The Effect of Hepatitis C Non-Structural Protein NS3/4A on the Translocation of Transcription Factors for Interferon Beta Expression

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Abstract

Hepatitis C Virus (HCV) infects liver cells and is estimated to infect 3% of the world's population. HCV is transmitted by contaminated blood and people who are infected with HCV can be asymptomatic. HCV can lead to cirrhosis of the liver and hepatocellular carcinoma. HCV is a single stranded RNA virus that belong to the *flavivirus* family and produces 10 viral proteins. These viral proteins aid in HCV replication and allow HCV to remain undetected by the immune system by inhibiting the production of interferon-beta.

The anti-viral response activates transcription factors such as ATF-2, NFκB, and IRF-3 that translocated into the nucleus and bind to the interferon gene to produce interferon. The HCV viral protein NS3/4A is known to inhibit IRF-3 movement into the nucleus, inhibiting the production of interferon. By blocking the production of interferon, HCV is able to hide from the immune system and establish a chronic infection. HCV viral protein NS5A is known to inhibit the movement of transcription factor NFKB into the nucleus, thus inhibiting the anti-viral response. We are interested to see if NS3/4A inhibits the anti-viral response by blocking the movement of both transcription factors IRF-3 and NFκB into the nucleus.

NS3/4A

Hepatitis C Virus makes 10 viral proteins once it infects a cell. Three of these proteins are structural proteins that are essential for virus assembly. Seven of these proteins are nonstructural proteins that aid the virus in replication and evading the anti-viral response. NS3/4A is one of these nonstructural proteins that is produced by HCV.

NS3/4A acts as a protease that cleaves the polycistronic strand of viral proteins made following translation of the viral genome. NS3/4A is also known to cleave the protein MAVS. When MAVS is cleaved from the mitochondria, MAVS becomes inactive. By inactivating MAVS, NS3/4A prevents the movement of transcription factor IRF-3 into the nucleus which inhibits the production of interferon. This blocks the aid In evading the anti-viral response as it blocks the movement of transcription factors by cleaving MAVS and inhibiting the production of interferon.

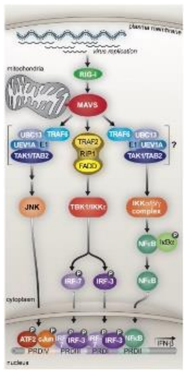


Figure 1: Production of interferon-beta through the activation of the RIG-I/MAVS pathway (1)

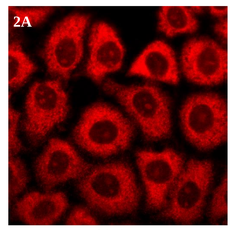
Hypothesis

Hepatitis C Virus nonstructural protein NS3/4A blocks the nuclear localization of both transcription factors NFκB and IRF-3 following infection.

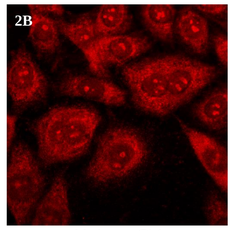
Results

Translocation of NFκB following Infection

Red = α-NFκB



HeLa



HeLa + SV

NS5A Inhibits Movement of NFκB

Red = α-NFκB Green = α-NS5A

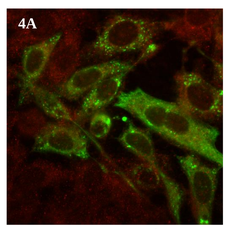
HeLaNS5A

HeLaNS5A + SV

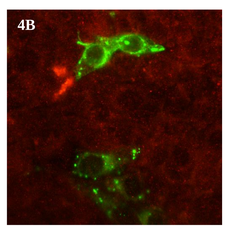
HeLaNS5A

NS3/4A Inhibits Movement of IRF-3 and NFκB

Red = α-IRF-3 Green = α-NS3/4A

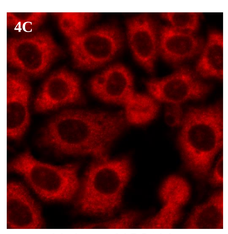


HeLa + NS3/4A

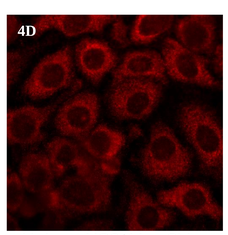


HeLa + NS3/4A + SV

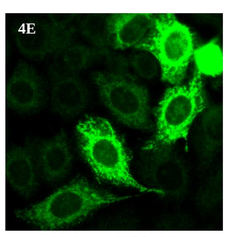
Red = α-NFκB Green = α-NS3/4A



HeLa + NS3/4A



HeLa + NS3/4A + SV



HeLa + NS3/4A

Discussion

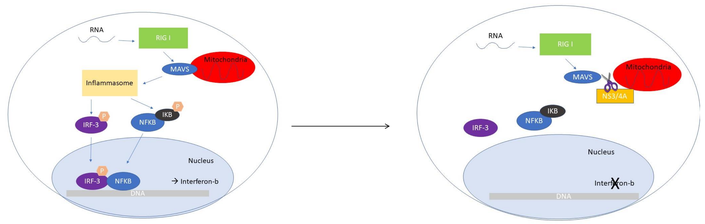
The movement of transcription factors can be observed using fluorescently labeled antibodies. The location of the transcription factor can be inferred from the location and intensity of the fluorescent signal.

In the control, uninfected HeLa cells showed localization of NFκB in the cytoplasm of the cell. When HeLa cells were infected with Sendai Virus, the localization of NFκB was observed in the nucleus of the cell (Figure 2A and 2B).

NS5A is known to inhibit the movement of NFκB into the nucleus following infection with Sendai Virus. This is observed in figures 3A and 3B as the nucleus of the infected cells remained darker than the cytoplasm.

Following infection, the movement of transcription factors NFκB and IRF-3 was inhibited in HeLa cells expressing NS3/4A (Figures 4B and 4D). To ensure these cells were expressing NS3/4A, they were also fluorescently labeled with an antibody for NS3/4A (Figure 4E).

Conclusions



Hepatitis C viral protein NS3/4A blocks the movement of transcription factors IRF-3 and NFκB into the nucleus of the cell following infection with Sendai Virus. By inhibiting the translocation of NFκB and IRF-3, HCV inhibits the production of interferon-beta which helps HCV evade the anti-viral response.

References

1. Mcwhirter, Sarah M. et al. "Connecting Mitochondria and Innate Immunity." Cell 122.5 (2005): 645-47. DOI: 10.106/j.cell.2005.08.026.

Acknowledgements

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