Hyaluronic Acid - Polyethyleneimine Conjugate for Targeted Intracellular Anti-VEGF siRNA Delivery

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Statement of Purpose: Hyaluronic acid (HA) is a negatively charged linear polysaccharide which has been used for controlled release formulation of various biopharmaceuticals [1]. Lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) is identified as one of various HA receptors [2]. In this work, polyethyleneimine (PEI)-HA conjugate was synthesized and used to prepare siRNA/PEI-HA complex for the target specific intracellular delivery of siRNA into HA receptor over-expressing tumor cells [3]. *In vivo* test was also carried out for the application to tumor treatment.

Methods:

Synthesis of PEI-HA conjugate: PEI-HA conjugate was synthesized via amide bond formation between the amine groups of PEI and the carboxyl groups of HA using EDC chemistry.

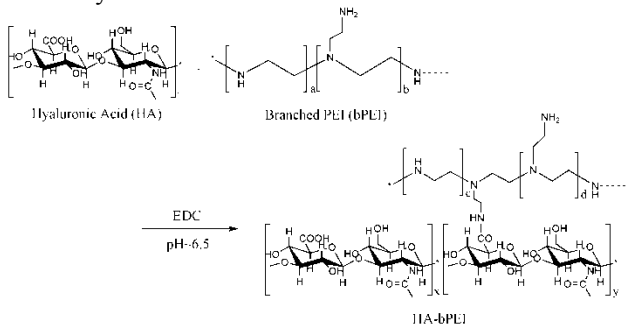

Figure 1. Schematic representation for the conjugation of HA to the branched PEI.

Intracellular delivery of FITC labeled siRNA/PEI-HA complex: B16F1 and HEK-293 cells were treated with fluorescein isothiocyanate (FITC) labeled siRNA/PEI-HA complex. The culture slide was incubated at 37°C and retrieved after 2 hours. Then, the cells were fixed with 1 wt% paraformaldehyde and observed with a con-focal laser scanning microscope after excitation at a wavelength of 543 nm.

Intratumoral injection of anti-VEGF siRNA/PEI-HA complex: In order to generate tumor, 5×10^5 B16F1 cells were injected to 6~8 week-old female C57BL/6 mice. After one week, anti-VEGF siRNA (siVEGF)/PEI, scrambled siVEGF (scVEGF)/PEI-HA and siVEGF/PEI-HA complexes (W/W ratio = 3) were prepared in 5% glucose solution and injected intratumorally (each 50 μL of the complex solution). The tumor volumes were measured every 2~3 days. The mice were sacrificed and anatomized in 2 weeks.

Results: Figure 2-A shows the con-focal microscopic images of FITC-siRNA/PEI-HA complex taken up to B16F1 cells. To the contrary, FITC-siRNA/PEI-HA complex was not readily taken up to the HEK-293 cells without HA receptors (Figure 2-B). According to *in vivo* test, intratumorally injected siVEGF/HA-PEI complex inhibited the angiogenesis in tumor tissues and suppress the tumor growth efficiently compared to the control of 5% glucose solution, siVEGF/PEI and scVEGF/PEI-HA complexes (Figure 3).

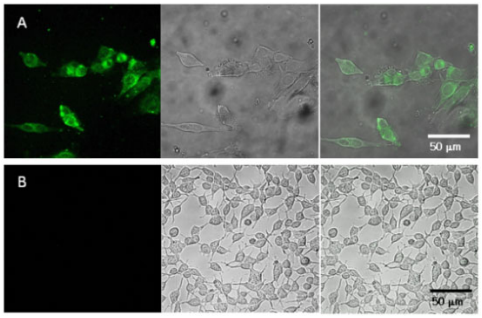

Figure 2. Confocal microscopic images of (A) B16F1 cells and (B) HEK-293 cells after incubation for 2 hours with FITC labeled siRNA complexed with PEI - HA conjugate.

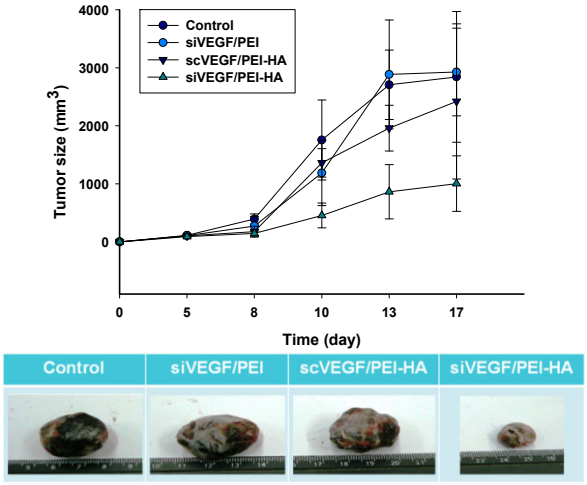

Figure 3. Tumor volume change with increasing time after intra-tumoral injection of a control of 5% glucose solution, siVEGF/PEI complex, scrambled siVEGF (scVEGF)/PEI-HA complex, and siVEGF/PEI-HA complex.

Conclusions: Target specific intracellular delivery of siRNA was successfully carried out using PEI-HA conjugate. FITC-siRNA/PEI-HA complex could be well up-taken to B16F1 cells through the LYVE-1 HA receptor mediated endocytosis. Furthermore, the intratumoral injection of siVEGF/PEI-HA resulted in the efficient inhibition of tumor growth.

References:

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