{51–53} Wan's group has shown that the sex of an animal can affect the gut microbiota, which is implicated in the dissimilar development of steatosis in both western-diet-fed mice and FXR knockout (KO) mouse models according to sex.⁵⁴ Decreased S24–7, in parallel with increased Bacteroidaceae, Rikenellaceae, Lactobacillaceae, and Verrucomicrobiaceae, has been observed in wild-type female mice compared to their male counterparts. However, these sex differences are abolished in FXR KO mice, indicating that sex difference in steatosis is FXR dependent.⁵⁴ Western-diet-fed male FXR KO mice develop advanced NASH with massive hepatic lymphocyte infiltration, and have decreased Firmicutes and increased Proteo-bacteria.⁵⁵ Broad-spectrum as well as a Gram-negative coverage antibiotics are useful in treating NASH in male FXR KO mice, but are relatively ineffective when FXR KO male mice are on a western diet.⁵⁵ In the Proteobacteria, the relative abundance of Heli-cobacteraceae and Desulfovibrionaceae substantially increases because of FXR inactivation. Consistently, antibiotic-reduced hepatic inflammation is accompanied by their reduction. In contrast, *Lactococcus*, *Lactobacillus*, and *Coprococcus* have a protective effect in hepatic inflammation.⁵⁵ The basic mechanisms of dysbiosis affecting liver disease are summarized in Fig. 1.

2.2. Gut microbiota and liver cirrhosis as well as cirrhosis-associated complications

2.2.1. Gut microbiota and liver cirrhosis—Liver cirrhosis is the end stage of chronic liver diseases and is characterized by fibrosis, abnormal hepatic architecture, and portal hypertension. Liver cirrhosis may lead to progressive hepatic failure and cancer. It has been shown that dysbiosis can affect clinical outcomes, including 90-day-hospitalization, organ failure, and death.^{56–58}

Cirrhosis-associated gut dysbiosis is accompanied by reduced Bacteroidetes, increased Proteobacteria at the phylum level, and reduced Lachnospiraceae as well as increased Enterobacteriaceae and Veillonelaceae at the family level⁵. Potentially pathogenic overgrowth of Enterobacteriaceae is linked to the severity of cirrhosis and its complications, such as hepatic encephalopathy.⁵ Chen *et al.*⁵⁹ demonstrated an increase in Proteobacteria and Fusobacteria, along with a decrease in Bacteroidetes and change in Firmicutes at the phylum level in fecal samples from cirrhotic patients. In addition, cirrhotic patients have increased fecal Entero-bacteriaceae, Veillonelaceae, and Streptococcaceae, and reduced Lachnospiraceae.⁵⁹ Moreover, several commensal genera, such as, *Coprococcus*, *Pseudobutyrvibrio*, and *Roseburia* in the Lachnospiraceae family, are beneficial to the host via production of SCFAs.⁵⁹

In 2014, Bajaj *et al.*⁵⁶ compared fecal microbiota analysis in cirrhotic patients and healthy controls. They reported that the reduction of autochthonous taxa, including Lachnospiraceae, Ruminococcaceae, and Clostridiales XIV, and increase of non-autochthonous taxa including Staphylococcaceae, Enter-ococcaceae, and Enterobacteriaceae, are linked to liver failure and plasma LPS levels in cirrhosis patients. In addition, Enterobacteriaceae and endotoxemia are enriched in patients with alcoholic compared with non-alcoholic cirrhosis.⁵⁶ Enterobacteriaceae are also frequently found in spontaneous bacterial peritonitis; an infection in decompensated cirrhosis.⁶⁰ Enterobacteriaceae are more abundant in patients

with decompensated cirrhosis compared to patients with compensated cirrhosis and healthy controls.⁵¹ The mucosal microbiota in the duodenum also differs markedly between cirrhotic patients and healthy controls.³⁴ Based on the predicted metagenomes analyzed, pathways related to nutrient absorption are enriched in the duodenal microbiota of patients with cirrhosis, while bacterial proliferation and colonization, including bacterial motility proteins and secretory systems, are over-represented in control subjects.³⁴

Bile acid pool size and composition are major regulators of microbiome structure.^{61,62} Increased primary bile acid, cholic acid (CA) can cause dysbiosis with a dramatic shift toward the Firmicutes, particularly *Clostridium* cluster XIVa and can increase production of deoxycholic acid (DCA).^{61,62} Cirrhosis-associated dysbiosis increases inflammation via metabolism, LPS, and translocation. Inflammation can suppress synthesis of bile acids in the liver.^{61,62} Secondary bile acids, which are generated by the Clostridiales cluster, are reduced in cirrhotic patients.^{63,64} Bile acids have an important role in the pathogenesis of cirrhosis,^{54,55,65} Reduced bile acid secretion facilitates oral microbiota migration to the distal gut and boosts SIBO. In contrast, activation of FXR stimulates bile acid excretion and induces production of antimicrobial peptides.^{66,67} The interaction between bile acids and microbiota plays an important role in cirrhosis.^{61,62} The data related to alteration of gut microbiota in cirrhotic patients are summarized in Table 2.^{34,56,57,59,64}

2.2.2. Gut microbiota and complications associated with liver cirrhosis—

Bacterial translocation (BT) plays a crucial role in the development of complications associated with hepatic cirrhosis.⁶⁸ By inoculating an equal amount of *Escherichia coli* (*E. coli*) into small and large intestines, it was found that BT predominantly occurs in the small intestine.⁶⁹ Consistently, the small intestine is a preferred site for BT in cirrhotic patients.⁷⁰ In addition, BT is closely associated with SIBO as well as intestinal barrier injury in cirrhotic rats.⁷¹

Spontaneous bacterial peritonitis is a common complication of liver cirrhosis because bacterial infections occur in cirrhotic patients with ascites.^{72–74} Most of the bacteria in patients with spontaneous bacterial peritonitis are *E. coli*, *Klebsiella pneumoniae*, coagulase-negative *Staphylococcus*, and *Enterococcus*.⁷⁴ *E. coli* is the predominant pathogen of spontaneous bacterial peritonitis.^{72–74}

Hepatic encephalopathy is a common complication of liver cirrhosis and a result of liver failure.^{75,76} Hepatic encephalopathy affects brain astrocytes, microglia, and neurons.^{75,76} A decrease in autochthonous bacteria and increase in Gram-negative bacteria are observed in cirrhotic patients with hepatic encephalopathy. It has been shown that elevated serum ammonia levels are linked to astrocytic impairment.⁷⁷ Moreover, ammonia-associated brain magnetic resonance imaging changes are associated with autochthonous taxa and *Enterobacteriaceae*, while white matter inflammatory changes are associated with oral taxa such as Porphyromonadaceae.⁷⁷ Only mucosal and not fecal microbiota is altered significantly in patients with hepatic encephalopathy. The Firmicutes phylum, including *Veillonella*, *Megasphaera*, *Bifidobacterium*, and *Enterococcus*, is highly enriched in hepatic encephalopathy, whereas *Roseburia* is more abundant in the non-hepatic encephalopathy group.⁷³

2.2.3. Gut microbiota and liver transplantation—Liver transplantation is one option used to treat cirrhosis or cirrhosis-associated complications,^{78,79}. Liver transplantation affects the recipient's microbiota (Table 3).^{79–81} Gut microbiota diversity is increased after liver transplantation, but does not reach the levels in healthy controls.⁸⁰ Alteration of Proteobacteria and Firmicutes links with improved cognitive level of patients with liver transplantation.⁸⁰ In 2016, the Wan laboratory established the relationship between intestinal microbiota and expression of hepatic genes in regenerating the liver, using partial hepatectomy mouse models.⁸² Removal of two-thirds of mouse liver led to rapid changes in gut microbiota, with increased Bacteroidetes S24–7 and Rikenellaceae as well as decreased Firmicutes Clostridiales, Lachnospiraceae, and Ruminococcaceae.⁸² The abundance of Ruminococcaceae, Lachnospiraceae, and S24–7 was closely linked with liver metabolism and immune functions.⁸² Hepatic secondary bile acids are positively correlated with Firmicutes and negatively with Bacteroidetes, while tauro-conjugated bile acids show positive correlations with Bacteroidetes and negative correlations with Firmicutes.⁸² Priming mice with all-trans retinoic acid lowers the ratio of Firmicutes to Bacteroidetes and increases hydrophilic bile acids, which is linked with facilitated metabolism and enhanced cell proliferation in regenerating mouse livers.⁸³

Bajaj *et al.*⁸¹ have reported the effect of liver transplantation on microbial composition and functionality in patients. Successful liver transplantation increases the microbial diversity accompanied by an increase in autochthonous and a decrease in potentially pathogenic taxa.⁸¹ The favorable changes in the gut microbiota also have the benefit of increasing fecal bile acids and urinary phenyl-acetylglutamine, accompanied with a reduction in serum ammonia and endotoxemia.⁸¹

2.2.4. Fungal dysbiosis and complications associated with liver cirrhosis—In addition to bacteria, microbiota includes archaea, protists, fungi, viruses, and bacteriophages.⁸⁴ A recent study showed fungal dysbiosis in cirrhotic patients. Bajaj *et al.*⁸⁵ have demonstrated a link between fungal and bacterial diversity in patients with liver cirrhosis, and Bacteroidetes/Ascomycota ratio can affect 90-day-hospitalization (Table 4). Moreover, *Candida* overgrowth and reduced intestinal fungal diversity are observed in patients with alcoholic cirrhosis (Table 4).⁸⁶

2.2.5. Oral microbiota and liver cirrhosis—Oral microbiota contributes to the progression of liver diseases. Elevated oral *Streptococcus* and *Veillonella* are found in cirrhotic patients.⁸⁷ Increased Enterobacteriaceae and Enterococcaceae, as well as reduced autochthonous bacteria, are found in patients with previous episodes of hepatic encephalopathy.⁸⁷ Oral microbiota has a significant impact on duodenal microbiota. At the genus level, the most distinctive taxa found in cirrhotic patients and controls include *Veillonella*, *Prevotella*, *Neisseria*, and *Haemophilus*, which are commonly found in the oral cavity.⁸⁸

Bajaj *et al.*⁸⁹ performed a direct comparison of the salivary microbiome between healthy controls and patients with cirrhosis. Relative abundance of potentially pathogenic taxa (*Prevotella* and Fusobacteriaceae) increased whereas autochthonous taxa (Lachnospiraceae and Ruminococcaceae) decreased in oral microbiota of cirrhotic patients with previous

hepatic encephalopathy.⁸³ Mi-crobes of oral origin can be present in the duodenum. Duodenal *Prevotella* and *Fusobacterium* are also increased significantly in, cirrhotic patients.³⁴ Proton pump inhibitors increase the microbiota of oral origin in patients with cirrhosis.⁹⁰ The removed pH barrier in the gastrointestinal tract allows the microbiota of oral origin to migrate along the gastrointestinal tract and even into feces.⁹⁰ Certain oral bacteria can produce high levels of hydrogen sulfide (H₂S) and methyl mercaptan (CH₃SH).⁹¹ Higher proportions of *Neisseria*, *Porphyromonas*, and *SRI* are linked to H₂S production that can damage deoxyribonucleic acid (DNA). *Prevotella*, *Veillonella*, *Atopobium*, *Megasphaera*, and *Selenomonas* are associated with production of CH₃SH, which contributes to development of hepatic encephalopathy.^{91,92} Literature related to oral microbiota and liver disease is summarized in Table 5.^{34,87,89,90}

2.3. Gut microbiota and HCC

Gut microbes are implicated in liver carcinogenesis.^{5,93,94} *Helicobacter* species are important pathogens that may be directly involved in the occurrence of liver cancer, and are found in human HCC specimens.⁹⁵ A human study has shown that *Helicobacter* is present in the liver of patients with primary liver carcinoma but not in controls without primary liver carcinoma.⁹⁶ However, *Helicobacter hepaticus* (*H. hepaticus*) is not present in HCC patients with chronic hepatitis B or C.⁹⁷

H. hepaticus infection promotes HCC in chemical and viral transgenic liver cancer models.⁹⁸ However, HCV transgene or *H. hepaticus* exposure alone is not sufficient to initiate liver cancer.⁹⁸ Moreover, increased risk of HCC is not dependent on translocation of *H. hepaticus* to the liver.⁹⁸ Gut *H. hepaticus* colonization induces nuclear factor κ -light-chain-enhancer of activated B cell signaling, which activates innate and Th1-type adaptive immunity.⁹⁸ Thus, *H. hepaticus* in the intestinal niche without translocation to the liver can change the immune signaling and play a synergistic role with chemical and viral carcinogenic factors.⁹⁸

Gut dysbiosis is found in patients with liver cirrhosis and HCC as well as animal models using streptozotocin-HFD, diethylnitrosamine (DEN), or carbon tetrachloride (CCl₄). Blooming of *E. coli* is found in cirrhotic patients who have HCC compared to those without HCC.⁹⁹ In the C57BL/6J mouse model of NASH and HCC induced by streptozotocin-HFD, a significant increase of *Atopobium* spp., *Bacteroides* spp., *Bacteroides vulgatus*, *Bacteroides acidifaciens*, *Bacteroides uniformis*, *Clostridium cocleatum*, *Clostridium xylanolyticum*, and *Desulfovibrio* spp. is associated with disease progression.¹⁰⁰ A significant reduction of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* species, along with increased *E. coli* and *Atopobium* cluster, has been found in rat models of HCC induced by DEN.¹⁰¹ Moreover, *Clostridium* spp. are reduced in CCl₄-induced liver carcinogenesis models.¹⁰² When DEN is used in combination with CCl₄, gut sterilization or TLR4 deletion reduces tumor number and volume but does not affect tumor incidence, while continuous low-dose LPS administration increases tumor number and size.¹⁰³

Changes in the microbiota of the tongue coating have been noted in patients with HCC.¹⁰⁴ Moreover, enrichment of tongue *Oribacterium* and *Fusobacterium* could be microbial biomarkers of HCC.¹⁰⁴ Microbial genes in the categories related to nickel/iron transport,

amino acid transport, energy-producing systems, and metabolism differ in abundance between HCC patients and healthy controls.¹⁰⁴

The steatohepatitis-inducing HFD (STHD-01) is a NASH-inducing HFD, which promotes HCC without chemical carcinogens.¹⁰⁵ A recent study revealed that gut bacteria associated with secondary bile acid production promote STHD-01-induced HCC development that can be prevented by antibiotics.^{105,106} In addition, *Prevotella* and *Oscilibacter*, producers of anti-inflammatory metabolites, can inhibit carcinogenesis. This anti-cancer effect may result from increased regulatory T (Treg) cells and reduced, migration of Th17 cells to the liver.⁹⁴ The gut microbiota plays a key role in HCC development and can potentially be used to treat HCC. Literature related to microbiota alteration in human HCC and animal model of HCC is summarized in Tables 6 and 7,^{95–97,99–102,104} respectively.

3. Gut microbiota-targeted therapy

Dysbiosis contributes to the development of liver diseases. Thus, restructuring the gut microbiota community to establish eubiosis can be effective in preventing or treating liver diseases.

3.1. Probiotics

Probiotics are live microorganisms that provide health benefits for the host when consumed in adequate amounts.¹⁰⁷ In addition to the beneficial effects on gastrointestinal diseases, probiotics also exert a beneficial effect in liver diseases.^{108–113}

Li *et al.*⁸³ reported that using Prohep for feeding reduces the liver tumor size in xenograft mouse models. Prohep consists of *Lactobacillus rhamnosus* (*L. rhamnosus*) GG, *E. coli* Nissle 1917, and heat-inactivated VSL#3. Prohep feeding increases the abundance of *Prevotella* and *Oscilibacter* and generates anti-inflammatory metabolites, which lead to reduced Th17 polarization and increased differentiation of Treg/Tr1 cells in the gut. In addition, *L. rhamnosus* GG protects mice from high-fructose-induced NAFLD and reduces cholesterol in HFD-fed mice.^{114,115} *Lactobacillus casei* shirota protects against NAFLD in multiple mouse NAFLD models via improved insulin sensitivity, reduced plasma LPS-binding protein, and inhibition of LPS/TLR4 signaling in the liver.^{116–118} Other probiotics such as *Lactobacillus plantarum* MA2, *Lactobacillus plantarum* NCU116, *Lactobacillus johnsonii* BS15, *Lactobacillus reuteri* GMNL-263, and *Lactobacillus gasseri* BNR17 also have protective roles in improving dyslipidemia and NAFLD.^{119–122} Moreover, *Bifidobacterium* prevents fat accumulation and increases insulin sensitivity in HFD-fed rats.¹²³ Probiotics of *Bifidobacterium* are superior to *Lactobacillus acidophilus* in decreasing hepatic fat accumulation.¹²⁴

Probiotics of *Clostridium butyricum* MIYAIRI 588, a butyrate-producing bacterium, reduce hepatic lipid droplets and improve insulin sensitivity in rats with HFD-induced NAFLD.¹²⁵ This strain also decreases hepatic lipids and LPS in rats with NAFLD induced by choline-deficient/L-amino acid-defined diet.¹²⁶ Kumar *et al.*¹²⁷ have demonstrated that probiotic-fermented milk and chlorophyllin significantly reduce the incidence of aflatoxin B1-associated HCC.

Although the health effects of probiotics are mainly obtained from animal studies, some consistent results have been generated in clinical studies. Administration of *L. rhamnosus* GG and a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophiles* has beneficial effects on obese children with NAFLD.^{128,129} VSL#3 im, proves liver function and increases glucagon-like peptide 1 levels in obese children with NASH.¹³⁰ Moreover, *L. rhamnosus* GG alters gut microbiota in patients with cirrhosis.¹³¹ Compared with placebo, *L. rhamnosus* GG increases the beneficial autochthonous Clostridiales Incertae Sedis XIV and Lachnospiraceae and reduces the abundance of Enterobacteriaceae and Porphyromonadaceae in patients with stable cirrhosis and minimal hepatic encephalopathy.¹³¹ Combination of *Bifidobacterium longum* and fructooligosaccharides (FOSs, a mixture of fermentable dietary fibers) improved minimal and overt hepatic encephalopathy in clinical studies.^{132,133} In addition, VSL#3 prevented hepatic encephalopathy in a randomized controlled clinical study.¹³⁴ Compared with baseline, 3 months treatment with VSL#3 increased psychometric hepatic encephalopathy scores and reduced the levels of arterial ammonia, SIBO, and orocecal transit time.¹³⁴ Over 6 months, VSL#3 treatment reduced the recurrence of hepatic encephalopathy in cirrhotic patients compared with the placebo-treated controls.¹³⁵ VSL#3 also decreased the hepatic venous pressure gradient, cardiac index, and heart rate, and increases systemic vascular resistance and mean arterial pressure in patients with cirrhosis and ascites.¹³⁶ This indicates that VSL#3 improves the hepatic and systemic hemodynamics in patients with cirrhosis.¹³⁶ A probiotics combination of eight strains of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, is also effective in preventing secondary hepatic encephalopathy in patients with cirrhosis.¹³⁷ However, in a study conducted by Solga *et al.*¹³⁸ 4 months supplementation with VSL#3 increased hepatic lipid content in four patients who already had steatosis. Another randomized double-blind study conducted by And reasen *et al.*¹³⁹ revealed that 4 weeks intake of *L. acidophilus* NCFM improved insulin sensitivity but did not affect systemic inflammatory response. Additionally, 6 weeks supplementation with *L. acidophilus* did not change serum lipids in volunteers who had elevated cholesterol.¹⁴⁰ More well-designed trials are needed to further study the effects of probiotics in preventing liver diseases.

3.2. Prebiotics

Prebiotics are food ingredients that selectively stimulate the growth or activity of beneficial microorganisms, such as bacteria and fungi.^{141,142} They can alter the composition and/or activity of gut microbiota. Prebiotics are useful in preventing NAFLD in laboratory animals and clinical studies.^{109,143–145}

Prebiotics of FOSs prevent NAFLD via restoring the gut microbiota composition and intestinal epithelial barrier function, leading to reduced serum LPS, hepatic inflammation, and hepatic cholesterol content in NAFLD mice.^{146–148,157} FOS supplementation significantly reduces serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in NASH patients.¹⁴³ Lactulose increases the growth of lactic acid bacteria and *Bifidobacterium*.¹⁴⁹ It also decreases serum LPS and hepatic inflammation in rats with NASH.¹⁵⁰ Chitin-glucan, a prebiotic from a fungal source, reduces hepatic triglyceride, body weight gain, and glucose intolerance via restoring *clostridial* cluster XIVa in HFD-induced obese mice.¹⁵¹ Treatment with isomaltooligosaccharides plus lycopene increases

adipose tissue fat mobilization, reduces body weight gain, and improves insulin sensitivity in HFD-induced NAFLD mice.¹⁵² Prebiotics have great potential for prevention of liver disease through improving metabolism and the intestinal barrier, as well as reducing endotoxemia.

3.3. Synbiotics

Synbiotics refer to the combination of probiotics and prebiotics in a form of synergism.¹⁵³ Synbiotics that consist of *Lactobacillus paracasei* B21060 plus arabinogalactan and FOSs reduce hepatic inflammation in diet-induced NAFLD.¹⁵⁴ Supplementation with seven probiotics consisting of *L. casei*, *L. rhamnosus*, *S. thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *B. longum*, and *L. bulgaricus* plus FOSs improves fasting blood glucose, serum triglycerides, and inflammatory cytokines in both lean and obese NAFLD patients.^{155,156} Compared to lifestyle intervention alone, synbiotics of *B. longum* plus FOSs have added benefits for NASH patients. This intervention reduces serum tumor necrosis factor α (TNF α), C-reactive protein, endotoxin, and AST.¹⁵⁷

Milk oligosaccharides (MOs) selectively increase the growth of *Bifidobacterium infantis* (*B. infantis*). Synbiotics of *B. infantis* and MOs prevent occurrence of cancer-prone NASH in western-diet-fed FXR KO male mice. *B. infantis* and MOs increase G protein-coupled bile acid receptor 1 (also known as Takeda G protein-coupled receptor, TGR5)-regulated signaling, thereby generating beneficial effects. *B. infantis* and/or MO treatment also improves ileal SCFA, signaling in western-diet-fed FXR KO mice. Furthermore, MOs alone and *B. infantis* plus MOs inhibit the growth of genus *Bilophila* and reduce the abundance of bacterial genes including dissimilatory sulfite reductase (*dsrA*) and methyl coenzyme M reductase A (*mcrA*), which are increased in mice with NASH.¹⁵⁹

3.4. Other approaches

3.4.1. Bacterial metabolite butyrate—Butyrate is generated by bacterial fermentation of non-digestible polysaccharides.^{15,160} Sodium butyrate treatment reduces inflammation and fat accumulation in diet-induced NAFLD, potentially via enriching beneficial bacteria *Christensenellaceae*, *Blautia*, and *Lactobacillus*.¹⁶¹ Additionally, butyrate supplementation reverses NASH via reducing hepatic β -muricholic acid (β -MCA) as well as DCA, which are implicated in the development of NASH in western-diet-fed FXR-KO mice.^{65,69} It has been shown that *Lactobacillus* and *Bifidobacterium* reduce adiposity and inflammation in NAFLD rats via butyrate production and butyrate receptor G-protein-coupled receptor 109A-regulated signaling.¹⁶⁰ Butyrate and its synthetic derivative, N-(1-carbamoyl-2-phenyl-ethyl) butyramide, reduce the intracellular lipid accumulation and oxidative stress in diet-induced insulin-resistant obese mice.¹⁶² Furthermore, sodium butyrate has a protective role in NAFLD pathogenesis via increased duodenal melatonin synthesis, as well as decreased hepatic inducible nitric oxide synthase in fructose-induced NAFLD mice.¹⁶³

3.4.2. Fecal microbiota transplantation—A randomized clinical trial in patients with cirrhosis and recurrent hepatic encephalopathy was conducted to compare the safety of fecal microbiota transplantation with no such intervention.¹⁶⁴ Fecal microbiota transplantation reduced hospitalization and improved cognition and dysbiosis in patients with cirrhosis with

recurrent hepatic encephalopathy, when compared with standard of care (SOC).¹⁶⁴ Fecal microbiota transplantation has protective effects in rats with CCl₄-induced hepatic encephalopathy.¹⁶⁵ Fecal microbiota transplantation reduces intestinal permeability and improves the TLR response of the liver, leading to improved cognitive function and reduced liver function indexes.¹⁶⁵

3.4.3. Diet—Diet is a contributing factor to liver diseases. Fructose-enriched diet alters liver metabolism and gut barrier function, increases endotoxemia, decreases *Bifidobacterium* and *Lactobacillus*, and eventually leads to NAFLD.¹⁶⁶ Long-term fructose consumption increases lipogenic enzymes via activation of sterol regulatory element binding protein-1c (SRFBP1c) and carbohydrate responsive element binding protein (ChREBP).¹⁶⁷ It promotes lipogenesis, hypertriglyceridemia, hepatic insulin resistance, and hepatic steatosis.¹⁶⁷ A diet rich in fermented milk, vegetables, cereals, coffee, and tea contributes to a higher microbial diversity in patients with cirrhosis.¹⁶⁸ Microbial diversity is an independent factor that reduces the risk of 90-day hospitalization.¹⁶⁸

4. Conclusions and perspectives

Gut microbiota plays a pivotal role in the pathogenesis of metabolic liver diseases. Re-establishing eubiosis using probiotics, prebiotics, and synbiotics, as well as natural products, is a promising avenue to prevent and treat liver diseases and, potentially, liver cancer. Although bile acid and SCFA-regulated pathways can explain how diet through gut microbiota affects health and disease processes, other molecular links remain to be uncovered. With the advancement of sequencing technology as well as cultural techniques, specific bacterial species and microbial functions can be uncovered to establish a causal relationship. There is no doubt that metabolomics and epigenetic genomics are powerful tools to elucidate the underlying mechanism for disease processes, leading to innovative treatment strategies. The generated information should have an impact on personalized nutrition as well as precision medicine.

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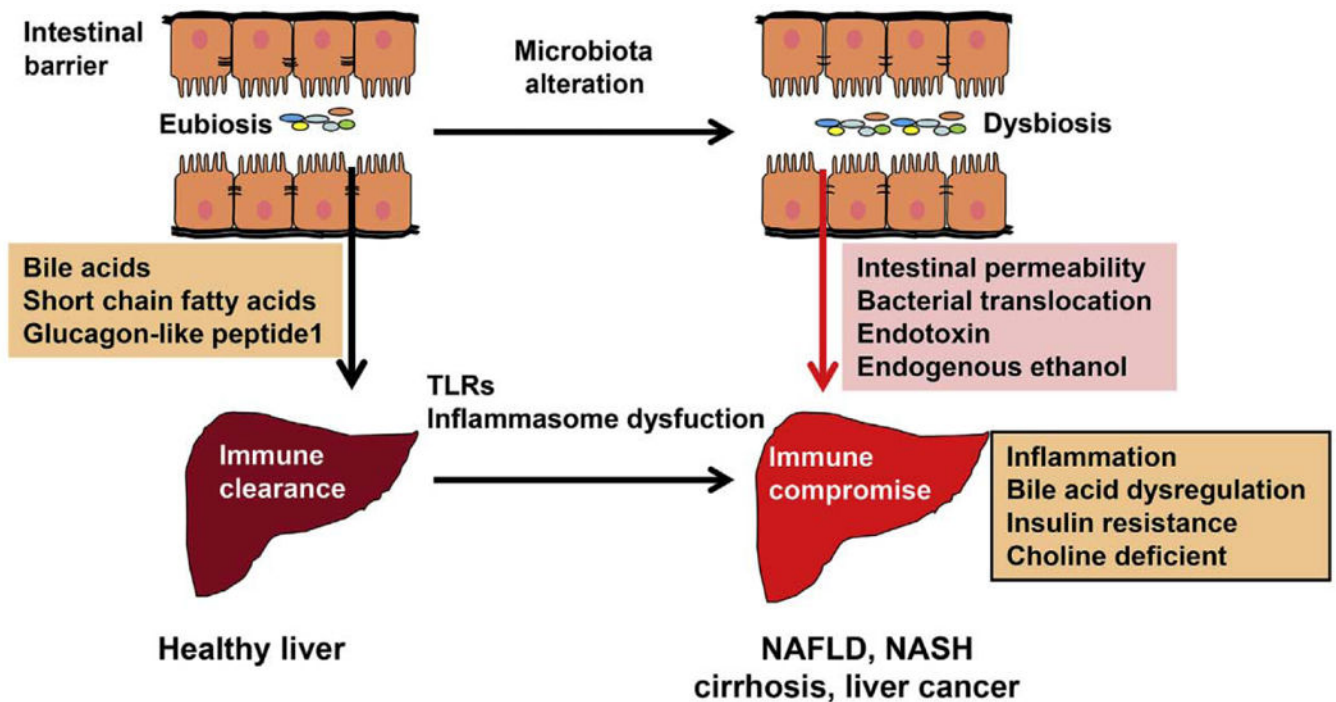


Fig. 1. The mechanisms by which gut microbiota affects liver health and diseases. Under healthy condition, intestinal barrier and integrity prevent the entry of bacterial products, such as endotoxin, from the gut into the portal circulation. Liver immune cells rapidly clear the microbial products and bacteria passing through the gut barrier, thereby establishing immune tolerance without inflammation. Gut microbiota contributes to improving insulin sensitivity, reducing inflammation, and hepatic lipid accumulation via modulating the productions of bile acids, short-chain fatty acids, glucagon-like peptide 1, etc. Factors such as antibiotics, injury, infection, and high-fat diet can cause dysbiosis. Dysbiosis increases endogenous ethanol, endotoxin, and intestinal permeability, thereby leading the translocations of bacteria and bacterial metabolites from the intestine to the liver. Bacteria and their metabolites can activate the innate immune system via toll-like receptors and cause inflammation and subsequent liver damage. Moreover, dysbiosis-associated bile acid dysregulation increases insulin resistance, hepatic lipid accumulation, and inflammatory signaling. Furthermore, dysbiosis converts choline to trimethylamine, which leads to choline deficiency. All these metabolites and factors contribute to liver diseases. Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; TLRs, Toll-like receptors.

Table 1

Gut microbiota alteration in NAFLD and NASH patients.

Authors	Population	N	Comparison	Implicated microbiota	Family	Genus	Methodology
				Phylum			
Boutsier <i>et al.</i> ¹⁴	F0/F1 fibrosis without NASH F0/F1 fibrosis with NASH	20 10	NASH vs no NASH	Bacteroidetes	Bacteroidaceae ↑	<i>Bacteroides</i> ↑	16S rRNA gene sequencing (Stool sample)
				Bacteroidetes	Prevotellaceae ↓	<i>Prevotella</i> ↓	
	F 2 fibrosis without NASH F 2 fibrosis with NASH	2 25	F 2 fibrosis vs F0/F1 fibrosis	Bacteroidetes	Bacteroidaceae ↑	<i>Bacteroides</i> ↑	
				Bacteroidetes	Prevotellaceae ↓	<i>Prevotella</i> ↓	
Firmicutes	Ruminococcaceae	<i>Ruminococcus</i> ↑					
Firmicutes	Erysipelotrichaceae ↓	N/A					
Mouzaki <i>et al.</i> ¹⁷	Steatosis patients NASH patients Healthy controls	11 22 17	NASH vs Healthy NASH vs Steatosis	Bacteroidetes ↓	N/A	N/A	Quantitative real-time PCR (Stool sample)
				Bacteroidetes ↓	N/A	N/A	
				Firmicutes	Lachnospiraceae	<i>Clostridium coccooides</i> ↑	
Shen <i>et al.</i> ¹⁸	NAFLD patients Healthy controls	25 22	NAFLD vs Healthy	Proteobacteria ↑	Enterobacteriaceae ↑	<i>Escherichia_Shigella</i> ↑	16S rDNA amplicon sequencing (Stool sample)
				Fusobacteria ↑	N/A	N/A	
				Firmicutes	Lachnospiraceae ↑	<i>Lachnospiraceae_Incenaes_Sedis</i> ↑	
				Firmicutes	Erysipelotrichaceae ↑	<i>Blautia</i> ↑	
				Firmicutes	Streptococcaceae ↑	<i>Clostridium_XVIII</i> ↑	
				Bacteroidetes ↓	Prevotellaceae ↓	<i>Streptococcus</i> ↑ <i>Prevotella</i> ↓	
	NAFLD patients with NASH NAFLD patients with fibrosis(F 2)	6 4	NASH vs no NASH F 2 fibrosis vs F0/F1 fibrosis	Firmicutes	Lachnospiraceae ↑	<i>Blautia</i> ↑	
				Proteobacteria	Enterobacteriaceae ↑	<i>Escherichia_Shigella</i> ↑	
Wang <i>et al.</i> ¹⁹	NAFLD patients Healthy controls	43 83	NAFLD vs Healthy	Bacteroidetes ↑	N/A	N/A	454 pyrosequencing of the 16S rRNA V3 region (Stool sample)
				Firmicutes ↓	Lachnospiraceae ↑	N/A	
				Proteobacteria (Gramnegative bacteria) ↓	Enterobacteriales	<i>Escherichia_Shigella</i> ↑	
Stamislawski <i>et al.</i> ²⁰	Adolescents exposure to gestational diabetes mellitus during singleton pregnancies	107	HFF vs non HFF	Proteobacteria	Desulfotribionaceae	<i>Bilophila</i> ↑	16S rRNA gene sequencing (Stool sample)
				Bacteroidetes	Prevotellaceae	<i>Paraprevotella</i> ↑	
				Proteobacteria	RF32	<i>Saturella</i> ↑	
				Bacteroidetes	Bacteroidaceae	<i>RF32</i> ↑	
				Firmicutes	Ruminococcaceae	<i>Bacteroides</i> (U-shaped pattern); ↑ or ↓	

Authors	Population	N	Comparison	Implicated microbiota	Family	Genus	Methodology
				Phylum			
Konikoff <i>et al.</i> ²³	Mild/moderate NAFLD (Stage 0–2 fibrosis)	72	Stage 0–2 fibrosis vs Stage 3 or 4 fibrosis	Proteobacteria ↑ Proteobacteria ↑	N/A	N/A	Whole genome shotgun sequencing of DNA (Stool sample)
	Advanced fibrosis (Stage 3 or 4 fibrosis)	14		Firmicutes ↓	Eubacteriaceae	<i>Eubacterium rectale</i> ↓	
				Firmicutes ↓	Ruminococcaceae	<i>Ruminococcus obeum</i> CAG:39 ↓, <i>Ruminococcus obeum</i> ↓	
Roma <i>et al.</i> ²⁴	NAFLD	30	NAFLD vs Healthy	Proteobacteria	Kiloniellaceae ↑	N/A	16S rRNA gene pyrosequencing (Stool sample)
	Healthy controls	30		Proteobacteria Firmicutes Firmicutes	Pasteurellaceae ↑ Lactobacillaceae ↑ Lachnospiraceae ↑	N/A <i>Lactobacillus</i> ↑ <i>Robinsoniella</i> ↑, <i>Roseburia</i> ↑, <i>Dorea</i> ↑	
				Firmicutes Firmicutes Bacteroidetes	Ruminococcaceae ↓ Veillonellaceae ↑ Porphyromonadaceae ↓	<i>Oscillibacter</i> ↓ N/A N/A	

Comparison of condition A vs condition B:

↑ signifies an increase in condition A relative to condition B.

↓ signifies a decrease in condition A relative to condition B.

(-) signifies no changes in condition A relative to condition B.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; HFF, hepatic fat fraction; N/A, not applicable.

Table 2

Gut microbiota alteration in cirrhotic patients.

Authors	Population	N	Comparison	Implicated microbiota		Methodology
				Phylum	Family	
Chen <i>et al.</i> ³⁴	Cirrhotic patients with HBV	24	Cirrhosis vs Healthy	Actinobacteria Firmicutes	Coriobacteriaceae Veillonellaceae	16S rRNA gene pyrosequencing (Mucosa of the distal duodenum sample)
	Cirrhotic patients with PBC	6		Proteobacteria	Pasteurellaceae	<i>Atopobium</i> [↑] <i>Dialister</i> [↑] , <i>Veillonella</i> [↑] and <i>Megasphaera</i> [↑] <i>Hemophilus</i> [↓] , <i>Neisseria</i> [↓] and <i>SR 1 genera incertae sedis</i> [↓]
	Healthy controls	28				
Bajaj <i>et al.</i> ⁵⁶	Patients with liver cirrhosis	219	Cirrhosis vs Healthy	Firmicutes	Lachnospiraceae [↓] , Ruminococcaceae [↓] and Clostridiales XIV [↓] ,	Multi-tagged pyrosequencing (Stool sample)
	Healthy controls	25		Firmicutes	Staphylococcaceae [↑] , Enterococcaceae [↑]	
				Proteobacteria Firmicutes Bacteroidetes	Enterobacteriaceae [↑] Veillonellaceae [↓] , Porphyromonadaceae [↓]	
Bajaj <i>et al.</i> ⁵⁷	Patients with liver cirrhosis	278 out of 335	Hospitalized vs non Hospitalized	Bacteroidetes	Bacteroidaceae [↓]	16S rRNA pyrosequencing (Stool sample)
	Non hospitalized patients with liver cirrhosis within 90 days	162		Firmicutes	Clostridiales XIV [↓] , Lachnospiraceae [↓] , Ruminococcaceae [↓]	
	Hospitalized patients with liver cirrhosis	94		Firmicutes Proteobacteria Bacteroidetes [↓] Bacteroidetes [↓]	Enterococcaceae [↓] Enterobacteriaceae [↓] BacterDitetes_Bacteroidaceae [↓] Bacteroidetes_Porphyrromonadaceae [↓]	

signifies a decrease in condition A relative to condition B.
Abbreviations: HBV, hepatitis B virus; PBC, primary biliary cirrhosis; DM, diabetes mellitus; N/A, not applicable.

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Table 3

Gut microbiota alteration and liver transplantation.

Authors	Population	N	Comparison	Implicated microbiota	Phylum	Family	Genus	Methodology
Sun <i>et al.</i> ⁷⁹	Post-LT patients Healthy controls	9 15	Post-LT vs Pre-LT	Proteobacteria Proteobacteria Actinobacteria Proteobacteria Proteobacteria Verrucomicrobia	Pasteurellaceae Enterobacteriaceae Micromonosporaceae Desulfobacteriaceae Eubacteriaceae Akkermaniaceae	<i>Actinobacillus</i> ↓ <i>Escherichia</i> ↓ and <i>Shigella</i> ↓ <i>Micromonosporaceae</i> ↑ <i>Desulfobacteres</i> ↑ the <i>Sarcina</i> genus of <i>Eubacteriaceae</i> ↑ <i>Akkermansia</i> ↑	MiSeq-PE250 sequencing of the V4 region of 16S rRNA (Stool sample)	
Bajaj <i>et al.</i> ⁸⁰	Outpatient patients with cirrhosis on the LT list Healthy controls	45 45	Improved cognition post-LT vs Pre-LT	Proteobacteria ↓ and Firmicutes ↑	N/A	N/A	N/A	Multitagged sequencing; 16s rRNA (V1-V2) sequencing (Stool sample)
			Not improved cognition after LT vs Healthy	Proteobacteria ↑ and Firmicutes ↓	N/A	N/A	N/A	
			post-LT vs Pre-LT	Firmicutes (-)	Ruminococcaceae ↑ and Lachnospiraceae ↑	N/A	N/A	
				Bacteroidetes (-)	N/A	N/A	N/A	
				Proteobacteria (-)	Enterobacteriaceae	<i>Escherichia</i> ↓, <i>Salmonella</i> ↓ and <i>Shigella</i> ↓		
			Pre-LT patients vs Healthy	Proteobacteria (-)	Enterobacteriaceae	<i>Escherichia</i> , ↑ <i>Shigella</i> ↑, <i>Salmonella</i> ↑		
			Post-LT patients vs Healthy	Firmicutes (-) Actinobacteria Bacteroidetes	Ruminococcaceae ↑ and Lachnospiraceae ↑ Bifidobacteriaceae ↓ Bacteroidaceae ↑	N/A N/A N/A		
Bajaj <i>et al.</i> ⁸¹	Patients with cirrhosis	40	Post-LT vs Pre-LT	Proteobacteria Proteobacteria Actinobacteria Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Bacteroidetes	Enterobacteriaceae Sutterellaceae Desulfovibrionales Bifidobacteriaceae Clostridiales Incertae Sedis XI Ruminococcaceae Clostridiales Incertae Sedis XIII Lachnospiraceae Streptococcaceae Clostridiaceae Rikenellaceae	<i>Shigella</i> ↓, <i>Escherichia</i> ↓, and <i>Salmonella</i> ↓ <i>Sutereella</i> ↑ <i>Bifidobacterium</i> ↓ <i>Desulfatibacter</i> ↑, and <i>Sporanaerobacter</i> ↑ <i>Clostridium</i> IV ↑, <i>Osdiiibacte</i> ↑, <i>Anaerovorax</i> ↑	Multitagged sequencing (Stool sample)	

Authors	Population	N	Comparison	Implicated microbiota	Family	Genus	Methodology
				Phylum		<i>Anaerostipes</i> [↑] , <i>Clostridium XIVb</i> [↑] , <i>Blautia</i> [↑] <i>Roseburia</i> [↑] , and <i>Dorea</i> [↑] <i>Streptococcus</i> [↑] <i>Butyrivococcus</i> [↑] , <i>Crostridium XIVa</i> [↑] <i>Alistipes</i> [↑]	

Comparison of condition A vs condition B:

- ↑ signifies an increase in condition A relative to condition B.
 - ↓ signifies a decrease in condition A relative to condition B.
 - (-) signifies no changes in condition A relative to condition B.
- Abbreviations: LT, liver transplantation; N/A, not applicable.

Table 4

Fungal dysbiosis in complications associated with liver cirrhosis.

Authors	Population	N	Comparison	Implicated microbiota		Methodology
				Phylum	Family	
Bajaj <i>et al.</i> ⁸⁵	Outpatients cirrhotics	77	Inpatients <i>vs</i> Outpatients	Basidiomycota ↓ Ascomycota	Bacteroidetes/Ascomycota ratio ↑ Saccharomycetaceae	N/A <i>Candida</i> ↑
	Inpatients cirrhotics	66	After antibiotics <i>vs</i> before antibiotics	Ascomycota	Saccharomycetaceae	<i>Candida</i> ↑
	Controls	26	Inpatients <i>vs</i> Outpatients	Ascomycota	Saccharomycetaceae	<i>Candida</i> ↑
				Proteobacteria	Enterobacteriaceae ↑	N/A
				Firmicutes	Enterococcaceae ↑	N/A
			Outpatients <i>vs</i> Controls	Basidiomycota ↓ Ascomycota	N/A Saccharomycetaceae	N/A <i>Candida</i> ↑
Yang <i>et al.</i> ⁸⁶	Healthy individuals	8	Patients <i>vs</i> Healthy individuals	Ascomycota	Enterobacteriaceae ↑	N/A
	Alcohol-dependent patients (nonprogressive liver disease)	10		Firmicutes	Enterococcaceae ↑	N/A
	Patients with alcoholic liver cirrhosis	4	Outpatients on antibiotics	Ascomycota ↑ Proteobacteria	Saccharomycetaceae Pasteurellaceae ↑	<i>Candida</i> ↑ N/A
				Ascomycota	Saccharomycetaceae	<i>Candida</i> ↑
				Ascomycota	Saccharomycetaceae	
				Ascomycota	Saccharomycetaceae	

Comparison of condition A *vs* condition B:

↑ signifies an increase in condition A relative to condition B.

↓ signifies a decrease in condition A relative to condition B.
Abbreviation: N/A, not applicable.

Table 5

Oral microbiota alteration in patients with cirrhosis.

Authors	Population	N	Comparison	Implicated microbiota			Methodology	
				Phylum	Family	Genus		
Chen <i>et al.</i> ³⁴	Cirrhotic patients	30	Patients vs Controls	Actinobacteria	Coriobacteriaceae	<i>Atopobium</i> ↑	16S rRNA gene pyrosequencing (Mucosal from the distal duodenum sample)	
				Firmicutes	Veillonellaceae	<i>Dialister</i> ↑, <i>Veillonella</i> ↑, and <i>Megasphaera</i> ↑		
	Healthy controls	28	Proteobacteria	Pasteurellaceae	<i>Hemophilus</i> ↓			
			Proteobacteria	Neisseriaceae	<i>Neisseria</i> ↓			
			undefined	undefined	<i>SR 1 genera incertae sedis</i> ↓			
Qin <i>et al.</i> ⁸⁷	Patients with cirrhosis	98	Patients vs Controls	Firmicutes	Streptococcaceae	<i>Streptococcus</i> ↑	Quantitative metagenomics (Stool sample)	
				Firmicutes	Veillonellaceae	<i>Veillonella</i> ↑		
	Healthy controls	83	Firmicutes	Enterococcaceae	N/A			
			Proteobacteria	Enterobacteriaceae	N/A			
			Proteobacteria	Enterobacteriaceae	N/A			
Bajaj <i>et al.</i> ⁸⁹	Patients with cirrhosis without HE	59	Patients vs Controls	Bacteroidetes	Prevotellaceae	<i>Prevotella</i> ↑	Quantitative metagenomics (Stool or saliva sample)	
				Fusobacteria	Fusobacteriaceae	N/A		
	Patients with cirrhosis with previous HE	43	Firmicutes	Lachnospiraceae	N/A			
			age-matched controls	32	Ruminococcaceae	↓		
					Proteobacteria	Enterobacteriaceae		N/A
Bajaj <i>et al.</i> ⁹⁰	Cirrhotic outpatients on PPI	59	PPI users vs Patients without PPI and Controls	Firmicutes	Enterococcaceae	↑	Multi-tagged sequencing (Stool sample)	
				Firmicutes	Streptococcaceae	↑		
	Cirrhotic outpatients not on PPI	78	Firmicutes	Lachnospiraceae	↓			
			Healthy controls	45	Firmicutes	Lachnospiraceae		↓
					Firmicutes	Lachnospiraceae		N/A

Authors	Population	N	Comparison	Implicated microbiota			Methodology
				Phylum	Family	Genus	
	Cirrhotic outpatients not on PPI	15	After vs Before PPI initiation	Bacteroidetes Firmicutes	Porphyromonadaceae ↑ Streptococcaceae ↑	N/A N/A	
	Patients with decompensated cirrhosis on chronic PPI	15	PPI withdrawal vs Pre-PPI therapy	Bacteroidetes Firmicutes	Porphyromonadaceae ↓ Streptococcaceae ↓, and Veillonellaceae ↓	N/A N/A	

Comparison of condition A vs condition B:

↑ signifies an increase in condition A relative to condition B.

↓ signifies a decrease in condition A relative to condition B.

Abbreviations: HE, hepatic encephalopathy; PPI, proton pump inhibitors; N/A, not applicable.

Table 6

Gut microbiota alteration in human HCC.

Authors	Population	N	Comparison	Implicated microbiota			Methodology
				Phylum	Family	Genus	
Nilsson <i>et al.</i> ⁹⁵	Liver specimens of patients with cholangiocarcinoma HCC human specimens Controls (liver tissue from patients with resected metastasés from colorectal cancers)	14 16 20	HCC or cholangiocarcinoma specimens <i>vs</i> Controls	Proteobacteria	Helicobacteraceae	<i>Helicobacter</i> spp. [†]	PCR and DNA sequencing (HCC human specimens)
Huang <i>et al.</i> ⁹⁶	HCC human specimens Controls without HCC	20 16	HCC specimens <i>vs</i> Controls	Proteobacteria	Helicobacteraceae	<i>Helicobacter pylori</i> [†]	PCR, DNA sequencing, and immunostaining (Liver specimens)
Krüttgen <i>et al.</i> ⁹⁷	Patients with viral-induced HCC Control patients	14 11	Patients with viral-induced HCC <i>vs</i> Control patients	Proteobacteria	Helicobacteraceae	<i>H. hepaticus</i> (no exist)	PCR (Stool sample)
Grat <i>et al.</i> ⁹⁹	Patients with HCC Non HCC patients	15 15	HCC <i>vs</i> non HCC	Proteobacteria	Enterobacteriaceae	<i>Escherichia coli</i> [†]	Culturing on enriching and selective agar media (Stool sample)
Lu <i>et al.</i> ¹⁰⁴	Early liver carcinoma patients with cirrhosis Healthy controls	35 25	HCC <i>vs</i> Healthy	Firmicutes Fusobacteria	Lachnospiraceae Fusobacteriaceae	<i>Oribacterium</i> changes <i>Fusobacterium</i> changes	16S rRNA gene sequencing (Tongue coat sample)

Comparison of condition A *vs* condition B:

[†] signifies an increase in condition A relative to condition B.
Abbreviation: HCC, hepatocellular carcinoma.

Table 7

Gut microbiota alteration in HCC animal models.

Authors	Model	Agent	Comparison	Implicated microbiota			Methodology
				Phylum	Family	Genus	
Xie <i>et al.</i> ¹⁰⁰	NASH-HCC C57BL/6J mouse model	STZ-HFD	NASH-HCC vs Controls	Actinobacteria Bacteroidetes	Coriobacteriaceae Bacteroidaceae	<i>Atopobium</i> spp. [↑] <i>Bacteroides</i> spp. [↑] <i>Bacteroides vulgatus</i> , [↑] <i>Bacteroides acidifaciens</i> [↑] and <i>Bacteroides uniformis</i> [↑]	16S rDNA gene pyrosequencing (Stool sample)
				Firmicutes	Clostridiaceae	<i>Clostridium cocleatum</i> , [↑] <i>Clostridium</i> and <i>xylanolyticum</i> [↑]	
				Proteobacteria	Desulfovibrionaceae	<i>Desulfovibrio</i> spp. [↑]	
Zhang <i>et al.</i> ¹⁰¹	Male Sprague-Dawley HCC rats	DEN	HCC rats vs Controls	Firmicutes Firmicutes Actinobacteria	Lactobacillaceae Enterococcaceae Bifidobacteriaceae	<i>Lactobacillus</i> [↓] <i>Enterococcus</i> [↓] <i>Bifidobacterium</i> [↓]	16S rRNA based quantitative real-time PCR (Stool sample)
Gómez-Hurtado <i>et al.</i> ¹⁰²	Female Balb/c fibrosis mice	CCl ₄	Fibrosis mice vs Controls	Firmicutes Firmicutes	Clostridiaceae Clostridiaceae	<i>Clostridia</i> spp. [↓] <i>Clostridium coccooides</i> [↓] <i>Clostridium leptum</i> [↓]	Quantitative real-time PCR (Stool sample)

Comparison of condition A vs condition B:

[↑] signifies an increase in condition A relative to condition B.

[↓] signifies a decrease in condition A relative to condition B.

Abbreviations: STZ-HFD, streptozotocin-high fat diet; DEN, diethylnitrosamine; HCC, hepatocellular carcinoma; CCL₄ carbon tetrachloride.