Editorial Commentary

Liver microbiome: an intrahepatic resident playing a role in liver diseases

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The commensal microbiome has been shown to exhibit intimate associations with human health and diseases. Harboring abundant microbes, the gut is involved in regulating the physical and pathological status of liver, including hepatic function, inflammation, immunity, metabolism, regeneration, etc. (1,2). Although it has been proposed in the gut-liver axis theory that the gut microbes and their metabolites could be disseminated into liver through the portal vein system, particularly in the conditions of gut microbiota dysbiosis and intestinal barrier dysfunction (3), the liver microbiome has only been confirmed and characterized in recent years. In this article, we reviewed the latest research advancements pertaining to the liver microbiome, discussed its potential clinical implications, and further explored the promising avenues for future research in this field (*Figure 1*).

Mouse models have shown that the liver microbiome changes with the host's age and physiological conditions (4), similar to the intestinal microbiome (5). It has been reported that the intrahepatic microbiome of patients with non-alcoholic fatty liver disease (NAFLD) or liver cancer differs from that of healthy controls, and there are also differences between the microbiome within liver tumors and adjacent tissues (6,7). As an intrahepatic resident, the liver microbiome is not a mere bystander in liver diseases, but plays a role that cannot be ignored.

Multi-omics research has preliminarily revealed the interactions between the host and intrahepatic microbiome. A study in patients with NAFLD not only found a correlation between the liver microbial signatures and host phenotypes and disease severity (6), but also discovered a link between the host genetics and the intrahepatic microbiome (8). Carriers of risk alleles such as *PNPLA3*-rs738409 and *TM6SF2*-rs58542926 have higher levels of *Gammaproteobacteria* in their liver tissues. A polygenic risk score based on the presence of risk/protection alleles in *PNPLA3*, *TM6SF2*, *MBOAT7*, *HSD17B13*, and *FGF21* can explain 7.4% of the differences in liver microbial composition at the genus level, suggesting the influence of host genetic backgrounds on the liver microbiome. Xue *et al.* initially revealed some microbes in hepatocellular carcinoma (HCC) that are correlated with tumor genetic and metabolic alterations (9). Their study found significant associations between 10 microbes, including *Alcaligenes*, that

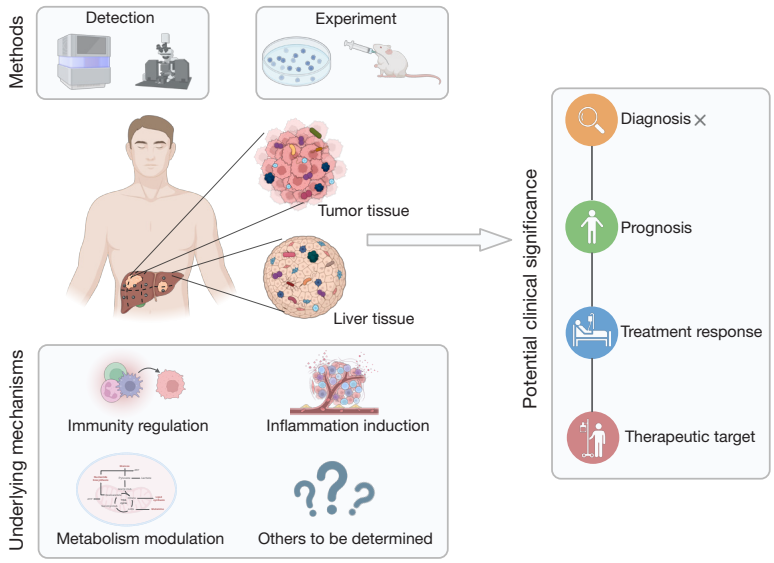

Figure 1 Illustration of the recent advancements pertaining to the liver microbiome and the potential clinical implications it holds for liver diseases.

are related to the tumor metabolome and the expression of 25 differentially methylated genes. By integrating transcriptome, methylation, and metabolome data, this study contributes to understanding the impact of liver microbiome on the HCC tumor microenvironment. Li *et al.* also discovered that the liver microbiome plays a role in shaping the tumor microenvironment in HCC (10). Based on microbial signatures obtained through metagenomic sequencing, 29 patients with hepatitis B virus (HBV)-related HCC were classified into two subtypes. One subtype was dominated by viruses, while the other subtype was characterized by a bacteria-dominated microbiome. Not only did these two subtypes differ in their tumor-associated microbiota composition, but they also exhibited distinct clinical features. The bacteria-dominated subtype is likely to be associated with poorer clinical outcomes, as patients classified as this subtype had larger tumors, a higher proportion of capsular invasion, and higher D-dimer levels. Interestingly, analyses based on RNA sequencing further revealed differences in the transcriptomic landscapes of the two subtypes, with the differentially expressed genes primarily enriched in immune and metabolic-related pathways. The bacteria-dominated subtype was infiltrated with higher levels of M2 macrophage, and exhibited higher activity in multiple metabolic pathways, including amino acid, carbohydrate, lipid, and energy metabolism. Notably, the level of amino acid metabolism was positively correlated with macrophage infiltration. Hence, it is likely that the liver microbiome influences the tumor immune microenvironment of HBV-related HCC by regulating metabolism.

Consistent with multi-omics studies, basic research demonstrates liver microbiome primarily exerts its influence on the host through the regulation of immunity and inflammation. A study in mice showed that normal immune function in liver relies on the intrahepatic microbiome, particularly *Bacteroidetes* species (4). Glycosphingolipids derived from these liver-enriched microbes activate liver natural killer T (NKT) cells, upregulating the CCL5 pathway and thus facilitating immune recruitment in this organ. Depletion of *Bacteroidetes* species leads to dysfunction of the immune network in liver, manifesting as a dramatic decrease in hepatic immune cells, impeded maturation of antigen-presenting cells, and a decline in adaptive immunity. As of now, the exact origin of the liver microbiome remains uncertain. Given the intimate anatomic and physiological relationships between the intestine and liver, it is plausible that the intrahepatic microbiome is at least partially populated from the gut through a selective process. In patients with colorectal cancer (CRC), harmful bacteria originating from the gut promote liver metastases through immune recruitment when gut vascular barrier is impaired in tumor tissues and normal intestinal mucosa (11). The translocation of *Escherichia coli* from the gut to

liver upregulates the expression inflammatory cytokines and chemokines, which facilitate the recruitment of macrophages and neutrophils. Additionally, it also upregulates the expression of genes related to extracellular matrix deposition. Altogether, the bacteria dissemination leads to the formation of inflammatory pre-metastatic niches in liver for CRC. In HCC patients with cirrhosis, it was found that tumor tissues were more abundant in *Stenotrophomonas maltophilia* than adjacent tissues. The enrichment of *Stenotrophomonas maltophilia* activates the senescence-associated secretory phenotype through the TLR4/NF-κB pathway, resulting in the formation and activation of the NLRP3 inflammasome and contributing to the progression of liver cirrhosis to HCC (12).

Increasing evidence suggests that the intrahepatic microbiome possesses potential clinical value. Huang *et al.* established a classifier based on liver microbiome, effectively distinguishing HCC tumor tissue from healthy liver tissue (7). As a result, they claimed that liver microbiome could aid in the diagnosis of HCC. However, the practical significance of this conclusion is rather limited as it relies on the acquisition of liver tissue, precluding its use as a non-invasive diagnostic tool. Interestingly, the liver microbiome exhibits implications in predicting patient prognosis, pointing to a potential novel avenue for prognostic assessment. Sun *et al.* discovered that the tumor-associated microbiome in HCC could categorize patients into two distinct subtypes, designated as hepatotype A and B, respectively (13). The subtype based on the microbiome emerged as an independent predictor of overall survival and recurrence-free survival among HCC patients who underwent surgical resection. Specifically, the hepatotype B, characterized by higher microbial diversity, exhibited significantly better prognoses compared to the hepatotype A. *Akkermansia* and *Methylobacterium* were more abundant in hepatotype B, and higher levels of these two bacterial genera suggested a more favorable prognosis.

Immunotherapy has transformed the landscape of treatment for liver cancer, yet only a fraction of patients exhibits favorable responses to immune checkpoint inhibitors (14). Studies have underscored the pivotal role of the gut microbiota in shaping tumor treatment outcomes, particularly in terms of patient responsiveness to immune checkpoint inhibitors. Given the pronounced disparities in localization and abundance between the liver and gut microbiome, their respective impacts on the liver immune microenvironment are presumably distinct. It is conceivable that the liver microbiome locally modulates the immune microenvironment, thereby exerting a potential influence on tumor immunotherapy. Therefore, it is worthwhile to delve into the intricate interplay between the liver microbiome and the immune system, with the goal of providing novel insights into this clinically significant question.

While we anticipate the emergence of liver microbiome as a novel prognostic or therapeutic response biomarker, it is imperative to recognize that our current understanding remains in its infancy. A significant obstacle in this field lies in the reliance on high-throughput sequencing techniques, as the sparse abundance of liver microbiome introduces a high risk of contamination during detection. Consequently, a pressing concern is the elimination of contaminant sequences that may arise during sampling, nucleic acid extraction, amplification, and sequencing processes. To tackle this challenge, establishing a meticulous and rigorous standardized protocol is paramount. Additionally, the development of rational and comprehensive decontamination algorithms will also be highly advantageous.

The current research on liver microbiota has yielded profound insights, yet there remains a vast territory to be explored. It is crucial for us to comprehend the complicated relationships between the liver microbiome and the host with enhanced spatial precision. New technologies, such as single-cell sequencing and spatial transcriptomics, can aid in precisely pinpointing the localization of liver microbiome and elucidating its intricate influence on the local microenvironment. Furthermore, considering the pivotal role that the evolution of the commensal microbiome plays in altering disease outcomes (15), it is essential to thoroughly investigate the composition, diversity, and genetic alterations of liver microbiome throughout the progression of liver diseases. Longitudinal studies encompassing multiple time points will provide us with a deeper comprehension. With the ongoing advancements in fecal microbiota transplantation, probiotic supplementation, and engineered bacteria, it is conceivable that the liver microbiome, in addition to serving as a biomarker for prognoses and therapeutic responses, may emerge as a novel therapeutic target. As research continues to unravel novel insights into the liver microbiome, it can be anticipated that new discoveries will further bolster our comprehension and refine treatment strategies for liver diseases accordingly.

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