Review Article

Gut microbiome in liver disease

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Abstract: A diverse community of microbes resides in the human intestine to maintain the homeostasis of metabolic status and immune response in the host. Dysbiosis of intestinal flora was noted in a wide range of disease, including obesity, autoimmune diseases, diabetes, carcinogenesis and liver diseases. Here we review the current knowledge of the gut flora under liver pathogenesis, including non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcohol-induced liver disease and hepatitis B virus (HBV)-induced liver cirrhosis. Liver malfunction leads to heavily altered genetic composition of gut microbial, while distinct bacterial species are present during liver pathogenesis, and the abundance of bacteria is correlated with grade of liver disease, suggesting the key role of "gut-liver axis". Further, we also discuss the high throughput sequencing technology with status of art as well as the future challenges in this emerging field of gut microbiome.

Keywords: Gut microbiome; liver disease; hepatitis B virus (HBV), liver cirrhosis

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Introduction

The human gut microbiota functions as a fundamental organ in maintenance of host homeostasis via gene modulation and microbe restructuring. It is an essential component for induction and control of metabolic homeostasis and host immune response. The intestinal microbial dysbiosis has been associated with various chronic disease, including obesity (1,2), autoimmune diseases (3), diabetes (4,5), carcinogenesis (6,7) and liver disease (8,9). Recent studies highlighted that liver diseases in human is associated with compositional changes in intestinal microbial community. In this review, we focus on current knowledge of intestinal microbial community in relation to gut-liver axis. Characterizing the nature of gut dysbiosis and hepatic immune response to gut-derived microbial factors is potentially relevant to the development of new therapies to treat chronic liver disease. We also discuss challenges and future directions in this emerging field.

The essential role of gut-liver axis in hepatic disease

At birth, the human gut consists of about 100 species of bacteria. Within the first 3 years, the gut bacteria further populate to around 1,000 species. Among them, *Bacteroidetes* and *Firmicutes* are two dominant groups, accounting for >90% of the total gut microbiota. Besides *Bacteroidetes* and *Firmicutes*, the other four main phyla in the intestinal microbeta are *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia*. As the anatomy of the liver closely interact with the gut, their components and metabolites of gut-derived bacteria, as well as nutrients and other signals are delivered to the liver via the portal circulation; while bile produced by the liver flows to the gut and influence the abundance and destruction of various organism in the latter's lumen, which is refer to "gut-liver axis". It was considered that the interaction between commensal and the liver significantly shapes hepatic innate and adaptive immunity (10,11) and determines the outcomes of liver pathophysiology.

Stimulation or inhibition of liver immunity might depend on the type and strength of the specific microbial signals to which hepatic cells have been exposed. Gut microbiota is able to act on the liver via both immune-tolerating and immune-stimulatory pathways. It was reported that upon exposure to low levels of Gram-negative bacteria-derived lipopolysaccharides (LPSs), liver sinusoid endothelial cells (LSECs), hepatic antigen-presenting cells, facilitates the secretion of IL-10 (12,13), resulting in liver tolerance. Nevertheless, liver tolerance can be overridden by regulating innate immunity pathway in the liver through gut microbiota. For example, the maturation of gut microbiota in the adult mice was able to stimulate liver immunity, resulting in rapid hepatitis B virus (HBV) clearance, while young mice without established gut bacteria failed to do so (14). Mouse Kupffer cells (KC) pretreated with bacterial CpG-DNA exhibited significantly elevated IL-6 production for subsequent LPS challenge (15). CpG-DNA/TLR9 pathway enabled HBV-specific cytotoxic $CD8^+$ T cell expansion which further eradicated HBV-infected hepatocytes during chronic infection (16). In response to microbial TLR ligands, such as LPS and CpG-DNA, KCs and LSECs can suppress Treg activity and promote $CD4^+$ T cell proliferation (17).

Besides host liver immunity, the microbial metabolites produced in dysbiotic intestinal environment play a significant role in liver pathogenesis (18). The serum lipid levels of phospholipids, free fatty acids and polyunsaturated fatty acids have significant correlation with specific fecal flora in liver cirrhosis. Blood metabolic molecules, including cytokines, amino acids and vitamins, are correlated with gut microbiota in probiotics-treated liver cirrhosis patients (19). Further, a compromised barrier induces inflammatory mediators flowing into systemic circulation and hence enhance the immune tolerance.

Non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH)

NAFLD is a comprehensive hepatic manifestation of the metabolic abnormalities, including fat deposition in hepatocytes without any inflammation or necrosis as well as NASH syndrome. It is estimated that 30% of NAFLD is NASH, a symptom of liver inflammation, fibrosis and cirrhosis. Animal studies suggested a direct link between the intestinal microbiota and fat deposition in the liver. Bacterial enzyme can contribute to the digestion of dietary polysaccharides which is otherwise indigestible and obtains calories (20). Enteric bacteria are able to suppress the synthesis of fasting-induced adipocyte factor (Fiaf) and secretion from the small intestine, leading to an accumulation of triglycerides in the liver (2,21). Further, liver inflammatory events could derive from pro-inflammatory reactions stimulated by bacterial products (22). The malfunction of murine inflammasomes results in an increase in *Bacteroidetes* along with reduced *Firmicutes*, leading to increased intestinal permeability and severe hepatic steatosis and inflammation (23). Microbiota from patients with NAFLD and NASH tends to harbor a declined abundance of *Ruminococcaceae* compared to healthy donors (8). Age also affect the constitution of gut microbial. Among children with obesity and NASH, *Escherichia* is the only large population of bacteria in the intestine that is significantly disproportionate (8). In contrast, adult NASH patients had an elevated proportion of *Clostridium coccoides* than NAFLD patients (9). However, other studies comparing specific bacterial compositions of adult patients with NAFLD versus those with NASH showed variable findings (8,9,24). It could be due to a small number of subjects included in the analysis, which were significantly varied in demographic factors and different status of liver pathogenesis.

Alcohol-induced liver disease

Alcohol induced liver cirrhosis (ALC) is a major leading cause of morbidity and mortality, especially in Western Countries. From preclinical and clinical studies, patients with chronic alcohol abuse is accompanied by microbiota dysbiosis and elevated intestinal bacterial load. In an ethanol intake mouse model, bacterial overgrowth was observed along the entire gastrointestinal tract; the dysbiosis was found with substantial reductions in probiotic bacteria such as *Lactobacillus*, *Pediococcus*, and *Lactococcus*. Administration of probiotic *Lactobacillus* alleviates symptoms of alcoholic liver disease in animal models (25). Indeed, a similar phenomenon was also noticed in several human studies. Probiotics is able to improve alcohol induced intestinal permeability and liver functional impair in patients (26). Quantitative analyses of bacterial cultures showed that both aerobic and anaerobic bacterial cultures of jejunal aspirates from patients who excessively intake alcohol suffered from bacterial overgrowth (27). Gut bacterial products like endotoxin might be largely responsible for gut inflammation and facilitate the development of alcohol-related liver

injury, including impaired motility of the small intestine (28), reduced bile flow (29), and altered secretion of IgA (29) and antimicrobial molecules (30).

Further, the functional composition of fecal microbiomes was greatly affected by alcoholic cirrhosis. A comprehensive study revealed that 92 genes were uniquely altered in response to alcohol consumption (31). Among them, four genes were found significantly ($P<0.05$) reduced in ALC than health individuals and chronic hepatitis B (CHB) patients, including carbohydrate metabolism gene (phosphogluconate dehydratase) as well as glycan biosynthesis and metabolism genes (beta-N-acetyl-D-hexosaminide N-acetylhexosaminohydrolase and N-acetylmuramoyl-L-alanine amidase). Notably, they all belong to metabolism related gene, suggesting that altered microbiome further modulates metabolic status within host.

HBV-induced liver cirrhosis

The infection of HBV is endemic in many Asian countries including China, which could progress to liver cirrhosis and hepatocellular carcinoma (HCC), affecting 400 million people and lead to more than 0.5 million deaths per year. Two independent studies showed a reduced functional diversity of fecal microbiota in patients with HBV infection induced liver cirrhosis (HBLC) was observed compared to healthy individuals (32,33). On one hand, *Bacteroidetes* consisted of 53% of the normal fecal microbiota but only 4% of fecal microbiota from HBLC patients, whereas *Proteobacteria*, which harbors most of the opportunistic pathogens, made up less than 5% of the normal fecal microbiota but increased to 43% of fecal microbiota from HBLC patients. Such trends observed at the phylum level were also confirmed at the family level. For example, the *Enterobacteriaceae*, *Veillonellaceae*, and *Streptococcaceae* families, usually less than 1% in normal fecal microbiota, were made up 18-39% of HBLC fecal microbiota.

Interestingly, the abundance of specific gut bacteria was associated with the status of liver cirrhosis. For example, remarkable absence of *Bacteroides* is negatively correlated with Child Turcotte Pugh (CTP) score, while high abundance of *Veillonella* genus or *Enterobacteriaceae* family is positively correlated with the CTP scores. Compared to ALC patients, patients with CHBL have similar fecal microbial communities, which is dramatically different with health individuals (34) except a significant increase in the family of *Prevotellaceae* compared to patients with CHBL and healthy donors (34).

In addition to the changes in composition in fecal microbiota, remarkable functional differences between patients' microbiota and the normal fecal microbiota were reported. Fecal microbiota from HBLC patients revealed an enrichment gene associated with material transport and metabolism but an absence of genes for metabolism related to cell cycle, while contained a less abundance of genes related to toxins depredation and nutrient absorption, compared to that of healthy individuals. It is speculated that the microbiota from HBLC patients harbored more fastidious bacteria that required more nutrients in the external environment for survival and growth.

Although HBV-related cirrhosis was the long-term outcome of a chronic HBV infection, the exact role of HBV-infection in dysbiosis of gut flora profile remains unclear. The interplay between gut microbiome and HBV infection in liver pathogenesis remains to be explored. A longitudinal analysis of phylogenetic diversity of fecal microbiota from HBV patients and their functional gene composition at the different stages of HBV infection is urgently required to understand the role of gut microbiome in the development of HBV disease progress. It will also facilitate the diagnosis and development of targeted treatments of HBV patients.

Culture-independent methods to study gut microbiota

While the cultivation and isolation of bacteria is the gold standard for the identification of microbes, the full diversity of gut micro flora had remained largely unexplored. Characterization and analysis of each species within gut microbiome have been greatly enhanced by advances in genomic technologies, especially massive parallel sequencing of 16S rRNA gene segments from bulk DNA extracted from stool sample (35), such as 454/Roche or Illumina pyrosequencing technology. 16S rRNA genes contain nine hypervariable regions containing considerable and diverse sequence among bacteria. Primers are designed based on conserved regions around hypervariable regions to enrich gene segments extracted from the uncultured bacterial mixture. The DNA fragment libraries generated from application are sequenced and compared with the reference 16S rRNA database, allowing identification of bacterial lineages and their abundance of each species within gut flora in a short time at an affordable price.

16S rRNA gene fragments can also be extracted from shotgun metagenomic sequences of stool DNA (36), which not only identify the bacterial species and characterize

diversity of natural bacterial communities, but also probe the functional genes within the microbial communities, while circumventing biased amplification of 16S genes. Essentially, the procedures for a metagenomic analysis is to directly extract DNA from clinical fecal samples and then construct a library of inserts that further to be multiplexed and sequenced. Ten millions of Illumina reads are typically generated to understand the full genetic property of clinical sample. Reads could be classified by searching against established bacterial database to determine the taxonomy. It should be noted that both approaches have their drawbacks. On one hand, as 16S rRNA genes could be unequally amplified, 16rRNA sequencing result could be biased; on the other hand, shotgun metagenomic sequencing technology might not be deep enough to identify the rare species in fecal microorganism.

Challenges and future directions

Currently, fecal samples are commonly used for these analyses because they are easy to obtain with a large amount, and theoretically a composite of bacteria collected throughout the whole gastrointestinal tract. However, stools contain a representative collection of the bacterial taxa could mainly derive from the lower gastrointestinal tract. Whether the fecal samples can represent the whole spectrum of gut microbiota remains to be explored, and spatial microbial diversity might not be captured by a fecal sample.

Although an association between intestinal dysbiosis and liver disease in patients was observed in both animal and human studies, the mechanism of interactions between liver and microbial communities remains to be defined, including a core set of gut microbes and functional biomarkers associated with patients with different status of liver disease. Whether restoring the homeostasis of gut microbial communities would ameliorate hepatic disease also need to be validated further.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2016.11.03). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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