A River From the Liver: Detecting Hepatocyte Genomic DNA in Urine

A major challenge in hepatology is the inaccessibility of the liver for disease monitoring. Intrahepatic events remain hidden as direct sampling (e.g., liver biopsies) becomes less frequent in clinical practice. This problem is particularly acute in patients with few symptoms and infrequent specialist management (e.g., chronic hepatitis B).

The most common workaround is to indirectly measure surrogate markers of liver disease progression in the circulation. However, this strategy cannot be used for many potentially informative molecules. One of the more elusive markers is genomic DNA from hepatocytes, which is generally secured within the nucleus of cells that are in turn locked up in the liver itself.

In this issue of *Hepatology Communications*, Lin et al.[1] discover that hepatocyte-derived cellular DNA can be detected in the urine of patients with hepatitis B virus (HBV) infection (Fig. 1). They provide strong evidence for this by measuring a form of the virus that only exists within hepatocyte genomic DNA, namely integrated HBV DNA. Integrated HBV DNA is also strongly associated with liver tumors (although the specific causative mechanism still eludes the field), providing additional clinical relevance of this study.

Abbreviations: HBV, hepatitis B virus; NGS, next-generation sequencing; PCR, polymerase chain reaction.

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Urine contains material from all bodily tissues. Thus, the authors used specifically targeted assays, first by isolating DNA from liver tissue or > 1 mL of urine, and then subjecting DNA extracts to:

1. A capture-sequencing approach enriching for HBV-DNA sequences, followed by next-generation sequencing (NGS) and bioinformatics analysis for integration junction sequences; or
2. A specific polymerase chain reaction (PCR) followed by Sanger sequencing (in cases where they had paired liver tumor tissue or for validation of integrations detected by NGS).

In the first half of their study, the authors showed that integrated HBV-DNA sequences detected in the liver tumor tissue by capture sequencing (approach 1) could also be detected by specific PCR (approach 2) in the urine of 5 of 8 patients with HBV. This is consistent with previous studies showing that integrated HBV-DNA sequences from tumors could be detected in the blood.[2]

The second half of the study analyzed the urine of patients with HBV in different stages of chronic HBV infection, ranging from precirrhosis, cirrhosis, and liver cancer (before and after resection). The authors could detect integrated HBV DNA in all but 1 patient using approach 1, with 88% of integrations verified by approach 2. No HBV-DNA integrations were detected in the urine samples of people without HBV infection. Sequence analysis showed that the detected integrations resembled the previously described genomic distribution with respect to the genomes of the host cell (mostly random across the host genome) and the virus (clustering close to the ends of the double-stranded linear HBV-DNA substrate that integrates).[3] Together, these results

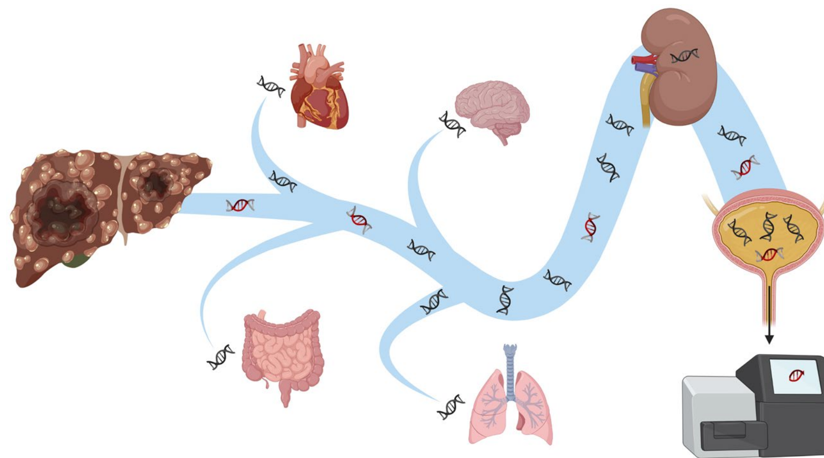

FIG. 1. Lin et al. have shown that hepatocyte-derived DNA fragments (denoted by integrated HBV-DNA sequences, red) can be detected in urine using specific NGS and direct PCR assays, presumably on the background of cellular DNA from other organs (black and gray). Created with BioRender.com.

suggest that the authors were detecting true integration events.

This proof-of-principle study is consistent with the previous work showing the presence of HBV DNA in the urine,[4–6] but importantly strengthens arguments that a source of this DNA is from intrahepatic virus (as opposed to circulating virus DNA). This study also expands the breadth of possible bodily compartments to sift for clinically useful biomarkers. Tracking changes in hepatocyte genomic DNA could lead to greater understanding and monitoring for the genomic and epigenetic changes that occur in the lead up to many liver diseases, including liver cancer. However, several issues stand in the way of this approach becoming a prognostic or diagnostic clinical marker.

First, numerous factors are likely to affect the levels of integrated HBV DNA in the urine. This could indeed be due to true differences in integrated HBV-DNA levels in the liver, but could also be affected by other hepatic factors (e.g., level of inflammation) or completely independent factors (e.g., hydration or kidney injury). The multitude of these factors likely contribute to the inability of this study to detect differences in numbers of integrations between patients with and without cirrhosis (the former likely have increased integration rates due to clonal expansion of hepatocytes with integrations resultant from chronic inflammation[7,8]).

Also, the techniques the authors have used in this study are, at best, semi-quantitative (if read depth can be used as a surrogate for frequency) and may not be able to determine copy numbers of particular integrations and thereby give indications about clonal expansion of hepatocytes, a risk factor associated with cancer. This could be addressed in future studies by using complementary techniques that can absolutely quantify HBV-DNA integrations, such as digital droplet PCR or inverse PCR.

However, even if the true intrahepatic integration levels could be quantified, it remains to be determined whether this is a clinically significant marker. Viral integrations occur in all stages of HBV infection,[7,8] remain after infection clearance,[9] and do not appear to be correlated to liver cancer progression (at least when analyzing the nontumor tissue).[8] It is possible that the urine could be used to assess cancer recurrence (as previous studies have done for HBV-DNA integrations in blood[2]). Theoretically, tumor-associated integrations could be identified from resection tissue (e.g., by NGS), and recurrence could be monitored by specific sequencing from DNA extracted from the urine. The sensitivity and specificity of this method would be important to measure in these future studies.

Finally, extension of these results to other liver diseases may prove difficult due to the lack of specific markers. Integrated HBV DNA is a special case in which hepatocytes are the only targets of HBV

infection (and therefore integration), so in itself is a specific indicator of its origin. However, other liver diseases (e.g., metabolic-associated fatty liver disease) still lack a suitable marker to filter for liver-originating sequences. This is particularly difficult due to the short length (<1 kbp) of the DNA fragments present in urine.

In summary, Lin et al. have shown that urine carries cellular DNA derived from hepatocytes. Further studies will determine whether we can divine clinically relevant information from this readily accessible compartment or whether the components in urine simply represent useless debris. Regardless, the authors have brightened the prospects for a painless, noninvasive avenue for monitoring of liver diseases with their innovative work. As such, there is hope that this work might lead to lower barriers for patient monitoring and therefore improve health outcomes in chronic liver disease.

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