Chapter

Microbiota, Inflammation, and Gut Barrier Dysfunction in HCC

Amit Kumar Ram, Gavin Wright and Balasubramaniyan Vairappan

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

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 microbiota 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, symbiotics, 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...
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...
Liver Pathology

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 m² 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-κB 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 enhanced 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. shermanii* | 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/acetylamino-fluorene-induced HCC rats induced intrinsic and extrinsic apoptosis and inhibited HCC progression |
| Dipito 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-acetylamino-fluorene-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 CCl₄-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-CCl₄- 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, CCl₄-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-CCl₄-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 (HepPten−/−), TAK-242 treatment for 28 days significantly reduced tumor burden and ameliorated HCC progression [93]. Eritoran tetrassodium 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 CCl4-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 polyphenols 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-1 expression 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

| Abbreviation | Definition |
|--------------|------------|
| ALT          | alanine transaminase |
| AMPs         | antimicrobial peptides |
| AST          | aspartate transaminase |
| BAs          | bile acids |
| BDL          | bile duct ligation |
| CCL          | chemokine ligand |
| CCl₄         | 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 |
Microbiota, Inflammation, and Gut Barrier Dysfunction in HCC
DOI: http://dx.doi.org/10.5772/intechopen.89578

Author details

Amit Kumar Ram†, Gavin Wright‡ and Balasubramaniyan Vairappan¶

1 Liver Diseases Research Lab, Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, India

2 Basildon University Hospital, Essex, United Kingdom

*Address all correspondence to: balasubramaniyan.v@jipmer.edu.in

†These authors have contributed equally to this work.

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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
References

[1] Ozakyol A. Global epidemiology of hepatocellular carcinoma (HCC epidemiology). Journal of Gastrointestinal Cancer. 2017;48(3):238-240

[2] Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nature Reviews. Immunology. 2016;16(6):341-352

[3] Nagpal R, Yadav H. Bacterial translocation from the gut to the distant organs: An overview. Annals of Nutrition and Metabolism. 2017;71(Suppl 1):11-16

[4] Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology. 2014;146(6):1513-1524

[5] Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut microbiota in health and disease. Physiological Reviews. 2010;90(3):859-904

[6] Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. Nutrients. 2014;7(1):17-44

[7] Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. Nature. 2016;535(7610):65-74

[8] Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307(5717):1915-1920

[9] Schneider KM, Albers S, Trautwein C. Role of bile acids in the gut-liver axis. Journal of Hepatology. 2018;68(5):1083-1085

[10] Wiest R, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. Hepatology. 2005;41(3):422-433

[11] Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology. 2011;54(2):562-572

[12] Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature. 2014;513(7516):59

[13] Lu H, Ren Z, Li A, Zhang H, Jiang J, Xu S, et al. Deep sequencing reveals microbiota dysbiosis of tongue coat in patients with liver carcinoma. Scientific Reports. 2016;6:33142

[14] Ren Z, Li A, Jiang J, Zhou L, Yu Z, Lu H, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. Gut. 2019;68(6):1014-1023

[15] Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. Hepatology. 2019;69(1):107-120

[16] Grąt M, Wronka KM, Krasnodębski M, Masior Ł, Lewandowski Z, Kosińska I, et al. Profile of gut microbiota associated with the presence of hepatocellular cancer in patients with liver cirrhosis. Transplantation Proceedings. 2016;48(5):1687-1691

[17] Fox JG, Feng Y, Theve EJ, Raczynski AR, Fiala JLA, Doernte AL, et al. Gut microbes define liver cancer risk in mice exposed to chemical and viral transgenic hepatocarcinogens. Gut. 2010;59(01):88-97

[18] Huang Y, Fan X-G, Wang Z-M, Zhou J-H, Tian X-F, Li N. Identification of helicobacter species in human
Microbiota, Inflammation, and Gut Barrier Dysfunction in HCC
DOI: http://dx.doi.org/10.5772/intechopen.89578

Liver samples from patients with primary hepatocellular carcinoma. Journal of Clinical Pathology. 2004;57(12):1273-1277

[19] Zhang H-L, Yu L-X, Yang W, Tang L, Lin Y, Wu H, et al. Profound impact of gut homeostasis on chemically-induced pro-tumorigenic inflammation and hepatocarcinogenesis in rats. Journal of Hepatology. 2012;57(4):803-812

[20] Dapito DH, Mencin A, Gwak G-Y, Pradere J-P, Jang M-K, Mederacke I, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell. 2012;21(4):504-516

[21] Berkes J, Viswanathan VK, Savkovic SD, Hecht G. Intestinal epithelial responses to enteric pathogens: Effects on the tight junction barrier, ion transport, and inflammation. Gut. 2003;52(3):439-451

[22] Pradere J-P, Dapito DH, Schwabe RF. The Yin and Yang of toll-like receptors in cancer. Oncogene. 2014;33(27):3485-3495

[23] Yu L-X, Yan H-X, Liu Q, Yang W, Wu H-P, Dong W, et al. Endotoxin accumulation prevents carcinogen-induced apoptosis and promotes liver tumorigenesis in rodents. Hepatology. 2010;52(4):1322-1333

[24] Wang L, Zhu R, Huang Z, Li H, Zhu H. Lipopolysaccharide-induced toll-like receptor 4 signaling in cancer cells promotes cell survival and proliferation in hepatocellular carcinoma. Digestive Diseases and Sciences. 2013;58(8):2223-2236

[25] Jing Y-Y, Han Z-P, Sun K, Zhang S-S, Hou J, Liu Y, et al. Toll-like receptor 4 signaling promotes epithelial-mesenchymal transition in human hepatocellular carcinoma induced by lipopolysaccharide. BMC Medicine. 2012;10:98

[26] Lu Y, Xu J, Chen S, Zhou Z, Lin N. Lipopolysaccharide promotes angiogenesis in mice model of HCC by stimulating hepatic stellate cell activation via TLR4 pathway. Acta Biochimica et Biophysica Sinica. 2017;49(11):1029-1034

[27] Peterson LW, Artis D. Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. Nature Reviews. Immunology. 2014;14(3):141

[28] Van der Sluis M, De Koning BAE, De Bruijn ACJM, Velcich A, Meijerink JPP, Van Goudoever JB, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology. 2006;131(1):117-129

[29] Velcich A, Yang W, Heyer J, Fragale A, Nicholas C, Viani S, et al. Colorectal cancer in mice genetically deficient in the Mucin Muc2. Science. 2002;295(5560):1726-1729

[30] Balda MS, Matter K. Tight junctions at a glance. Journal of Cell Science. 2008;121(Pt 22):3677-3682

[31] Ram AK, Pottakat B, Vairappan B. Increased systemic zonula occludens 1 associated with inflammation and independent biomarker in patients with hepatocellular carcinoma. BMC Cancer. 2018;18:572

[32] Capaldo CT, Nusrat A. Cytokine regulation of tight junctions. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2009;1788(4):864-871

[33] Guo S, Al-Sadi R, Said HM, Ma TY. Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane
expression and localization of TLR-4 and CD14. The American Journal of Pathology. 2013;182(2):375-387

[34] Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012;482(7384):179-185

[35] Inagaki T, Moschetta A, Lee Y-K, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proceedings of the National Academy of Sciences. 2006;103(10):3920-3925

[36] Gadaleta RM, van EKJ, Oldenburg B, Willemsen ECL, Renooij W, Murzilli S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut. 2011;60(4):463-472

[37] Kakiyama G, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. Journal of Hepatology. 2013;58(5):949-955

[38] Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013;499(7456):97-101

[39] Yamada S, Takashina Y, Watanabe M, Nagamine R, Saito Y, Kamada N, et al. Bile acid metabolism regulated by the gut microbiota promotes non-alcoholic steatohepatitis-associated hepatocellular carcinoma in mice. Oncotarget. 2018;9(11):9925-9939

[40] Dossa AY, Escobar O, Golden J, Frey MR, Ford HR, Gayer CP. Bile acids regulate intestinal cell proliferation by modulating EGFR and FXR signaling. The American Journal of Physiology-Gastrointestinal and Liver Physiology. 2015;310(2):G81-G92

[41] Raimondi F, Santoro P, Barone MV, Pappacoda S, Barretta ML, Nanayakkara M, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. The American Journal of Physiology-Gastrointestinal and Liver Physiology. 2008;294(4):G906-G913

[42] Münch A, Ström M, Söderholm JD. Dihydroxy bile acids increase mucosal permeability and bacterial uptake in human colon biopsies. Scandinavian Journal of Gastroenterology. 2007;42(10):1167-1174

[43] Murakami Y, Tanabe S, Suzuki T. High-fat diet-induced intestinal hyperpermeability is associated with increased bile acids in the large intestine of mice. Journal of Food Science. 2016;81(1):H216-H222

[44] Verbeke L, Mannaepts I, Schierwagen R, Govaere O, Klein S, Vander Elst I, et al. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. Scientific Reports. 2016;6:33453

[45] Cipriani S, Mencarelli A, Chini MG, Distritti E, Renga B, Bifulco G, et al. The bile acid receptor GPBAR-1 (TGR5) modulates integrity of intestinal barrier and immune response to experimental colitis. PLoS One. 2011;6(10):e25637

[46] Li J, Sung CYJ, Lee N, Ni Y, Pihlajamäki J, Panagiotou G, et al. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. Proceedings of the National Academy of Sciences. 2016;113(9):E1306-E1315

[47] Fotiadis CI, Stoidis CN, Spyropoulos BG, Zografos ED. Role of probiotics, prebiotics and synbiotics in
chemoprevention for colorectal cancer. World Journal of Gastroenterology. 2008;14(42):6453-6457

[48] Shukla S, Shukla A, Mehboob S, Guha S. Meta-analysis: The effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy. Alimentary Pharmacology and Therapeutics;33(6):662-671

[49] Pinzone MR, Celesia BM, Di Rosa M, Cacopardo B, Nunnari G. Microbial translocation in chronic liver diseases. International Journal of Microbiology. 2012. DOI: 10.1155/2012/694629. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3405644/

[50] Riggio O, Varriale M, Testore GP, Di Rosa R, Di Rosa E, Merli M, et al. Effect of lactitol and lactulose administration on the fecal flora in cirrhotic patients. Journal of Clinical Gastroenterology. 1990;12(4):433-436

[51] Chen C, Li L, Wu Z, Chen H, Fu S. Effects of lactitol on intestinal microflora and plasma endotoxin in patients with chronic viral hepatitis. The Journal of Infection. 2007;54(1):98-102

[52] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. Journal of Hepatology. 2014;60(5):940-947

[53] Zong D-W, Guo C-Y, Cheng H-T, Hu H-T, Xiao J-C, Li H-L. Influence of lactulose on interventional therapy for HCC patients with hepatocirrhosis and hypersplenism. Asian Pacific Journal of Tropical Medicine. 2016;9(2):193-196

[54] Yu J, Zhang W, Zhang R, Ruan X, Ren P, Lu B. Lactulose accelerates liver regeneration in rats by inducing hydrogen. The Journal of Surgical Research. 2015;195(1):128-135

[55] Bakker-Zierikzee AM, Alles MS, Knol J, Kok FJ, Tolboom JJM, Bindels JG. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable bifidobacterium animalis on the intestinal microflora during the first 4 months of life. The British Journal of Nutrition. 2005;94(5):783-790

[56] Mack DR. Probiotics. Canadian Family Physician. 2005;51(11):1455-1457

[57] Arthur JC, Gharraibeh RZ, Uronis JM, Perez-Chanona E, Sha W, Tomkovich S, et al. VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. Scientific Reports. 2013;3:2868

[58] Dhiman RK, Rana B, Agrawal S, Garg A, Chopra M, Thumburu KK, et al. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: A randomized, controlled trial. Gastroenterology. 2014;147(6):1327-1337.e3

[59] Alisi A, Bedogni G, Baviera G, Giorgio V, Porro E, Paris C, et al. Randomised clinical trial: The beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Alimentary Pharmacology and Therapeutics. 2014;39(11):1276-1285

[60] Shi D, Lv L, Fang D, Wu W, Hu C, Xu L, et al. Administration of Lactobacillus salivarius LI01 or Pediococcus pentosaceus LI05 prevents CCl4-induced liver cirrhosis by protecting the intestinal barrier in rats. Scientific Reports. 2017;7(1):6927

[61] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, Puri P, Sterling RK, et al. Randomised clinical trial: Lactobacillus GG modulates gut microbiome, metabolome and endotoxemia in patients with cirrhosis. Alimentary
Liver Pathology

Pharmacology and Therapeutics. 2014;39(10):1113-1125

[62] Kumar M, Verma V, Nagpal R, Kumar A, Gautam SK, Behare PV, et al. Effect of probiotic fermented milk and chlorophyllin on gene expressions and genotoxicity during AFB₁-induced hepatocellular carcinoma. Gene. 2011;490(1-2):54-59

[63] El-Nezami HS, Polychronaki NN, Ma J, Zhu H, Ling W, Salminen EK, et al. Probiotic supplementation reduces a biomarker for increased risk of liver cancer in young men from southern China. The American Journal of Clinical Nutrition. 2006;83(5):1199-1203

[64] Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: Effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatology (Baltimore, Md.). 2004;39(5):1441-1449

[65] Bakhshimoghaddam F, Shateri K, Sina M, Hashemian M, Alizadeh M. Daily consumption of synbiotic yogurt decreases liver steatosis in patients with nonalcoholic fatty liver disease: A randomized controlled clinical trial. The Journal of Nutrition. 2018;148(8):1276-1284

[66] Chiu W-C, Huang Y-L, Chen Y-L, Peng H-C, Liao W-H, Chuang H-L, et al. Synbiotics reduce ethanol-induced hepatic steatosis and inflammation by improving intestinal permeability and microbiota in rats. Food and Function. 2015;6(5):1692-1700

[67] Abdel-Hamid NI, El-Azab MF, Moustafa YM. Macrolide antibiotics differentially influence human HepG2 cytotoxicity and modulate intrinsic/extrinsic apoptotic pathways in rat hepatocellular carcinoma model. Naunyn-Schmiedeberg’s Archives of Pharmacology. 2017;390(4):379-395

[68] Sidhu GS, Go A, Attar BM, Mutneja HR, Arora S, Patel SA. Rifaximin versus norfloxacin for prevention of spontaneous bacterial peritonitis: A systematic review. BMJ Open Gastroenterology. 2017;4(1):e000154

[69] Moreau R, Elkrief L, Bureau C, Perarnau J-M, Thévenot T, Saliba F, et al. Effects of long-term Norfloxacin therapy in patients with advanced cirrhosis. Gastroenterology. 2018;155(6):1816-1827.e9

[70] Gómez-Hurtado I, Gimenez P, García I, Zapater P, Francés R, González-Navajas JM, et al. Norfloxacin is more effective than Rifaximin in avoiding bacterial translocation in an animal model of cirrhosis. Liver International. 2018;38(2):295-302

[71] Koo HL, DuPont HL. Rifaximin: A unique gastrointestinal-selective antibiotic for enteric diseases. Current Opinion in Gastroenterology. 2010;26(1):17-25

[72] Patidar KR, Bajaj JS. Antibiotics for the treatment of hepatic encephalopathy. Metabolic Brain Disease. 2013;28(2):307-312

[73] Vlachogiannakos J, Saveriadis AS, Viazis N, Theodoropoulos I, Foudoulis K, Manolakopoulos S, et al. Intestinal decontamination improves liver haemodynamics in patients with alcohol-related decompensated cirrhosis. Alimentary Pharmacology and Therapeutics 2009;29(9):992-9

[74] Vlachogiannakos J, Viazis N, Vasiadoulou P, Vafiadis I, Karamanolis DG, Ladas SD. Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. Journal of Gastroenterology and Hepatology. 2013;28(3):450-455
[75] van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. The New England Journal of Medicine. 2013;368(5):407-415

[76] Bastard QL, Ward T, Sidiropoulos D, Hillmann BM, Chun CL, Sadowsky MJ, et al. Fecal microbiota transplantation reverses antibiotic and chemotherapy-induced gut dysbiosis in mice. *Scientific Reports*. 2018;8(1):6219

[77] Wang W-W, Zhang Y, Huang X-B, You N, Zheng L, Li J. Fecal microbiota transplantation prevents hepatic encephalopathy in rats with carbon tetrachloride-induced acute hepatic dysfunction. *World Journal of Gastroenterology*. 2017;23(38):6983-6994

[78] Bajaj JS, Kassam Z, Fagan A, Gavis EA, Liu E, Cox IJ, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology* (Baltimore, Md.). 2017;66(6):1727-1738

[79] Fecal Microbiota Transplantation for Decompensated Cirrhosis—Full Text View—ClinicalTrials.gov. 2018. Available from: https://clinicaltrials.gov/ct2/show/NCT03014505

[80] Fecal Microbiota Transplantation (FMT) in Nonalcoholic Steatohepatitis (NASH). A Pilot Study—Full Text View—ClinicalTrials.gov. 2018. Available from: https://clinicaltrials.gov/ct2/show/NCT02469272

[81] Vrieze A, Van Nood E, Holleman P, Salojärvi J, Kootte RS, Bartelsman JFW, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*. 2012;143(4):913-916.e7

[82] Philips CA, Pande A, Shasthy SM, Jamwal KD, Khillan V, Chandel SS, et al. Healthy donor fecal microbiota transplantation in steroid-ineligible severe alcoholic hepatitis: A pilot study. *Clinical Gastroenterology and Hepatology*. 2017;15(4):600-602

[83] Schwartz M, Gluck M, Koon S. Norovirus gastroenteritis after fecal microbiota transplantation for treatment of *Clostridium difficile* infection despite asymptomatic donors and lack of sick contacts. *The American Journal of Gastroenterology*. 2013;108(8):1367

[84] Gupta S, Allen-Vercoe E, Petrof EO. Fecal microbiota transplantation: In perspective. *Therapeutic Advances in Gastroenterology*. 2016;9(2):229

[85] Seki E, De Minicis S, Österreich Ch, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-β signaling and hepatic fibrosis. *Nature Medicine*. 2007;13(11):1324-1332

[86] Domingues MM, Inácio RG, Raimundo JM, Martins M, Castanho MARB, Santos NC. Biophysical characterization of polymyxin B interaction with LPS aggregates and membrane model systems. *Biopolymers*. 2012;98(4):338-344

[87] Piazza M, Rossini C, Della Fiorentina S, Pozzi C, Comelli F, Bettoni I, et al. Glycolipids and benzylammonium lipids as novel antiseptis agents: Synthesis and biological characterization. *Journal of Medicinal Chemistry*. 2009;52(4):1209-1213

[88] Barochia A, Solomon S, Cui X, Natanson C, Eichaker PQ. Eritoran tetrasodium (E5564) treatment for sepsis: Review of preclinical and clinical studies. *Expert Opinion on Drug Metabolism and Toxicology*. 2011;7(4):479-494
Liver Pathology

Matsunaga N, Tsuchimori N, Matsumoto T, li M. TAK-242 (resatorvid), a small-molecule inhibitor of toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. Molecular Pharmacology. 2011;79(1):34-41

Patra MC, Choi S. Recent progress in the development of toll-like receptor (TLR) antagonists. Expert Opinion on Therapeutic Patents. 2016;26(6):719-730

Oya S, Yokoyama Y, Kokuryo T, Uno M, Yamauchi K, Nagino M. Inhibition of toll-like receptor 4 suppresses liver injury induced by biliary obstruction and subsequent intraportal lipopolysaccharide injection. The American Journal of Physiology-Gastrointestinal and Liver Physiology. 2013;306(3):G244-G252

Salama M, Elgamal M, Abdelaziz A, Ellithy M, Magdy D, Ali L, et al. Toll-like receptor 4 blocker as potential therapy for acetaminophen-induced organ failure in mice. Experimental and Therapeutic Medicine. 2015;10(1):241-246

Nguyen J, Jiao J, Smoot K, Watt GP, Zhao C, Song X, et al. Toll-like receptor 4: A target for chemoprevention of hepatocellular carcinoma in obesity and steatohepatitis. Oncotarget. 2018;9(50):29495-29507

McDonald K-A, Huang H, Tohme S, Loughran P, Ferrero K, Billiar T, et al. Toll-like receptor 4 (TLR4) antagonist eritoran tetrasodium attenuates liver ischemia and reperfusion injury through inhibition of high-mobility group box protein B1 (HMGB1) signaling. Molecular Medicine. 2015;20(1):639-648

Kitazawa T, Tsujimoto T, Kawaratani H, Fukui H. Salvage effect of E5564, toll-like receptor 4 antagonist on d-galactosamine and lipopolysaccharide-induced acute liver failure in rats. Journal of Gastroenterology and Hepatology. 2010;25(5):1009-1012

Kuo W-T, Lee T-C, Yu LC-H. Eritoran suppresses colon cancer by altering a functional balance in toll-like receptors that bind lipopolysaccharide. Cancer Research. 2016;76(16):4684-4695

Opal SM,Laterre P-F, Francois B, LaRosa SP, Angus DC, Mira J-P, et al. Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: The ACCESS randomized trial. Journal of the American Medical Association. 2013;309(11):1154-1162

Sun L, Dai JJ, Hu WF, Wang J. Expression of toll-like receptors in hepatic cirrhosis and hepatocellular carcinoma. The Genetics and Molecular Research. 2016;15(2). DOI: 10.4238/gmr.15027419

Ma Z, Zhang E, Yang D, Lu M. Contribution of toll-like receptors to the control of hepatitis B virus infection by initiating antiviral innate responses and promoting specific adaptive immune responses. Cellular and molecular immunology. 2015;12(3):273-282

Akira S, Sato S. Toll-like receptors and their signaling mechanisms. Scandinavian Journal of Infectious Diseases. 2003;35(9):555-562

Chen L, Xu Y-Y, Zhou J-M, Wu Y-Y, Zhu Y-Y. TLR3 dsRNA agonist inhibits growth and invasion of HepG2.2.15 HCC cells. Oncology Reports. 2012;28(1):200-206

Akira S, Takeda K. Toll-like receptor signalling. Nature Reviews. Immunology. 2004;4(7):499-511

Mohamed FE, Al-Jehani RM, Minogue SS, Andreola F, Winstanley A, Olde Damink SWM, et al. Effect of toll-like receptor 7 and 9 targeted therapy
to prevent the development of hepatocellular carcinoma. Liver International. 2015;35(3):1063-1076

[104] Zou H, Wang W-K, Liu Y-L, Braddock M, Zheng M-H, Huang D-S. Toll-like receptors in hepatocellular carcinoma: Potential novel targets for pharmacological intervention. Expert Opinion on Therapeutic Targets. 2016;20(9):1127-1135

[105] So EY, Ouchi T. The application of toll like receptors for cancer therapy. International Journal of Biological Sciences. 2010;6(7):675-681

[106] Lopes JAG, Borges-Canha M, Pimentel-Nunes P. Innate immunity and hepatocarcinoma: Can toll-like receptors open the door to oncogenesis? World Journal of Hepatology. 2016;8(3):162-182

[107] Kalaitzakis E. Gastrointestinal dysfunction in liver cirrhosis. World Journal of Gastroenterology. 2014;20(40):14686-14695

[108] Pardo A, Bartoli R, Lorenzo-Zúñiga V, Planas R, Viñado B, Riba J, et al. Effect of cisapride on intestinal bacterial overgrowth and bacterial translocation in cirrhosis. Hepatology. 2000;31(4):858-863

[109] Madrid AM, Hurtado C, Venegas M, Cumsille F, Defilippi C. Long-term treatment with cisapride and antibiotics in liver cirrhosis: Effect on small intestinal motility, bacterial overgrowth, and liver function. The American Journal of Gastroenterology. 2001;96(4):1251-1255

[110] Sandhu BS, Gupta R, Sharma J, Singh J, Murthy NS, Sarin SK. Norfloxacin and cisapride combination decreases the incidence of spontaneous bacterial peritonitis in cirrhotic ascites. Journal of Gastroenterology and Hepatology. 2005;20(4):599-605

[111] Kassahun WT, Guenl B, Ungemach FR, Jonas S, Abraham G. Expression and functional coupling of liver β2 - adrenoceptors in the human hepatocellular carcinoma. Pharmacology. 2012;89(5-6):313-320

[112] Thiele M, Albillos A, Abazi R, Wiest R, Gluud LL, Krag A. Non-selective beta-blockers may reduce risk of hepatocellular carcinoma: A meta-analysis of randomized trials. Liver International. 2015;35(8):2009-2016

[113] Dietrich P, Moleda L, Kees F, Müller M, Straub RH, Hellerbrand C, et al. Dysbalance in sympathetic neurotransmitter release and action in cirrhotic rats: Impact of exogenous neuropeptide Y. Journal of Hepatology. 2013;58(2):254-261

[114] Leithead JA, Rajoriiya N, Tehami N, Hodson J, Gunson BK, Tripathi D, et al. Non-selective β-blockers are associated with improved survival in patients with ascites listed for liver transplantation. Gut. 2015;64(7):1111-1119

[115] Reiberger T, Ferlitsch A, Payer BA, Mandorfer M, Heinisch BB, Hayden H, et al. Non-selective betablocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. Journal of Hepatology. 2013;58(5):911-921

[116] Wang F, Liu H, Wang F, Xu R, Wang P, Tang F, et al. Propranolol suppresses the proliferation and induces the apoptosis of liver cancer cells. Molecular Medicine Reports. 2018;17(4):5213-5221

[117] Nkontchou G, Aout M, Mahmoudi A, Roulot D, Bourcier V, Grando-Lemaire V, et al. Effect of long-term propranolol treatment on hepatocellular carcinoma incidence in patients with HCV-associated cirrhosis. Cancer Prevention Research (Philadelphia, Pa.). 2012;5(8):1007-1014

[118] Chang P-Y, Chung C-H, Chang W-C, Lin C-S, Lin H-H, Dai M-S,
et al. The effect of propranolol on the prognosis of hepatocellular carcinoma: A nationwide population-based study. PLoS One. 2019;14(5):e0216828

[119] Larrosa M, Yañez-Gascón MJ, Selma MV, González-Sarrías A, Toti S, Cerón JJ, et al. Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. Journal of Agricultural and Food Chemistry. 2009;57(6):2211-2220

[120] Gao F, Deng G, Liu W, Zhou K, Li M. Resveratrol suppresses human hepatocellular carcinoma via targeting HGF-c-met signaling pathway. Oncology Reports. 2017;37(2):1203-1211

[121] Bishayee A, Dhir N. Resveratrol-mediated chemoprevention of diethylnitrosamine-initiated hepatocarcinogenesis: Inhibition of cell proliferation and induction of apoptosis. Chemico-Biological Interactions. 2009;179(2):131-144

[122] Vairappan B, Sundhar M, Srinivas BH. Resveratrol restores neuronal tight junction proteins through correction of ammonia and inflammation in CCL4-induced cirrhotic mice. Molecular Neurobiology. 2019;56(7):4718-4729

[123] Lee RH, Cho JH, Jeon Y-J, Bang W, Cho J-J, Choi N-J, et al. Quercetin induces antiproliferative activity against human hepatocellular carcinoma (HepG2) cells by suppressing specificity protein 1 (Sp1). Drug Development Research. 2015;76(1):9-16

[124] Ji Y, Li L, Ma Y-X, Li W-T, Li L, Zhu H-Z, et al. Quercetin inhibits growth of hepatocellular carcinoma by apoptosis induction in part via autophagy stimulation in mice. The Journal of Nutritional Biochemistry. 2019;69:108-119

[125] Teng C-F, Yu C-H, Chang H-Y, Hsieh W-C, Wu T-H, Lin J-H, et al. Chemopreventive effect of phytosomal curcumin on hepatitis B virus-related hepatocellular carcinoma in a transgenic mouse model. Scientific Reports. 2019;9(1):10338

[126] Tang Y. Curcumin targets multiple pathways to halt hepatic stellate cell activation: Updated mechanisms in vitro and in vivo. Digestive Diseases and Sciences. 2015;60(6):1554-1564

[127] Wang L, Phan DD, Zhang J, Ong P-S, Thuya WL, Soo R, et al. Anticancer properties of nimbolide and pharmacokinetic considerations to accelerate its development. Oncotarget. 2016;7(28):44790-44802

[128] Ravitha K, Vidya Priyadarshini R, Anitha P, Ramalingam K, Sakthivel R, Purushothaman G, et al. Nimbolide, a neem limonoid abrogates canonical NF-κB and Wnt signaling to induce caspase-dependent apoptosis in human hepatocarcinoma (HepG2) cells. European Journal of Pharmacology. 2012;681(1-3):6-14

[129] Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. Journal of Hepatology. 2014;60(1):197-209

[130] Toffanin S, Cornella H, Harrington A, Llovet JM. HCC is promoted by bacterial translocation and TLR-4 signaling: A new paradigm for chemoprevention and management. Hepatology. 2012;56(5):1998-2000

[131] Verbeke L, Farre R, Verbinnen B, Covens K, Vanuytsel T, Verhaegen J, et al. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. The American Journal of Pathology. 2015;185(2):409-419

[132] Piglionica M, Cariello M, Moschetta A. The gut-liver axis in hepatocarcinoma: A focus on the nuclear receptor FXR and the enterokine FGF19.
Microbiota, Inflammation, and Gut Barrier Dysfunction in HCC
DOI: http://dx.doi.org/10.5772/intechopen.89578

Current Opinion in Pharmacology. 2018;43:93-98

[133] Degirolamo C, Modica S, Vacca M, Tullio GD, Morgano A, D’Orazio A, et al. Prevention of spontaneous hepatocarcinogenesis in farnesoid X receptor–null mice by intestinal–specific farnesoid X receptor reactivation. Hepatology. 2015;61(1):161-170

[134] Úbeda M, Lario M, Muñoz L, Borrero M-J, Rodríguez-Serrano M, Sánchez-Díaz A-M, et al. Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. Journal of Hepatology. 2016;64(5):1049-1057

[135] Mookerjee RP, Mehta G, Balasubramaniyan V, Mohamed FEZ, Davies N, Sharma V, et al. Hepatic dimethylarginine-dimethylaminohydrolase1 is reduced in cirrhosis and is a target for therapy in portal hypertension. Journal of Hepatology. 2015;62(2):325-331

[136] Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. Journal of Hepatology. 2000;32(5):742-747

[137] Ma TY, Iwamoto GK, Hoa NT, Akotia V, Pedram A, Boivin MA, et al. TNF-α-induced increase in intestinal epithelial tight junction permeability requires NF-κB activation. The American Journal of Physiology-Gastrointestinal and Liver Physiology. 2004;286(3):G367-G376

[138] Mankertz J, Amasheh M, Krug SM, Fromm A, Amasheh S, Hillenbrand B, et al. TNFα up-regulates claudin-2 expression in epithelial HT-29/B6 cells via phosphatidylinositol-3-kinase signaling. Cell and Tissue Research. 2009;336(1):67-77

[139] Balasubramanian V, Mehta G, Jones H, Sharma V, Davies NA, Jalan R, et al. Post-transcriptional regulation of hepatic DDAH1 with TNF blockade leads to improved eNOS function and reduced portal pressure in cirrhotic rats. Scientific Reports. 2017;7(1):17900

[140] Osanai M, Nishikiori N, Murata M, Chiba H, Kojima T, Sawada N. Cellular retinoic acid bioavailability determines epithelial integrity: Role of retinoic acid receptor α agonists in colitis. Molecular Pharmacology. 2007;71(1):250-258

[141] Ukena SN, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, et al. Probiotic Escherichia coli Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. PLoS One. 2007;2(12):e1308

[142] Nunes C, Freitas V, Laranjinha J. Modulation of tight junctions in intestinal epithelial cells by red wine polyphenols. Free Radical Biology and Medicine. 2018;120:S149

[143] Mayangsari Y, Suzuki T. Resveratrol ameliorates intestinal barrier defects and inflammation in colitic mice and intestinal cells. Journal of Agricultural and Food Chemistry. 2018;66(48):12666-12674

[144] Chen M, Hou P, Zhou M, Ren Q, Wang X, Huang L, et al. Resveratrol attenuates high-fat diet-induced non-alcoholic steatohepatitis by maintaining gut barrier integrity and inhibiting gut inflammation through regulation of the endocannabinoid system. Clinical Nutrition. 2019. DOI: 10.1016/j.clnu.2019.05.020

[145] Wang J, Ghosh SS, Ghosh S. Curcumin improves intestinal barrier function: Modulation of intracellular signaling, and organization of tight junctions. The American Journal of Physiology-Cell Physiology. 2017;312(4):C438-C445