HEPATITIS C VIRUS PROTEIN NS5A BLOCKS SENDAI VIRUS-MEDIATED NUCLEAR TRANSLOCATION OF THE TRANSCRIPTION FACTOR NF-κB

by

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Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Biology

Texas Christian University

Fort Worth, TX

May 2, 2016

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Abstract

Hepatitis C Virus (HCV) is an enveloped, positive-stranded RNA virus that infects liver cells, leading to liver damage and cancer. HCV infection causes Hepatitis C in an estimated 170 million people worldwide. Currently, there are treatment options for Hepatitis C but treatment is not 100% effective and instigates severe side effects. Further research into the life cycle and pathogenesis is necessary to develop better treatments and potentially a vaccine. HCV evades the host cell immune response through multiple mechanisms, one of which is through the inhibition of interferon-B expression by NS5A: K2040. When a cell detects viral infection, three transcription factors translocate from the cytoplasm into the nucleus to bind to the IFNβ promoter: ATF-2, IRF-3, and NF-κB. Binding to the promoter turns on the IFNβ gene and leads to IFNB expression. Therefore, HCV NS5A: K2040 could potentially prevent IFNβ expression through inhibiting the translocation of one or more of the transcription factors. Using immunofluorescence analysis in HeLa cells, the cellular location of transcription factors before and after viral infection was examined. The results indicated that NS5A does not block the translocation of ATF-2 or IRF-3. However, NS5A prevented translocation of NF-κB indicating that NS5A attenuates IFNβ expression through inhibition of the translocation of NF-κB.

Acknowledgements

I would like to thank the people that made completion of this thesis possible. First, I would like to thank Dr. Akkaraju for providing me with the opportunity to participate in research and learn through his guidance, knowledge, and patience. I would also like to thank Dr. Misamore and Dr. Strzhemechny for serving on my thesis committee and proving their feedback and suggestions. I could not have completed me research without the members of my lab both past and present who helped maintain the research facility, collaborated with me, and overall, created an enjoyable atmosphere. I would also like to thank the members of my family for their words of encouragement. Additionally, funding for my research was supported by a grant from the Science and Engineering Research Center (SERC). SERC also provided me the experience of presenting my research in a poster format at the Student Research Symposium (SRS). Thank you SERC for your continued support of TCU students and our education. Lastly, TCU has given me the opportunity to participate in research and receive an education, for which I am extremely grateful!

Table of Contents

Introduction

Hepatitis C Virus (HCV) is an enveloped, positive-stranded RNA virus within the family *Flavivirdae*. It is estimated that 130-170 million people, or about 2% of the world's population, are chronically infected with HCV (Scheel et al., 837). HCV is blood-borne and can be transmitted through donated blood or organs, mother to child, or sharing needles for IV drug use. In approximately 75-85% of cases, HCV chronically infects hepatocytes causing Hepatitis C. HCV infection can lead to liver cirrhosis or cancer and is the most common indication for liver transplantation in the United States (Foy et al., 1145).

HCV infection was previously treated with pegylated inferferon-α (peg-IFN-α) and ribavirin. The treatment lead to virologic cure in 50% of patients but peg-IFN-a caused severe side effects such as flu-like and neuropsychiatric symptoms, autoimmune diseases, and hemolytic anemia. Triple combination therapy, adding one of two direct-acting antivirals protease inhibitors, was approved in 2011 and increased cure rates to 70%. However, problems with current therapy options such as severe side effects, drug-drug interactions, and resistance still persist (Scheel et al., 837). Further understanding of the HCV lifecycle will allow for the development of improved treatment options and potentially a vaccine.

The HCV particle is icosahedral in shape, packaging the 9.6 kb ssRNA genome. The genome is composed of one large open reading frame (ORF) flanked by 5' and 3' untranslated regions. Translation of the ORF at an internal ribosome entry site (IRES) gives rise to a polyprotein, which is processed to produce nine viral proteins: three structural (C, E1, and E2) and seven nonstructural (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Scheel et al., 837). The nonstructural proteins consist of a capsid protein, protein C, and two envelope glycoproteins, E1 and E2. E1 and E2 interact with the co-receptors SR-BI and CD81 to facilitate viral entry.

HCV enters hepatocytes through clathrin-mediated endocytosis and the low pH of endosomes facilitates fusion. Currently, E1 and E2 are being explored as potential fusogens for this process (Scheel et al., 837).

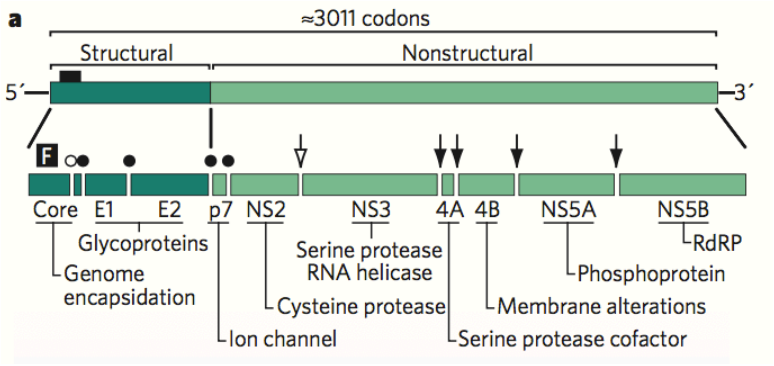

Figure 1. The HCV genome (Lindenbach et al., 933)

In the cytoplasm, the HCV genome is translated by host ribosomes and the resulting polyprotein is cleaved by signal peptidase and HCV NS2 and NS3/4A (Lindenbach et al., 933). NS4B alters cytoplasmic membranes to form a membranous web to serve as a scaffold for replication complex assembly. NS5B encodes the RNA-dependent RNA polymerase (RdRP) responsible for replicating the HCV genome. Replication and assembly are regulated by the phosphoprotein NS5A (Lindenbach et al., 933).

The host cell may detect viral infection through pathogen recognition receptors (PRRs), which detect pathogen-associated molecular patterns (PAMPs) presented by the virus. Examples of PAMPs are double-stranded RNA, nucleic acid products of viral replication, or polyuridine signatures. Retinoic acid-induced gene I (RIG-I) is a PRR that recognizes viral dsRNA, such as in HCV infection. RIG-I interacts with the protein mitochondrial antiviral signaling (MAVS), propogating a signal transduction pathway to induce IFNB expression (Li et al., 17717). The signal transduction pathway leads to the activation of transcription factors, causing translocation of the transcription factors from the cytoplasm into the nucleus. The transcription factors ATF-2, IRF-3, and NF-κB create an enhancesome

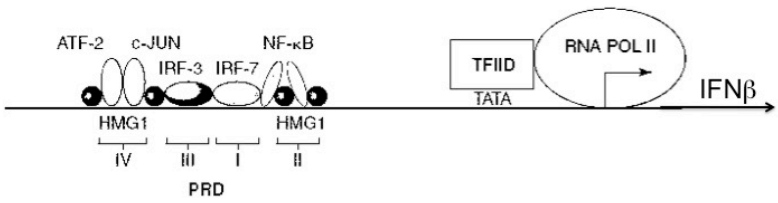

Figure 2. Promotor region for the IFN-B gene. (Acheson, 2011).

on the IFNβ promoter, stimulating IFNβ transcription and expression. IFN-B is secreted from the cell and binds to IFNβ receptors in a paracrine and autocrine manner. Binding of IFNβ receptors leads to activation of Jak-STAT, which causes a signal cascade leading to the expression of IFN-stimulated genes (ISGs) (Gale Jr et al., 939). Expression of ISGs fights viral infection through preventing viral replication and spread.

One product of the ISGs is the protein kinase R (PKR). PKR is an enzyme activated by dsRNA, as seen in viral infection, that phosphorylates eIF-2. Phosphorylated eIF-2 binds to eIF2B and the complex functions to reduce protein synthesis within the cell. Protein synthesis is further reduced by destruction of RNA by RNAse L, an enzyme activated by 2'5'-oligoadenylates. OAS proteins are products of ISGs and produce 2'5'-oligoadenylates when activated by dsRNA. Inhibition of protein synthesis is non-specific, disabling both the virus and the host cell. The host cell may also be killed as a preventative measure through IFNβ induced stimulation of p53 activity, leading to initiation of apoptosis (de Veer et al., 912). Therefore, IFNβ expression is tightly regulated because cells cannot function and thrive without protein synthesis. In addition, interferons and related cytokines are the cause of fever, headache, and flu-like symptoms associated with infection.

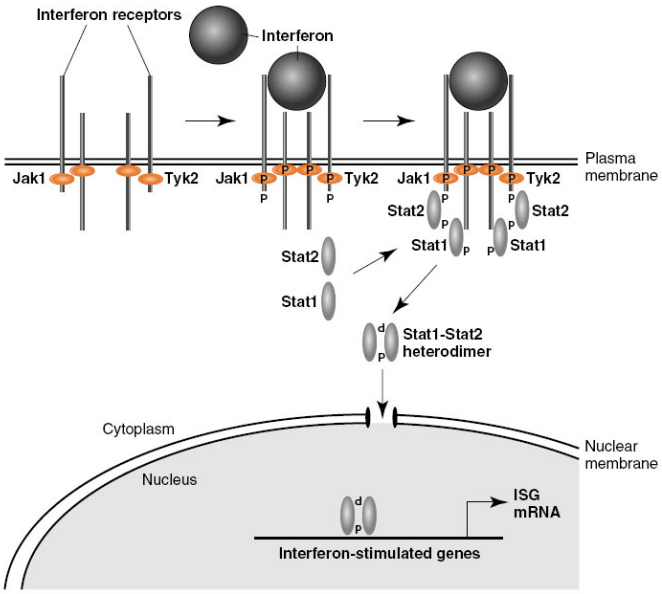

Figure 3. Activation of interferon-stimulated genes by interferon receptors. (Acheson, 2011).

The ability of HCV to chronically infect hepatocytes can be attributed to its ability to attenuate the host cell antiviral response. It is necessary for MAVS to be in close proximity to the

mitochondria to perform its signaling function. The HCV protease NS3/4A has been shown to cleave MAVS at the N-terminal domain, causing the disassociation of MAVS with the mitochondria. MAVS is rendered unable to propagate the signal and important downstream signaling proteins such as IkB kinase and TBK1 are not activated. The end result is inhibition of RIG-I-mediated induction of the expression of IFNβ (Li et al., 17717).

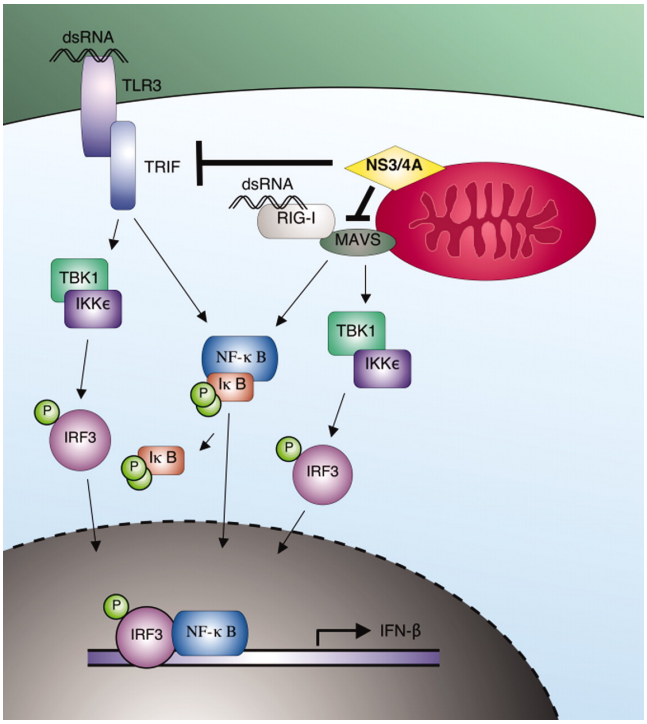

Figure 4. Attenuation of IFN-β activation by Hepatitis C Virus (Seth et al., 2006).

Previous research has indicated that the HCV non-structural protein NS5A, specifically the K2040 mutant, also inhibits the expression of IFNβ but the exact mechanism is unknown. K2040 is an insertion of a lysine within the NS5A coding sequence (Sumpter et al., 2004). One way in which NS5A could attenuate expression is through inhibiting the translocation of transcription factors specific to the IFNβ promoter: IRF-3, ATF-2, and NF-κB. Promoter binding of all three transcription factors is necessary for gene expression. Without promoter binding of IRF-3, ATF-2, and/or NF-κB, the IFNB gene would not be turned on, rendering the host cell unable to respond to viral infection. The purpose of this study was to test the hypothesis that NS5A blocks IFNβ expression through inhibiting one or a combination of the transcription factors.

Materials and Methods

Cell Culture and Splitting

HeLa cells and HeLa NS5A 10A cells were grown in 25 cm^3 tissue culture flasks. The flasks were filled with 6 mL of complete medium (Sigma-Aldrich®) and placed in a CO_2 treated incubator. When the cells became confluent and began to overgrow the surface area of the flask, the cells were split in a hood to prevent contamination. Medium was aspirated from the flasks and replaced by 1 mL 10x PBS. The PBS was rotated around the flask and then aspirated and replaced by 1 mL trypsin. The trypsinized flask was placed in the incubator for approximately 2 minutes, or enough time so that the cells began to detach themselves from the bottom of the flask. Using a 1 mL pipette, the cells were titrated multiple times and then 0.2 mL of the cell solution was transferred to a new 25 cm^3 tissue culture flask containing 6 mL of complete medium.

Plating of Cells

The same procedure was used for both HeLa cells and HeLa: NS5A K2040 cells. Cells were plated for use in experiments when the tissue culture flasks became confluent. The cell splitting procedure was used except 3 mL of trypsin was added to the flask before titration to quench the trypsin and prevent cell damage. A sample of the cell solution was added to a hemocytometer in order to calculate the cell density. Using this measurement, the volume of solution needed to plate 5,000 cells was determined. One cover slip was placed in each well of a 6-well tray. Sterilized tissue culture was rinsed on top of each

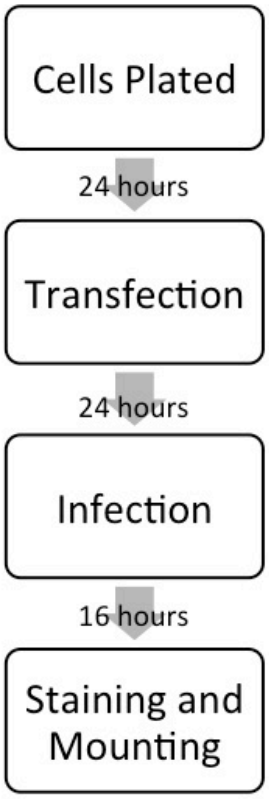

Figure 5. Timeline of experimental design.

cover slip and removed. The calculated volume of cell solution was then added to each well on top of the coverslips. The tray was placed in the incubator for approximately 20 minutes before 2 mL of complete medium was added to each well.

Transfection of Cells with NS3/4A

The following procedure was used in experiments in which cells were transfected with the plasmid expressing NS3/4A. Transfection occurred approximately 24 hours following plating of cells and 24 hours prior to cell infection. LyoVec™ (InvivoGen) solution was brought to room temperature and vortexed for approximately 30 seconds. 3 ug of NS3/4A DNA was added to 100 μL LyoVec™ and incubated for 30 minutes at room temperature. 100 μL of solution containing 3 μg of DNA was prepared per well requiring transfection. The solution was then added to the wells.

Infection with Sendai Virus

Infection occurred approximately 24 hours after cell plating or transfection and 16 hours prior to cell staining. Sendai Virus (Charles River Laboratory, Cantell strain), further noted as SV, was stored at -80°C. SV was removed from the freezer and transferred to the hood in a container filled with ice to prevent thawing. The vial of SV was allowed to thaw such that the required volume of solution was obtainable. Either 5 μL or 10 μL of SV, at a titer of 6,000 HAU/mL, was added to each well depending on the particular experimental design.

Treatment with TNF-α

Similar to SV, TNF-α (Sigma-Aldrich®) was stored at -80°C and transported on ice when used for experimentation. The vial was thawed for the minimum necessary amount of time to obtain the volume needed. Under the laminar flow hood to prevent contamination, the appropriate volume of TNF- α (10 μg/mL) was added to the medium of the wells using a micropipette.

Either 2 μL or 10 μL, equating to a concentration of 10 ng/mL or 15 ng/mL, was used depending on the particular experimental design.

Preparation of Antibody Solutions

Antibody solutions were prepared the day staining took place. In two tubes, 500 mL per well of PBS was added: one tube for the primary antibody solution and one tube for the secondary antibody solution. Antibodies were stored in a refrigerator and placed on ice when needed to prepare the solutions. Using a micropipette, each antibody was added to their respective tubes at a concentration of 1/250 for primary antibodies (Santa Cruz Biotechnology) and 1/500 for secondary antibodies (Life Technologies, Alexa Fluor®). The tubes were stored in the refrigerator until use.

Cell Staining and Mounting

Cells were removed from the incubator and placed on a lab bench for the staining process. All incubations were conducted at room temperature on the lab bench. Medium was removed from the wells and the cells were rinsed with PBS. 4% paraformaldehyde was then added to each well, ensuring each coverslip was fully immersed. After 30 minutes of incubation, the 4% paraformaldehyde was removed and replaced by 10% horse serum in PBS. The 10% horse serum in PBS was removed after 1 hour of incubation followed by rinsed with PBS. The primary antibody solution was added and the cells incubated for 1 hour. After removal of the primary antibody solution, the cells were rinsed three times with 0.5% Tween in PBS solution. The secondary antibody solution was then added and the cells incubated for 1 hour at room temperature covered from the light. Cells were covered at this step to prevent photo-bleaching of the fluorescently-conjugated secondary antibodies. Again, the cells were rinsed with 0.5% Tween in PBS solution three times after removal of the antibody solution. The coverslips were

then mounted on slides using Fluoromount-G® (Southern BioTec) and sealed with nail polish. The slides were stored covered at room temperature until use in microscopy.

Immunofluorescence Analysis

Slides were viewed using a Leica 500 Confocal microscope. The confocal microscope was set to detect wavelengths of 488 nm and wavelengths of 546 nm, specific to the fluorescently conjugated secondary antibodies used in the particular experiment. Cellular location of the proteins of interest was concluded based on where the respective antibodies were detected. The absence of color, meaning no detection of a fluorescent antibody, was interpreted as the protein of interest not being present in that area of the cell.

Results

NS5A Does Not Inhibit the Translocation of ATF-2

To determine if NS5A inhibits the translocation of ATF-2, two groups of three treatments were analyzed. The two groups were HeLa cells and HeLa: NS5A K2040 cells and the treatments were 5 μL SV, 10 μL SV, or 0 μL SV as a control. The different concentrations of virus were used to determine the amount of virus needed to stimulate translocation of ATF-2. HeLa cells were used to demonstrate the appearance of the expected translocation and as a tool of comparison for HeLa: NS5A K2040 cells. Coverslips were placed in each well of a six well tray and 10,000 cells were placed in each well (Table 1). The cells were infected with SV approximately 24 hours after plating and 16 hours prior to staining. The samples were stained with primary antibodies against ATF-2 and NS5A and the respective secondary antibodies. Untreated HeLa cells did not show nuclear localization of ATF-2 while HeLa cells treated with both concentrations of ATF-2 appeared to have nuclear localization. NS5A was not detected in any of the HeLa cell samples. HeLa: NS5A K2040 cells produced similar results to that of HeLa cells, with nuclear localization of ATF-2 occurring after treatment with SV.

Table 1. Experimental design of ATF-2 translocation studies. Each section represents one well and one sample. Primary antibodies were used as indicated.

HeLa (α-ATF-2, α-NS5A)	HeLa + 5 μL SV (α-ATF-2, α-NS5A)	HeLa + 10 μL SV (α-ATF-2, α-NS5A)
HeLa: NS5A K2040 (α-ATF-2, α-NS5A)	HeLa: NS5A K2040 + 5 μL SV (α-ATF-2, α-NS5A)	HeLa: NS5A K2040 + 10 μL SV (α-ATF-2, α-NS5A)

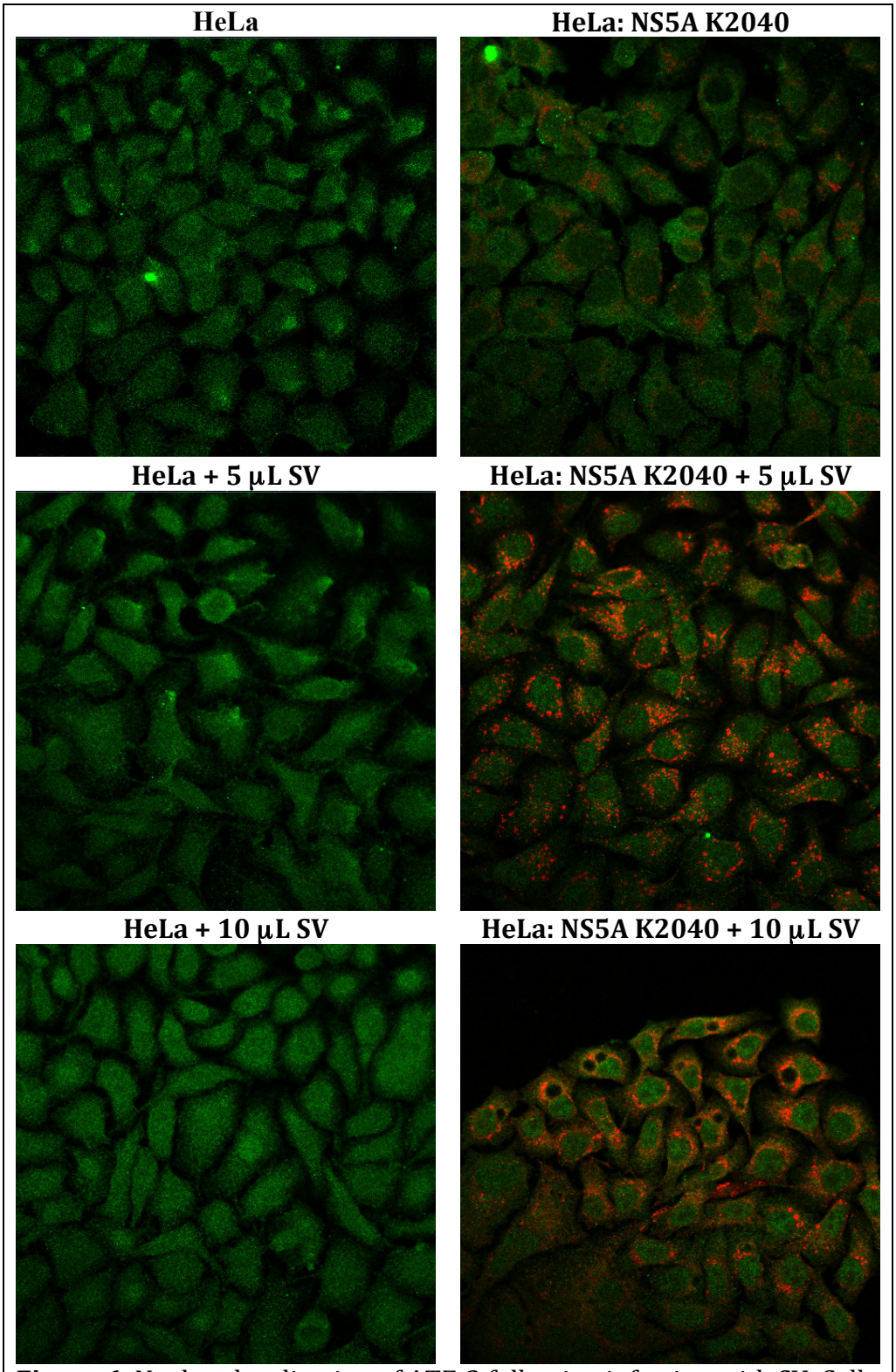

Figure 6. Nuclear localization of ATF-2 following infection with SV. Cells were stained with primary antibodies against ATF-2 (green) and NS5A (red).

NS5A and NS3/4A Do Not Inhibit the Translocation of IRF-3

Since the translocation of ATF-2 was determined to not be inhibited by NS5A, IRF-3, a different transcription factor for the interferon-β promoter, was studied. To determine if the HCV viral proteins NS5A and NS3/4A block translocation of IRF-3, three groups of HeLa cells were examined before and after infection with SV (Table 2). The three groups were HeLa cells, the cell line HeLa: NS5A K2040, and HeLa cells transfected with NS3/4A. HeLa cells not expressing HCV proteins were examined as a means of comparison to the effects of NS3/4A and NS5A. In a six well tray containing one coverslip per well, 5,000 HeLa cells were plated in four of the wells and 5,000 HeLa: NS5A K2040 cells were plated in two of the wells. Approximately 24 hours later, two of the wells containing HeLa cells were transfected with a plasmid expressing the NS3/4A gene. Half of the wells, one of each cell type, were infected with 10 uL SV 16 hours following transfection and the cells were stained and mounted 24 hours after infection. The cells were stained with antibodies against IRF-3 and either NS3/4A or NS5A, respectively. IRF-3 appeared to be located in the cytoplasm of the three cell types prior to infection. After infection with SV, nuclear localization of IRF-3 was observed in the three cell types (Figure 7).

Table 2. Experimental design of IRF-3 translocation studies. Each square represents one well of a six well tray and one sample. Primary antibodies were used as indicated.

HeLa (α-IRF-3, α-NS3/4A)	HeLa + NS3/4A (α-IRF-3, α-NS3/4A)	HeLa: NS5A K2040 (α-IRF-3, α-NS5A)
HeLa + SV (α-IRF-3, α-NS3/4A)	HeLa + NS3/4A + SV (α-IRF-3, α-NS3/4A)	HeLa: NS5A K2040 + SV (α-IRF-3, α-NS5A)

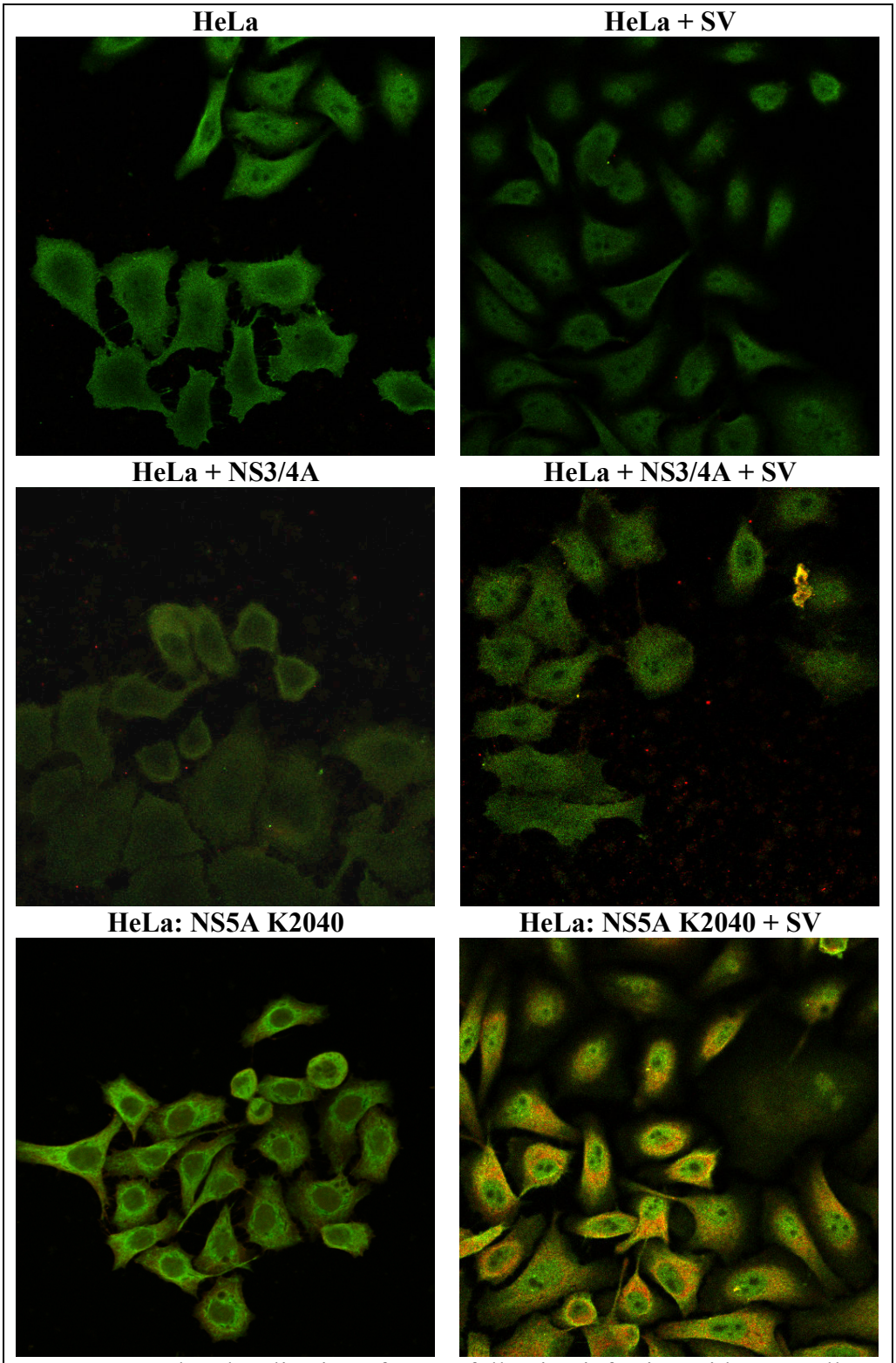

Figure 7. Nuclear localization of IRF-3 following infection with SV. Cells were stained with primary antibodies against IRF-3 (green) and either NS5A (red) or NS3/4A (red).

NS5A Blocks the Translocation of NF-κB

Having shown that neither IRF-3 nor ATF-2 function is blocked by NS5A, a third transcription factor activating the interferon-β promoter, NF-κB, was examined as a potential target of NS5A. In a six well tray containing one coverslip per well, 5,000 HeLa cells were plated in two wells and 5,000 HeLa: NS5A K2040 cells were plated in two wells (Table 3). One well of each cell type was infected with SV approximately 48 hours after plating and 16 hours prior to staining. HeLa cells were stained with primary antibodies against NFκB and NS5A and respective secondary antibodies HeLa: NS5A cells were stained with primary antibodies against NF-κB and NS5A and respective secondary antibodies. Nuclear localization of NF-κB following infection with SV was observed in HeLa cells. However, nuclear localization was not observed in HeLa: NS5A K2040 cells infected with SV (Figure 8).

Table 3. Experimental design of NF-κB translocation studies. Each square represents one well of a six well tray and one sample. Primary antibodes were used as indicated.

HeLa (α-NF-κB, α-NS5A)	HeLa + SV (α-NF-κB, α-NS5A)
HeLa: NS5A K2040 (α-NF-κB, α-NS5A)	HeLa: NS5A K2040 + SV (α-NF-κB, α-NS5A)

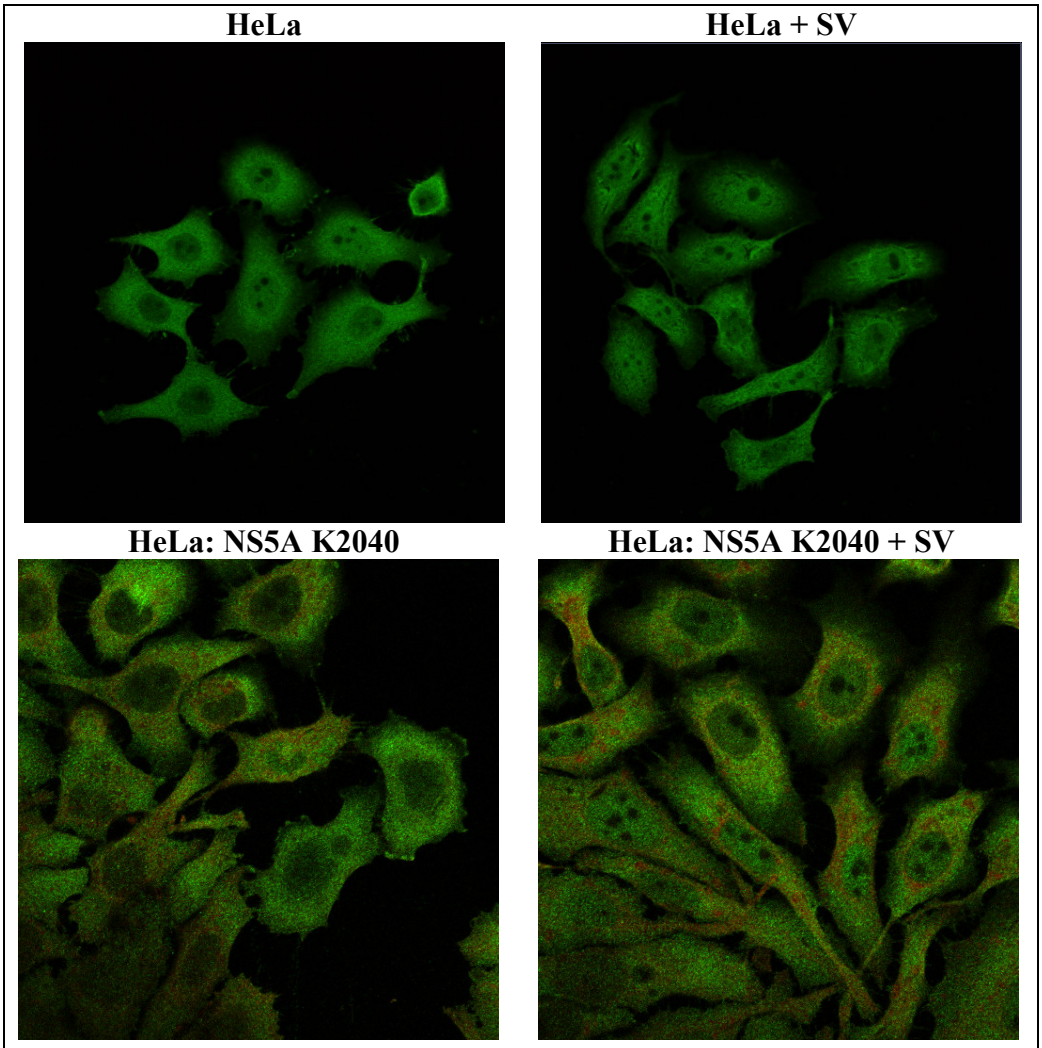

Figure 8. Nuclear localization of NF-κB following infection with SV. Cells were stained with antibodies against NF-κB (green) and either NS3/4A (red) or NS5A (red).

NS3/4A Blocks the Translocation of NFκB

Due to the finding that expression of NS5A blocks the translocation of NF-κB, a second HCV protein, NS3/4A, was examined. To determine if NS3/4A blocks NF-κB translocation, similarly to NS5A, experiment 3 was repeated with two additional samples of HeLa cells transfected with a plasmid expressing the NS3/4A gene. Prior to infection, all three cells types showed cytoplasmic localization of NF-κB. HeLa cells demonstrated nuclear localization of NF-κB following infection with SV. In support of the findings in experiment 3, HeLa: NS5A K2040 cells did not display translocation of NF-κB. Additionally, HeLa cells expressing NS3/4A presented with inhibited translocation of NF-κB, comparable to that of HeLa: NS5A K2040 cells.

Table 4. Experimental design of NF-κB translocation studies. Each square represents one well of a six well tray and one sample. Primary antibodes were used as indicated.

HeLa (α-NF-κB, α-NS5A)	HeLa: NS5A K2040 (α-NF-κB, α-NS5A)	HeLa + NS3/4A (α-NF-κB, α-NS3/4A)
HeLa + SV (α-NF-κB, α-NS5A)	HeLa: NS5A K2040 + SV (α-NF-κB, α-NS5A)	HeLa + NS3/4A + SV (α-NF-κB, α-NS3/4A)

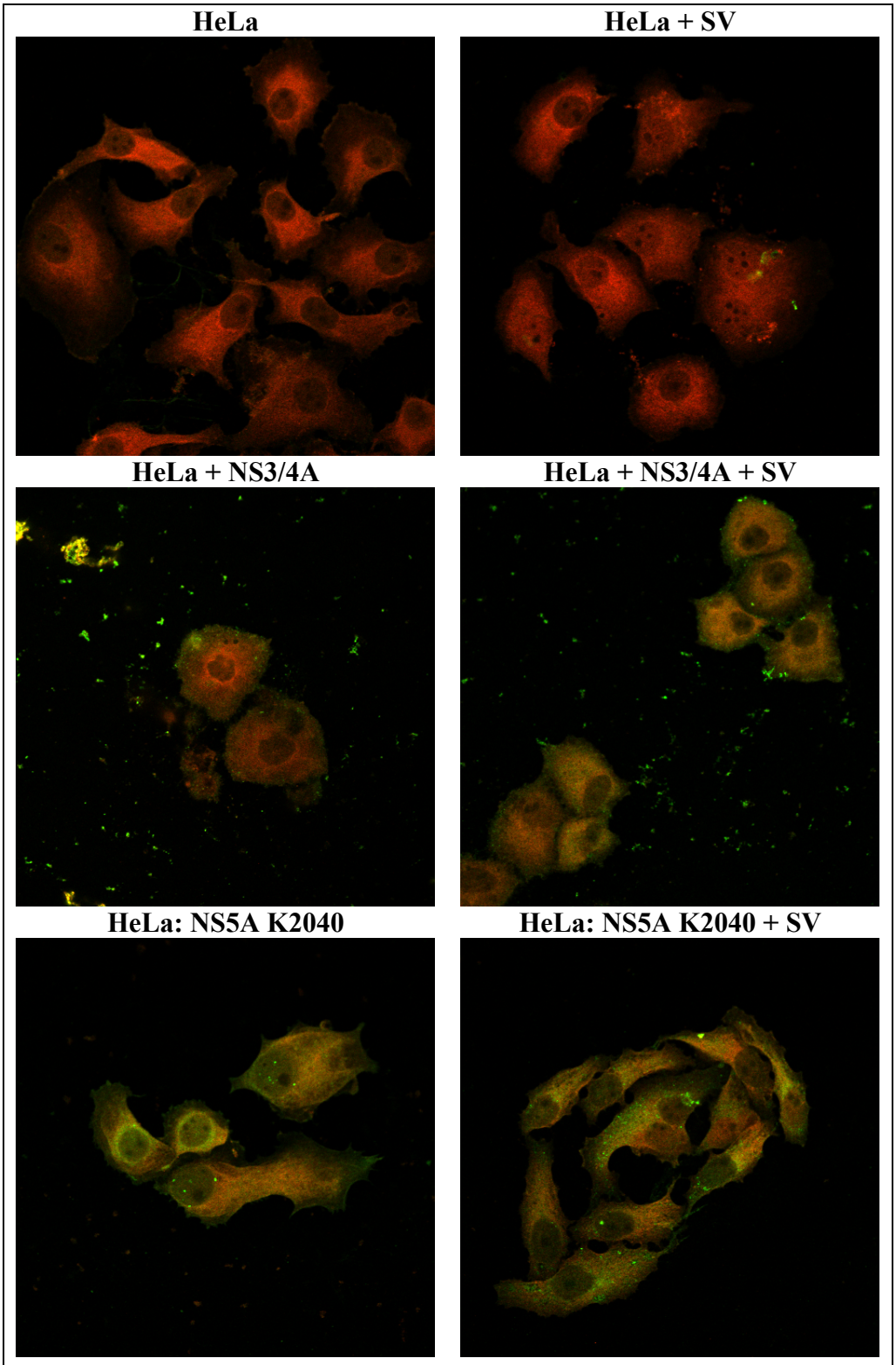

Figure 9. Translocation of NF-κB following infection with SV. Cells were stained with antibodies against NF-κB (red) and either NS3/4A (green) or NS5A (green).

NS5A Does Not Block TNF-α-Induced Translocation of NF-κB

In previous experiments in which NS5A was shown to block the translocation of NF-κB, infection with SV was used to induce translocation. However, the detection of dsRNA by RIG-I is not the only activator for NF-κB. Tumor necrosis factor alpha (TNF-a) is also capable of inducing NF-κB activation and subsequent translocation but through a different signal transduction pathway than SV (Ruland, 709). To determine if NS5A also blocks TNF-α-induced translocation of NF-κB, HeLa cells and HeLa: NS5A K2040 cells were treated with TNF-α. Three wells of HeLa cells and three wells of HeLa: NS5A K2040 cells were plated on coverslips. Two different concentrations of TNF-α were tested, 2 μL and 10 μL, and both treatments were performed 6 hours prior to cell staining. Two different concentrations were used to determine the threshold for NF-κB activation and ensure translocation. Primary antibodies against NF-κB and NS5A were used with their respective secondary antibodies. NF-κB was detected in the cytoplasm of untreated cells. In both cell types, translocation of NF-κB was observed at either concentration.

Table 5. Experimental design of NF-κB translocation studies. Each square represents one well of a six well tray and one sample. Primary antibodes were used as indicated.

HeLa (α-NF-κB, α-NS5A)	HeLa + 2 μL TNF-α (α-NF-κB, α-NS5A)	HeLa + 10 μL TNF-α (α-NF-κB, α-NS5A)
HeLa: NS5A K2040 (α-NF-κB, α-NS5A)	HeLa: NS5A K2040 + 2 μL TNF-α (α-NF-κB, α-NS5A)	HeLa: NS5A K2040 + 10 μL TNF-α (α-NF-κB, α-NS5A)

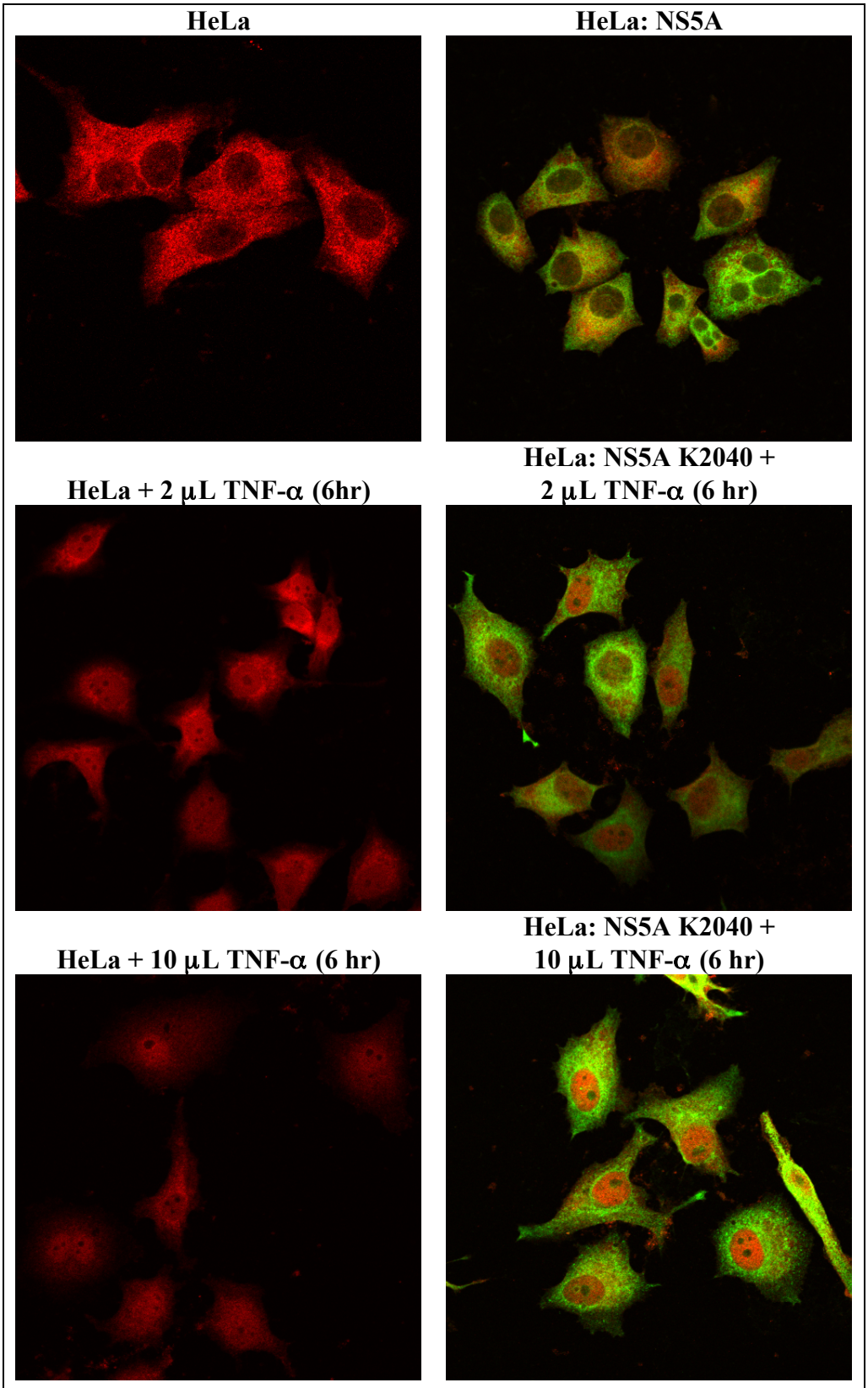

Figure 10. Translocation of NF-κB stimulated by TNF-α. Cells were stained with antibodies against NF-κB (red) and NS5A (green).

Discussion

Hepatitis C Virus impacts millions of people worldwide and is an imposing problem to public heath. Current treatment options for Hepatitis C are not satisfactory and oftentimes patients feel as though the side effects are worse than the disease itself. In order to develop better therapeutic options and a vaccine, the lifecycle and pathogenicity of HCV must be studied further. Additionally, the mechanisms by which HCV evades the host response should be examined. PRRs detect PAMPs on viruses and activate transcription factors that in turn translocate into the nucleus. Once in the nucleus, the transcription factors ATF-2, IRF-3, and NF-κB bind to the IFNβ promoter and turn the gene on. IFNβ is then secreted from the cell and binds to IFNβ receptors on surrounding cells and the host cell to induce the antiviral state. ISGs are expressed, increasing cellular defense mechanisms such as the RIG-I pathway. The HCV protein NS5A has been shown to attenuate IFNβ expression but the mechanism has not been characterized. The focus of this research was to test the hypothesis that NS5A inhibits IFNβ expression through blocking the translocation of one or more of the IFNβ promoter transcription factors: ATF-2, IRF-3, and NF-κB.

To test the hypothesis, immunofluorescence analysis was performed on HeLa cells. HeLa cells expressing NS5A K2040 or HeLa cells transfected with a plasmid expressing HCV NS3/4A were used to study the effects of HCV proteins. The cells were stained with fluorescently conjugated antibodies specific to proteins of interest, such as IRF-3 or NS5A, and the cellular location of the proteins was determined visually using a confocal microscope. In order to stimulate translocation, infection with Sendai Virus and treatment with TNF-α was used. The indication of nuclear localization of ATF-2 and IRF-3 in HeLa: K2040 cells infected with SV suggests that NS5A does not inhibit the translocation of neither ATF-2 nor IRF-3. However,

examining the translocation of ATF-2 presented challenges because in some experiments nuclear localization of ATF-2 was observed in HeLa cells prior to infection with SV. Therefore, it cannot be said conclusively whether NS5A does not block translocation of ATF-2 or if ATF-2 was in the nucleus before NS5A could exert its effects. Studying IRF-3 and NFkB did not present this challenge. In addition to NS5A, HCV NS3/4A was also shown to not inhibit translocation of IRF-3. This conclusion is made based on observed nuclear localization of IRF-3 following SV infection in HeLa cells transfected with NS3/4A.

The hypothesis was supported by experiments examining the translocation of NF-κB. In HeLa: NS5A K2040 cells infected with SV, nuclear localization of NF-κB was not observed while nuclear localization of NF-κB was observed in HeLa cells infected with SV. Additionally, NF-κB translocation was not exhibited by HeLa cells transfected with NS3/4A. Therefore, it can be concluded that both HCV NS5A and NS3/4A inhibit the translocation of NF-κB to attenuate expression of IFNB. To determine if NS5A also inhibits NF-κB translocation in the TNF-α pathway, the translocation of NF-κB was examined in HeLa cells and HeLa: NS5A K2040 cells treated with TNF-α. Nuclear localization of NF-κB was observed in HeLa cells and HeLa: NS5A K2040 cells treated with TNF-α at both concentrations. It can be concluded that TNF-α–induced translocation of NF-κB is not inhibited by NS5A. The incongruity between the results observed when cells were treated with SV versus TNF-α can provide insight into the step in which NS5A blocks NF-κB translocation. NS5A most likely exerts its effects on a point in the SV signal transduction pathway that is not shared by the TNF-α pathway. Further research into the function of NS5A should focus on the SV signal transduction pathway. Isolating the specific points at which NS5A, and other HCV proteins, attenuate the host response will be useful in determining better treatment options for the millions of people infected with HCV.

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