Gut-liver axis in liver disease: From basic science to clinical treatment (Review)

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Abstract. Incidence of a number of liver diseases has increased. Gut microbiota serves a role in the pathogenesis of hepatitis, cirrhosis and liver cancer. Gut microbiota is considered 'a new virtual metabolic organ'. The interaction between the gut microbiota and liver is termed the gut-liver axis. The gut-liver axis provides a novel research direction for mechanism of liver disease development. The present review discusses the role of the gut-liver axis and how this can be targeted by novel treatments for common liver diseases.

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Abbreviations: GM, gut microbiota; HBV, hepatitis B; HCV, hepatitis C; HCC, hepatic cell carcinoma; ALD, alcoholic liver disease; NAFLD, non-alcoholic fatty liver disease; FXR, farnesoid X receptor; PAMP, pathogen-associated molecular pattern; FMT, fecal microbiota transplantation; BA, bile acid; HSC, hepatic stellate cell; SBP, spontaneous bacterial peritonitis; HE, hepatic encephalopathy; SIBO, small intestinal bacterial overgrowth; LPS, lipopolysaccharide; DCA, deoxycholic acid; NASH, non-alcoholic steatohepatitis; DMR, duodenal mucosal surface replacement

Key words: gut-liver axis, liver disease, gut microbiota, clinical treatment

1. Introduction

The liver is the organ that interacts most with the digestive system. As such, the liver is exposed to numerous gut microbiota (GM). The GM is a diverse ecosystem that contains bacteria, protozoa, archaea, fungi and viruses. There are $>1x10^{14}$ species of microorganisms in the human gastrointestinal tract, including $\sim1x10^{4}$ species of bacteria (1). The unique microenvironment and physicochemical barriers of each region of the digestive system determine the growth of specific microbiota. It is widely recognized that transgenes serve a role in the physiological and pathological aspects of human health, particularly liver health (2,3).

Previous studies have reported the role of the GM in occurrence and development of a number of liver diseases (such as hepatitis, alcoholic liver disease (ALD), non-alcoholic liver disease (NAFLD), liver fibrosis, cirrhosis, and liver cancer, etc.) (4,5). In recent years, researchers have assessed the gut-liver axis (6-10). For example, *Pseudomonas aeruginosa* can significantly inhibit NAFLD-HCC progression by secreting acetate salts (7). The present review aimed to assess the previous research to provide a broader understanding of this axis and discuss the mechanism of the gut-liver axis in a number of common types of liver diseases, highlighting the potential drugs and treatment methods targeting GM in clinical treatment of liver disease.

2. Overview of liver disease

The term 'liver disease' covers a range of illnesses, including acute problems caused by harmful agents such as viruses, poisons, alcohol and pharmaceutical agents, as well as chronic liver disease, which that may result in cirrhosis. Any type of cirrhosis increases the risk of developing hepatic cell carcinoma (HCC), a primary liver cancer; this risk is higher if the cirrhosis is caused by hepatitis B (HBV) or hepatitis C (HCV)

infection (11,12). Most types of liver diseases (such as hepatitis, ALD, NAFLD, focal liver disease, and some types of liver cancer) (13-18) can be conservatively treated with non-surgical treatment methods), including targeted therapy (19,20), immunotherapy (21), radiotherapy (22), etc. Acute liver failure is associated with rapid and massive injury to hepatocytes, which is rare, but has a high incidence and mortality rate (23). HCV infection causes ~290,000 deaths worldwide each year and is the primary cause of liver cirrhosis and associated complications (i.e. decompensated cirrhosis) (24). Other data indicate that globally, the number of HBV-related deaths is expected to reach 1,109,500 by 2030 (25). ALD is the most common cause of liver cirrhosis worldwide. According to WHO data in 2018, the global prevalence of alcohol use disorders was 5.1% (26). A modeling study suggests that if current drinking trends are not controlled for, the age-specific mortality rate (ASDR) associated with ALD in the United States is expected to increase from 8.2 deaths per 100,000 patients per year in 2019 to 15.2 deaths per 100,000 patients per year in 2040 (27). Other data indicate that globally, the number of HBV-related deaths is expected to reach 1,109,500 by 2030 (25). ALD is the most common cause of liver cirrhosis worldwide. According to WHO data in 2018, the global prevalence of alcohol use disorders was 5.1% (26). Primary liver cancer is the seventh most common cancer in the world and the second most common cause of cancer death. HCC is the main type of liver cancer worldwide, accounting for approximately 75% of the total. It is the primary cause of diagnosis and death in liver cancer cases (28).

3. Overview of the gut-liver axis

The gut and liver communicate with each other. The liver can regulate gut function through the bile ducts. The intestine can regulate liver function through the portal vein. In addition, these two organs can indirectly affect each other's function through the whole body blood circulation. The liver delivers bile salts and antimicrobial molecules such as immunoglobulin A and angiopoietin to the gut via the biliary tract. This maintains the GM by modulatin microbiota growth (29). Bile acids (BAs) exert direct antibacterial effects by disrupting the cell membranes of intestinal bacteria and causing membrane protein degradation (30). Moreover, BAs indirectly regulate the composition of the GM by activating BA receptors in the intestine, particularly farnesoid X receptor (FXR) encoded by Nuclear Receptor Subfamily 1 Group H Member 4 (31). Liu *et al* (32) reported that activated FXR is involved in the expression of gut tight junction markers (claudin1 and zonula occludens-1), maintaining BA homeostasis and inhibiting the expression of inflammatory factors, thereby inhibiting bacterial overgrowth and mucosal damage in the ileum. Mice lacking FXR exhibit an increase in harmful bacteria in the ileum and damage to the epithelial barrier. BAs also regulate hepatic BA synthesis, glucose and lipid metabolism and dietary energy use via nuclear receptors such as the FXR and the G protein-coupled BA receptor (TGR5). FXR receptors inhibit the expression of BA synthase by binding to endogenous BAs, which provides negative feedback to regulate BA synthesis (33). For example, cholesterol 7-α hydroxylase 1 and cytochrome P450 Family 27 Subfamily A Member 1 are enzymes required for the synthesis of BAs, with their expression significantly reduced upon FXR stimulation (34). BAs can interact with nuclear transcription factors in the promoter region of gluconeogenesis-associated genes via the FXR-small heterodimer partner-dependent pathway and inhibit their expression (35). TGR5 belongs to the G protein-coupled receptor superfamily. The activated TGR5 receptor is associated with energy expenditure of the body. According to Watanabe's research report, treating brown adipocytes and human skeletal muscle cells with BA can increase the activity of TGR5 in cells, thereby upregulating the expression of cAMP-dependent type 2 deiodinase (DIO2). This enzyme catalyzes the deiodination of prothyroid hormones to triiodothyronine (T3), thereby enhancing oxygen and energy consumption in key thermogenic tissues such as brown adipose tissue and skeletal muscle (36,37). In terms of regulating blood sugar, previous studies have reported that the liver decreases hepatic gluconeogenesis through the BA FXR signal, which induces hepatic glycogen synthesis to regulate blood glucose levels (33). The GM and its metabolites, such as lipopolysaccharides (LPS), short-chain fatty acids (SCFAs), and tryptophan metabolites, are transported to the liver via the portal vein, inducing a local inflammatory response and exacerbating hepatic necrosis (38-40). In addition, metabolites produced by the liver, such as free fatty acids (FFAs), inflammatory factors, choline metabolites, and ethanol metabolites, can enter the systemic circulation, thereby prolonging the gut-liver axis and exerting systemic effects on multiple organs throughout the body, including the gut. For example, butyrate in the blood can enhance gut barrier function and reduce the translocation of gut microbial toxic metabolites to extraintestinal sites (41,42). Ethanol and acetaldehyde can increase intracellular calcium ion (Ca^{2+}) concentration and disrupt the integrity of gut epithelial tight junctions (43).

GM in the gut-liver axis. The human microbiota, which includes bacteria, fungi, viruses, archaea and protozoa, is a collection of microorganisms that live in humans. The term 'human microbiome' refers to genes carried by these microbes and the surrounding environment in which they live and interact (44). The human microbiota, especially in the gastrointestinal tract, serves a role in human health. As the gastrointestinal tract makes direct contact with the liver through the portal vein, GM can directly affect liver (45).

The number of GM is in the order of $1x10^6$ times higher than the number of human cells and GM total weight is estimated to be ~2 kg. The GM comprises ~3,000,000 genes, which is 150 times that of the entire human genome (46). GM is composed of >1,000 species, distributed in more than 50 different phyla (47); *Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, Tenericutes, Actinobacteria,* and *Verrucomicrobia* are the most advantageous phyla, making up to 90% of the total microbial population in humans. Among them, *Bacteroidetes* and *Firmicutes* are the most dominant phyla (44).

GM are concentrated within the lumen of the gut and adhere to the mucosal surface. The location and diameter of the gut lumen vary, and the types and abundance of GM present also vary. The bacterial population density in the jejunum and ileum is higher than that in the stomach cavity and duodenum. However, the most densely populated area is the colon, which contains ~1,000 colony-forming units per milliliter/ml and is

mainly composed of anaerobic bacteria such as *Bacteroides, Porphyromonas, Bifidobacterium, Lactobacillus* and *Clostridium*. In the colon, the ratio of anaerobic to aerobic bacteria is 100:1-1,000:1. This is influenced by changes in optimal growth conditions for these bacteria, which are caused by local colonic lesions (48-50). Microbes are concentrated in the lumen of the gut wall or adhere to the surface of the mucous membrane.

GM induces inflammation and immune stress. The gastrointestinal tract contains a large number of microorganisms, particularly bacteria, which are a source of pathogen-associated molecular patterns (PAMPs) and metabolites (51). Under normal conditions, small amounts of GM and metabolites enter the liver and are rapidly cleared. However, when the normal gut barrier permeability is increased, as in gut ecological dysbiosis, large amounts of GM and GM metabolites enter the liver, leading to activation of the immune cascade in the liver and production of pro-inflammatory cytokines (52,53). Increased gut permeability is attributed to tight junction disruption, potentially from the pathological change in the composition of GM and its metabolites or their induced immune cascade and inflammatory response (54-57). Dendritic cells form an extensive network under the gut epithelium. Dysregulated GM stimulates immature dendritic cells to produce IL-23, which promotes the secretion of cytokines IL-17A and IL-22 by interacting with surface receptors on activated $CD4^+$ T cells, thereby inducing a local gut inflammatory response (58,59). Moreover, dendritic cells and macrophages produce cytokines including IL-1β, IL-6, IL-18 and TNF to exacerbate the inflammatory response (60). Large amounts of pro-inflammatory cytokines affect tight junctions between gut epithelial cells, in addition to increasing the inflammatory burden. IL-1β recruits granulocytes to infiltrate foci of infection and directly disrupt the junctions and tightness of gut epithelial cells (61). TNF-α promotes myosin light chain kinase (MLCK) protein expression level in the gut epithelium (62). Previous studies have shown that MLCK triggers perijunction actinosin ring (PAMR) contraction, leading to increased permeability of tight junctions adjacent to gut epithelial cells (63). Moreover, TNF-α and IL-1β induce endoplasmic reticulum stress, which affects gut epithelial cells and alters proteins in the apical and basal lateral membranes, including E-calmodulin. This further disrupts the tight junctions of the gut epithelium (64). Release of these cytokines can activate natural killer cells, which bind to epithelial cells, releasing toxic particles (such as perforin, and granzymes) (65,66) and inducing apoptosis in epithelial cells. Furthermore, dendritic cells phagocytose antigens, which activate T cells and promote the differentiation of T helper 0 (Th0) cells into Th1, Th2 and Th17 cells. Th1 cells induce cytotoxic T cells to activate, proliferate and attack infected gut epithelial cells. IL-18 is a pro-inflammatory cytokine that promote the secretion of significant amounts of interferon-γ (IFN-γ) by Th1 cells. IFN-γ induces apoptosis in gut epithelial cells, thereby compromising the integrity of the gut epithelium (67-69). Th2 cells activate B cells, causing B cell proliferation and differentiation into plasma cells that secrete IL-4, IL-5 and IL-13 (70). These factors are believed to be associated with local eosinophil and mononuclear infiltration, increased mucus production, and epithelial cell proliferation and hypertrophy in the gastrointestinal tract (71). In addition, IL-13 activates STAT6 in epithelial cells and affects tight junctions in gut epithelium (72). IL-13 can also induce apoptosis of gut epithelial cells and further increase gut permeability (73,74). Th17 cells secrete IL-17A to mediate inflammatory responses (75). Large amounts of pro-inflammatory cytokines affect tight junctions between gut epithelial cells, in addition to increasing the inflammatory burden.

Increased gut permeability results in a heightened passage of GM and metabolites, such as LPS and endotoxins, into the portal circulation (76). Metabolites produced by GM, including trimethylamine and alcohol, exert direct toxic effects on the liver, while PAMPs- the distinctive molecular structures of GM-induce liver injury through the activation of the innate immune system (77). GM is detected by pathogen recognition receptors in the liver, encompassing toll-like receptors (TLRs) and inflammasomes. TLRs are present on hepatic sinusoidal cells, such as Kupffer cells and hepatic stellate cells, and they identify PAMPs located on cell membranes (78,79). TLR-mediated signaling pathways lead to sustained production of inflammatory cytokines, which cause or exacerbate liver injury (80) (Fig. 1).

Circadian regulation of GM promotes liver metabolism. The 'biological clock' is an intrinsic rhythm formed by organisms to adapt to changes in the surrounding environment. The ability of the circadian clock to persist in the absence of environmental cues provides internal temporal organization, allowing rhythmic activities to occur at characteristic times during the circadian cycle (81). The mammalian biological clock is composed of a number of core transcriptional regulators, including Brain and muscle arnt-like protein 1, Clock Circadian Regulator (CLOCK), period and cryptochrome. Disturbances in the biological clock are associated with the progression of a number of diseases, including fatty liver disease, heart disease, diabetes and cancer (82-85). However, the specific pathological mechanisms have not been fully identified. Previous studies have suggested that the GM may serve a link between circadian rhythm disorders and disease progression, which may be associated with the role of GM on host immune system function and metabolism (54,86,87).

Although GM is not directly affected by external environmental, self-regulation of the biological clock, host activity, and metabolic patterns, particularly changes in eating patterns, can induce rhythmic oscillations in the abundance of GM and GM metabolite. For example, Thaiss *et al* (88) reported rhythmic changes in GM composition over a 24-h period by analyzing fecal microbiota of mice. The abundance of *Lactobacillus reuteri* in the mouse gut increased during the light and decreased during the dark period. By contrast $Per1/2^{-/-}$ mice, which lack a functional host biological clock, exhibit almost complete loss of this GM abundance variation. GMs with different compositions secrete different metabolites. GM affects host metabolism and energy homeostasis by metabolite signaling. Previous studies have reported an increase in body fat percentage and insulin resistance in mice fed without transgenic genes under the same feeding conditions compared to normal mice (89,90). Further research reports indicate that differences exist in the composition of GM between obese and normal mice (91,92). For example, compared with

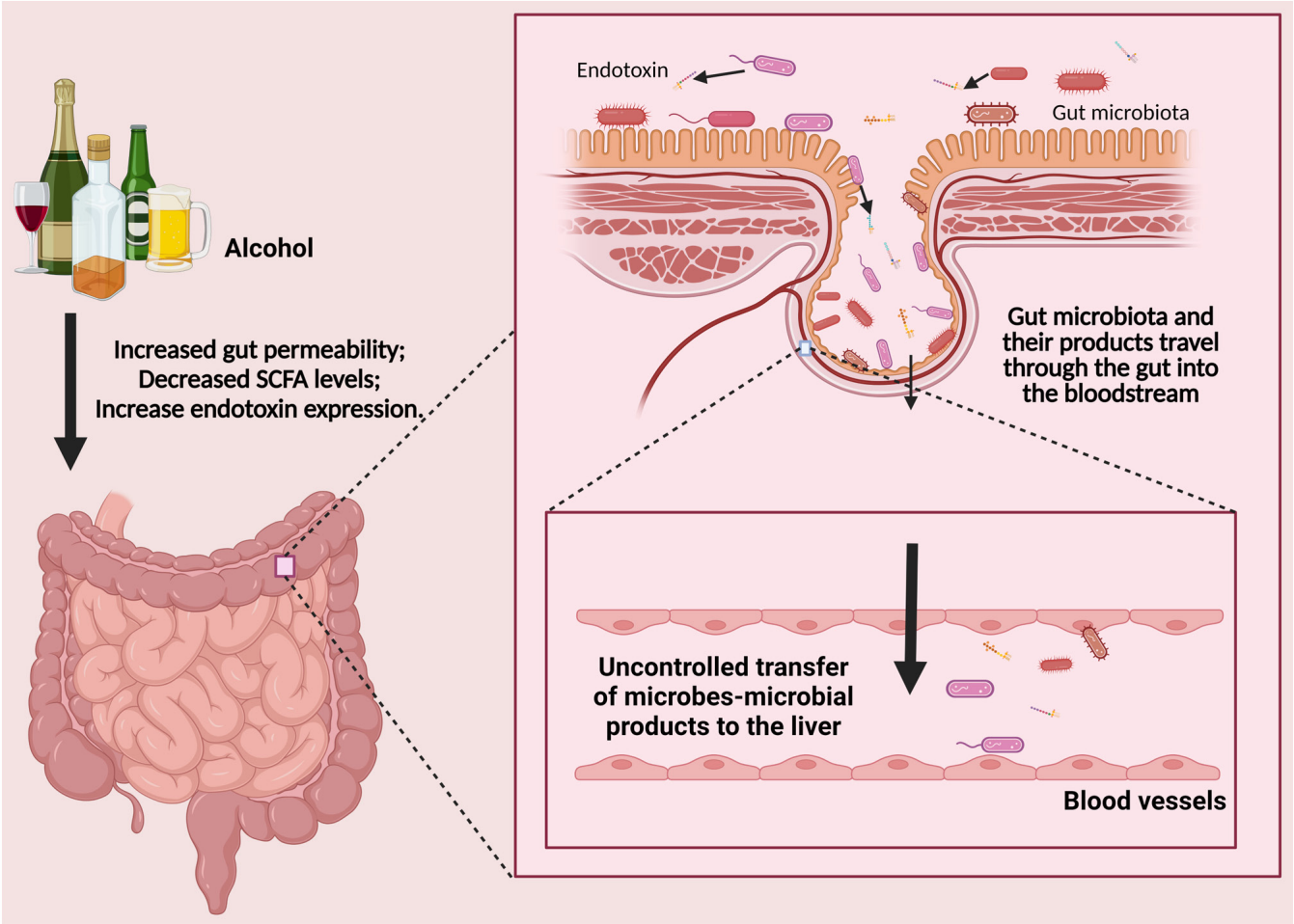

Figure 1. Gut microbiota of human is disordered. Disordered gut microbiota releases a large number of toxic metabolites, including LPS and deoxycholic acid. These toxic metabolites transfer from the gut to the liver and combine with HSC in the liver, leading to the transformation of HSC into SASP and promoting release of inflammatory cytokines, growth factors, chemokines and tumor-associated factors. Toxic metabolites of gut microbiota entering the liver can also block the apoptosis of liver cells and pre-cancerous cells. DCA, deoxycholic acid; LPS, lipopolysaccharide; HSC, hepatic stellate cell; TLR4, toll-like receptor 4; SASP, senescence-associated secretory phenotype.

the normal feed group, the high-fat feed group had higher abundance of *Lachnospiraceae* and *Blautia* in the gut of mice, while the abundance of *Lactobacillus*, *Faecalibaculum*, *Lachnoclostridium*, *Bacteroides* and *Desulfovibrio* was lower (92). The aforementioned research indicates that GM is an important environmental factor affecting energy collection and storage in the host. Turnbaugh *et al* (93) reported that the increased ability of the GM of obese mice to obtain energy from the diet is associated with a reduced abundance of *Bacteroidetes* and an increased abundance of *Firmicutes* in the GM. This change has also been confirmed in humans (94). *Akkermansia muciniphila*, *Bifidobacterium longum*, *Clostridium leptum* group, *Faecalibacterium prausnitzii* and Faecalibacterium and *Dorea* were suggested to serve a role in the regulation of blood glucose; changes in the abundance of these genera can lead to dysregulation of glucose metabolism and the progression of type 2 diabetes in humans (95).

4. Role of the gut-liver axis in liver disease

Association between GM and viral hepatitis. Viral hepatitis is the most common type of hepatitis worldwide. Hepatitis is defined as inflammation of the liver tissue. More than 300 million people worldwide are affected by viral hepatitis infections, which has a notable negative impact on public health and the economy and leads to high mortality (96). Hepatitis A, B, C, D and E are the five most common types of viral hepatitis. HBV and HCV often lead to chronic infections, and in severe cases, may lead to cirrhosis and liver cancer, affecting 257 million and 71 million people worldwide, respectively (97,98). Chou *et al* (99) reported that GM serves a role in age-dependent immunity of mice against HBV infection. After 6 weeks of infection, normal adult mice with mature GM completely eliminate HBV, but young mice without GM remain positive for HBV. Following clearance of the GM of adult mice, their resistance to HBV decreased. Another study reported that after treatment with fecal microbiota transplantation (FMT), patients with hepatitis B e-antigen (HBeAg) positivity showed a significant decrease in their blood HBeAg levels, indicating a weakened viral replication activity (100).

Previous studies have reported that the GM composition of patients with hepatitis changes compared with healthy individuals (101-103). For example, the levels of *Bacteroides* in the GM of patients with HBV-related cirrhosis is low, at 4 vs. 53% in

healthy patients and the level of *Proteus* is high at 43 vs. 4% for healthy patients (104). Furthermore, Bajaj *et al* (105) reported the unique composition of GM in patients with HCV. GM of these patients predominantly comprises *Enterobacteriaceae*, *Clostridium* and *Ruminococcaceae* genera. A previous study compared the GM of stage 4 HCV patients with that of healthy individuals, revealing that the relative abundance of Bacteroides in the GM of HCV patients increased, whereas the abundance of Firmicutes decreased (106). Using high-throughput 16S rRNA gene sequencing, Aly *et al* (106) reported that the GM of patients with HCV exhibits high level of *Proctor* and levels of *Acinetobacter*, *Vibrio* and *Lactobacillus* were also increased compared with healthy patients. However, the probiotic genus, *Bifidobacterium* were found exclusively in the GM of HCV patients, while no *Bifidobacterium* were detected in the GM of healthy individuals. In conclusion, because viral hepatitis is associated with GM composition, GM could be targeted in the development of novel hepatitis treatment strategies in future.

Association between GM and ALD/NAFLD. ALD is the most prevalent form of chronic liver disease, affecting 150 million people worldwide (107). Alcoholic steatohepatitis (ASH), which is characterized by hepatic inflammation, may develop from alcoholic fatty liver. ASH is characterized by acute inflammatory response with neutrophils and hepatocellular damage, whereas cirrhosis involves chronic architectural remodeling with fibrosis and regenerative nodules (108). Chronic ASH can lead to fibrosis and cirrhosis. In 2019, ~371,964 people exhibited alcoholic cirrhosis-related mortality, accounting for 25% of all liver cirrhosis-associated mortalities. There were 90,741 mortalities due to alcohol-related HCC (109). Moreover, ASH can directly lead to liver failure, and severe ASH may lead to high mortality rates (110).

Chronic alcohol consumption directly or indirectly alters composition of GM and leads to changes in human and animal gut development (111). Chronic ethanol feeding leads to a decrease in abundance of *Bacteroidetes* and *Firmicutes* phyla in the GM and an increase in the proportion of *Gram-negative Proteobacteria* and *Gram-positive Actinobacteria* phyla (112). Alcohol can directly and indirectly increase the permeability of the gut wall, as dysfunctional GM and its metabolites enter the liver and activate Kupffer cells by binding with TLR4 or TLR9, inducing Kupffer cells to secrete pro-inflammatory cytokines. The GM of healthy individuals produces SCFAs by breaking down dietary fiber and resistant starch in the gastrointestinal tract. SCFA plays a role in gastrointestinal physiology, immune function, and host metabolism (113). Alcohol use is associated with lower levels of gut SCFA, suggesting that GM may serve a role in development and progression of liver disease (114-116) (Fig. 2). SCFA in the mouse gut helps alleviate the progression of ALD, which may be related to the regulatory effect of SCFA on the liver immune microenvironment (114).

Furthermore, prevalence of NAFLD increases with obesity and has replaced alcoholic hepatitis as the most common type of chronic liver disease worldwide (117). Obesity is associated with dysbiosis of the GM and GM modulation has potential for prevention and treatment of NAFLD (118). According to mouse studies and fecal transplantation trials, GM serves a key role in the development of NAFLD (119,120). Ecological dysbiosis of GM and its metabolites promotes the signal cascade reaction in the liver during translocation (such as activation of TLR and NLRP3 signaling pathway) promotes the secretion of cytokines such as TNF-α and IL-1β, and leads to steatosis and inflammation in the liver of susceptible mice (121). Regardless of the amount of alcohol consumed, GM produce endogenous ethanol, particularly when sugar-rich foods are consumed (122). Ethanol synthesized by GM activates TLRs in the liver, promoting cytokine synthesis and secretion and altering BA profile in obese humans and mice (123-125).

In conclusion, as GM contributes to the development of ALD and NAFLD, the GM may be a potential therapeutic target for ALD and NAFLD. Using MIYAIRI 588, a butyrate-producing probiotic, to treat NAFLD in rats significantly improves liver lipid deposition, insulin resistance, serum endotoxin levels, and the liver inflammation index (126). Additionally, a meta-analysis indicated that probiotic therapy significantly reduces blood ALT, AST, total cholesterol (T-chol), high-density lipoprotein (HDL), and TNF-α levels in NAFLD patients, while also improving insulin resistance (127). Kirpich *et al* (128) showed that short-term oral supplementation with *Bifidobacterium* and *Lactobacillus plantarum 8PA3* can improve the dysbiosis of GM in patients with ALD, and reduce serum levels of ALT, AST, gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase, and total bilirubin.

Association between GM and cirrhosis. Cirrhosis is histological development of regenerative nodules surrounded by fibrous bands, which arises from chronic liver injury and leads to portal hypertension and end-stage liver disease (129). The mechanism underlying cirrhosis has been extensively studied (130-132). Cirrhosis is caused by abnormal accumulation of the extracellular matrix (ECM) (mainly composed of fibrous collagen, elastin, and matrix proteins) under chronic (133). Excessive deposition of ECM can lead to the replacement of the normal structure of liver lobules with fibrous tissue, resulting in the destruction of the normal liver architecture and the formation of fibrous septa and nodules (133). It is hypothesized that the first step of cirrhosis involves the production of oxygen-free radicals and inflammatory substances that damage liver cells and recruitment of Kupffer and inflammatory cells. Additionally, elevated levels of oxygen free radicals and inflammatory substances in liver tissue can cause hepatic stellate cells (HSC) to differentiate into myofibroblasts, the primary source of ECM (134). The most common causes of cirrhosis are viral hepatitis, NASH and ALD (135). According to a 2023 survey, cirrhosis is frequently attributed to alcohol use disorders (~45% of cases), HCV (41%), and NAFLD (26%) (136). The 2019 Global Burden of Disease Study estimated global deaths related to cirrhosis as follows: 395,000 from HCV-associated cirrhosis, 331,000 from HBV-related cirrhosis, 372,000 from alcohol-related cirrhosis, and 134,000 from NASH-related cirrhosis (137). Cirrhosis is the 11th most common cause of mortality and the third most common cause of mortality among people aged 45-64 years. Together with liver cancer, cirrhosis accounts for 3.5% of all deaths worldwide (138). To date, there is no consensus regarding the treatment of cirrhosis, with current methods limited to controlling symptoms and complications, as well slowing the progression of cirrhosis. If the liver is severely damaged, liver transplantation may be the only treatment option (139,140).

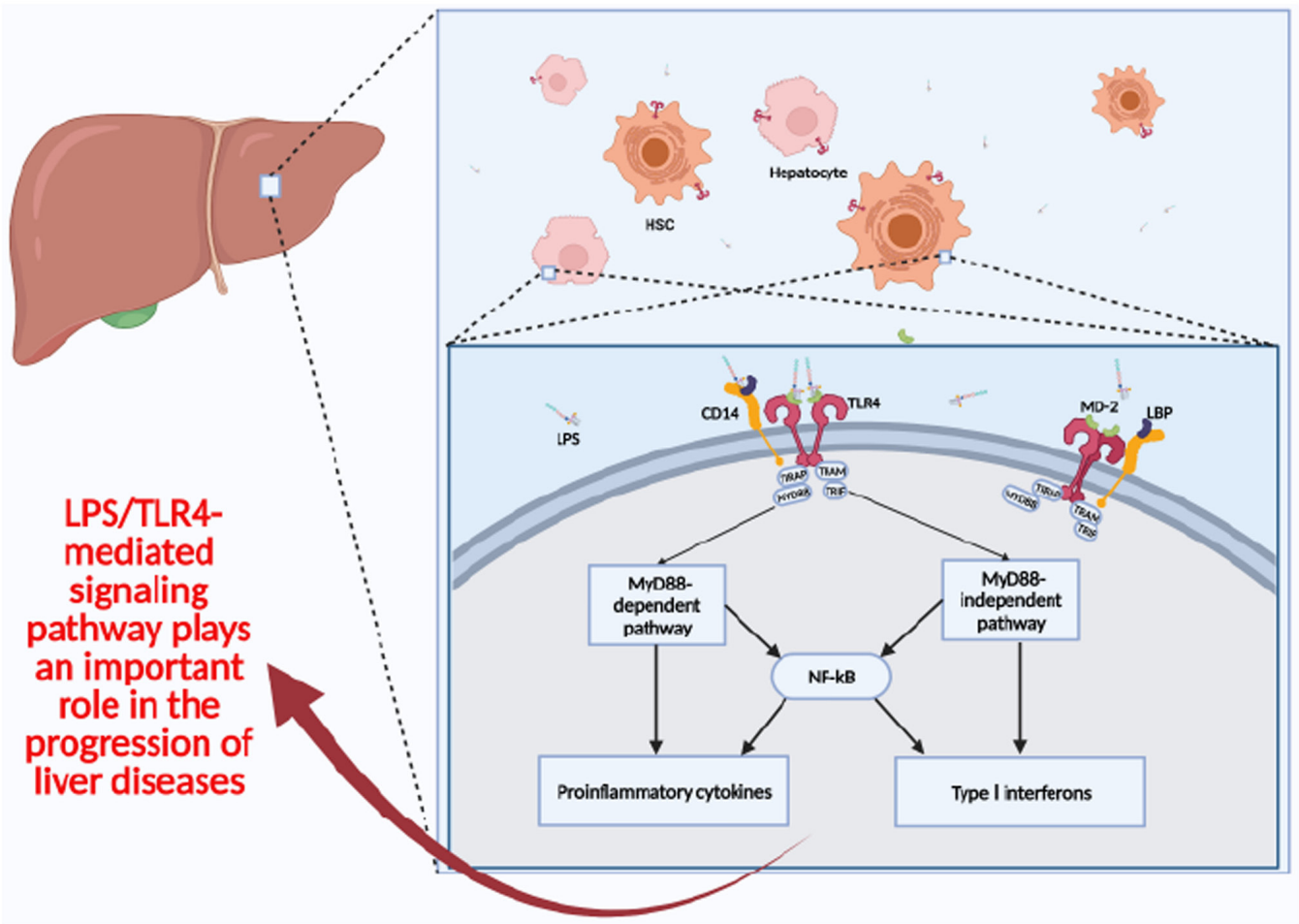

Figure 2. Alcohol changes the composition of gut microbiota, whereby a dysfunctional gut microbiota secretes a large number of toxic metabolites, including endotoxins. Alcohol and toxic metabolites destroy function of the gut wall and increase its permeability. Dysfunctional gut microbiota and their metabolites enter blood through the gut wall and enter the liver through circulation of the blood. At the same time, the imbalance of gut microbiota caused by alcohol stimulation decreases levels of anti-inflammatory protective factors including SCFAs, which further exacerbates the disease process. SCFA, short chain fatty acid.

The gut-liver axis serves a role in progression of cirrhosis. The gut epithelium is a single-cell layer that serves as a selective permeation barrier, facilitating the absorption of nutrients, electrolytes, and water, while effectively defending against intracavitary toxins, antigens, and GM (141). Previous studies have demonstrated that gut permeability in patients with cirrhosis is increased, which has been reported to be related to the degree of endotoxemia (142,143). A prospective study demonstrated higher serum endotoxin levels in patients with cirrhosis compared to healthy individuals (144). Subsequent studies have indicated that increased gut permeability facilitates the translocation of intestinal-derived endotoxins (LPS) into the bloodstream (145). In addition, the increase in gut permeability promotes pathological translocation of GM and its metabolites into liver, leading to the activation of numerous inflammatory cytokine signaling pathways in the liver, driving immune dysfunction associated with inflammation and cirrhosis (146). Moreover, cytokines secreted by immune cells can both reduce (i.e. TNFα and IFNγ) gut barrier function and enhance (i.e. TGFβ and IL-10) gut barrier function. The immune response may lead to ecological imbalance or microbial changes in feces, intestinal mucosa, ascites, liver, serum and saliva (147). Ecological imbalance is related to gut barrier dysfunction as GM and its products regulate the barrier function by affecting epithelial inflammatory reaction and mucosal repair functions (148). Further research has shown that gut permeability can be directly regulated by GM through the release of soluble peptides or toxins, which in turn regulate the expression of gut tight junction proteins, including integrated membrane proteins, junction complex proteins, and cytoskeletal structural proteins (149). Additionally, other metabolites of GM, such as SCFAs, BA metabolites, conjugated FAs, indole derivatives, and polyamines, can regulate the expression of gut tight junction proteins by binding to receptors such as FXR, G protein-coupled bile acid receptor (TGR), and aromatic hydrogen receptor (AHR) on the surface of gut epithelial cells (150).

Small intestinal bacterial overgrowth (SIBO) is frequently observed in patients with liver cirrhosis and is more prevalent in those with advanced cirrhosis. Spontaneous bacterial peritonitis (SBP) and hepatic encephalopathy (HE) are two frequent complications of liver cirrhosis, both closely associated with increased patient mortality (136). Increasing evidence suggests that the imbalance of intestinal ecology in patients with liver cirrhosis is closely related to disease progression (143,151). Chang *et al* (152) reported that 70% of patients with SBP

cirrhosis exhibit SIBO, whereas only 20% of patients with non-SBP cirrhosis had SIBO. In patients with a history of SBP, intestinal peristalsis is impaired and may contribute to development of SIBO. Corradi *et al* demonstrated that the control of SIBO with antibiotic treatment may mitigate the progression of spontaneous bacterial peritonitis in cirrhotic rats (153). HE is associated with the GM (154). A randomized controlled trial demonstrated that FMT improved the dysbiosis of GM in patients with liver cirrhosis and delayed the progression of HE (155). Bajaj *et al* (156) suggested that the sigmoid microbiota of patients with cirrhosis exhibits a low abundance of autochthonous genera including *Dorea*, *Subdoligranulum*, and *Incertae Sedis other* and a high abundance of potentially pathogenic bacteria, including *Enterococcus*, *Burkholderia*, *Proteus* and *Clostridium*. Other studies have reported that the abundance of *Veillonella*, *Megasphaera*, *Dialister*, *Atopobium* and *Prevotella* increased in the GM of patients with cirrhosis (143,157). Changes in fungi in GM have also been reported in alcohol-related cirrhosis (158). Compared with healthy individuals, the diversity of gut fungi in patients with ALD is decreased, demonstrated by the overgrowth of Candida (74). Candida hemolysin is a peptide toxin secreted by *Candida* that can transfer from the gut to the bloodstream and translocate to the liver, causing direct damage to liver cells. β-glucan is a cell wall component of many symbiotic fungi. Serum levels of β-glucan were significantly elevated in alcohol-fed mice. Concentration of serum β-glucan is closely related to gut integrity, inflammation, and the severity of liver disease (159). β-glucan can stimulate immune cells to produce a strong immune response, which is an important cause of disease exacerbation (160). By analyzing the composition and abundance of GM, the etiology of cirrhosis can be effectively determined, guiding treatment (161). Controlling the abnormal growth of GM can effectively manage the progression of cirrhosis. Oral *probiotic lactobacilli* can regulate the decrease in *Enterococcal* abundance, which is associated with reducing the severity of liver injury (162).

Association between GM and liver cancer. Primary liver cancer is the sixth most common malignancy and the fourth highest cause of cancer-associated mortality worldwide. The most common type of primary liver cancer is HCC, according to the World Health Organization (163). A number of risk factors are associated with liver cancer, notably HBV, HCV, alcohol abuse and aflatoxins in the diet (164).

Based on studies in human and animal models, GM may contribute to development of HCC (91,101,120,143,157,165-190) (Table I). Patients with HCC and mice treated with diethylnitrosamine exhibit dysbiosis of the GM. The reduction of *Lactobacillus*, *Bifidobacterium* and *Enterococcus* in the gut of rats with liver cancer, as well as proliferation of *Escherichia coli*, suggests an imbalance in composition of GM in liver cancer (191). Lipopolysaccharide (LPS), a metabolite of the GM, binds to TLR4 on HSCs, triggering the activation of HSCs, which leads to the development of liver fibrosis and cirrhosis (Fig. 3). Dapito *et al* (192) reported that activation of TLR4 accelerates HCC progression by promoting cell proliferation and inhibiting apoptosis. Furthermore, Mou *et al* (193) reported the role of the LPS/TLR4 signaling pathway in regulating liver fibrosis progression in rats. Neomycin is an antibiotic that inhibit the overgrowth of GM. Yu *et al* found that the accumulation of LPS and the expression of TLR4 were reduced in the liver of liver cancer mice treated with neomycin (194). Further studies using antibiotics in mouse models are needed to understand the disease progression in the absence of GM. Dapito *et al* (192) demonstrated that removal of the GM by antibiotics protected mice from liver fibrosis and HCC. This may be due to antibiotics reducing the quantity of GM, decreasing the secretion of toxic metabolites such as LPS, thereby lessening the degree of liver damage.

Yoshimoto *et al* (195) reported that deoxycholic acid (DCA), a metabolite of the GM known to induce DNA damage, is more frequently produced in the gut of obese mice. As a result of elevated levels of DCA in the gut-liver axis, HSCs exhibit a senescence-related secretory phenotype and release inflammatory factors and pro-tumor chemicals (196). Further research has shown that DCA can activate the NF-κB signaling pathway, TNF signaling pathway, and NLRP3 signaling pathway in HSC cells, triggering a large secretion of downstream factors such as IL-1 β, IL-6, TNF, ROS, etc. that promote damage. Exposure of the liver to these damaging factors can easily lead to HCC. These findings suggest that GM metabolites contribute to development of obesity-induced HCC in animals.

5. Clinical treatment of liver disease based on the liver-gut axis

FMT. FMT is an increasingly popular method of altering GM composition during disease. FMT involves transplantation of GM obtained from the stool of a healthy donor into the gastrointestinal tract of a patient (197-199). In most cases, this therapy is used to treat gastrointestinal diseases caused by activity of pathogenic or conditionally pathogenic microorganisms (200). Recent studies have reported that this approach has potential for clinical application in treatment of liver disease (201,202).

FMT can be administered through three channels: Oral, through the upper gastric portion; nasal, via nasogastric tubes; and rectal through colonoscopy or enema (203). Fecal suspension perfusion in the rectum using colonoscopy is considered to be the best method for FMT (204). By contrast, FMT using the upper gastric route, including nasogastric tube, nasogastric or upper endoscopy exposes the entire gastrointestinal tract to donor stool and can lead to pulmonary or gastrointestinal complications due to the presence of large numbers of pathogenic bacteria in the upper GI and respiratory tracts (205). The use of fecal microbiota in oral capsules has also been reported (206). In a randomized controlled study of 22 obese patients (206), FMT capsules exhibited no significant side effects and significantly improved composition of the GM of patients and decreased metabolic levels of taurocholic, which cause damage to the liver (207,208) and BAs.

Phage therapy. Phages are viruses that specifically infect bacteria. In the early days of phage therapy application, infectious phage agents were commonly used to treat diseases caused by bacterial infections including *Staphylococcus, Streptococcus, Vibrio, Klebsiella, Enterobacter, Shigella, Escherichia, Pseudomonas* and *Providencia*, which have the advantage over antibiotics of targeting specific bacterial

Table I. Changes in gut microbiota in patients with hepatic cell carcinoma.

Liver disease	Decreased	Increased	(Refs.)
Alcohol-related liver disease	*Bacteroidetes*	*Proteobacteria*	(177-180)
	Bacteroidaceae	*Enterobacteriaceae*	
	Firmicutes	*Streptococacceae*	
	Lactobacillaceae	*Veillococcaceae*	
	Lachnospiraceae	*Candida*	
Non-alcoholic fatty liver disease	*Firmicutes*	*Enterobacteriaceae*	(120,181-184)
	Lachnospiraceae	*Proteobacteria*	
	Ruminococcaceae	*Bacteroidetes*	
		Prevotellaceae	
		Rikenellaceae	
		Lactobacillaceae	
Cirrhosis	*Bacteroidetes*	*Proteobacteria*	(143,185-187)
	Bacteroidaceae	*Enterobacteriaceae*	
	Firmicutes	*Streptococaceae*	
	Lachnospiraceae	*Clostridiacceae*	
	Ruminococcaceae	*Veillococcaceae*	
		Fusobacteria	
		Fusobacteriaceae	
Hepatocellular carcinoma	*Verrucomicrobia*	*Actinobacteria*	(91,101,188-190)
	Phascolarctobacterium	*Gemmiger*	
	Ruminococcus	*Parabacteroides*	
	Bifidobacterium	*Klebsiella*	
	Escherichia-Shigella	*Haemophilus*	
	Enterococcus	*Bacteroides*	
		Ruminococcaceae	
		Faecalibacterium	
		Ruminococcus	
		Ruminoclostridium	

species or strains while self-replicating and spreading to infect other target bacterial cells (209). Notably, phage therapy has the ability to edit the GM. In two randomized placebo-controlled trials (study nos. NCT03269617 and NCT04511221), phages improved the GM profile by targeting specific bacterial genera, modulated the overall metabolism and reduced the incidence and severity of gastrointestinal discomfort (210-212).

Specific GM serve a role in the pathogenesis of a number of types of liver diseases; therefore, phage therapy capable of eliminating specific GM has potential value in treatment of liver disease. In humanized mice colonized with bacteria from the feces of patients with alcoholic hepatitis, phage therapy targeting lysogenic *Enterococcus faecalis* decreases mortality as well as ethanol-induced liver injury, steatosis, inflammation and fibrosis (213). However, to the best of our knowledge, there are no clinical trials to validate the safety and efficacy of phage therapy in treatment of human liver disease. Despite this, phage therapy has been suggested as potential novel therapy for the treatment of liver disease (214).

Engineered bacterial therapy. Bacteria can acquire the ability to transcribe and translate various genes through gene editing technology. Through oral administration and other delivery methods, these genetically modified bacteria can reach the human intestine and colonize it. These genetically engineered bacteria can express specific enzymes, thereby promoting the conversion of toxic metabolites in the intestine to non-toxic products (215). Hyperammonemia is associated with liver disease, and the intestine is the primary source of systemic ammonia (NH3) (216). Kurtz *et al* (217) developed an engineered bacterium called *SYNB1020* that can colonize the intestine via oral administration. This bacterium can convert NH3 in the intestine into l-arginine, thereby reducing blood ammonia levels. In a mouse model of hyperammonemia, *SYNB1020* treatment increases survival rate. Moreover, *SYNB1020* has good tolerability in a phase I clinical trial of hyperammonemia disease (217). Thus, *SYNB1020* warrants further clinical development.

E. coli Nissle 1917 is a traditional probiotic with a well-established safety record, which has been widely used in the production of therapeutic agents, delivery carriers, and microbial platforms in industrial production (218). Lynch *et al* (219) genetically engineered *E. coli Nissle 1917* to overexpress catalase and superoxide dismutase; inflammatory response of colonic tissue in mice with inflammatory bowel disease model significantly reduced following oral

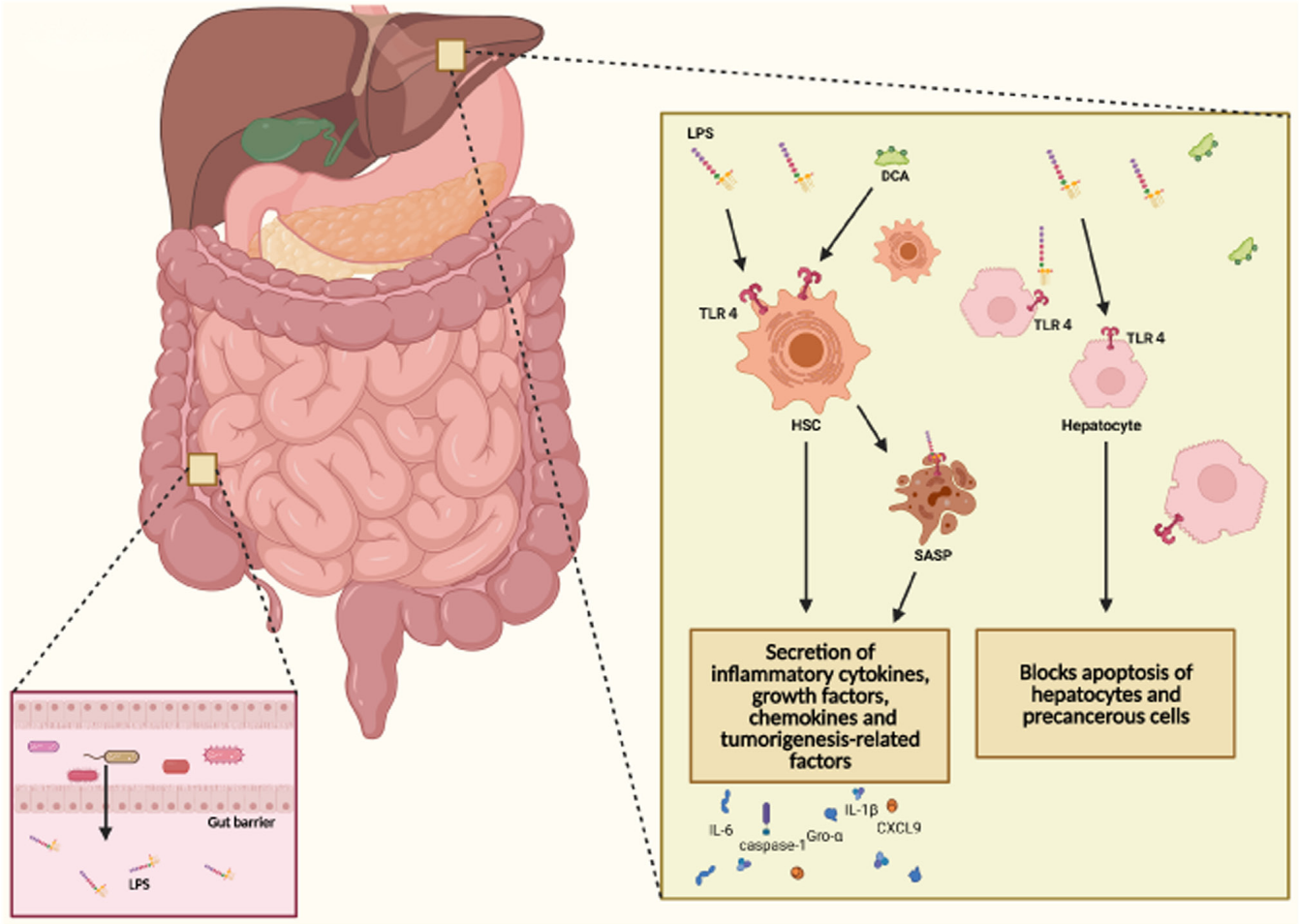

Figure 3. LPS/TLR4 signaling pathway serves a role in progression of liver disease. LPS from the gut binds with TLR4 on hepatocytes and the HSC surface, which activates downstream signaling pathways including the MyD88-dependent and -independent signaling pathway and promotes synthesis and secretion of pro-inflammatory cytokines and type I interferons. TLR4, toll-like receptor 4; LPS, lipopolysaccharide; HSC, hepatic stellate cell; LBP, lipopolysaccharide-binding protein.

administration and adjusted the composition of GM and repaired the gut epithelial barrier.

Duodenal mucosal surface replacement (DMR). As the most proximal gut segment, the duodenal mucosa has a unique chemosensory capacity to detect luminal contents and rapidly release bioactive mediators and hormones with local and systemic effects. These bioactive compounds include GM and GM metabolites, which are recognized by metabolite-sensing receptors (220). Intestinal luminal chemosensing involves the regulation of gut function and the systemic regulation of metabolism, energy balance, and food intake (221). Pharmacological modulation targeting the duodenum can maintain metabolic homeostasis in obesity, diabetes and NAFLD (222,223). Duodenal mucosal surface reconstruction (DMR) is a novel surgical procedure that, under endoscopic guidance, involves the introduction of a catheter with a balloon into the duodenum. The balloon is then expanded to segment the duodenum, and the duodenal mucosa is separated by injecting saline into the submucosal layer, followed by mucosal ablation using circulating hot and cold water. The ablation range covers all duodenal mucosa from 1 cm distal to the main papilla to the ligament of Treitz. After mucosal regeneration, the formation of new gut cells and the re-establishment of a healthy neuroendocrine axis can restore gut function and provide a healthy gut environment (224-226). In a randomized controlled clinical trial (227), DMR reported safety and efficacy in glycemic control and liver fat content in type 2 diabetes. However, in another clinical trial, DMR did not improve NASH (228).

6. Conclusion

The gut-liver axis underscores the connection between gut health and liver function. Numerous types of liver diseases, including NAFLD, ALD, HE and HCC, are influenced by changes in GM. This axis is a target for clinical applications, aiding in diagnosis, prognosis and the development of treatment. GM analysis offers insights into the mechanisms and phenotypes of liver disease. Modulating BA signaling and fecal transplantation or probiotics from human sources show potential as treatments. However, safety evaluation data for these methods are still insufficient. Further research is needed to ensure their efficacy and safety, bridging the gap between animal models and clinical practice to prevent progression of early liver disease.

In summary, the correlation between the gut and liver serves as a pathway for exploring the clinical treatment of liver disease. The function of a complete gut barrier, GM, and their associated metabolites communicates complex host-microbial interactions that can maintain health or promote disease. However, more research is needed to elucidate the changes in gut microbiota abundance in patients with different types of liver diseases, as well as the specific signaling pathways involved, and how to regulate GM in a way that minimizes side effects, to develop better clinical treatments for liver diseases. However, the present review has limitations. The impact of environmental exposure and lifestyle factors on the occurrence and development of liver disease was not discussed. Finally, GM could serve as a diagnostic tool and therapeutic target in patients with liver disease.

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Availability of data and materials

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Authors' contributions

JW and SC conceived the study and drafted the manuscript. XW constructed the figures and table and revised the manuscript. EZ and BC revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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