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Polymeric and liposomal nanoparticles as gene delivery systems

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There has been increasing attention focused on the development of safe and efficient nonviral gene transfer vectorsystems that are either polycationic polymers or cationic lipids. Researches related to nonviral gene carriers comprising chemicallysynthesized molecules have increased enormously to overcome and eventually, to replace the viral vectors. Various polymers from synthetic to naturally occurring ones have been introduced and tested for their suitability in the field of gene therapy. Among them, dendrimers are still very attractive to many scientists for the design of gene carriers because of their well-defined structure and ease of control of surface functionality. In addition, cationic lipids are widely used because it is possible to design and synthesize various kinds of derivatives that are outstanding in the aspects of transfection efficiency, biodegradability and low toxicity. However, there are still many issues for such classical synthetic vectors about the gene transfer potency, the half-life in the blood stream, and the biocompatibility deserving less cytotoxicity.

In this talk, it will be presented the characteristics of some nonviral vectors developed in our group and some recent research projects. It was tried to couple linear PEG withthe globular macromolecule, poly(L-lysine) dendrimer (PLLD) or polyamidoamine (PAMAM) dendrimer, to form an ABA type linear-dendritic hybrid block copolymer. It was performed that the characteristics of nanoparticle formation of the copolymers with plasmid DNA *via* supramolecular self-assembly and the application to practical *in vitro* tests. In addition, the work concerning the preparation of stabilized plasmid DNA-lipid nanoparticles using a novel pH-sensitive PEG-lipid and application to gene delivery system will be presented and discussed.

First, a barbell-like ABA-type triblock copolymer, poly(L-lysine) dendrimer-block-

poly(ethylene glycol) block-poly(L-lysine) dendrimer (PLLD-PEG-PLLD), was synthesized by the liquid-phase peptide synthesis method. The self-assembling complex formation of the third and fourth generation of the copolymer with plasmid DNA was studied. H-1 NMR and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) were used for the characterization of the synthesized copolymer. The self-assembling behavior of the 3rd and 4th generations of the copolymer with plasmid DNA was investigated by electrophoretic mobility shift assay, DNase I protection assay, and ethidium bromide exclusion assay. We observed great differences in the self-assembling ability of the 3rd and 4th generations of the polymer. This suggests that the number of positively charged amines per polymer molecule should be an important factor for the potential for self-assembling complex formation with DNA. Atomic force microscopy (AFM) and zeta potentials were used for evaluating the shape, size distribution, and surface charge of the complexes at various charge ratios. From AFM images, it was observed that the shape of the complex was nearly spherical and its size was about 50-150 nm in diameter. The in vitro cytotoxicity of the copolymer was compared with that of poly(L-lysine), poly(D-lysine), and polyethylenimine.

Second, another novel triblock copolymer, PAMAM-*block*-PEG-*block*-PAMAM was synthesized and applied as a gene carrier. PAMAM dendrimer is proven to be an efficient gene carrier itself, but it is associated with certain problems such as low water-solubility and considerable cytotoxicity.Therefore, we introduced PEG to engineer a nontoxic and highly transfection efficient polymeric gene carrier because PEG is known to convey water-solubility and biocompatibility to the conjugated copolymer. This copolymer could achieve self-assembly with plasmid DNA, forming compact nano-sized particles with a narrow size distribution. Fulfilling our expectations, the copolymer was found to form highly water-soluble polyplexes with plasmid DNA, showed little cytotoxicity despite its poor degradability, and finally achieved high transfection efficiency comparable to PEI in 293 cells. Consequently, these data show that an approach involving the introduction of PEG to create a tree-like cationic copolymer possesses a great potential for use in gene delivery systems.

Third, The acid-labile poly(ethylene glycol) diorthoester distearoylglycerol lipid (POD)

was used with a cationic lipid-phosphatidylethanolamine mixture to preparestabilized plasmid-lipid nanoparticles (POD SPLP) that could mediate gene transfer in vitro by a pH triggered escape from the endosome. Nanoparticles of 60 nm diameter were prepared at pH 8.5 using a detergent dialysis method. The DNA encapsulation efficiency in the nanoparticles was optimal between 10 and 13 mol % ratio of cationic lipid and at a POD content of 20 mol %. The apparent zeta? potential of the nanoparticles at 1 mM salt and pH 7.5 was positive, indicating cationic lipid on the external surface. However, the external layer of the nanoparticles was depleted in the cationic component compared to the starting mole ratio. Low pH sensitivity of the POD SPLP was characterized by a lag phase followed by a rapid collapse; at pH 5.3 the nanoparticles collapsed in 100 min. Nanoparticles prepared from a pH-insensitive PEG-lipid, PEG-distearoylglycerol had similar physicochemical characteristics as the POD SPLP but did not collapse at low pH. The POD SPLP had up to 3 orders of magnitude greater gene transfer activity than did the pH-insensitive nanoparticles. Both the pH-sensitive and pH-insensitive nanoparticles were internalized to a qualitativelysimilar extent in a punctate pattern into cultured cells within 2 h of incubation with the cells; thus, increased gene transfer of the POD SPLP was dueto a more rapid escape from the endosome rather than to greater cell association of these nanoparticles. These results suggest that the pH-sensitive stabilized plasmid-lipid nanoparticles may be a useful component of a synthetic vector for parenterally administered gene therapy.