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Synthesis, characterization and transfection efficiency of artificial virus Nanoparticles Based on Modified Polyethylenimines as a Promising non-viral gene Carrier

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Abstract – PEI polymers were modified by adding hydrophobic chains to the primary amines of PEI in different degrees of grafting using bromoacetic acid derivatives with different lengths. These polymers were complexed with plasmid DNA and the resulting nanoparticles were characterized by dynamic light scattering and EtBr-DNA binding assay to determine particle sizes and complex formation respectively. Cytotoxicity and transfection efficiency of the polymers were also tested in cultured Neuro2a cell line. pRL-CMV plasmid DNA and luminometer was employed to quantify the level of transgene expression. Grafting of dextran onto the primary amines of modified-PEI was found to strongly influence physico–chemical properties of PEI as well as of polyplexes formed with DNA depending on the degree of grafting.

Many factors affect both transfection efficiency and cytotoxicity of polyethylenimine (PEI), a widely-used polycationic vector for non-viral gene and oligonucleotide delivery [1]. Various strategies have been examined to improve its vector properties. This study was done in order to enhance biophysical and biological properties of branched-chain 10kDa PEI with relatively low toxicity by a step by step conversion method. At initial step, PEI was altered by substitution of various percentages of its primary amines with carboxylate-terminated short, moderate and long alkyl chains, by reaction with bromoacetic, 6-bromohexanoic, 10-bromodecanoic and 16-bromohexadecanoic acids. To restore the amine content of the polymer, in the second step of modifications a series of modified PEI were synthesized by coupling of oligoamines to alkylcarboxylated-PEI. Finally to avoid non-specific interaction with serum and improve stability of polyplexes, the biocompatible water soluble dextran polysaccharide was grafted onto the oligoamine-alkylcarboxylated-PEI. These polymers were complexed with plasmid DNA at different C/P ratios and the resulting nanoparticles were characterized by dynamic light scattering and EtBr-DNA binding assay to determine particle sizes and complex formation respectively. Cytotoxicity and transfection efficiency of the polymers were also tested in cultured Neuro2a and HepG2 cell lines. pRL-CMV plasmid DNA and luminometer was employed to quantify the level of transgene expression in cells.

DNA condensation measured by ethidium bromide exclusion revealed that resulted polymers could form polyplexes with plasmid DNA and they have the ability to condense DNA in relatively low amounts of polymers. Particles size measurement of polyplexes showed that they form particles in the size range of below 180 nm. Transfection experiments were performed on HepG2 hepatocytes expressing high levels of asialoglycoprotein receptors and on Neuro2a mouse neuroblastoma cells lacking this receptor. For both cell lines, a decrease in transfection efficiency was found for all polymers. The cytotoxicity of modified-PEI was reduced compared to unmodified PEI as documented by MTS assay.

Toxicity of the conjugates was reduced compared to PEI. As a consequence, the transfection efficiency was decreased in both receptor positive and negative cells. This is most probably caused by a steric hindrance during complex formation between Dex-OA-PEI and DNA, as well as reduced polyplex–cell interactions due to carbohydrate conjugation. These changes in the structure might be the cause that an optimal and a careful degree of grafting should be existed to enhance the efficiency of transgene expression [2].

References

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