The optimization of the RSAD2 siRNA concentration in Huh-7 cells treated with Interferon alpha

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Background: Small interfering RNAs (siRNAs) have been used as a remarkable tool for knock downing of specific genes expression in experimental studies. RSAD2 (Radical SAM- domain-containing 2) belongs to the family of Interferon Stimulated Genes (ISG) which is highly induced by type I, II, and III Interferons(IFN).Interferon alpha has been approved as the first drug used for treatment of viral Hepatitis infection.RSAD2 gene plays a fundamental role in restricting replications of different viruses including HCV and also may be a key factor during infection of Hepatitis B virus. The aim of this study was to determine the optimal concentration of siRNA which is needed for inhibition of the RSAD2 gene expression in HuH-7 cells.

Material and Methods: Huh-7 cells were seeded at 4×10^4 cells/cm^2 for 24 hours.RSAD2 pooled of four different siRNAs was prepared according to the manufacture's instruction. The transient transfection was performed using Lipofectamine 2000 transfection reagent at concentrations of 10nM to 80nM of pooled siRNA. Transfected cells were treated with Interferon alpha 24 hours post transfection. RNAs of the cells were extracted after 72 hours and the Real-Time PCR was performed.

Results: The results were evaluated by statistical methods. The ability of different siRNA concentrations targeting RSAD2 gene was examined. 40nM of pooled siRNA was suitable and sufficient for inhibiting the expression of the RSAD2 gene in Huh-7 cells treated with Interferon alpha.

Conclusion: In this study we conducted a dose dependent optimization for RSAD2 gene silencing in Huh-7 cells. These findings can be helpful for further studies of HBV and HCV replication, Interferon cell signaling and the substantial role of RSAD2 gene during these hepatic infections.

Keywords:siRNA, RSAD2, HBV, Interferon alpha, HCV