Target Specific Intracellular Delivery of siRNA Using Hyaluronic Acid – Reducible PEI Conjugate

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Statement of purpose: A small interfering RNA (siRNA) is a short double-stranded RNA molecule [1], which has been regarded as novel potential therapeutics for the treatment of various diseases by specific gene silencing of the complementary mRNA. High molecular weight (MW) polyethyleneimine (PEI) has been exploited to make a complex with siRNA for the intracellular delivery and endosomal escape by its proton sponge effect [2]t. However, the inherent cytotoxicity and low serum stability of PEI have limited its wide applications to *in vivo* gene therapy [3]. In this work, to alleviate these problems, relatively non-toxic low MW PEI of 2,000 Da was crosslinked with cystamine bisacrylamide (CBA) and conjugated with hyaluronic acid (HA) in the forms of HA-g-(PEI-SS) and HA-b-(PEI-SS). The target specific intracellular delivery of siRNA/(PEI-SS)-HA complex by HA receptor mediated endocytosis and the following gene silencing were assessed in B16F1 cells with HA receptors.

Methods:

Synthesis of HA-g-(PEI-SS) and HA-b-(PEI-SS): PEI-SS was synthesized by Michael addition between CBA and branched PEI with a MW of 2,000 Da. Then, HA-g-(PEI-SS) was prepared by amide bond formation between the primary amine groups of PEI-SS and the carboxyl groups of HA. In contrast, HA-b-(PEI-SS) was prepared by reductive amidation of HA with primary amine groups of PEI-SS.

Flow cytometry (FACS) analysis: In order to investigate the target specific intracellular delivery of siRNA, B16F1 cells were transfected with Cy3-siRNA /(PEI-SS)-HA complexes. After 3 hours, the cells were harvested, washed, and fixed for FACS analysis.

Cytotoxicity and gene silencing efficiency: *In vitro* cytotoxicity of siRNA/(PEI-SS)-HA complex was assessed by MTT assay in B16F1 cells. *In vitro* gene silencing efficiency was evaluated by the co-transfection of pVMC-luc and siRNA/(PEI-SS)-HA complex.

Results: The cytotoxicity of reducible PEI-SS and HA-(PEI-SS) conjugate was as ignorable as low molecular weight PEI likely due to the degradation of PEI-SS to low MW PEI in the endosome. The HA-(PEI-SS) conjugates could make a complex with siRNA by the electrostatic interaction between negatively charged siRNA and positively charged PEI in HA-(PEI-SS). Figure 1 shows the flow cytometric analysis of B16F1 cells transfected with Cy3-siRNA/(PEI-SS)-HA complexes demonstrating the target specific intracellular delivery of siRNA by HA receptor mediated endocytosis. The resulting siRNA/(PEI-SS)-HA complex showed an excellent *in vitro* gene silencing efficiency in the range of 50~80% in the absence and presence of 50 vol% serum (Figure 2).

On the basis of *in vitro* test results, *in vivo* gene silencing tests using anti-VEGF siRNA will be carried out for the treatment of various angiogenic diseases including age-related macular degeneration and cancers, especially the liver cancer with over-expressed HA receptors.

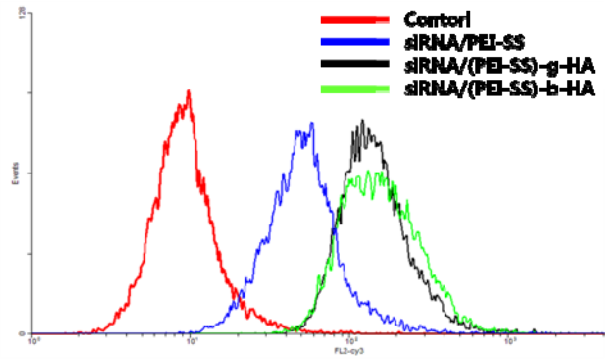

Figure 1. The flow cytometry (FACS) analysis of B16F1 cells transfected with Cy3-siRNA/(PEI-SS)-HA complex. The control represents a fluorescence emitted from B16F1 cells without treatment.

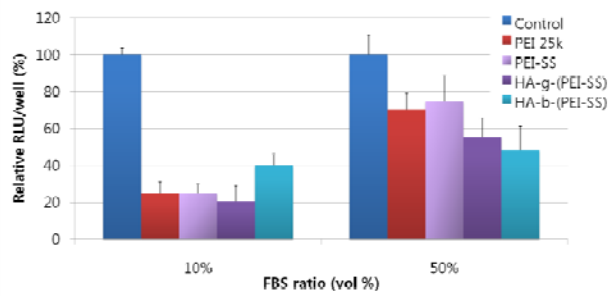

Figure 2. Effect of serum concentration up to 50 vol% on Luc-gene silencing of siRNA/(PEI-SS)-HA complex in luciferase gene expressing B16F1 cells.

Conclusions: We successfully developed HA-g-(PEI-SS) and HA-b-(PEI-SS) conjugates for target specific intracellular delivery of siRNA by HA receptor mediated endocytosis. The cytotoxicity of HA-(PEI-SS) conjugate was remarkably reduced in comparison to high MW PEI. The gene silencing efficiency of siRNA/(PEI-SS)-HA complex was maintained in the range of 50~80% in the absence and presence of 50 vol% serum. The novel HA-(PEI-SS) conjugate will be investigated further for the development of anti-angiogenic therapeutics.

References:

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3. Cho, K. C. Macromol. Res. 2006;14;348-353.