Activation of MxA Gene by the Hepatitis C Virus F Protein

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Background & Objectives: The hepatitis C virus possesses an alternative reading frame overlapping the core gene, which product is known as core+1, or alternative reading frame or F protein. We still know little of the functional properties of this F protein. Previous reports suggest that HCV core protein interacts with IFN signaling pathway by modulating MxA gene expression. Thus, here we investigated whether the F and core proteins have both shared function in MxA expression regulation. MxA antiviral protein is one of the best characterized IFN-inducible gene product acts by affecting viral nucleocapsid transport and RNA synthesis.We examined the effect of core+1 protein of HCV on MxA expression in the IFN α treated Huh-7 cells.

Methods: Recombinant pcDNA (+) 3.1 vector harboring Core+1 gene was constructed and transiently transfected into Huh-7 cells by electroporation. Expression analysis was assessed via western blotting (WB) and immunofluorescence microscopy (IFM). Transfected Huh-7 cells (with or without IFN-α treatment) were used for RNA extraction. Quantitative Real Time PCR (RT-qPCR) using SYBR green was performed to examine the effect of core+1 protein on MxA gene expression. Fold induction was then calculated by the Δct Methods using GAPDH mRNA level to normalize values. Untransfected cells and IFN-α untreated cells were used as controls.

Results: Construction of appropriate expression vectors for HCV Core+1 was confirmed by restriction analysis and DNA sequencing. WB and IFM analysis confirmed proper expression of the core+1 protein inside the transfected hepatic cells. Results of RT-qPCR indicated that MxA mRNA was induced by the Core+1 protein in the Huh-7 cells.

Conclusion: These results suggest that the Core+1 protein may share some properties identified previously for the HCV core protein.

Keywords: Hepatitis C Virus; Core+1; MxA