SCIENCE CHINA
Life Sciences

• INSIGHT •

November 2011 Vol.54 No.11:1064–1067
doi: 10.1007/s11427-011-4245-z

Current progress of polymeric gene vectors

ZENG Xuan, SUN YunXia, ZHUO RenXi & ZHANG XianZheng*

Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Wuhan 430072, China

Received October 26, 2011; accepted November 30, 2011

Citation: Zeng X, Sun Y X, Zhuo R X, *et al*. Current progress of polymeric gene vectors. Sci China Life Sci, 2011, 54: 1064−1067, doi: 10.1007/s11427-011-4245-z

After over 40 years of progress, gene therapy provides great opportunities for treating diseases from various genetic disorders, infections and cancers. The success of gene therapy largely depends on the availability of suitable gene vectors. As an attractive alternative to virus-based gene therapy, non-viral gene delivery system has been developed and investigated due to their merits including low immunogenecity, convenient operability, and large-scale manufacturability [1]. Because polycations can condense with DNA as a result of electrostatic interactions, form nanosize polyplexes, and protect DNA from degradation by DNase, cationic polymer becomes a major type of non-viral gene delivery vectors (Figure 1) [2]. A wide range of polymeric vectors have been developed and investigated in the past decade, such as polyethylenimine (PEI)-based vectors, poly(L-lysine) (PLL)-based vectors, dendrimer-based vectors, polypeptide-based vectors, and chitosan-based vectors [3]. However, unlike viral vectors that have the ability to infect host cells and overcome cellular barriers through the course of evolution, nonviral gene vectors exhibit significantly reduced transfection efficiency as they are obstructed by various extra- and intracellular barriers, including serum proteins in blood stream, cell membrane, endosomal compartment and nuclear membrane [4].

As a result, numerous studies have focused on designing polymer carriers that have smart molecular structure, present good biocompatibility, avoid both *in vitro* and *in vivo* barriers, and achieve successful delivery of genetic material [1]. To circumvent different systemic and cellular obstacles during gene delivery, proper functional groups were conjugated to polymeric vectors. The current modification of polymeric vectors has shown great improvements in enhancing intracellular gene transfer efficiency and attaining tumor targeted gene delivery [4]. For example, receptor-mediated cell uptake can quickly deliver ligand-targeted polyplexes into endosomes, membrane active compounds (lipids and peptides) can enhance the release of endocytosed materials. Moreover, nuclear localization signal peptides can enhance both the nuclear transport and expression of DNA. Here, current research progress regarding polymeric gene vectors that mainly published in Chinese magazines from 2010 was reviewed, and future trends for the polymer-based gene delivery systems would also be delineated.

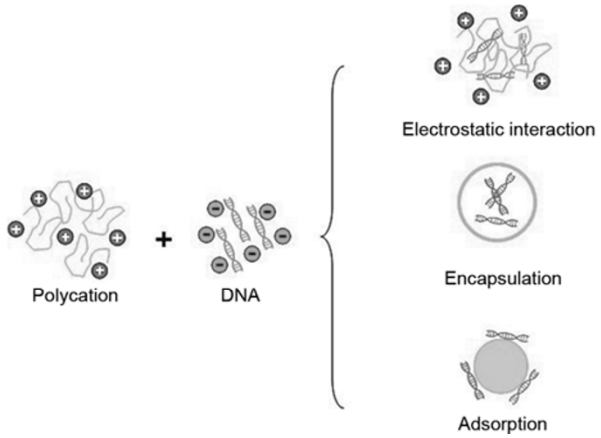

Figure 1 Three main strategies employed to package DNA [4].

*Corresponding author (email: xz-zhang@whu.edu.cn)

1 Commonly used polymeric gene vectors

1.1 Polyethyleneimine (PEI) based vectors

Among all the commonly used polymeric gene vehicles, polyethyleneimine (PEI) is the most prominent one because of its strong buffering capability, high DNA binding capability as well as high transfection efficiency (e.g., 25 kD PEI has been considered as the gold standard of gene transfection). However, high molecular weight and charge density of PEIs also brought high cytotoxicity, which seriously limited their clinical application [5]. On the contrary, low molecular weight PEIs (MW<2000) have relatively low cytotoxicity, but present unsatisfactory gene transfer capability. To overcome this inconsistency, an extensive variety of modifications has been used and could be divided into two main approaches.

The first approach is surface modification of PEI using biodegradable or biocompatible polymers (e.g., PEG, cyclodextrin, dextran) in order to minimize the toxicity of PEI molecules and enhance their serum stability [1]. Pei *et al.* used polyethyleneimine-g-methoxy poly(ethylene glycol) (PEI-g-MPEG) to mediate interleukin-10 (IL-10) gene delivery into rat dorsal root ganglion cells [6]. As compared with Lipofectamine2000, higher IL-10 expression and lower cytotoxicity could be found in PEI-g-MPEG/IL-10 complexes transfected cells after 48 h, indicating that PEI-g-MPEG could be a potential candidate for gene delivery during the therapy of neuropathic pain. Deng's group [7] applied PEG-g-PEI/DNA to transfect mesenchymal stem cells. Results indicated that PEG-g-PEI could completely protect DNA against DNase I, formed PEG-g-PEI/DNA nanoparticles of 100–150 nm and obtained higher transfection efficiency and lower toxicity than cationic liposome.

The other approach is to synthesize various biodegradable PEI compounds via reducible disulfide linkages or ester conjugation. These compounds present high positive charges, but can degrade into nontoxic low molecular weight PEIs in the physiological environment. Zhuo's group [4] has developed a series of disulfide crosslinked PEIs (Figure 2). Recently, they designed a biotinylated transferrin/avidin/biotinylated disulfide containing PEI bioconjugates (TABP-SS) mediated p53 gene delivery system [8]. As compared with jetPEI, this system exhibited much lower cytotoxicity, higher transfection efficacy, and induced more obvious apoptosis in HepG2 and HeLa cells due to the specific interactions between transferrin ligands and their receptors on tumor cells. Chen's group [9] crosslinked low molecular weight polyethylenimine-poly(γ-benzyl L-glutamate) (PEI-PBLG) to obtain CBA-PEI-PBLG(CPP). This compound has good reducible property, and also has good biocompatibility because of introducing PBLG segment. CPP/DNA complexes showed higher transfection efficiencies and lower cytotoxicity in HeLa cells. Wang's group [10] developed cyclic phosphoester monomer ethyl ethylene phosphate

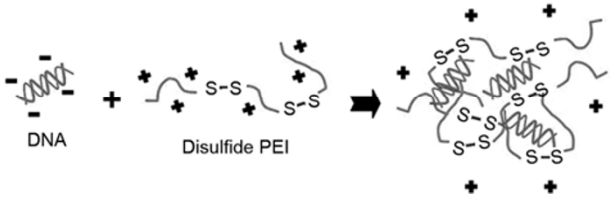

Figure 2 Formation of disulfide containing PEI/DNA complexes [4].

(EEP) modified PEI (PEI-g-EEP) for gene delivery. They found that transfection efficiency of PEI-g- EEP/DNA complexes was correlated to modification degrees with phosphoester. When the modification of phosphoester to PEI was moderate, PEI-g-EEP/DNA complexes exhibited comparable or even higher transfection ability than PEI/DNA complex at its optimal N/P ratio in the absence of serum; otherwise, transfection efficiency reduced dramatically.

Small interfering RNA (siRNA), as a potential tool for gene therapy, also requires suitable vehicles for stable complexation, protection, low cytotoxicity and high efficiency of gene knockdown. Xiao *et al.* [11] synthesized (dextran-hexamethylenediisocyanate)-g-polyethylenimine ((Dex-HMDI)-g-PEI) as a siRNA vector. They found that the complexation of siRNA with the polycation can significantly knock down the expression of enhanced green fluorescent protein (EGFP).

1.2 Poly(amidoamine) dendrimer based vectors

Besides PEI, various other polymers have been synthesized and investigated for gene transfer. Dendrimers are perfect monodisperse macromolecules with a regular and highly branched three-dimensional architecture and may be synthesized to reach nanometric sizes [1]. Their well-defined architectures, highly-branched structures, high density of functional terminal groups, and controllable molecular weights offer great potential for gene delivery. Poly(amidoamine) (PAMAM) dendrimer is the most common class of dendrimers suitable for gene transfer. Wen *et al.* [12] conjugated histidine to the surface of PAMAM G4 using aminolysis reaction to construct a PAMAM derivative (His-PAMAM G4) as a new gene vector. Experimental results revealed that His-PAMAM G4 could sufficiently condense DNA, reduce toxicity and enhance transfection efficiency in serum as compared to PAMAM G4. Zhuo's group [13] found that PAMAM with pendant aminobutyl group demonstrated higher transfection efficiency and percentage of nuclear localization than 25 kD PEI. This compound also exhibited better tissue compatibility, reflected by no or less inflammatory response in the site of muscle injection. Recently, Gu's group [14] reviewed the development of peptide dendrimers with emphasis on their applications both in diagnostics and in therapy.

1.3 Polypeptide based vectors

Among various non-viral gene delivery methods, using natural or artificial polypeptides with certain biological functions is considered as one promising approach. Unlike other vectors, peptides have low cytotoxicity, greater biodegradability, and also serve different functions. For example, lysine and/or arginine-rich cationic peptides can condense DNA into compact particles, TAT-based cell-penetrating peptides can disrupt the endosomal membrane, nuclear localization signal (NLS) peptides can traffic DNA to the nucleus, and RGD-rich peptides can target polyplexes to specific receptors [15]. These properties may all be part of a single peptide sequence or a combination of peptides chemically conjugated to form a vector capable of packaging and targeting DNA for efficient delivery.

Recently, Zhuo's group [16] introduced an iodine atom to nuclear localization signal (PKKKRKV) by chemically attaching 2-iodobenzoic acid to the peptide to obtain NLS-I peptide for cell targeting and nuclear transport. They found that cell internalization and nuclear accumulation of NLS-I was markedly increased compared to NLS in MCF-7 cells, and gene expression by PEI1800/DNA/NLS-I complexes exhibited much enhanced efficiency (up to 130-fold). This study demonstrates an alternative method to construct non-viral delivery system for targeted gene transfer into breast cancer cells. They also synthesized arginine-rich amphiphilic lipopeptides with hydrophobic aliphatic tails ($C_{12}GR_8GDS$ and $C_{18}GR_8GDS$) as functional gene vectors [17]. They found that these lipopeptides exhibited very low cytotoxicity even at high concentration, and could be specifically recognized by cancer cells due to the incorporation of arginine-glycine-aspartic acid (RGD) sequences (specifically recognized by integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ over-expressed on cancer cells).

Chen's group [18] grafted RGD peptides to the carboxyl groups of poly(glutamic acid) to get a targeting shielding carrier (PGA-RGD) for PEI gene carrier. The experiment results showed that PGA-RGD can effectively shield PEI/DNA complex particles. For PGA-RGD/PEI/DNA shielding system, although its surface charges were decreased by PGA-RGD shielding, its gene transfection efficiency was effectively improved because of the specific binding affinity between RGD and receptors on tumor endothelial cell membranes.

Chen's group [19] indicated that multifunctional and multiplexed nanoparticles, as the next generation of nanoparticles, are now being extensively investigated and are promising tools to achieve personalized and tailored cancer treatments. In a recent review from Zhuo's group [20], they reported that stimulus-responsive polymeric nanoparticles, which can change their structures, shapes, and properties after being exposed to external signals (including pH, temperature, magnetic field, photo, etc.), are considered as the most promising materials for biomedical applications including drug delivery, gene delivery and imaging. To increase the *in vivo* stability of polycation gene carriers, Chen's group [21] synthesized a pH-sensitive shielding system, γ-benzyll-glutamate-co-glutamate acid polymer (PGA(60)). This compound showed pH sensitivity at about pH 6.0, and could efficiently shield the positive charge of DNA/PEI complexes. The transfection efficiency was improved when the positive charge was partly shielded by PGA(60). As the suitable pH sensitive range, PGA(60) may be a potential shielding system for polycation gene carriers to be used *in vivo*. Xu and Lu synthesized a series of multifunctional spermine-based pH-sensitive amphiphilic carriers, which exhibited pH-sensitive cell membrane disrupting activity and low cytotoxicity [22]. These compounds resulted in more than 400 times higher luciferase transfection efficiency than that of Lipofectamine-2000 in U87 cells.

2 Future trends

Although intensive studies have been made on nonviral gene vectors and numerous research papers were published in recent years, their commercial development and human clinical application were still limited. Therefore, great emphasis should be placed on *in vivo* trials and development of new commercial transfection agents during the next decade. In order to obtain the permission for clinical application of non-viral gene vector mediated gene therapy, we need to make sustained efforts to combine those efficient synthetic vectors with therapeutic genes, make clinical safety assessment, and provide indications how animal models correlate with clinical experience.

Currently, we have to confront challenges associated with cell targeting specificity, gene transfer efficiency, gene expression regulation, and vector safety. Hence, the successful non-viral vectors for future gene therapy must be multifunctional systems, and will be more efficient in targeting and transfection. As a result, peptide-base vectors have great potential for gene delivery as numerous new functional peptides will be continuously discovered, and they have the ability to achieve these goals alone or in combination with other systems. Moreover, polymeric vectors should preferably be biocompatible and biodegradable to prevent carrier-induced toxicities and the accumulation of carrier components in the host. Accordingly, more efforts should be made in order to facilitate DNA release where low Mw polymers are crosslinked or linearly linked together by degradable linkages to form a high MW polymer that can eventually degrade to its lower MW components. It is very important for us to remember the mechanisms, applications and limitations of current gene delivery systems, and get more novel information from chemical, medical, biological and various fields so as to design innovational successful gene vectors.

This work was supported by Ministry of Science and Technology of China (Grant No. 2011CB606202), National Natural Science Foundation of China (Grant No. 20974083), and the Fundamental Research Funds for the Central Universities.

1 Mintzer M A, Simanek E E. Nonviral vectors for gene delivery. Chem Rev, 2009, 109: 259–302
2 Pack D W, Hoffman A S, Pun S, *et al*. Design and development of polymers for gene delivery. Nat Rev Drug Discov, 2005, 4: 581–593
3 Wonga S Y, Peletb J M, Putnam D. Polymer systems for gene delivery-past, present, and future. Prog Polym Sci, 2007, 32: 799–837
4 Zhang X Z, Zeng X, Sun Y X, *et al*. Bioactive materials in gene therapy. In: Zhao X, Courtney J M, Qian H, eds. Bioactive Materials in Medicine Design and Applications. UK: Woodhead Press, 2011. 179
5 McLachlan G, Davidson H, Holder E, *et al*. Pre-clinical evaluation of three non-viral gene transfer agents for cystic fibrosis after aerosol delivery to the ovine lung. Gene Ther, 2011, 18: 996–1005
6 Pei Y Y, Wang W W, Liu X G, *et al*. *In vitro* evaluation of interleukin-10 gene delivery into dorsal root ganglion cells mediated by PEI-g-MPEG (in Chinese). Acta Polym Sin, 2010, 1: 79–86
7 Chen X A, Zhou Y F, Deng Y B. Plasmid encapsulated polyethylene glycol grafted polyethylenimine nanoparticles for gene delivery into mesenchymal stem cells (in Chinese). Sci Sin Vitae, 2010, 40: 694–703
8 Xiao W, Sun Y X, Cheng H, *et al.* Inhibition of enhanced green fluorescent protein expression by (dextran-hexamethylenediisocyanate)-g-polyethylenimine/siRNA complexes. J Microencapsul, 2010, 27: 447–452
9 Zeng X, Sun Y X, Qu W, *et al*. Biotinylated transferrin/avidin/biotinylated disulfide containing PEI bioconjugates mediated p53 gene delivery system for tumor targeted transfection. Biomaterials, 2010, 31: 4771–4780
10 Guo Z P, Tian H Y, Xia J L, *et al*. Bioreducible crosslinked low molecular weight branched PEI-PBLG as an efficient gene carrier. Sci China Chem, 2010, 53: 2490–2496
11 Du J Z, Tang L Y, Yuan Y Y, *et al*. Phosphoester modified poly(ethylenimine)as efficient and low cytotoxic genevectors. Sci China Chem, 2011, 54: 351–358
12 Wen Y T, Pan S R, Guo Z H, *et al*. Histidine modified PAMAM as gene vector for enhancing gene transfection efficiency in serum. Chin J Biomed Eng, 2010, 29: 129–136
13 Peng L, Gao Y, Xue Y N, *et al*. Cytotoxicity and *in vivo* tissue compatibility of poly(amidoamine) with pendant aminobutyl group as a gene delivery vector. Biomaterials, 2010, 31: 4467–4476
14 Gu Z W, Luo K, She W C, *et al*. New-generation biomedical materials: peptide dendrimers and their application in biomedicine. Sci China Chem, 2010, 53: 458–478
15 Martin M E, Rice K G. Peptide-guided gene delivery. AAPS J, 2007, 9: E18–E29
16 Wang H Y, Li C, Yi W J, *et al*. Targeted delivery in breast cancer cells via iodine: nuclear localization sequence conjugate. Bioconjug Chem, 2011, 22: 1567–1575
17 Chen J X, Wang H Y, Quan C Y, *et al*. Amphiphilic cationic lipopeptides with RGD sequences as gene vectors. Org Biomol Chem, 2010, 8: 3142–3148
18 Lin L, Tian H Y, Xia J L, *et al*. Synthesis and characterization of targeting shielding system for PEI gene carrier (in Chinese). Sci Sinca Chim, 2011, 41: 368–373
19 Li X H, Zhang C, Le Guyader L, *et al*. "Smart" nanomaterials for cancer therapy. Sci China Chem, 2010, 53: 2241–2249
20 Li Y Y, Dong H Q, Wang K, *et al*. Stimulus-responsive polymeric nanoparticles for biomedical applications. Sci China Chem, 2010, 53: 447–457
21 Xia J L, Chen J, Tian H Y, *et al*. Synthesis and characterization of a pH-sensitive shielding system for polycation gene carriers. Sci China Chem, 2010, 53: 502–507
22 Xu R Z, Lu Z R. Design, synthesis and evaluation of spermine-based pH-sensitive amphiphilic gene delivery systems: Multifunctional non-viral gene carriers. Sci China Chem, 2011,54: 359–368