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Recent progress in cationic polymeric gene carriers for cancer therapy

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In recent years, various carriers for gene delivery have been developed for biomedical applications. Among all kinds of gene carriers, cationic polymeric carriers for delivery therapeutic gene as non-viral carriers have received growing interests due to their improved high transfection efficiency with the relative safety. In particular, the advancement of novel polymeric gene carriers has gained much progress in the development of effective anticancer therapy. Herein, this review focused on the development of cationic polymeric carriers for cancer therapy, including polyethylenimine (PEI), polyamidoamine (PAMAM) dendrimers, polylysine (PLL), chitosan and modified cationic polymers. And recent progresses in the development of novel polymeric carriers for gene delivery, such as targeted gene carriers, responsive gene carriers and multifunctional gene carriers, were summarized. Finally, the future perspectives in the development of novel polymeric carriers for delivery gene were presented.

polymeric gene carriers, cancer therapy, stimuli-responsive carriers, multifunctional gene carriers

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1 Introduction

Gene therapy has shown an attractive strategy for treating many diseases, such as genetic disorders, malignant tumors, AIDS, cardiovascular and neuronal diseases [1–3]. Despite the progress research, delivery of the therapeutic gene (e.g. RNA interference, plasmid DNA and antisense oligonucleotides) into targeted cells is still a formidable challenge. Therefore, the efficient and safe gene carriers are needed for therapeutic gene delivery.

Gene carriers as one of key technologies for gene therapy are generally divided into two major types, viral vectors and non-viral vectors. Viral vectors have proven to be the effective gene delivery system and the successfully used in clinical trials [4,5]. However, some challenges have restricted their application in gene therapy, such as, their intrinsic immunogenicity, limited gene packing capacity, their potential side effects and the cost of production [6,7]. In contrast, non-viral vectors are more attractive due to their relative safety with low immunogenicity, high biocompatibility, the flexibility to package of nucleic acids, and easy to design and produce with low cost [8].

Among various kinds of non-viral carriers, cationic polymers for gene delivery have received growing interests due to their improved high transfection efficiency with relative safety [9–11]. The complexes (cationic polymers/therapeutic gene) are based on the electrostatic interact ions which are formed between the positively charged cationic polymers and negatively charged therapeutic gene. Herein, this review illustrated the development of cationic polymers for gene delivery to cancer therapy.

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2 The types of cationic gene carriers based on polymers

Cationic polymers have been extensively utilized as gene delivery carriers due to their condensing ability. Hereinafter, various types of cationic polymers (Figure 1) were investigated such as polyethylenimine (PEI), polyamidoamine (PAMAM) dendrimers, polylysine (PLL), chitosan and modified cationic polymers.

2.1 PEI

Within the cationic polymeric delivery systems, PEI reported first in 1995 is one of the most studied cationic carriers for gene delivery [8,12]. PEI, as gold standard for gene transfection, has high gene encapsulation efficiency both *in vitro* and *in vivo* [8,13]. However, cytotoxicity of high molecular weight PEI is the main hindrance on its application in gene delivery due to its high positive charge density, which could cause self-aggregation and adherence on the surface of cells [13,14]. Therefore, many approaches have been taken into account to reduce the cytotoxicity of PEI for practical application of gene delivery.

For the purpose of decreasing cytotoxicity of PEI, one of the most extensively strategy was to introduce the neutral hydrophilic polyethylene glycol (PEG), which could shield the cationic charge of PEI and reduce non-specific interactions of the complexes with blood components [15]. Consequently, PEG-PEI was synthesized, and could really reduce the cytotoxicity and prolonged blood circulation time in the bloodstream. However, the PEG block may reduce the transfection efficiency by decreasing the positive charge density compared to non-PEGylated PEI. Tian *et al*. [16] reported a novel rapid pH-responsive polymer (PELG) as the shielding system to shield PEI25k/DNA. The surface zeta potential of ternary polyplex (PELG/PEI/pDNA) could change from a negative to positive charge in tumor extracellular environment, which exhibited high transfection efficiency and good inhibition effects in HeLa xenograft tumors.

Another method to decrease the cytotoxicity of PEI is using the low molecular weight (M_W) PEI, which has lower toxicity profiles but is less efficient for transfection compared with the high molecular weight PEI [17]. Therefore, many studies had been conducted to compensate the cell transfection with cross-link or modified lower M_W of PEI. Li *et al*. [18] prepared several cationic polymers (DA-PEI, DS-PEI and DO-PEI) based on low molecular weight PEI (M_W=600) linked with diglycidyladipate or its analogs. The transfection efficiency of DS-PEI *in vitro* was about 5 times higher compared to that of the PEI/DNA polyplex in A549 cells. Meanwhile, the cytotoxicity of cross-linked PEI polymers is lower than that of 25 kDa PEI in HEK293 cells. Liu *et al*. [19] de-

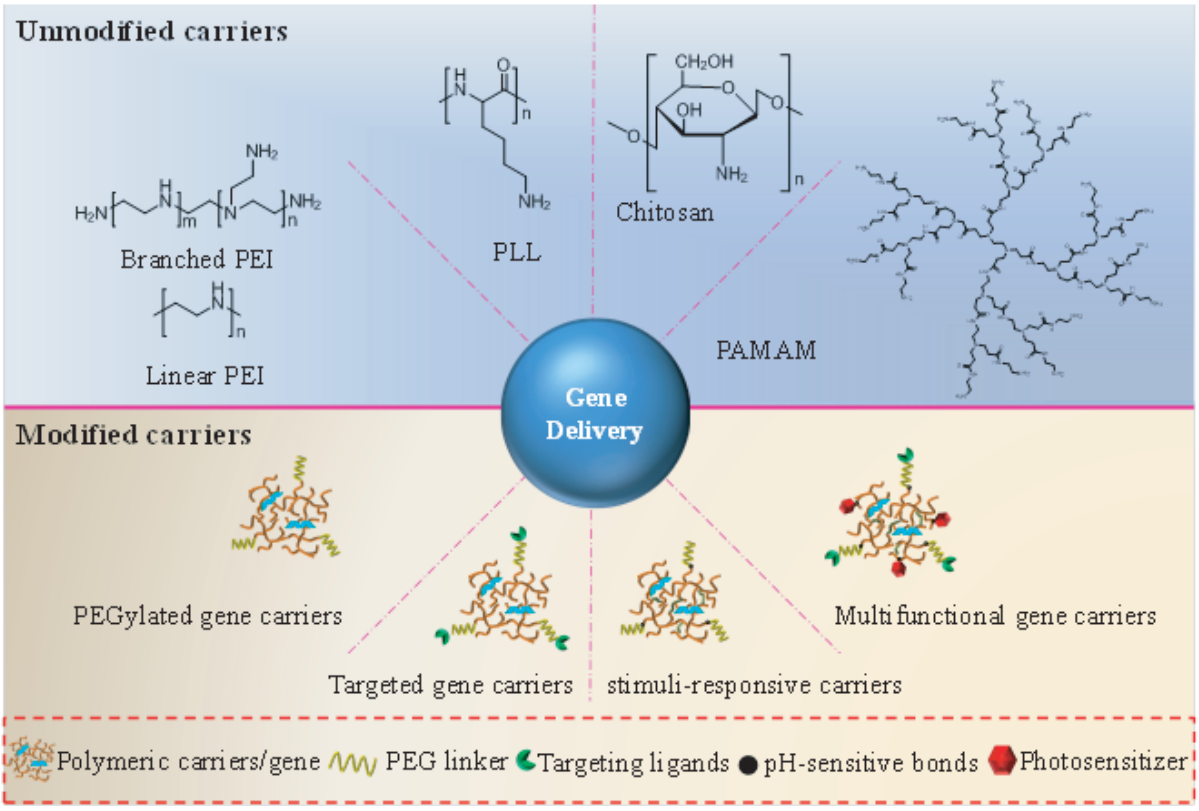

Figure 1 The structures of unmodified cationic polymers and modified cationic polymers for gene delivery (color online).

veloped branched disulfide-bonded PEI (lPEI2200-SS) using linear PEI (M_W=2200) as survivin-targeted siRNA carrier for murine breast cancer therapy. The cellular uptake of lPEI-SS/siRNA polyplexes was significantly enhanced as the higher branching degree of lPEI-SS than lPEI. Moreover, the lPEI-SS/siRNA polyplexes showed significant anti-tumor effects *in vitro* and *in vivo* on 4T1 cells and 4T1 murine breast cancer models.

2.2 PAMAM dendrimers

PAMAM dendrimers are the most investigated carriers for gene delivery among dendritic polymers, which were first developed in 1985 by Tomalia *et al*. [20,21]. Structurally, PAMAM dendrimers consist of a core; interior layers (generations) and terminal functional groups. The shapes of PAMAM dendrimers depend on the number of generations. The planar, elliptical shapes are the lower generation dendrimers (G0–G4), while the spherical conformations are the higher generations (G5–G10) due to the densely packed branches [22]. PAMAMs have large number of secondary amine groups and tertiary amine groups, which could be protonated at physiological pH, thus the PAMAM dendrimers could bond siRNA/DNA through the electrostatic interaction to form complexes, and could induce high transfection efficiency. Furthermore, PAMAM has the ability to escape from endosome due to the buffer capacity of the high density of amine groups [23].

PAMAM dendrimers are known to be highly effective in transfecting siRNA/DNA into various cells [24]. However, the higher dendrimer generations (e.g., G7, G9) possess higher transfection efficiency with relative toxicities. To balance between the transfection efficiency and cytotoxicity, the lower generations (<G4) were selected by many researchers for gene delivery [21]. And the PAMAM dendrimers were slightly changed with chemical modification as a method to improve the transfection efficiency [25]. Additionally, PEG chains were conjugated in the PAMAM dendrimers to reduce the toxicity and increase the circulation time *in vivo* [26,27].

To decrease the toxicity of the active groups on dendrimer surface, G5 and G6 PAMAM dendrimers were modified by polyethylene glycol (M_W=5000) at different molar ratios. PEG conjugation PAMAM dendrimers (PEG-PAMAM) could significantly decreased cytotoxicities *in vitro* and *in vivo* compared with unmodified PAMAM dendrimers [28]. Wang *et al*. [29] constructed a pH-sensitive gene delivery system based on folic acid-PEG-chitosan-PAMAM-plasmid DNA complexes (FPCPHDs) for targeting cancer cells. FPCPHDs could enhance gene transfection and expression with minor toxic effects on KB cells, and FPCPHDs could escape from endosomes rapidly. Furthermore, FPCPHDs could increase red fluorescence protein (RFP) expression at the tumor site in S180 xenograft nude mice [11]. In another study, Liu *et al*. synthesized a hydroxyl terminal PAMAM dendrimer (PAMAM-OH) by attaching to *S*-methyl-*L*-cysteine (SMLC) via an acid-labile ester bond, and a folic acid (FA) were attached to the PAMAM dendrimer by a polyethylene glycol (PEG) linker [30]. The modified PAMAM (PAMSPF) showed less cytotoxic and higher transfection efficiency in KB and HepG2 cells compared with PAMAM. Additionally, PAMSPF/DNA exhibited long circulation time and led to high targeting of tumor sites *in vivo* study.

2.3 PLL

PLL is synthesized by polymerization of *N*-carboxy-anhydride of lysine with the advantage of biocompatible and biodegradable profiles, and is suitable for application *in vivo* [31,32]. PLL contains a large number of amino groups and has the ability to pack siRNA/DNA into complexes under physiological conditions. However, the application of PLL has been hampered due to their relative low transfection efficiency and high toxicity, especially high molecular weight (M_W=25000 Da), and low M_W (5000 Da) of PLL was inefficient for gene transfection [33]. To address the above problems, much progress has been achieved through modifying PLL.

Early studies showed that PLL (M_W=3000 Da) could promote DNA-mediated transfection effectively through conjugating to *N*-glutarylphosphatidylethanolamine (NGPE) [34]. And PLL (M_W=14000 Da) modified with PLGA could decrease the toxicity of PLL about five times [35]. Also, PLL with low and high M_W (4000 and 25000 Da) was modified by several endogenous lipids with substituting 10% of ε-NH_2. These results showed that the transfection efficiency correlated closely with the degree of substitution. And the lipid-modified high MW PLL was found to be more effective in bone marrow stromal cells (BMSC). Additionally, the transgene expression of lipid-modified PLL was significantly increased by about 20%–25% compared to unmodified PLL and commercial Lipofectamine-2000 [36]. Thiersch *et al*. [37] synthesized peptide-modified PLL-*g*-PEG polymers for improving the transfection efficiency of HIF-1α plasmid DNA and decreasing the cytotoxicity of PLL. The above results showed that the modified PLL with peptide could strongly enhance transfection efficiency *in vitro*, and the cell viability of peptide-functionalized PLL-*g*-PEG polymers was about 90%–100%, which indicated that the cytotoxicity of PLL could be deceased by peptide or PEG modification.

2.4 Chitosan

Chitosan as a natural linear alkaline polysaccharide is obtained from the deacetylation of chitin [38]. It has been widely used for siRNA/DNA delivery, as the primary amine groups of chitosan can be protonated under physiological

conditions. Chitosan as gene carriers owns lots of advantages, such as relative low cytotoxicity, high cellular permeability and high biocompatibility [39]. However, the transfection efficiency of chitosan is relative low, which can be influenced by the degree of deacetylation and molecular weight [40]. To improve gene transfection efficiency, chitosan was conjugated with cationic polymers, peptides, and lipid chains [41–43]. Furthermore, the hydrophilicity and stability of chitosan would be improved by inducing the PEG [44].

To enhance the chitosan-mediated gene delivery, chitosan could be modified with many methods by some researchers. For this purpose, Yu *et al*. [45] constructed a chitosan-graft-PEI-eprosartan (CPE) conjugate using molecular-weight chitosan backbone for delivering vascular endothelial growth factor (VEGF) plasmid, and CPE/VEGF complexes had high transfection efficiency in AT1R-overexpressed H9C2 cells. Li *et al*. [46] developed a pH-sensitive agmatine-chitosan bioconjugate (CS-DM-Agm) for condensing herapeutic VEGF siRNA. The CS-DM-Agm bioconjugates had high transfection efficiency, especially in the pH 6.5 environment. In another study, low-molecular-weight chitosan was grafted with 18-carbon chain, and the modified chitosan could significantly enhance gene transfection in human embryonic kidney cells without affecting the low cytotoxicity of chitosan [47].

For facilitating the effective usage of chitosan in gene delivery, PEGylated chitosan was used to improve its solubility and stability in water solution [48]. Du *et al*. [49] synthesized PEG-*N*-chitosan and PEG-*N*,*O*-chitosan to improve the solubility of chitosan, thus the solubility was obviously improved and all PEGylated chitosan was water-soluble, even with the low degree of substitution. In addition, the solubility was further improved with increasing degree of substitution of PEG grafts. Sun *et al*. [50] constructed poly(ethylene glycol)-modified chitosan (PEG-CS) for survivin targeted siRNA delivery, and the PEG-CS could effectively improve the solubility of chitosan, and then enhance the transfection efficiency of surviving siRNA in 4T1 tumor cell lines.

3 The progress of functional gene carriers based on polymers

In recent years, great attention has been focused on the functional gene carriers (Figure 1), such as targeted gene carriers, stimulus-responsive gene carriers and multifunctional gene carriers [51,52]. Carriers could be conjugated to some cell-recognizable functional ligands for targeted delivery, for instance, Arg-Gly-Asp (RGD) peptide, folic acid and hyaluronic acid (HA) [53,54]. In addition, with the purpose of changing the solubility, alteration of the hydrophilic, conformation and release ability of carries, some stimuli-sensitive/responsive carriers were developed through changing their properties to response to stimuli, such as temperature, pH, light irradiation, ionic strength and reactive oxygen species (ROS) [55–57]. Furthermore, carriers with two or more functions were developed for gene delivery [58]. The usages of the targeted or stimuli-responsive carries provide a promising approach for gene delivery. And the delivery carriers become an active participant instead of passive delivery, which is beneficial in the optimization of therapy.

3.1 Targeted gene carriers

Delivery gene based on polymer nanoparticles to the tumor site by systemic circulation has two targeting strategies, passive targeting and active targeting. In passive targeting, nanoparticles with gene could be accumulated in tumor tissue due to the enhanced permeability and retention (EPR) effects [59]. However, poorly targeting accumulation is large challenge using EPR effect, and active targeting delivery could enhance gene efficacy through the increasing gene accumulation in tumor site and reduce the non-specific toxicity. Wide ranges of targeting ligands are currently being utilized for tumor targeting, such as folic acid, RGD peptides, antibody and aptamer [60–63].

A ternary copolymer conjugated polyethylenimine-graft-polycaprolactone-block-poly-(ethylene glycol)-folate (PEI-PCL-PEG-Fol) was prepared by Liu and co-workers [64] (Figure 2), which could enhance uptake of therapeutic siRNA in folate receptor overexpressing cell lines due to the folic acid ligand. PEI-PCL-PEG-Fol/siRNA micelleplexes increased cellular uptake and gene knockdown in SKOV-3 cells, and the deposition of siRNA in the tumors exhibited 110-fold higher than that treated with nontargeted PEI-PCL-PEG. Nam and co-workers [65] designed a novel carrier, using chitosan, PEI and a targeting ligand (HPOCP polyplexes), which was used for targeting human epidermal growth factor receptor2 (HER2/neu). This result showed that HPOCP polyplexes could enhance the cellular uptake due to the present targeting ligand. Another type of targeting nanoparticles for gene delivery is based on the use of aptamer. Askarian *et al*. [66] developed PLL-alkyl-PEI copolymers conjugated to AS1411 aptamer, which was used to target cancer cells. And the gene transfection was enhanced by 1.8–5 folds in cancer cells by introducing aptamer in PLL-alkyl-PEI copolymers. Overall, the targeted gene delivery platforms are the promising approaches and provide a new option for personalized medicines for cancer therapy.

3.2 pH-responsive gene carriers

It is well known that extracellular pH of solid tumors is more acidic (pH 6.5–6.8) than normal tissues, which is due to the increasing lactic acid production of glycolysis from cancer cells [67]. Therefore, some pH-sensitive nanoparticles for

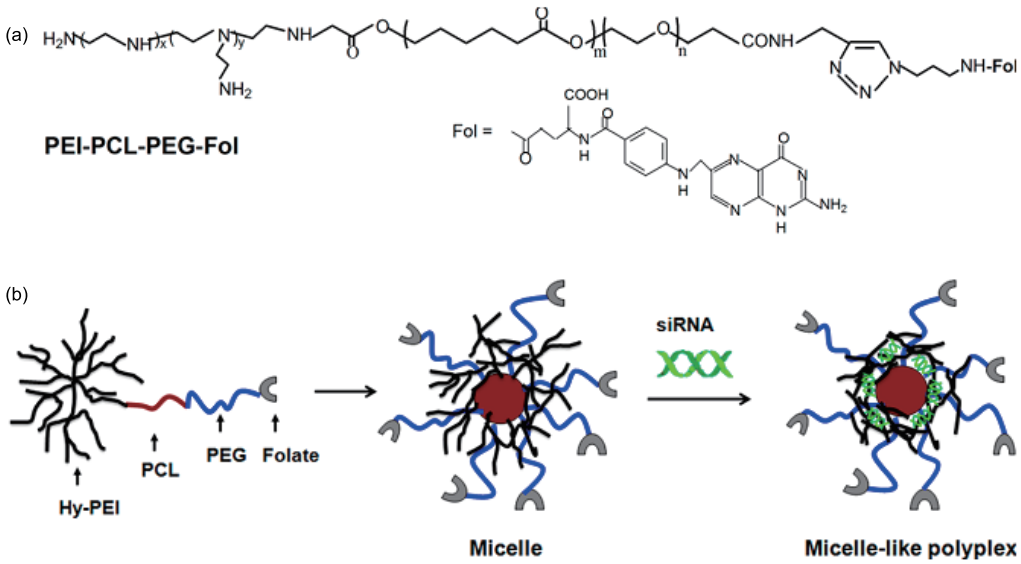

Figure 2 (a) Structure of PEI-PCL-PEG-Fol; (b) schematic illustration of the micelle-like polyplex formation with siRNA [64] (color online).

gene delivery were exploited for tumor targeting delivery using the acidic tumor microenvironments [68–70].

From the previous studies, PEGylation of cationic carriers could enhance stability and biocompatibility *in vivo* [71]. However, it inhibited cellular uptake and endosomal escape significantly in cancer cells. To solve this problem, some stimuli-responsive shell-detachable carriers were developed for gene delivery. Zhao *et al*. [72] developed sheddable ternary complexes for efficient and safe gene delivery. Plasmid DNA (pDNA) was complexed with cationic amphiphilic PEI-PTMC nanoparticles to form a stable core, and then a pH-sensitive charge-conversional PEG layer was coated on the surface of nanoparticles to form ternary complexes (PEI-PTMC/DNA/PEG-DA) by electrostatic interaction. At the tumor site, the charge-reversal PEG was detached from nanoparticles via electrostatic repulsion, and the positively charged complexes re-exposed, which was beneficial for cellar uptake and endosomal escape as the abundant amine in PEI. In addition, PEI-PTMC/DNA/PEG-DA complexes could improve the tumor targeting and gene transfection efficiency in HeLa tumor-bearing BALB/c mice.

Another type of pH-sensitive nanoparticle was charge-reversal PEG corona introduced by covalent bonds [73]. Sun *et al*. [74] constructed a simple acid-sensitive bridged copolymer for tumor targeting delivery of siRNA. A tumor-pH-labile polymeric nanoparticles (PEG-*Dlink*$_m$-R9-PCL) was synthesized by the same group with an acid-labile linker between PEG and PDLLA copolymers. When the PEG-*Dlink*$_m$-R9-PCL nanoparticles arrived at the tumor sites, the PEG layer was detached from nanoparticles, and surface charge was increased. Thus the cellular uptake and antitumor activities were improved both *in vitro* and *in vivo* with an excellent safety profile. Furthermore, an ultrasensitive pH triggered charge/size dual-rebound gene delivery system was developed (Figure 3) [56]. PEG crosslinking could shield the surface positive charges (PEI/DNA) and tighten the complex particles, which could induce to decreasing cytotoxicity and prolonging circulation. When the nanoparticles arrived at tumors area, the PEG shielding could be rapidly peeled off, which could enhance the tumor cell uptake efficiency. Thus the ultrasensitive pH triggered charge/size dual-rebound gene delivery system showed outstanding antitumor therapeutic efficacy.

3.3 Temperature-responsive gene carriers

Usually, a low critical solution temperature (LCST) is used for obtaining the solubility behavior of temperature-responsive nanoparticles by changing temperature. The solubility of temperature-responsive nanoparticles is increased by reducing the temperature below the LCST [75,76]. Among varieties of temperature-responsive polymers, poly(*N*-isopropylacrylamide) (PNIPAm) is most commonly used for gene delivery [77,78].

Feng and co-workers [79] developed an efficient temperature-responsive gene carrier for delivery therapeutic plasmid DNA (pDNA). The cationic block copolymer gene delivery system was prepared via mixing pDNA and copolymers at 25 °C. When the temperature increased from 25 to 37 °C, the tolerability against nuclease and resistance to protein adsorption were enhanced by the mixed polyplex micelles (MPMs), thus the gene transfection efficiency of MPMs was significantly increased *in vitro* and *in vivo*. Wang and co-workers reported a thermo-responsive polymer (G5-PBA$_{30}$-pNIPAM$_{35}$) consisting of dendrimer, poly(*N*-isopropylacrylamide) (pNIPAM) and phenylboronic acid (PBA) (Figure 4) [57]. The complexes of G5-PBA$_{30}$-pNIPAM$_{35}$ and siRNA were stable

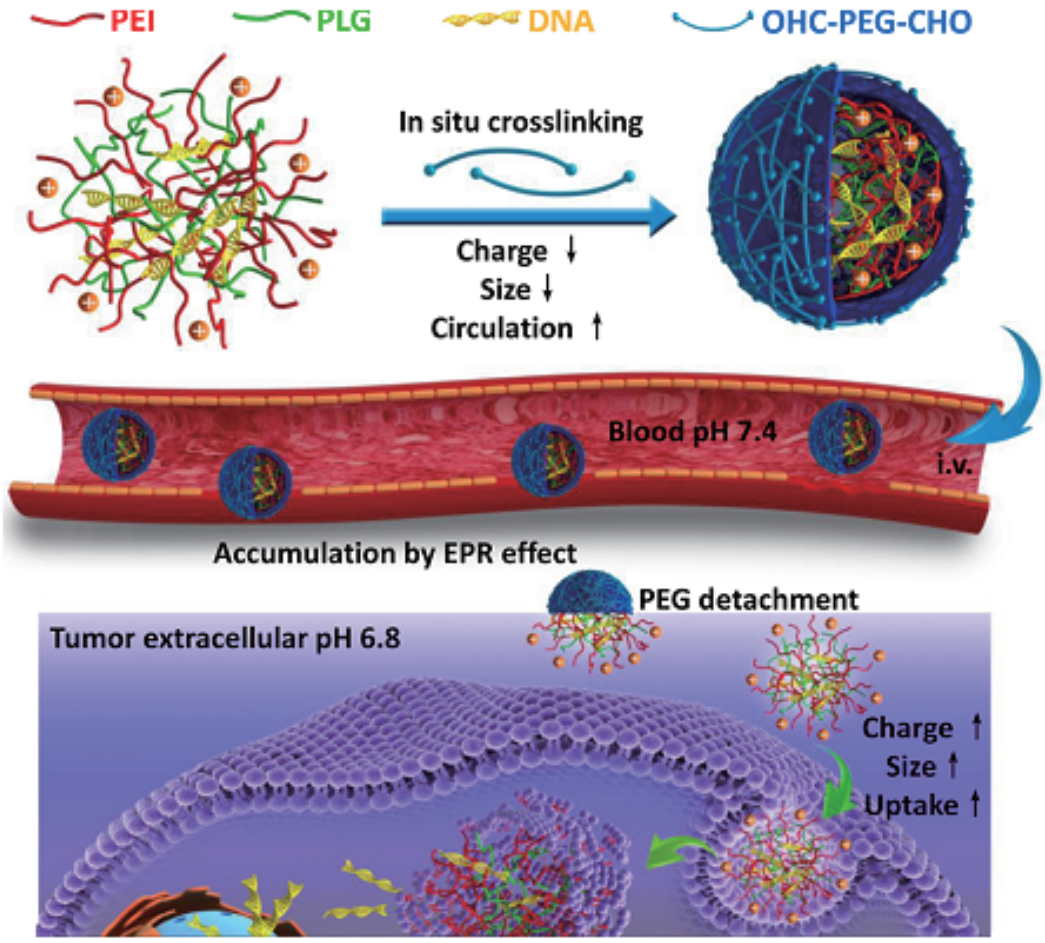

Figure 3 The complex nanoparticles (NPs) was formed through complex gene, PEI and PLG, and the NPs were further tightened by PEG formed Schiff bases. The Schiff base bonds between PEG and PEI was cleavable in acidic pH of tumor area, thus the PEG shielding could be rapidly peeled off. The cell uptake efficiency was enhanced in tumor area and antitumor therapeutic efficacy was improved [56] (color online).

and the complexes size was about 200 nm at 37 °C. The complexes were unstable and the complex size was significantly increased when the surrounding temperature changed to 4 °C, thus siRNA could be released from complexes in cooling solution below its LCST, and gene silencing efficacy was significantly increased by cool treatment with minimal toxicity on HeLa cells.

One of the major challenges in hindering *in vivo* applications of temperature-responsive polymers is simultaneous achievement of efficient gene transfection activity and prolonged retention in blood circulation in target tissues. Li *et al*. [80] constructed novel rod-shaped ternary polyplex micelles (TPMs) for delivering the plasmid DNA containing the thermo-responsive formation with PEG-shielding. The results showed that the TPMs exhibited distinct temperature-responsive formation with facile increasing from room temperature to body temperature.

3.4 Light-responsive gene carriers

It is known that cells activity gradually decreases with increasing of temperatures due to enzyme deactivation. When the temperature is above 43 °C, enzymatic activities of most cells are drastically reduced and ultimately lead to cells apoptosis [81]. Especially, cancer cells are relatively more sensitive to temperature than normal cells [82]. Therefore, some light-responsive polymers upon illumination of UV/visible or near-infrared (NIR) light are developed for photothermal therapy. NIR light-responsive polymers with the wavelengths of 700–1000 nm are more suitable for biomedical applications *in vivo* than UV/visible-responsive polymers, as NIR light can penetrate the skin with less damage to the irradiated site [83].

A novel light-responsive cationic polymeric carrier, consisting of cell-penetrating, helical polypeptides and light-sensitive 4,5-dimethoxy-2-nitrobenzyl-glutamate (DMNBLG), was designed by Yin and co-workers [84]. When the illumination of light was applied after transfection, the cytotoxicity was reduced and the intracellular gene release was enhanced, as the helical conformation of the polypeptide was transformed to the helix-disrupted conformation and the charge

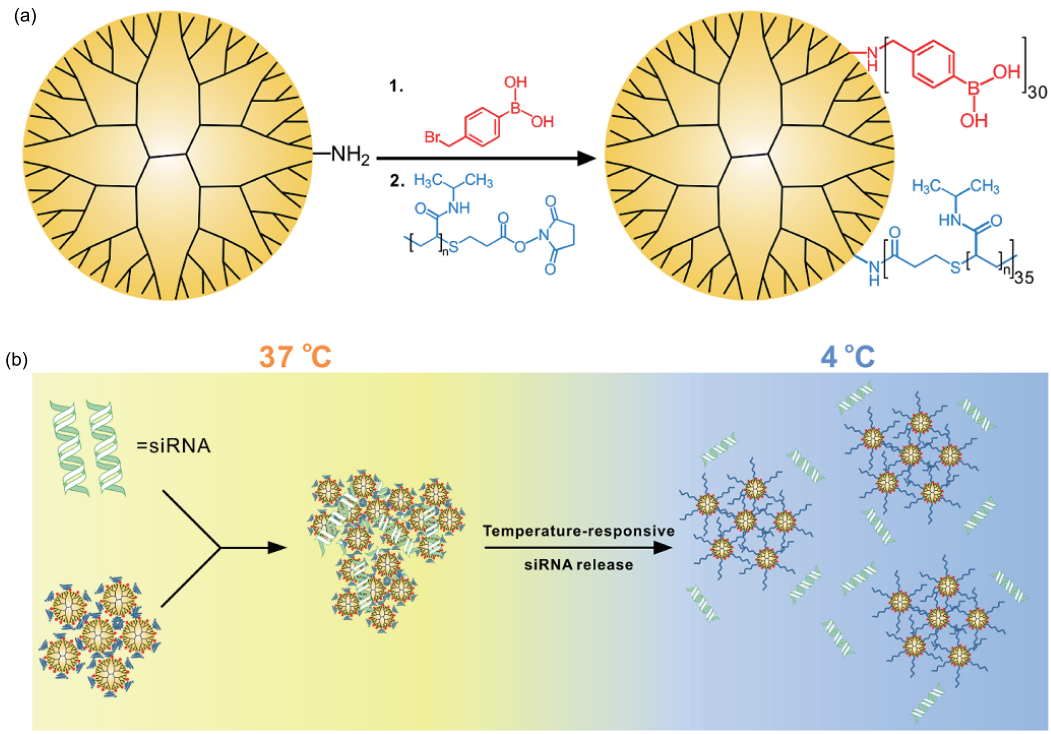

Figure 4 (a) Synthesis process of G5-PBA$_{30}$-pNIPAM$_{35}$; (b) the mechanism of temperature-responsive siRNA release [57] (color online).

density was reduced in response to external light-triggers. Feng *et al.* [85] constructed a new light-responsive carrier, PEG and PEI co-conjugated ultra-small nano-GO (NGO-PEG-PEI), for DNA and siRNA delivery. The plasmid DNA transfection efficiency of NGO-PEG-PEI was remarkably enhanced under NIR laser irradiation, as the cell membrane permeability was increased by the mild photothermal heating. Moreover, the siRNA could be delivered into cells in the presence of light irradiation, and the target gene was significantly down-regulated.

3.5 Redox-responsive gene carriers

The level of glutathione (GSH) in intracellular (10 mM) is higher than that in the extracellular fluid (2–10 μM), and a higher concentration of GSH in tumor tissues have been found than in normal tissues by researchers [86,87]. Therefore, some polymers were designed based on the different concentration of GSH between tumor tissue and normal tissue. The most common example of using this difference is constructed polymers using disulfide bonds, which could be transformed to thiols in the presence of GSH in tumor tissue [88,89].

Wei *et al.* developed a new amphiphilic block copolymer with a disulfide bond for efficient *in vivo* plasmid delivery, consisting of poly(e-caprolactone) (PCL), oligoamine tetraethylenepentamine (TEPA), poly(glycidyl methacrylate) (PGMA), and oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA) [90]. This result showed that the disulfide bridges in copolymer/pDNA could be degraded by glutathione (GSH) in the cytosol, and then hydrophobic PCL core was detached, thus the release of nucleic acid (pDNA) could be facilitated. Furthermore, the transfection efficacy was studied in mouse brains, the transfection activity in reducible block formulation was 15.6-fold higher than that in non-reducible block analogue. Jia and co-workers [91] constructed PEG-ss-chitosan oligosaccharide (COS)-ss-PEI copolymers for effective gene delivery. The PEG-ss-COS-ss-PEI copolymers could effectively condense DNA and showed high transfection efficiency in HeLa cells. The PEG-ss-COS-ss-PEI copolymers were rapidly unpacked in the presence of GSH, and then DNA was effectively released from copolymers.

Owing to the oncogenic transformation, the level of reactive oxygen species (ROS) is higher in cancer cells than in normal cells, including H_2O_2, superoxide and hydroxyl radical [92,93]. Hence, some ROS-responsive polymeric carriers which can target and deliver gene into ROS-overproducing cancer cells were developed [94,95]. ROS-responsive thioketal-based poly(amino thioketal) PATK was constructed for gene delivery by Shim and co-workers [94]. Thioketal linkages in PATK could be degraded under ROS conditions, thus the intracellular release of the complexed DNA could be enhanced in prostate cancer cells. Therefore, the high levels of intracellular ROS can be used for efficient gene delivery in tumor cells.

3.6 Multifunctional gene carriers

Some carriers were developed for gene delivery to overcome various barriers in biomedical applications, such as multi-stimuli-responsive carriers, multifunctional gene carriers [58,96]. A dual stimuli-responsive gene carrier composed of phenylboronic acid (PBA), sugar-installed PEI and PEG was constructed by Kim and co-workers [97], which was triggered in acidic endosomal pH or intracellular ATP. This dual stimuli-responsive carrier for anti-angiogenic gene delivery had high transfection efficiency *in vitro* and provided high tumor targeting ability, which was beneficial for tumor growth inhibition [97]. Another dual stimuli-responsive carrier polymer nanoparticles was developed by Yang and co-workers [98] with photo- and pH-responsive polypeptides (PPPs). The PPP-NPs could efficiently deliver siRNA into the target cancer cells. Upon the NIR light illumination, the siRNA loaded PPP-NPs could selectively accumulate at the lowered pH tumor sites.

Huang and co-workers [60] developed a multifunctional carrier for gene delivery, consisting of targeting ligand (FR), gene therapy and photothermal therapy. The microRNA-181b inhibitor (anti-miR-181b) was loaded into PEI-modified and FR-targeted PEGylated gold nanocages (AuNCs) (PTPAuNCs).The results showed that anti-miR-181b/PTPAuNCs could effectively enhance the cellular uptake and significantly inhibit proliferation of cancer cells under laser illumination *in vitro*. Furthermore, the anti-miR-181b/PTPAuNCs could be effectively delivered into target tumor site, and the tumor volumes were significantly decreased upon near-infrared radiation in SMMC-7721 tumor-bearing nude mice. Liu *et al*. [99] synthesized a multifunctional cationic gene delivery system (PCD-SS-PDMAEMA/PEG-FA), which had two functionalized modules, reduction-triggered degradability and cell-targeting ability. The PCD-SS-PDMAEMA could be degraded by cleaving disulfide linkers and then trigger DNA release in reductive microenvironment, the PEG-FA could specifically and effectively target the folate-receptor positive cells.

4 Conclusions and perspective

An overview of emerging trends in the gene delivery carriers for cancer therapy was provided systematically in this review. However, there are still many challenges that need to be addressed. One of the main difficulties is that the polymeric carriers with gene are usually efficient *in vitro* but inefficient *in vivo* as the poor permeability in tumor tissues. In addition, the cytotoxicities of polymeric carriers should be addressed in the future study.

The "intelligent" design of polymeric carriers for gene delivery is one of the effective strategies for enhancing therapeutic effect *in vivo* in the future study. The changeable sizes and properties of "intelligent" carriers can be designed by using different microenvironments of tumor tissues, such as low pH, hypoxic status, high concentration of proteases, and redox conditions. Another strategy for improving therapeutic effect *in vivo* is to design delivery gene carriers for targeting tumor-associated fibroblasts in the tumor stoma via some ligands or antibodies, thus the tumor microenvironments is modulated and then lead to enhanced therapeutic effect. In addition, the combined approaches for co-delivery two or more therapeutic genes using delivery carriers offers the opportunities for enhancing the cancer therapy and reducing the cytotoxicities *in vivo*. All these factors, such as the selection of therapeutic agents, loading capacity and release kinetics, need to be considered comprehensively when designing new carriers for gene co-delivery. In conclusion, with the development of highly effective and safe carriers for cancer therapy, the strategies for cancer therapy will be improved rapidly in the future due to the design of personalized biomedicine based on individual patient profiles.

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Conflict of interest The authors declare that they have no conflict of interest.

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