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A new dimension in viral hepatitis research

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A new dimension in viral hepatitis research

Perspective on

Cross et al. "Characterization of HBV and co-infection with HDV and HIV through spatial transcriptomics"

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Abbreviations:

HBV, Hepatitis B virus

HDV, Hepatitis delta virus

HIV, human immune deficiency virus

CHB, chronic hepatitis B

HCC, hepatocellular carcinoma

WHO, World Health Organization

ATAC-Seq, assay for transposase accessible chromatin - sequencing

ChIP-Seq, chromatin immunoprecipitation - sequencing

Abstract

Chronic hepatitis B (CHB) is the leading cause of hepatocellular carcinoma (HCC) and a significant global health issue, affecting over 296 million people worldwide, with 15 million people co-infected with Hepatitis Delta virus (HDV) suffering accelerated disease progression.

Recent advances in single-cell sequencing and spatial transcriptomics offer promising insights to improve the understanding of the liver's immune responses and HBV-infected cell distribution, with the final goal being the achievement of an HBV "functional cure".

In this issue of *eGastroenterology*, Cross et al. utilized the GeoMx nanostring Digital Spatial Profiling (DSP) technology to study gene expression in liver tissues of three patients (one HBV-monoinfected, one HBV/HDV co-infected, and one HBV/HIV co-infected). Unlike other spatial transcriptomics techniques, GeoMx DSP allows targeted selection of specific tissue regions (ROIs) for analysis, enabling precise gene expression mapping. The study revealed spatially distinct transcriptomic signatures related to immune features and viral burden, identifying a component of under-investigated immune cells. Despite the small sample size, this proof-of-concept study demonstrates the feasibility of spatial transcriptomics in analyzing HBV infections. Future advances, such as integrating viral proteins and nucleic acids, will enhance understanding of spatial viral replication. Challenges in tissue processing, data analysis, and costs remain before spatial transcriptomics can be applied as a diagnostic tool, but ongoing multi-omics approaches offer promise for improved diagnosis and therapy.

Chronic hepatitis B (CHB) is the first cause of hepatocellular carcinoma (HCC) and a major public health problem, being the most prevalent chronic infectious disease worldwide with more than 296 million individuals concerned (1). Fifteen million chronic HBV carriers are also co-infected with Hepatitis Delta virus (HDV), resulting in an accelerated progression to cirrhosis and HCC. The management of HBV/HDV co-infected patients is limited by the low response rate to interferon therapy and Bulevirtide, an entry inhibitor, has received a conditional approval from EMA (2). Current treatments against CHB induce viral suppression but require life-long regimens in most patients. “HBV functional cure”, defined as sustained loss of HBV surface antigen (HBsAg) and HBV DNA off-therapy, is the holy grail of current therapeutic development, since it is associated with improved liver disease outcomes (3). Eradicating or silencing of HBV genomes coupled to re-educating the liver micro-environment are considered key to establish a sustained immune control of the infection. The WHO estimates that 8% of persons with HIV are also co-infected with HBV, a condition associated with accelerated liver disease progression and mortality (4). Paradoxically, despite the HIV-induced immunosuppression, rates of HBV functional cure are higher in HIV/HBV co-infected persons compared to people infected with HBV alone (5)

A major obstacle to the development of innovative and efficient therapeutic interventions against CHB is represented by the lack of comprehensive investigation on the interplay between intrahepatic viral burden and immune responses in relationship with liver microenvironment.

While it appears evident that the distribution of HBV-infected cells is not homogeneous across the liver lobule, recent attempts using multiplex immunofluorescence approaches have provided limited information on the interplay between viral burden and liver microenvironment (6,7).

The implementation of fine liver aspirates (FNAs) coupled to single cell sequencing or high-sensitive techniques for HBV detection has recently paved the way to groundbreaking studies on liver HBV persistence and immune responses (8–10). However, FNAs do not preserve liver tissue architecture, thus missing the liver spatial information.

Spatial transcriptomic has gained momentum in the last quinquennium (11), becoming increasingly used either to enrich information provided by bulk RNA-seq or single cell RNA-seq approaches through deconvolution methods (12,13), or to provide, by itself, a spatial reference for single cell expression, cell to cell communication insights (14) and help researchers to go from “causative loci” to “causative location”, when dealing with pathological phenotypes.

In this issue of eGastroenterology, Cross et al. applied a spatial transcriptomic approach, using the GeoMx nanostring Digital Spatial Profiling (DSP) technology to investigate the gene expression patterns associated to selected topographies of HBV infected cells in the liver of three representative chronically infected patients (one HBV-monoinfected, one HBV/HDV co-infected and one HBV/HIV co-infected) (15).

Differently from other sequencing-based spatial transcriptomic techniques (16,17), which provide a fixed grid of capture spots, the GeoMX DSP platform allow the manual definition of specific areas of the tissue (i.e. Regions Of Interest – ROIs), where barcodes attached to tissue mRNAs can be released and aspirated by a microcapillary pipette. These barcodes can be collected and converted into a next-generation sequencing compatible library for direct counting. In this way, barcodes (a surrogate for bound detection oligonucleotides) can be mapped to ROIs to provide a digital readout of gene expression within the tissue context (18). ROIs can be selected by the user to leverage any a-priori knowledge of the tissue/phenotype, providing a “target sequencing”-like approach (18). Though the resolution achieved at the time of writing is still in the order of 50-100 cells per ROI (almost an order of magnitude bigger than the 10x Visium, its direct competitor (17)), the targeted ROI selection strategy allows for the precise capture of gene expression profile of histologic structure of interest. Moreover, fluorescently labeled antibodies can be used to segment discontinuous cells (e.g. HBsAg staining and CD45 staining in Cross et al.) to perform differential expression analysis. Conversely, this approach is not suited to obtain a map of gene expression across the entire tissue and could introduce bias due to the manual selection of ROIs. Like its 10x counterpart, the expression profiles generated by the GeoMX DSP still represent a composite, bulk-like,

collection of cells, therefore it is imperative to apply computational approaches to deconvolve the cellular composition of the generated profiles.

The proof-of-concept study presented by Cross et al. provides an example of a state of the art analysis workflow: 1) differentially expressed genes (DE) are identified comparing ROIs in a 'case-control' style, 2) Pathways over representation analysis is performed exploiting the Gene Ontology datasets, 3) A weighted gene correlation network analysis (WGCNA) is applied to the expression values to identify cluster of genes 'moving together', 4) and a cell deconvolution method is used to increase the resolution to the picture the authors are providing.

This kind of workflow should be regarded as the standard approach to provide a solid starting point to investigate a tissue micro-environment also in small collections of samples.

This proof-of-concept study has the merit of demonstrating the feasibility and utility of spatial transcriptomic (ST) in analyzing gene expression patterns, immune cell composition and biological processes at high resolution within the liver microenvironment. The use of anti-HBsAg and CD45 immunostaining to drive ROI selection allowed the identification of tissue regions with different balance between viral burden and extent of immune infiltration. Although the small number of samples and the high intra- and inter-sample variability severely limit the generalization of the conclusions, it is worth noting that spatially discrete transcriptomic signatures relating to immune features could be identified. Moreover, insights were provided on under-investigated immune subsets, namely γδT cells, αβT cells, mature B cells and inflammatory macrophages, whose proportions differed between samples. Further advances in the interpretations of these type of results will come once multiple viral proteins (e.g. HBsAg, HBcAg, HDV antigens) and even viral nucleic acids would be included in the workflow, thus allowing a precise understanding of spatial distribution of cells harboring active viral replication.

Besides the results provided by Cross et al in their study, which, limited in sample size as it is, represents the first application of the GeoMx DSP technology on HBV samples with co-infection, a question is still lingering: how can we leverage the insights provided by spatial transcriptomic, to improve diagnosis and eventually tailor therapeutic interventions?

Many challenges remain to be addressed so that ST approaches could become a proper diagnostic tool, mainly in the tissue processing and data analysis, but also from a cost-related side. On a technical side, there is still the need of independent assessment of performances, to quantify the contribution of biological and technical sources of variability. Moreover, the need for benchmarks to ensure the stability of the platform and compatibility of data collected at different times. Additionally, the data analysis has a long turnaround time, more consistent with the research time-span, that with the one required for a laboratory developed test.

Ideally, the best approach would be to apply ST techniques to representative cohorts, increasing, for example, the power to identify meaningful inter-groups variation by sampling different regions from the same sample, generating expression profiles for a bigger subpopulation of cells (obviously, this calls for the additional control of any batch effect introduced, with additional steps of data normalization or QC). Results from the analyses performed on cohorts, also related to response to treatments, could then inform researcher and guide them to identify novel diagnostic biomarkers or drug targets that could be validated using techniques already available in a laboratory contest, providing than an instrument that will be cost-effective and more likely to be included in the diagnostic process (Figure 1).

The work from Cross et al. testifies of the “spatial” revolution occurring in the hepatology field (14,19,20) and expected to transform basic and translation research also in viral hepatitis. The use of FFPE material opens the possibility of investigating large stored collections of samples. The implementation of multi-omics approaches, coupling proteomics, transcriptomics, metabolomics and their integration with other modalities such as ATAC-seq or ChIP-seq is already on the way and is expected to provide unprecedented insights into virus-host interactions in the liver.

Competing interests:

M.C. has nothing to declare

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Patient and Public Involvement

Patients' involvement was not applicable to this type of manuscript.

Ethics Statement

The manuscript does not involve any human participants or animal subjects.

Contributorship:

MC and BT draft the manuscript, finalized it and created the figure. BT is the guarantor for the manuscript.

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Figure 1. Workflow aiming at implementing spatial approaches into clinical development. Created with Biorender.com