Hepatocellular Carcinoma Management's Gut Microbiota

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Liver cancer, predominantly hepatocellular carcinoma (HCC), is the third leading cause of cancer-related deaths worldwide. Emerging data highlight the importance of gut homeostasis in the pathogenesis of HCC. Clinical and translational studies revealed the patterns of dysbiosis in HCC patients and their potential role for HCC diagnosis. Research on underlying mechanisms of dysbiosis in HCC development pointed out the direction for improving the treatment and prevention. Despite missing clinical studies, animal models showed that modulation of the gut microbiota by probiotics may become a new way to treat or prevent HCC development.

hepatocellular carcinoma dysbiosis microbiota probiotics

1. Introduction

Liver cancer, predominantly hepatocellular carcinoma (HCC), is a substantial health burden worldwide. In 2017, with an estimation of 803,400 (753,100 to 856,700) cases, the age-standardized years lived with disability (YLDs) rate increased by 8.1% when compared with that in 2007 [1]. With a new death of 830,180 cases in 2020, liver cancer represents the third (8.3%) leading cause of cancer-related deaths worldwide [2]. Due to the low screening rate in high-risk populations and inadequate sensitivity of the present diagnostic technology (imaging and serum alpha-fetoprotein [AFP] quantification), HCC is usually diagnosed at the late stages, leading to low accessibility of curative therapy and high mortality. Early diagnosis and better prevention and treatment are the goals pursued by doctors and patients together. In terms of diagnostic technology, sensitive and specific biomarkers for early diagnosis of HCC are still lacking. As for prevention and treatment of HCC, apart from etiological treatment of HCC, such as anti-hepatitis B virus (HBV) in HBV-HCC, extra measures are in great need.

Approximately 4×10^{13} microbial cells spanning ~3×10^{3} species inhabit the human body. The vast majority (97%) of them are bacteria in the colon, and the remaining include extracolonic bacteria and Archaea and eukaryotes such as fungi [3][4]. Gut and liver are closely related, not only anatomically but also functionally. The liver receives blood from the gut through the portal vein, while the gut receives bile from the liver through the bile duct. Blood from the gut brings nutrition, microbial metabolite, and microbe-associated molecular patterns (MAMPs). MAMPs may elicit inflammatory responses by activating pattern recognition receptors (PRRs) in the liver, contributing to the progression of liver diseases and development of HCC. Bile acids, important components in bile, are synthesized from cholesterol in the liver, then metabolized by gut bacteria. They can shape the composition and function of the intestinal microbiota. Mutual interplay of bile acids and gut microbiota regulates many physiological processes [5][6]. Emerging data highlight the importance of gut homeostasis in the pathogenesis of HCC. Clinical and translational

studies revealed the patterns of dysbiosis in HCC patients, indicating the diagnostic value of the dysbiosis in early diagnosis of HCC. Mechanism research demonstrates that gut microbiota plays an important role in liver tumorigenesis, which suggests the possibility of preventing and treating HCC by modulating gut microbiota.

Although the relationship between gut bacterial microbiota and fibrosis/liver cirrhosis is of importance to understand between gut bacterial microbiota and HCC, previous reviews have discussed this topic in detail [7][8]. Therefore, in the present review, we only focus on the alteration of gut bacterial microbiota in HCC patients and the underlying mechanisms of dysbiosis in HCC development. Meanwhile, diagnostic value of gut dysbiosis and therapeutic potential by targeting gut dysbiosis in HCC were discussed.

2. Gut Microbiota Changes in HCC Patients

Gut bacteria dysbiosis in HCC patients has been reported in many countries and regions recently (**Table 1**). Both stool and blood samples possess the value of diagnosing and assessing dysbiosis in HCC patients.

Table 1. Gut bacteria dysbiosis in HCC patients.

Patients/Control	Increased Microbiota	Decreased Microbiota	Reference
cirrhotic HCC/cirrhosis	Escherichia coli.		[9]
HCC/NC	Escherichia coli., Enterococcus	Bifidobacterium, Lactobacillus	[10]
HCC/cirrhosis HCC/cirrhosis HCC/control	Actinobacteria Gemmiger, Parabacteroides, Paraprevotella, Clostridium_XVIII Klebsiella and Haemophilus	Ruminococcus, Oscillibacter, Faecalibacterium, Clostridium IV, and Coprococcus	[11]
HCC/NC NBNC-HCC/NC HBV-HCC/NC NBNC-HCC/NC HBV-HCC/NC	Lactobacillus, Bifidobacterium Proteobacteria Escherichia-Shigella, Enterococcus Faecalibacterium, Ruminococcus, Ruminoclostridium	Firmicutes Proteobacteria Faecalibacterium, Ruminococcus, Ruminoclostridium	[12]
HCC/NC	Proteobacteria (Enterobacte, Haemophilus)		[13]
NAFLD-HCC/NAFLD-cirrhosis	Bacteroides, Ruminococcaceae	Bifidobacterium	[14]
cirrhotic HCC/cirrhosis	Erysipelotrichaceae Odoribacter, Butyricimonas	Leuconostocaceae Fusobacterium, Lachnospiraceae	[15]
NAFLD-HCC/NAFLD-	Enterobacteriaceae Bacteroides caecimuris, Veillonella	Eubacteriaceae	[16]

Patients/Control	Increased Microbiota	Decreased Microbiota	Reference
cirrhosis	parvula, Clostridium bolteae, and Ruminococcus gnavus		
HCC/NC	Proteobacteria Staphylococcus, Acinetobacter, Klebsiella, Trabulsiella	Pseudomonas	[17]

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NBNC, non-hepatitis B virus non-hepatitis C virus; NC, normal control.

In the early stage, the number of colony-forming units per gram (cfu/g) of wet feces was adopted to analyze the gut bacterial change in HCC patients. Fecal counts of Escherichia coli (E. coli.) increased in 15 cirrhotic HCC patients, when compared to 15 etiology and model for end stage liver disease (MELD) score-matched cirrhosis patients [9]. E. coli. and Enterococcus increased, while Bifidobacterium and Lactobacillus significantly decreased in 20 HCC patients vs. 20 normal controls [10].

3. Mechanism Linking Gut Dysbiosis to HCC

3.1. Mechanisms Other Than Bile Acids Dysregulation

More than a decade ago, a mouse model tested the hypothesis that specific intestinal bacteria promote liver cancer in a chemical and viral transgenic mouse model [18]. Underlying mechanisms linking gut dysbiosis to HCC attracted the attention of scientists in the field. So far, leaky gut (a failing gut barrier), bile acids dysregulation, bacterial translocation, endotoxemia and subsequent promotion of liver inflammation, fibrosis, proliferation, and immune suppression have been identified to contribute to the development of HCC in the setting of chronic liver diseases (Figure 1). The concept of the gut–liver axis, bidirectional relationship between the gut and its microbiota, provides the possibility of preventing and treating HCC by targeting gut and its microbiota [16].

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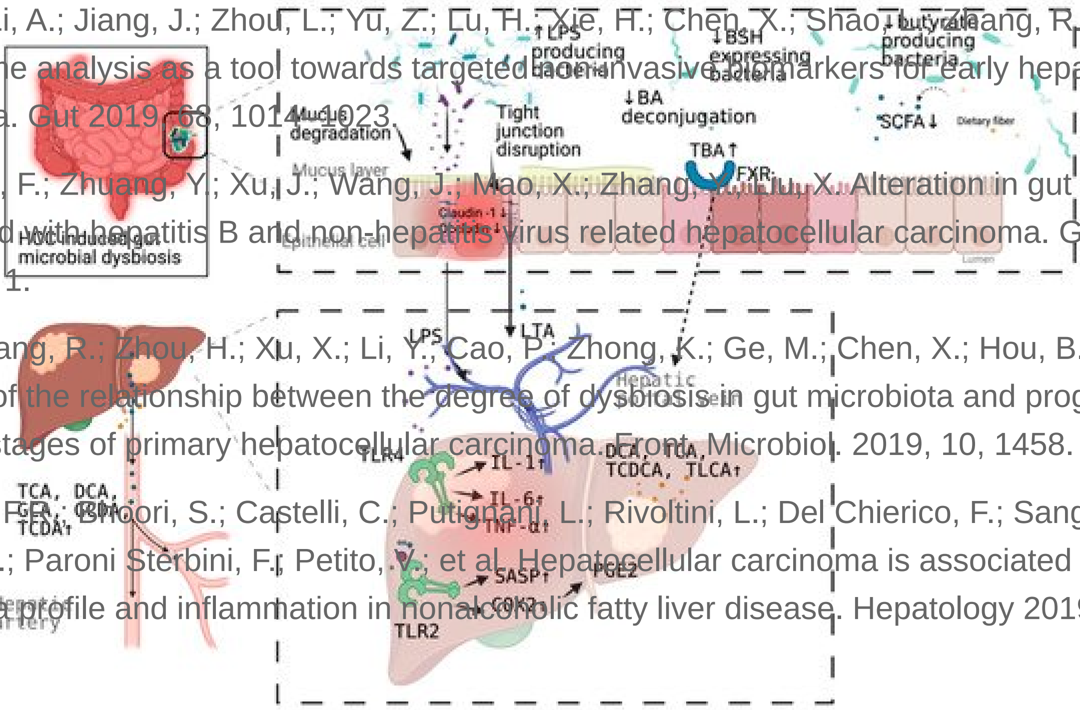

Figure 1. Schematic representation of the mechanism of the promotion and progresssion of HCC by gut microbiota. BA, bile acid; TBA, total bile acid; LPS, Lipopolysaccharides; BSH, bile salt hydrolase; LTA, Lipoteichoic acid; SCFA, short chain fatty acid; DCA, deoxycholic acid; TCA, taurocholic acid; GCA, glycocholic acid; GCDA, glycochenodeoxycholic acid; TCDA, taurochenodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid (TLCA); PGE2, prostaglandin E2; COX2, cyclooxygenase-2; SASP, senescence associated secretory phenotype; TLR, toll-like receptor, FXR, farnesoid X receptor. Figure created with BioRender.com (San Francisco, CA, USA).

3.2. Bile Acids Dysregulation in HCC

Emerging evidence indicates the association between bile acid–bacterial microbiota crosstalk and the development of HCC. Bile acids, synthesized from cholesterol in the liver, are metabolized by gut bacteria and subsequently sensed by two major sensing receptors, farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (GPBAR1) (transmembrane G-protein-coupled receptor 5, (TGR5)). Bile acids in a variety of species of amphipathic acidic steroids and have both protective and pathogenic roles in liver diseases. Hydrophilic bile acids, such as ursodeoxycholic acid (UDCA), and its taurine-conjugated form tauroursodeoxycholic acid (TUDCA), show profound cytoprotective properties [5], while excessive production of hydrophobic bile acids is cytotoxic and promotes hepatocyte injury [19].

FXR, a nuclear receptor, activation, is involved in regulating the antibacterial defense in the small intestine [20], preventing chemically-induced intestinal inflammation [21] and modulating liver regeneration [22]. FXR and epidermal growth factor receptor (EGFR) signaling is involved in cholangiocellular proliferation by bile acids [23]. In addition, FXR and small heterodimer partner (SHP) can regulate protein N-glycan modifications in the liver [24]. TGR5, a plasma membrane receptor, is expressed in sinusoidal endothelial cells, Kupffer cells, cholangiocytes and

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activated hepatic stellate cells, modulating microbial sensing, and regeneration, biliary secretion, and gallbladder filling [25].

Multiple genera of the gut microbiota are involved in bile acid metabolism, including Bacteroides, Clostridium, Lactobacillus, Bifidobacterium, and Listeria in bile acid deconjugation; Bacteroides, Eubacterium, Clostridium, Escherichia, Egghertella, Eubacterium, Peptostreptococcus, and Ruminococcus in oxidation and epimerization of hydroxyl groups; and Clostridium and Eubacterium in 7-dehydroxylation [26]. Dysbiosis of gut microbiota can affect bile acids homeostasis theoretically. Indeed, clinical studies and animal models demonstrated the dysbacteriosis of some of these above-mentioned bacteria, dysregulation of bile acids in multiple samples (liver tissue, serum, feces, and urine), and the association between the above two abnormalities [27][28][29][30][31][32][33].

4. Microbial Dysbiosis in HCC Diagnosis

Both fecal and circulating microbial dysbiosis have the potential value in diagnosis of HCC (**Table 2**).

Table 2. Diagnostic value of microbiota and metabolites in HCC.

Microbiota [1]	Patients/Control	AUC	95% CI	Sensitivity	Specificity	Reference
Escherichia coli	HCC/cirrhosis	0.742	0.564–0.920	66.7%	73.3%	[9]
30 OTUs markers	HCC/non-HCC	0.806	0.745–0.868	-	-	[11]
Enterococcus	Cirrhotic HCC/cirrhosis	0.868	-NA	95.8%	69.2%	[34]
Enterococcus	Non-cirrhotic HCC/cirrhosis	0.899	NA	100%	78.3%	
Limnobacter	Non-cirrhotic HCC/cirrhosis	0.858	NA	62.5%	91.3%	
Phyllobacterium	Non-cirrhotic HCC/cirrhosis	0.868	NA	75.0%	91.3%	
5 OTUs markers (serum)	HCC/control	0.879	NA	72.9%	85.0%	[17]
Phe-Trp + GCA (serum)	HCC/cirrhosis	0.807	0.753–0.861	92.1%	52.8%	[28]
Phe-Trp + GCA +AFP (serum)	HCC/cirrhosis	0.826	0.774–0.877	77.9%	76.4%	

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Microbiota [1]	Patients/Control	AUC	95% CI	Sensitivity	Specificity	Reference
CDCA + LPC 20:5 + succinyladenosine + uridine (serum)	HCC/cirrhosis	0.938	-	93.3%	86.7%	[29]

[1] feces sample is used if not specified. AFP, alpha-fetoprotein; AUC, area under the curve; CDCA, chenodeoxycholic acid; CI, confidence interval; GCA, glycocholate; HCC, hepatocellular carcinoma; LPC, lysophosphatidylcholine; NA, not available; OTU, operational taxonomic unit; Phe-Trp, phenylalanyl-tryptophan. - NA: failed to find out the 95%CI from the paper.

5. Targeting Microbial Dysbiosis in HCC Treatment and Prevention

The clear role microbial dysbiosis in the development of HCC, offers multiple pathways and targets for HCC treatment and prevention theoretically.

For example, PGE2 and its receptor may be novel therapeutic targets for noncirrhotic NASH-associated HCC [35]. Blocking DCA production or reducing gut bacteria efficiently prevents HCC development in obese mice [36]. Gut sterilization can prevent HCC in a mouse model, suggesting that the intestinal microbiota and TLR4 represent therapeutic targets for HCC prevention in advanced liver disease. TLR antagonists can block the propagation of downstream cytokine release [37][38]. Reduction of HCC development by modulating gut microbiota was showed in animal models [37][39][36]. Antibiotics can be used to eliminate disease-promoting bacteria and decrease release of MAMPs and metabolites from a leaky gut. FXR agonists can modulate various downstream immune-related pathways.

Fecal microbiota transplantation (FMT) is the transfer of stool from a healthy donor into the gastrointestinal tract, aiming to gain a therapeutic benefit by changing or normalizing the recipient's gut microbiota directly. FMT has been approved for treating recurrent and refractory Clostridium difficile infection (CDI) by the United States Food and Drug Administration. In the field of treating liver diseases, FMT can improve neurocognitive function and reduce the readmission of patients with hepatic encephalopathy (HE), despite the small scale of study and absence of long-term follow-up [40]. What is more gratifying is that microbiota originating from donors was found in human recipients one year after FMT [40]. However, clinical study regarding FMT in the treatment and prevention of HCC is still missing.

Probiotics can be used to restore normal microbiota composition, suppress the growth of pathogenic microorganisms, and interact with the mucosal system, which affects the systemic immunity. Administration of a commercial probiotic compound VSL#3 (VSL Pharmaceuticals, Fort Lauderdale, FL, USA) dramatically suppressed penicillin-increased HCC formation in rats [41]. A mouse model demonstrated that the efficacy of a novel probiotic mixture (Prohep) slows down the tumor growth significantly and reduces the tumor size and weight by 40% compared with the control [42]. Notably, Prohep limits tumor growth by reducing angiogenesis, and so forth lead to

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hypoxia-induced cell death in tumor. This indicates that combining Prohep with drugs of other mechanisms, such as immunotherapy, may play a synergistic therapeutic effect.

Given the BA-bacterial microbiota crosstalk in the development of HCC, restoring bile acids homeostasis by modulating gut microbiota or targeting directly bile acids may be effective strategies on preventing and treating HCC. Treatment with antibiotics dramatically reduced the accumulation of secondary bile acids and significantly suppressed tumor developments in the HFD mouse model [43]. An obese mouse model showed that blocking DCA production or reducing gut bacteria by oral antibiotic caused a marked reduction of HCC development in obese mice [36]. Treatment with antibiotics significantly attenuated liver pathology and suppressed tumor development in a new class NASH-inducing HFD mouse model [43]. In addition, oral administration of cholestyramine, bile acid sequestrant to enhance intestinal excretion of hydrophobic bile acids, significantly prevent HCC in a mouse model [33]. Depleting Gram-positive bacteria by vancomycin treatment can induce hepatic NKT cell accumulation and suppress liver tumor growth in multiple mouse models, while feeding secondary bile acids or colonization of bile acid-metabolizing bacteria can reverse both NKT cell accumulation and inhibition of liver tumor growth in mice [44].

Together, targeting microbial dysbiosis to treat and prevent HCC seems promising. However, there is no clinical data in this regard currently.