In vitro and *in vivo* siRNA Delivery Using Modified Hyperbranched PEI

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Statement of Purpose: RNA interference (RNAi) has become widely used for knocking down the expression of a specific target gene. It can achieve gene silencing by the level of post-transcriptional. RNAi is the specificity of siRNA-mediated inhibition of gene expression and it can efficiently identify and silence homologous mRNA. siRNA-mediated targeted gene silencing technology is the most effective way to treat a variety of gene-related diseases. The application of siRNA is largely dependent on the development of gene carriers, which must be administered safely and efficiently with repeatability and lower cytotoxicity.

Vascular endothelial growth factor (VEGF) is a multifunctional angiogenic growth factor that is a primary stimulant of the development and maintenance of a vascular network in the vascularization of solid tumors. Angiogenesis for tumor growth was reported to be suppressed efficiently by down-regulating the gene expression of VEGF. There have been many reports on therapeutic application of VEGF siRNA (siVEGF) for the treatment of cancer.[1-3]

In search for effective non-viral gene vectors for the delivery of siRNA, polyethylenimine (PEI) is the most popularly used as a classic polycationic gene carrier because of its high transfection efficiency owing to a unique proton sponge effect. However, the major limitation of PEI is high cytotoxicity. In order to decrease the toxicity of PEI and enhance its gene transfection efficiency, we investigated PEI-PBLG as a gene delivery system, which was designed and synthesized by grafting hydrophobic poly(g-benzyl L-glutamate) segment (PBLG) to hyperbranched polyethylenimine (PEI-PBLG).[4] In this study, PEI-PBLG was utilized as a siRNA delivery system both *in vitro*[5] and *in vivo*.

Methods:

Materials: Commercial branched PEI, Lipofectamine^{TM}2000 , Luciferase siRNA (siLUC) and VEGF siRNA (siVEGF) were applied in this study. Stably luciferase transfected CT26 cells were used *in vitro* and mice experiments.

Methods: The cytotoxicity of materials was test by MTT assay. Flow cytometric and confocal microscopic analyses exhibited the cellular uptake of polymer/siRNA complexes. *In vitro* siRNA gene silencing experiments were carried out in stably transfected CT26/Luc cells using siLUC and negative siRNA as controls. The relative luciferase activity was related to untreated control cells. For the *in vivo* studies, Balb/c mice bearing subcutaneous tumors were constructed by injecting subcutaneously 50μL of 1×10^6 stably transfected CT26 cells. Tumor treatment started after 10 days when the tumor size became approximately 70 mm^3. After the local administration of polymer/siVEGF complexes, the regression of the tumors could be observed and measured.

Results: *In vitro* studies indicated that PEI-PBLG could efficiently deliver siRNA to cells to silence the target gene. Markedly, PEI-PBLG caused lower cytotoxicity in comparison with unmodified PEI. The siRNA complexed with PEI-PBLG showed a remarkable knockdown of target luciferase gene in stable expressing luciferase CT26 cells while the Lipofectamine^{TM}2000 and unmodified PEI could only achieve knockdown rates of 57.92% and 15.31%, respectively. CLSM and Flow cytometric assay also indicated that PEI-PBLG induced higher cell uptake efficiency than other commercial reagents. PEI-PBLG was shown to be a promising siRNA carrier *in vitro*.

In vivo tumor treatment also exhibited that PEI-PBLG/siVEGF complex could efficiently deliver siVEGF to tumors and achieved obviously therapeutic effect for the treatment of tumor.

Conclusions: PEI-PBLG was successfully developed as a non-viral siRNA delivery system. Hyperbranched PEI modified with PBLG significantly reduced the cytotoxicity by shielding the positive charge of PEI. Gene silencing efficiency of PEI-PBLG polymers was more than 4 times higher than that of PEI-25K. PEI-PBLG had high internalization efficiency in siRNA delivery process compared with the common commercial transfected reagents lipofectamine^{TM}2000 and PEI-25K. Furthermore, PEI-PBLG/siVEGF complex has been successfully applied to the treatment of tumor dramatically retarding the tumor growth. All the results suggested that PEI-PBLG copolymer had a potential to be used as a low toxic, highly effective siRNA delivery carrier.

References:

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