The Effect of Hepatitis C Virus Non-Structural Protein NS5A on Antiviral Gene Expression

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Abstract

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 (IFNβ). Once IFNβ 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 IFNβ. Without IFNβ, the host is unable to fight off the HCV infection, which allows the infection to become chronic.

Interferon-β and ISGs

RNA viruses induce IFNβ gene expression via the RIG-I/MAVS pathway.[1] IFNβ activates the JAK-STAT pathway in an autocrine and paracrine fashion to activate ISG expression.[2] This study focuses on the ISGs MX1, OAS1, and TRIM14.

- **MX1** – Decreased liver damage is associated with specific haplotypes of the *MX1* gene,[3] and increased sustained response to HCV treatment is associated with higher expression of *MX1* mRNA.[4]
- **OAS1** – Inhibition of HCV infection occurs in cultured cells expressing *OAS1*.[5]
- **TRIM14** – Degradation of HCV NS5A occurs in cultured cells expressing *TRIM14*.[6]

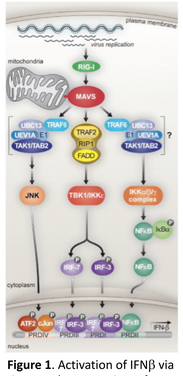


Figure 1. Activation of IFNβ via the RIG-I/MAVS pathway.[1]

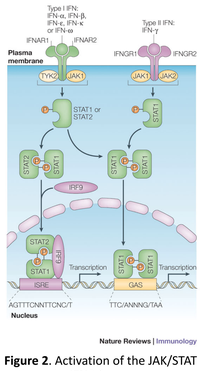


Figure 2. Activation of the JAK/STAT pathway by IFNβ.[2]

Hypothesis

We are attempting to optimize the technique to measure virus-induced ISG expression. We will then test the hypothesis, that the differences in levels of replication between the two mutants of NS5A is due to differential inhibition of SV-induced IFNβ gene expression. Cells expressing NS5A 10A should have lower levels of antiviral gene expression (e.g. the ISGs listed above), and cells expressing NS5A H27 should have higher levels of antiviral gene expression.

References

1. Mcwhirter, Sarah M. et al. "Connecting Mitochondria and Innate Immunity." Cell 122.5 (2005): 645-47. DOI: 10.1016/j.cell.2005.08.026 .
2. Platanias, Leonidas C. "Mechanisms of Type-I- and Type-II-interferon-mediated Signalling." *Nature Reviews Immunology* 5.5 (2005): 375-86. DOI: 10.1038/nri1604.
3. Yee, Leland J. et al. "Myxovirus-1 and Protein Kinase Haplotypes and Fibrosis in Chronic Hepatitis C Virus." *Hepatology* 46.1 (2007): 74-83. DOI: 10.1002/hep.21636.
4. Tang, J., and R. A. Kaslow. "Pharmacogenomic Perspectives of Chronic Hepatitis C Virus (HCV) Infection." *The Pharmacogenomics Journal* 4.3 (2004): 171-74. DOI: 10.1038/sj.tpj.6500246.
5. Wong, Mun-Teng, and Steve S-L Chen. "Emerging Roles of Interferon-stimulated Genes in the Innate Immune Response to Hepatitis C Virus Infection." *Cell Mol Immunol Cellular and Molecular Immunology* 13.1 (2014): 11-35. DOI: 10.1038/cmi.2014.127.
6. Wang, Shanshan et al. "TRIM14 Inhibits Hepatitis C Virus Infection by SPRY Domain-dependent Targeted Degradation of the Viral NS5A Protein." *Scientific Reports* 6 (2016): 32336. DOI: 10.1038/srep32336.
7. Bartenschlager, Ralf et al. "The Molecular and Structural Basis of Advanced Antiviral Therapy for Hepatitis C Virus Infection." Nature Reviews Microbiology 11.7 (2013): 482-96. DOI: 10.1038/nrmicro3046.

HCV NS5A Mutants

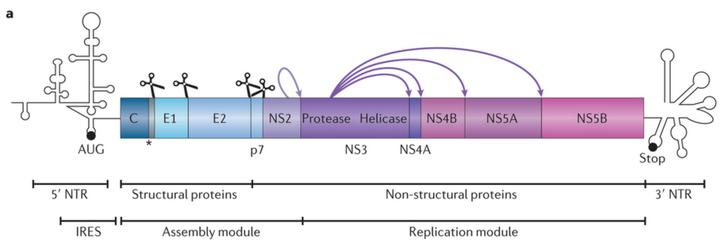


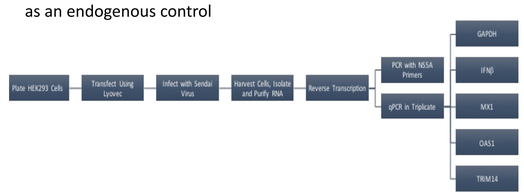
Figure 3. Structure of the HCV genome.[7]

HCV produces non-structural proteins, such as NS5A, that aid in viral replication and are capable of blocking IFNβ.[7] As a RNA virus, mutations in the genome are relatively common. This study focuses on two mutant forms of HCV NS5A.

- **NS5A 10A**
  - K2040 mutant – Lysine deletion
  - Leads to increased levels of viral replication
- **NS5A H27**
  - L2198S mutant – Lysine to serine substitution
  - Leads to decreased levels of viral replication

Experimental Design

- **Experiment Setup**
  - Day 1: Plate HEK 293 cells (10^6) on 10cm dishes with 10mL DMEM
  - Day 2: Transfect cells with 2μg NS5A 10A DNA/dish using LyoVec
  - Day 3: Infect cells with 20μL Sendai Virus (SV, 4000 HAU/mL) to trigger the IFNβ 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 IFNβ, MX1, OAS1, and TRIM14, using GAPDH as an endogenous control



Results and Discussion

NS5A 10A Blocks NF-κB Activation

Red = α-NFκB Green = α-NS5A

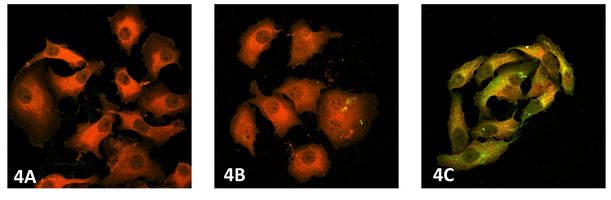


Figure 4. Nuclear localization experiments using confocal microscopy. **(A)** NF-κB is not in the nucleus in unstimulated HeLa cells. **(B)** NF-κB moves into the nucleus when infected with SV. **(C)** NF-κB does not move into the nucleus in SV-infected cells when NS5A 10A is present. Courtesy of Josey Richards.

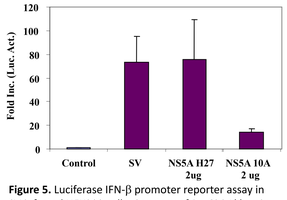


Figure 5. Luciferase IFN-β promoter reporter assay in SV-infected HEK293 cells. Courtesy of Dr. Giri Akkaraju.

- *TRIM14* expression was unaffected by SV infection, which may be a factor of infection time.
- To determine if gene expression results were influenced by the transfection process, the qPCR and Luc-IFNβ promoter experiments were repeated in HeLa cells stably expressing NS5A 10A, and yielded similar results to the experiments with HEK293 transfected cells shown in Figure 6.
- The effect of NS5A 10A on antiviral gene expression may be tissue-specific.

Virus Infection-Induced IFNβ and ISG mRNA Levels were not Inhibited by NS5A 10A

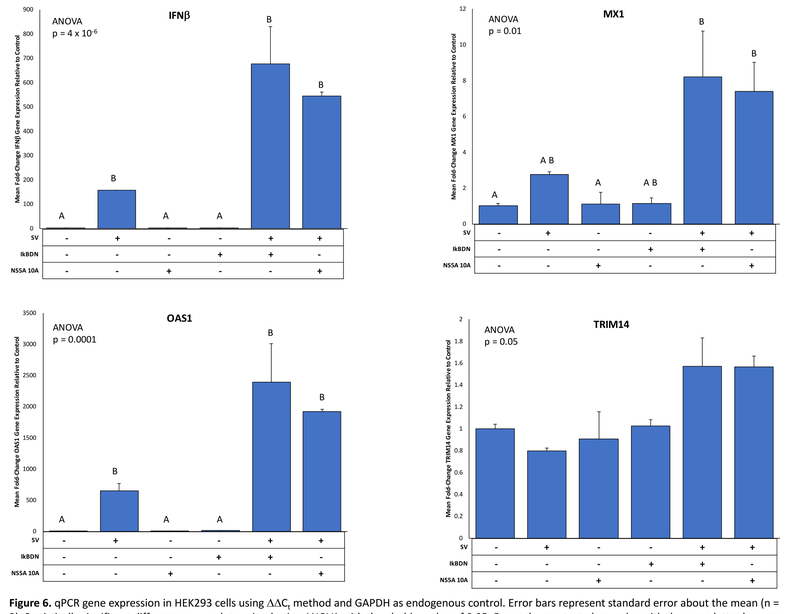


Figure 6. qPCR gene expression in HEK293 cells using $\Delta\Delta C_t$ method and GAPDH as endogenous control. Error bars represent standard error about the mean (n = 2). Statistically significant differences were determined using ANOVA with threshold p-value of 0.05. For each gene tested, samples with the same letter have no statistically significant differences between ΔC_t values, and samples with different letters have statistically significant differences.

Acknowledgements

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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.

Future Direction

- Confirm expression of NS5A 10A in transfected cells, and test effect on gene expression of IFNβ and ISGs
- Repeat qPCR experiments in Huh7 cell line to test if ISG 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