Single cell RNA Sequencing of an *In Vitro* Model for Studies of Hepatitis B Virus

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Introduction

- Hepatitis B virus (HBV) represents an enormous public health burden with 296 million people chronically carrying the infection, which culminates in 820,000 HBV-associated deaths annually. Chronic HBV infection is the leading cause of hepatocellular carcinoma worldwide.
- Although infectious *in vivo*, there is a lack of convenient and efficient HBV infectable *in vitro* models.
- We previously demonstrated that culture with human serum (HS), instead of conventional fetal bovine serum (FBS), could shift Huh7.5 hepatoma cells towards a differentiated (more hepatocyte-like) phenotype and altered expression of one third of genes.

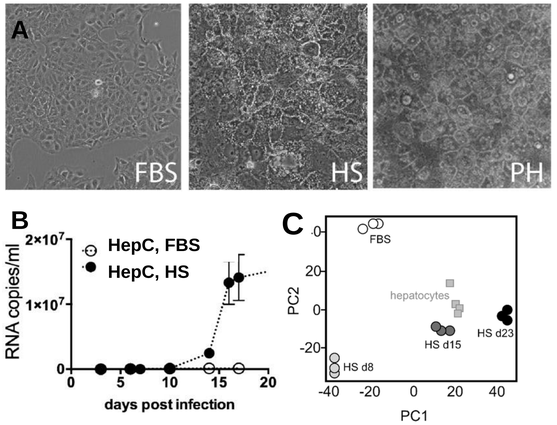

Fig. 1: Huh7.5 cells cultured in HS take on differentiated phenotypes. Huh7.5 cells cultured in HS for 21 days **A)** resemble primary hepatocytes (PH) and **B)** secrete more Hepatitis C virus compared to cells in FBS (Steenbergen *et al.*, Hepatology 2013). **C)** Principal component analysis of primary human hepatocytes, and Huh7.5 cells cultured in FBS or HS for various durations (Steenbergen *et al.,* Sci Rep 2018).

Objective and Hypothesis

- **Objective:** Create and characterize an improved model to study Hepatitis B virus (HBV) infection.
- **Hypothesis:** Culturing Huh7.5-NTCP cells in HS will reconstitute hepatocyte-like phenotypes. This differentiation will support enhanced HBV infection efficiency.

Results

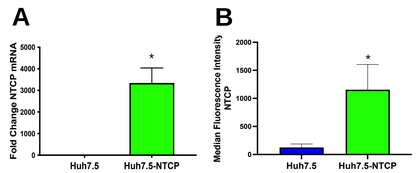

Fig. 2: Establishment of NTCP over-expression in Huh7.5 cells (Huh7.5-NTCP). NTCP measured by **(A)** qPCR (mRNA) and **(B)** flow cytometry (protein) of Huh7.5 and Huh7.5-NTCP cells, respectively.

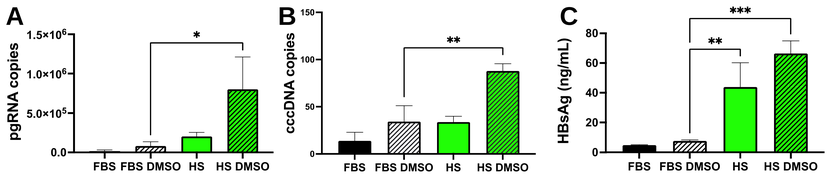

Fig. 3: Enhancement of HBV infection in Huh7.5-NTCP cells cultured with HS. A) pgRNA (copies/10ng total RNA), **B)** cccDNA (copies/10ng total DNA), and **C)** HBV surface antigen (HBsAg) from infected Huh7.5-NTCP cells cultured in FBS or HS, with or without 2% DMSO. ANOVA: *, p<0.03,; **, p<0.002; ***, p<0.0002; n=3.

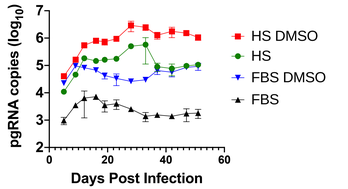

Fig. 4: Long-term infection with HBV. Cells were infected in FBS or HS, with or without 2% DMSO. n=3.

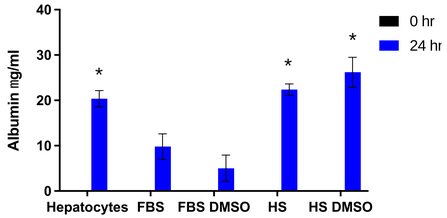

Fig. 5. Increased secretion of albumin, a hepatocyte marker, in Huh7.5-NTCP cells. Secreted albumin from cultured primary human hepatocytes and Huh7.5-NTCP cells supplemented with FBS, HS, and/or DMSO. Two-way ANOVA: *, p < 0.01 compared to FBS. n=3.

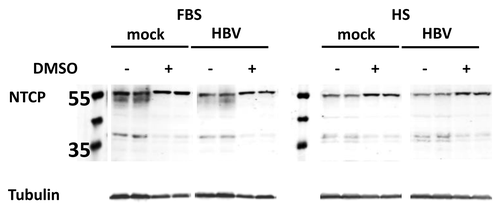

Fig. 6: NTCP glycosylation altered in DMSO or HS. NTCP in Huh7.5-NTCP cells that were uninfected (mock) or HBV infected and supplemented with FBS, HS, and/or DMSO. The 37kDa and 55kDa bands represent unglycosylated and fully glycosylated NTCP, respectively.

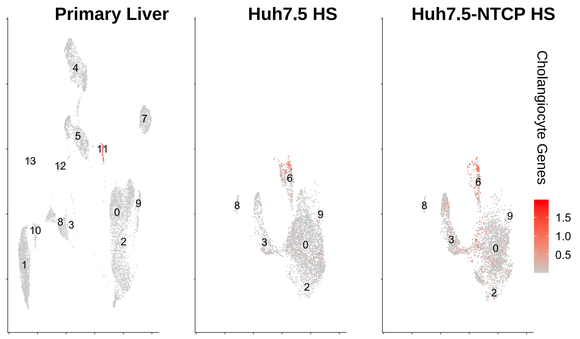

Fig. 7: Huh7.5 and Huh7.5-NTCP cells cultured with HS contain a cell subtype expressing cholangiocyte genes. Single cell RNA sequencing analysis of primary liver tissue (MacParland *et al.*, Nat Commun 2018), and Huh7.5 and Huh7.5-NTCP cells supplemented with HS. Uniform Manifold Approximation Projections depict expression level of cholangiocyte genes (red = higher cholangiocyte marker gene expression) in single cells.

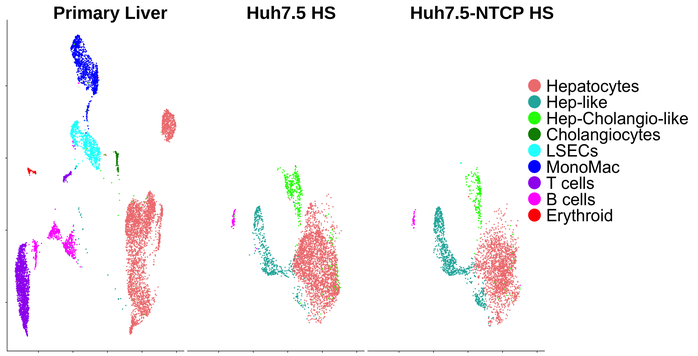

Fig. 8: Delineated cell subtypes in primary liver tissue, and Huh7.5 and Huh7.5-NTCP cells cultured with HS supplementation. Single cell RNA sequencing analysis was conducted on primary liver tissue (MacParland *et al.*, Nat Commun 2018), and Huh7.5 and Huh7.5-NTCP cells supplemented with HS. Uniform Manifold Approximation Projections depict labelled cell subtypes determined using cell marker genes.

Conclusions

- Developed a novel model for studying HBV
- Our HS culture method enhances HBV infection compared to culture in FBS.
- HS alone supports robust HBV infection without requiring the addition of DMSO.
- HS differentiation induces increased expression of hepatocyte markers, such as albumin secretion.
- HS and DMSO supplementation may affect glycosylation of NTCP.
- Huh7.5-NTCP cells cultured in HS supplemented culture contain cell subtypes, including a hepatocyte-cholangiocyte-like cell subtype.

Acknowledgments

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Reference*:

Connie Le, Reshma Sirajee, Rineke Steenbergen, Michael A. Joyce, William R. Addison, D. Lorne Tyrrell. *In vitro* infection with hepatitis B virus using differentiated human serum culture of Huh7.5-NTCP cells without requiring dimethyl sulfoxide. *Viruses,* 2021, 13(1):97. https://doi.org/10.3390/v13010097