Proteomics Study Reveals a Hydroxysteroid Dehydrogenase (HSD) as a Sex- based Biomarker in Hepatitis C Virus-Induced Cirrhosis

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Introduction

Hepatitis C virus (HCV) infection- related inflammation, liver fibrosis and cirrhosis often lead to development of hepatocellular carcinoma (HCC).[1] In the United States, 4.6 million people are infected with HCV.[2] Studies show that chronic HCV infections are more prevalent in males and progress more rapidly to cirrhosis and cancer development as compared to females. In contrast, pre-menopausal females and women on hormone replacement therapy have been associated with less-severe disease through all stages of HCV infection.[3] . Estrogen Receptor signaling is known to regulate inflammation and immunity. We have previously identified sex-based differences in the expression of estrogen receptors (ERs) in normal livers and dysregulated mRNA and protein expression of ER subtypes in both HCV-related cirrhosis and HCC suggesting a possible role in its pathogenesis.[4]

The study investigates the enzyme families including hydroxysteroid dehydrogenases (HSDs). HSDs contribute largely to the synthesis and degradation of steroid hormones such as testosterone and estrogen sex-hormones, as well as cholesterol and fatty acid metabolism.[5] Chronic inflammation due to HCV infection in the liver may alter HSD enzyme regulation and alter hormone metabolism. Estrogen Receptor signaling is known to regulate inflammation and immunity.

Methods

Explant Liver Tissue Preparation:

Male and female deidentified human explant liver tissue (normal and HCV cirrhosis) obtained from the NIH Liver Tissue Cell Distribution System at the University of Minnesota. The study included 10 males and 10 females in the normal control group, and 10 males and 10 females in the HCV cirrhosis experimental group. There were 40 total samples considered in the study. This study qualified for exempt IRB.

Table 1: Age range of normal and diseased subjects in liver tissue samples

	Number of Subjects	Number of Subjects from Males	Age Range (years)	Number of Subjects from Females	Age Range (years)
Normal Control	20	10	26-70	10	36-72
HCV Cirrhosis	20	10	46-57	10	39-61

Proteomics Study:

Total protein from tissue extracts was reduced, alkylated, and purified by chloroform/methanol extraction prior to digestion with sequencing grade modified porcine trypsin (Promega). Tryptic peptides were then separated by reverse phase using an UltiMate 3000 RSLCnano system (Thermo). Eluted peptides were ionized by electrospray followed by mass spectrometric analysis on an Orbitrap Exploris 480 mass spectrometer (Thermo). To assemble a chromatogram library, six gas-phase fractions were acquired on the Orbitrap Exploris with 4 m/z DIA) using a staggered window pattern from narrow mass ranges using optimized window placements. For wide-window acquisitions, the Orbitrap Exploris was configured to acquire a precursor scan (385-1015 m/z, 60,000 resolution, normalized AGC target 100%, maximum injection time 50 ms) followed by 50x 12 m/z DIA spectra using a staggered window pattern with optimized window placements. Precursor spectra were acquired after each DIA duty cycle.

Statistical Analysis:

Following data acquisition, we searched the data using an empirically corrected library and performed quantitative analysis to obtain a comprehensive proteomic profile. Proteins were identified and quantified using EncyclopeDIA and visualized with Scaffold DIA using 1% false discovery thresholds at both the protein and peptide level.

Protein exclusive MS1 intensity values were assessed for quality using ProteiNorm (ref) to systematically evaluate several normalization methods including log2 normalization (Log2), median normalization (Median), mean normalization (Mean), variance stabilizing normalization (VSN) [6], quantile normalization (Quantile) [7], cyclic loess normalization (Cyclic Loess) [8], global robust linear regression normalization (RLR) [1], and global intensity normalization (Global Intensity) [1]. The individual performance of each method was evaluated by comparing of the following metrices: total intensity, Pooled intragroup Coefficient of Variation (PCV), Pooled intragroup Median Absolute Deviation (PMAD), Pooled intragroup estimate of variance (PEV), intragroup correlation, sample correlation heatmap (Pearson), and log2-ratio distributions. The normalized data was analyzed using Linear Models for Microarray Data (limma) with empirical Bayes (eBayes) smoothing to the standard errors (ref). Proteins with an FDR adjusted p-value < 0.05 and a fold change > 2 were considered to be significant.

Hypothesis

We hypothesized that chronic HCV infection leads to dysregulated HSDs in male HCV cirrhosis patients leading to the development of HCC.

Objective

Identify and profile HSD protein expression in pre-malignant male and female HCV cirrhosis donor liver tissues to determine sex-based differences in disease progression.

Results

4,443 proteins belonging to different protein families that were differentially expressed in HCV cirrhosis and controls were profiled. Within the HSD protein cohort 7 exhibited differential expression in the liver cirrhosis group compared to healthy controls. Our research focused on protein HSD17B13.

Table 2: P-Values and Log Fold-Change Amounts For Each Sample Group Comparison

HSD17B13	HCV Cirrhosis Male v. Normal Control Male	HCV Cirrhosis Female v. Normal Control Female	HCV Cirrhosis Male v. HCV Cirrhosis Female	Normal Control Male v. Normal Control Female
P-value	0.075	0.238	0.003	0.979
LogFC	-1.365	0.896	2.241	0.020

Figure 1: Differential Expression of Basal Estrogen Receptors ∝ and β in Normal Male and Female Liver Tissues (Iyer JK et al., 2017)

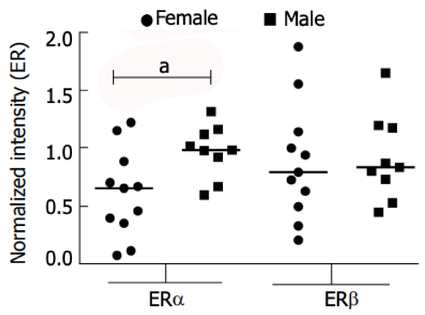

Figure 2: Differential Expression of Protein HSD17B13 Across Normal Controls and HCV Cirrhosis Liver Tissues

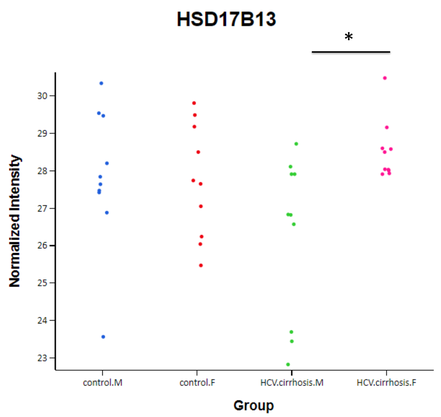

Table 2: Highlights significant difference in HSD17B13 gene expression in male and female HCV cirrhosis groups.
Figure 1: Demonstrates significantly higher basal expression of liver ER∝ in males as compared to females.
Figure 2: HSD17B13 expression was significantly downregulated in HCV cirrhosis males compared to HCV cirrhosis females.

- Of these 7 proteins, only HSD17B13 demonstrated a significant sex-based differential expression between male and female HCV cirrhosis groups.
- HCV cirrhosis males demonstrated decrease with a positive logFC value of 2.241 ($p < 0.01$) when compared to HCV cirrhosis females.
- HSD17B13 showed downregulation in HCV cirrhosis males compared to normal control males, but not significantly ($p=0.075$).
- No differences were observed in the remaining experimental groups (HCV F vs. normal control F and normal control M vs. normal control F).

Conclusions

HSD17B13 protein levels were significantly downregulated in HCV cirrhosis males when compared to HCV cirrhosis females.

HSD17B13 protein may serve as a sex-based biomarker in liver cirrhosis and cancer development.

To the best of our knowledge this is the first report showing sex-based differences in HSD proteins in premalignant HCV-related cirrhosis.

Further Studies

Reduced HSD17B13 protein levels in HCV Cirrhosis males need further investigation.

Including more subjects will prove to be useful in confirming these observations.

mRNA expression of HSD17B13 by qPCR will be performed in order to confirm our data further in these liver tissues.

Immunohistochemistry (IHC) studies will confirm the expression of HSD17B13 protein in various cells of HCV cirrhosis liver tissues.

Using **clinical data,** we aim to correlate liver inflammation parameters with HSD protein expression in these liver tissues to confirm their clinical relevance as a pre-cancerous marker.

References

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Acknowledgments

Thank you OSU-CHS Office of Research and the Research Committee for facilitating the research program. These research efforts were made possible through OSU-CHS intramural grant (RK and AK), Cancer sucks Inc., Bixby OK (RK), and IDeA National Resource for Proteomics (R24GM137786)(LS and RK).
Liver tissues were obtained from NIH Liver Tissue and Cell Distribution System (LTCDS; Contract No. HHSN276201200017C).