Editorial

Nonviral gene delivery system

Gene therapy has been investigated a lot in both basic research and clinical trials.[1] The first antisense oligodeoxyribonucleotide (ODN) drug, Vitravene (Fomivirsen), was approved by the United States Food and Drug Administration (FDA) in 2005.[2] After this approval, more and more clinical trials are conducted, not only for ODNs, but also for other nucleic acids drugs, such as plasmid vectors and small interference RNAs (siRNAs).

However, delivery efficiency is a big barrier for the clinical application of gene drugs. It is necessary to overcome their large molecular weight, large size, and negative charge. Nuclease-mediated degradation is also an issue, decreasing the performance of gene drugs. Currently, there are two major categories of methods for gene delivery, viral vectors and nonviral carriers. Viral vectors have higher delivery efficiency than nonviral carriers; whereas nonviral carriers are less toxic and immunogenic. Another important feature for the nonviral delivery system is that they offer delivery on genes with various sizes, which facilitates the potential application of oligonucleotides, such as antisense ODNs and siRNAs.

A nonviral delivery system is a strategy of utilizing natural or synthetic compounds to formulate gene drugs. Cationic lipids are the most commonly used gene delivery agents so far. Cationic lipids are usually used to form lipid/nucleic acid lipoplexes or cationic liposomes to encapsulate nucleic acids. The application of cationic lipids in gene delivery has been almost 25 years since 1987. *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethlyl ammonium chloride (DOTMA) was firstly used to deliver both DNA and RNA in mouse, rat, and human cell lines.[3] However, many other cationic lipids did not show high efficiency *in vivo*, such as 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) and 1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide (DMRIE). Fortunately, more and more novel cationic lipids are synthesized and studied. It was reported recently that stable nucleic acid/novel cationic lipid particles (SNALPs) can inhibit hepatitis B virus (HBV) replication in mice after systemic administration.[4] Another group also successfully encapsulated ApoB-specific siRNAsin SNALPs. These SNALPs were injected intravenously to cynomolgus monkeys and showed significant silencing on ApoB mRNA.[5]

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Cationic lipids can also be utilized to formulate cationic liposomes to deliver nucleotides. Pyridinium lipids formulated cationic liposomes were used to deliver genes to silence TGF-β1 mRNA, and successfully increased transfection efficiency for both siRNAs and plasmids *in vitro*.[6] Targeting ligands can also been conjugated to cationic liposomes to enhance their transfection ability. It was observed that hepatic fibrosis in rats was almost cured after administrating complexes of vitamin-A-coupled liposomes and anti-gp46 siRNA.[7]

Another important nucleic acid delivery agents are cationic polymers, including polyethyleneimine (PEI),[8] poly(L-lysine) (PLL),[9] poly(amidoamine) (PAMAM) dendrimer,[10] polyallylamine,[11] and methacrylate/methacrylamide polymers.[12] Compared to cantionic lipids, polymeric carriers usually have lower toxicity, but also less transfection efficiency.

PEIs with either a branched or a linear form are the most frequently used cationic polymer. These polymers have a broad molecular range from less than 1 kDa to 1.6×10^3 kDa. However, the practical molecular weight range for PEIs applied in gene delivery is from 5 to 25 kDa, because high molecular weight PEIs is much more cytotoxic to the cells and bodies than low molecular weight.[13-16] To overcome the toxicity, many strategies were employed. In 2003, low molecular weight PEIs (800 Da) were coupled together to form 14–30 kDa PEIs, which remain essentially nontoxic but with higher transfection efficiency.[17] Other strategies to reduce the toxicity are synthesizing graft copolymers with PEI and linear poly(ethylene glycol) (PEG).[18,19] Actually, PEI-g-PEG block copolymers could not only reduce the toxicity, but also reduce the diameter of final complexes.[19]

Dendrimers belong to a specific category of cationic polymers. Their unique architecture is similar to branches in the trees, and gives dendrimers' various distinctive properties, such as enhanced viscosity in solution and enlarged surface area, which can significantly increase the loading of nucleic acids in complexes. Polyamidoamine (PAMAM) dendrimers are studied a lot recently because of their good water solubility and nontoxicity. The ODN–PAMAM dendrimer complexes showed good silencing effects with very little cytotoxicity in D5 mouse melanoma and Rat2 embryonal fibroblast cell lines, compared to Lipofectamine and DEAE dextran complexes.[20] Besides good delivery efficiency on ODNs, dendrimers also showed excellent performance on siRNA delivery.[21]

Nevertheless, the toxicity of most cationic lipids and polymers limits their clinical applications. Therefore, bioconjugation of nucleic acids to nonionic carriers also plays an important role in current research. The attempt of conjugating ODNs to asialoglycoprotein (ASGP) by sulfosuccinimidyl

6-[3′-(pyridyldithio)propionamido]hexanoate (sulfo-LC-SPDP) showed efficient delivery.[22] However, it will disturb the bioability of gene drugs if nucleic acids are directly conjugated to the carriers. To solve this issue, carbohydrate or amino acid clusters were usually applied as spacing units. GFLG linkers, a lysosomally degradable tetrapeptide, were used as spacers in a study published in 2009.[23] Yang *et al*. conjugated ODNs to M6P-GFLG-HPMA-GFLG-ONP and showed great delivery efficiency. Hepatic accumulation of conjugated ODNs almost reached 80% of the total injected dose, compared to only 45% for free ODNs.[23] For siRNA conjugation, applicable carriers are usually including cholesterol[24] and PEG.[25] It is different from ODN delivery that the site for siRNA conjugation is very critical. The 3′- or 5′-terminus of the sense strand is generally used for conjugation, because it is necessary to keep the binding ability of the sense strand of siRNA to target mRNA strand.[26]

Nonviral gene delivery systems showed less toxicity and immunogenicity, compared to viral vectors. However, transfection efficiency of nonviral carriers is orders of magnitude lower than viral vectors. Therefore, only 24% of clinical trials conducted so far employed nonviral methods, whereas 67% were using viral vectors (http://www.wiley.com/legacy/wileychi/genmed/clinical/). Fortunately, intensive studies have been done to identify pivotal factors to improve gene delivery by nonviral carriers.[27] We hope more and more nonviral delivery systems can be employed in clinical trials in recent future.

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