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Review Article

Gut microbial dysbiosis associates hepatocellular carcinoma via the gut-liver axis

Jian-Wen Jiang[a,b,c], Xin-Hua Chen[a,b], Zhigang Ren[d], Shu-Sen Zheng[a,b,*]

[a] Department of Hepatobiliary and Pancreatic Surgery, Key Laboratory of Combined Multi-organ Transplantation, Ministry of Public Health, First Affiliated Hospital, Zhejiang University School of Medicine, #79 Qingchun Road, Hangzhou 310003, China
[b] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Zhejiang University, Hangzhou 310003, China
[c] Health Management Center, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China
[d] Department of Infectious Disease, Precision Medicine Center, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. Gut microbiota has been demonstrated to play a critical role in liver inflammation, chronic fibrosis, liver cirrhosis, and HCC development through the gut-liver axis.

Data sources: Recently there have been several innovative studies investigating gut microbial dysbiosis-mediated enhancement of HCC through the gut-liver axis. Literatures from January 1998 to January 2018 were searched in the PubMed database using the keywords “gut microbiota” and “hepatocellular carcinoma” or “liver cancer”, and the results of experimental and clinical studies were analyzed.

Results: Gut microbial dysbiosis accompanies the progression of alcoholic liver disease, non-alcoholic fatty liver disease and liver cirrhosis, and promotes HCC progression in an experimental mouse model. The immune system and key factors such as Toll-like receptor 4 are involved in the process. There is evidence for gut microbial dysbiosis in hepatitis virus-related HCC patients.

Conclusions: Gut microbial dysbiosis is closely associated with hepatic inflammation disease and HCC through the gut-liver axis. With the enhanced understanding of the interactions between gut microbiota and liver through the gut-liver axis, new treatment strategies for HCC are being developed.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world; the incidence is listed as the 7th among all tumors, and its mortality ranks as the 3rd of all cancers [1]. Causes of HCC include hepatitis virus, chemical toxicants and metabolites that may cause cell damage, gene mutation, and cancerous transformation [2]. In the United States, obesity and diabetes are the major risk factors of HCC [3], but in China, the major etiology is hepatitis B virus (HBV) infection. It is estimated that about 466 000 HCC new cases and 420 000 HCC caused-deaths occur annually in China. [1]. Thus, there is an urgent need to investigate the novel pathogenesis of HCC and its treatment strategies.

A large number of microflora (10^{14}) with complex structures and functions colonize the human gut [4]. There is a symbiotic and co-evolution relationship between the gut microflora and the host; gut microbiota affects many aspects of human health, including nutrition, metabolism, and immunity, and is closely associated with human health and disease [5–7]. Eckburg et al. [8] proposed that the gut microbiome is equivalent to an important metabolic "organ" of the human body.

Gut microbiota has been identified not only as crucial determinants of intestinal inflammation, but also as key players in chronic inflammatory liver diseases [9]. Recent experimental and clinical studies have shown that there is a complex link between gut microbiota and HCC. We reviewed the recent literature focusing on gut microbiota and hepatic inflammatory disease and HCC, and found that gut microbiota works as an unignorable factor in the progression of hepatic inflammatory disease and in the formation of tumor micro-environments during the onset and progression of HCC. Gut microbial dysbiosis may promote hepatic inflammatory disease and HCC via the gut-liver axis primarily through the lipopolysaccharide (LPS)–Toll-like receptor 4 (TLR4)-NF-κB

* Corresponding author at: Department of Hepatobiliary and Pancreatic Surgery, Key Laboratory of Combined Multi-organ Transplantation, Ministry of Public Health, First Affiliated Hospital, Zhejiang University School of Medicine, #79 Qingchun Road, Hangzhou 310003, China.
E-mail address: shusenzheng@zju.edu.cn (S.-S. Zheng).

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signaling pathway, as well as through metabolites such as deoxycholic acid. Ren et al. [10] first reported gut microbial dysbiosis in HBV-related HCC (HBV-HCC) patients.

The anatomical and physiological relationship between gut microbiota and liver

The gut and the liver are closely related in their origin and anatomy from the embryonic phase. They both originate from the fore-gut, sharing a common origin. The precursor of the gut-associated lymphoid tissue originates from the developing liver. The liver and the gut coordinate and influence each other, and the gut-liver axis integrates their functions. About 70% of liver blood is supplied by the portal vein, which contains a large amount of metabolites from the digestive tract, and intestinal microbial antigens and metabolites.

In 1998, Marshall proposed the gut-liver axis theory [11]. The anatomical relationship between the liver and the gut provides the basis for the gut-liver axis. The intestinal barrier can prevent substances such as bacteria and toxins from passing into the blood circulation by means of mechanical, biological, immune, and chemical barriers of intestine. Normal gut barrier function and liver detoxification are essential for the stability of the human internal environment [12].

The liver and the gut microbiota are also functionally linked. In physiological conditions, the liver clears the intestinal bacteria, antigens, inflammatory factors, maintains active function of inflammatory cells and immune homeostasis [13,14]. Gut microbiota interferes with liver metabolism, oxidation, proinflammatory cytokines, hepatic lipogenesis, and bile acid production, which in turn further modifies gut microbiota.

Liver disease or injury can aggravate gut microbial dysbiosis. For instance, liver cirrhosis reduces gastric acid, decreases bile acid secretion, and weakens bowel movement, which together contribute to the overgrowth of intestinal bacteria and gut microbial dysbiosis [15,16]. Moreover, inflammatory factors produced by the liver immune system increase the permeability of the intestinal mucosa [17,18]. In contrast, intestinal bacteria and their products pass through the portal vein system into the liver when intestinal barrier function is compromised, which further damages the liver immune system. Hepatic Kupffer cells and stellate cells are then activated. They release a series of inflammatory factors, aggravating intestinal mucosal injury [19,20], triggering liver cell steatosis and necrotic inflammation, thereby promoting liver fibrosis and cirrhosis [20]. Ren et al. [21] also applied the gut-liver axis theory to investigate the relationship between gut microbiota and hepatic graft injury. They proposed that the improvement of hepatic injury could ameliorate gut barrier function and promote gut microbial restoration, which might further benefit hepatic function by positive feedback via the gut-liver axis.

Gut microbial dysbiosis is associated with chronic liver disease progression

Chronic liver diseases mainly include chronic hepatitis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and liver cirrhosis. Recent studies have indicated that gut microbial alterations contribute to the onset and progression of chronic hepatitis B [22], ALD [23], and NAFLD [24] through the gut-liver axis. During liver disease progression, gut microbial dysbiosis may promote the production of microbial metabolites such as LPS, bacterial DNA, and deoxycholic acid, which may enter the hepatic sinusoids through the portal vein, and cause chronic liver inflammation, even fibrosis and cirrhosis [9].

Hepatitis virus-related liver disease

The gut microbiota of chronic hepatitis B patients is significantly different from that of healthy controls. *Actinomyces, Clostridium sensu stricto,* unclassified *Lachnospiraceae* and *Megamonas* are increased significantly; *Alistipes, Asaccharobacter, Bacteroides, Butyricimonas, Clostridium cluster IV,* and *Escherichia/Shigella* are decreased significantly [25] (Table 1). Compared with hepatitis B carriers, in patients with decompensated hepatitis B cirrhosis, the dominant intestinal flora of *Faecalibacterium prausnitzii, Enterococcus faecalis* and *Enterobacteriaceae* were significantly increased. Conversely, *Bifidobacteria* and *Lactic acid bacteria* were significantly decreased. The *Bifidobacteria/Enterobacteriaceae* (B/E) ratio also decreased significantly. The gene diversity index was increased, and the levels of fecal secretory IgA, and serum tumor necrosis factor (TNF-α) were significantly increased [22]. These factors of gut microbial dysbiosis may influence disease progression. In a mouse model of liver disease induced by frog virus 3 [26], sterile mice avoided the development of viral liver disease. Meanwhile, for normal mice, polymyxin B protected the mice against liver disease, which indicates the necessity of gut microbiota for the development of viral liver disease.

As for chronic hepatitis C patients, Inoue et al. [27] found that they had lower bacterial diversity, a decrease in the order *Clostridiales*, and an increase in *Streptococcus* and *Lactobacillus*. Hepatitis C virus (HCV) infection was associated with gut microbial dysbiosis, even in patients with mild liver disease. Moreover, the overgrowth of *Viridans group streptococci* may account for hyperammonemia in chronic hepatitis C and liver cirrhosis. Because the gut microbiota could be therapeutically altered, it is potentially possible to reduce the complications of chronic hepatitis C by modulating gut microbial dysbiosis.

Alcoholic liver disease

ALD is closely related to gut microbiota changes. Mutlu et al. [23] found that *Bacteroidetes* in fecal samples were significantly decreased in patients with alcoholic cirrhosis. In the Tsukamoto-French model of ALD, bacterial overgrowth and dysbacteriosis were mainly manifested by a significant reduction in the proportion of probiotics such as *Lactobacillus, Pediococcus, Leuconostoc*, and *Lactococcus* [28,29]. Meanwhile, quantitative PCR also confirmed a reduction in the number of intestinal *Lactobacillus* in alcohol-related diseases [30]. Clinical trials have also indicated that taking probiotics is beneficial for patient's ALD [31]. Gut microbial dysbiosis initiates and mediates increased intestinal permeability in ALD patients. Intestinal sterility protects the intestinal barrier from leakage and prevents bacterial translocation [32,33]. Gut bacteria can metabolize ethanol and produce acetaldehyde. Ethanol and the metabolic derivative acetaldehyde can destroy the integrity of intestinal tight junctions [34]. Meanwhile, gut microbiota can also synthesize ethanol, which has a detrimental effect on the intestinal barrier [35]. Thus, as a cause for liver injury, alcohol induced gut microbial dysbiosis and increased bacterial translocation contribute to the development of ALD.

NAFLD/NASH

With obesity more prevalent in the developed world, NAFLD has become the most common chronic liver disease [36]. A large number of studies have confirmed that inflammation and gut microbial dysbiosis participate in the development of NAFLD and NASH. Le Roy et al. [37] highlighted that different composition of intestinal microbiota could determine the response of mice to a high-fat diet, and changes in intestinal microbiota promoted the

Table 1
Changes in gut microbiota with different liver diseases.

Liver diseases	Changes in gut microbiota	References	Clinical significance
Chronic B hepatitis	Increased in taxonomy *Actinomyces* *Clostridium sensu stricto* *unclassified Lachnospiraceae* *Megamonas* Decreased in taxonomy *Alistipes* *Asaccharobacter* *Bacteroides,Butyricimonas* *Clostridium IV* *Escherichia/Shigella* *Parabacteroides,* *Ruminococcus* unclassified *Bacteria* unclassified *Clostridiales,* unclassified *Coriobacteriaceae* unclassified *Enterobacteriaceae* unclassified *Lachnospiraceae* unclassified *Ruminococcaceae*	[25]	Gut microbial dysbiosis may influence disease progression.
Chronic hepatitis C	*Streptococcus* ↑ *Lactobacillus*↑ *Enterobacteriaceae*↑ *Viridans streptococci*↑ order *Clostridiales*↓ *Lachnospiraceae*↓ *Ruminococcaceae*↓	[27]	HCV infection was associated with gut microbial dysbiosis, even in patients with mild liver disease.
Alcoholic liver disease	*Enterobacteriaceae* ↑ *Proteobacteria* ↑ *Fusobacteria*↑ *Lachnospiraceae*↑ *Erysipelotrichaceae*↑ order *Clostridiales*↓ *Lactobacillus*↓ *Pediococcus*↓ *Leuconostoc*↓ *Lactococcus* ↓	[28–30,46]	Alcohol contributes to the development of ALD by inducing gut microbial dysbiosis and increasing bacterial translocation.
NAFLD	*Escherichia_Shigella*↑ *Lachnospiraceae Incertae Sedis genera*↑ genus *Blautia*↑ *Prevotella genera*↓ families *Prevotellaceae*↓ families *Ruminococcaceae*↓	[24,40]	NAFLD patients and healthy subjects harbor varying gut microbiota decreased levels of *Prevotella* might be detrimental for adults with NAFLD.
NASH (including fibrosis)	family *Lachnospiraceae*↑ genus *Escherichia_Shigella*↑ family *Enterobacteriaceae*↑ genus *Blautia*↑ proportion of alcohol-producing bacteria↑ *Bacteroides*↑ *Ruminococcus*↑ *Clostridium* ↓ *Prevotella*↓	[24,39,40]	The increased level of the genus *Blautia*, the family *Lachnospiraceae*, the genus *Escherichia Shigella*, and the family *Enterobacteriaceae* may be a primary contributor to NAFLD progression.
Liver cirrhosis	(family) *Enterobacteriaceae* ↑ *Streptococcaceae*↑ *Veillonellaceae*↑ *Pasteurellaceae*↑ *Fusobacteriaceae*↑ *Lachnospiraceae* ↓ *Bacteroidaceae* ↓ (genus) *Streptococcus*↑ *Veillonella*↑ *Clostridium*↑ *Prevotella*↑ *Bacteroides*↓ *Eubacterium*↓ *Alistipes*↓	[44,45]	Gut microbial dysbiosis was associated with liver cirrhosis development.
DEN-induced HCC rat	*Escherichia coli* ↑ *Atopobium cluster* ↑ *Lactobacillus species*↓ *Bifidobacterium species*↓ *Enterococcus species*↓	[51,52,72]	Gut microbial dysbiosis and LPS activate TLR4 on resident liver cells, which promotes tumor progression.

(*continued on next page*)

Table 1 (*continued*)

Liver diseases	Changes in gut microbiota	References	Clinical significance
Obesity-induced HCC rat	*Gram-positive bacteria*↑ lipoteichoic acid polysaccharide (LTA)↑ cluster XI of the genus Clostridium↑ *Clostridium* cluster XIVa↑	[54,59]	Deoxycholic acid is a risk factor leading to obesity-associated HCC. The hepatic translocation of obesity-induced lipoteichoic acid (LTA) promotes HCC development by creating a tumor-promoting microenvironment.
HCC patients	Genera producing lipopolysaccharide↑ *Escherichia coli* ↑ Butyrate-producing bacterial genera↓	[10,64]	The increased genera producing lipopolysaccharide promotes tumor progression. Cirrhotic patients with abundant intestinal *E. coli* are more susceptible to the development of HCC.

progression of NAFLD. Mouzaki et al. [38] reported on gut microbial dysbiosis in patients with NAFLD and NASH; the abundance of *Clostridium* in these patients was significantly decreased compared with that of healthy people. Zhu et al. [39] reported on the intestinal microbiota of patients with NASH: the proportion of alcohol-producing bacteria in the intestine was increased, resulting in elevated blood ethanol concentrations. Ethanol could induce chronic hepatocellular inflammatory damage. They showed that alcohol-producing bacteria are involved in the pathogenesis of NASH, and ethanol metabolism was involved in oxidative stress and liver inflammatory process.

Shen et al. [24] evaluated the relationship between gut microbial dysbiosis and severe NAFLD lesions, including NASH and fibrosis. They found that NAFLD patients had lower gut microbiota diversity and *Prevotella* abundance; whereas NASH patients had higher abundance of genus *Blautia* and *Lachnospiraceae* family. NASH patients with significant fibrosis had a higher abundance of genus *Escherichia Shigella* and *Enterobacteriaceae* family compared to those with F0/F1 fibrosis (Table 1). Boursier et al. [40] found that *Bacteroides* abundance was significantly increased in NASH and patients with fibrosis stage (F) $\geq$ 2, whereas *Prevotella* abundance was decreased. *Ruminococcus* abundance was significantly higher in F $\geq$ 2 patients. Importantly, *Bacteroides* abundance was independently associated with NASH and *Ruminococcus* with F $\geq$ 2 fibrosis. NAFLD severity is associated with gut dysbiosis, and gut microbial analysis could predict NAFLD severity. These studies suggest that targeting gut microbiota is a new strategy to treat NAFLD/NASH.

Gut microbial dysbiosis is associated with the progression of precancerous disease to HCC

Most cases of HCC are the result of chronic inflammatory disease processes in the liver with a long-term consequence of chronic liver injury, inflammation, fibrosis, and cirrhosis. Moreover, about 80%–90% of HCC cases occur in advanced fibrotic or cirrhotic livers. About one in three patients with compensated liver cirrhosis will develop HCC in their life time [2,41]. Therefore liver cirrhosis can be considered as the precancerous disease of HCC.

The pathological hallmark of liver cirrhosis is that hyperplasia of tissue and collagen deposition replaces normal liver tissue. Liver chronic injury leads to a decrease in the clearance function of LPS, bacteria, and metabolites, and a decrease in bile and bile acid secretion [42]. These factors directly affect the stability and function of the gut microbiota. In contrast, the development of chronic liver diseases is usually associated with inflammation and bacterial infections. Clinical research including 169 chronic liver disease patients showed that elevated levels of endotoxin were found in 27%, 85%, and 41% of patients with chronic hepatitis, chronic hepatitis with acute exacerbation, and cirrhosis, respectively. Plasma endotoxin concentrations correlated with the degree of liver dysfunction according to Child-Pugh scores, which suggests that the elevated endotoxin is linked to deteriorate liver disease [43].

Gut microbial dysbiosis can promote the progression of liver cirrhosis [44,45] and its complications [46] through the gut-liver axis. Sandler et al. [47] found that compared with uninfected individuals, HCV- and HBV-infected individuals had higher plasma levels of LPS, intestinal fatty acid binding protein, and IL-6. They concluded that LPS-induced local and systemic inflammation is associated with liver cirrhosis and the degree of inflammation could predict the progression to end-stage liver disease in patients with HBV or HCV infection.

Chen et al. [44] analyzed the intestinal microflora changes in patients with cirrhosis. *Bacteroidetes* were significantly reduced, while *Proteobacteria* and *Fusobacteria* were significantly increased. At the family level, *Enterobacteriaceae, Veillonellaceae, a*nd *Streptococcaceae* were significantly enriched. *Streptococcaceae* were positively correlated with Child-Pugh scores, while *Lachnospiraceae* were significantly and negatively correlated with Child-Pugh scores. These disorders of the intestinal microflora may affect the prognosis of patients with cirrhosis.

With the progression of liver cirrhosis, gut microbial dysbiosis is also significantly aggravated, and these aggravations are closely related to the complications of liver cirrhosis and patient prognosis. During liver cirrhosis, gut microbial dysbiosis can lead to increased levels of endotoxin, increased systemic inflammatory response, elevated levels of blood ammonia, and even the development of hepatic encephalopathy and infection such as spontaneous bacterial peritonitis [48].

Intestinal decontamination with non-absorbable antibiotics (rifaximin) is an effective treatment for subclinical and apparent hepatic encephalopathy [49]. Bajaj et al. [46] studied the bacterial structure of 219 patients with cirrhosis and found that the degree of gut microbial dysbiosis and its gradual aggravation were associated with the progression of liver cirrhosis, and were also positively correlated with the level of endotoxin. In the longitudinal comparison, the gut microbiota of the patients with cirrhosis before and after infection was significantly different; the degree of gut microbial dysbiosis was significantly positively correlated with the incidence of organ failure and death.

Gut microbial dysbiosis promotes HCC progression in an animal model

Recent studies in animal models indicate that gut microbial dysbiosis promotes HCC development through the gut-liver axis. Fox et al. [50] showed that gut microbial dysbiosis was closely related to the risk of liver cancer formation in mice exposed to carcinogenic chemicals or hepatitis virus transgenes. In 2010, Yu et al. [51] firstly reported LPS-induced HCC development. They found that TLR4 on the membrane of bone marrow-derived hepatic Kupffer cells is the target of LPS in a diethylnitrosamine (DEN)-induced HCC mouse model. Kupffer cells produce inflammatory cytokines TNF-α and IL-6, which produce paracrine activated tumor cytokines through the LPS-TLR4-NF-κB signaling pathway, then activate precancerous hepatocellular proliferation and induce HCC. These data indicated that LPS is a pathological mediator of inflammation-associated HCC. The regulation of gut microflora and

decrease of LPS production may decrease the development of HCC from liver cirrhosis.

In 2012, Dapito et al. [52] found that intestinal microbial dysbiosis increases DEN-induced HCC in mice. Non-bone marrow-derived hepatic stellate cells and hepatocytes are the main target of LPS. TLR4 on the membrane of hepatic stellate cells mediated regenerative feedback, which lead to compensatory proliferation of liver cells after injury. They found that TLR4 and the intestinal microbiota were not necessary for HCC initiation but required for HCC promotion, and that TLR4 and the intestinal microbiota promoted hepatomitogen epiregulin expression, hepatic proliferation and prevention of hepatic apoptosis. However, to induce intestinal aseptic state by using antibiotic can effectively inhibit the development of HCC restricted to late stages of hepatocarcinogenesis. Dapito et al. indicated that the intestinal microbiota and TLR4 represent therapeutic targets for HCC prevention in advanced liver disease. Darnaud et al. [53] summarized the above experimental results and suggested a treatment to prevent progression of HCC by targeting intestinal microbiota.

In 2013, Yoshimoto et al. [54] reported that gram-positive bacteria were significantly more prevalent in obese mice than in healthy mice. The concentration of gut deoxycholic acid was significantly increased in obese mice. Deoxycholic acid is an obesity-induced gut microbial metabolite in bile acid metabolism, which is toxic and causes liver cell DNA damage and liver cell aging [55,56]. The aging cells secrete aging-related secretory phenotype [57], and thus promote the oncogenesis of HCC. These data suggested deoxycholic acid is a risk factor leading to obesity-associated HCC.

Bisfructose anhydride III can inhibit the key enzymes (7a-hydroxylase) in the bile acid synthesis, and treatment of obese mice with bisfructose anhydride III significantly reduced the aging hepatic stellate cells. In contrast, the long-term use of deoxycholic acid on non-obese mice can also promote the development of HCC [58]. Loo et al. [59] found that hepatic translocation of obesity-induced lipoteichoic acid creates a tumor-promoting microenvironment for HCC. These results indicated the importance of the gut-liver axis in the development of obesity-associated HCC and suggested that gut microbiota may be therapeutic target for obesity-associated HCC.

Gut microbial dysbiosis is associated with hepatitis virus related HCC progression

Most of the HCC patients in China have HBV infection, and the chronic liver disease course usually continues for decades driven by a vicious cycle of liver injury, inflammation and regeneration. HBV itself cannot induce hepatic diseases, but causes liver inflammatory injury through the host immune system. HBV DNA can be integrated into the hepatocytes, and the virus can escape from the host immune system, triggering a continuous immune response in the host liver cells which leads to chronic hepatitis, fibrosis, and liver cirrhosis.

Liver cirrhosis can damage liver function, congest intestinal venous circulation, increase exudation, change intestinal microbial composition, and contribute to gut microbial dysbiosis [60]; unfortunately, gut microbial dysbiosis further deteriorates the immunity, metabolism, nutrition, inflammatory status of the liver because of increased intestinal permeability, bacterial overgrowth, or impaired clearance of microbial products by Kupffer cells. Complex network interaction between liver chronic inflammation induced by HBV infection and gut microbial dysbiosis may promote a tumor micro-environment in the liver, and participate in the progression of HBV-HCC. The gut microbial dysbiosis may be a synergistic factor for HBV carcinogenesis through the gut-liver axis.

Chou et al. [61] found that the maturation of gut microbiota is an important condition to clear HBV. They found that an immuno-tolerance pathway against HBV prevailed in young mice, before the establishment of gut microbiota. Sterilization of gut microbiota in mice from 6 to 12 weeks of age using antibiotics prevented adult mice from rapidly clearing HBV. By means of the matured gut microbiota, the adult mice stimulated liver immunity and cleared HBV rapidly through a TLR4-dependent pathway after HBV infection. Ren et al. [62] proved that fecal microbiota transplantation induces hepatitis B virus e-antigen (HBeAg) clearance in patients with positive HBeAg after long-term antiviral therapy, indicating that the adjustment of gut microbial dysbiosis is critical for the treatment of chronic hepatitis with positive HBeAg. Lu et al. [63] found that HBV-HCC patients with liver cirrhosis have oral microbial dysbiosis. The oral special bacteria may provide novel and non-invasive potential diagnostic biomarker for HCC. Grąt et al. [64] found that the number of fecal *Escherichia coli* (*E. coli*) in HCC patients was significantly higher than those in liver cirrhosis patients. Cirrhotic patients with abundant intestinal *E. coli* are more susceptible to the development of HCC. Ren et al. [10] found that in HBV-HCC patients, fecal microbial diversity was increased from cirrhosis to early HCC with cirrhosis, but it was decreased from healthy controls to cirrhosis. The genera producing LPS were increased, while the butyrate-producing bacterial genera were decreased in early HCC versus healthy controls.

The occurrence of HCC is a long-term chronic process, and HBV-HCC is usually accompanied by hepatitis and cirrhosis. Based on the above research, it is presumed that the maturation of gut microbiota could help mice to clear HBV rapidly after HBV infection, to regulate gut microbial dysbiosis by fecal microbiota transplantation could help patients to control the progress of chronic HBV-related inflammation. There is gut microbial dysbiosis in HBV-HCC patients. These data suggest that gut microbiota may be preventive and treatment target for HBV-HCC.

HCV affects 130–210 million people worldwide, and HCV infection is a major risk factor for liver cirrhosis and HCC [65]. Compared to HBV infection, HCV infection is more likely to cause cirrhosis and liver cancer, because about 90% HCV-related HCC (HCV-HCC) derived from advanced fibrosis or cirrhosis [66]. Inoue et al. [27] found that Streptococcus salivarius was increased in HCV-HCC patients in liver cirrhosis, implying that *Streptococcus salivarius* might enhance the progresses from chronic hepatitis to liver cirrhosis and to HCC. *Streptococcus salivarius* could downregulate the innate immune responses of human epithelial cells, probably promoting HCV-HCC progression.

In patients with viral hepatitis, antiviral treatment against hepatitis B and hepatitis C might prevent chronic inflammation and disease development, but for the majority of patients who have developed cirrhosis accompanied by gut microbial dysbiosis and increased translated LPS and metabolites, it may be necessary to modulate gut microbial dysbiosis to prevent the progression of liver cirrhosis and HCC.

Key functional bacteria in gut microbiota may be therapeutic targets for HCC

The relationship between gut microbiota and HCC is complex; the immune system could influence the development of HCC. T helper 17 (Th17) cells are a novel subset of T helper cells which can produce pro-inflammatory and pro-angiogenic factors such as IL-17A and IL-22. IL-17A may play pro-tumor roles by promoting tumor angiogenesis and secreting angiogenic mediators and cytokines [67]. Zhang and Liao et al. separately found that Th17 cells were increased in tumors and blood of HCC patients and that the levels of Th17 cells were positively correlated with poor survival [68,69]. However, the amount of Th17 cells in the healthy liver is very low; they cannot be produced in the gut unless they interact with gut microbiota [70]. Therefore, it is postulated that

gut microbiota attenuate the progression of HCC by modulating the production of Th17 in the gut.

Li et al. [71] reported that the new probiotic combination such as Prohep (*Lactobacillus rhamnosus GG*), viable *Escherichia coli Nissle* 1917 (EcN), and heat-inactivated *VSL#3* (1:1:1) can effectively inhibit the growth of HCC in mice. The new probiotic combination in Prohep may promote the growth of *Prevotella* and *Oscillibacter* and decrease the growth of segmented filamentous bacteria which are commensals that induces IL-17A production and exacerbate hepatocellular damage. *Prevotella* and *Oscillibacter* could produce anti-inflammatory metabolites, which subsequently decreased the Th17 differentiation and improved the differentiation of anti-inflammatory Treg/Type 1 regulatory T cells in the gut. In addition, the antitumor function of Prohep may be related to the secretion of anti-inflammatory IL-10 secretion.

Zhang et al. [72] found that probiotics can improve the intestinal microenvironment, reduce intestinal inflammation, and HCC. Probiotics not only inhibit the transfer of pathogen-associated molecular patterns from gut to liver, but also activate damage associated molecular patterns, such as high mobility group proteins 1, decrease inflammation and anti-inflammatory cytokines, and then slow the tumor-related inflammation in the liver. The destruction of intestinal permeability promotes the progress of HCC, however, probiotics improve intestinal stability and inhibit the progression of HCC. Wan and El-Nezami [73] summarized the possible mechanisms of probiotics against HCC, which include binding/absorption of carcinogens, improvement of intestinal barrier function, modulation of short chain fatty acid (SCFA) production, regulation of Th17 response, and enhancement hypoxia-induced cell death.

Aging, antibiotics, smoking, hormones, diet, and other factors may cause gut microbial dysbiosis, which may then activate pattern recognition receptors and TLRs in the innate immune system and alter the balance of adaptive immune responses. Certain members of the gut microbiota may alter the balance between gut microbiota and the immune system, then promote chronic inflammation and tumorigenesis [74]. Increasing medical studies [50–52,54,59] have demonstrated the role of gut microbiota in HCC progression and tumor response to treatment. Therefore, the manipulation of gut microbiota may represent a novel way to prevent or treat HCC.

Different tumor treatments may alter the composition of the gut microbiota, and the changed gut microbiota may also affect the effects of various therapies [75]. Gut microbiota participates in antitumor therapy. Viaud et al. [76] found that cyclophosphamide (CTX) can cause changes in the composition of mouse gut microbiota and the transfer of certain Gram-positive bacteria to secondary lymphoid organs. The transferred bacteria stimulate the host to produce a specific subset of “pathogenic” Th17 cells and memory Th1 immune responses, then stimulate an antitumor (namely metastasizing B16F10 melanomas and non-metastasizing MCA205 sarcomas) immune response. However, CTX is ineffective in sterile mice or mice that have killed Gram-positive bacteria with antibiotics. Adoptive transfer of “pathogenic” Th17 cells partially restored the antitumor efficacy of cyclophosphamide, which indicates that the gut microbiota regulates the anti-cancer immune effect of CTX [76]. Daillère et al. [77] found that *Enterococcus hirae* and *Barnesiella intestinihominis* were important for the anticancer effect of CTX; *Enterococcus hirae* shifted from the small intestine to the secondary lymphoid organs increased the intratumoral CD8/Treg ratio, *B. intestinihominis* enriched in the colon promoted the penetration of IFN-γ-producing $\gamma\delta$T cells in the tumor. *E. hirae* and *B. intestinihominis* specific-memory Th1 cell immune responses could selectively predict prognosis for advanced lung and ovarian cancer patients treated with CTX.

Checkpoint inhibitors are novel drugs that can activate the immune system against tumors and have a significant effect on the treatment of tumors. Researchers found that the drug did not work in some patients. The gut microbiota composition in this part of the patients was found to be abnormal. It is speculated that the gut microbial dysbiosis precludes the patient normal antitumor immune response [78]. Gut microbial dysbiosis results in reduced efficacy of immunotherapy and platinum therapy for subcutaneous tumors. In antibiotic-treated or sterile mice, tumor-infiltrating myeloid-derived cells respond very rarely to tumor treatment. In order for antitumor therapy to achieve the desired therapeutic effect, a complete commensal flora is required to regulate the function of myeloid-derived cells in the tumor microenvironment [79].

These above studies indicate that the gut microbiota helps the host form an antitumor immune response. Key functional bacteria may become therapeutic targets for HCC treatment. Although some studies have found a correlation between bacteria and cancer, there is no definitive evidence that these bacteria directly cause tumors. To demonstrate the causal relationship between tumor-associated bacteria and tumors, better experimental design is needed, such as the transfer of tumor-associated bacteria to sterile animals for in-depth studies [80].

The development of gene genomics and metabolomics technology promote the progress of HCC research

The development of the Next Generation Sequencing (NGS) and metabolomics technology open new era for gut microbiota research. MetaHIT (Metagenomics of the Human Intestinal Tract) analyzed the gut microbiota of 124 individuals, completed the sequence assembly and functional annotation of 33,00,000 microbial genes by using NGS [81]. Qin et al. [82] established the first gut microbiota gene data set for Chinese people with diabetes by using NGS and found moderately dysregulated intestinal microbiota, which showed decreased production of butyrate bacteria and an increased number of opportunistic pathogens. Karlsson et al. [83] established an European women gut microbiota gene set by using NGS, and identified a gut microbiota model based on metagenome analysis. Qin et al. [45] constructed gut microbiota gene set of 98 patients with cirrhosis in China by using NGS, and obtained 2.69 million non-redundant genes; 75,245 genes with significant differences in cirrhosis and healthy volunteers could be categorized into 66 gene clusters. Among these genes, 28 gene clusters were enriched in cirrhosis patients and 38 were clustered in healthy volunteers.

Metabolomics uses liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, and nuclear magnetic resonance spectroscopy for qualitative and quantitative study of biological body fluids and their endogenous metabolites. It reveals the biological overall state at specific time and in a specific environment [84]. The interaction between gut microbiota and the human body is very complicated, so to combine biomechanics, transcripts, proteomics, and metabolomics to do integrative omics has become a trend in the field of human microbiota and disease research [85]. Integrative omics study will open new avenues for understanding the relationship between gut microbiota and HCC in depth.

The outlook for translational medical research of gut microbiota and HCC

Most HCC cases are the consequence of long-term chronic liver disease. Chronic liver diseases, such as ALD, NAFLD, NASH, and cirrhosis, can cause gut microbial dysbiosis through liver-intestinal circulation and bile acid secretion. At the same time, the gut microflora and their products (LPS, DNA, and metabolites) interact with the liver and host through the portal system, and may

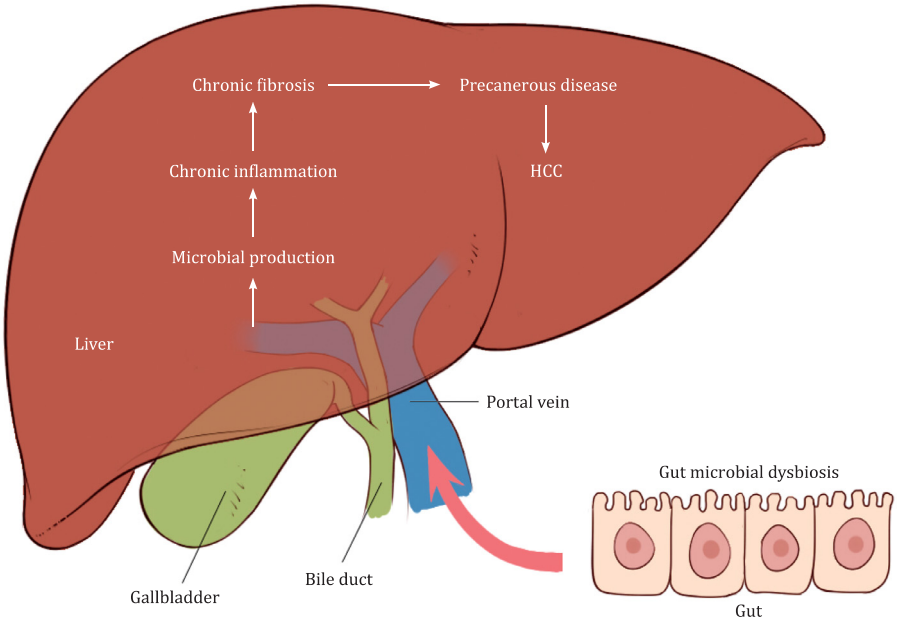

Fig. 1. Gut microbial dysbiosis is associated with chronic liver diseases and HCC via the gut-liver axis. Chronic liver disease such as ALD, NASH, NAFLD, and cirrhosis cause gut microbial dysbiosis through liver-intestinal circulation and bile acid secretion. Gut microflora and their products (LPS, DNA, and metabolites) interact with the liver and host through the portal system, promoting the development of liver inflammation, fibrosis, cirrhosis, and even HCC.

promote the development of liver inflammation, fibrosis, cirrhosis, and even HCC (Fig. 1).

There are many unknown aspects of the interaction between gut microbiota and HCC. With progress of the study of the diversity of intestinal microbiota, research should focus on the function of microflora species. It is important to conduct integrative metagenomics and metabolomics research of intestinal microbiota in different cohorts such as hepatitis B carriers, chronic hepatitis B patients, liver cirrhosis patients, and HCC patients with healthy people as controls.

HBV-HCC animal models are still lacking. Although a DEN-induced animal HCC model may partially imitate the role of gut microbiota on HBV-HCC, its scientific quality needs to be carefully verified. In addition, in order to explain the pathophysiological mechanisms of gut microbiota during the progress of HCC, it is necessary to find and characterize the potential cancer-causing strains, or similar strains, and use pure metabolic compounds to carry out simultaneous experiments.

Sterile animals can exclude the original intestinal microflora and the impact of the external environment, so they are good models to study the effect of gut microbiota on embryo development, immunity, and metabolism. It is imperative to use sterile animal models to verify whether the gut microbiota of HCC patients have carcinogenic effect, and to explore new causes and mechanisms of HCC development.

The gut microbiota is of great significance for HCC development, and could be potential target for adjuvant treatment against HCC. Integrative omics study would help researchers in understanding and analyzing the mechanism underlying the effects of gut microbiota, which involves the immune system and key factors such as TLR4. With enhanced understanding of the connection between gut microbiota and the liver through the gut-liver axis, new opportunities would rise for the development of diagnosis and treatment strategies for HCC.

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Contributors

JJW, CXH and RZ wrote the main body of the article under the supervision of ZSS. All authors contributed to the design and interpretation of the study and to further drafts. ZSS is the guarantor.

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Ethical approval

Not needed.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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