Chapter

Microbiota, Inflammation, and Gut Barrier Dysfunction in HCC

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Abstract

Hepatocellular carcinoma (HCC), which represents 90% of all primary liver cancers, is the fifth most common cancer and the third cause of cancer mortality rate. It is a complex disease with a poor prognosis. Incidence and mortality rates are increasing in many geographical regions, indicating a need for better management strategies. Chronic inflammation is the major driving factors for HCC development, which typically develops on the background of chronic liver disease (CLD). Currently, a large body of literature has focused on the key role of the gut-liver axis as the major pathophysiological mechanism of hepatic disease severity and HCC development. This chapter will describe the role of gut microbiota, inflammation, and intestinal barrier dysfunction-associated mechanism in the progression of HCC. In particular, enteric dysbiosis, tight junction, and inflammatory mediators in the pathogenesis of liver cancer will be discussed. Furthermore, this chapter will identify the possible potential therapeutic approach for the control of gut bacterial overgrowth, inflammation and restoration of eubiosis, and tight junction integrity in HCC.

Keywords: gut-liver axis, HCC, inflammation, microbiota, zonula occludens-1

1. Introduction

Highlights

- Gut dysbiosis, inflammation, and increased intestinal permeability are synergistically contributed to the pathogenesis of hepatocellular carcinoma.
- Previous studies in animal models suggest that targeting the gut-liver axis can inhibit HCC development.
- Targeting the gut-liver axis with probiotics, antibiotics, FMT, TLR4 antagonists, FXR agonists, and natural compounds could be the promising strategies for HCC prevention.

Hepatocellular carcinoma (HCC) is a heterogeneous type of tumor that is likely to develop on the background of an inflammatory milieu in patients with advanced liver disease. It is the third leading cause of cancer death globally and is more prevalent in men than in women [1]. Over the past two decades, there is increasing evidence from studies suggesting a causal link between gut microbiota in the progression of HCC. Normal commensal gut microbiota acts as an important source

of energy and is pivotal to host metabolism and innate immunity [2]. Not unsurprisingly therefore, alteration to gut microbial composition has been linked to the promotion of chronic inflammatory bowel disease (IBD) via local effects. However, activation of such inflammatory effects can have a broader response across all organ beds such as the liver, kidney, brain, heart, and the hematopoietic system and have been strongly associated with carcinogenesis [3]. Anatomical considerations provide us with a logical understanding on why gut microbiosis may have such an impact on disease development, especially in the liver. Since the liver is anatomically connected to the intestine via the portal vein, it is the first organ to receive nutrient-rich blood and also the first target of gut microbiota. Furthermore, the liver can elicit an inflammatory response through microbe-associated molecular patterns (MAMPs) and pattern recognition receptors (PRRs). Though translocation of gut microbiota from the intestinal lumen to the systemic circulation is counterchecked by multilayer intestinal epithelium, any change in its integrity can initiate inflammation and contribute to fibrosis and thus chronic liver disease (CLD) progression and thus a precursor to HCC development, which is itself usually only seen in the context of cirrhosis, the most advanced form of CLD [4]. In this chapter, we summarize the available literature on the key role of gut microbiome in HCC pathogenesis and novel therapeutic approaches developed to target these processes.

2. Gut microbiota

The gut microbiota resides in the gastrointestinal (GI) tract. The human gut harbors complex and diverse microbial community of 100 trillion microorganisms with more than 2000 distinct species of bacteria, in addition to fungi protozoans and viruses. These microorganisms are collectively called gut microbiota, which comprises of commensals, beneficial microbiota, and opportunistic pathogens residing in what is a complex and dense microenvironment. Immediately after birth commensal bacteria colonize the intestine and predominantly comprise *Proteobacteria*, *Lactobacillus*, and *Actinobacteria*, but as we mature into adults *Bacteroidetes* and *Firmicutes species* predominate [5]. The composition of microbiota also varies from the small intestine to the distal colon, due largely to the effects of nutrient availability, intestinal pH, and motility. Moreover the overall composition of the microbial community in the gut is further individualized by any alteration in our diet, age, lifestyle, disease, and also medication exposure [6]. A symbiotic relationship exists between gut microbiota and the human host, which are critical to our maintenance of health. For example, gut microbiota are involved in the metabolism of bile acids, synthesis of vitamins, digestion of complex polysaccharides, and production of short-chain fatty acids (SCFAs) [2]. SCFAs are a vital source of energy for enterocytes, which are integral in maintaining gut barrier integrity. In addition, gut microbiota are also involved in the development of local and innate immunity providing defense against not only the pathogenic invasion but also systemic infection [7]. Experimental studies from rodents and humans have demonstrated that the gut microbiota is involved in the progression of HCC by increasing LPS-mediated pro-inflammatory microenvironment in the liver.

3. Gut-liver axis

Gut microbiota are known to influence multiple extraintestinal organs; however the importance of the gut-liver axis has understandably received greater attention in recent years. The gut and liver share anatomy from the embryonic phase, with bidirectional interaction through the portal vein. The symbiotic relationship between the

gut microbiota and the liver is modulated by the nutrition, immune, metabolic, and neuroendocrine crosstalk between them and thus shapes human health and disease [8]. Functionally, gut and liver coordination influences our physiology. The liver receives 70% of the blood supply from the gut via the portal circulation. The nutrient-rich blood from the gut is effectively processed by the liver and delivered to systemic circulation for normal body growth. In turn, the liver synthesizes bile acids (BAs) and other mediators, like IgA, which influence intestinal microbial composition and barrier integrity, thereby maintaining intestinal homeostasis [8]. Bile acids are involved in energy homeostasis by regulating the metabolism of glucose and lipids and also help in conjugation and detoxification process as well as maintenance of intact intestinal epithelia. Bile acids also regulate microbial composition via antimicrobial peptides production; in turn, microbiota influences the bile acid pool in the intestine as they are involved in secondary bile acid production [9]. IgA secreted from the liver and intestine prevents growth and invasion of pathogenic bacteria to maintain normal gut-liver homeostasis [7]. In normal physiological conditions, translocation of gut bacteria and their metabolites is tightly regulated by the intestinal epithelial barrier, and if any reaches the liver, it is eliminated by hepatic Kupffer cells. Any breach in barrier integrity resulting from intestinal inflammation allows microbiota to pass through the portal vein to potentially trigger hepatic immune cells to enact an inflammatory response (from hepatic stellate cells and Kupffer cells), which may result in necrotic inflammation and hepatic fibrosis contributing to worsening fibrosis and thus liver disease progression [10]. Accumulating evidence suggests gut dysbiosis, bacterial endotoxin, and increased intestinal permeability are hallmark features of CLD and positively correlate with disease severity. These factors play a crucial role in the pathogenesis of not only CLD but have also been shown to promote HCC through various mechanisms (**Figure 1**).

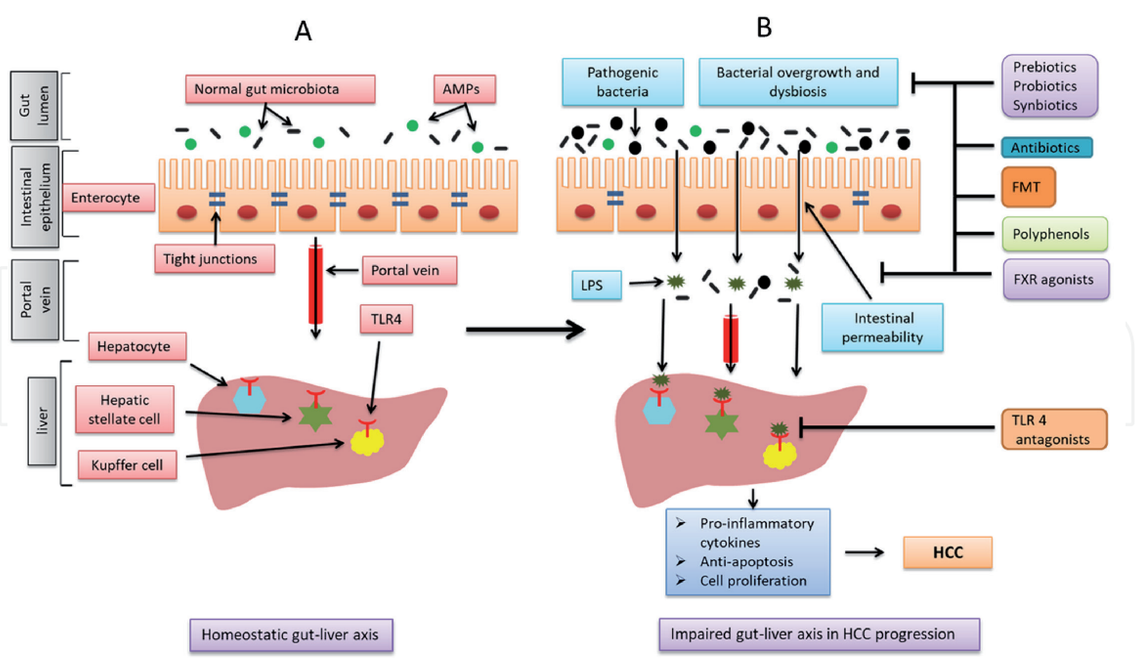

Figure 1.
An overview of homeostatic and impaired gut-liver axis in HCC progression. (A) In homeostatic condition gut lumen contains normal gut flora which is restricted by tightly closed intestinal epithelial cells to prevent its translocation to the liver. (B) Increased bacterial overgrowth in gut lumen and increased intestinal permeability promotes HCC progression through binding of LPS to toll-like receptor 4 (TLR4) which are present on Kupffer cells, hepatocytes, and HSC to elicit the release of pro-inflammatory cytokines and activation of proliferative and anti-apoptotic signals. Prebiotics, probiotics, synbiotics, FMT, and polyphenols can be used to restore eubiosis, while the use of antibiotics can potentially eliminate pathogenic bacteria and endotoxin release. FXR agonists can attenuate intestinal permeability and prevent bacterial translocation. TLR4 antagonists prevent binding of LPS to TLR4 and suppress cancer-promoting signals.

4. Gut dysbiosis

Dysbiosis defines any change in the typical gut microbial composition found in health. Several lines of evidence suggest that gut bacterial dysbiosis is a pathogenic factor in the progression of HCC whatever the trigger for CLD (e.g., alcohol, non-alcoholic steatohepatitis (NASH), viral hepatitis, etc.). The role of gut dysbiosis in the propagation of progressive CLD is likely triggered by the formation of microbial metabolites such as LPS, bacterial DNA, and deoxycholic acid, which causes chronic inflammation in the portal circulation and thus the liver. In cirrhotic patients overgrowth of the pathogenic bacteria such as *Enterobacteriaceae*, *Veillonellaceae*, and *Streptococcaceae* and decreased *Lachnospiraceae* have been observed to correlate with the child-turcotte-pugh (CTP) score, clinically used to assess the severity of cirrhosis [11]. Moreover, in cirrhotic patients studies have found an increase in both the oral and gut levels of the same microbial species suggesting invasion from the mouth to intestine [12]. It is therefore postulated oral bacterial overgrowth may have a profound effect on intestinal bacterial communities and thus CLD pathogenesis and HCC development. This concept is supported by a recent study that showed a high level of oral microbiota *Oribacterium* and *Fusobacterium* in HCC patients [13]. The alteration in gut microbiota composition in HCC is summarized in **Table 1**.

Microbial gene signatures that relate to energy production, nickel/iron transport, and amino acid transport appear to be altered in HCC patients when compared to healthy controls [13]. Moreover, when compared to cirrhotic patients, fecal samples from HCC patients have shown increased growth of phylum *Actinobacteria* and 13 genera including *Gemmiger* and *Parabacteroides* [14]. They also found a decrease in butyrate-producing bacteria and an increase in LPS-producing bacteria in HCC patients when compared to healthy controls [14]. A study conducted by *Ponziani* et al. in NAFLD-related HCC showed increased fecal *Bacteroides* and *Ruminococcaceae*, whereas reduced *Akkermansia* and *Bifidobacterium* were negatively correlated with intestinal inflammatory marker fecal calprotectin level [15]. This study also showed increased intestinal permeability in these patients accompanied by an increase in plasma level of IL8, IL13, CCL3, CCL4, and CCL5, showing the evidence that alteration in gut microbiota profile is associated with systemic inflammation that may contribute to HCC pathogenesis [15]. In another study, *E. coli* growth in fecal samples was significantly elevated in HCC patients compared to matched cirrhotic patients [16]. Interestingly, inoculation of AFB1 and/or *Helicobacter hepaticus* in *Helicobacter*-free C3H/HeN mice was associated with HCC progression [17]. This observation may suggest that neither direct bacterial translocation nor hepatocyte injury is necessary for HCC development. In clinical studies utilizing liver HCC tumor biopsy tissue, some authors report the presence of *Helicobacter* ssp. DNA, whereas other investigators failed to correlate the presence of *Helicobacter* ssp. DNA with HCC progression [18]. In DEN-treated rats, fecal and cecal samples show an increase of pathogenic bacterial species like *E. coli*, *Atopobium*, *Collinsella*, *Eggerthella*, and *Corynebacterium*; in contrast there was a decline in the numbers of beneficial bacteria like *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterococcus* spp. [19]. Although the exact mechanism by which gut microbiota promotes HCC has not been firmly established, studies in murine models indicated LPS-TLR4 axis plays a crucial role in the progression of HCC [19, 20]. Zhang et al. suggest that gut dysbiosis merely promotes HCC by increasing LPS levels and that conversely probiotics may suppresses tumor growth [19]. Similarly, Dapito et al. propose that gut microbiota only has a role in the promotion of HCC rather than its initiation [20]. The ability of pathogenic bacteria to disrupt TJs protein thereby increasing intestinal permeability has also been postulated as another mechanism by which microbiota may promote CLD and HCC [21]. However further preclinical and clinical studies

Authors	Sample type	Changes in fecal microbiota composition	Clinical implication in HCC
Ren et al. [14]	Feces	*Klebsiella i*	• Increase in lipopolysaccharide-producing bacteria promotes HCC progression • Decrease in butyrate-producing bacteria promotes intestinal mucosal injury which contributes to HCC development
Ponziani et al. [15]	Feces	*Bacteroides* ↑ *Ruminococcaceae* ↑ *Enterococcus* ↑ *Phascolarctobacterium* ↑ *Oscillospira* ↑ *Bifidobacterium* ↓ *Blautia* ↓	• Increase in Gram-negative bacteria promotes HCC progression • Decrease in beneficial bacteria was correlated with intestinal inflammation favoring local microenvironment for HCC development
Grat et al. [16]	Feces	*Escherichia coli* ↑	• Increase in *pathogenic E. coli* contributes to HCC progression
Huang et al. [18]	HCC liver biopsy	*Helicobacter pylori* ↑	• Helicobacter linked to hepatocarcinogenesis by colonizing the liver
Zhang et al. [19]	Feces	*Escherichia coli* ↑ *Atopobium cluster* ↓ *Prevotella* ↓ *Bacteroides* ↑ *Lactobacillus* spp. ↑ *Bifidobacterium* spp. ↑ *Enterococcus* spp. ↓	• Alteration in gut microbiota profile promoted tumor formation in DEN-induced HCC rats
Yoshimoto et al. [38]	Feces	*Clostridium* cluster XI and XIVa ↑	• Increase in deoxycholic acid -producing bacteria accelerated HCC progression in DMBA-HFD-induced HCC rats • Deoxycholic acid is a risk factor for obesity-induced HCC development

Table 1.
Gut microbiota dysbiosis in HCC.

are needed to establish the causal link between gut microbiota and HCC progression and to further delineate the molecular mechanisms involved.

5. Inflammation as a key player: triggered by LPS-TLR4 mediated pathway

HCC is arising in an inflammatory environment of the CLD, and therefore, neutralizing inflammation with anti-inflammatory agents may reduce the incidence and recurrence of HCC. Much attention has been focused on the potential involvement of the toll-like receptors (TLR) signaling pathway in the development of liver inflammation and associated HCC progression. Gut-derived endotoxin initiates the innate immune system such as TLRs, which recognize bacterial products and are predominantly expressed throughout the gut-liver axis. In addition, TLR4 plays a central role through LPS (a component of Gram-negative bacteria)-induced hepatic inflammation, while TLR 2 senses component of Gram-positive bacteria such as

peptidoglycan [22]. In this context, Yu et al. identified that increased activation of the TLR 4-LPS axis correlated with intestinal permeability in DEN-induced acute liver failure (ALF), which directly regulates pro-survival molecules and enhances hepatocyte proliferation [23]. Interestingly, in mice models the use of antibiotics and/or TLR4 genetic ablation prevented tumor growth and multiplicity [19, 23]. Dapito et al. showed a close link between gut microbiota and LPS-TLR 4 axis in HCC progression in a chimeric mice model [20]. This study also showed in DEN-CCl4 treated mice (with histological CLD) that low-dose LPS treatment triggered TLR4 activation and increased rate of tumor formation, whereas gut sterilization prevented HCC progression rather than regression of the established tumor [20]. Taken together these studies would therefore suggest that gut microbiota may not have a role in HCC initiation but may instead have a tumor-promoting effect through TLRs signaling pathways [20].

In respect of HCC promotion, multiple downstream targets of the LPS-TLR4 axis have been identified in both in vitro and in vivo studies. HSCs, Kupffer cells, and hepatocytes show TLR4 expression and thus are sensitive to LPS challenge [24]. Dapito et al. showed that TLR4 activation in HSCs, hepatocytes, and non-bone-marrow-derived resident cells promotes hepatocarcinogenesis by upregulating epiregulin (hepatomitogen) and inhibiting cleaved caspase 3 via NF-κB activation [20]. Moreover, Yu et al. showed hepatic Kupffer cells as the chief target for LPS-induced TLR4 activation by increasing pro-inflammatory cytokines such as TNF-α and IL-6 production [23]. Similarly, in vitro studies have shown evidence of LPS-TLR4-promoted HCC cell proliferation via NF-κB, MAPK, and STAT3 mediated signaling pathways [24]. LPS-TLR4 has also been shown to promote epithelial-to-mesenchymal transition (EMT) in HCC by upregulating NF-κB- and JNK/MAPK-mediated expression, while NF-κB and JNK/MAPK signaling blockade inhibited EMT occurrence [25]. Similarly, LPS-TLR4 axis is also known to enhance angiogenesis in HCC mice model via production of pro-angiogenic factors by HSCs in tumor stroma [26]. However further studies incorporating TLR4 deletion are needed to better understand its role in hepatocyte proliferation and distinguish paracrine signaling from HSCs and Kupffer cells in HCC progression.

6. Intestinal epithelial barrier dysfunction: role of tight junction proteins

Commensals and opportunistic pathogens are kept in check within the intestinal lumen by a single layer of intestinal epithelial cells (IEC) which spans almost 400 m^2 in surface area [27]. The gut barrier is highly dynamic in nature in which IEC is capable of self-renewal every 4–7 days with constant changes in intestinal luminal content. IEC are predominantly composed of absorptive enterocytes, which have metabolic and digestive functions. It also has secretory functions enacted by cell types such as enteroendocrine, goblet, and paneth cells which are specialized for maintaining digestive, immune, and epithelial barrier function [27]. Enteroendocrine cells connect the central and enteric neuroendocrine system via the secretion of various digestive hormones like gastrin, cholecystokinin, incretin, etc. The highly glycosylated mucins secreted by goblet cells form the first line of defense against microbial invasion and when compromised may predispose to disease as is evident in Mucin 2-deficient mice which are susceptible to colitis and inflammation-induced colorectal cancer [28, 29]. The intestinal barrier function is further strengthened by antimicrobial peptides (AMPs) including defensin, cathelicidin, and lysozyme [27]. These AMPs disrupt bacterial cell membranes and prevent adherence to gut mucosa.

Apart from IEC, the gut barrier is primarily maintained by tight junction (TJs) components (e.g., claudin, occludin, zonula occludens, and other junctional adhesion molecules (JAMs)) preserving intact epithelia which in turn regulate the paracellular movement of solutes, water, and other nutrients while restricting the entry of bacteria from the lumen to systemic circulation [30]. The mechanism of increased intestinal permeability is poorly understood. Growing evidence suggests that inflammation and TJ protein disruption are two of the key players driving increased intestinal permeability. In our previous study, we found increased systemic ZO-1 level in HCC patients reflecting increased intestinal permeability [31]. Moreover, plasma ZO-1 level was positively correlated with the inflammatory marker hs-CRP and with disease severity, suggesting inflammation drives intestinal permeability associated with HCC progression [31]. Bacterial overgrowth leads to increased production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, which is mainly mediated through the TLR4-NF-kB signaling pathway, thereby promoting intestinal inflammation and HCC progression [19]. These pro-inflammatory cytokines have a direct effect on TJ proteins like claudin and ZO-1, leading to enhance intestinal permeability [32]. Furthermore, in both in vivo and in vitro models, LPS dose dependently increases intestinal permeability via upregulating TLR4-mediated CD14 expression in enterocytes [33]. Similarly, NLRP6-deficient mice show altered microbiota and enhanced colonic inflammation through the chemokine (C-C motif) ligand CCL5 [34]. This results in increased intestinal permeability to microbial products and thus increases hepatic inflammation and progression from NAFLD to NASH [34]. Moreover, enteric pathogens such as *Escherichia coli* and *Clostridium difficile* are increased in CLD and are capable of increased intestinal permeability by modulating TJ integrity [11, 21].

Bile acids are another key player for maintaining gut barrier function by promoting intestinal epithelial cell proliferation and microbiota composition [35]. There is clear evidence that bile acids have both direct antimicrobial effect and an indirect effect through FXR-induced AMPs and thus control growth and adhesion of intestinal bacteria. In fact, decreased bile acids pool in the intestine is associated with bacterial overgrowth and inflammation [36]. The study by Kakiyama et al. showed that cirrhosis reduced bile acids entering the intestine causing bacterial dysbiosis by reducing beneficial bacteria such as Gram-positive *Blautia and Ruminococcaceae* and increasing pathogenic bacteria like *Enterobacteriaceae* [37]. In a NASH-induced CLD/HCC mouse model, increased Gram-positive *Clostridium* clusters (XI and XVIa) were positively correlated with increased serum deoxycholic acid (DCA) [38]. Notably, *Clostridium clusters* are capable of synthesis of secondary bile acid DCA via 7α-dehydroxylation of primary bile acids. DCA is a DNA-damaging agent and a known pro-carcinogen shown to affect various cancer signaling pathways. In this context, a study by *Yoshimoto* et al. revealed DCA activates a senescent-associated secretory phenotype in HSCs, thereby producing various pro-inflammatory and pro-tumorigenic factors promoting HCC development in mice, while antibiotic treatment and/or blocking DCA production prevented HCC development [38]. Similarly, in a HCC mouse model induced by steatohepatitis-inducing high-fat diet (STHD-01), increased hepatic and fecal bile acids concentrations were observed [39]. In this model, DCA activated mTOR and promoted HCC development. However, following antibiotic treatment, there was a decrease in HCC progression suggesting an interrelationship between BA metabolism, gut microbiota, and HCC development [39].

Bile acids maintain homeostatic IEC proliferation via EGFR- and FXR-dependent pathways, which helps the continuous regeneration of enterocytes and maintain intact epithelia [40]. Several studies demonstrate that the intestinal bile acid pool also regulates TJ protein distribution and expression. In Caco-2 cell

monolayers, incubation with dihydroxy bile acids decreased transepithelial resistance (TER) and was accompanied by increased phosphorylation and redistribution of occludin [41]. In human colonic biopsies, DCA induces Cr-EDTA permeability altering TER and increasing translocation of *E. coli*. Several in vivo studies have also elucidated the role of bile acids in the regulation of TJ permeability [42]. In HFD mice increased intestinal bile acid pool was associated with increased intestinal permeability with decreased expression of TJ proteins ZO-2 and JAM-A [43]. Similarly, in bile duct ligated (BDL) rats where intestinal BA delivery was prevented, there was increased bacterial translocation and increased intestinal permeability with decreased expression of claudin-1 and occludin. Conversely, the above effects were ameliorated by FXR activation [44]. These studies highlighted the protective role of FXR in the maintenance of intestinal barrier integrity; however, it is unclear whether these effects were from direct activation of FXR on TJ proteins or indirect effects from altered mucosal immune cells. In addition to FXR, another bile acid receptor TGR5 also modulates barrier permeability and TJ protein expression. In TGR5 null mice, increased intestinal permeability due to alteration of TJ protein expression develops colitis [45]. Therefore, the quantity and composition of BA pool in the intestine represent an important factor in the regulation of gut microbial community and gut barrier integrity.

7. Therapeutic approach controlling gut microbiota, gut dysbiosis, and inflammation to prevent HCC

7.1 Prebiotics, probiotics, and synbiotics

Traditionally, HCC is cured based on the available treatment options such as surgical treatment, chemotherapy, and local ablation therapy; however, patients are facing many problems including the poor hepatic reserve [46]. Furthermore, the possible therapeutic interventions targeting the gut-liver axis in HCC are summarized in **Table 2**.

Prebiotics are non-absorbant and nondigestible food ingredients which promote growth or activity of beneficial bacteria like *Bifidobacteria* and *Lactobacilli* and inhibit the growth of potentially pathogenic bacteria [47]. Currently, prebiotics like lactulose, lactitol, fructo-oligosaccharides, and galacto-oligosaccharides are commercially available [48]. Synthetic disaccharides like lactulose and lactitol are extensively used for the treatment of hepatic encephalopathy in CLD patients as ammonia detoxifying agents [48]. Also, these disaccharides are metabolized by colonic bacteria to produce lactic acid and acetic acid due to which pH in the gut lumen is decreased [49]. Low pH enhances the growth of non-urease-producing *lactobacilli* and inhibits pathogenic urease-producing bacteria [50]. Chen et al. showed that in chronic viral hepatitis patients, lactitol administration significantly decreased plasma endotoxin levels and increased the growth of beneficial *Lactobacilli* and *Bifidobacteria* species [51]. In contrast, Bajaj et al. reported lactulose administration in patients with HE did not improve dysbiosis and increased growth of Gram-negative bacteria such as *Enterobacteriaceae and Bacteroidaceae* [52]. This indicates the pattern of gut microbiota abundance is the major determinants of disease severity [52]. In HCC patients administration of lactulose (30 mL/day) for 14 days significantly reduced ALT and bilirubin levels, while antioxidant enzyme SOD, anti-inflammatory markers IFN-γ and IL-4, and blood cells CD4(+)/CD8(+) were found to be increased suggesting its ability to reduce inflammation and restore oxidation/antioxidant system imbalance [53]. Similarly in partial hepatectomized rats, administration of lactulose induces liver regeneration by

Authors	Intervention class	Medication	Desired effect in HCC
Zong et al. [53]	Prebiotics	Lactulose	• Lactulose treatment to HCC patients increased antioxidant enzymes and anti-inflammatory markers while improving tumor immunity and overall prognosis
Zhang et al. [19]	Probiotics	VSL#3	• VSL#3 treatment to DEN-induced HCC rats reduced tumor number and multiplicity by ameliorating hepatic and intestinal inflammation and improving intestinal permeability and restoring eubiosis
El-Nezami et al. [63]	Probiotics	*Lactobacillus rhamnosus LC 705* and *Propionibacterium freudenreichii subsp. shermani*	• Treatment to AFB1-induced HCC patients reduced aflatoxin-DNA adduct excretion in urine
Li et al. [46]	Probiotics	Prohep	• Treatment with Prohep to DEN-induced HCC mice reduced tumor size and angiogenic factors *VEGFA* and *TEK*
Kumar et al. [62]	Probiotics	Combination of probiotic fermented milk and chlorophyllin	• Treatment to AFB1-induced HCC rats reduced DNA damage, oncogenic signal, and tumor incidence
Yoshimoto et al. [38]	Antibiotics	4Abx (ampicillin, metronidazole, vancomycin, neomycin)	• Treatment with 4Abx in DMBA-HFD-induced HCC rats reduced tumor number and tumor size
Abdel-Hamid et al. [67]	Antibiotics	Clarithromycin and azithromycin	• Treatment to DEN-CCL4/acetylaminofluorene-induced HCC rats induced intrinsic and extrinsic apoptosis and inhibited HCC progression
Dapito et al. [20]	Antibiotics	Rifaximin	• Rifaximin treatment to DEN-CCL4 induced HCC rats reduced tumor number
Nguyen et al. [93]	TLR4 antagonists	TAK-242	• TAK-242 treatment to transgenic HCC mice reduced tumor burden and ameliorated hepatic steatosis and fibrosis
Nkontchou et al. [117]	Nonselective β-blockers	Propranolol	• Long-term treatment with propranolol reduced HCC incidence in HCV-related cirrhosis patients
Chang et al. [118]	Nonselective β-blockers	Propranolol	• Treatment with propranolol reduced mortality risk and improved survival in unresectable/metastatic HCC patients
Bishayee et al. [121]	Natural compound	Resveratrol	• Resveratrol treatment to DEN-induced HCC rats reduced hepatic nodules and prevented HCC progression
Ji et al. [124]	Natural compound	Quercetin	• Quercetin treatment to DEN-acetylaminofluorene-induced HCC rats reduced number of nodules
Teng et al. [125]	Natural compound	Curcumin	• Treatment with curcumin to HBV-related transgenic HCC mice reduced hepatic nodules and suppressed HCC progression

Table 2.
Therapeutic intervention targeting the gut-liver axis in HCC.

inducing hydrogen and abrogating oxidative stress (heme oxygenase-1, SOD-2) and inflammation (IL-6 and TNF-α) [54]. Moreover, mixed diets of galacto-oligosaccharides and fructo-oligosaccharides to infants were increased the growth of *Bifidobacterium*; however, this formulation has not been tried in patients with liver disease [55].

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit for the host [56]. Probiotic supplementation was also shown to restore intestinal dysbiosis in CLD patients [48]. Furthermore, mice treated with probiotic such as VSL#3 significantly reduces *Clostridium* spp. and modified gut microbiota [57]. In cirrhotic patients, VSL#3 supplementation for nearly 6 months was shown to reduce the risk of hospitalization and improved CTP and MELD score [58]. Similarly, in NASH-associated obese children, treatment with VSL#3 over the period of 4 months reduces fatty liver and improved lipid profile and insulin sensitivity [59]. The other probiotics such as *Lactobacillus salivarius* LI01 and *Pediococcus pentosaceus* LI05 reduced inflammation, protected the intestinal barrier, prevented bacterial translocation, restored eubiosis, and attenuated hepatic fibrosis in CCl_4-induced cirrhotic rats [60]. Similarly, *Lactobacillus* GG (LGG) supplemented with standard diet in cirrhosis patients show significantly reduced blood endotoxemia and TNF-α, thereby restoring eubiosis [61]. However, in HCC, probiotic usage is meager, and only a few studies have identified the therapeutic potential. VSL#3 (contains three strains of *Bifidobacteria*, four strains of *Lactobacilli*, and one strain of *Streptococcus thermophilus*) treatment to DEN-induced rat hepatocarcinogenesis has shown to attenuate HCC progression, reduce tumor number and multiplicity, ameliorate hepatic and intestinal inflammation, and thus restore gut dysbiosis [19]. Li et al. identified that subcutaneous administration of Prohep (a novel probiotic mixture of *Lactobacillus rhamnosus GG*, *Escherichia coli Nissle 1917*, and heat-inactivated VSL#3) reduced the tumor size and HCC growth [46]. Prohep improves beneficial bacteria such as *Prevotella* and *Oscillibacter* and control tissue inflammation as evidenced by decreased T helper 17 cells in the gut, thereby attenuating the progression of HCC [46]. Moreover, in aflatoxin B1-induced HCC rats, treatment with probiotic fermented milk and chlorophyllin significantly reduced tumor incidence by decreasing the expression of cyclin D1, bcl-2, and c-myc proto-oncogenes [62]. Similarly, aflatoxin-induced HCC patients treated with dietary supplementation of probiotics (using viable *Lactobacillus rhamnosus LC 705* and *Propionibacterium freudenreichii subsp. shermani)* decreased the urinary excretion of aflatoxin-DNA adduct (AFB-N7 guanine) and improved HCC [63]. Thus, probiotic supplementation could be beneficial to cirrhotic patients with the potential to progress to HCC.

Synbiotics are combined form of prebiotics and probiotics, which contains four fermentable fibers (symbiotic 2000) and four freeze-dried non-urease-producing lactic acid bacteria. Synbiotic administration to cirrhotic patients results in decreased plasma endotoxin and ammonia levels and increased fecal *Lactobacillus* spp. [64]. Interestingly 50% of these patients have improved child-turcotte-pugh score compared to placebo [64]. Moreover, in NAFLD patients synbiotic supplementation has shown to have beneficial effects by improving lipid profile, glucose homeostasis, hepatic marker enzymes, and inflammatory markers [65]. Synbiotic (FloraGuard) administration also has a protective role in alcohol-induced liver injury in rats [66]. In addition, the synbiotic treatment restored ethanol-induced intestinal permeability and increased the growth of beneficial bacteria *Bifidobacterium* spp. and *Lactobacillus* spp. [66]. Currently, studies are lacking for the use of synbiotics in chronic liver diseases or HCC prevention.

8. Antibiotics

Several studies have postulated that antibiotic treatment may cause gut microbiota dysbiosis. It may represent effective strategies to prevent the tumor-promoting gut microbiota, its metabolites DCA, and pro-inflammatory signal inducer LPS which all have a role in the progression of CLD and HCC. In DEN-CCl_4- and DMBA-HFD-induced HCC mice model, the oral antibiotics ampicillin, metronidazole, neomycin, and vancomycin significantly reduced the tumor number and size [20, 38]. In addition, this antibiotic combination also reduced the liver fibrosis and improved liver histology in cirrhosis. Moreover, the effectiveness of antibiotics administration was enhanced when given at late-stage HCC in mice rather than earlier stage [20]. In another study conducted in DEN-induced HCC rats, clarithromycin and azithromycin suppressed HCC progression through extrinsic and intrinsic apoptotic pathways, whereas erythromycin aggravated HCC and did not show antitumorigenic effect [67]. Vancomycin is an antibiotic used to treat a bacterial infection, which effectively prevented the mouse model of HCC; however, long-term administration to CLD patients develops potential side effects [38]. Many studies have concluded that norfloxacin and rifaximin treatments to cirrhotic patients have beneficial effects [68]. In a double-blind placebo-controlled clinical trial, long-term oral administration of norfloxacin to cirrhotic patients markedly reduces Gram-negative bacteria in the fecal matters and lowers the spontaneous bacterial peritonitis (SBP) [69]. Furthermore, CCl_4-induced cirrhotic animals showed decreased SBP and inflammation following norfloxacin treatment [70]. Norfloxacin was identified as a promising antibiotic in regulating gut microbiota overgrowth and prevention of BT in both cirrhotic humans and rodents; indeed its effect on HCC patients remains unidentified. Rifaximin is a broad spectrum oral antibiotic having potential bactericidal activity against aerobic and anaerobic Gram-negative bacteria [71]. It is an excellent choice of drug to cure HE in advanced cirrhotic patients [72]. The study conducted by *Vlachogiannakos* et al. showed treatment with rifaximin for 4 weeks significantly decreased portal pressure and LPS levels in decompensated cirrhotic patients [73]. Long-term treatment with rifaximin reduces SBP occurrence, variceal bleeding, HRS, and HE with an overall improvement in survival rate in cirrhotic patients [74]. Similarly, in murine DEN-CCl_4-induced HCC mouse model, rifaximin treatment was shown to ameliorate HCC progression [20]. Although rifaximin is clinically used for prevention of HE and other complications in cirrhotic patients, its role in HCC prevention in humans is further warranted.

9. Fecal microbiota transplantation (FMT)

Fecal microbiota transplantation is currently being used for the treatment of *Clostridium difficile* infection [75]. The enteric dysbiosis was restored to normal gut flora following FMT from healthy donor to *Clostridium difficile*-infected patients [75]. Moreover, in mice with gut dysbiosis induced by antibiotics and chemotherapy, treatments were reversed by FMT [76]. In experimental cirrhosis with hepatic encephalopathy, FMT improves liver function and HE grade by limiting inflammation and improving tight junction integrity [77]. In this context, Bajaj et al. reported that FMT in cirrhotic patients with recurrent HE improves cognition, restores dysbiosis, and improves MELD score compared to standard care in those patients [78]. A number of clinical trials are ongoing in patients with NASH and cirrhosis for the efficacy of FMT [79, 80]. Vrieze et al. reported in patients with severe metabolic syndrome that treatment with FMT from a healthy donor

improves liver biochemistry, peripheral insulin resistance, and restoration of eubiosis [81]. Furthermore, in alcoholic hepatitis patients, FMT treatment significantly reduced liver disease severity and HE occurrence [82]. FMT recipients also showed an increase in beneficial bacteria like *Actinobacteria* and *Bifidobacterium longum* and decrease in *Pseudomonas* and *E. coli* with an increase in bile secretion [82]. Collectively these findings indicated that FMT may restore gut dysbiosis and reduce complications in cirrhotic patients and thus attenuate HCC development. However, scarce literature supporting the beneficial effect of FMT and long-term study is needed to prove permanent colonization in altered gut microbiota environment in cirrhotic patients. Moreover, there is a chance of inducing viral infection and transmission of other pathogens through FMT which may have deleterious effect and immunosuppression in advanced liver disease patients [83, 84].

10. TLR agonist or antagonist

Numerous studies have shown that LPS-TLR4 is a key inflammatory pathway in the progression of CLD and has a tumor-promoting effect on HCC [20, 23, 85]. Therefore blocking this pathway might represent a promising treatment approach in controlling cirrhosis and HCC progression. Several TLR4 antagonists have been developed toward controlling LPS-activated TLR4-mediated inflammatory responses which include polymyxin B [86], glycolipids interfering CD14-LPS interaction [87], eritoran [88], resatorvid (TAK242) [89], and thalidomide [90]. In BDL-induced cirrhotic rats, intravenous administration of TAK-242 significantly reduces plasma transaminases and inflammatory cytokines [91]. It also ameliorates acetaminophen-induced acute liver failure [92]. Similarly, in transgenic HCC mice (Hep*Pten*$^{-}$), TAK-242 treatment for 28 days significantly reduced tumor burden and ameliorated HCC progression [93]. Eritoran tetrasodium protects against liver ischemia/reperfusion injury by inhibiting inflammatory response induced by high-mobility group box protein B1 (HMGB1) [94]. Similarly in D-galactosamine- and LPS-induced acute liver failure, treatment with eritoran significantly reduces inflammation and hepatic marker enzymes [95]. Eritoran treatment also decreases proliferation and induces apoptosis in tumor cells in a chemically induced mouse model of colorectal carcinoma [96]. Although resatorvid and eritoran showed the beneficial effect in improving the survival of murine sepsis model, it failed to do so in patients with severe sepsis [97]. Several TLRs have been upregulated in HCC [98]. In addition, TLRs are also abundantly expressed on immune cells which recognize various pathogens such as HBV which upon activation induces an innate immune response [99]. All the TLRs are activated by two independent pathways: MyD88-dependent (except TLR3) and MyD88-independent (TLR3 and TLR4) pathway [100]. Activation of TLR3-TRIF signaling pathway leads to apoptotic activity independently and, in turn, also activates IRF3 transcription factor to produce interferons [100]. TLR3 agonist BM-06 (synthetic dsRNA) significantly inhibited HCC cell proliferation, increased apoptosis, and decreased cell invasion and migration with increased antiviral IFN level [101]. Activation of TLR9 leads to phosphorylation of NF-κB with increased production of pro-inflammatory cytokine TNF-α, IL-6, and IL-10 [102]. Mohamed et al. showed that inhibition of TLR7 and TLR9 with antagonist IRS-954 or chloroquine significantly reduces HCC cell proliferation, angiogenesis with increased apoptosis [103]. This was further supported by tumor xenograft and DEN-induced rat HCC model in which chloroquine treatment reduces HCC incidence [103]. Although growing evidence shows TLRs as an important mediator of HCC progression, the molecular mechanism for disease progression is not completely understood. Therefore, further research needs to be

done for the use of TLR agonist or antagonist as a drug target for HCC prevention since TLRs are also involved in both cancer-promoting mechanism and immune-modulator which is responsible for an innate immune response against tumor cells and HBV and HCV infection [99, 104–106].

11. Prokinetics

Bacterial overgrowth due to impaired gut motility has been reported in cirrhotic patients [107]. Cisapride, a prokinetic drug, has shown beneficial effects not only by regulating intestinal motility but also inhibiting bacterial overgrowth and preventing bacterial translocation in both rodent models and liver cirrhotic patients [108, 109]. Cisapride in combination with norfloxacin significantly reduces the incidence of SBP in high-risk cirrhotic patients [110]. Although the mechanism of impaired gastrointestinal motility in cirrhotic patients is unclear, increased adrenergic activity may be responsible for the altered motility.

12. Nonselective beta-blockers (NSBB)

Nonselective β-blockers are prevalently used in decompensated chronic liver disease patients to reduce morbidity and mortality. It is used as bleeding prophylaxis in cirrhotic patients with esophageal varices. NSBB also antagonizes β-adrenoceptors. The β-adrenergic receptor pathway is involved in maintaining normal physiological functions. The catecholamines such as epinephrine and norepinephrine are the key ligands for β-adrenoceptors (β1 and β2). Furthermore, increased expression of β-adrenoceptors was observed in HCC cell membranes compared to healthy liver cells; however, the mechanisms remain unclear [111]. Catecholamines exhibit pro-carcinogenic effects in gastric, pancreatic, and breast cancer, which is antagonized by NSBB. Its beneficial effects to reduce the risk of HCC have also been identified by the very recent observational and experimental trials [112]. Moreover, in ovarian and breast cancer patients, NSBB treatment was shown to reduce cancer formation and growth. In cirrhotic patients, increased catecholamines results in disease severity, and thus, NSBB treatment may be potent to inhibit carcinogenesis in cirrhosis [113]. A recent study by Leithead et al. showed NSBB is safe and may confer benefit in patients with ascites complicating the end-stage liver disease [114]. In addition, Reiberger et al. observed that NSBB treatment ameliorates intestinal permeability and reduces BT in cirrhotic patients [115]. In a recent study, Wang et al. showed propranolol induces apoptosis and suppresses proliferation of liver cancer cells [116]. Of note, long-term treatment with propranolol in patients with HCV-related cirrhosis reduces the incidence of HCC [117]. Similarly, in patients with unresectable/metastatic HCC cohort, propranolol treatment significantly reduces mortality risk and improved overall survival suggesting β-blockers might be another therapeutic approach in HCC prevention [118].

13. Natural compounds

Treatment with natural compounds and food ingredients like polyphenols represents another therapeutic approach in the restoration of eubiosis, modulation of gut microbiota, reduction of inflammation, prevention of cirrhosis, and thus the progression of HCC. Many experimental data have shown evidence that flavonoids are proficient to alter gut microbial composition and restoration of

eubiosis in chronic liver diseases. Proanthocyanidin improves beneficial microbiota *Bacteroides* such as *Lactobacillus* spp. and *Bifidobacterium* spp. composition and reduces intestinal inflammation and oxidative damage, thereby attenuating experimental colon cancer. Resveratrol, a flavonoid, is shown to modulate intestinal microbiota with a profound increase in *Bifidobacterium* spp. and *Lactobacillus* spp. and reduce systemic and colonic inflammation in rats [119]. Resveratrol also shown to have anticancer activity in HCC cell lines; inhibit proliferation, viability, invasion, and metastasis; and induce apoptosis [120]. Its anticancer property was also studied in DEN-induced hepatocarcinogenesis [121]. We found resveratrol treatment to cirrhotic mice attenuated systemic inflammation and ammonia levels and altered neuronal TJ proteins, thereby preventing secondary complications such as HE in cirrhosis [122]. Quercetin, a bioactive flavonoid, was shown to inhibit human HCC cell proliferation, migration, and invasion and trigger apoptosis both in vivo and in vitro [123]. Its profound antitumor effect was also shown in xenograft and DEN-induced HCC rodent models [124]. Curcumin, a powerful antioxidant, has a wide range of bioactive properties. Previous studies have shown evidence that curcumin has HCC chemoprevention in preclinical models as well as patients with HCC [125, 126]. Moreover, nimbolide from the leaf of the neem tree (*Azadirachta indica*) is another potential natural compound having antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer properties [127]. In vitro study in HCC cell line (HepG2) shows that nimbolide induces cell apoptosis by abrogating NF-κB and Wnt signaling pathway [128]. Currently, our lab is focusing on anticancer effects of nimbolide and its molecular mechanisms in an experimental hepatocarcinogenesis. Of note, these natural compounds have a wide variety of biological activities on gut microbiota and may preserve gut barrier integrity, microbial metabolites, TJ integrity, and mucosal immunology. Indeed, further human studies are warranted to see the effect of natural compounds on gut microbial modulation and prevention of HCC.

14. Targeting gut epithelial barrier to prevent HCC

Gut epithelial barrier acts as a fence for translocation of gut microbiota and its metabolite into the systemic circulation which is the major driving factor for CLD progression and HCC development [129]. Therefore, primarily targeting or restoring the gut epithelial barrier is an interesting therapeutic approach in HCC pathogenesis. Compelling evidence has shown that targeting gut microbiota (restoring eubiosis) directly or receptor-mediated pharmacological intervention using TLR4 antagonist or FXR agonist might improve epithelial barrier function [130, 131]. FXR is a BA receptor which is widely expressed throughout the gut-liver axis. Decreased BA is associated with intestinal bacterial overgrowth, increased gut permeability, and bacterial translocation in rodents [38]. FXR controls hepatic inflammation, promotes liver regeneration, and suppresses HCC formation mainly through enterokine FGF19 [132]. FXR null mice have an intestinal barrier dysfunction and high occurrence of HCC, whereas reactivation of FXR inhibited HCC through FGF15-cyp7a1 axis [133]. In this context, FXR agonist obeticholic acid (OCA) prevents gut barrier dysfunction and BT in cholestatic rats [131]. Furthermore, in CCl_4-induced cirrhotic rats, OCA treatment significantly reduces BT and inhibits intestinal inflammation by restoring intestinal TJ proteins such as ZO-1 and occludin and antimicrobial peptides [134]. OCA treatment also improves hepatic inflammation and decreased portal pressure in BDL cirrhotic rats [135]. Consequently, OCA treatment may prevent HCC by limiting intestinal inflammation and improving gut barrier dysfunction in advanced liver disease.

Increased TNF-α production in mesenteric lymph nodes by monocyte is the major factor responsible for increased intestinal permeability in cirrhosis and HCC [19, 136]. TNF-α decreases ZO-1 expression through NF-κB and MLCK activation [137]. TNF-α also downregulated occludin expression in Caco-2 enterocyte by targeting PI3k/Akt signaling [138]. In BDL rats, treatment with infliximab (IFX, a monoclonal antibody against TNF) significantly reduced portal pressure and attenuated inflammation [139]. Therefore modulating intestinal TJs protein with anti-TNF-α therapy may restore intestinal integrity. However, due to the immunosuppressive activity of TNF inhibitors, it may lead to systemic infection in cirrhosis patients. Hence, detailed knowledge for local inhibition is required for improvement in gut barrier dysfunction without affecting innate immune response.

Retinoic acid can modulate TJs proteins. In a mice model of colitis characterized by gut permeability, treatment with retinoic acid enhances barrier function by upregulating TJs proteins claudin-1, claudin-4, and ZO-1 [140]. However, the effect of retinoic acid in the preservation of intestinal integrity has not been tested in cirrhosis and HCC. Modulation of the epithelial barrier with probiotics seems to be beneficial. In this regard, *Zhang* et al. showed probiotics VSL#3 (combination of *S. thermophilus*, four *Lactobacillus* species, and three *Bifidobacterium* species) treatment restores intestinal permeability and dysbiosis and prevented HCC progression in rats [19]. Similarly, probiotics like *E. coli Nissle1971* (ECN) was also shown to enhance intestinal TJs integrity by upregulating ZO-1 expression in the mouse model of DSS-induced colitis [141].

In addition, treatment with red wine polyphenol promoted barrier function by significantly increasing mRNA expression of TJ proteins occludin, claudin-5, and ZO-1 in cytokine-stimulated HT-29 colon epithelial cells [142]. Resveratrol also preserves TJ barrier integrity and diminishes intestinal permeability by upregulating occludin, ZO-1, and claudin-1expression and thus abrogating intestinal inflammation and oxidative stress both in vivo and in vitro [143]. Similarly in NAFLD mice model, treatment with resveratrol enhances barrier function by increasing mRNA expression of TJ proteins ZO-1, occludin, and claudin-1 in the intestinal mucosa [144]. Curcumin also modulates intestinal barrier integrity and attenuates paracellular permeability and organization of TJs [145]. Thus, targeting TJ proteins, which maintain intact intestinal epithelia, is another area of therapeutic approach for the control of intestinal permeability in cirrhosis and HCC.

15. Clinical value

HCC is the end-stage liver disease, which mostly develops on the background of cirrhosis. As discussed above, the gut-liver axis plays a significant role in the progression of CLD and ultimately HCC and would be a potentially significant therapeutic target in the prevention of HCC. There have been several preclinical and human studies demonstrating an association between gut dysbiosis and HCC progression. However, from the animal studies, it is unclear whether gut microbiota initiates HCC or acts with other precipitating factors like chronic inflammation in the progression of HCC. Modulation of gut dysbiosis with prebiotics and probiotics, FMT, or preventing bacterial overgrowth with antibiotics may therefore prevent HCC. Moreover, in cirrhotic patients these interventions appeared to prevent secondary complications and improved survival. We may therefore speculate that the above interventions might prevent HCC development in high-risk cirrhotic patients. Moreover, we should consider trialing these therapies in HCC patients with unresectable tumors, which might improve survival time and secondary complications. Furthermore interventions that restore intestinal barrier integrity may prevent gut BT and may consider another line of therapy in HCC.

16. Conclusion

In conclusion, there is growing evidence to suggest gut microbiota may play a significant role in the progression of CLD and thus HCC, which is likely to involve multiple pathways ranging from gut dysbiosis, endotoxemia, inflammation, loss of TJ integrity, and intestinal permeability. It is therefore suggested that the use of agents that have the potential to target microbial dysbiosis and restore intestinal epithelial barrier integrity may prevent bacterial translocation and ultimately delay HCC progression. However, whether bacterial overgrowth and/or intestinal permeability act independently or synergistically as causal pathogenic factors to influence the inflammatory milieu so closely associated with HCC progression remains unclear. Further research is therefore warranted to better understand the molecular pathways involved and guide the development of novel therapeutic interventions that can be taken to clinical trial to limit CLD/HCC progression through the targeting of dysbiosis and its effect on inflammation and intestinal permeability.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Abbreviations

ALT	alanine transaminase
AMPs	antimicrobial peptides
AST	aspartate transaminase
BAs	bile acids
BDL	bile duct ligation
CCL	chemokine ligand
CCl_4	carbon tetrachloride
CD	cluster of differentiation
CLD	chronic liver disease
CTP	child-turcotte-pugh
DCA	deoxycholic acid
DEN	diethylnitrosamine
DMBA	2,4-dimethoxybenzaldehyde
ERK	extracellular signal-regulated kinase
FMT	fecal microbiota transplantation
HCC	hepatocellular carcinoma
HFD	high-fat diet
HSCs	hepatic stellate cells
IBD	inflammatory bowel disease
IEC	intestinal epithelial cell
IL	interleukin
JNK	c-Jun N-terminal kinase
LBP	lipopolysaccharide binding protein
LPS	lipopolysaccharides
MAMPs	microbe-associated molecular patterns
MAPK	mitogen-activated protein kinase
MELD	model for end-stage liver disease
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis

NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NLRP	nucleotide-binding oligomerization domain, leucine-rich repeat- and pyrin domain-containing
NSBB	nonselective beta-blockers
OCA	obeticholic acid
PRRs	pattern recognition receptor
SCFAs	short-chain fatty acids
STHD	steatohepatitis-inducing high-fat diet
TB	total bilirubin
TGR5	takeda G-protein-coupled receptor 5
TLR	toll-like receptors
TNF-α	tumor necrosis factor alpha
TJs	tight junctions
WHO	World Health Organization
ZO	zonula occluden

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