Systemic targeted siRNA delivery with a multifunctional carrier

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Statement of Purpose: Our primary goal of this study was to develop efficient and targeted delivery systems for systemic and cell-specific delivery of therapeutic siRNA. We reported here peptide targeted siRNA delivery systems from a multifunctional carrier EHCO, which showed pH-sensitive amphiphilicity and promoted endosomal-lysosomal escape of the siRNA delivery systems (1). An anti-hypoxia inducible factor 1α (HIF-1α) siRNA was used as a therapeutic siRNA to study the delivery efficiency and therapeutic efficacy of the targeted delivery systems. The overexpression of HIF-1α is associated with tumor malignancy and poor prognosis. HIF-1α expression represents an angiogenic switch that promotes cancer cell survival under hypoxic conditions by elevating glycolysis and angiogenesis. Systemic and specific in vivo delivery of anti-HIF-1α siRNA is crucial to effectively treat solid tumors with RNAi

Methods: The targeted siRNA delivery systems were prepared by the incorporation of RGD-PEG-MAL or bombesin-PEG-MAL (2.5% mole ratio based on EHCO) to the surface of the siRNA/EHCO nanoparticles by the reacting with the unreacted surface thiols. The nanoparticles were also labeled with fluorescein by reacting fluorescein-5-maleimide with the surface thiols to allow us to determining cellular uptake of targeted siRNA delivery system. Cellular uptake of the peptide targeted siRNA nanoparticles was evaluated by flow cytometry in human glioma U87 cells in comparison with pegylated and unmodified nanoparticles. The endosomal-lysosomal escape of the siRNA/EHCO nanoparticles, pegylated nanoparticles, bombesin and RGD targeted nanoparticles with a PEG spacer has been studied using confocal microscopy. The efficiency of the systemic and targeted delivery of therapeutic siRNA of the peptide-targeted delivery systems via intravenous injection was evaluated in nude mice with human glioma U87 xenografts with an anti-HIF-1α siRNA as a model siRNA based on its antitumor efficacy.

Results: Both RGD and bombesin targeted siRNA/EHCO nanoparticles resulted in specific receptor mediated cellular uptake as shown by flow cytometry. Confocal microscopic studies showed that the siRNA/EHCO nanoparticles, pegylated nanoparticles, and bombesin and RGD targeted nanoparticles with a PEG spacer escaped from the endosomal-lysosomal compartments. The endosomal-lysosmal escape was promoted by the change of amphiphilic properties of EHCO at the endosomal-lysosomal pH.

Figure 1 shows the relative tumor growth after the treatments with siRNA/EHCO nanoparticles, pegylated nanoparticles, and bombesin and RGD targeted nanoparticles of an anti-HIF-1α siRNA. The mice treated with the targeted siRNA nanoparticles of both peptides had slower tumor growth than those treated with non-targeted delivery system and free siRNA during the treatment. The mice treated with RGD targeted siRNA nanoparticles had slower tumor growth after the treatment than the other treatment groups. The results indicate that tumor specific peptide targeted EHCO nanoparticles can efficiently deliver siRNA into tumor tissue after systemic administration, resulting in tumor growth inhibition. It appears that RGD targeted siRNA nanoparticles are more effective than bombesin targeted nanoparticles in tumor inhibition, most likely because they bind to different targets. Further studies are needed to understand the difference of two peptides for *in vivo* tumor targeting.

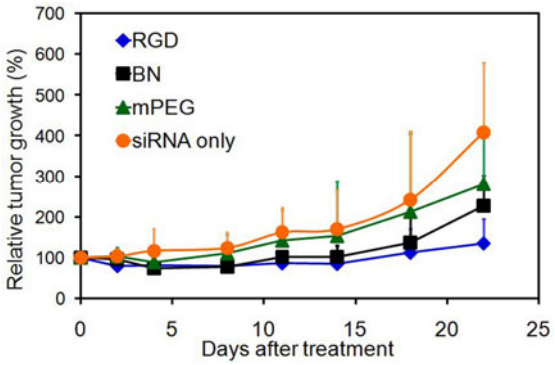

Figure 15. Tumor growth curve of the mice treated free siRNA, pegylated and targeted anti-HIF-1α siRNA/EHCO nanoparticles at a siRNA dose of 2 mg/kg. The treatment was initiated at 21 days after subcutaneous inoculation of U87 cells. The mice were treated again on day 2, 4, 8, 12 and 15 after the initial treatment (day 0).

Conclusions: The peptide targeted siRNA delivery systems with the multifunctional carrier EHCO can specifically deliver siRNA to target cells and escape from the endosomal-lysosomal compartments due to the pH-sensitive amphiphilicity of EHCO. Systemic administration of the targeted siRNA delivery systems of an anti-HIF-1α siRNA resulted in significant tumor growth inhibition at a low siRNA dose. The targeted delivery systems with the multifunctional carrier EHCO are promising for systemic and targeted delivery of therapeutic siRNA.

References: 1. X.-L. Wang, S. Ramusovic, T. Nguyen, Z.-R. Lu. Novel polymerizable surfactants with pH sensitive amphiphilicity and cell membrane disruption for efficient siRNA delivery. Bioconjugate Chem. 2007, 18, 2169-77.