

Investigating the Effect of RNAi Mediated Silencing of Gene NPC1L1 on Cholesterol Absorption in an in Vitro Cell based Model System

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ABSTRACT

RNA interference is a precise and powerful technology with enormous applications in functional genomics. It has revolutionized studies pertaining to regulation of gene expression providing a better understanding of molecular mechanisms involved in development of diseases such as cancer, cardiovascular disease (CVD) and many more. The present study here is an attempt to define the role of Niemann-Pick C1-like 1 (NPC1L1), a cholesterol transporter and a key regulator in cholesterol uptake as well as intracellular cholesterol trafficking. Four different siRNAs were designed against the gene (targeting different regions), synthesized by in vitro transcription and validated in HepG2 cell based assay system. The inhibitory effect of the four siRNA molecules individually and in different combinations was compared in this model system to analyse the impact of siRNAs on the function of the gene as cholesterol transporter. The pooling of siRNAs lead to approximately 78% decrease in the percentage of cholesterol absorbed. This cell based assay validated the gene silencing effect of siRNAs against NPC1L1. Thus, establishing its role in cholesterol absorption. So it can be concluded that knocking down the expression of NPC1L1 can significantly reduce the uptake of cholesterol. On the same time this can be used as the basis to design further experiments for better understanding of the mechanism of cholesterol absorption by NPC1L1 along with other transport proteins. Moreover this could also provide a lead to new line of treatment based on gene silencing against cardiovascular diseases.

Keywords: RNA interference, cholesterol homeostasis, NPC1L1, Insilco analysis