EFFECT OF THE HEPATITIS C VIRUS PROTEIN, NS5A, ON SENDAI VIRUS-MEDIATED ACTIVATION OF TRANSCRIPTION FACTOR, IRF-3

By

Eli A. Reynolds

Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Biology

Texas Christian University

Fort Worth, Texas

May 6, 2019

THE EFFECT OF HEPATITIS C VIRUS PROTEIN, NS5A, ON SENDAI VIRUS-MEDIATED ACTIVATION OF TRANSCRIPTION FACTOR, IRF-3

Project Approved:

Supervising Professor: Giridhar Akkaraju, Ph. D.

Department of Biology

Matthew Chumchal, Ph. D.

Department of Biology

David E. Minter, Ph. D.

Department of Chemistry

Acknowledgements:

My Honors project would not have been possible without the contribution from many people. First, I would like to thank Dr. Giridhar Akkaraju. He has served as my primary contact throughout this entire process. I appreciate the way he designed the research process to give me a direct say in the direction of the project. I can honestly say he has been a great mentor to me throughout my college years. I would also like to thank my lab mates for creating a lab environment that was both fun and challenging. I felt constantly encouraged by the group whether it was in lab meetings or individually. Additionally, I am thankful for my Honors Committee and their willingness to help create a project that I am proud to turn in. Last, I would like to thank TCU for fostering an environment where professors and students can work together in such impactful ways.

TABLE OF CONTENTS

Abstract:

Hepatitis C is a disease of the liver that is caused by the Hepatitis C virus. The Hepatitis C virus (HCV) chronically infects between 130-170 million people in the world making it a significant health burden. The HCV is 9.6 kb single-stranded RNA virus and a member of the *Flaviviridae* family of viruses which include Zika and Dengue. It is a smaller virus with a mature virion size between 50-80 nm. With a specific tropism for liver cells, the diseases of Hepatitis C are accordingly associated with the liver. The two predominant diseases related to HCV infection are cirrhosis and hepatocellular carcinoma. These are both a result of chronic infection which occurs in about 80% of cases. In order to establish a chronic infection, the virus has evolved the ability to inhibit the innate immune response leading to a greater likelihood of reproduction and survival. Our specific interest is the mechanism by which HCV evades the host immune response. In previous studies we have shown that NS5A 10A, a mutant protein of NS5A, inhibits the activation of the IFN-β promoter which serves a key role in the innate immune response. However, the specific mechanism of this inhibition is not fully understood. In this paper we investigate precisely how the NS5A 10A protein interferes with the activation of the IFN-β promoter through studying the effect of NS5A on the transcription factors that activate the IFN-β promoter.

Introduction

The hepatitis C virus (HCV) chronically infects 130-170 million people on the planet. The virus develops into a chronic infection in over 80% of cases. Chronic infection by HCV can cause serious complications like cirrhosis and hepatocellular carcinoma, which are often caused by the hyperinflammation of the liver resulting from the infection. In fact, about a quarter of all cirrhosis and hepatocellular carcinoma cases in the world are due to hepatitis C infection (1). The geographic areas that experience the burden of HCV infection are highly variable. Areas of particularly high incidence include some African countries, Eastern Europe and Russia. In contrast, those with some of the lowest rates of infection include the United Kingdom and Scandinavia (3). The current combinatory treatment of hepatitis C through antivirals like sofosbuvir and daclatasvir along with others is effective. However, similar to other chronic viral infections like HIV, drug resistance is a problem (2). Therefore, the high global burden of disease, the surfacing of antiviral resistance, and the severe complications of a chronic infection (often ending in liver transplantation) highlight the need for continued understanding of HCV.

The HCV was discovered in 1989 to be the cause of non-A, non-B post-transfusion hepatitis indicating that it is a blood-borne virus. This discovery was monumental at the time when the hepatitis C disease had perplexed scientists for years. The HCV sprang onto the global scene for two principal reasons: widespread availability of injectable therapies (medical transmission) and drug use. Some of the predominant modes of transmission involve a contaminated needle seen in both drug-using communities or in medical instrument cases. In these cases, the contaminated needles introduce a sufficient viral load to facilitate the success

of the virus in the body. The distribution of injectable and drug induced transmission varies across locations. The injectable therapy (medical contamination) is the predominant cause in developing countries while drug use is the predominant source of transmission in developed countries (4). Other potential modes of transmission include perinatal transmission which occurs in about 5% of pregnancies with HCV. In this case, HCV RNA must be present in the blood at a sufficient level in order to spread. The spread of HCV through sexual activity is still debated.

Virus Structure:

THE HCV is a member of the *Flaviviridae* family of viruses which are known to cause serious disease and; as described above, diseases associated with HCV are no exception. HCV has a 9.6-kb single-stranded RNA genome and the mature virion is between 50-80 nm. The genome possesses a 5' untranslated region followed by a long open reading frame which is translated into three structural proteins (core, E1, and E2) and seven nonstructural proteins: p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (5) (Figure 1).

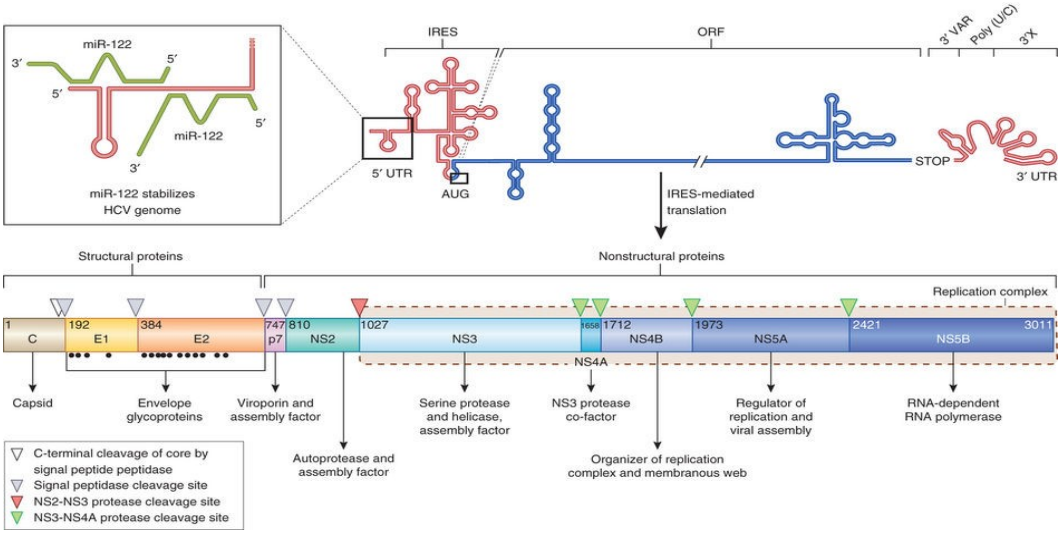

Figure 1. The HCV genome. https://www.nature.com/articles/nrmicro3506

Viral Life Cycle:

In order to understand the functions of these proteins it is effective to look at them in the context of the overall virus life cycle. Further understanding of the life cycle provides opportunities for the development of effective drugs against HCV, especially in an age of increasing resistance of HCV against direct-acting antivirals. HCV has a specific tropism for liver cells. The virus initially associates with the liver cells as a result of a low-affinity binding to LDL receptors or glycosaminoglycans. This low-affinity interaction then leads to the high-affinity interaction of E1-E2 with the co-receptors SR-BI and CD81 (6). Once the virus has closely associated with the liver cell, it will be brought into the cell via clathrin-mediated endocytosis. Upon a drop in pH during the endosomal stage, the virus genome is released into the cytoplasm. There is active research underway to further understand this mechanism of entry so that the mature virion may be targeted for neutralization. However, there has not been much success in this particular area of research because HCV can infect neighboring cells intracellularly and therefore prevents neutralization in the extracellular space by antibodies (7). Thus, the majority of research into pharmaceutical anti-viral targets comes from an understanding of the viral life cycle once the genome has been released into the cytoplasm. Upon release, the genome undergoes the first round of translation utilizing its internal ribosomal entry site (nucleotide sequence part of the 5' untranslated region) to be recognized by host ribosomes for translation (8). The genome is then synthesized into a long polyprotein (9). This non-functional polyprotein is cleaved by both viral (NS3 and its associated NS4A co-factor) and cellular proteases promoting the maturation and activation of the different

functions of the viral proteins. Once cleavage has occurred, the functional proteins are then able to initiate HCV replication. The viral replication complex associates with the endoplasmic reticulum (ER) derived membrane. Within this complex, the proteins NS3, NS4A, NS4B, NS5A, and NS5B combine to form a functional replication complex. Notable functions within this complex include the NS5B protein which is the RNA-dependent RNA polymerase, the NS3 protein which binds nucleic acids to build the viral genome, and the NS5A protein, the specific protein of interest in our project. The NS5A protein regulates the balance between RNA replication and downstream processes such as formation of the mature virion (10). After many rounds of HCV genome replication the last stage of the viral life cycle begins which encompasses the assembly and release of the mature virion. The NS5A protein, specifically the C-terminal domain III, initiates this assembly of the virus through association with the core proteins E1 and E2 (11). The mechanism of genome incorporation for HCV is not very well understood. However, upon formation of a mature virion it is likely that the release is facilitated through the VLDL pathway (12) which then allows HCV to infect surrounding liver cells.

Innate Immune Response:

Fortunately, the host has evolved mechanisms to recognize that viruses such as HCV have invaded the body. These mechanisms include signal transduction pathways that comprise of pattern recognition receptors (PRRs), a successive signal transduction pathway, activation of genes associated with immune function, and then a successive response from surrounding cells as a result of this activation. These innate pathways serve essential functions in fighting off viral infection. An example of an innate immune response pathway is the RIG-I pathway (Figure 2).

RIG-I, a pattern recognition receptor (PRR), recognizes dsRNA which is found in the secondary structure of viruses like HCV, and then upon recognition of the foreign DNA initiates a cellular response. The activated RIG-I activates MAVS, a signal transducer, that then ultimately initiates the movement of transcription factors into the nucleus to activate the IFN-β promoter (Figure 3, Page 11). The IFN-β gene product will then initiate the antiviral state in the surrounding cells to promote general defense (13).

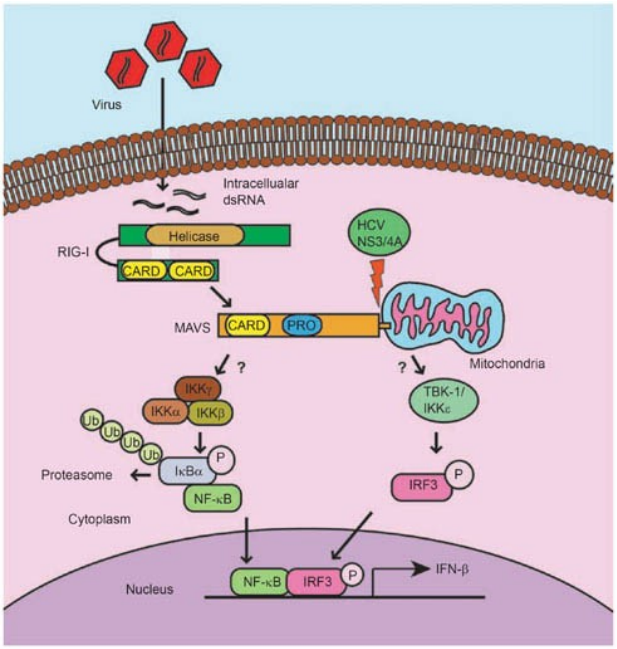

Figure 2. RIG-I signal transduction pathway. https://www.researchgate.net/figure/The-RIG-I-MAVS-signaling-pathway-RIG-I-is-a-receptor-for-intracellular-dsRNA-It_fig2_3829744

The antiviral state involves three essential proteins: RNA-dependent protein kinase (PKR), 2',5'-oligoadenylate synthetase (OAS) and RNase L. The PKR protein, when activated by a foreign genome, halts host translation through phosphorylation and subsequent inactivation of eIF2α, a translation initiation factor. 2', 5'-Oligoadenylate synthetase, upon activation by a foreign genome, produces 2',5'-oligoadenylate which then activates RNase L. The activation of RNase L leads to the degradation of all RNA within the cell. Overall, the result of this antiviral

state is the cessation of both cellular and viral protein production which prevents the successful reproduction of the virus.

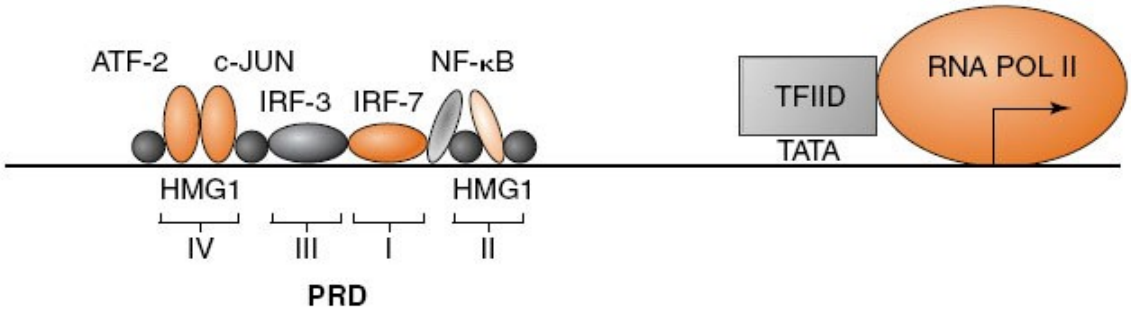

Figure 3. The distinct domains of the IFN-β promoter. Source: Giridhar Akkaraju.

Viral Evasion Strategies:

If the host were completely efficient in its recognition of pathogen-associated molecular patterns (PAMPs) then the story would be over. However, viruses have evolved the capability to disrupt the host's recognition of PAMPs and the subsequent antiviral state. For example, many viruses evade the RIG-I receptor mediated pathway. Some viruses such as Dengue utilize sequestering mechanisms in order to hide themselves from the RIG-I protein which ultimately prevents activation of the innate response (14). In addition to sequestering, viruses have evolved mechanisms to inhibit PRRs directly. A noteworthy example of this is the ability of enteroviruses to cleave MDA5 and RIG-I which accordingly inhibits activation (15). Viruses also possess the ability to target adaptor proteins within the host signal transduction pathway. An example of this is the ability of the adenovirus oncoprotein E1A to bind to STING to prevent induction of type I IFN (18). In summary, viruses have evolved effective mechanisms to target the recognition and successive signal transduction promoting interferon production. However, they have also evolved efficient mechanisms to target the antiviral response. One of the primary targets is the PKR protein. Targeting this protein inhibits the ability of the host to

shutdown protein synthesis and therefore allows the virus to utilize the host mechanisms to replicate.

For our project we were specifically interested in elucidating how the HCV evades the innate immune response. One significant mechanism involves the cleavage of MAVS, a signal transducer in the RIG-I pathway, by the nonstructural protein, NS3/4A (16). The cleavage of MAVS prevents downstream activation of the IFN-β promoter thereby reducing its ability to alert the surrounding cells of the foreign element in the body.

We were interested in investigating how the NS5A 10A protein, a mutant of NS5A, helps HCV evade the host immune response. Mutants of the NS5A protein are of specific interest because they are associated with drug resistance and are consistently found in more virulent strains. This observation directly leads to our investigation into why these mutants are more virulent. The NS5A protein possesses three domains. The N-terminal contains Domain I while Domains II and III are located in the C-terminal (17). It is these different domains that are essential for its function in the HCV replicase.

Hypothesis:

While understanding its function in the replicase is important, we focused on the ability of NS5A mutants to subvert the innate immune response which is associated with the success of the more virulent and resistant strains of hepatitis C. We specifically studied how one virulent mutant labeled in our lab as NS5A 10A (K2040) contributed to the subversion of the immune response. To investigate this, we studied the mutants effect on the Interferon-Beta (IFN-β) response, a key component in host immunity.

We hypothesized that the NS5A 10A mutant of the hepatitis C virus attenuates the innate immune response by preventing activation of the IFN-β promoter by blocking the entry of transcription factors into the nucleus.

Materials and Methods

Cell Culture:

Human embryonic kidney (293 HEK) cells were used in our experiments. They were graciously given by Dr. James Chen from the University of Texas Southwestern. The 293 HEK cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing FBS, Penicillin, Glutamine, and non-essential amino acids. They were stored in an incubator set to 37 °C, 5% CO_2, 95% air. tissue culture incubator and grown in a T25 flask. Cells were split and plated when they reached between 60-80% confluence. For cell splitting, the medium was aspirated from the T25 flask. After aspiration, 1xPBS (Phosphate-buffered Saline) was added in order to wash the cells. The 1xPBS was then aspirated. Next, to detach the cells 1 mL of 37 °C 0.05% Trypsin-EDTA was added for 2 minutes. A microscope was used to ensure that the cells detached. After the 2 minutes passed, the trypsin-cell mixture was triturated to break up the clusters of cells and then 3 mL of DMEM was added in order to quench the trypsin. The trypsin-cell-DMEM mixture was then counted using a Bright-line hemocytometer and plated in a 24-well plate with ~50,000 cells/well.

Plasmids:

Plasmids were utilized for both the reporter genes and the inhibitory proteins in the study. The preparation of each plasmid was accomplished according to the QIAprep Spin Midiprep protocol. The following plasmids were a kind gift from Dr. James Chen at the

University of Texas Southwestern Medical Center: PRD-II Luc, IFN-β-Luc, RL-CMV, EF-IRES-p-NS5A 10A, IκBΔN, and NS3/4A. The IRF-3-Luc plasmid was a generous gift from Dr. Raven Kok from the University of Hong Kong. The IFN-β promoter consists of multiple domains. Two of these domains are the NF-κB or PRD-II domain and the IRF-3 domain. To study the specific mechanism of inhibition of the NS5A 10A protein, the PRD-II domain and the IRF-3 domain were isolated and inserted before the luciferase reporter gene.

Luciferase Assay:

The cells were plated as described above. The day after plating, the cells were transfected. Transfection utilized LyoVec (InVivogen), a lyophilized cationic lipid-based transfection reagent, to facilitate the entry of DNA into the 293 HEK cells. The reporter plasmids used in transfection included IFN-β Luc and RL-CMV. In addition to the reporter plasmids, the cells were transfected with the DNA of interest (NS5A 10A and/or NS3/4A). The day after transfection, I infected designated wells with 4 μL of Sendai Virus (SV, 4000 HAU/mL). On the final day, the 293 HEKs were harvested and gene expression was measured. The medium was removed, 1x PBS was added to wash the cells, and finally 100 μL of Passive Lysis Buffer (PL Buffer) was added and the PL Buffer was shaken for 15 minutes. The 100 μL lysates in the wells were then transferred to centrifuge tubes. Twenty μL of the lysates were then transferred into new centrifuge tubes. To measure gene expression, 50 μL of the Luciferase Assay Reagent (LAR-II) was added to the lysates to measure expression of the IFN-β Luc or IRF-3 Luc gene. After the measurement of the luciferase gene, the Renilla luciferase reaction is initiated by adding 50 μL of the Stop & Glo reagent which measures the expression of the RL-CMV-Luc gene. This reaction is used as a control for transfection and ultimately to standardize the luciferase

activation between the lysates. The resulting activation data were analyzed in Excel by comparing (+SV) to the control cells (-SV).

Results

NS5A 10A inhibits Sendai Virus induced activation of transcription factor NF-κB but does not inhibit activation of the IRF-3 response element in the IFN-β promoter

While we have previously shown that NS5A 10A inhibits the IFN-β promoter (Figure 4), we were specifically interested in the mechanism by which NS5A 10A inhibits its activation. There are several transcription factors that activate the IFN-β promoter. Therefore to understand more fully how NS5A 10A interferes with IFN-β production, it is necessary to investigate more specifically which transcription factors NS5A 10A targets to accomplish its inhibition. Previous research by a former student demonstrated that NS5A 10A prevents activation of the PRD-II portion of the IFN-β promoter (Figure 5). The ability of NS5A 10A to inhibit the PRD-II portion of the promoter suggests the mutant interferes with the movement and activation of the NF-κB transcription factor.

Since inhibition of NF-κB by NS5A had already been demonstrated, our specific interest centered on the effect of the NS5A 10A protein on the IRF-3 transcription factor. In order to test this, IRF-3 binding domain of the IFN-β promoter was isolated and ligated to the luciferase reporter gene to make the IRF-3-Luc reporter gene. This was done by Dr. Raven Kok and her research team at the University of Hong Kong which was then kindly given to us. Upon transfection of NS5A 10A, the activation of the IRF-3-Luc was not inhibited suggesting that the NS5A 10A protein does not inhibit the IRF-3 transcription factor (Figure 6). This finding helps us

narrow the role of NS5A 10A in specifically targeting the NF-κB transcription factor while the IRF-3 transcription factor does not appear to be affected.

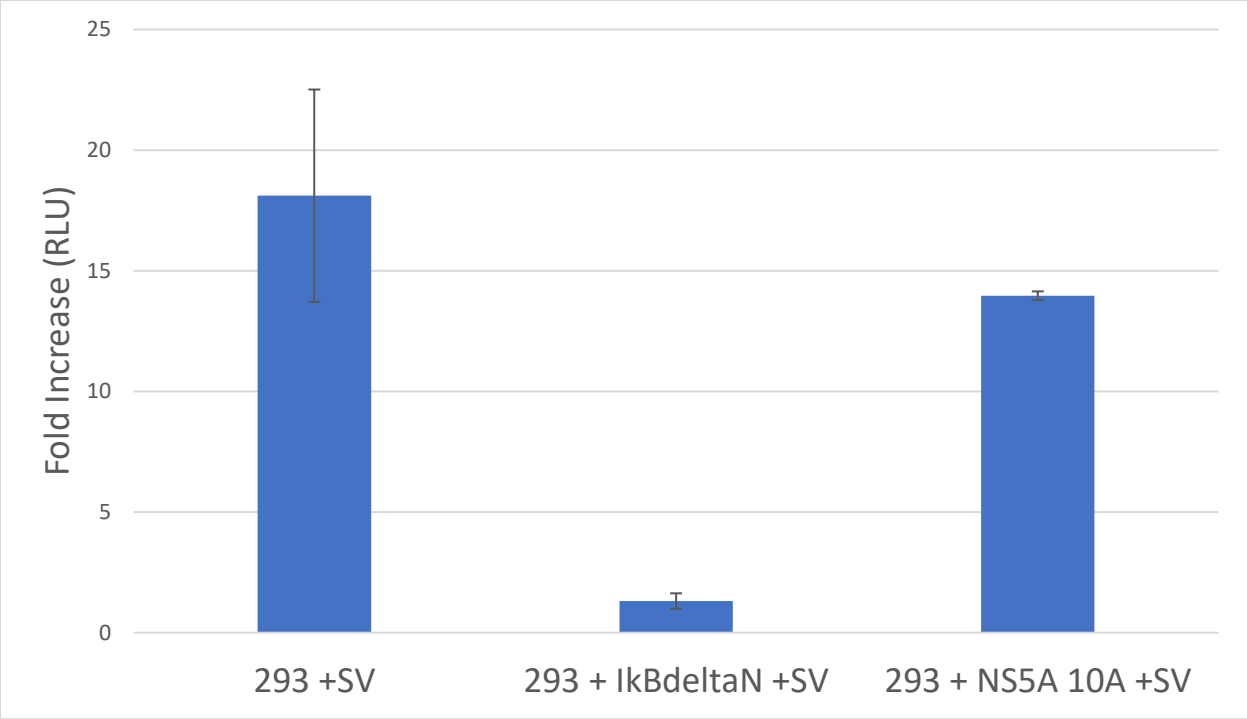

Figure 4. Effect of NS5A 10A on the IFN-β promoter. The IκBΔN serves as a control since it is a known inhibitor of the IFN-β promoter.

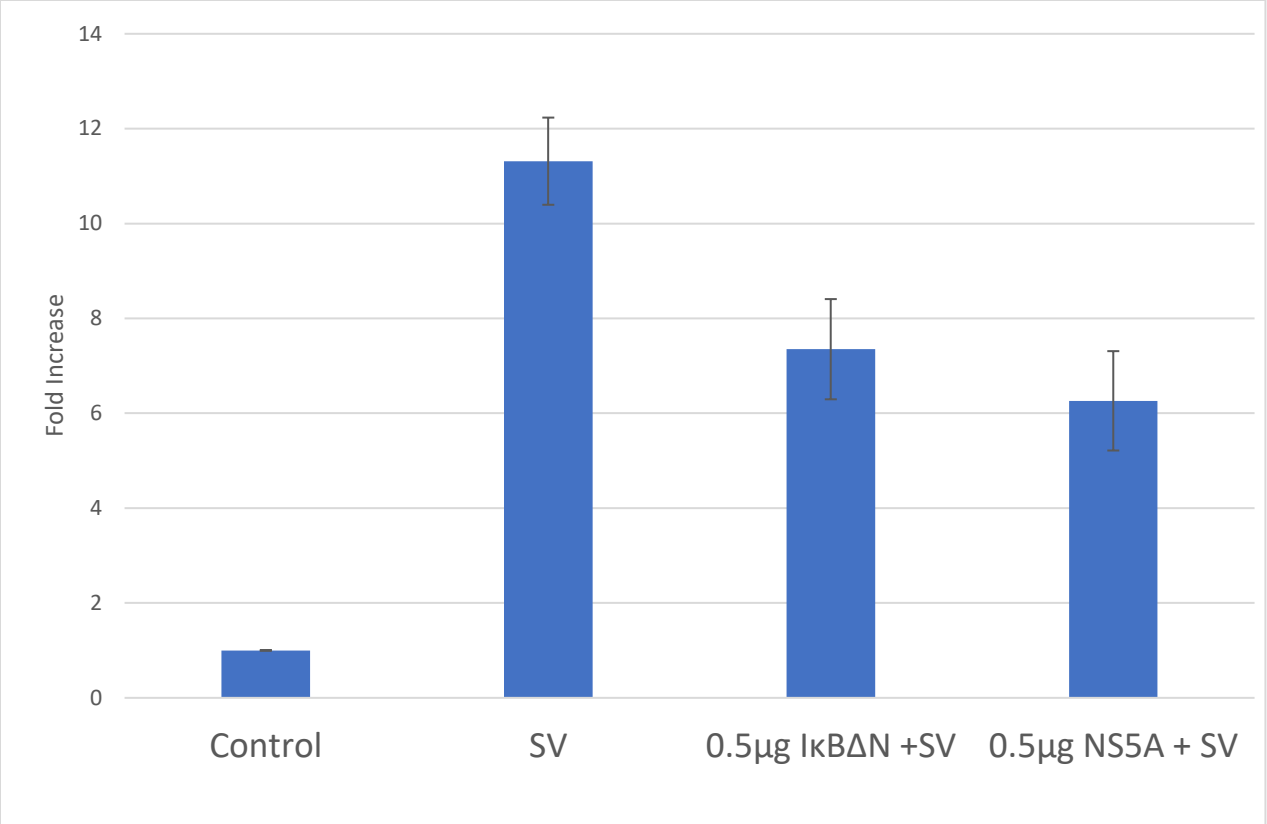

Figure 5. Luciferase Assay showing the effect of NS5A 10A on the NF-κB portion of the IFN-β. IκBΔN serves as the control as a known inhibitor of NF-κB. Error bars are derived from S.D.

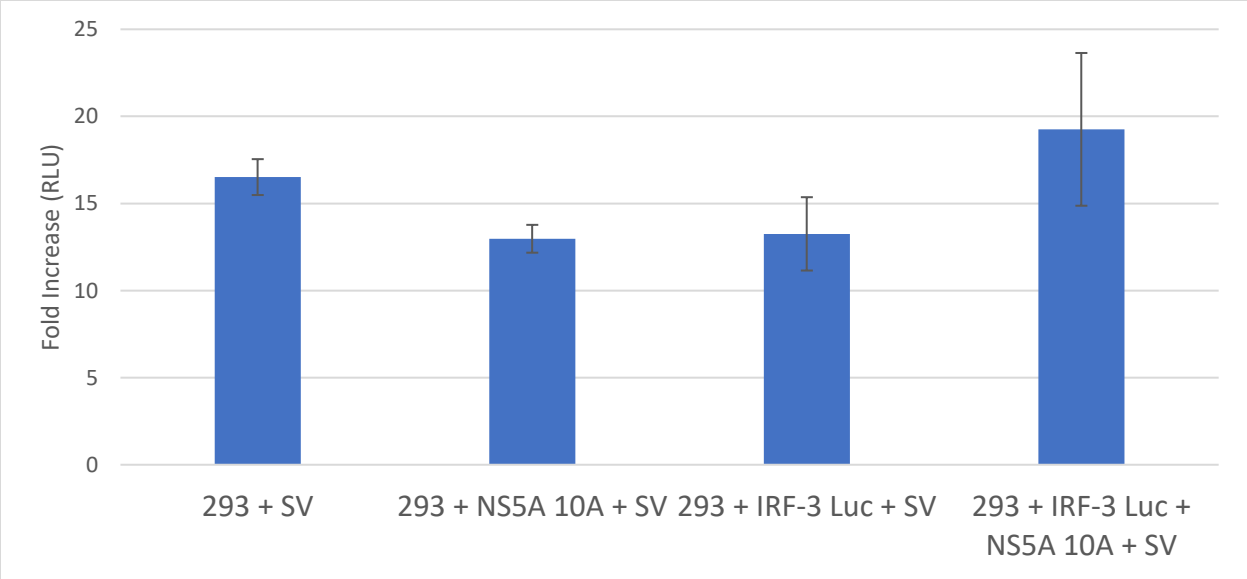

Figure 6. Luciferase Assay showing SV-induced activation of the IFN-β promoter and the IRF-3 promoter. Error bars are shown.

Daclatasvir does not inhibit ability of NS5A to inhibit the innate immune response

The current treatment for Hepatitis C infection is a combination therapy of Sofosbuvir and Daclatasvir (Dac). Dac is a known inhibitor of the NS5A protein and its function in the viral replicase. However, it is unknown the effect of Dac on the ability of NS5A to target and prevent its inhibition of the IFN-β promoter. Upon transfection of NS5A 10A and then successive treatment with Dac before infection with SV, it was expected that Dac would restore activation of the IFN-β promoter. However, that is not what is seen. Upon treatment with Dac, there is no increase in activation of the IFN-β promoter (Figure 6) suggesting that while Dac targets the replicative function of NS5A it does not target its ability to inhibit the innate immune response.

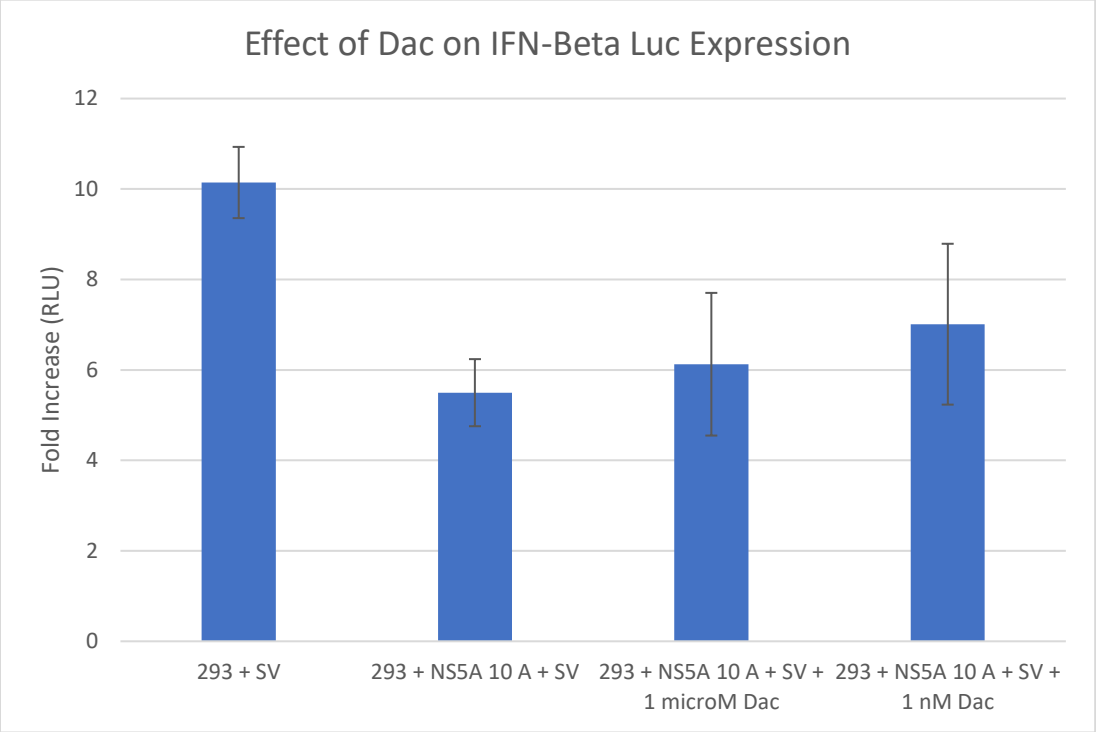

Figure 6. Cells were treated with increasing concentration of Dac 6 hours before infection. The figure displays activation of the IFN-β promoter. Error bars are shown.

NS3/4A does not inhibit activation of PRD-II

To understand further the mechanism of action of NS3/4A, PRD-II, the NF-κB binding domain of the IFN-β promoter was isolated. Previously, studies have shown that the NS3/4A protein cleaves MAVS (16). However, the exact downstream effects are not well understood. We were interested in investigating which transcription factors were affected by the cleavage of MAVS. In order to do this, NS3/4A was co-transfected with the PRD-II-Luc reporter plasmid. The results showed that the NS3/4A protein did not inhibit the activation of PRD-II (Figure 7). This indicates that the NS3/4A viral protein targets MAVS and prevents successive activation of the IRF-3 promoter (19), but it does not appear to have an effect on the NF-κB transcription factor.

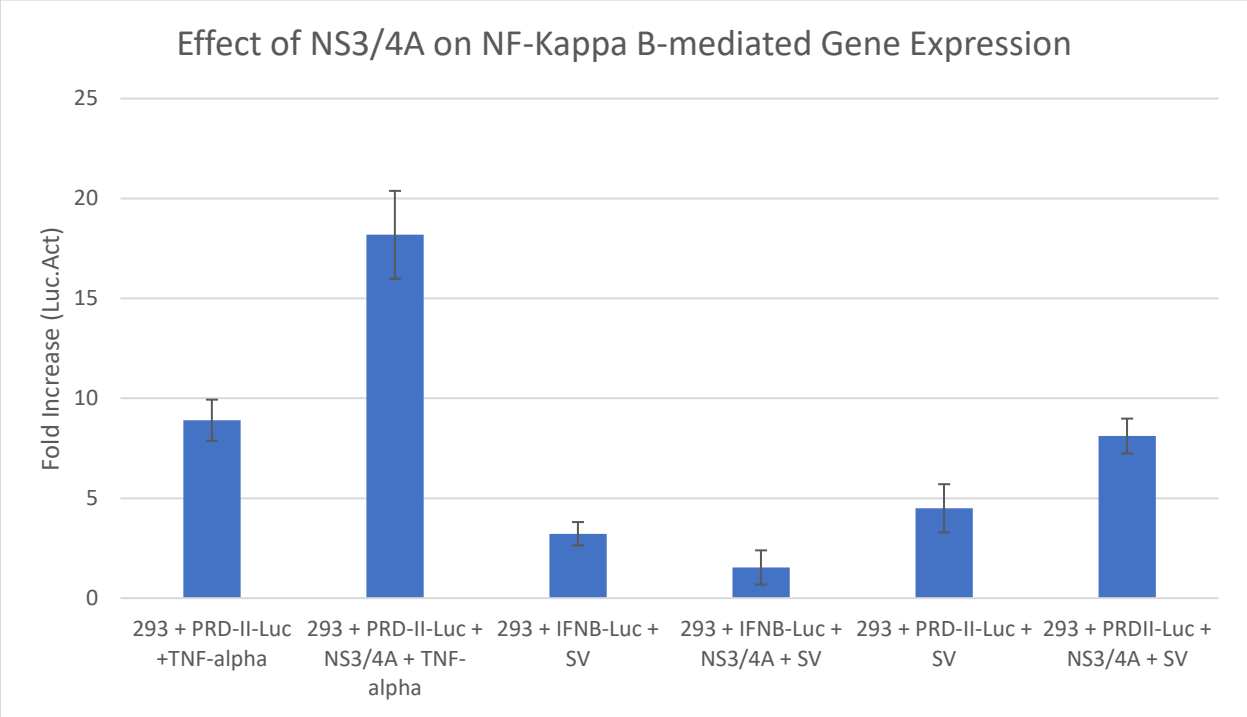

Figure 7. Luciferase Assay showing the effect of the NS3/4A protein on the activation of PRD-II-Luc and IFN-β by SV. TNF-alpha served as the known activator of the NF-κB transcription factor.

Discussion

The Hepatitis C virus encodes nine viral proteins that serve in the replication of the virus and attenuation of the innate immune response. We know that the virus utilizes two distinct proteins to inhibit the activation of the IFN-β promoter, NS3/4A and NS5A 10A, which indicates this function is important for the virus to persist and replicate successfully in the body. The inhibition of the IFN-β production–the first line of defense against infection–allows the virus to hide from the immune system. This prevents the body from mounting a sufficient response to target the virus therefore facilitating the establishment of chronic infection.

We investigated more specifically the mechanisms by which the Hepatis C virus targets the innate immune response. Studying the effect of the viral protein on the IFN-β promoter has furthered our understanding of the mechanism by which both HCV proteins NS3/4A and NS5A 10A targets the activation of the cytokine response. The NS3/4A viral protease is known to cleave MAVS preventing the successive signal transduction pathway. We studied the effects of NS3/4A on the transcription factors downstream of MAVS. A previous study by an honors student showed that the protease targets the movement of IRF-3 into the nucleus and found that NS3/4A inhibited the movement of IRF-3 into the nucleus. So our interest then turned to the effect of NS3/4A on the movement of the NFκB transcription factor into the nucleus. To study this, we isolated the PRD-II portion of the IFN-β promoter and found that NS3/4A did not inhibit the activation of PRD-II. Consequently, it does not have an effect on the NF-κB transcription factor and works solely on the IRF-3 activation pathway.

In addition to further illuminating the mechanism of NS3/4A, we were interested in understanding how the NS5A 10A protein targets the immune response. Previously, it was known that NS5A 10A inhibits the transcription factor NF-κB. However, our specific interest

focused on its effect on the IRF-3 transcription factor. To investigate this, we isolated the IRF-3 portion of the IFN-β promoter and measured its activation in the presence of NS5A 10A. Our results showed that NS5A 10A has no effect on the Sendai-virus mediated activation of the IRF-3 promoter suggesting NS5A 10A does not target the IRF-3 transcription factor. Understanding the mechanisms of action of the two proteins further illuminates the two-pronged approach used by HCV to evade the body's immune response: NS3/4A targets IRF-3 while NS5A 10A targets NF-κB.

Understanding how HCV utilizes this two-pronged approach to hide from the innate immune system further elucidates potential new targets for novel drugs. Additionally, our research sheds light on how the strains containing a mutant NS5A could be more virulent than others and might be associated with resistance.

Furthermore, the knowledge of how Hepatitis C evades the innate immune response can be utilized to study how other viruses in the *Flaviviridae* family such as Zika establish a successful infection in the body. The findings outlined in this study can guide further research into Zika virus and the mechanism by which it is able to evade the host immune response.

References:

1. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. (2006). The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cnacer worldwide. *J Hepatol*. 529-538.
2. Rong L, Dahari H, Ribeiro RM, Perelson AS. (2010). Rapid emergence of protease inhibitor resistance in hepatitis C virus. *Sci Transl Med. ; 2(30):30ra32; 2(30):30ra32*
3. Alter MJ, Hutin YJ, Armstrong GL (2000). Epidemiology of hepatitis C. San Diego: Academic Press; 2000. pp. 169-183.

4. Des Jarlais DC, Diaz T, Perlis T Vlahov D, Maslow C, Latka M, Rockwell R, Edwards V, Friedman SR. (2003). Variability in the incidence of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus infection among young injecting drug users in New York City. *Am J Epidemiol. 2003 Mar 1: 157(5):467-71.*
5. Kato N. (2000). Genome of the human hepatitis C virus (HCV): gene organization, sequence diversity, and variation. *Microb. Comp Genomics. 2000:5(3):129-51. Review.*
6. Pileri, P. et al. (1998) Binding of hepatitis C virus to CD81. *Science* 282, 938-941.
7. Timpre, J.M. (2008) Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology* 47, 17-24.
8. *Honda M. Ping LH, Rinbrand RC, Amphlett E, Clarke B, Rowlands D, Lemon SM.* (1996). Structural requirements for initiation of translation by internal ribosome entry within genome-length hepatitis C virus RNA. 222(1):31-42.
9. Alvisi G, Maden V. Bartenschlager R. (2011). Hepatitis C virus and host cell lipids: an intimate connection. RNA Biol. 8(2):258-69
10. Moradpour D., F. Penin, and C.M. Rice. (2007). Replication of hepatitis C virus. Nat. Rev. Microbiol. 5:452-463.
11. Nicole Appel, Margarita Zayas, Sven Miller, Jacomine Kriinse-Locker, Tortsen Schaller, Peter Friebe, Stephanie Kallis, Urike Engel, and Ralf Bartensschlager. (2008) Essential role of domain III of nonstructural protein 5A for hepatitis C virus infectious particle assembly. 4(3):e1000035.
12. Coller K.E. (2012). Molecular determinants and dynamics of hepatitis C virus secretion. PloS Pathog. 8, e1002466.
13. Liu, Sigi & Cai, Xin & Wu, Jiaxi & Cong, Xiang & Li, Tuo & Du, Fenghe & Ren, Junyao & Wu, Youtong & Grishin, Nick and J Chen. (2015). Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. Science. 347. 10.1126/science.aaa2630.
14. Welsch S, Miller S, Romero-Brey I, Merz A, Bleck CK, Walther P, Fuller SD, Anthony C, Kriinse-Locker J, Bartenschlager. (2009). Composition and three-dimenstional

architecture of the dengue virus replication and assembly sites. Cell Host Microbe. 5(4):365-75

15. Feng Q, Langereis MA, Lork M, Mguyen M, Hato SV, Lanke K, Emdad L, Bhoopathi P, Fisher PB, Lloyd RE, van Kuppeveld FJ. (2014). Enterovirus 2Apro targets MDA5 and MAVS in infected cells. J. Virol. 88(6):3369-78.
16. Chen J, Xiao-Dong Li, Lijun Sun, Rashu B, Set, Gabriel Pineda. (2005). Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. 102(49):17717-17722.
17. Timothy L. Tellinghuisen, Joseph Marcotrigiano, and Charles M. Rice. (2006). Structure of the Zinc-Binding Domain of an Essential Replicase Component of Hepatitis C Virus Reveals a Novel Fold. Nature.
18. Lau L, Gray EE, Brunette RL, Stetson DB. (2015). DNA-tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway. Science. 350(6260):568-71
19. Gagné B, Tremblay N, Park AY, Baril M, Lamarre D. (2017). Importin b1 targeting by hepatitis C virus NS3/4A protein restricts IF3 and NF-kB signaling of IFN-b antiviral response. 18(6):362-377.