Hepatitis C Virus (HCV) is a bloodborne RNA virus that infects ~3 million people in the United States and ~140 million people worldwide. Once infected, only 15-25% of patients are able to clear this virus from their systems without treatment, leaving 75-85% of affected individuals with a chronic, life-long infection. Chronic HCV is often asymptomatic until decades after infection, so many patients are unaware of the need for treatment until damage has already reached advanced stages. Long term HCV infection can lead to several serious diseases, including chronic hepatitis, liver cirrhosis, and liver cancer. In the US, chronic HCV infection is the leading cause for liver transplants. As a RNA virus, mutations in the HCV genome are relatively common. Currently, there are 6 genotypes and at least 50 subtypes of the virus, which can affect response both to pharmaceutical treatment and to the host innate immune response.

When HCV infects a cell, the cell fights the infection by turning on the expression of antiviral genes, such as interferon-β (IFN). Once IFN is produced, it is secreted from the cell, and in turn activates expression of interferon-stimulated genes (ISGs) in the same cell and surrounding cells, thereby triggering the host innate immune response. HCV produces proteins that are capable of blocking (IFN). Without IFN, the host is unable to fight off the HCV infection, which allows the infection to become chronic.

RNA viruses induce IFN gene expression via the RIG-I/MAVS pathway. IFN activates the JAK-STAT pathway in an autocrine and paracrine fashion to activate ISG expression. This study focuses on the ISGs MX1, OAS1, and TRIM14.

• MX1 – Decreased liver damage is associated with specific haplotypes of the MX1 gene, and increased sustained response to HCV treatment is associated with higher expression of MX1 mRNA.

• OAS1 – Inhibition of HCV infection occurs in cultured cells expressing OAS1.

• TRIM14 – Degradation of HCV NS5A occurs in cultured cells expressing TRIM24.

Interferon-β and ISGs

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HCV NS5A Mutants

HCV produces non-structural proteins, such as NS5A, that aid in viral replication and are capable of blocking IFN. As a RNA virus, mutations in the genome are relatively common. This study focuses on two mutant forms of HCV NS5A:

- NS5A 10A
- NS5A H27

HCV NS5A 10A mutant – Leucine deletion
- Leads to increased levels of viral replication

HCV NS5A H27 mutant – Lysine to serine substitution
- Leads to decreased levels of viral replication

HCV NS5A 10A protein is not in the nucleus in unstimulated HeLa cells. Activation of the JAK/STAT pathway induced ISG expression. We will then test IFN mRNA levels via qPCR and perform RT-PCR to quantify mRNA levels in transfected cells.

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Experimental Design

- • Experiment Setup
  - Day 1: Plate HEK 293 cells (10%) on 10cm dishes with 10mL DMEM
  - Day 2: Transfect cells with 2ug NS5A 10A DNA/dish using Lipofectamine
  - Day 3: Infect cells with 20uL Sendai Virus (SV) 4000 HAU/mL to trigger the IFN antiviral pathway
  - Day 4: Harvest cells

- • Analysis
  - Isolate RNA and analyze for purity and quality
  - Perform RT-PCR and q-RT-PCR
  - Confirm NS5A expression in transfected cells
  - Measure gene expression in triplicate of IFN, MX1, OAS1, and TRIM14, using GAPDH as an endogenous control

Results and Discussion

Hepatitis C Virus is an RNA virus capable of establishing a chronic liver infection, which can eventually lead to liver cancer. HCV produces proteins capable of both aiding in viral replication and in blocking the host immune response, allowing for the establishment of this chronic infection. The Akkaraju Lab studies the HCV NS5A protein to better understand how NS5A affects host immunity.

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References

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Future Direction

- Confirm expression of NS5A 10A in transfected cells, and test effect on gene expression of IFN and ISGs
- Repeat qPCR experiments in Huh7 cell line to test IFN response is different in hepatocytes relative to HEK293 or HeLa cells
- Test different SV infection times needed to induce TRIM14 expression
- Repeat experiments with NS5A H27