Liver Research 3 (2019) 3–18

Contents lists available at ScienceDirect

Liver Research

journal homepage: http://www.keaipublishing.com/en/journals/liver-research

Review Article

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

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ARTICLE INFO

Article history:
Received 5 July 2018
Received in revised form
5 November 2018
Accepted 12 February 2019

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

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.

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 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

☆ Edited by Peiling Zhu and Genshu Wang.

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https://doi.org/10.1016/j.livres.2019.02.001

Table 1
Gut microbiota alteration in NAFLD and NASH patients.

Authors	Population	*N*	Comparison	Implicated microbiota			Methodology
				Phylum	Family	Genus	
Boursier *et al.*[14]	F0/F1 fibrosis without NASH	20	NASH *vs* no NASH	Bacteroidetes	Bacteroidaceae↑	*Bacteroides*↑	16S rRNA gene sequencing (Stool sample)
	F0/F1 fibrosis with NASH	10		Bacteroidetes	Prevotellaceae↓	*Prevotella*↓	
	F ≥ 2 fibrosis without NASH	2	F ≥ 2 fibrosis vs F0/1 fibrosis	Bacteroidetes	Bacteroidaceae↑	*Bacteroides*↑	
	F ≥ 2 fibrosis with NASH	25		Bacteroidetes	Prevotellaceae↓	*Prevotella*↓	
				Firmicutes	Ruminococcaceae	*Ruminococcus*↑	
				Firmicutes	Erysipelotrichaceae↓	N/A	
Mouzaki *et al.*[17]	Steatosis patients	11	NASH *vs* Healthy	Bacteroidetes↓	N/A	N/A	Quantitative real-time PCR (Stool sample)
	NASH patients	22	NASH *vs* Steatosis	Bacteroidetes↓	N/A	N/A	
	Healthy controls	17		Firmicutes	Lachnospiraceae	*Clostridium coccoides*↑	
Shen *et al.*[18]	NAFLD patients	25	NAFLD *vs* Healthy	Proteobacteria↑	Enterobacteriaceae↑	*Escherichia_Shigella*↑	16S rDNA amplicon sequencing (Stool sample)
	Healthy controls	22		Fusobacteria↑	N/A	N/A	
				Firmicutes	Lachnospiraceae↑	*Lachnospiraceae_Incertae_Sedis*↑, *Blautia*↑	
				Firmicutes	Erysipelotrichaceae↑	*Clostridium_XVIII*↑	
				Firmicutes	Streptococcaceae↑	*Streptococcus*↑	
				Bacteroidetes↓	Prevotellaceae ↓	*Prevotella* ↓	
	NAFLD patients with NASH	6	NASH *vs* no NASH	Firmicutes	Lachnospiraceae↑	*Blautia* ↑	
	NAFLD patients with fibrosis (F ≥ 2)	4	F ≥ 2 fibrosis *vs* F0/F1 fibrosis	Proteobacteria	Enterobacteriaceae ↑	*Escherichia_Shigella*↑	
Wang *et al.*[19]	NAFLD patients	43	NAFLD *vs* Healthy	Bacteroidetes↑	N/A	N/A	454 pyrosequencing of the16S rRNA V3 region (Stool sample)
	Healthy controls	83		Firmicutes↓	Lachnospiraceae↑	N/A	
				Proteobacteria (Gramnegative bacteria ↑)	Enterobacteriales	*Escherichia_Shigella*↑	
Stanislawski *et al.*[20]	Adolescents exposure to gestational diabetes mellitus during singleton pregnancies	107	HFF *vs* non HFF	Proteobacteria	Desulfovibrionaceae	*Bilophila*↑	16S rRNA gene sequencing (Stool sample)
				Bacteroidetes	Prevotellaceae	*Paraprevotella*↑ *Suturella*↑	
				Proteobacteria	RF32	*RF32*↑	
				Bacteroidetes	Bacteroidaceae	*Bacteroides* (U-shaped pattern; ↑or↓)	
				Firmicutes	Ruminococcaceae	*Oscillospira* ↓	
Del Chierico *et al.*[21]	NAFLD patients	61	NAFLD *vs* Healthy	Proteobacteria	Bradyrhizobiaceae	*Bradyrhizobium* ↑	454 pyrosequencing of the16S rRNA V1—V3 region (Stool sample)
	Healthy controls	54		Firmicutes	unassigned	*Anaerococcus*↑	
				Firmicutes	unassigned	*Peptoniphilus*↑	
				Actinobacteria	Propionibacteriaceae	*Propionibacterium acnes*↑	
				Firmicutes	Lachnospiraceae	*Dorea*↑	
				Firmicutes	Ruminococcaceae	*Ruminococcus*↑	
				Firmicutes	Ruminococcaceae	*Oscillospira* ↓	
				Bacteroidetes	Rikenellaceae	*Rikenellaceae* ↓	

Zhu *et al.*[22]	NASH patients	22	NASH or Obese *vs* Healthy	Actinobacteria↓	Bifidobacteriaceae↓	*Bifidobacterium*↓	16S rRNA pyrosequencing (Stool sample)
	Obese patients	25		Bacteroidetes↑	Bacteroidaceae (−)	*Bacteroides* (−)	
	Healthy controls	16		Bacteroidetes↑	Porphyromonadaceae (−)	*Parabacteroides* (−)	
				Bacteroidetes↑	Porphyromonadaceae	*Porphyromonas* (−)	
				Bacteroidetes↑	Prevotellaceae↑	*Prevotella*↑	
				Bacteroidetes↑	Rikenellaceae↑	*Alistipes*↑	
				Firmicutes↓	Clostridiales family XI (−)	*Anaerococcus* (−)	
				Firmicutes↓	Peptoniphilaceae	*Finegoldia* (−)	
				Firmicutes↓	Peptoniphilaceae	*Peptoniphilus*↑	
				Firmicutes↓	Lachnospiraceae↓	*Blautia*↓	
				Firmicutes↓	Clostridiaceae	*Clostridium* (−)	
				Firmicutes↓	Lachnospiraceae	*Coprococcus*↓	
				Firmicutes↓	Eubacteriaceae	*Eubacterium*↓	
				Firmicutes↓	Lachnospiraceae	*Roseburia*↓	
				Firmicutes↓	Ruminococcaceae	*Ruminococcus* (−)	
				Firmicutes↓	Ruminococcaceae↓	*Faecalibacterium* (−)	
				Firmicutes↓	Clostridiaceae	*Oscillospira*↓	
				Firmicutes↓	Ruminococcaceae	*Ruminococcus*↓	
				Firmicutes↓	Veillonellaceae (−)	*Acidaminococcus* (−)	
				Firmicutes↓	Veillonellaceae (−)	*Dialister* (−)	
				Firmicutes↓	Veillonellaceae (−)	*Megamonas* (−)	
				Proteobacteria ↓	Alcaligenaceae↓	N/A	
				Proteobacteria ↓	Campylobacteraceae (−)	*Campylobacter* (−)	
				Proteobacteria ↓	Enterobacteriaceae↑	*Escherichia*↑	
Koniffkoff *et al.*[23]	Mild/moderate NAFLD (Stage 0−2 fibrosis)	72	Stage 0−2 fibrosis *vs* Stage 3 or 4 fibrosis	Proteobacteria↑	N/A	N/A	Whole genome shotgun sequencing of DNA (Stool sample)
	Advanced fibrosis (Stage 3 or 4 fibrosis)	14		Firmicutes ↓	Eubacteriaceae	*Eubacterium rectale*↓	
				Firmicutes ↓	Ruminococcaceae	*Ruminococcus obeum* CAG:39↓, *Ruminococcus obeum*↓	
Roma *et al.*[24]	NAFLD	30	NAFLD *vs* Healthy	Proteobacteria	Kiloniellaceae↑	N/A	16S rRNA gene pyrosequencing (Stool sample)
	Healthy controls	30		Proteobacteria	Pasteurellaceae↑	N/A	
				Firmicutes	Lactobacillaceae↑	*Lactobacillus*↑	
				Firmicutes	Lachnospiraceae↑	*Robinsoniella*↑, *Roseburia*↑, *Dorea*↑	
				Firmicutes	Ruminococcaceae↓	*Oscillibacter*↓	
				Firmicutes	Veillonellaceae↑	N/A	
				Bacteroidetes	Porphyromonadaceae↓	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.

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 wadworthia* increases in response to a western diet or HFD.[27,28] *Bilophila wadworthia* 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 $F \geq 2$ have higher abundance of *Bacteroides* and lower abundance of *Prevotella* compared to those without NASH. Patients with $F \geq 2$ 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 $>10^5$ 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 Proteobacteria.[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 Helicobacteraceae 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 level.[5] 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 Enterobacteriaceae, Veillonelaceae, and Streptococcaceae, and reduced Lachnospiraceae.[59] Moreover, several commensal genera, such as

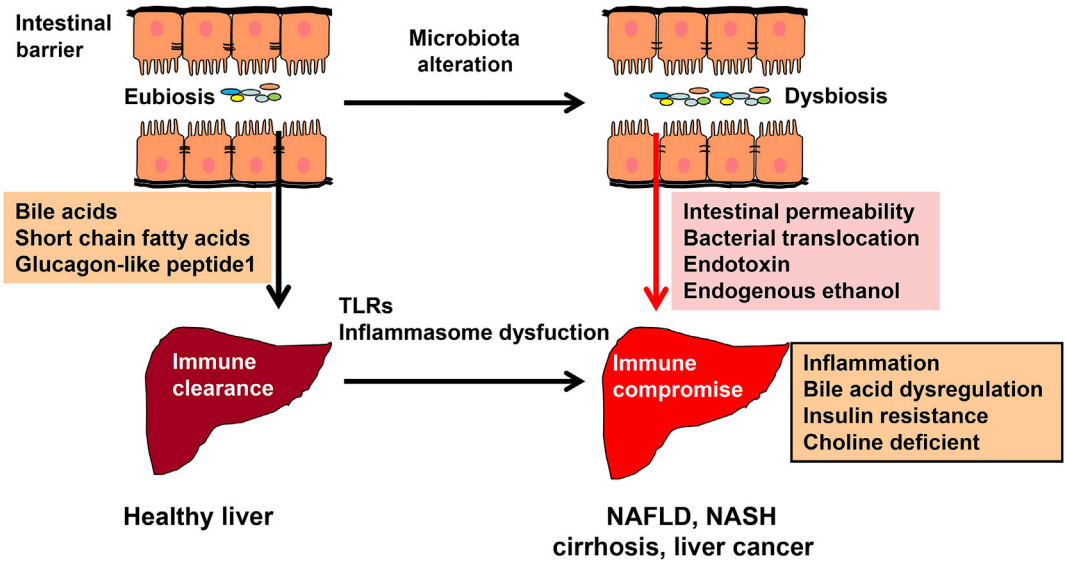

Fig. 1. The mechanisms by which gut microbiota affects liver health and diseases. Under healthy condition, intestinal barrier and integrity prevent the entry of bacterial products, such as endotoxin, from the gut into the portal circulation. Liver immune cells rapidly clear the microbial products and 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.

Coprococcus, *Pseudobutyrivibrio*, 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, Enterococcaceae, 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*,

Table 2
Gut microbiota alteration in cirrhotic patients.

Authors	Population	*N*	Comparison	Implicated microbiota			Methodology
				Phylum	Family	Genus	
Chen *et al.*[34]	Cirrhotic patients with HBV	24	Cirrhosis *vs* Healthy	Actinobacteria Firmicutes	Coriobacteriaceae Veillonellaceae	*Atopobium*↑ *Dialister*↑, *Veillonella*↑ and *Megasphera*↑	16S rRNA gene pyrosequencing (Mucosa of the distal duodenum sample)
	Cirrhotic patients with PBC	6		Proteobacteria	Pasteurellaceae	*Hemophilus*↓, *Neisseria*↓ and *SR 1 genera incertae sedis*↓	
	Healthy controls	28					
Bajaj *et al.*[56]	Patients with liver cirrhosis	219	Cirrhosis *vs* Healthy	Firmicutes	Lachnospiraceae↓, Ruminococcaceae↓ and Clostridiales XIV ↓,	N/A	Multi-tagged pyrosequencing (Stool sample)
	Healthy controls	25		Firmicutes	Staphylococcaceae↑, Enterococcaceae↑	N/A	
				Proteobacteria	Enterobacteriaceae↑		
				Firmicutes	Veillonellaceae↓,	N/A	
				Bacteroidetes	Porphyromonadaceae↓	N/A	
Bajaj *et al.*[57]	Patients with liver cirrhosis	278 out of 335	Hospitalized *vs* non Hospitalized	Bacteroidetes	Bacteroidaceae↓	N/A	16S rRNA pyrosequencing (Stool sample)
	Non hospitalized patients with liver cirrhosis within 90 days	162		Firmicutes	Clostridiales XIV↓, Lachnospiraceae↓, Ruminococcacae↓	N/A	
				Firmicutes	Enterococcaceae↓	N/A	
				Proteobacteria	Enterobacteriaceae↓	N/A	
	Hospitalized patients with liver cirrhosis	94		Bacteroidetes↓	Bacteroidetes_Bacteroidaceae↓,	N/A	
				Bacteroidetes↓	Bacteroidetes_ Porphyromonadaceae↓	N/A	
				Firmicutes	Firmicutes_Lactobacillaceae↑	N/A	
				Firmicutes	Firmicutes_Enterococcaceae↑	N/A	
				Firmicutes	Firmicutes_Clostridiales XIV↓	N/A	
				Firmicutes	Firmicutes_Lachnospiraceae↓	N/A	
				Firmicutes	Firmicutes_Ruminococcaceae↓	N/A	
				Proteobacteria	Proteobacteria_ Enterobacteriaceae↑	N/A	
				Proteobacteria	Proteobacteria_ Pasteurellaceae↑	N/A	
	Non DM DM	191 87	DM *vs* non DM	Bacteroidetes Proteobacteria	Bacteroidetes_Bacteroidaceae↓ Firmicutes_Eubacteriaceae↓	N/A N/A	
				Firmicutes	Firmicutes_Ruminococcaceae↑	N/A	
				Firmicutes	Firmicutes_Veillonellaceae↓	N/A	
				Firmicutes	Firmicutes_Streptococcaceae↓	N/A	
				Actinobacteria	Actinobacteria_ Streptomycetae↓	N/A	Mucosal sample
				Firmicutes	Firmicutes_Clostridiacaeae↓	N/A	
				Bacteroidetes	Bacteroidetes_Prevotellaceae↑	N/A	
				Fusobacteria	Fusobacteria_ Fusobacteriaceae↑	N/A	

Chen *et al.*[59]	Patients with liver cirrhosis	36	Cirrhosis *vs* Healthy	Bacteroidetes↓	Enterobacteriaceae↑	N/A		The 16S rRNA V3 region pyrosequencing; Real-time PCR (Stool sample)
	Healthy controls	24		Proteobacteria ↑	N/A	N/A		
				Fusobacteria↑	N/A	N/A		
				Firmicutes	Veillonellaceae↑	N/A		
				Firmicutes	Streptococcaceae↑	N/A		
				Firmicutes	Lachnospiraceae↓	*Coprococcus*↓, *Pseudobutyrivibrio*↓ *Roseburia*↓		
Kakiyama *et al.*[64]	Early cirrhotics	23	Cirrhosis *vs* Healthy	Firmicutes	Lachonospiraceae↓, Ruminococcaceae↓	N/A		Culture-independent multitagged-pyrosequencing (Stool sample)
				Firmicutes	Lachnospiraceae	*Blautia* ↓		
	Advanced cirrhotics	24		Bacteroidetes	Rikenellaceae↓	N/A		
	Healthy controls	14		Proteobacteria	Enterobacteriaceae↑	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: HBV, hepatitis B virus; PBC, primary biliary cirrhosis; DM, diabetes mellitus; N/A, not applicable.

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 Ruminococcacea, 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 phenylacetylglutamine, 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.[89] Microbes of oral origin can be present in the duodenum. Duodenal *Prevotella* and *Fusobacterium* are also increased significantly in

Table 3
Gut microbiota alteration and liver transplantation.

Authors	Population	*N*	Comparison	Implicated microbiota			Methodology
				Phylum	Family	Genus	
Sun *et al.*[79]	Post- LT patients	9	Post-LT *vs* Pre-LT	Proteobacteria Proteobacteria	Pasteurellaceae Enterobacteriaceae	*Actinobacillus*↓ *Escherichia*↓ and *Shigella*↓	MiSeq-PE250 sequencing of the V4 region of 16S rRNA (Stool sample)
	Healthy controls	15		Actinobacteria Proteobacteria Proteobacteria Verrucomicrobia	Micromonosporaceae Desulfobacteraceae Eubacteriaceae Akkermansiaceae	*Micromonosporaceae*↑ *Desulfobacterales*↑ *the Sarcina genus of Eubacteriaceae*↑ *Akkermansia*↑	
Bajaj *et al.*[80]	Outpatient patients with cirrhosis on the LT list	45	Improved cognition post-LT *vs* Pre-LT	Proteobacteria↓and Firmicutes↑	N/A	N/A	Multitagged sequencing; 16s rRNA (V1−V2) sequencing (Stool sample)
	Healthy controls	45					
			Not improved cognition after LT *vs* Healthy	Proteobacteria↑and Firmicutes↓	N/A	N/A	
			post-LT *vs* Pre-LT	Firmicutes (−)	Ruminococcaceae↑and Lachnospiraceae↑	N/A	
				Bacteroidetes (−) Proteobacteria (−)	N/A Enterobacteriaceae	N/A *Escherichia*↓, *Salmonella*↓ and *Shigella*↓	
			Pre-LT patients *vs* Healthy Post-LT patients *vs* Healthy	Proteobacteria (−) Firmicutes (−)	Enterobacteriaceae Ruminococcaceae↑and Lachnospiraceae↑	*Escherichia*,↑*Shigella*,↑*Salmonella*↑ N/A	
				Actinobacteria Bacteroidetes	Bifidobacteriaceae↓ Bacteroidaceae↑	N/A N/A	
Bajaj *et al.*[81]	Patients with cirrhosis	40	Post-LT *vs* Pre-LT	Proteobacteria	Enterobacteriaceae	*Shigella*↓, *Escherichia*↓, and *Salmonella*↓	Multitagged sequencing (Stool sample)
				Proteobacteria Proteobacteria Actinobacteria Firmicutes	Sutterellaceae Desulfovibrionales Bifidobacteriaceae Clostridiales Incertae Sedis XI	*Suterella*↑ *Bilophila*↑ *Bifidobacterium*↓ *Desulfatibacter*↑, and *Sporanaerobacter*↑	
				Firmicutes Firmicutes Firmicutes	Ruminococcaceae Clostridiales Incertae Sedis XIII Lachnospiraceae	*ClostridiumIV*↑, *Oscillibacte*↑, *Anaerovorax*↑ *Anaerostipes*↑, *Clostridium XIVb*↑, *Blautia*↑, *Roseburia*↑, and *Dorea*↑	
				Firmicutes Firmicutes Bacteroidetes	Streptococcaceae Clostridiaceae Rikenellaceae	*Streptococcus*↑ *Butyricicoccus*↑, *ClostridiumXIVa*↑ *Alistipes*↑	

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	Genus	
Bajaj *et al.*[85]	Outpatients cirrhotics	77	Inpatients *vs* Outpatients	Basidiomycota↓	Bacteroidetes/Ascomycota ratio↑	N/A	Metagenomics (Stool sample)
				Ascomycota	Saccharomycetaceae	*Candida*↑	
	Inpatients cirrhotics	66	After antibiotics *vs* before antibiotics	Ascomycota	Saccharomycetaceae	*Candida*↑	
	Controls	26	Inpatients *vs* Outpatients	Ascomycota	Saccharomycetaceae	*Candida*↑	
				Proteobacteria	Enterobacteriaceae↑	N/A	
				Firmicutes	Enterococcaceae↑	N/A	
			Outpatients *vs* Controls	Basidiomycota↓	N/A	N/A	
				Ascomycota	Saccharomycetaceae	*Candida*↑	
			Inpatients *vs* Controls	Proteobacteria	Enterobacteriaceae↑	N/A	
				Firmicutes	Enterococcaceae↑	N/A	
			Outpatients on antibiotics	Ascomycota↑	Saccharomycetaceae	*Candida*↑	
				Proteobacteria	Pasteurellaceae↑	N/A	
Yang *et al.*[86]	Healthy individuals	8	Patients *vs* Healthy individuals	Ascomycota	Saccharomycetaceae	*Candida*↑	Illumina MiSeq platform sequencing of the V4 region of 16S rRNA (Stool sample)
	Alcohol-dependent patients (nonprogressive liver disease)	10					
	Patients with alcoholic liver cirrhosis	4					

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.

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 (H_2S) and methyl mercaptan (CH_3SH).[91] Higher proportions of *Neisseria*, *Porphyromonas*, and *SR1* are linked to H_2S production that can damage deoxyribonucleic acid (DNA). *Prevotella, Veillonella*, *Atopobium*, *Megasphaera*, and *Selenomonas* are associated with production of CH_3SH, 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 (CCl_4). 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 *Atopobiumcluster*, has been found in rat models of HCC induced by DEN.[101] Moreover, *Clostridium* spp. are reduced in CCl_4-induced liver carcinogenesis models.[102] When DEN is used in combination with CCl_4, 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

Table 5
Oral microbiota alteration in patients with cirrhosis.

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

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 *Oscillibacter* 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 improves 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 fructo-oligosaccharides (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

Table 6
Gut microbiota alteration in human HCC.

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

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 *et al.*[100]	NASH-HCC C57BL/6J mouse model	STZ-HFD	NASH-HCC *vs* Controls	Actinobacteria Bacteroidetes Firmicutes Proteobacteria	Coriobacteriaceae Bacteroidaceae Clostridiaceae Desulfovibrionaceae	*Atopobium* spp.↑ *Bacteroides* spp.↑ *Bacteroides vulgatus*,↑ *Bacteroides acidifaciens*,↑and *Bacteroides uniformis*↑ *Clostridium cocleatum*, ↑ *Clostridium* and *xylanolyticum*↑ *Desulfovibrio* spp.↑	16S rDNA gene pyrosequencing (Stool sample)
Zhang *et al.*[101]	Male Sprague-Dawley HCC rats	DEN	HCC rats *vs* Controls	Firmicutes Firmicutes Actinobacteria	Lactobacillaceae Enterococcaceae Bifidobacteriaceae	*Lactobacillus*↓ *Enterococcus*↓ *Bifidobacterium*↓	16S rRNA based quantitative real-time PCR (Stool sample)
Gómez-Hurtado *et al.*[102]	Female Balb/c fibrosis mice	CCl_4	Fibrosis mice *vs* Controls	Firmicutes Firmicutes Firmicutes	Clostridiaceae Clostridiaceae Clostridiaceae	*Clostridia* spp.↓ *Clostridium coccoides*↓ *Clostridium leptum*↓	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; CCl_4, carbon tetrachloride.

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 Andreasen *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 isomalto-oligosaccharides 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 (TNFα), 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.[158] *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 CCl_4-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 (SREBP1c) 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.

Authors' contributions

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

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the USA National Institutes of Health (NIH), USA grants U01CA179582 and R01 CA222490. We also thank Michelle Nguyen (Department of Medical Pathology and Laboratory Medicine, University of California, Davis, Sacramento, CA, USA) and Mindy Huynh (Department of Dermatology, University of California, Davis, Sacramento, CA, USA) for reviewing the manuscript.

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