

Host Response to Hepatitis Delta Virus Infection

Johanna Lee, Lucas Yoshida, Takeshi Saito, MD, Ph.D.

Dept of Medicine, Division of Gastrointestinal and Liver Diseases, Bridge Institute, University of Southern California, Los Angeles, CA, USA



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Background

HDV (Hepatitis D Virus) is a satellite virus of HBV. It infects 10-20 million people worldwide with a mortality rate of up to 20%, thereby posing a significant threat to public health. To date, the exact mechanism by which HDV exhibits strong cytotoxicity is not well understood. Thus, furthering our understanding of HDV-host interaction is essential for the development of novel therapeutic strategies that mitigate the disease burden.

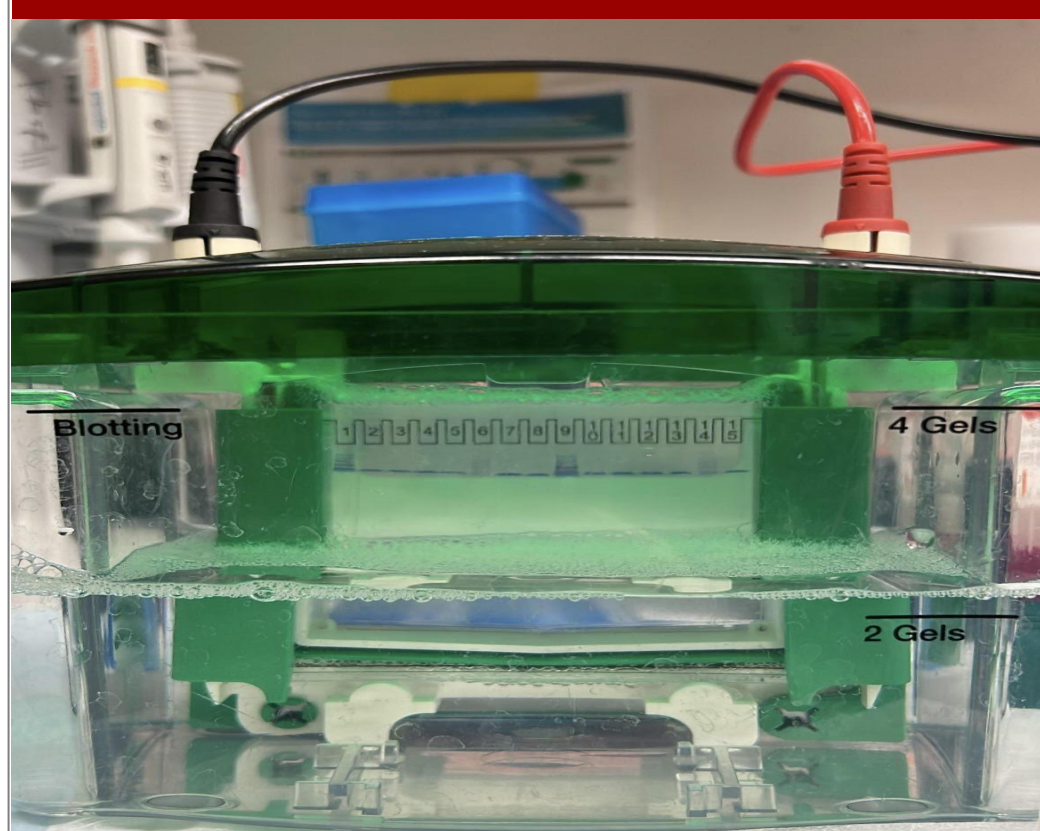
Objective

Using in vitro cultured primary human hepatocytes (PHH), the host response in HDV infection will first be analyzed via 2D SDS-PAGE protein mass spectrometry. Then, the function of the hits identified in the protein mass spectrometry will be determined using publically available databases such as UniProt and NCBI. The result of the mass spectrometry analysis will be confirmed by conventional Western Blotting analysis. To achieve this goal, the protein concentration of the crude cell lysates will be measured using Bradford Assay. Then, SDS PAGE will be used to separate proteins by molecular weight, which will then be transferred onto a PVDF membrane and probed with a specific primary antibody against the protein of interest (POI). Afterwards, the abundance of POI will be analyzed through the capturing of the chemiluminescence signal by a CCD camera.

Protein Quantification via Bradford Assay

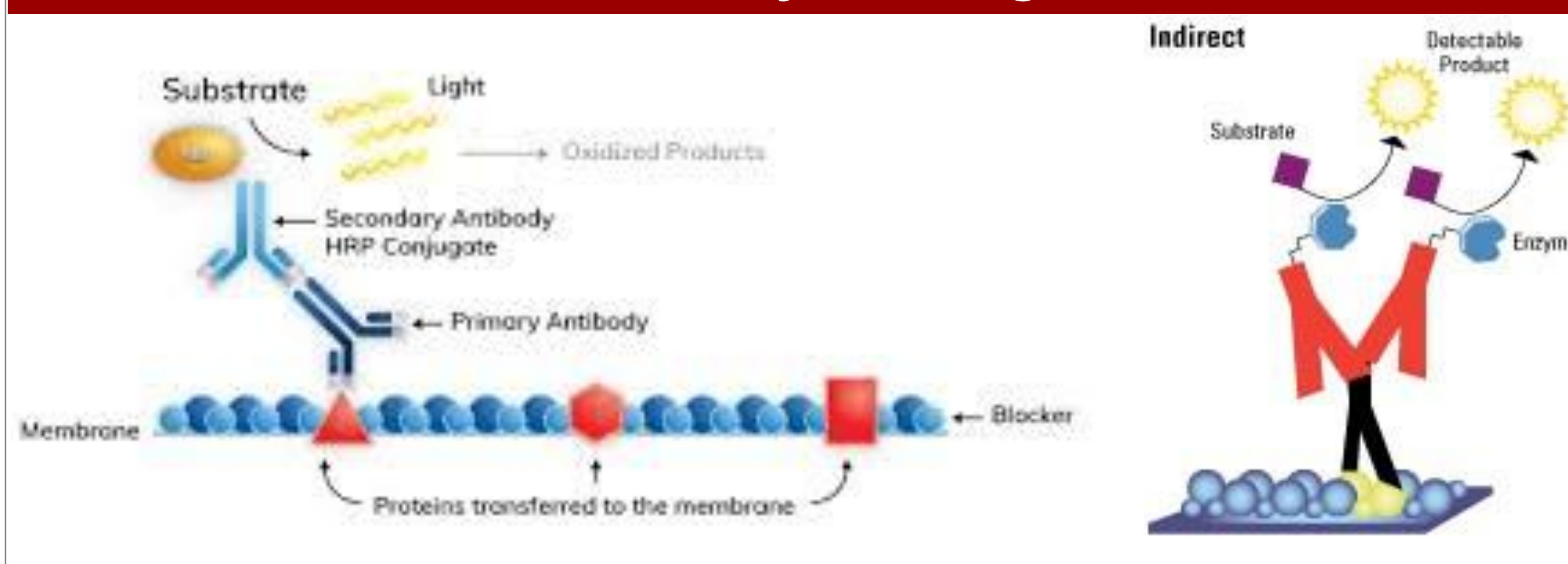
Prior to protein quantification in our samples, we verified the optimum range of detection using serially diluted Bovine Serum Albumin (BSA) with a known concentration. Based on the results, we determined that the range of protein concentration of 2ug/uL-4ug/uL will provide us with accurate readings. Therefore, all our samples will be adjusted to fall within this range.

SDS-PAGE

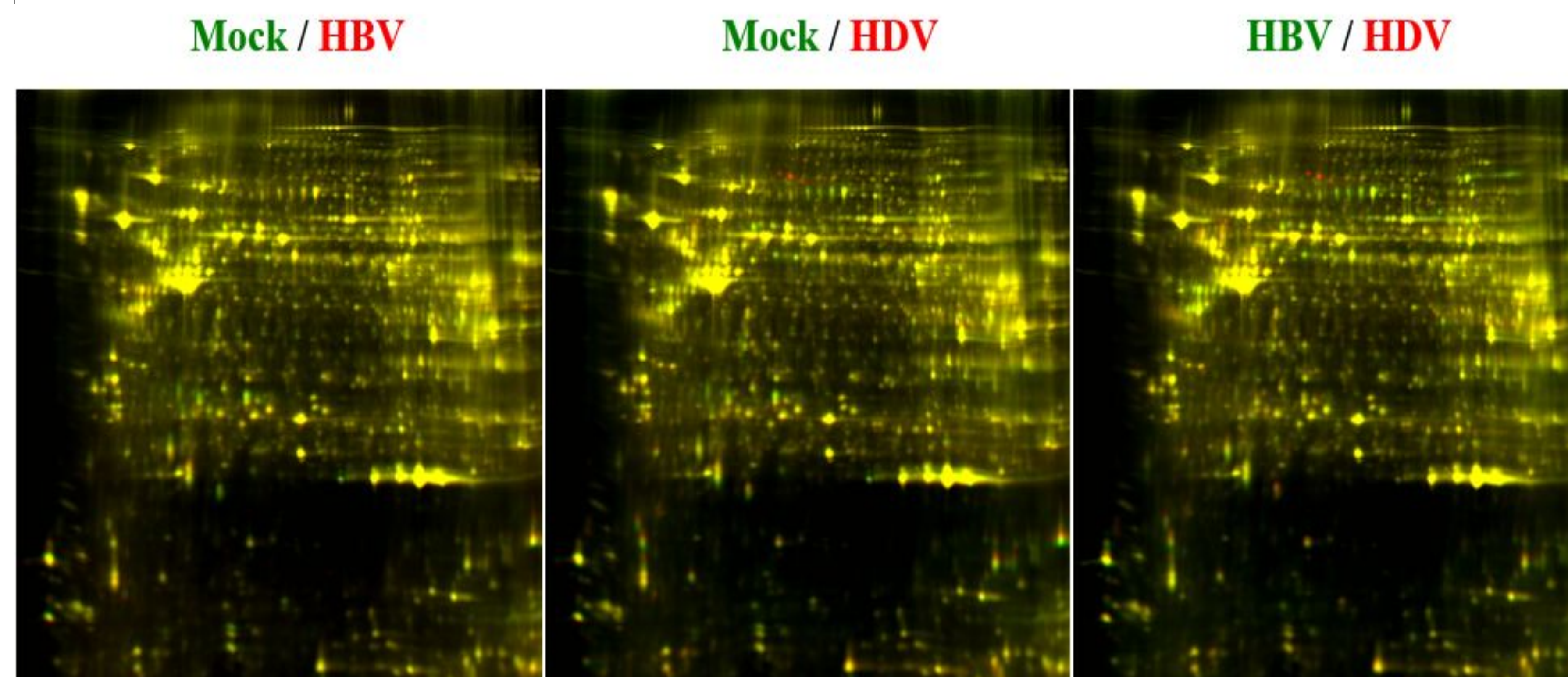


- Samples are subject to separation by molecular weight with SDS PAGE
- Negatively charged proteins migrate towards the positive charge at the bottom, spreading out the denatured proteins by molecular weight

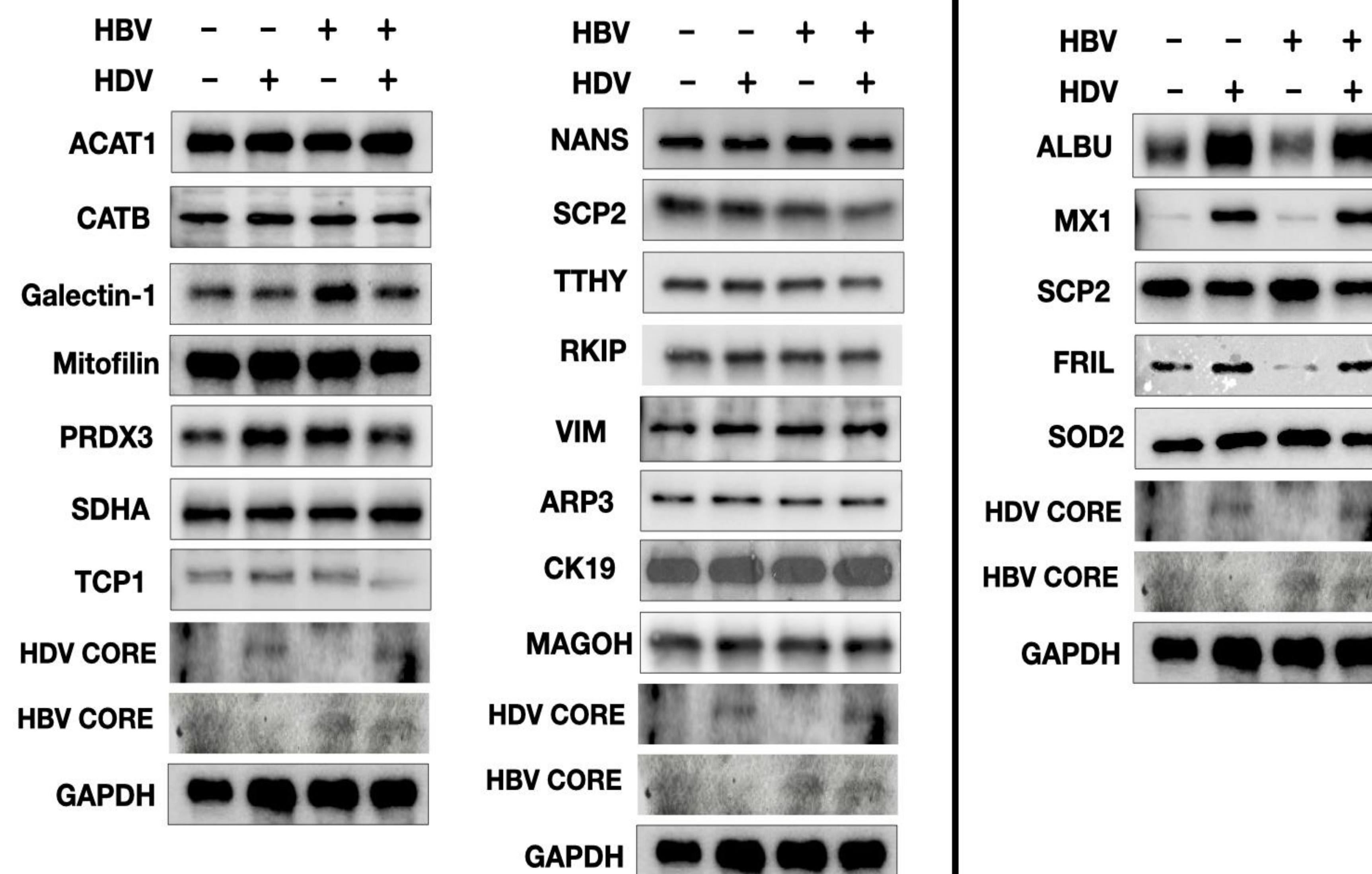
Antibody Probing



2D SDS-PAGE



Western Blot Data



Gene	HDV	HBV	HBV+HDV	Function
ALBU	↑		↑	Prevents fluid from leaking out of blood vessels into tissues
MX1	↑		↑	IFN stimulated gene, anti-viral response
SCP2		↑		Mediates cholesterol
FRIL	↑		↑	Iron storage function
SOD2		↑		Plays an antioxidant role in mitochondria

Summary

- The 2D SDS-PAGE mass spec identified 89 hits.
- Of those 89 hits, the top 32 proteins were subjected to the confirmatory Western Blotting analysis.
- Among those 32 proteins tested in the secondary Western Blotting analysis, 16% (5 molecules) were confirmed to be true hits.
- These true hit molecules were ALBU, MX1, SCP2, FRIL, and SOD2.
- 4 of these true hits (ALBU, MX1, FRIL, and SOD2) were upregulated in human hepatocytes infected with HDV.
- 1 of these true hits (SCP2) was downregulated in human hepatocytes infected with HDV.
- HBV, a secondary control, does not alter the expression abundance of true hits identified in HDV infected cells with the exception of FRIL downregulated in HBV infected cells.

Conclusion

- The primary screening with 2D SDS-PAGE identified 89 differentially expressed proteins in human hepatocytes infected with HDV.
- The secondary Western Blotting confirmatory analysis identified ALBU, MX1, FRIL, SCP2, and SOD2 as true hits.
- This study identified a high rate of false positivity with the primary screening method (protein mass spec with 2D SDS-PAGE), therefore, highlighting the importance of secondary confirmation with Western Blotting analysis.

Future Direction

- The potential antiviral and proviral role of the true hits will first be determined through literature review.
- With a gain of function approach (overexpression) and loss of function approach (gene silencing), the significance of true hits will be confirmed using human hepatocytes that are infected with HDV.

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CONTACT US

bridge.usc.edu/bugs, johannalee4806@gmail.com,
lucasyoshida295@berkeley.edu,
Takeshi.Saito@med.usc.edu