Identifying Effective siRNAs for Polymer-Mediated Combinatorial Delivery to Drug Sensitive and Resistant Breast Cancer Cells

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Statement of Purpose: Chemotherapy and small molecule signal transduction inhibitors are the first line therapy in most cancers, including breast cancer. However, resistance to these therapeutic agents develops inevitably in a short period [1]. Kinases act as signal transducers and are involved in many mechanisms crucial for cell survival, so that they have been employed as targets for small molecule inhibitors. As an alternative approach, RNA interference (RNAi) is a highly promising therapeutic modality; rather than inhibiting protein activity, RNAi eradicates protein mRNA, preventing the synthesis of the target proteins in the first place. RNAi, however, requires suitable carriers that will effectively deliver synthetic short interfering RNA (siRNA) to cells. Lipid-substituted polymers were previously shown to be promising for delivery of individual siRNA molecules. To improve therapeutic effects, however, it might be possible to deliver combinational (dual) delivery of siRNA to inhibit cell survival mechanisms more effective. In this study, we explored the possibility of delivering combinations of siRNA for superior responses. Our goal was to determine if dual siRNA delivery with polymeric carriers was able to curb uncontrolled cell proliferation especially in drug resistant breast cancer cells.

Methods: A library of 719 siRNAs targeting kinases and a library of 446 siRNAs against apoptosis proteins were repeatedly screened using a clinically-promising delivery system composed of lipid-substituted polymers [3]. The MDA-MB-231 and MDA-MB-435 cells served as the breast cancer model. Drug-resistant phenotypes were developed by long-term cultures with drugs. siRNA complexes were prepared with the polymers and delivered to cells in 96-well plates. Cell viability was evaluated after 72 hr using MTT assay, and "hits" were selected based on statistical criteria, where 8 kinases were selected based on following criteria: i) Common hits in both cell lines; ii) Significance in literature; iii) Potential synergistic effect with an siRNA against the anti-apoptotic protein Mcl-1. Dose-response curves were explored for selected targets to determine the most potent siRNAs. The most promising targets were then selected for simultaneous silencing with Mcl-1 siRNA *in vitro* and *in vivo*. Validation studies were performed with real-time PCR to ensure specificity of target silencing.

Results: Delivering the kinase siRNA library to MDA-MB-231 cells provided 36 hits, 8 of which were common with the 44 hits in MDA-MB-435 cells. Among the selected targets, Mitogen-activated protein kinase 3 (MAP2K3) was the most potent in decreasing cell viability, while Ribosomal protein S6 kinase (RPS6KA5) showed the most promising potential as a candidate for simultaneous silencing with Mcl-1. In fact, combinational silencing with Mcl-1 and RPS6KA5 *in vitro* caused a 90% drop in cell viability in MDA-MB-435 cells, more so than the delivery of single siRNAs (**Figure 1**). This combination was evaluated in a xenograft model in nude mice; a significant inhibition of tumor growth was observed for Mcl-1 silencing alone and Mcl-1/RPS6KA5 double silencing compared to Mcl-1 silencing alone. Moreover, breast cancer cells displaying drug resistance gave a similar response to wild-type of cells (**Figure 1**), indicating that siRNA therapy is not affected by phenotypic changes associated with drug resistance.

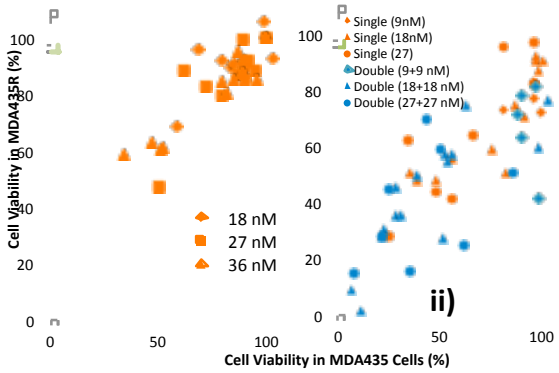

Figure 1. Cell viability after siRNA delivery as a single agent (left) or a combination of two siRNAs (right). Combinatorial delivery retarded cell growth *in vitro* at low doses (18 nM).

Conclusions: Lipid-substituted polymers were combined with siRNA libraries for effective delivery to breast cancer cells. Effective delivery of siRNA was possible whether the cells displayed drug resistance or not, and there was a strong correlation in RNAi activity between the native and drug-resistant cells. Carefully selected combinations of siRNAs were ideal for delivery with lipid-substituted polymers and most effective combination was also to control tumor growth in animal models. The methodological approach described here provides strong siRNA leads to combine with polymeric carriers to undertake cancer therapy.

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