for certain target sites, there is no correlation between the optimal length of siRNAs and shRNAs. Overall, our results highlight the importance of obtaining empirical data on the optimal si/shRNA format for any particular target site, and provide a set of optimized molecules targeting the Gag coding sequence of HIV-1 RNA for further development.

593. Targeted Histidinylated Lipopolyplexes for siRNA Delivery in 4T1 Mammary Carcinoma Cells

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Breast cancer is the leading cause of death among women aged 35 to 50 years. siRNA-based therapies aiming at reducing the overexpression of oncogenes and genes involved in tumor progression are developed for the treatment of cancer. However, clinical applications are delayed due to the absence of very efficient and safe delivery systems. For this purpose, we develop siRNA lipopolyplexes (LPRi) which are ternary complexes between cationic liposomes comprising imidazole/imidazolium lipophosphoramidates, histidinylated-lPEI cationic polymer and siRNA (1). Compared to commercial reagents, LPRi containing anti-eGFP or anti-Luc siRNA give in vitro higher specific inhibition (80% at 50nM siRNA) of reporter gene expression in cultures of eGFP-HeLa, Luc-B16F10 and Luc-4T1 cancer cells. Transfection of the 4T1 mammary carcinoma cells line with anti-survivin siRNA induces 30% reduction in cell viability. In vivo imaging after endotracheal administration of LPRi comprising polymer or liposomes labeled with a near-infrared fluorophore indicates a sustained delivery in the lung of the formulation until 24 hours post injection.

For in vivo application, PEGylated LPRi has been armed with uPA peptide or folate which binds to the urokinase receptor (uPAR) and the folate receptor (FR), respectively. These targeted LPRi exhibit a 200 nm size and a reduced positive zeta potential. When tested for their uptake efficiency in 4T1 cells, targeted LPRi show a higher endocytosis compared to untargeted LPRi. When transfected with 50nM anti-Luc siRNA formulated with LPRi containing 2% Folate or 5% uPA LPRi, a better specific inhibition (54% and 40%, respectively) of luciferase expression in Luc-4T1 cells is obtained compared to untargeted LPRi (32%). Therapeutic applications of these new targeted siRNA formulations are now tested by using the murine orthotopic 4T1 breast cancer model.

(1) Lipopolyplexes comprising imidazole/imidazolium lipophosphoramidate, histidinylated polyethyleneimine and siRNA as efficient formulation for siRNA transfection, Gonçalves et al., Int. J. Pharm., 2014; 460: 264-272.

594. PDGF-D Inhibition by Using Chitosan:siRNA Complexes in Breast Cancer Model of Rat

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Cancers are characterized by the uncontrolled cell growth and angiogenesis. Expression of growth factors like platelet-derived growth factor (PDGF), an angiogenic molecule, increased in many types of human tumors incluiding breast cancer. PDGFs have been implicated in the pathogenesis and angiogenesis of different tumor types.

PDGFs are growth factor family consist of 5 disulfide-linked dimers (PDGF-A to D). Among them PDGF-D has been recently found to be overexpressed in many tumor cells such as breast cancer and plays an important role in tumorgenesis. Overexpression of PDGF-D led to increased angiogenesis. Recently, RNAi offers a promising approach to develop a powerful class of inhibitors used to angiogenesis with siRNA as the therapeutic agent. However, siRNA has poor cellular uptake so, suitable delivery system is required. Among the different non-viral vectors, chitosan is an attractive gene delivery system.

The aim of this project is to investigate anti-angiogenesis and tumor inhibition effects of PDGF-D targeting siRNA complexed with chitosan in breast cancer model of rat.

siPDGF-D was complexed with chitosan at different N/P ratios (5/1 to 50/1) and the characterization of these complexes (size, surface charge and stability) was done. Chitosan/siPDGF-D complexes were injected intratumorally to rats bearing breast tumor. Tumor volumes were measured until 28 weeks. The PDGF-D mRNA expression level during the experiment were determined by RT-PCR. Apoptosis in tumor and treatment groups was studied by TUNEL method. To investigate the effect of complexes on the PDGF-D expression, PDGF-D was analyzed by western blotting.

The size of complexes changed between 179.1- 342.0 nm depending on their different N/P ratios (5/1- 50/1). The surface charge of complexes was -0.20 mV to +23.79 mV. Tumor volume of rats decreased 95% after siRNA treatment. At untreated rat groups, tumor volume reaches to 593 mm^3 after 28 days but in treated groups tumor volume (29 mm^3) has not increased. The relative PDGF-D mRNA level in tumor tissue after injection of complexes was markedly reduced in comparison with the level of the control group. PDGF-D mRNA expression was reduced approximately 71% by chitosan complexes containing siPDGF-D. In TUNEL study, apoptotic index was high in treated group compared to tumor group. Western blot analysis showed decrease in PDGF-D protein level in complex group, compared with the tumor control group. Similar results were also obtained with RT-PCR. Free siRNA PDGF-D injections showed lower inhibition effect than complex forms.

These results showed that the chitosan/siRNA complexes targeting to PDGF-D have a highest suppresive effect on protein and mRNA expressions and tumor volume in the breast cancer model of rats and chitosan is an effective vector for siRNA delivery.

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595. miR-199 Inhibits Tumor Growth and Enhance Chemosensitivity in Osteosarcoma

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Dysregulation of miRNAs is involved in osteosarcoma (OS). Here, we demonstrate that miR-199 is decreased in specimens of OS patients with a poor chemoresponse compared to those with a good chemoresponse. In addition, our clinical data show that decreased miR-199 was associated with poor survival in OS patients. Overexpression of miR-199 inhibited cell growth and chemoresistance. In contrast, inhibition of miR-199 or overexpression of target genes stimulated OS cell growth and chemoresistance both in vitro and in vivo. Taken together, these findings suggest that miR-199 is a tumor suppressor miRNA and induction of miR-199 is a potential strategy to inhibit OS progression.