Dysbiosis and liver diseases (Review)

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Abstract. Dysbiosis, a qualitative and quantitative aberrancy of gut microbiota, has attracted marked attention. At present, advances in molecular biological techniques have made it possible to analyze gut microbiota at the DNA and RNA levels without culturing, and methods such as 16S ribosomal RNA targeting analysis and metagenomic analysis using next-generation sequencers have been developed. The relationship between gut microbiota and various diseases has been extensively examined. Gut microbiota are essential for the immune system, energy intake and fat storage, and humans use them to build complex immune regulatory mechanisms and to obtain energy from food. The liver is the first organ to be nourished by the portal blood flow of intestinal origin, and liver diseases can be strongly influenced by various factors of intestinal origin, such as intestinal bacteria, bacterial components, and intestinal bacterial metabolites. Rigorous research has revealed that the composition of the gut microbiota is altered and the diversity of bacteria is reduced in liver diseases. Significance of various factors transported to the liver by portal vein blood flow from the intestine has been extensively investigated. Gut microbiota in liver disease can be associated with disease progression regardless of disease etiology and even with carcinogenesis. The relationship between gut microbiota and liver diseases (hepatitis virus-related diseases, autoimmune liver diseases, alcoholic liver disease, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, liver cirrhosis and hepatocellular carcinoma) and the treatments of dysbiosis (antibiotics, prebiotics, probiotics and fecal microbiota transplantation) in liver disease are outlined based on the current evidence.

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Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibody; CLD, chronic liver diseases; DAMP, damage-associated molecular pattern; DCA, deoxycholate; FMT, fecal microbiota transplantation; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HFD, high-fat diet; HSC, hepatic stellate cell; LC, liver cirrhosis; LPS, lipopolysaccharide; LTA, lipoteichoic acid; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PAMP, pathogen-associated molecular pattern; PBC, primary biliary cholangitis; PI3K, phosphoinositide 3-kinase; PSC, primary sclerosing cholangitis; RCT, randomized controlled trial; SASP, senescence-associated secretory phenotype

Key words: dysbiosis, liver disease, molecular mechanism, disease progression, carcinogenesis

Contents

1. Introduction: Gut microbiota and liver

A large number of bacteria live in various parts of the human body (skin, oral cavity, pharynx, upper respiratory tract, stomach, small intestine, colon). The gastrointestinal tract contains ~100 trillion intestinal bacteria of ~1,000 species (weighing ~1.5 kg), which live in symbiosis with humans. The majority of intestinal bacteria are found in the colon (1-4). Dysbiosis, a qualitative and quantitative aberrancy of gut microbiota, has attracted marked attention. At present, advances in molecular biological techniques have rendered it possible to analyze gut microbiota at the DNA and RNA levels without culturing, and methods such as 16S ribosomal RNA (16S rRNA) targeting analysis and metagenomic analysis (analysis of the entire genetic information of bacteria that constitute the gut microbiota) using next-generation sequencers have been developed (5). The relationship between gut microbiota and various diseases has been extensively reported (1-4).

Gut microbiota has been revealed to play important roles not only in digestion but also in immunity and metabolism. Gut microbiota is essential for the immune system, energy intake and fat storage, and humans use them to build complex immune regulatory mechanisms and to obtain energy from food (1-4). With proper diet, the gut microbiota can trigger changes in the balance of short-chain fatty acids, which are used as an energy source (3). Gut microbiota can be said to be an organ in itself. More than 99% of gut microbiota belong to four phylums: *Firmicutes* phylum (gram-positive bacteria), *Bacteroidetes* phylum (gram-negative bacteria), *Proteobacteria* phylum (such as *Escherichia coli, Salmonella, Vibrio and Helicobacter*), and *Actinobacteria* phylum (such as *Bifidobacteria*) (6). The composition of gut microbiota markedly changes with aging (7). There are several patterns of aging-related changes in the gut microbiota, including a group that decreases with aging (*Actinobacteria*), a group that increases with aging (*Bacteroidetes*), a group that is more prevalent only in adults (*Firmicutes*), and a group that is more prevalent in infants and the elderly (*Proteobacteria*) (7). The composition of gut microbiota can also change with food intake (8). The intestines of people who regularly consume an abundance of vegetables, fish, and fiber are likely to be rich in bacteria that help reduce inflammation, while the intestines of meat-lovers are likely to be rich in bacteria that promote inflammation (8). Long-term improvement of eating habits can improve the balance of intestinal microflora.

Rigorous research in recent years has revealed that the composition of the gut microbiota is altered and the diversity of bacteria is reduced (dysbiosis) in obesity, inflammatory bowel diseases, and liver diseases compared with healthy individuals (2,4). At present, such changes (i.e., dysbiosis) have been noted in colorectal cancer (9), type 2 diabetes (10), irritable bowel syndrome (11), atherosclerotic heart diseases (12,13), allergic diseases (14), autism (15), and even neurological diseases (16) (Fig. 1). Thus, aberrancies in the balance of the gut microbiota can disrupt host homeostasis and lead to a variety of diseases. The liver is the first organ to be nourished by the portal blood flow of intestinal origin, and liver diseases are considered to be strongly influenced by various factors of intestinal origin, such as intestinal bacteria, bacterial components, and intestinal bacterial metabolites (17). Various factors, including pathogen-associated molecular patterns (PAMPs), which are transported to the liver by portal vein blood flow, have attracted particular attention and their significance has been extensively investigated (17).

Hepatic stellate cells (HSCs) and hepatic macrophages are important cells that are affected by intestinal bacteria and metabolites in the development of chronic liver diseases (CLDs). Firstly, HSCs become activated under chronic liver injury or *in vitro* culture conditions, and change to a myofibroblast-like cell morphology with high expression of α-smooth muscle actin (α-SMA), and induce liver fibrosis by producing high levels of extracellular matrix such as collagen (18). In addition, HSCs can be activated by various stimuli such as reactive oxygen species (ROS), damage associated molecular patterns (DAMPs), cytokines, and chemokines (18). Activated HSCs persistently express signals related to cell proliferation, fibrosis, and growth factors. Therefore, it is known that they play an important role in the formation of the cancer microenvironment, which supports the growth and development of cancer cells by inducing angiogenesis and fibrosis (19). Secondly, liver macrophages include Kupffer cells and monocyte-derived macrophages: during inflammation, activated liver macrophages produce various secretory factors and induce influx of bone marrow-derived monocytes and neutrophils, and also activate HSCs to induce liver fibrosis (19). In addition, activated liver macrophages produce matrix metalloprotease, an extracellular matrix-degrading enzyme, and express tumor necrosis factor-related apoptosis inducing ligand (TRAIL), which induces apoptosis in liver parenchymal cells. Therefore, it is attracting attention as a therapeutic target for liver fibrosis (20).

This review outlined the relationship between gut microbiota and liver diseases: hepatitis virus-related liver diseases, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), autoimmune liver diseases, alcoholic liver disease, liver cirrhosis (LC) and hepatocellular carcinoma (HCC), and also outlined the treatments of dysbiosis (antibiotics, prebiotics, probiotics and fecal microbiota transplantation) in liver disease. The present review included mainly original studies and review articles regarding dysbiosis and liver disease between 1995-2021. In total 113 studies were included.

2. Hepatitis C virus and gut microbiota

A decrease in the diversity of gut microbiota has been reported in the intestinal microflora of patients with chronic hepatitis C virus (HCV) infection (21,22). The gut bacteria of patients with HCV infection exhibit an increase in harmful bacteria, a decrease in beneficial bacteria, and a decrease in bacterial species (21,22). Changes in the gut microbiota in patients with HCV are common and are caused by antibody-producing cells derived from B lymphocytes (22). According to the analysis of the gut microbiota of chronic hepatitis C patients in Egypt, where HCV infection is the highest in the world, *Prevotella*, *Faecalibacterium*, *Acinetobacter*, *Veillonella* and *Phascolarctobacterium* are increased in the intestinal microflora (21). As the disease progresses, changes in the gut microbiota become clearer, and it has been reported that patients with chronic HCV infection along with LC have clearly lower diversity of gut microbiota than those without LC (23,24). Furthermore, Inoue *et al* analyzed the gut microbiota of hepatitis C patients by fibrosis progression and reported that: i) changes in gut microbiota were already observed even in HCV carriers with normal liver function [persistent normalized alanine aminotransferase, (PNALT)], ii) as the disease condition worsens from PNALT, chronic hepatitis, LC, and HCC, the occupancy rate of indigenous bacteria in the intestinal flora decreases, the number of bacterial species comprising the flora decreases, and the pH of the stool increases, making it easier to develop dysbiosis at a high rate with the progression of liver fibrosis and iii) as hepatitis C progresses, there is an aberrant increase in *Streptococcus salivarius* in the intestinal flora, and these bacteria can degrade urea in the intestinal tract to produce ammonia, resulting in a high pH of the stool (24). Thus, in patients with HCV, close correlation between the degree of liver fibrosis and gut microbiota changes has been identified, however, correlation between HCV viral load and gut microbiota changes is unknown.

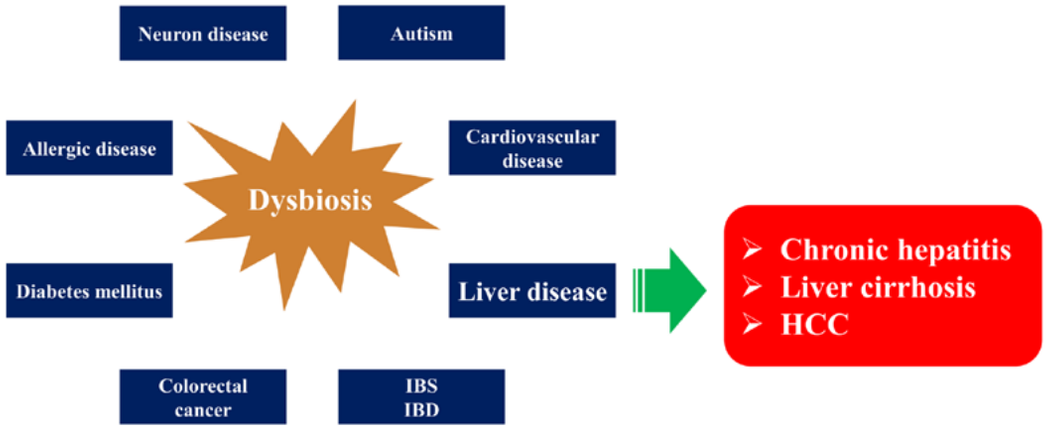

Figure 1. Dysbiosis can cause allergic, neurological, cardiovascular, metabolic, colorectal, liver disease (hepatitis, liver cirrhosis and hepatocellular carcinoma) and cancer. IBS, irritable bowel syndrome; IBD, inflammatory bowel disease; HCC, hepatocellular carcinoma.

3. Hepatitis B virus and gut microbiota

Only ~5-10% of adults develop chronic hepatitis B infection from acute hepatitis B virus (HBV) infection, but 90% of newborns and 30-50% of children aged 1-5 years fail to eliminate HBV from their bodies (25,26). In addition to the maturation of the immune system, the gut microbiota has also been implicated in the age-related differences in HBV viral elimination capacity (27). As aforementioned, the composition of gut microbiota markedly changes with aging (7). Adult mice with stable gut microbiota can eliminate HBV virus within 6 weeks after infection, but when dysbiosis is induced by antibiotics, viral elimination becomes impossible, suggesting the importance of anti-HBV activity by regulation of the immune system through gut microbiota (28).

The gut microbiota of patients with chronic HBV infection and HBV-related LC have been reported to be characterized by a decrease in *Bifidobacteria* and lactic acid-producing bacteria and an increase in *Enterococcus* and *Enterobacteriaceae* (29,30). Wei *et al* reported a decrease in *Bacteroidetes* (4 vs. 53%) and an increase in *Proteobacteria* (43 vs. 4%) in a comparison of the gut microbiota of patients with HBV-related LC and healthy subjects (31). In a recent study, gut microbiota composition in the three different stages (i.e., chronic hepatitis B, LC and HCC) of HBV-related CLD patients and healthy individuals was compared (32). The β-diversity (diversity differences between the two samples) demonstrated a separate clustering of healthy individuals and HBV-CLD patients, and gut microbiota of healthy individuals was more consistent, whereas those of chronic hepatitis B, LC and HCC varied substantially (32). The abundance of *Firmicutes* was lower, and that of *Bacteroidetes* was higher in patients with chronic hepatitis B, LC and HCC than in healthy individuals. Metagenomic analysis of microbial communities demonstrated an increase in glycan biosynthesis and metabolism-related genes in HBV-CLD compared with healthy individuals. Their results denoted that HBV-CLD can be associated with gut dysbiosis, with features including an increase in potential harmful bacteria (*Bacteroidetes*) or related genes and a decrease in potential beneficial bacteria (*Firmicutes*) or related genes (32).

4. Autoimmune liver diseases and gut microbiota

Autoimmune hepatitis (AIH) is a typical autoimmune disease frequently observed in middle-aged or older women, and its association with gut microbiota has received marked attention (33). A recent study in mice revealed that *Enterococcus gallinarum*, an intestinal bacterium, causes AIH when it migrates from the intestine to the liver (34). In humans, *Enterococcus gallinarum* was detected in the liver of AIH patients, but not in healthy controls. Manfredo Vieira *et al* used fluorescence to track bacteria in mice and identified that *Enterococcus gallinarum* was present in lymph nodes, liver and spleen in AIH patients (35). Interestingly, the secretion of immune signals associated with AIH such as the induction of TH17 cells was increased by *Enterococcus gallinarum* in these organs, but the presence of other types of bacteria in these organs did not cause AIH (34). It has also been reported that the diversity of gut microbiota is decreased in AIH patients (36). *Bifidobacterium*, which is associated with disease activity in AIH, has been reported to be decreased (37). Gut microbiota has also been revealed to be involved in AIH exacerbations. The exacerbation of AIH is triggered by interleukin (IL)-18, which is induced by TLR ligands derived from gut microbiota (38,39).

Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by progressive destruction of the intrahepatic bile ducts, leading to bile stasis, LC, and liver failure. $CD4^+$ and $CD8^+$ T lymphocytes directly target bile duct epithelial cells (40-42). The involvement of microorganisms such as *Escherichia coli* in the etiology or pathogenesis of PBC has been known for a long time, and vaginal or urinary tract infections in particular have been cited as risk factors for PBC (40-42). The major corresponding antigen of anti-mitochondrial antibodies (AMAs) is pyruvate dehydrogenase complex E2 component (PDC-E2), and as AMAs and autoreactive T cells of PBC patients cross-react with PDC-E2 derived from enteric bacteria such as *Escherichia coli*, autoimmunity by molecular homology has been postulated as a mechanism of PBC development (40-42). One of the histological features of PBC is granuloma formation, which is a tissue reaction caused by immune response to foreign antigens including microorganisms. Molecular biological identification of microorganisms in granulomas by PBC revealed genes derived from enteric

bacteria such as *Propionibacterium acnes* (43). PBC, as well as AIH, has been indicated to be associated with dysbiosis (44), and although intestinal bacterial diversity is decreased in patients with PBC, it improves with ursodeoxycholic acid (UDCA), the standard treatment for PBC (44). Dysbiosis can be a poor prognostic factor for PBC (45).

Primary sclerosing cholangitis (PSC), an intractable autoimmune disease for which there are few effective treatments other than liver transplantation, is often associated with inflammatory bowel disease such as ulcerative colitis, and the post-transplant recurrence rate was lower in patients who underwent total colectomy before liver transplantation (46). It has also been reported that oral administration of vancomycin to PSC patients resulted in improvement in hepatobiliary enzyme levels and liver histological findings (47). These findings indicated that inflammation of the gastrointestinal tract and gut microbiota may be involved in the pathogenesis and prognosis of PSC (48,49). Nakamoto *et al* found that three species of enteric bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterococcus gallinarum*), which cause activation of Th17 cells in the liver, were present in the stool of PSC patients with a high probability in the mesenteric lymph nodes (50). Th17 cells are closely associated with chronic inflammation in autoimmune diseases (51). Moreover, it was revealed that *Klebsiella* disrupts the intestinal barrier in mice, migrates to lymph nodes outside the intestinal tract, and induces an excessive immune response in the liver (50). Furthermore, the Th17 immune response in the mouse liver was attenuated to ~30% by the elimination of *Klebsiella* by antibiotics. These findings may lead to the development of new therapeutic and diagnostic agents against PSC targeting gut microbiota (50).

5. Alcoholic liver disease and gut microbiota

In alcoholic liver injury, alterations in gut microbiota have been recognized as an important risk factor for disease progression, along with alcohol consumption and genetic factors (52,53). Alcohol has been revealed to induce dysbiosis in animal models and humans (54), and alcohol and its degradation products disrupt tight junctions in the intestinal epithelium, increasing intestinal permeability (i.e., leaky gut) and inflammatory responses (55). In humans, a decrease in butyrate-producing *Clostridiales* and an increase in inflammation-inducing *Enterobacteriaceae* have been observed with alcohol consumption, and in patients who progress to cirrhosis, an increase in oral indigenous bacteria and a decrease in numerous bacteria such as *Bacteroidales* in the intestine have been reported (56). It has been reported that intestinal bacteria-derived PAMPs such as lipopolysaccharide (LPS) are increased after heavy alcohol intake (57). Chronic alcohol intake also alters the production of short-chain fatty acids (SCFAs) as an energy source. A decrease in SCFAs has been observed in the intestinal tract of rats after alcohol intake (58). In a previous study using a rat model of alcoholic liver injury, it was reported that antibiotics suppressed alcoholic liver injury by inhibiting LPS (59).

6. Non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and gut microbiota

NAFLD is currently one of the most important issues in liver disease, with a prevalence of 25% worldwide. NAFLD is recognized as one of the major risk factors for HCC and is expected to become the most common indication for liver transplantation in the near future (60,61). A total of ~20% of patients with NAFLD may progress to NASH with chronic inflammation, and then to LC and HCC (61,62). The histological picture of NASH is predominantly neutrophilic, and the involvement of endotoxins derived from gram-negative bacteria has been considered for its pathological development. Obesity induces dysbiosis of gut microbiota, leading to a decrease in diversity and an increase in the *Firmicutes* to *Bacteroidetes* ratio (63). The increased *Firmicutes* to *Bacteroidetes* ratio is also observed in diabetic patients (10). Dysbiosis in NAFLD and NASH patients increases intestinal permeability and causes stress on the liver by various gut microbiota-derived PAMPs (64).

It has been revealed that LPS in portal blood reaches the liver and increases TNF-α production in Kupffer cells via TLR4 signaling enhancement. Using mouse models, it has been revealed that Kupffer cell-derived TNF-α signaling also plays an important role in the pathogenesis of NASH (65,66). Furthermore, leptin, is a hormone secreted by adipocytes, and its main function is to suppress appetite by acting on the appetite center in the hypothalamus of the brain (67). Obesity in NAFLD is often accompanied by hyperleptinemia. Leptin-signal transducer and activator of transcription 3 (STAT3) signaling enhances CD14 expression in Kupffer cells, and the resulting increased sensitivity of Kupffer cells to LPS is one of the mechanisms of NAFLD pathogenesis (68). In addition, alcohol-producing bacteria are increased in NASH patients, and blood ethanol levels are predominantly elevated, causing oxidative stress and inflammation to the liver (69).

Liver fibrosis progression and gut microbiota in NAFLD patients have also been studied (aforementioned in the Introduction section). Gut microbiota-derived LPS activates TLR4 signaling in HSCs in addition to Kupffer cells, and decreases downstream transforming growth factor (TGF)-β pseudo-receptor Bambi expression, which enhances the sensitivity of HSCs to TGF-β, resulting in their activation and development of hepatic fibrosis (70). This hepatic fibrosis was inhibited by the suppression of LPS from the intestinal tract by intestinal treatment with antibiotics (70). NAFLD patients often consume high-fat and high-cholesterol diets, which accumulate free cholesterol in HSCs of NAFLD livers, resulting in further enhancement of LPS/TLR4 signaling in HSCs and exacerbation of NAFLD fibrosis (70,71). Furthermore, inflammation of the intestinal tract causes increased intestinal permeability. When NAFLD mice with a high-fat diet (HFD) were treated with dextran sulfate sodium to induce colitis, inflammation and fibrosis of the NAFLD liver deteriorated, along with an increase in blood endotoxin levels (72).

7. Liver cirrhosis (LC), hepatocellular carcinoma (HCC) and gut microbiota

In LC patients, pathogenic *Enterobacteriaceae* increase in proportion to the degree of progression, and there is an increase in LPS concentration in the portal vein (73). LPS exacerbates liver fibrosis (74). The formation of HCC was accelerated in a carbon tetrachloride (CCL4)-induced cirrhosis mouse model by continuous administration of low

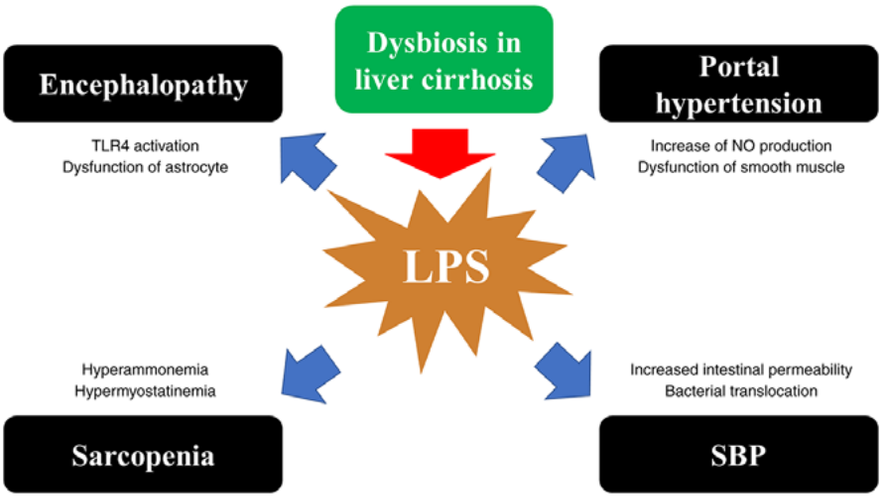

Figure 2. Cirrhosis-related complications and LPS caused by cirrhosis-related dysbiosis. LPS can cause and deteriorate hepatic encephalopathy, portal hypertension, sarcopenia and spontaneous bacterial peritonitis. LPS, lipopolysaccharide; SBP, spontaneous bacterial peritonitis.

concentrations of LPS. Activation of LPS/TLR4 signaling also promotes hepatocellular carcinogenesis by inducing cell proliferation and anti-apoptotic signaling in liver parenchymal cells through growth factors such as IGF-1 and epiregulin, which exacerbates inflammation (75,76). These observations strongly indicated that the induction of inflammation by LPS/TLR4 signaling may promote the formation of HCC predisposing to LC. Moreover, it has also been reported that the gut microbiota of LC patients has an increased number of oral commensals such as *Villonella*, *Streptococcus*, and *Prevotella*, in addition to the *Proteobacteria* phylum, which are gram-negative bacteria that produce LPS (77).

Ammonia is mainly produced in the intestinal tract as a byproduct of protein digestion and intestinal bacterial metabolism, flows into the portal vein, and is metabolized as urea in the liver through the urea cycle. In advanced cirrhosis, the function of the urea cycle is impaired, and ammonia enters the systemic circulation as a result of inadequate metabolism. Ammonia removal beyond the metabolic capacity of the liver depends on the kidney, brain, and skeletal muscle (78). In the brain, astrocytes detoxify ammonia by producing glutamine from ammonia and glutamate via the glutamine synthesis pathway. The swelling of astrocytes by the glutamine produced in this process is one of the causes of brain edema and encephalopathy (79). Gut dysbiosis can be associated with the incidence and severity of neuroinflammation and encephalopathy (79). LC patients with dysbiosis are prone to sarcopenia with high levels of myostatin (a myokine that inhibits muscle protein synthesis) in muscle caused by hyperammonemia due to harmful bacteria in the intestine (80-82). In LC patients, LPS causes swelling and dysfunction of astrocytes from activation of TLR4 in microglia and endothelial cells, inducing hepatic encephalopathy (83,84). Dysbiosis may also cause neuroinflammation, leading to encephalopathy (85). LPS exacerbates portal hypertension from increased NO production (increased NO production increases portal pressure while increasing hepatic portal blood flow) and vascular smooth muscle dysfunction (86). LPS also increases intestinal permeability, predisposes to bacterial translocation, and causes spontaneous bacterial peritonitis (SBP) (87) (Fig. 2).

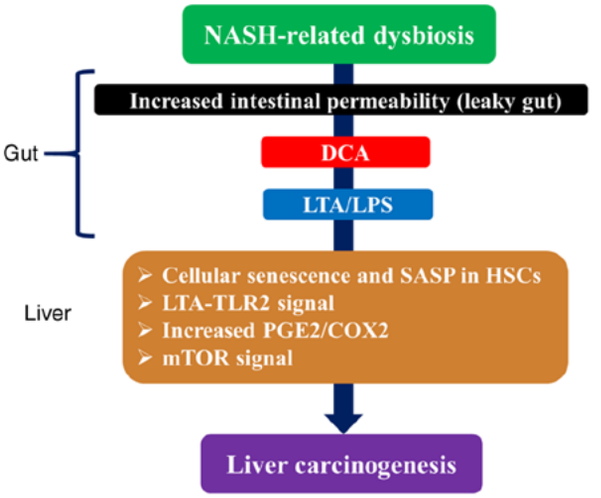

Figure 3. Nonalcoholic steatohepatitis-related dysbiosis and liver carcinogenesis through gut-liver axis. NASH, non-alcoholic steatohepatitis; DCA, deoxycholate; LTA, lipoteichoic acid; LPS, lipopolysaccharide; SASP, senescence-associated secretory phenotype; HSC, hepatic stellate cell.

Similar to hepatitis virus-related HCC, NASH-related HCC in most cases develops through chronic hepatitis, liver fibrosis, and LC. However, some cases have been reported to develop HCC without LC (88). Deoxycholate (DCA), a secondary bile acid converted by gut microbiota, was reported to be important in the formation of this non-cirrhotic NASH-related HCC (89,90). In obese mice treated with the carcinogen DMBA at birth and with HFD, HCC was revealed to develop in all mice. It was revealed that DCA, which increased with obesity, created a microenvironment for the development of HCC by inducing cellular senescence and senescence-associated secretory phenotype (SASP; a phenomenon in which senescent cells that accumulate in the body with aging are highly expressed and secrete a variety of inflammatory proteins) in HSCs through gut-liver circulation (89,90). It has recently been revealed that senescent cells secrete pro-inflammatory cytokines, and the accumulation of senescent cells with aging is considered to be a trigger for the functional decline of organs and tissues,

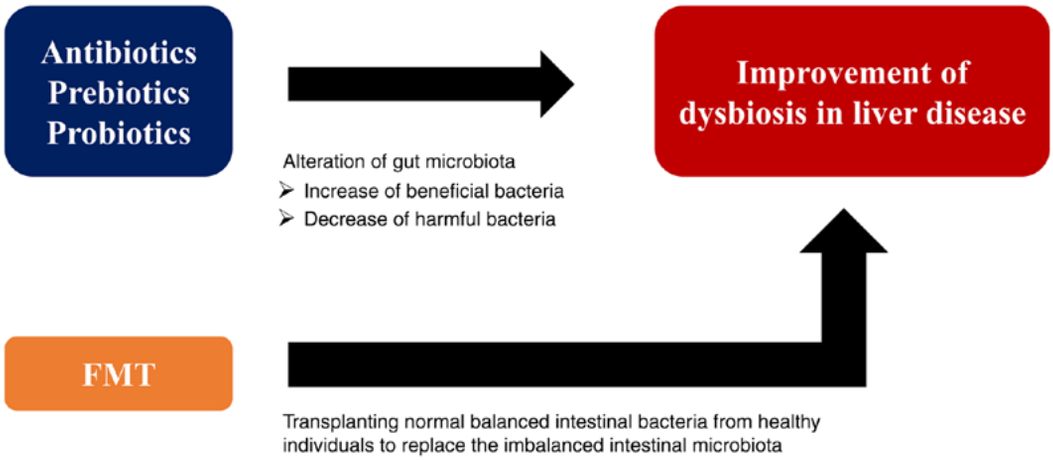

Figure 4. Improvement of dysbiosis by pharmacological therapies and fecal microbiota transplantation. FMT, fecal microbiota transplantation.

resulting in various aging-related diseases (91,92). In addition, long-term HFD treatment alters the gut microbiota, induces the growth of gram-positive bacteria such as *Clostridium* and excessive DCA production, and induces the translocation of lipoteichoic acid (LTA; a component of gram-positive bacteria) into the liver due to the breakdown of the intestinal barrier, thereby promoting the progression of HCC through the activation of LTA/TLR2 signaling. LTA, along with DCA, enhances SASP production in HSCs and increases COX-2-mediated production of prostaglandin E_2 (PGE_2) and expression of TLR2 (89,90). It has been reported that DCA levels in the blood of NASH patients are elevated (93). Furthermore, high expression of COX-2 and excessive PGE_2 production were observed in HSCs in patients with non-cirrhotic NASH-related HCC, indicating that a similar mechanism functions in humans (89). Conversely, it has also been suggested that DCA promotes the progression of HCC by activating mTOR signaling (94) (Fig. 3). DCA was revealed to activate mTOR and act in a phosphoinositide 3-kinase (PI3K)-dependent manner (94). It is well known that alterations in the PI3K/Akt/mTOR pathway are an important contributor to tumorigenesis.

8. Targeting gut microbiota for the treatment of liver diseases

Antibiotics, prebiotics and probiotics. As an example of antibiotic therapy, antibiotics such as rifaximin have been revealed to be effective in the treatment of liver diseases associated with the small intestine bacterial overgrowth (SIBO) (95,96). Dysbiosis caused by severe alcoholic hepatitis can be reversed by rifaximin therapy by reducing *Veillonella* (97). In addition, rifaximin may not affect systemic inflammation (98). Rifaximin therapy can ameliorate endotoxemia and encephalopathy without affecting gut microbiota in decompensated LC subjects (99). Prebiotics contain food components that are not easily digested and absorbed in the upper part of the gastrointestinal tract, and they promote intestinal peristalsis and the growth of specific intestinal bacteria (100). Pectin, as one of the prebiotics, has been revealed to prevent liver diseases by promoting the growth of *Bacteroides* and inhibiting the decrease of *Bacteroides* caused by alcohol consumption, and is expected to be applied as a therapeutic agent (101). Probiotics refer to living microorganisms that provide health benefits to humans, and the anti-obesity effects of *Bifidobacterium breve* administration have been reported in mice and humans (102,103). This mechanism is considered to include the possibility that *Bifidobacterium breve* promotes fatty acid degradation by inducing β-oxidation in the liver and inhibiting the reduction of intestinal barrier function caused by a HFD (104). Probiotics are expected to be most effective against CLDs by strongly affecting the gut-liver axis. A meta-analysis of the effects of probiotics on NAFLD and NASH reported that probiotic therapy lowered alanine aminotransferase (ALT), total cholesterol, and TNF-α and improved insulin resistance in patients with NAFLD and NASH (105). In addition, when probiotics and prebiotics were combined in patients with NAFLD, ALT level and fatty liver were greatly improved (106,107). In a study using an aflatoxin-induced HCC rat model, it was reported that probiotic fermented milk and chlorophyllin revealed tumor growth by suppressing the expression of c-Myc, Bcl-2, cyclin D1, and Ras p21 (108). Dapito *et al* also reported that inactivation of TLR4 by antibiotics reduced HCC by 80-90% (75). Thus, animal models indicated that regulation of the gut microbiota may be a preventive strategy for HCC.

Fecal microbiota transplantation. Fecal microbiota transplantation (FMT) is a method of attempting to treat various diseases by transplanting normally balanced intestinal bacteria from healthy individuals to replace the imbalanced intestinal microbiota. In 2013, van Nood *et al* reported a randomized controlled trial (RCT) of FMT for recurrent *Clostridium difficile* infection, and since then, the clinical application of FMT has been attracting attention. According to RCTs and systematic reviews of recurrent *Clostridium difficile*, 60-90% of patients were cured without recurrence by single FMT (109-111).

There are several studies on FMT in liver diseases. FMT altered the gut microbiota of mice with high sensitivity to ethanol and improved alcoholic liver injury (3). FMT can also improve cirrhosis-related neuroinflammation in mice (112). In humans, a pilot study was conducted in 8 male patients with severe alcoholic hepatitis. The results revealed that FMT was effective and safe in treating hepatic damage within 1 week after FMT, and eventually exhibited improvement in severe

hepatic damage and survival even after 1 year (61). In addition, in a previous study of FMT vs. standard therapy in 20 male LC patients associated with recurrent hepatic encephalopathy, FMT from donors was associated with improvement of dysbiosis, improved cognitive function and shorter hospital stay in the recipients compared with the standard therapy group (113). Further clinical trials are underway to determine whether FMT can be safely used to treat CLDs.

9. Final remarks

In recent years, it has become clear that gut microbiota is closely related to the pathogenesis of various liver diseases, and research on the mechanism of promotion or suppression of HCC via the gut-liver axis has become a fascinating topic. The components of gut microbiota such as LPS and LTA are associated with liver fibrosis and HCC progression. In addition, gut-microbiota-derived metabolites such as secondary bile acids and fatty acids, cellular senescence and SASP are also closely related to liver pathology. Elucidation of the detailed molecular mechanisms of the effects of gut microbiota-derived substances via the gut-liver axis will lead to the development of advanced methods for the treatment and prevention of liver diseases. FMT is gaining attention as a treatment that can improve dysbiosis as well as antibiotics, prebiotics and probiotics (Fig. 4). However, numerous issues remain to be clarified, such as the administration method, long-term benefits, and side effects of FMT. It is anticipated that more evidence will be generated in the future.

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Authors' contributions

HN wrote the article. SF, AA, KY, HO, SN and KH edited and reviewed the article. Literature research was performed by all the authors. All authors read and approved the final manuscript.

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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