Therapeutic Response in a Xenograft Mice Model by Folated PEG-PCL-PEI/TAM67 complexes

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Statement of Purpose: Gene therapy comprises a novel form of molecular medicine that will have a major impact on human health in next century. The needs for safe and efficient methods for gene delivery still remain a critical obstacle to the routine clinical implementation of human gene therapy. Polyethylenimine (PEI) is one of the successful polymers used for gene delivery because of density of primary, secondary and tertiary amines although several groups have reported that PEI is toxic in many cell lines. Various studies have shown that genes have been successfully delivered to the cells in *in vitro* and *in vivo* by exploiting receptor-medicated endocytosis. Our studies have been performed to improve gene delivery efficiency and to reduce their cytotoxicity by synthesizing a gene carrier based on low molecular weight PEI and biodegradable polycaprolactone (PCL). This also introduced biodegradable ester bond leading to low cytotoxicity compared with PEI 25K. In continuation with above approach, we coupled folic acid moiety to poly (ester amine)s (PEAs) with PEG as a spacer for receptor-mediated (FR) endocytosis for cancer therapy.

Methods: In our previous study[1], we synthesized PEAs by Michael addition reaction, to improve gene delivery efficiency and to reduce their cytotoxicity, based on low molecular weight PEI and biodegradable PCL. Folate conjugated-PEA (Fol/PEG/PCL/PEI-1.2) (FP-PEA) was synthesized by coupling folic acid with PCL/PEI copolymers with dicyclohexyl carbodimide (DCC)/N-hydroxyl succinamide (NHS) chemistry using bifunctional PEG (MW: 2000 Da). The complexation of FP-PEA/pDNA was characterized by gel retardation assay, dynamic light scattering (DLS) and transmission electron microscopy (TEM) to determine the complex forming ability, particle sizes and morphology, respectively. FP-PEA and non-folate PEA (P-PEA) were analyzed for their cytotoxicity and transfection efficiency on cultured KB and A549 cell lines *in vitro*. Tumor volume was drastically decreased in xenograft mice when the polyplexes containing FP-PEA and a therapeutic gene, TAM-67 gene were injected by intratumoral injection.

Results: After the successful synthesis of PEA by Michael addition reaction, folate moiety was coupled to it using DCC and NHS. Synthesis of FP-PEA was confirmed by ^{1}H NMR spectroscopy. The polymer showed suitable biophysical characteristics and excellent transfection efficiency in KB and A549 cells compared with PEI 25K. FP-PEAs showed typical receptor mediated enhanced transfection than P-PEAs or PEI 25K in the cell lines containing folate receptors such as KB and A549 cells. Remarkable decrease in tumor volume after the intratumoral injection of polyplexes in xenograft mice indicated the *in vivo* success of FP-PEAs through folate receptor mediated-endocytosis. Furthermore, antitumor activity with PEA without folic acid moiety (P-PEA) proved not to be effective against xenograft mice model with KB cells when administered at the same dose to that of FP-PEA. Taken together, these results indicate that FP-PEA is highly effective gene carrier capable of producing therapeutic benefit in xenograft mice model without any sign of toxicity.

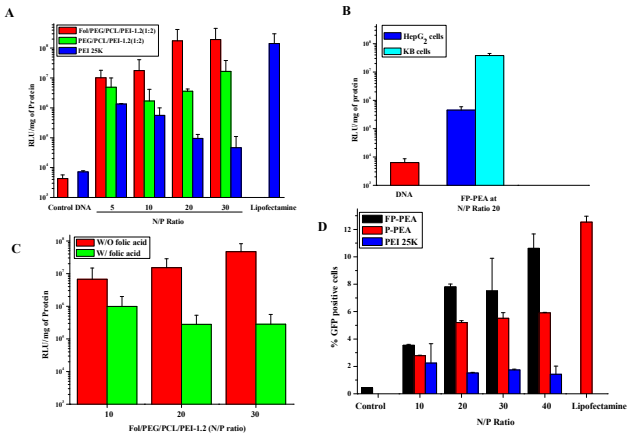

Figure 1. Transfection efficiency of FP-PEA by luciferase assay A) in KB cell line, B) competition assay using FR^+ KB cells and FR^- HepG2 cells. C) Folate competition assay using 800 µM free folic acid in KB cells. D) pEGFP-N_2 expressed in KB cells by flow cytometry.

Conclusions: The approach described in this work represents an easy and efficient method to get fairly stable gene delivery system with folate receptor mediated endocytosis. We have shown that FP-PEA/TAM67 complexes could inhibit tumor growth through diverse functions such as angiogenesis and apoptosis etc thus demonstrating the target specific gene delivery. We propose that biocompatible FP-PEA system is fit for repeated administration to maintain sustained gene expression, thereby opening the possibility for cancer gene therapy. Taken together, these results indicate that FP-PEA is highly effective gene carrier capable of producing therapeutic benefit in xenograft mice model without any sign of toxicity.

References: 1. Arote R. et al. Biomaterials 2007; 28:735-744.