Lipid Modified Polymeric Carriers for Delivery of siRNA to Lymphoma Cells

+[1]Sahin, B; [1]Landry, B; [1]Gul-Uludag, H; [2]Jiang, X; [1]Uludag, H
+[1]University of Alberta, Edmonton, AB, Canada; [2]Terry Fox Laboratories, BC Cancer Agency, Vancouver, BC, Canada

Introduction

RNA interference (RNAi) is a natural mechanism that regulates gene expression and also acts as a defense mechanism against double stranded RNA viruses. Whether it is endogenous (miRNA) or exogenous (viral genome or siRNA), the double stranded RNA is incorporated into a complex called RNA induced silencing complex (RISC), directly or after being processed by dicer, unwinded to give a single stranded RNA and then the mRNA that has complementary sequence to the RNA incorporated to the complex is cleaved by the RISC [1]. Short interfering RNA (siRNA) is a synthetic exogenous short (20-22 base pairs) RNA duplex designed to use the RNAi mechanism to achieve gene silencing. When siRNA is introduced to the cytosol of the cell, it is incorporated into RISC, so the protein is stopped from being translated [1]. As the siRNA induced silencing is sequence specific, it is a potent treatment for cancer as silencing of specific-cancer related genes can induce apoptosis in only cells in the cancerous state and spare the normal cells [2].

For the silencing to take place, siRNA needs to be delivered into the cytoplasm, where it will be incorporated into the RISC. For this purpose, a delivery system is necessary as the siRNA is not only negatively charged, hindering its passage through the cell membrane, but also unstable in serum [4]. Viral carriers are subjected to some safety concerns making non-viral carriers such as polycationic polymers a much better and safer way to deliver siRNA into the cell [5,6]. Polyethyleneimine (PEI) is a polycationic polymer that readily polyplexes with negatively charged nucleic acids in salt solution due to electrostatic forces, masks the negative nature of the molecule, and assists in internalization [4]. The commonly used 25 kDa PEI is overly toxic to cells. Lower MW PEI (2 kDa), however, is non-toxic but also ineffective in intracellular delivery of siRNA. Substitution of 2 kDa PEI with lipids such as caprylic acid (CA) and lineloic acid (LA) is applied to maximize the efficiency of intracellular delivery and minimize the cytotoxicity of the carrier [7].

Lymphoma, being one of the most common cancers in children and young adults, is an important health issue, as even though the survival rate is high, the short and long-term effects of conventional drug therapy constitute a major problem in patients' adult life [8]. The use of molecularly targeted *siRNA* and *polymer-based carriers* as a treatment for cancer is hypothesized to reduce the negative effects of drug treatment on the patient while increasing the survival rate. The aim of this work is to develop an effective non-viral carrier that will deliver siRNA in lymphoma cells upon.

Materials and Methods

Hut78 Cutaneous T-cell Lymphoma cells that grow in suspension were used as *in vitro* model, and GFP expressing Hut78 cells were used to establish the method of delivery as the detection of the changes in the fluorescence of GFP is easily quantified using flow cytometry.

The in-house carriers (previously described in [6]) were tested against several commercial reagents obtained from commercial sources for their delivery and silencing efficiency and their toxicity on Hut78 cells. Also centrifugation with complexes was tried to increase efficiency. Dose response studies were performed on potential cytotoxic targets; KSP, STAT3, CDK18, Bcl11b, MAP, RPS, PI3K and STK. Polymer toxicity and siRNA efficiency were quantified using cell counts with Tryphan blue and hemocytometer, AlamarBlue®, MTT and apoptosis assays. All studies were performed in triplicates and scrambled siRNA was used as control.

Results

A select set of lipid modified PEI polymers previously synthesized in Uludag Lab using several lipids including caprylic (CA), myristic (MA), palmitic (PA), stearic (SA), oleic (OA), and linoleic acid (LA), at different levels of substitutions were inspected for their ability to deliver and silence GFP in Hut78 cells, and out of the whole polymer library Caprylic acid (C8:0) and Linoleic acid (C18:2) modified PEI were chosen for further studies due to their stability and superior efficiency respectively. Even though the delivery efficiency of CA and LA substituted polymers were lower then that of 25PEI, they showed a significantly lower toxicity and higher efficiency in terms of decreasing the GFP fluorescence than the 25 kDa PEI as well as lipofectamine-RNAiMAX®, the most effective commercial agent for siRNA delivery to Hut78 cells. Centrifugation of the cells after addition of siRNA-polymer complexes significantly increased the silencing efficiency of PEI-LA up to a threshold while there were no significant changes in 25PEI and PEI-CA.

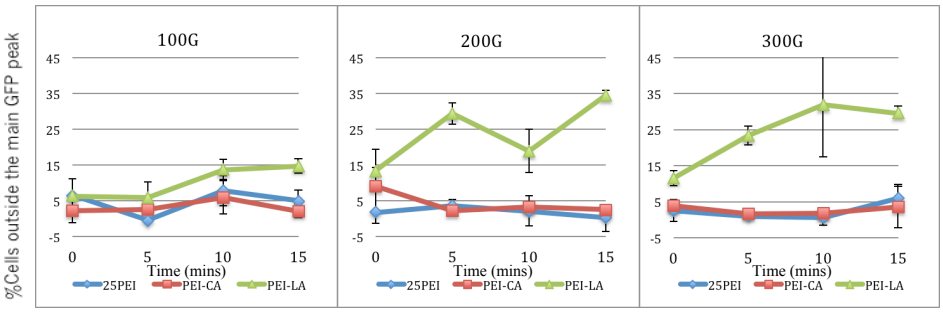

Figure 1. Percentage of cells dropping out of the main GFP peak as a function of centrifugation time at 100, 200 and 300G values

Among the potential cytotoxic targets, PI3K and CDK18 showed decreased cell viability compared to control and other siRNAs; however, the apoptosis assays failed to support the results from the viability assays.

Discussion

Lipid modified PEI polymers have better silencing and lower toxicity than 25 kDa PEI and other commercial carriers in Hut78 lymphoma cells, which makes them suitable candidates for therapeutic siRNA delivery for lymphoma. However, potent targets whose down-regulation would cause apoptosis in lymphoma cells are yet to be identified via screening siRNA libraries and inspecting dose dependent response of the cells to the treatment. With further investigation and achieving such efficiency in animal models, the method has the potential to be used in successful and safe treatment of lymphoma.

References

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