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# Gut Microbiota Modulation: A Viable Strategy to Address Medical Needs in Hepatocellular Carcinoma and Liver Transplantation

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ABSTRACT

Hepatocellular carcinoma (HCC) is the most common malignancy of the liver, posing a significant threat to public health. Although liver transplantation (LT) is an effective treatment for HCC, ischemia–reperfusion (I/R) injury, transplant rejection, and complications after LT can greatly reduce its effectiveness. In recent years, transplant oncology has come into being, a comprehensive discipline formed by the intersection and integration of surgery, oncology, immunology, and other related disciplines. Gut microbiota, an emerging field of research, also plays a crucial role. Through the microbiome–gut–liver axis, the gut microbiota has an impact on the onset and progression of HCC as well as LT. This review summarizes the mechanisms by which the gut microbiota affects HCC and its bidirectional interactions with chronic liver disease that can develop into HCC as well as the diagnostic and prognostic value of the gut microbiota in HCC. In addition, gut microbiota alterations after LT were reviewed, and the relationship between the gut microbiota and liver I/R injury, the efficacy of immunosuppressive drugs used, and complications after LT were discussed. In the era of LT oncology, the role of the gut microbiota in HCC and LT should be emphasized, which can provide new insights into the management of HCC and LT via gut microbiota modulation.



## 1. Introduction

Liver cancer is one of the most common malignancies worldwide and poses a huge medical burden. Liver cancer can be divided into two categories: primary and secondary. Primary liver cancer is a highly dangerous malignant tumor that accounts for the vast majority of liver cancer cases. Hepatocellular carcinoma (HCC) is the most common primary liver cancer, accounting for approximately 90% of all cases [1]. Despite progress in its prevention, diagnosis, prognosis, and treatment, the morbidity and mortality of HCC continue to increase [2]. Patients diagnosed with HCC are often diagnosed at an advanced stage. Although they receive systemic therapy, their overall response rate is poor.

Orthotopic liver transplantation (OLT) is the best treatment option for patients with end-stage liver disease, especially for patients with decompensated liver function who are not suitable for surgical resection and local ablation. HCC can be cured by liver transplantation (LT). In 1996, Mazzaferro et al. [3] first proposed the selection of patients with small HCC with cirrhosis for LT, establishing the Milan criteria as a result. Transplant recipients who meet the Milan criteria have achieved long-term survival; however, due to the strictness of the criteria, many patients with HCC are deemed unsuitable for transplantation. In response to this, extensions of the Milan criteria, such as the Hangzhou, Valencia, University of California, and University Clinic of Navarra criteria, have been successively proposed [4–6]. With the continuous

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strengthening of the concept of multidisciplinary comprehensive treatment, transplantation oncology has emerged over time. In 2014, Professor Hibi et al. from Kumamoto University in Japan [7] proposed the concept of liver transplant oncology. The development of LT oncology brings together the strength of multiple disciplines, including surgery, oncology, immunology, pharmacology, and imaging [8,9]. Several studies have found that the gut microbiota plays an important role in transplantation oncology. In the era of LT oncology, the role of the gut microbiota cannot be ignored.

The gut microbiota is crucial for human health. As a "virtual metabolic organ," the gut microbiota can interact with and influence various organs and different systems, including the brain, kidney, cardiovascular system, and skeletal system [10,11]. Although the liver does not interact directly with microbiota, it is anatomically connected to the gut. The discovery of the gut–liver axis has greatly improved our understanding of the role that the gut microbiota plays in the onset and progression of liver disease [12,13]. The gut microbiota can transfer microbial components and metabolites to the liver via the portal vein, thereby influencing liver function. For instance, short-chain fatty acids (SCFAs), the main microbial metabolites of dietary fibers in the intestine, can suppress hepatic inflammation by inducing regulatory T cells (Tregs) [14]. The gut microbiome-mediated 7α-dehydroxylation of primary bile acids (BAs) can produce secondary BAs, which induce DNA damage and regulate liver anti-tumor immune surveillance [15]. Other substances, such as lipopolysaccharide (LPS) and lipoteichoic acid, can also influence liver function [16]. Therefore, the gut microbiota is important for maintaining normal liver function. Furthermore, the gut microbiota has been found to not only serve as an emerging tool for the diagnosis and prognosis of HCC because of its operational simplicity and precision of analysis but has also been reported to exert pathological effects on HCC and LT by regulating metabolism and immunity [17,18].

In this review, the gut microbiota is briefly introduced. Then, the mechanisms by which the gut microbiota affects HCC and its bidirectional interaction with chronic liver disease, which can evolve into HCC, are summarized as well as the diagnostic and prognostic value of the gut microbiota in HCC. In addition, the role of the gut microbiota in LT is discussed. The objective of this review is to summarize achievements in the field and gain insights into the management of HCC and LT via the modulation of the gut microbiota.

## 2. Gut microbiota

The gastrointestinal tract is the largest microbial system in the human body, expanding over an area of 250–400 $m^2$ [19]. As many as 10–100 trillion microbes have been estimated to live in the human gastrointestinal tract [20]. The collective genome of the human gastrointestinal tract, known as the "microbiome," is 150 times larger than the human genome [21]. A recent study compiled and analyzed 204 938 genomes and 170 602 708 genes from the human gut microbiome dataset and generated a Unified Human Gastrointestinal Genome and Protein catalog [22]. As the most complete catalog of human gut microbiota sequences available, this information provides an opportunity to enhance our understanding of the human gut microbiota.

The gut microbiota contains several types of microbes, including bacteria, fungi, archaea, and viruses [23]. Bacteria in the gut microenvironment are divided into seven major groups (Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, and Cyanobacteria), with Bacteroidetes and Firmicutes accounting for over 90% [24]. Compared to bacteria, fungi and archaea have received less attention but have also formed a complete research system. Although fungi only account for 0.1% of gut microbes, the crucial role of gut fungi cannot be ignored, especially *Candida* and *Saccharomyces* [25]. In addition, archaea are considered stable commensals in the gastrointestinal tract that can be involved in several physiological activities [26].

The gut microbiota connects various parts of the body into an organized system through pathways such as the gut–liver and gut–brain axes, which form a delicate symbiotic relationship in the human body. When the human body is in good condition, good and bad microbes depend on and restrict each other and can be found in a relatively balanced state, providing a natural defense system for the maintenance of human health. However, dysbiosis of the gut microbiota can cause damage to the body, which is closely related to the progression of a variety of metabolic, immune, and neurological diseases, including diabetes [27], hypertension and atherosclerosis [28], inflammatory bowel disease [29], and autism [30]. Therefore, the gut microbiota plays an important role in health and disease.

## 3. Gut microbiota and HCC

Despite a 2% annual decline in the incidence of liver cancer in recent decades, the mortality rate of liver cancer continues to rank second among the different types of cancer [2], with 412 216 deaths in China and 32 332 deaths in the United States [31], posing a huge threat to global public health. HCC is the most common type of primary liver cancer and one of the leading causes of cancer death worldwide. It is characterized by a poor prognosis, with a five-year survival rate of 18% [32,33]. Furthermore, there is increasing evidence of a strong link between HCC and the gut microbiota (Fig. 1).

### 3.1. Dysbiosis, leaky gut, and HCC

The intestinal tract serves as the primary site for the digestion and absorption of nutrients. It also functions as an immune barrier, and the intestinal immune system is divided into innate, adaptive, and mucosal immune systems [34]. The intestinal mucosa is directly connected to the outside world, coming into contact with various organic and inorganic substances in the intestine. This makes the intestinal mucosal epithelium the first protective barrier against pathogens. However, gut microbiota dysbiosis leads to increased intestinal permeability and the disruption of the intestinal mucosal barrier, with mucosal barrier disruption typically resulting in the breakdown of intestinal barrier tight junctions, known as leaky gut syndrome [35]. A strong link has been reported between gut microbiota imbalance and leaky gut. In addition, gut microbiota dysbiosis and leaky gut can lead to microbial translocation and increased liver exposure to microbiota-derived products and metabolites [36], which may cause liver cirrhosis. Liver cirrhosis is associated with the progression of early liver disease and can eventually cause HCC [37]. Therefore, dysbiosis of the gut microbiota, leaky gut, and HCC are closely related.

Gut microbiota dysbiosis, leaky gut, and HCC have a complex relationship with each other. Gut microbiota dysbiosis can cause leaky gut, which can aggravate the imbalance. In addition, intestinal leakage can also mediate the occurrence and development of HCC through the liver–intestine axis. Intestinal bacterial metabolites regulate intestinal permeability and inflammation through Toll-like receptor 4 (TLR4)-mediated pathways [38]. In a mouse model of diethylnitrosamine (DEN) plus carbon tetrachloride ($CCl_4$)-induced HCC, ligands derived from the bacterial gut microbiota were triggers that promoted TLR4-dependent tumors. TLR4-positive HCC cells have dramatically improved invasion and migratory capabilities [39]. Additionally, TLR4 signaling-induced leaky guts may enhance HCC progression. Moreover, LPS can cause

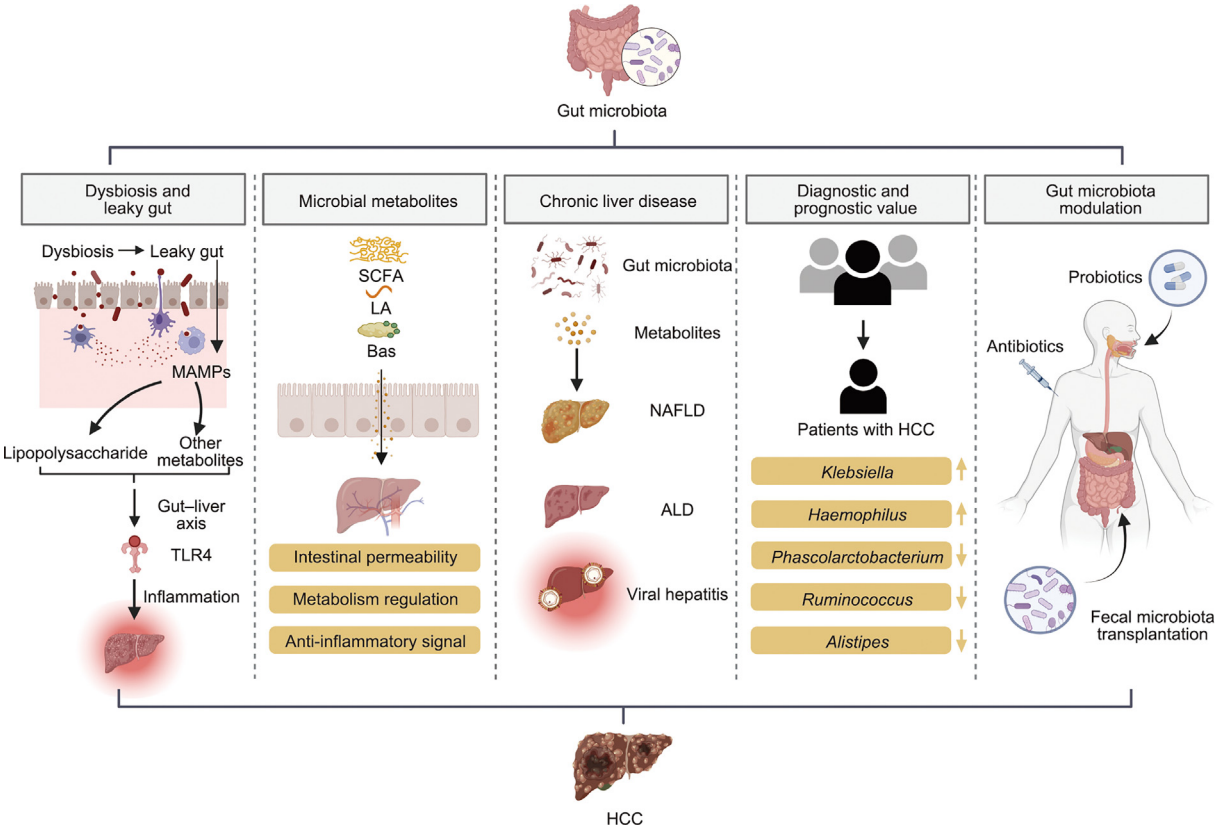


**Fig. 1.** Gut microbiota and HCC. MAMP: microbiota-associated molecular pattern; TLR4: Toll-like receptor 4; LA: lactic acid; NAFLD: non-alcoholic fatty liver disease; ALD: alcoholic liver disease.

inflammation through TLR4 and is the most commonly used marker of microbiota-associated molecular pattern (MAMP). After oral antibiotic mixtures were used to eradicate intestinal bacteria and lower LPS levels in mice, substantial tumor regression occurred [40]. Furthermore, calprotectin, which is mainly derived from neutrophils, has direct antibacterial effects and plays a role in the innate immune response. Calprotectin is present in various body fluids and is a useful surrogate marker for inflammatory responses [41]. Lower percentages of *Akkermansia* and *Bifidobacterium* were detected in the feces of HCC patients, whereas the concentration of calprotectin was significantly increased, which represents a more intense inflammatory reaction and hepatocyte injury [42]. Overall, it is speculated that, in the case of a leaky gut, bacteria can translocate to the upper gastrointestinal tract, from which the generated LPS enters the liver, thereby activating the corresponding immune response, resulting in the compensatory proliferation of hepatocytes, and ultimately inducing HCC.

### 3.2. Gut microbial metabolites and HCC

Through portal vein circulation, gut-derived metabolites, cellular components, hormones, and other substances enter the liver and interact with the immune cells [43]. The effects of compounds generated by microbes vary. Some contribute to inflammation, hepatitis, liver cirrhosis, fibrosis, and even cancer, while others maintain barrier integrity and prevent pathogen penetration. Currently, SCFAs, lactic acid (LA), and BAs are the main gut microbial metabolites that have been studied [44].

#### 3.2.1. SCFAs

SCFAs, also known as volatile fatty acids, are the main products of the fermentation of undigested carbohydrates, such as oligosaccharides, non-starch polysaccharides, and resistant starch, by anaerobic bacteria in the colon and include acetic acid, propionic acid, and butyric acid. In human metabolism, SCFAs are of great importance in maintaining the normal operation of the large intestine and the morphology and function of colonic epithelial cells [45].

Several studies have suggested that SCFAs can efficiently promote Tregs during active immune responses and control cancer progression by downregulating cancer related pathways. McBrearty et al. [46] found that SCFA-fed HBx transgenic mice exhibited normal liver tissue development and inhibited HCC growth. *Lactobacillus reuteri* plays an anticancer role by inhibiting the production of specific cytokines by type 3 congenital lymphocytes; however, the level of *Lactobacillus reuteri* is significantly reduced in patients with HCC, and SCFA supplementation can play a role similar to *Lactobacillus reuteri*, thus controlling the progression of HCC [47]. In contrast, SCFAs exceeding the threshold concentration and tolerated by the host have been shown to cause HCC [48,49]. Therefore, the specific mechanism by which SCFAs affect HCC needs to be further clarified, and further studies are urgently required.

#### 3.2.2. Lactic acid

LA, a metabolic mediator, can determine the activity and function of immune cells. In the tumor microenvironment, the combined accelerated metabolism of tumor cells and cancer-associated fibroblasts creates an immunological environment that supports tumor development. Tumor tissues deplete local energy, forcing neighboring immune cells to process high concentrations of metabolites, such as LA, in the absence of nutrients, leading to immune suppression and tumor growth [50]. De la Cruz-López et al. [51] concluded that sodium lactate can inhibit several

glycolytic enzymes of $CD4^+$ T cells and reduce the expression of glucose flow, resulting in the accumulation of T cells at inflammatory sites. In addition, the level of LA increases significantly with the progression of liver disease and can be used as an effective tool for early HCC diagnosis [52]. Gu et al. [53] demonstrated that LA produced by over-glycolytic HCC cells can stimulate extracellular signal-regulated kinase (ERK) phosphorylation in the co-cultured human hepatic stellate cell line LX2 and the leukemic monocyte cell line THP1 non-tumor cells through NDRG3 and MCT1, which promotes HCC cell malignancy and stemness. As an important metabolite in cancer metabolic reprogramming, the question of how the gut microbial LA intervenes in HCC requires further investigation.

#### 3.2.3. Bile acids

BAs are endogenous steroid molecules synthesized from cholesterol, which is the main component of bile. BAs can regulate the differentiation and function of T cells, including inflammatory T helper 17 (TH17) cells and anti-inflammatory Tregs [54]. Conde de la Rosa et al. [55] demonstrated that the level of total hepatic BAs in HCC patients is significantly increased, and BAs can stimulate the generation of tumor-initiating stem cells. Ma et al. [15] also showed that the synthesis of BAs can mediate the upregulation of C–X–C motif ligand 16 (CXCL16) in mice, control the accumulation of wild-type natural killer T cells, regulate the expression of CXCL16 messenger RNA (mRNA) in human hepatic sinusoid endothelial cells, and play anti-HCC and cancer cell metastasis role. Moreover, the metabolic regulator sirtuin 5 (SIRT5) has been reported to inhibit HCC immune escape by mediating the metabolism of BAs, which suggests a strategy for HCC treatment using BAs chelators [56]. Furthermore, deoxycholic acid, a secondary BA produced by the dihydroxylation of primary BAs produced by certain strains of the intestinal *Clostridium* cluster, can stimulate cellular functions related to inflammation and tumorigenesis through the senescence secretome, exacerbating the development of HCC [57]. Generally, the accumulation of BAs plays a key role in HCC development because it can cause cell damage. Thus, changing the type or amount of BAs in patients with HCC can reduce inflammation and represents a potential effective method for alleviating disease progression.

Overall, more studies are needed to investigate the precise mechanism by which gut microbial metabolites affect HCC as they may reveal two aspects of the control of HCC development. In addition, the effects of other gut microbial metabolites on HCC, such as choline metabolites, phenolic derivatives, and indole derivatives, should also be explored.

### 3.3. Gut microbiota and chronic liver disease

HCC usually develops as a result of chronic liver disease, with approximately 80%–90% of HCC cases occurring alongside advanced liver fibrosis or cirrhosis; thus, cirrhosis is the greatest risk factor for the development of HCC [58]. The main causes of cirrhosis are non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and viral hepatitis, which are also closely associated with HCC [59]. The role of intrahepatic microbiota in liver diseases has been emphasized in pathogenesis and tumorigenesis. Liu et al. [60] found that the abundance of *Stenotrophomonas maltophilia* was higher in the intrahepatic microbiota of patients with HCC and cirrhosis, which can promote the progression of HCC by stimulating the senescence-associated secretory phenotype and inducing the secretion of inflammatory factors in the liver. Through the gut–liver axis, the gut microbiota can also interact with the host in the human body and function in the onset and progression of liver inflammation, fibrosis, and cirrhosis [61]. Understanding the alterations in the gut microbiota during cirrhosis and their relationship with chronic liver disease will provide new strategies for the prevention and treatment of HCC (Table 1 [62–85]).

#### 3.3.1. Non-alcoholic fatty liver disease

NAFLD includes a range of liver diseases, from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and eventually HCC. The link between the gut microbiota and NAFLD was first revealed in the early 1880s. Drenick et al. [86] demonstrated that both liver steatosis and bacterial overgrowth were observed in patients with NAFLD and that the use of metronidazole significantly decreased steatosis, suggesting that intestinal bacteria are closely related to NAFLD. Wang et al. [62] found that compared with healthy controls (HCs), the levels of Bacteroidetes were higher but Firmicutes were lower in the feces of patients with NAFLD, and the ratio of Firmicutes to Bacteroidetes decreased significantly. Furthermore, Leung et al. [63] found that *Methanobrevibacter*, *Phascolarctobacterium*, *Slackia*, and *Dorea formicigenerans* may be risk factors for NAFLD. *Eubacterium rectale* and *Bacteroides vulgatus* were shown to be the most abundant organisms in mild/moderate NAFLD, whereas *Bacteroides vulgatus* and *Escherichia coli* were the most abundant in advanced fibrosis [64]. Yu et al. [65] also found that the abundance of *Veillonella*, *Collinsella*, *Latilactobacillus*, *Dialister*, and *Bifidobacterium* increased gradually with the progression of NAFLD. Rapid developments have enabled the relationship between the gut microbiota and NAFLD to be studied extensively.

Dysbiosis of the gut microbiota and NAFLD is closely linked. Bacterial toxic substances and metabolic mediators are generated in high doses during dysbiosis and accumulate in the intestinal tract, which may promote liver inflammation and NAFLD formation. Björkholm et al. [66] found that in the absence of the gut microbiota, high expression of constitutively active receptors can affect the levels of BAs, bilirubin, and steroid hormones, stimulating the development of NAFLD. In C57BL/J6 mice, high fructose consumption can lead to small intestinal bacteria overgrowth and increase intestinal endotoxin translocation, thereby resulting in the development of NAFLD [67]. Thuy et al. [68] regulated the gut microbiota and reduced intestinal permeability by controlling the intake of fructose and carbohydrates in NAFLD patients and concluded that dietary fructose intake can increase intestinal endotoxin translocation, which may contribute to the development of NAFLD. Additionally, the gut microbiota can regulate energy balance and affect NAFLD through intercommunication pathways. Two mechanisms have been found to stimulate fatty acid oxidation, reduce fat storage, and protect germ-free (GF) mice from diet-induced obesity, including elevated levels of fasting-induced adipose factor and increased 5′-adenosine monophosphate (AMP)-activated protein kinase activity [69].

The composition of the gut microbiota in patients with NAFLD differs from that in the healthy population. Furthermore, changes in energy and metabolites, endotoxin-mediated inflammation, and increased intestinal permeability are currently believed to be the main mechanisms by which the gut microbiota is involved in the pathogenesis and progression of NAFLD. Studying the role of the gut microbiota in NAFLD can better guide the prevention and treatment of HCC.

#### 3.3.2. Alcoholic liver disease

Chronic alcohol consumption can cause damage to multiple organs, especially the liver, which is involved in alcohol metabolism [87]. The intestinal oxidation of alcohols results in an increase in acetaldehyde. Acetaldehyde can cause significant alterations in the quality and quantity of the gut microbiota. By detecting alterations in the gut microbiota, the connection between chronic ethanol feeding and the symbiotic bacterial microbiota was investigated in an ALD mouse model, showing that Bacteroidetes and Firmicutes decreased and Proteobacteria and Actinobacteria

**Table 1**
Gut microbiota and chronic liver disease.

| Authors | Gut microbiota | Associated disease | Outcomes | Reference |
|---|---|---|---|---|
| Wang et al. | Bacteroidetes and Firmicutes | NAFLD | Higher levels of Bacteroidetes and lower levels of Firmicutes in the feces of NAFLD patients | [62] |
| Leung et al. | *Methanobrevibacter*, *Phascolarctobacterium*, *Slackia*, and *Dorea formicigenerans* | NAFLD | *Methanobrevibacter*, *Phascolarctobacterium*, *Slackia*, and *Dorea formicigenerans* may be risk characteristics of NAFLD | [63] |
| Loomba et al. | *Eubacterium rectale, Bacteroides vulgatus,* and *Escherichia coli* | NAFLD | Higher *Eubacterium rectale and Bacteroides vulgatus* abundance in mild/moderate NAFLD and *Bacteroides vulgatus* and *Escherichia coli* in advanced fibrosis | [64] |
| Yu et al. | *Veillonella*, *Collinsella*, *Latilactobacillus*, *Dialister*, and *Bifidobacterium* | NAFLD | The abundance of *Veillonella*, *Collinsella*, *Latilactobacillus*, *Dialister*, and *Bifidobacterium* increased gradually with the progress of NALFD | [65] |
| Björkholm et al. | — | NAFLD | High expression of constitutive active receptors can affect stimulate the development of NAFLD in the absence of the gut microbiota | [66] |
| Bergheim et al. | — | NAFLD | Markedly reduced hepatic lipid accumulation after concomitant treatment with antibiotics, which may restrict the development of NAFLD | [67] |
| Thuy et al. | — | NAFLD | Dietary fructose intake can increase intestinal endotoxin translocation, which may contribute to the development of NAFLD | [68] |
| Bäckhed et al. | — | NAFLD | Increased fatty acid metabolism and phosphorylated AMP-activated protein kinase in GF mice | [69] |
| Bull-Otterson et al. | Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria | ALD | Decreased Bacteroidetes and Firmicutes, and increased Proteobacteria and Actinobacteria were features of ALD | [70] |
| Llopis et al. | *Faecalibacterium* | ALD | Key deleterious species were associated with sAH while the *Faecalibacterium* genus was associated with noAH | [71] |
| Brandl et al. | — | ALD | ALD is associated with disturbance of BA homeostasis | [72] |
| Jiang et al. | Parvoviridae and Herpesviridae | ALD | Mammalian viruses increased in fecal samples from ALD patients | [73] |
| Hsu et al. | *Propionibacterium*, *Lactobacillus*, and *Leuconostoc* phages | ALD | ALD may be associated with intestinal virome | [74] |
| Yan et al. | Bacteroidetes and Verrucomicrobia | ALD | Bacterial translocation and overgrowth may contribute to the development and progression of ALD | [75] |
| Bode et al. | — | ALD | Gut-derived endotoxin can function in the development of ALD | [76] |
| Llorente et al. | *Enterococcus* | ALD | Gastric acid-induced *Enterococcus* overgrowth can aggravate the liver injury, steatosis, inflammation and fibrosis, thus leading to ALD | [77] |
| Duan et al. | *Enterococcus faecalis* | ALD | Bacteriophages targeting cytolytic *Enterococcus faecalis* can abrogate ethanol-induced liver injury and steatosis, thus alleviating ALD | [78] |
| Everard et al. | *Akkermansia muciniphila* | ALD | *Akkermansia muciniphila* can promote barrier function by improving mucus production, and protect against ALD | [79] |
| Grander et al. | *Akkermansia muciniphila* | ALD | Treatment with *Akkermansia muciniphila* can protect against experimental ALD | [80] |
| Liu et al. | *Faecalibacterium*, *Ruminococcus*, and *Ruminoclostridium* | HBV | The species richness of fecal microbiota in B-HCC patients was much higher than NBNC-HCC patients and HCs | [81] |
| Wang et al. | — | HBV | Intestinal barrier disruption secondary to zonulin pathway disorder may contribute to the deterioration of HBV infection | [82] |
| Sultan et al. | *Prevotella, Succinivibrio, Catenibacterium, Megasphaera*, and Ruminococcaceae | HCV | Increase in the abundance of *Prevotella, Succinivibrio, Catenibacterium, Megasphaera*, and Ruminococcaceae was shown in treatment-naive HCV | [83] |
| Pérez-Matute et al. | *Lachnospira* | HCV | The low abundance of *Lachnospira* may be another cause of persistent injury and inflammation in HCV-infected patients | [84] |
| Heidrich et al. | *Lactobacillus* | HCV | *Lactobacillus* increased in HCV-infected patients | [85] |

AMP: 5′-adenosine monophosphate; GF: germ-free; sAH: severe alcoholic hepatitis; noAH: without alcoholic hepatitis; HBV: hepatitis B virus; B-HCC: HBV-related HCC; NCNB-HCC: non-HBV non-hepatitis C virus related HCC; HC: health control; HCV: hepatitis C virus.

increased significantly after chronic feeding with ethanol [70]. After transplanting GF mice with the gut microbiota of patients with or without alcoholic hepatitis (AH), liver inflammation in mice harboring the gut microbiota from an alcoholic with severe AH was found to be more severe than that in mice harboring the gut microbiota from a patient without AH (noAH), suggesting that the microbiota from noAH patients may reverse ALD [71]. In addition, patients with ALD have been shown to have reduced hepatic cholic acid synthesis and increased serum BAs, suggesting that ALD is associated with a disturbance of BA homeostasis [72]. Furthermore, Jiang et al. [73] reported that mammalian viruses, such as Parvoviridae and Herpesviridae, increased in fecal samples from ALD patients. Hsu et al. [74] also found that *Propionibacterium*, *Lactobacillus*, and *Leuconostoc* phages decreased in patients with alcohol use disorders, revealing that ALD may be associated with intestinal virome. Thus, there is a close correlation between the gut microbiota and ALD.

Recent evidence suggests a role for the gut microbiota and its metabolites in the pathophysiology of ALD. Alcohol abuse can lead to intestinal bacterial overgrowth and translocation, which may be the result of reduced bactericidal c-type lectins, Reg3b and Reg3g. This suggests that bacterial translocation and overgrowth are the main contributors to the development and progression of alcoholic steatohepatitis [75]. Dysbiosis of the gut microbiota was also found to induce mucosal alterations and enhance intestinal permeability, resulting in endotoxemia [88]. The endotoxemia of patients with alcoholic cirrhosis was significantly higher than that of patients with non-alcoholic cirrhosis, which showed that gut-derived endotoxins play a role in the development of ALD [76]. In addition, hepatic macrophages and Kupffer cells can recognize *Enterococcus* and induce the secretion of interleukin (IL)-1β, which leads to ethanol-induced liver inflammation and hepatocyte injury. Llorente et al. [77] found that inhibiting gastric acid-induced *Enterococcus* overgrowth could aggravate liver injury, steatosis, inflammation, and fibrosis in mice. The isolation of highly strain-specific *Enterococcus faecalis* bacteriophages may enable the direct editing of the gut microbiota. Bacteriophages targeting the cytolytic *Enterococcus faecalis* abrogate ethanol-induced liver injury and steatosis [78].

Furthermore, the gut microbiota and its metabolites can be used for the treatment of ALD. The administration of tributyrin or glycerol in mice receiving long-term intragastric ethanol administration was found to inhibit the decrease in the expression of tight junction proteins and the destruction of intestinal integrity, thus alleviating intestinal barrier damage [89]. *Akkermansia muciniphila* can promote barrier function by improving mucus production [79]. Grander et al. [80] found that patients with alcoholic steatohepatitis showed a decrease in *Akkermansia muciniphila* abundance, and *Akkermansia muciniphila* administration in ALD mice protected against steatosis, neutrophil infiltration, and liver injury. Therefore, *Akkermansia muciniphila* supplementation may be used for the treatment of ALD.

Dysbiosis of the gut microbiota increases the susceptibility of patients to ALD through intestinal barrier damage, toxins, and molecular metabolism, ultimately increasing the risk of developing HCC. Interventions targeting the gut microbiota and its metabolites can be used to treat ALD by introducing new strategies for the prevention of HCC.

#### 3.3.3. Viral hepatitis

Dysbiosis of the gut microbiota is associated with viral hepatitis, represented by hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Chronic HBV infection is a high-risk factor for HCC. HBV is believed to mediate chronic liver damage through abnormal immune attack, leading to chronic necroinflammation and hepatocellular regeneration, which are the main causes of HCC [90]. 16S ribosomal RNA (rRNA) analyses of HCs, HBV-related HCC (B-HCC) individuals, and non-HBV non-HCV-related HCC (NBNC-HCC) individuals showed that the species richness of fecal microbiota in B-HCC patients was much higher than that in the other two groups. However, the feces of patients with NBNC-HCC were found to contain more potential pro-inflammatory bacteria and decreased levels of *Faecalibacterium, Ruminococcus*, and *Ruminoclostridium*, which further reduced the levels of SCFAs [81]. Wang et al. [82] assessed zonulin levels in HCs, HBV-related cirrhosis patients, and HBV-associated HCC patients and found that the zonulin levels were significantly higher in patients with HBV-related cirrhosis and HBV-associated HCC, suggesting that intestinal barrier disruption secondary to zonulin pathway disorder may be a contributing factor to the deterioration of HBV infection.

HCV is also a major cause of HCC [91]. The carcinogenesis of the HCV-infected liver can be attributed to damage to hepatocytes carrying the virus, which can cause excessive cell proliferation. In addition, chronic inflammation and oxidative stress may lead to the accumulation of cancer-related gene mutations in hepatocytes [92]. The diversity of HCV microbiota also changes. For instance, Sultan et al. [83] found that treatment-naive HCV showed an increase in the abundance of *Prevotella, Succinivibrio, Catenibacterium, Megasphaera*, and Ruminococcaceae, whereas Pérez-Matute et al. [84] showed that 15 bacterial genera differed in HCV patients, and the degree of liver fibrosis in HCV patients could lead to differences in the composition of the gut microbiota. Pérez-Matute et al. [84] also found that the low abundance of *Lachnospira* in HCV-infected patients may be another cause of persistent injury and inflammation. In addition, Heidrich et al. [85] found that *Lactobacillus* increased in HCV-infected patients.

HBV and HCV can induce liver injury through the gut–liver axis. The gut microbiota can mediate a persistent inflammatory response by regulating metabolism and aggravating or slowing the occurrence of viral hepatitis, thus affecting the development of HCC.

### 3.4. Diagnostic and prognostic value of the gut microbiota in HCC

HCC is highly recurrent and can develop into new HCC tumors after resection or ablation [93]. Clinically, most patients are already in an advanced stage when diagnosed with HCC, and their prognosis is generally poor. Therefore, early detection and treatment are the major strategies for the treatment of HCC. The gut microbiota has the advantages of being non-invasive, highly efficient, and accurate in disease diagnosis. Alterations in the gut microbiota composition are prominent features of HCC and advanced liver disease that may evolve into HCC. Controlling the relationship between the gut microbiota and the stage of HCC lesions is an important breakthrough for the early prediction of HCC.

In the first global report on the characterization of the gut microbiota in patients with early HCC using MiSeq sequencing in a large cohort, the microbial diversity of early HCC was found to be significantly higher than that of cirrhosis, especially Actinobacteria. Thirteen genera, including *Gemmiger* and *Parabacteroides*, were enriched in early HCC and liver cirrhosis. Compared with the HCs, the beneficial bacteria (*Ruminococcus*, *Alistipes*, and *Phascolarctobacterium*) producing butyrate decreased in early HCC, whereas the harmful bacteria (*Klebsiella* and *Haemophilus*) producing LPS increased. The effectiveness of microbiota alterations in the diagnosis of HCC has been tested in clinical practice, with the results showing a high accuracy for patients with HCC in northwest and central China [17]. Deng et al. [94] showed that the abundance of Bacteroidetes decreased and that of Actinobacteria increased in patients with HCC compared with that in healthy controls and patients with cholangiocarcinoma. Albhaisi et al. [95] also found that the abundance of *Enterococcus*, *Salmonella*, *Clostridium XIVb*, *Clostridium IV*, *Lactonifactor*, and *Eggerthella* was lower in cirrhosis patients who developed HCC in the future. In addition, sex differences in the gut microbiota were also demonstrated in a spontaneous HCC mouse model. Alterations in the relative abundance of anaerobic and facultative anaerobic bacteria may be used as predictors of HCC in females, whereas *Paraprevotella*, Paraprevotellaceae, and *Prevotella* have the potential to predict HCC in males [96]. Furthermore, Liu et al. [97] found that the diversity of gut fungi changed in patients with HCC and that the abundance of *Candida albicans* was significantly increased. The abnormal colonization of *Candida albicans* is related to the development of HCC, which may provide new insights into the prediction and treatment of HCC. However, the clinical application of the gut microbiota in the prediction of HCC has not been fully investigated, and further studies are needed.

The clinical value of the gut microbiota in HCC is not only reflected in the diagnosis but also in the prognosis. According to clinicopathological characteristics, HCC can be divided into small HCC and non-small HCC, with the former showing a better prognosis. 16S rRNA sequencing was performed on fecal samples collected from patients with HCC associated with the HBV and HCs. *Bacteroides*, *Lachnospiracea incertae sedis*, and *Clostridium XIVa* were found to be enriched in patients with small HCC, while the endotoxin activity produced by these strains was weak, also predicting moderate inflammatory response and controlled tumor development [18]. The gut microbiota can also influence the clinical response to immunotherapy in patients with HCC. Immune checkpoint inhibitors (ICIs) have been widely researched in the field of cancer treatment, and anti-programmed cell death protein-1 (PD-1) immunotherapy has shown encouraging effects in sorafenib-refractory HCC [98]. Studies have shown that the prevalence of Proteobacteria, especially *Escherichia coli*, may inhibit the effects of anti-PD-1 therapies. Patients with HCC who received ICI showed an association between gut microbiota diversity and response to treatment. A high abundance of *Faecalibacterium* was found to significantly prolong progression-free survival (PFS) after ICI treatment, whereas a high abundance of Bacteroidales had the opposite effect [99]. Moreover, the presence of probiotics, such as *Lactobacillus*, *Bifidobacterium dentium*, and *Streptococcus thermophilus*, can be beneficial for ICIs and inhibit the immune escape

of cancer cells, which may also have implications for HCC prognosis [100]. Thus, the gut microbiota can play a prognostic role in many aspects of HCC, ranging from distinguishing the prognosis of small and non-small HCC to influencing immunotherapy efficacy.

### 3.5. Gut microbiota modulation in HCC

With the development of studies on the gut microbiota and HCC, regulation of the gut microbiota may be a new and important adjunct to current anticancer treatments, and preventive approaches based on the gut microbiota and liver are promising research directions. Gut microbiota modulation has been explored, including via antibiotics, probiotics, and fecal microbiota transplantation (FMT).

#### 3.5.1. Antibiotics

In 1928, the discovery of penicillin represented a breakthrough in medical history and catalyzed the development of antibiotics [101]. The regulation of the gut microbiota by antibiotics is a proven strategy that can suppress pro-inflammatory signals by eliminating bacteria with a high translocation capacity or eliminating cancer-promoting chemicals by reducing the number of bacteria producing specific metabolites. Vancomycin is a first-generation glycopeptide antibiotic that acts by inhibiting cell wall formation in Gram-positive bacteria [102]. Singh et al. [103] revealed that vancomycin can suppress secondary BAs and SCFAs, which can halt the development of liver cancer in insulin-fed TLR5-deficient mice. However, its use in the treatment of HCC is not recommended because of its potential side effects. In addition, norfloxacin administration selectively eliminated aerobic Gram-negative bacilli from fecal flora and significantly reduced the recurrence of spontaneous bacterial peritonitis in liver cirrhosis [104]. However, a major disadvantage of norfloxacin administration is that drug resistance can easily develop, making it difficult to meet the long-term demand for HCC prevention [105]. Furthermore, Fujinaga et al. [106] found that rifaximin can reduce intestinal permeability by reducing portal endotoxins and inhibiting the LPS–TLR4 signaling pathway, which can significantly reduce liver fibrosis. Rifaximin has the advantage of not having strong side effects on the gut microbiota. In contrast to norfloxacin, no clinically relevant drug resistance to rifaximin has been reported, suggesting that it may be suitable for long-term treatment [107]. However, the effects of norfloxacin and rifaximin on HCC development remain to be explored further.

#### 3.5.2. Probiotics

Several studies have confirmed the efficacy and mechanism of probiotics in preventing HCC by regulating the composition of the gut microbiota, improving intestinal barrier function, and regulating local and systemic immunity. Probiotic VSL#3 has been shown to reduce the severity of liver disease and hospitalization in patients with cirrhosis and is widely commercialized [108]. Prohep, a probiotic mixture composed of *Lactobacillus rhamnosus* GG, *Escherichia coli* Nissle 1917, and VSL#3, can successfully inhibit angiogenesis, regulate the subpopulation of $CD4^+$ T cells, and increase SCFA-producing bacteria, ultimately reducing tumor weight and size by 40% in mice [109]. Probiotics can also reduce HCC incidence by mediating epigenetic regulation in the host. Heydari et al. [110] demonstrated that *Lactobacillus acidophilus* and *Bifidobactrum bifidioum* can negatively regulate the expression of oncogenic microRNAs in HCC cancer mice. Furthermore, Mihailović et al. [111] found that the probiotic *Lactobacillus paraplantarum* BGCG11 can reduce DNA damage and increase the activation of pro-survival protein kinase B (Akt), which may inhibit cell carcinogenesis. Probiotic supplementation can control HCC development by downregulating TLR-induced liver inflammation. In the DEN-induced HCC model, probiotic supplementation restored intestinal homeostasis, reduced exotoxin levels, and inhibited tumor cell proliferation [112]. In addition, *Lactobacillus plantarum* can significantly decrease the expression of TLR4, CXCL9, and phosphatidylinositol-3,4,5-trisphosphate-dependent RAC exchanger 2 (PREX-2) and prevent carcinogenesis of liver cirrhosis [113]. Furthermore, several studies have shown that probiotics can prevent HBV and HCV infection and improve liver function during infection [114,115]. Interestingly, only high doses of probiotics were effective, suggesting that the adequate intake of probiotics may lead to the competitive exclusion of disease-promoting bacterial strains [116]. Overall, we can conclude that probiotics have the potential to be adjuncts in the treatment of HCC.

#### 3.5.3. Fecal microbiota transplantation

FMT is defined as the transfer of healthy gut bacteria via donor stool to a patient with the aim of obtaining therapeutic benefits by directly altering or normalizing the gut microbiota of recipients. Zhou et al. [117] showed that FMT not only increases the level of butyrate and reduces the level of endotoxin but also improves intrahepatic immunity in an NASH mouse model induced by a high-fat diet. Wang et al. [118] also suggested that FMT could prevent intestinal mucosal barrier damage and limit the systemic inflammatory response in mice with hepatic encephalopathy. Furthermore, no infections related to FMT were observed in immunocompromised patients with *Clostridium difficile* infection who received FMT, demonstrating the value of FMT in clinical practice [119].

FMT may delay the development of HCC and can reconstruct intestinal immune microecology, which may improve the efficacy of HCC immunotherapy. Nevertheless, one risk of FMT is the possibility of disease transmission, and the highly dynamic composition of living microorganisms is another major source of uncertainty. Additionally, FMT is susceptible to external factors, such as diet and drugs, and researchers should focus on manipulating the gut microbiota with greater precision. More studies are needed before FMT can be employed for the treatment of HCC.

## 4. Gut microbiota and LT

LT is a therapeutic option for patients with end-stage liver disease and acute liver failure (ALF), which can prolong the long-term survival rate and effectively improve the prognosis of patients [120,121]. In LT, allografts can bring donor immune cells into recipients, which leads to gut microbiota alterations in recipients [122]. Extensive research has shown that the gut microbiota and LT are closely correlated.

In this section, the alterations in the gut microbiota after LT are summarized, and the relationship between the gut microbiota and liver ischemia–reperfusion (I/R) injury, the efficacy of immunosuppressive drugs used, and complications after LT are also discussed (Fig. 2).

### 4.1. Gut microbiota alterations after LT

The gut microbiota and its alterations are crucial in LT. After rats received LT, researchers found that the number of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, significantly decreased, whereas the numbers of harmful bacteria, such as Enterobacteriaceae and *Enterococcus*, significantly increased [123]. Wu et al. [122] further found that the levels of the above strains, except for *Enterococcus*, returned to normal over time after LT. This change may be caused by the introduction of donor microorganisms into the recipient by the graft. In addition, Bajaj et al. [124] also showed alterations in gut microbial function after

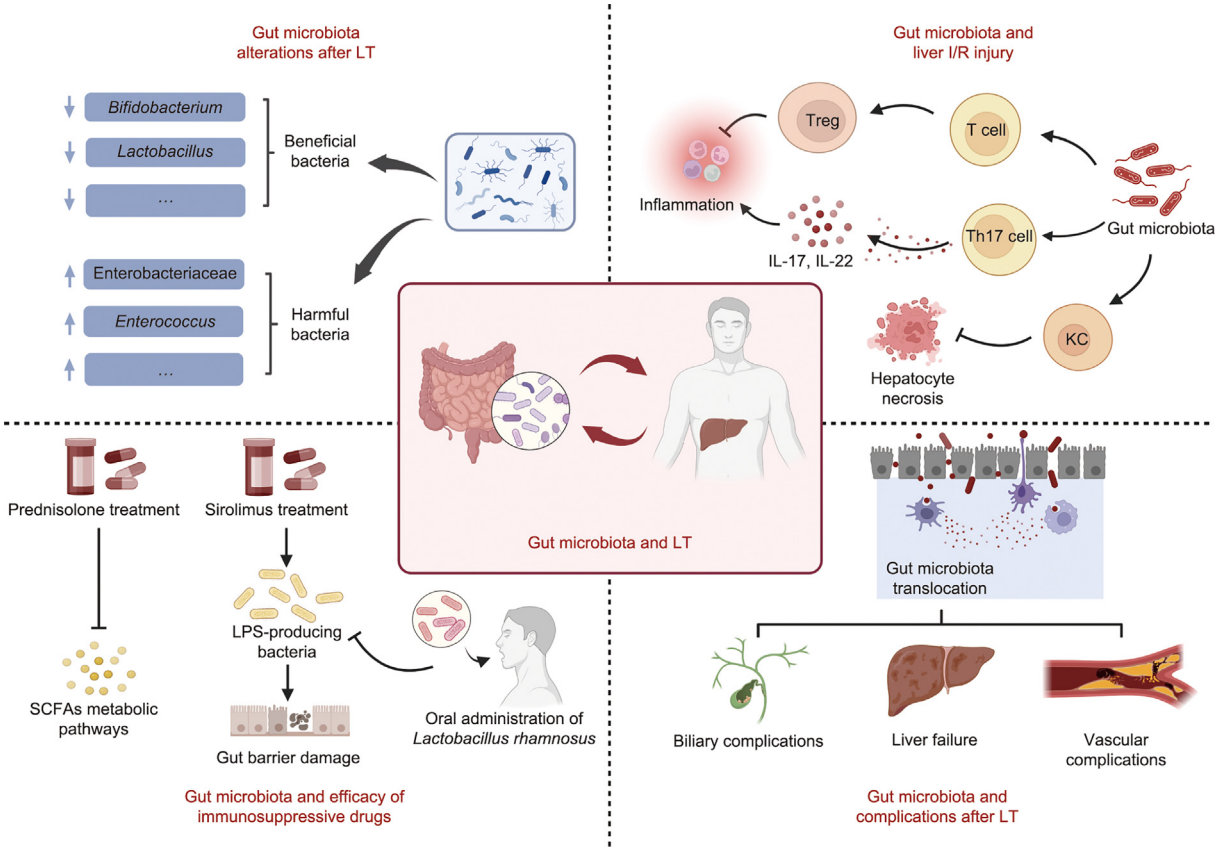


**Fig. 2.** Gut microbiota and LT. KC: Koffler cell.

LT. The relative abundance of Enterobacteriaceae was significantly reduced, whereas that of Ruminococcaceae and Lachnospiraceae increased after LT. They proved that endotoxin synthesis, ammonia, BA modulation, and methylamine metabolism were significantly improved, suggesting that the liver plays a central role in influencing the function of the gut microbiota.

The composition of the gut microbiota in patients before and after LT changes significantly, and improvement in liver function after LT is closely related to alterations in the gut microbiota.

### 4.2. Gut microbiota and liver I/R injury

Liver I/R injury is closely related to the gut microbiota [125]. Tregs can help improve the tolerance of liver allografts by modulating immune effects and preventing the development of I/R injury-induced acute cellular rejection [126,127]. It has been confirmed that intestinal *Bacteroides fragilis* and capsular polysaccharides induce $CD4^+$ T cells to differentiate into Tregs after LT dysregulation, preventing increased inflammation and suppressing excessive immune responses [128]. As key mediators of autoimmune diseases, Th17 cells secrete IL-17, IL-22, and IL-26, which may trigger a strong inflammatory response and aggravate I/R injury [129]. The presence of segmented filamentous bacteria in the gastrointestinal tract of mice can specifically induce Th17 cell differentiation and inhibit the invasion of intestinal pathogens [130]. 3-oxolithocholic acid and isolithocholic acid can also regulate the Th17/IL-17 signaling axis, and there is a negative correlation between them [131]. Moreover, Corbitt et al. [132] found that the number of Koffler cells (KCs) is determined by gut bacteria and constitutive MAMP exposure through portal venous blood, and the depletion of gut bacteria can lead to KCs deficiency, which affects liver reperfusion injury. Inactivated KCs can abrogate I/R injury and the gut microbiota can significantly affect the response of KCs to I/R injury [133]. Furthermore, Nakamura et al. [134] reported that the levels of sinusoidal congestion, edema/vacuolization, and hepatocellular necrosis were reduced in mice with gut microbiota modified with antibiotics after liver allotransplantation, indicating a decrease in I/R injury. *Clostridium* and *Bacteroides* can produce SCFAs, which induce the expression of IL-10 and downregulate the expression of claudin-2 in the small intestine, thereby preventing I/R injury, intestinal apoptosis, and inflammation in intestinal TLR9-deficient mice [135,136].

Generally, the severity of I/R injury is directly related to the recovery and maintenance of organ function after LT, and the gut microbiota, as the core regulator of I/R injury, can provide a feasible way to improve the prognosis of LT.

### 4.3. Gut microbiota and efficacy of immunosuppressive drugs

Solid organ transplant recipients must take anti-rejection drugs after transplantation to prevent immune rejection [137]. The efficacy of immunosuppressive drugs and the composition of the gut microbiota can influence each other, with advances being made in this research field.

Sirolimus can inhibit the activation and proliferation of T lymphocytes stimulated by antigens and cytokines and is considered a promising immunosuppressive agent [138]. Compared to the control, the relative abundance of Proteobacteria significantly increased in mice treated with sirolimus, resulting in intestinal barrier damage and metabolic disorders after LT. Oral intervention with *Lactobacillus rhamnosus* HN001 alleviated sirolimus-induced adverse reactions [139]. In addition, combined immunosuppressive therapies, including prednisolone, mycophenolate mofetil, and tacrolimus, inhibit *Clostridium* and promote the proliferation

of *Escherichia coli* in the intestinal tract of mice [140]. Swarte et al. [141] demonstrated a strong correlation between immunosuppressive therapies and dysbiosis; they also showed that the more serious the extent of dysbiosis, the higher the mortality after transplantation.

Therefore, the dysbiosis observed in post-transplant recipients can be attributed to a complex interaction between anti-rejection drugs and the gut microbiota.

### 4.4. Gut microbiota and complications after LT

Although LT can effectively treat patients with end-stage liver disease and ALF, the occurrence of postoperative complications reduces the therapeutic effect [142,143]. The gut microbiota plays a role in the pathogenesis and development of complications after LT. There are differences in the gut microbiota and metabolites between patients with complications after LT and those without complications, suggesting that the gut microbiota and metabolites can be used to predict the occurrence of complications after LT. Moreover, several studies have revealed the mechanism by which the gut microbiota and metabolites mediate complications after LT. Therefore, early intervention of the gut microbiota and metabolites using probiotics after LT can prevent the occurrence of complications, which sheds new light on the therapeutic potential of gut microbiota modulation.

In this section, complications after LT, such as biliary complications, liver failure, and vascular complications, are introduced. Gut microbiota alterations, their mediating mechanisms, and potential preventive methods are presented (Table 2 [144–170]).

#### 4.4.1. Biliary complications

Biliary complications are the most common complications after LT, with an incidence of 30%, and include bile leakage, bile duct stenosis, biliary sludge formation, and biliary tract infection [171]. Lichtman et al. [144] showed that rats with intestinal bacterial overgrowth displayed extrahepatic and intrahepatic bile duct injury. *Veillonella*, *Streptococcus*, and *Enterococcus* are enriched in patients with primary sclerosing cholangitis (PSC) [145]. Moreover, the analysis of intestinal fungi in patients with PSC revealed increased *Exophiala* and decreased *Saccharomyces cerevisiae* [146]. Gut dysbiosis was also observed in patients with primary biliary cholangitis (PBC) [147]. The gut microbiota and metabolites may be used to predict complications after LT. This suggests that biliary complications after LT can be regulated by the gut microbiota, showing a close relationship.

A bidirectional regulatory relationship exists between the gut microbiota and BAs, which may reveal the triggering mechanism of biliary complications. After LT, bile is released into the intestine, which alters the pH of the intestinal environment and inhibits the growth of harmful microorganisms. However, the gut microbiota, which is associated with the onset and development of cholestatic liver disease, can modulate the action of BA-activated receptors and mold the BA pool. By directly inducing the production of mitochondrial reactive oxygen species and subsequent mitochondrial oxidative stress, hydrophobic BAs can initiate apoptosis, which is a key mechanism in cholestatic liver disease following LT [148]. In addition, bacterial dysregulation can promote BA-induced cell death in mouse hepatocytes, increase intestinal permeability, activate inflammation, and induce cholestatic liver disease [149].

Ursodeoxycholic acid (UDCA) treatment may be used to prevent biliary complications after LT. Tabibian et al. [150] revealed that UDCA, a commensal microbial metabolite, can abrogate cholangiocyte senescence, suggesting the significance of commensal microbiota and its metabolites in biliary injury. Gut dysbiosis caused by PBC can also be partially ameliorated by UDCA treatment [147].

#### 4.4.2. Liver failure

ALF, chronic liver failure, and acute-on-chronic liver failure (ACLF) are all significant side effects of LT. Previous research has established that gut microbiota translocation is a key factor in the development of systemic inflammation and liver failure [172].

The severe hepatotoxicity caused by acetaminophen (APAP) is one of the main causes of ALF. Several studies have shown that APAP-induced acute liver injury exhibits diurnal changes as well as changes in the composition and function of the gut microbiota, suggesting that ALF is associated with the gut microbiota [151,152]. Furthermore, gut microbiota modulation may effectively prevent the occurrence of ALF after LT. 1-phenyl-1,2-propanedione generated by *Escherichia coli*, *Citrobacter freundii*, *Clostridium difficile*, and *Enterococcus faecalis* can synergistically improve APAP-induced ALF, while yeast can protect the liver against such damage [153]. *Bifidobacterium adolescentis* CGMCC 15058 and *Bifidobacterium longum* R0175 were also found to inhibit bacterial translocation and inflammation during ALF and maintain intestinal mucosa integrity, which can effectively reduce or prevent ALF symptoms [154,155]. In addition, the use of probiotic bacteria, such as *Saccharomyces boulardii*, significantly improved gut dysbiosis and alleviated ALF in a *D*-galactosamine-induced liver injury mouse model [156].

The gut microbiota and metabolites may predict the complications of ACLF after LT. The analysis of the gut microbiota in liver cirrhosis patients with and without ACLF revealed that the intestinal ecology in ACLF patients was severely dysregulated, and dysbiosis of the gut microbiota on admission may predict an increased risk of extrahepatic organ failure, ACLF, and death [157]. Chen et al. [158] demonstrated that patients with ACLF also had gut dysbiosis. The abundance of Bacteroidaceae, Ruminococcaceae, and Lanchnospiraceae was lower, while that of Pasteurellaceae, Streptococcaceae, and Enterobacteriaceae was higher. They also found that the relative abundance of Pasteurellaceae could be used to predict the mortality rate of ACLF patients [158]. In addition, the relationship between the gut microbiota and ACLF can provide guidance on how to prevent the occurrence of complications after LT through the modulation of the gut microbiota. Moreau et al. [159] revealed the relationship between the accumulation of blood metabolites and alterations in major metabolic pathways, intense systemic inflammation, and organ failure in patients with ACLF. Moreover, intestinal bacterial infection and alcohol abuse are the main causes of hepatocyte injury in patients with ACLF. Alcohol abuse can exacerbate intestinal barrier disruption, and bacteria trigger a strong inflammatory response through pathogen-related molecular patterning, ultimately leading to ACLF [160].

In summary, the gut microbiota and metabolites may be used to predict the complications of liver failure after LT, and specific interventions on the gut microbiota will promote or inhibit ALF and ACLF.

#### 4.4.3. Vascular complications

Hepatic artery thrombosis is an LT complication that can terminate liver graft dysfunction or even ischemic necrosis. In addition to governing the gut barrier, several metabolites from the gut microbiota can reach distant regions in the body [173,174], and the gut microbiota is closely associated with vascular complications after LT.

Thrombosis of the arterial system is based on atherosclerotic plaque rupture. Ott et al. [161] found bacterial DNA in atherosclerotic plaques, supporting the hypothesis of multiple bacterial colonization in arterial lesions. Furthermore, *Acinetobacter*, *Acidovorax*, and *Neisseria polysaccharea* were found to be most prevalent in symptomatic atherosclerotic plaques, and asymptomatic plaques had a higher abundance of Porphyromonadaceae, Bacteroidaceae,

**Table 2**
Gut Microbiota and Complications after LT.

| Authors | Gut microbiota | Associated complications | Outcomes | Reference |
|---|---|---|---|---|
| Lichtman et al. | — | Biliary complications | Intestinal bacterial overgrowth displayed extrahepatic and intrahepatic bile duct injury | [144] |
| Little et al. | *Veillonella*, *Streptococcus*, and *Enterococcus* | Biliary complications | *Veillonella*, *Streptococcus*, and *Enterococcus* were considered to be enriched in PSC patients | [145] |
| Lemoinne et al. | *Exophiala* and *Saccharomyces cerevisiae* | Biliary complications | PSC patients revealed increased *Exophiala* and decreased *Saccharomyces cerevisiae* | [146] |
| Tang et al. | *Faecalibacterium* and Enterobacteriaceae | Biliary complications | Gut dysbiosis was found in PBC patients and partially relieved by treatment with UDCA | [147] |
| Li et al. | — | Biliary complications | Hydrophobic BAs can start apoptosis, which is a key mechanism of cholestatic liver disease following LT | [148] |
| Isaacs-Ten et al. | — | Biliary complications | Absence of the gut microbiota can protect mice from cholestatic-mediated liver injury and inflammation. | [149] |
| Tabibian et al. | — | Biliary complications | UDCA can abrogate cholangiocyte senescence | [150] |
| Kim et al. | — | Liver failure | ALF is associated with the gut microbiota | [151] |
| Thaiss et al. | — | Liver failure | ALF is associated with the gut microbiota | [152] |
| Gong et al. | — | Liver failure | Saccharomyces cerevisiae can reduce intestinal 1-phenyl-1,2-propanedione levels and markedly alleviate APAP-induced liver damage and ACLF | [153] |
| Li et al. | *Bifidobacterium adolescentis* CGMCC 15058 | Liver failure | *Bifidobacterium adolescentis* CGMCC 15058 can effectively reduce or prevent ALF symptoms | [154] |
| Wang et al. | *Bifidobacterium longum* R0175 | Liver failure | *Bifidobacterium longum* R0175 can effectively reduce or prevent ALF symptoms | [155] |
| Yu et al. | *Saccharomyces boulardii* | Liver failure | *Saccharomyces boulardii* significantly improved gut dysbiosis and alleviated ALF | [156] |
| Bajaj et al. | Proteobacteria and Firmicutes | Liver failure | Taxa belonging to phylum Proteobacteria (Enterobacteriaceae, Campylobacteriaceae, and Pasteurellaceae) and Firmicutes (Enterococcaceae and Streptococcaceae) may related to ACLF | [157] |
| Chen et al. | Bacteroidaceae, Ruminococcaceae, Lanchnospiraceae, Pasteurellaceae, Streptococcaceae, and Enterecoccaceae | Liver failure | The abundance of Bacteroidaceae, Ruminococcaceae, and Lanchnospiraceae was lower, while that of Pasteurellaceae, Streptococcaceae, and Enterecoccaceae was higher | [158] |
| Moreau et al. | — | Liver failure | The relationship between blood metabolite accumulation and metabolic pathway alteration and intense systemic inflammation in ACLF was revealed | [159] |
| Moreau et al. | — | Liver failure | Patients with ACLF had more associated bacterial infections than patients without ACLF | [160] |
| Ott et al. | — | Vascular complications | Bacterial DNA was present in atherosclerotic plaques | [161] |
| Mitra et al. | *Acinetobacter*, *Acidovorax*, *Neisseria polysaccharea*, Porphyromonadaceae, Bacteroidaceae, Micrococcaceae, and Streptococcaceae | Vascular complications | Distinct groups of microbial agents might play different roles during the development of atherosclerotic plaques | [162] |
| Bennett et al. | — | Vascular complications | Gut microbial TMAO can cause atherosclerosis | [163] |
| Carnevale et al. | *Escherichia coli* | Vascular complications | LPS from *Escherichia coli* localizes in human atherosclerotic plaque and may trigger atherosclerotic damage | [164] |
| Zhu et al. | — | Vascular complications | TMAO can promote the formation of thrombosis by directly enhancing the activity of platelets | [165] |
| Duerschmied et al. | — | Vascular complications | Serotonin can promote the formation of thrombosis by directly enhancing the activity of platelets | [166] |
| Jäckel et al. | — | Vascular complications | The gut microbiota can regulate hepatic von Willebrand factor synthesis and arterial thrombus formation | [167] |
| Li et al. | *Akkermansia muciniphila* | Vascular complications | *Akkermansia muciniphila* can reverse the progression of atherosclerotic lesions | [168] |
| Wu et al. | — | Vascular complications | The inhibition of LPS is considered an effective way of treating thrombosis | [169] |
| Mathew et al. | — | Vascular complications | Butyrate plays a certain therapeutic role in atherosclerosis | [170] |

PSC: primary sclerosing cholangitis; PBC: primary biliary cholangitis; UDCA: ursodeoxycholic acid; APAP: acetaminophen; ACLF: acute-on-chronic liver failure; TMAO: metabolite trimethylamine N-oxide.

Micrococcaceae, and Streptococcaceae [162]. In animal models, Bennett et al. [163] demonstrated that the gut microbial metabolite trimethylamine N-oxide (TMAO) can cause atherosclerosis. LPS can translocate from the gut microbiota to atherosclerotic plaques, and the level of LPS is positively correlated with the degree of atherosclerosis [164]. Therefore, the gut microbiota and metabolites may serve as predictive markers for vascular complications after LT.

In addition, the destruction of liver vascular endothelial function and increased platelet activity are associated with the possibility of thrombosis [175]. TMAO, serotonin, and other metabolites can promote thrombosis by directly enhancing the activity of platelets [165,166]. Extensive bidirectional interactions exist between the inflammatory and clotting systems. The inflammatory mechanism can increase platelet activity, trigger coagulation activation, and cause thrombosis [176]. The gut microbiota can regulate liver endothelial cell homeostasis in a TLR2-dependent manner, thus regulating von Willebrand factor and ultimately affecting platelet deposition in the extracellular matrix [167]. These findings explain the mechanisms of the gut microbiota, platelet function, and thrombosis risk and provide insights into the connection between the gut microbiota and vascular complications after LT.

Gut microbiota modulation may also play a role in the prevention of vascular complications after LT. Li et al. [168] indicated that *Akkermansia muciniphila* could reverse the progression of atherosclerotic lesions. Furthermore, LPS can induce inflammation and oxidative stress, which can affect the function of vascular endothelial cells and have a direct impact on thrombosis; the inhibition of LPS is considered an effective way to treat thrombosis [169]. Butyrate plays a therapeutic role in atherosclerosis by inhibiting oxidative and inflammatory responses during vascular smooth muscle cell proliferation [170].

## 5. Conclusions

The liver and intestine are closely related to physiological functions. Nutrients and toxins originating from the intestine first enter the liver through the portal vein and are then transported to the entire body after removing harmful substances from the liver. Some gut-derived immune cells and released cytokines can also enter the liver through the portal vein to achieve a remote echo of gut–liver immunity. The liver transmits substances to the intestine by secreting BAs, regulating hormone levels and immune responses, and affecting intestinal homeostasis.

In recent years, an increasing number of studies have focused on the role of the gut microbiota in the balance between host health and disease. Gut microbiota imbalance is related to the occurrence and development of HCC. Intestinal leakage caused by alterations in the gut microbiota can aggravate the translocation of various bacterial and gut microbial metabolites to the liver, which affects the development of HCC. The gut microbiota also functions in chronic liver disease, which may evolve into HCC. Furthermore, alterations in the composition of the gut microbiota can be used as effective diagnostic and prognostic markers for HCC. In addition, gut microbiota modulation through antibiotics, probiotics, FMT, and other methods can significantly improve liver function, reduce liver injury, and prevent chronic liver disease and HCC. LT is an effective treatment for HCC and its technology is relatively mature, with the gut microbiota composition changing in patients after treatment. Although LT continues to be plagued by postoperative rejection and various complications, its prognosis may be improved via the modulation of the gut microbiota. However, studies on the gut microbiota of patients with HCC receiving LT remain scarce.

To summarize, gut microbiota modulation is a viable strategy to address the medical needs in HCC and LT, particularly now, in the era of liver transplant oncology. However, studies on the gut microbiota in HCC and LT remain scarce and need to be promoted further. Unremitting efforts should be made to apply preclinical research in clinical practice to truly benefit patients.

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## Authors' contribution

Ze Xiang and Xuyong Wei had the idea for the article; Jian Wu and Jiarui Li performed the literature search and data analysis; Xiao Xu and Shusen Zheng drafted and critically revised the work.

## Conflict of with ethics guidelines

Ze Xiang, Jian Wu, Jiarui Li, Shusen Zheng, Xuyong Wei, and Xiao Xu declare that they have no conflict of interest or financial conflicts to disclose.

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