Lipid-Modified Polymers to Undertake siRNA Delivery to Suspension-Growing Chronic Myeloid Leukemia Cells

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Statement of Purpose: Chronic Myeloid Leukemia (CML) is a disease of the hematopoietic cells characterized by the rearrangement of chromosome 9 and 22 that gives raise to *bcr-abl* fusion oncogene. This gene is translated into a protein with constitutive tyrosine kinase activity, which causes uncontrolled proliferation and differentiation in hematopoietic cells [1]. Due to improved knowledge of CML at a molecular level, RNA interference (RNAi) has become a promising therapy that can be used for down-regulating the aberrant proteins in CML. In order to implement RNAi, however, a carrier that delivers the siRNA through the cell membrane in a safe and efficient manner is needed. Lipid substituted low molecular polyethylenimines (<2 kDa) have been developed in our lab to achieve non-viral delivery of siRNA and gene down-regulation in attachment-dependent and leukemia cell lines [2,3]. The purpose of this study was to evaluate the most suitable lipid-substituted polymers for siRNA delivery and silencing in the difficult-to-transfect (suspension growing) CML cells.

Methods: Polyethyleneimine (0.6-2 kDa; 0.6PEI, 1.2 PEI and 2PEI) was substituted with caprylic acid (CA; C8), palmitic acid (PA; C16), oleic acid (OA; C18), and lineloic acid (LA; C18), as described in [2]. Polymer-siRNA complexes were prepared by adding polymer solutions (1 mg/mL in water) to siRNA solutions (150 mM NaCl) at various polymer:siRNA ratios and incubated for 30 min before addition to the cells. Comparison was performed against native 25 kDa PEI (25PEI), 2PEI and Lipofectamine™ 2000 as indicated. Binding and dissociation studies of the polymer-siRNA complexes were determined by semi-quantitative EMSA [3]. Chronic myeloid leukemia cells used were K562 cells. siRNA delivery ability was quantitatively measured by flow cytometry using FAM-labelled siRNA. Where indicated, a comparison of delivery with commercial carriers was also performed. Suppression of protein expression was determined by using GFP-positive K562 cells (with GFP-specific siRNA,) and using the target bcr-abl (with specific siRNA) and quantitative PCR (qPCR).

Results: We first compared siRNA uptake between suspension-growing K562 cells and attachment-dependent breast cancer cells (MDA 231) with different polymers. The siRNA uptake was significantly lower (>5-fold) in K562 cells as compared to MDA231 cells, indicating general difficulty of delivering siRNA into leukemic cells with non-viral vectors. There were little silencing of the reporter GFP gene in K562 cells; even the linoleic acid-substituted 2PEI, which was previously found to be successful in acute myeloid leukemia cells [3], was not effective in K562 cells. This was unlike the commercial liposome Lipofectamine™ 2000, which gave significant (~50%) silencing of GFP in K562 cells. The 25PEI also gave some silencing, but significant toxicities were evident with both 25PEI and Lipofectamine™ 2000. However, PA-substituted polymers prepared from smaller PEIs (0.6 and 1.2 kDa) were found to be effective in silencing GFP gene in K562 cells (**Figure 1**). The most effective silencing was obtained with 1.2 kDa PEI modified with 1.98 PA/polymer (highest modification achieved with this MW polymer). The level of silencing was equivalent to 25PEI without significant cell death associated with 25PEI. Finally, PA-substituted polymer was able to lower bcr-abl mRNA levels in K562 cells with specific siRNA, resulting in increased apoptosis.

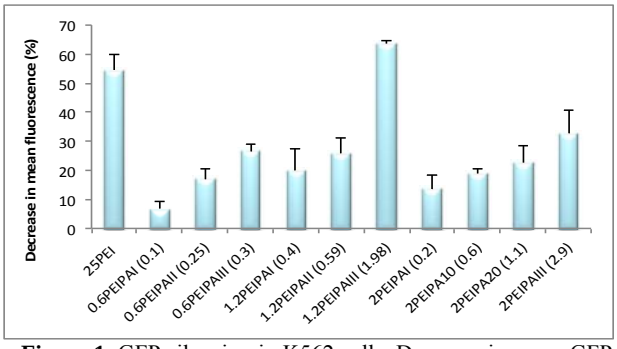

Figure 1. GFP silencing in K562 cells. Decrease in mean GFP fluorescence was expressed as a percentage of control siRNA treated cells (number of PAs/PEI in parenthesis).

Conclusions: We conclude that although the LA-substituted 2PEI was highly efficient for both plasmid DNA[3] and siRNA delivery in attachment-dependent cells and some suspension cells (e.g., THP-1) [3], the efficiency of this carrier for delivery of siRNA in K562 cells was low in comparison with 25PEI and Lipofectamine. Other lipid-based carriers, such as PA and OA substituted PEIs, were found to be more promising and are now being explored for improved siRNA delivery in CML cells for a CML therapy. The reasons behind their better efficacy are being actively investigated.

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References: [1]. I. Sloma et al., Leukemia. 2010, 24: 1823-33. [2]. H. Aliabadi et al., Macromol. Biosci. 2011, 11: 662-72. [3]. B. Landry, et al., PLoS ONE. 2012, 7:e44197. [4]. K.C. Bahadur et al., Acta Biomater. 2011, 7: 2209-17.