The Effect of Hepatitis C Virus Non-Structural Protein NS5A on Transcription Factor NF-κB

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Abstract
Hepatitis C Virus (HCV) evades the immune system by blocking the production and release of Interferon-β, allowing the virus to chronically infect liver cells. Our laboratory has shown that the HCV non-structural protein NS5A plays a necessary role in blocking Interferon-β expression, but the specific mechanism by which it does so is unknown. Elucidating the means by which HCV interferes with the cell’s antiviral response will allow for the development of better treatment options for those infected by Hepatitis C.

Our research focuses on the effects of NS5A on the activation and nuclear translocation of the three transcription factors that bind to and activates the Interferon-β promoter. HeLa cells expressing HCV NS5A were treated with Sendai virus (SV) or TNF-α and stained with fluorescent antibodies specific to the three transcription factors. Fluorescent microscopy was then used to show that NS5A specifically inhibits the translocation of NF-κB. To measure the activation of NF-κB, a Dual Luciferase Assay was performed by transfecting 293HEK cells with an NF-κB-sensitive promoter attached to the Luciferase reporter gene. Then, the cells were co-transfected with NS5A and treated with SV or TNF-α. The activation of NF-κB was analyzed using a luminometer.

Hepatitis C Virus
Hepatitis C Virus is a single stranded RNA virus capable of infecting liver cells. The CDC estimates that 3.5 million people in the United States are chronically infected with Hepatitis C Virus (HCV). HCV’s ability to chronically affect liver cells can be attributed to its ability to evade the host cell immune system. This study focuses on the mechanism by which HCV interferes with the Interferon-β-signaling pathway in particular.

Interferon-β
Interferon-β (IFN-β) is an essential component of the host cell defense against viral infection. Virus-induced transcription of IFN-β is enhanced by the transcription factors ATF-2, IRF-3, and NFκB. All three of these transcription factors need to bind to the promoter before the IFN-β gene is expressed. The activity of transcription factor NFκB can be measured using the PRDII element of the IFN-β promoter attached to the Luciferase reporter gene.

Hypothesis
The Hepatitis C non-structural protein NS5A blocks Interferon-β expression by inhibiting the activation and nuclear translocation of transcription factor, NFκB.

Results
HCV NS5A Inhibits SV-Induced Activation of NFκB

Red = α-NFκB Green = α-NS5A

Red = α-NFκB Green = α-NS5A

HCV NS5A Does Not Inhibit TNF-induced Activation of NFκB

Red = α-NFκB Green = α-NS5A

HCV NS5A Does Not Inhibit SV-induced Activation of ATF-2 and IRF-3

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HCV NS5A Does Not Inhibit SV-induced Activation of ATF-2 and IRF-3

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Conclusions
• HCV NS5A 10A mutant (K2040) inhibits virus infection-mediated activation of the IFNβ promoter.
• Specifically, NS5A 10A blocks the activation of NFκB response element in the IFNβ promoter.
• NS5A 10A prevents virus infection-induced nuclear translocation of transcription factor NFκB.
• NS5A 10A does not block the entry of the transcription factors IRF3 or ATF-2 into the nucleus.
• NS5A 10A does not inhibit the TNFα-induced translocation of NFκB and activation of NFκB responsive gene expression.
• This narrows down the mechanism of NS5A-mediated inhibition of the host response to proteins that are specific to the dsRNA-induced RIG-1 mediated antiviral signaling pathway.

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