Proteomics Study Reveals a Gender-based Ribosomal Inflammatory Biomarker in Hepatitis C Virus Induced Cirrhosis

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INTRODUCTION

According to the CDC, Hepatitis C Virus (HCV) impacts almost 2.4 million Americans and is a main contributor to the development of Hepatocellular Carcinoma (HCC). Clinical evidence suggests that pre-menopausal women, with elevated levels of circulating estrogen, clear HCV infection faster than males[2], and show low incidence of HCC. Our studies have shown gender-based differential estrogen receptor expression in the normal liver, which could contribute to protection in pre-menopausal women against chronic liver diseases including HCC[3,4]. Identifying gender-specific biomarkers that can be used for earlier diagnoses and prognosis of the disease and help in best treatment strategies. Current research has focused on ribosomal proteins as potential biomarkers. Ribosomal protein (RP) genes play an important role in cellular apoptosis, cell proliferation, and cancer development[5,6]. Ribosomal gene FAU (Finkel-Biskis-Reilly murine sarcoma virus) has recently been identified as an apoptosis regulatory gene. FAU expression has been shown to be downregulated in ovarian, breast, and prostate cancer (4,5,6).

OBJECTIVES

- **We hypothesize that Chronic HCV infection may induce gender based differential inflammation leading to dysregulation of RPs**
- The purpose of this study was to identify early cancer biomarkers in HCV-related cirrhosis by analyzing liver proteins-specifically focusing on ribosomal family of proteins (RPs)

METHODS

Samples: Deidentified discarded Normal and HCV cirrhosis liver tissues were obtained from National institutes of Health Liver Tissue and Cell Distribution System located at the University of Minnesota, Minneapolis, MN. Samples were then packaged and sent to NIH IDeA National Resource for Quantitative Proteomics core lab for DIA proteomic analysis.
End-stage liver disease patients with HCV-cirrhosis (N=20: 10 M, 10 F) were used for diseased samples
Normal patients with no HCV diagnosis were used as normal controls (N=20: 10 M, 10 F).
DIA Proteomics: 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. . Proteins were identified and quantified using EncyclopeDIA and visualized with Scaffold DIA using 1% false discovery thresholds at both the protein and peptide level.
This study was conducted under exempt IRB exemptions

RESULTS

- Total number of identified proteins: 4,443
- Total number of Ribosomal Proteins (RP): 132
- Total number of **gender**-based significant RP: 13

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

Group:	No. of subjects	No. of females	Age range (yr.)	No. of males	Age range (yr.)
Normal	20	10	36 - 72	10	26-70
HCV	20	10	39 - 61	10	41-57

Figure 1: Differential expression of ERα at basal level in normal liver tissues (Male v. Female) (Iyer JK et al., 2017)

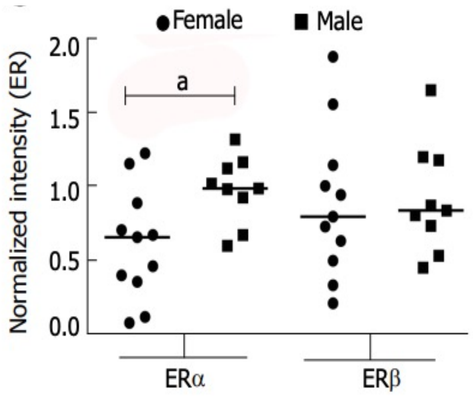
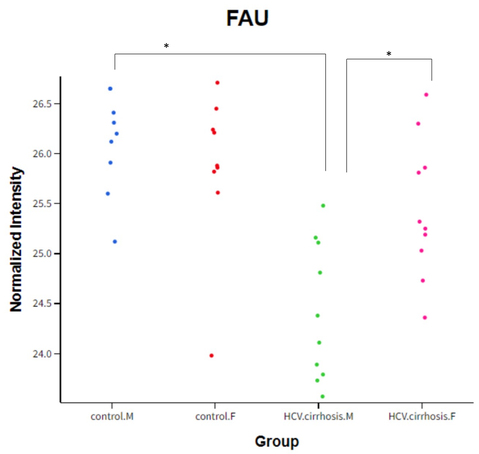


Figure 2: Altered expression of FAU observed in males with HCV-related cirrhosis when compared to females



* Indicates Significance

Table 2: P-values and significance levels among experimental groups

Experimental Group Comparison and Significance				
Group:	HCV Cirrhosis M vs. Normal M	HCV Cirrhosis F vs. HCV Cirrhosis M	HCV F vs. Normal Control F	Normal Control M vs. Normal Control F
P-Value	0.00000241	0.00124717	0.17771874	0.44053286
Significance	✓	✓	NS	NS

CONCLUSION

- FAU was significantly **downregulated** in HCV males compared to HCV female liver tissues.
- FAU was significantly **downregulated** in HCV male liver tissues compared to normal control males.
- FAU RP downregulation may affect inflammation and cirrhosis development in males with HCV cirrhosis.
- **FAU could serve as a valuable prognostic biomarker to monitor HCC disease progression and response to treatment.**

FUTURE DIRECTIONS

- The role of ribosomal proteins, specifically FAU protein, will need further investigation to be confirmed as a potential HCV cirrhosis-induced HCC biomarker.
- mRNA gene expression and cellular protein expression by Immunohistochemistry (IHC) studies will be performed with increased sample sizes to confirm these results.
- We will correlate the clinical data (liver inflammation parameters) with FAU protein expression in these liver tissues to confirm their clinical relevance as pre-cancerous marker.

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ACKNOWLEDGEMENTS

OSU-CHS intramural grant (**RK and AK**);NIH Liver Tissue and Cell Distribution System (LTCDS; Contract No. HHSN276201200017C) Cancer sucks Inc., Bixby OK(RK).IDeA National Resource for Proteomics (R24GM137786)(**LS and RK**).