# IL-33 Expression is Altered in Hepatitis C Virus-Related Pathologies

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## INTRODUCTION

- At least 71 million individuals worldwide suffer from chronic hepatitis C virus (HCV) infection (1)
- This infection can cause fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (HCC) due to the chronic inflammatory response
- IL-33 is a cytokine and nuclear factor implicated in numerous inflammatory and fibrotic diseases (2) including HCC (3)
- The role of IL-33 in HCV infection and HCV-mediated HCC is unknown, as is the mechanism behind the malignant transformation

## OBJECTIVES

- Confirm the presence of IL-33 in human liver tissues
- Determine the cellular location of IL-33 expression
- We hypothesized that IL-33 expression is altered in patients with HCV/cirrhosis and HCV/HCC as compared to normals

## MATERIALS/METHODS

**Samples:** Study was conducted on paraffin-embedded human liver tissues from the NIH Liver Tissue and Cell Distribution System with IRB approval

- Male end-stage liver disease patients with either HCV/cirrhosis (N=7) or HCV/HCC (N=7) were selected if they had no coinfection with HBV or HIV and no history of drug or alcohol abuse
- Normals were patients with no HCV or HCC diagnosis (N=6)

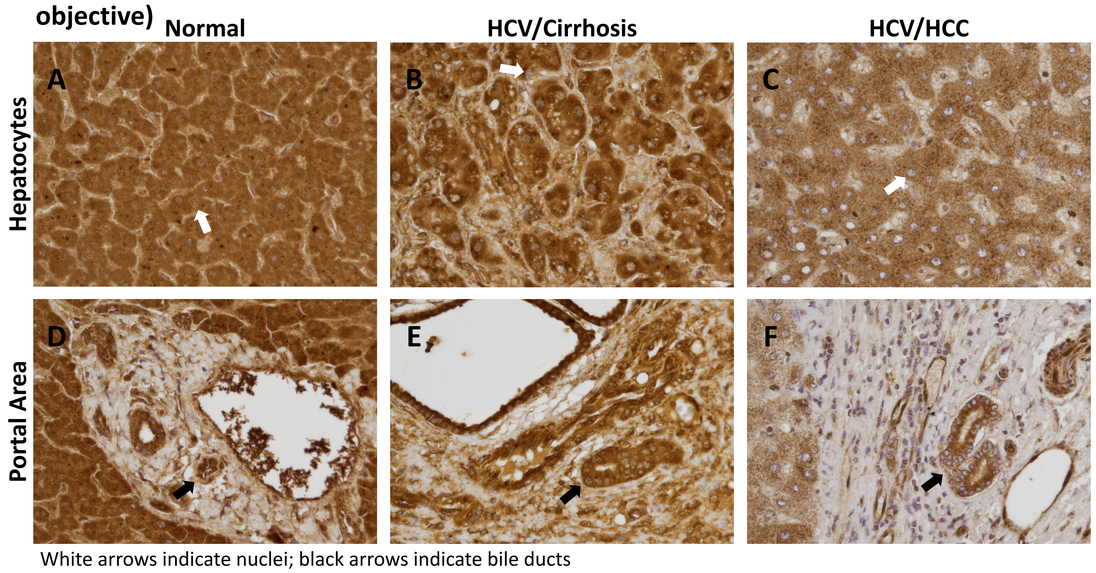
**IHC:** We performed immunohistochemistry (IHC) according to standard protocol. We used a nonenzymatic approach for antigen retrieval. We used human IL-33 as our primary antibody (R and D Systems AF3625) and anti-goat IgG as our secondary antibody (ImmPress Reagent Kit MP-7405). The tissues were stained with DAB (Vector Labs ImmPACT DAB kit SK-4105 ) and counterstained with hematoxylin

**Image Analysis:** Protein positive staining and cellular localization were observed at 20X objective with the Nikon Eclipse Ts2R camera and the NIS Elements Basic Research Software. MATLAB was used (4) to analyze all images and provide an accurate quantification of IL-33 signal strength in energy units/pixel

**Statistical Analysis:** Data obtained from image analysis were analyzed statistically using GraphPad Prism software v7.04. Statistical significance between appropriate groups was determined by the non-parametric Kruskall-Wallis test. Post-hoc analysis was performed using Dunn's multiple comparisons test

## RESULTS

**Figure 1: Immunohistochemical staining of IL-33 in human liver tissues (40X objective)**




White arrows indicate nuclei; black arrows indicate bile ducts

- IL-33 expression was found in human hepatocytes (Figure 1A-C) and in the portal area (Figure 1D-F) across all patients groups
- All normal patients (N=6) showed nuclear expression of IL-33 compared to 57% (N=4/7) HCV/cirrhosis and 29% (N=2/7) HCV/HCC patients
- As compared to normal tissue, IL-33 expression in hepatocytes was increased in HCV/cirrhosis patients and slightly decreased in HCV/HCC patients (Figure 2)
- In the portal area, IL-33 expression was similar across all patient groups (Figure 3)

**Figure 2: IL-33 Expression in Hepatocytes**

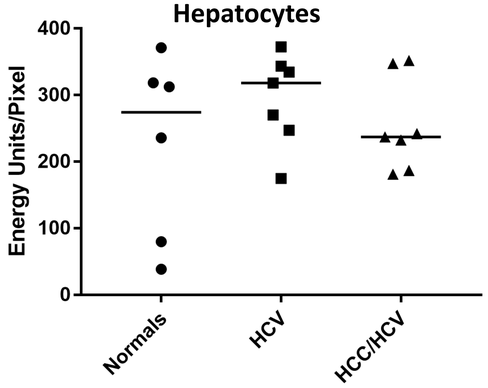
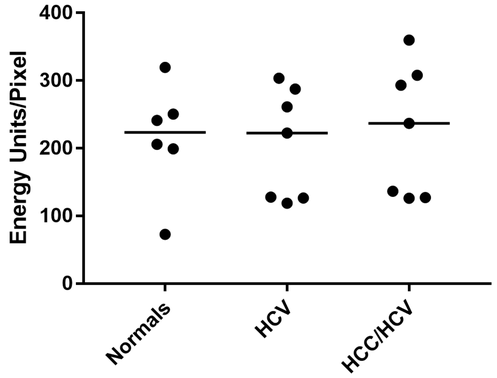



**Figure 3: IL-33 Expression in Portal Area**




## CONCLUSION

- IL-33 is expressed in human hepatocytes and portal areas
- Differential nuclear expression of IL-33 seems to be correlated with worsening disease state
- IL-33 expression as measured by energy units/pixel is different according to disease state

## FUTURE WORK

- We will further investigate the differential expression of IL-33 in the cytoplasm and nucleus by western blot
- RT-qPCR will be used to confirm IL-33 mRNA expression in the liver tissues
- IHC will be performed with the aim of staining the IL-33 receptor, ST2, in an attempt to further ascertain the main area of activity of IL-33
- We will study the correlates of IL-33 with that of various cancer markers, especially NF-κB, p65, and cyclin D1

## REFERENCES

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