Editorial Commentary

Novel biomarkers for hepatocellular carcinoma detection and treatment

Hongyuan Yi, Haifeng Xu

Department of Hepatic Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

Correspondence to: Haifeng Xu, MD. Department of Hepatic Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 1 Shuaifuyuan, Dongcheng District, Beijing 100730, China. Email: xuhf781120@sina.com.

Comment on: Yang J, He Q, Lu F, *et al.* A distinct microbiota signature precedes the clinical diagnosis of hepatocellular carcinoma. Gut Microbes 2023;15:2201159.

Keywords: Hepatocellular carcinoma (HCC); biomarkers; gut microbiome; oral microbiome; early detection

Submitted Sep 09, 2024. Accepted for publication Sep 20, 2024. Published online Sep 26, 2024.
doi: 10.21037/hbsn-24-517
View this article at: https://dx.doi.org/10.21037/hbsn-24-517

Hepatocellular carcinoma (HCC) ranks as the sixth most prevalent malignant tumor globally and is the third leading cause of cancer-related mortality, with its incidence and mortality rates demonstrating a consistent annual increase (1). The primary reason for this occurrence lies in the insidious onset of the disease, leading to late-stage diagnosis in a majority of patients, resulting in limited treatment options and poor efficacy. Consequently, emphasis on prevention and early detection is imperative for enhancing the prognosis of HCC patients. Furthermore, the identification of biomarkers with heightened sensitivity and specificity holds particular significance (2).

In recent years, there has been considerable attention focused on the association between microorganisms and tumorigenesis, with particular emphasis on the gut microbiome's role in cancer as a prominent subject of contemporary investigation (3). The structural and physiological interconnection linking the gut and liver is termed the "enterohepatic axis", through which various elements such as nutrients, toxins, gut microbiota, microbial components, and metabolites intricately interact to influence the development of diverse liver diseases (4). Additionally, the oral microbiota represents the second largest microbial community in the human body, akin to the intestinal microbiota. Research has demonstrated a close association between dysbiosis in oral microbial communities and oral, gastrointestinal, and hepatic pathologies (5-7). Therefore, the identification of microbial markers from the oral-intestinal-liver axis for the diagnosis of liver cancer represents a feasible approach.

Several prior studies have demonstrated a close correlation between specific bacterial populations in the oral and intestinal microbiota and the development of HCC. An imbalance in the gut microbiome, along with increased intestinal permeability, facilitates the translocation of gut microbes and their associated metabolites into the bloodstream, thereby hastening the progression of chronic liver disease (CLD) and elevating the risk of liver cancer (8). The intestinal microbiota of hepatitis B virus-chronic liver disease (HBV-CLD) patients has been demonstrated to undergo alterations, characterized by a decrease in the levels of *Bifidobacterium* and *Shigella*, and an overabundance of *Streptococcus* (9). Research has demonstrated a significant decrease in the diversity of intestinal microbes in cirrhosis patients compared to healthy controls, while the diversity of intestinal microbes is higher in HCC patients than in cirrhosis patients (10). The gut microbiome of cirrhosis patients exhibits specific changes, including an increased abundance of *Fusobacteria*, *Proteobacteria*, *Enterococcus*, and *Streptococcaceae* dominating the composition, while the abundance of *Bacteroidetes*, *Ruminococcus*, *Roseburia*, *Veillonellaceae*, and *Lachnospiraceae* is relatively reduced. Importantly, these alterations are not linked to the etiology of cirrhosis (11).

The development of HCC is associated with an increase in *Bacteroides* and *Ruminococcaceae* in the intestinal tract, as well as a decrease in protective bacteria such as *Bifidobacterium*. The phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* have been identified as the predominant components of the gut microbiome in HCC patients, and overexpression of *Bacteroides* in early-stage liver cancer may be linked to the progression of HCC (12). Moreover, recent studies have reported the presence of microbial colonization in various tumor tissues. Huang *et al.* identified the existence of *Staphylococcus aureus*, *Rothia*, *Bacillus*, and *Corynebacterium* in fresh HCC tissues, indicating the presence of microorganisms within HCC tissues. Furthermore, the genera *Bacillus*, *Acidobacteria*, *Parcubacteria*, *Saccharibacteria*, and *Gammaproteobacteria* are considered to be indicative of HCC (13).

The translocation of oral microorganisms to the intestines forms the basis of the oral-gut microbiome axis. Oral microorganisms can migrate to the intestines through various pathways, either by direct invasion via the digestive tract or by entering the systemic circulation through the periodontal ligament and subsequently colonizing the intestines. Once established in the intestines, they persist and contribute to the activation of the intestinal immune system and chronic inflammation (14). Research has indicated a reduction in the diversity of oral microorganisms in patients with chronic hepatitis, irrespective of the presence of cirrhosis, and a significant increase in the ratio of *Firmicutes* to *Bacteroidetes* (15). Patients with cirrhosis exhibit a significant dysbiosis of oral microbiota, characterized by an increase in potentially pathogenic bacteria such as *Enterococcaceae* and *Enterobacteriaceae*, alongside a reduction in autochthonous families (16).

Patients with HCC present a significant dysbiosis of tongue microbiota, accompanied by a notable increase in microbial diversity. The predominant bacterial communities in the oral microbiota of HCC patients consist primarily of the phyla *Dermatophytes*, *Actinobacteria*, *Clostridia*, and *Firmicutes*; whereas in the healthy control group, the dominant bacterial communities are primarily observed to be from the phyla *Gammaproteobacteria* and *Bacteroidetes*. Furthermore, differences in the abundance of *Clostridium* and *Parvimonas* species contribute to distinguishing HCC patients from healthy controls (17).

These findings provide compelling evidence supporting the quest for potential biomarkers for early detection of liver cancer.

The study conducted by Yang *et al.* employed machine learning technology to identify distinct microbial composition features in HCC before clinical diagnosis, which can be utilized for early detection (18). Significant differences were observed between HCC patients and healthy individuals in both oral and intestinal microbiomes, with *Streptococcus* being particularly prominent. Thus, the focused analysis of oral and fecal microbial diversity validated the potential of the oral-intestinal tumor microbiome as a method for early liver cancer screening.

In their study design, Yang *et al.* demonstrated, through a retrospective cohort and prospective validation cohort, that the combination of specific oral microbiome and gut microbiome species can serve as an effective method for diagnosing liver cancer. They also characterized the distribution features of the microbiome in various ecological niches of liver cancer patients, encompassing the oral cavity, gut, and tumor tissue.

Considering the disease progression from HBV infection to cirrhosis and ultimately to HCC, the study also encompassed patients with HBV infection to investigate potential changes in microbial communities as the disease advances. Subsequent analyses involved 16S rRNA sequencing and metagenomic sequencing to explore microbial patterns in the oral and intestinal cavities, as well as 5R 16S rDNA sequencing to examine microbial patterns in tumor and adjacent tissues.

In the comparative analysis of fecal microbiome profiles from different subjects in the retrospective cohort, researchers observed a gradual shift in the proportions of multiple microbial genera during the progression of HCC. Notably, there was a significant accumulation of *Streptococcus* and *Shigella*-related genera, indicating a marked increase in abundance. This suggests that these changes may be associated with specific alterations in intestinal hypoxia and permeability observed in HCC patients.

In the validation cohort, researchers observed a high abundance of microbial communities, including *Enterococcus* species, *Shigella* species, and *Escherichia coli* in both liver cancer tissues and adjacent non-cancerous tissues. This further confirms the presence of bacteria in liver cancer and suggests that they may originate from the oral cavity or intestines. This observation represents a significant distinction from non-cancerous tissues.

Based on the results of microbial sequencing, it has been determined that liver cancer patients harbor diverse ecological niches of oral-intestinal-tumor microbial communities, providing a more precise physiological basis for their potential use as diagnostic markers. Subsequently,

the findings from the retrospective cohort were validated using machine learning and random forest analysis in a prospective cohort. This validation identified 10 oral bacterial genera and 9 fecal bacterial genera capable of distinguishing HCC from controls, along with their respective area under the curve (AUC) values. Integration with influential classification units further increased the AUC, and when combined with serum alpha-fetoprotein (AFP) levels, there was a significant improvement in model performance AUC, indicating that combining oral and fecal microbiota with serum AFP can significantly enhance the prediction accuracy of HCC.

In conclusion, Yang *et al.* identified the presence of the liver microbiome through both retrospective and prospective cohort studies, indicating potential bacterial translocation or cross-contamination in liver cancer patients and uncovering possible relationships between the cancer ecosystem, oral microbiota, and intestinal microbiota. This research revealed the potential of the oral-intestinal-tumor microbiome for early detection of HCC, offering a promising and reliable approach for early disease detection while providing a novel perspective on microbial involvement mechanisms in liver cancer pathogenesis. Additionally, validation from longitudinal cohorts demonstrated the potential of combining oral and fecal microbiomes to differentiate HCC from healthy individuals.

However, this study is subject to certain limitations. The samples collected are predominantly static and lack dynamic follow-up. Additionally, they originate from the same hospital and ethnic group, thus lacking diversity in terms of region and ethnicity. Therefore, future research and validation should encompass diverse ethnic groups from different regions to further ascertain the generalizability of the screening method.

However, it is undeniable that an increasing number of researchers are demonstrating a growing interest in the application of intestinal flora and microorganisms for disease prediction, as well as recognizing their potential in early diagnosis and treatment. This article introduces a novel concept and approach for early screening of HCC, offering the prospect of new biomarkers to enhance the prognosis and treatment of the disease.

Acknowledgments

Funding: This work was supported by the Chinese Academy of Medical Sciences, the Central Level Public Welfare Research Institutes Basic Research Expenses (No. 2023-RW320-05).

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *HepatoBiliary Surgery and Nutrition*. The article did not undergo external peer review.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-24-517/coif). 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.

References

1. Singal AG, Kanwal F, Llovet JM. Global trends in hepatocellular carcinoma epidemiology: implications for screening, prevention and therapy. Nat Rev Clin Oncol 2023;20:864-84.
2. Xie DY, Zhu K, Ren ZG, et al. A review of 2022 Chinese clinical guidelines on the management of hepatocellular carcinoma: updates and insights. Hepatobiliary Surg Nutr 2023;12:216-28.
3. Sun L, Li J, Feng Y, et al. Gut microbiome evolution impacts the clinical outcomes of diseases. Hepatobiliary Surg Nutr 2023;12:261-3.
4. Ohtani N, Hara E. Gut-liver axis-mediated mechanism of liver cancer: A special focus on the role of gut microbiota. Cancer Sci 2021;112:4433-43.
5. Albuquerque-Souza E, Sahingur SE. Periodontitis, chronic liver diseases, and the emerging oral-gut-liver axis. Periodontol 2000 2022;89:125-41.
6. Kitamoto S, Nagao-Kitamoto H, Hein R, et al. The

Bacterial Connection between the Oral Cavity and the Gut Diseases. J Dent Res 2020;99:1021-9.
7. Kuraji R, Sekino S, Kapila Y, et al. Periodontal disease-related nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: An emerging concept of oral-liver axis. Periodontol 2000 2021;87:204-40.
8. Myojin Y, Greten TF. The Microbiome and Liver Cancer. Cancer J 2023;29:57-60.
9. Shen Y, Wu SD, Chen Y, et al. Alterations in gut microbiome and metabolomics in chronic hepatitis B infection-associated liver disease and their impact on peripheral immune response. Gut Microbes 2023;15:2155018.
10. Zheng R, Wang G, Pang Z, et al. Liver cirrhosis contributes to the disorder of gut microbiota in patients with hepatocellular carcinoma. Cancer Med 2020;9:4232-50.
11. Trebicka J, Macnaughtan J, Schnabl B, et al. The microbiota in cirrhosis and its role in hepatic decompensation. J Hepatol 2021;75 Suppl 1:S67-81.
12. Komiyama S, Yamada T, Takemura N, et al. Profiling of tumour-associated microbiota in human hepatocellular carcinoma. Sci Rep 2021;11:10589.
13. Huang JH, Wang J, Chai XQ, et al. The Intratumoral Bacterial Metataxonomic Signature of Hepatocellular Carcinoma. Microbiol Spectr 2022;10:e0098322.
14. du Teil Espina M, Gabarrini G, Harmsen HJM, et al. Talk to your gut: the oral-gut microbiome axis and its immunomodulatory role in the etiology of rheumatoid arthritis. FEMS Microbiol Rev 2019;43:1-18.
15. Ling Z, Liu X, Cheng Y, et al. Decreased Diversity of the Oral Microbiota of Patients with Hepatitis B Virus-Induced Chronic Liver Disease: A Pilot Project. Sci Rep 2015;5:17098.
16. Bajaj JS, Betrapally NS, Hylemon PB, et al. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. Hepatology 2015;62:1260-71.
17. Lu H, Ren Z, Li A, et al. Deep sequencing reveals microbiota dysbiosis of tongue coat in patients with liver carcinoma. Sci Rep 2016;6:33142.
18. Yang J, He Q, Lu F, et al. A distinct microbiota signature precedes the clinical diagnosis of hepatocellular carcinoma. Gut Microbes 2023;15:2201159.

Cite this article as: Yi H, Xu H. Novel biomarkers for hepatocellular carcinoma detection and treatment. HepatoBiliary Surg Nutr 2024;13(5):901-904. doi: 10.21037/hbsn-24-517