Targeted capture single-cell sequencing provides a new perspective for HBV persistent infection

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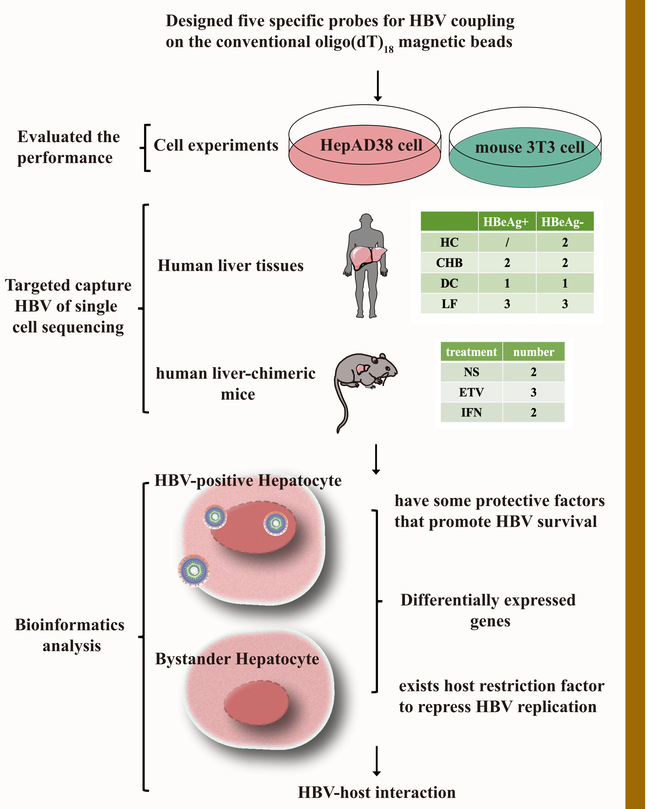

Introduction

At present, the characteristics of HBV-positive hepatocytes have not been fully elucidated because the overwhelming number of host transcripts in current single-cell RNA sequencing (scRNA-seq) techniques using the poly(A) tail significantly reduces the capture rate of virus [1].

Aim

To improve the capture rate of virus, we designed five specific probes for HBV coupling on conventional oligo(dT)18 magnetic beads to target enrichment for HBV sequences, evaluated its performance, and characterize the features of HBV-positive hepatocytes.

Method

- The performance of targeted capture single-cell sequencing methods was assessed using the HBV-positive HepAD38 cell line.
- Fourteen human liver tissues were analyzed, including healthy liver donors (n=2), treatment-naïve chronic hepatitis B (n=4), decompensated cirrhosis (n=2), and liver failure (n=6), as well as antiviral therapy-treated humanized liver-chimeric Tet-uPA-Rag2^{null}-γc^{null} mice (Hu-URG) [2] with saline solution (n=2), entecavir (n=3), and peg-interferon (n=2).
- We analyzed differential gene expression analysis within the same subcluster of hepatocytes, at different disease stages, and after antiviral treatment, and show the characteristics of HBV-positive hepatocytes.

Results

- *We established a single-cell sequencing method to quantitatively measure the levels of the three HBV mRNA transcripts (S, X, pgRNA), rcDNA, and cccDNA in individual cells.*
- *Hepatocytes positive for HBV exhibit significant heterogeneity, where subclusters Hep_Hp and Hep_MT are more favorable for HBV replication. HBV-positive hepatocytes upregulate genes reflecting liver synthetic functions, such as albumin, apolipoprotein, and fibrinogen, while downregulating factors associated with mRNA splicing, such as DDX5, DDX17, and SRSF2.*
- *GSEA enrichment analysis shows that HBV-positive hepatocytes are involved in interferon-alpha response and interferon-gamma response. Conversely, in Hu-URG mice, the differentially expressed genes in HBV-positive hepatocytes are completely reversed after antiviral treatment.*

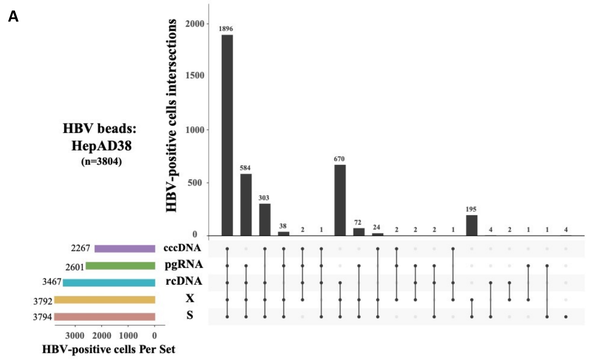

Fig.1 UpSetR plots depicting the unique and shared number of cells for each probe with HBV magnetic beads. The top number above each bar represents the number of cells that are unique to each group. Bars represent the number of cells shared between probes indicated by linked dots below the x-axis.

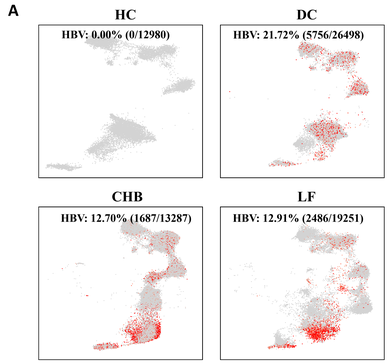

Fig.2 FeaturePlot visualizes HBV UMI counts for patients from Healthy Control (n=2), Chronic hepatitis B (n=4), decompensated cirrhosis (n=2), Liver failure (n=6). The deeper the red color of each point reflects the higher content of HBV.

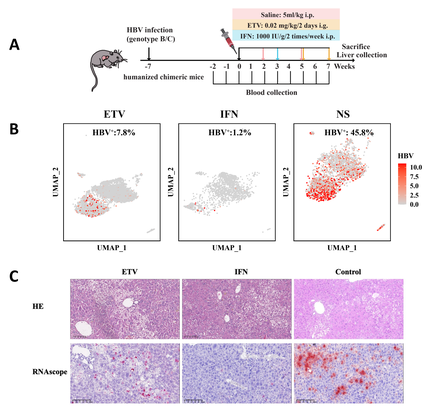

Fig.3 (A) Experimental design of the Hu-URG mouse (n=7). (B) The UMAP plot reveals the content of HBV UMIs in human hepatocytes within Hu-URG mice, where a deeper red indicates a higher content of HBV. (C) Representative images of HE staining and in situ hybridization staining (HBV RNAscope) of liver sections of Hu-URG mice.

Conclusions

We have established a method for targeted capture of single-cell transcriptomics at single-cell resolution that detects HBV mRNA/DNA and have uncovered the characteristics of HBV-positive hepatocytes.HBV-positive hepatocytes exhibited upregulation of genes reflecting liver synthesis and metabolism, alongside the downregulation of factors associated with mRNA splicing.

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