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Serum-resistant complex nanoparticles functionalized with imidazole-rich polypeptide for gene delivery to pulmonary metastatic melanoma

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ABSTRACT

To enhance serum-resistance and overcome the lysosomal barrier are effective and feasible strategies to increase the transfection efficiency of non-viral gene delivery system. For the systemic delivery of therapeutic gene, we previously developed self-assemble carboxymethyl poly(L-histidine) (CM-PLH)/poly(β-amino ester) (PbAE)/pDNA ternary complex nanoparticles based on electrostatic coating as an effective pDNA carrier. Recharging cationic PbAE/pDNA polyplexes with CM-PLH was a promising method to reduce the cytotoxicity and enhance the stability *in vivo* of positive charged polyplexes. In the present study, the transfection activities of ternary complex nanoparticles were further evaluated *in vitro* and *in vivo*. The transfection efficiency of ternary complex nanoparticles showed significant serum-resistance (CM-PLH-containing (51.9 ± 4.35)% in 50% FBS > CM-PLH-free (14.7 ± 5.66)% in 50% FBS), cell line dependent (HEK293 > MCF-7 > COS7 > B16F10 > A549 > Hela > SPC-A1 > CHO > SKOV3) and incubation period dependent (24 h, 20 h, 16 h > 12 h > 8 h > 4 h > 2 h > 1 h > 0.5 h). After transfected with ternary complex nanoparticles loading pGV240-MDA-7/IL-24, the B16F10 cells exhibited significant apoptosis and proliferation inhibition due to the expression of IL-24. Moreover, in the pulmonary metastatic melanoma model, ternary complex nanoparticles loading pGV240-MDA-7/IL-24 showed significant antitumor therapeutic efficacy *in vivo*. These results suggested that CM-PLH/PbAE/pDNA ternary complex nanoparticles were promising and challenging gene vector for practical application.

1. Introduction

Gene therapy is the insertion, alteration or removal of genes within individual's cells and biological tissues to treat variety diseases including genetic and acquired diseases, including cancer. Therapeutic plasmid DNA (pDNA) and small interfering RNA (siRNA) represent promising new anticancer agents (Scholz and Wagner, 2012). However, a significant bottleneck associated with gene therapy is the development of effective gene delivery system. Viral and non-viral gene delivery systems are generally two categories for the delivery of nucleic acids in gene therapy (Boulaiz et al., 2005). Both *in vitro* and *in vivo* the transfection efficiency of viral vectors is indeed encouraging, but the security problems greatly limit its applications (Cristiano, 1998; Lundstrom and Boulikas, 2003). Therefore, non-viral vectors have gained more attention because of their obvious advantages (Schatzlein, 2001).

One significant concern in non-viral gene vector research is that exploring reliable cationic polymer, which must be labeled on the characteristics such as biodegradable, biocompatibility, low toxicity, high transfection efficiency and efficient delivery gene *in vivo* (De Smedt et al., 2000; Shi et al., 2010). Poly(β-amino ester) (PbAE) is degradable cationic polymer synthesized by Michael addition of amines to diacrylate esters which is considered as an attractive hotspot in the field of non-viral gene delivery system due to its good biocompatibility and relatively low cytotoxicity (Akinc et al., 2003; Jere et al., 2008). Because of the high positive charge density, PbAE can complex with pDNA self-assembly based on electrostatic adsorption in slightly acidic, aqueous suspension (Gu et al., 2012). Many candidates led to high transfection efficiency and low toxicity in malignant cells compared to commercial agents Lipofectamine™ 2000 (Anderson et al., 2004; Zugates et al., 2007). Nevertheless, PbAE had poor transfection ability when applied alone or without modifications *in vivo* (Devalapally et al., 2007;

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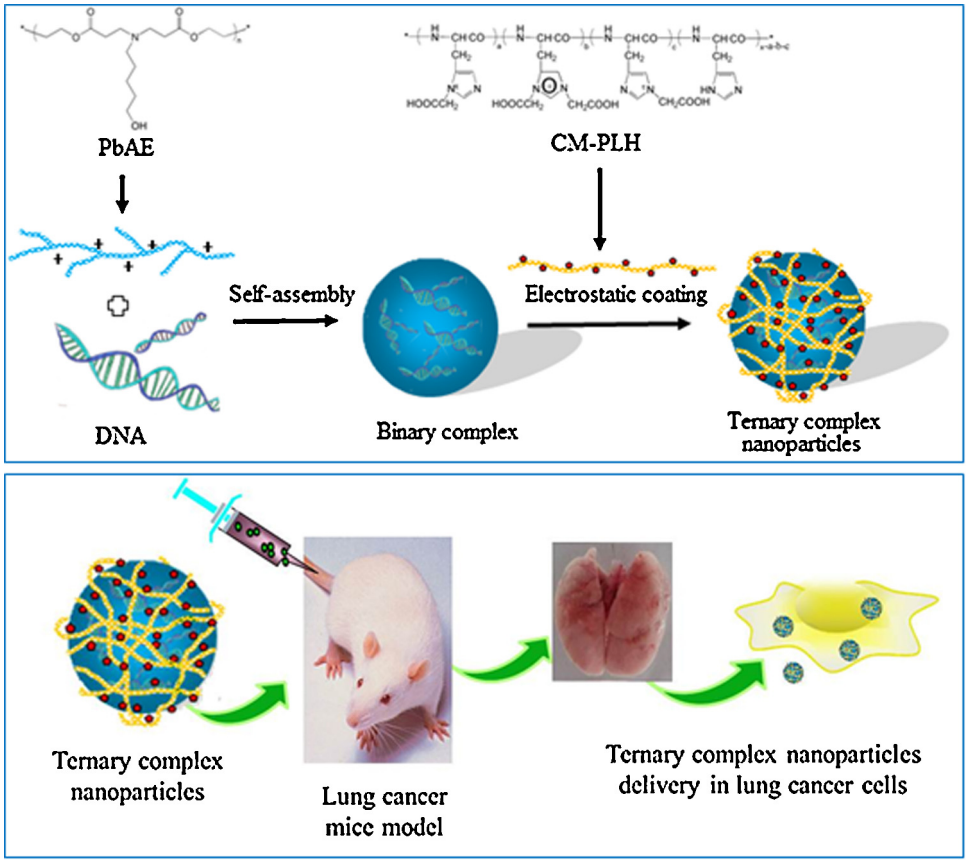

Fig. 1. Formation of CM-PLH/PbAE/DNA ternary complexes nanoparticles based on electrostatic coatings and transfection process *in vivo*.

Fields et al., 2012; Kamat et al., 2013; Tzeng et al., 2012). The relatively low transfection level of PbAE/pDNA complex nanoparticles is possibly due to poor stability in serum, short circulation time and inadequate escape of the complexes from endosomes (Midoux and Monsigny, 1999).

It was reported that heterocyclic imidazole groups containing polymers possess large capacity of proton buffering at endosomal/lysosomal pH and promising transfection efficiency (Ghosn et al., 2010; Gu et al., 2012; Liu et al., 2013; Midoux and Monsigny, 1999; Pack et al., 2000; Pires et al., 2011; Yang et al., 2008). As a polypeptide, poly(L-histidine) has many imidazole groups with a pK_a around 6.0, which can absorb protons and possess buffering capacity in the endosomal pH range (pH 5–6.5) and lead to the osmotic swelling and more escape of pDNA (Asayama et al., 2004, 2009, 2012). The introduction of the anionic carboxymethyl groups, therefore, improved the water solubility of the poly(L-histidine) at physiological pH. Furthermore, CM-PLH exhibited hemolytic activity at endosomal/lysosomal pH as well as no significant effect on a rapid aggregate formation of serum proteins at physiological pH (Asayama et al., 2008).

Recharging cationic complexes with highly charged polyanion is a useful method for achieving a longer circulation time for gene delivery to tumor *via* the enhanced permeability and retention (EPR) effect (Harris et al., 2010; Luo et al., 2012; Tian et al., 2011). In order to increase the transfection ability and stability of the PbAE/pDNA complex nanoparticles *in vivo*, we previously introduced CM-PLH into the binary complex nanoparticles to form non-covalent ternary complex nanoparticles by electrostatic assembling as non-viral gene delivery system. The ternary complex nanoparticles were confirmed to perturb the endosomal membrane and CM-PLH/PbAE support the endosomal escape of plasmid DNA by their proton sponge effect, which led higher transfection efficiency than Lipofectamine™ 2000. Based on these results, we further verified serum-resistance and anti-tumor ability of the ternary complex nanoparticles *in vitro* and *in vivo* in the present study. CM-PLH formed a fixed aqueous layer on the surface of nanoparticles and provided a steric barrier to avoid interactions with plasma proteins, resulting in escape from trapping by the reticuloendothelial system (RES) after *in vivo* administration. Therefore, the ternary complex nanoparticles displayed cancer sites after systemic injection owing to the EPR. The schematic representation of formation of CM-PLH/PbAE/pDNA ternary complex nanoparticles we constructed and transfection process *in vivo* are shown in Fig. 1.

2. Materials and methods

2.1. Materials

PbAE and CM-PLH were synthesized in our laboratory according to a previously reported method. Lipofectamine™ 2000 transfection kit was obtained from Invitrogen Corporation (Carlsbad, CA, USA) and used as suggested by the manufacturer. The Dulbecco's Modified Eagle's Medium (DMEM) with high glucose, RPMI 1640 medium, fetal bovine serum (FBS), trypsin–EDTA solution (0.25%) and penicillin–streptomycin were obtained from Gibco-BRL (Burlington, ON, Canada) (USA). Goat anti-IL-24 and biotinylated rabbit anti-goat secondary antibody were purchased from R&B Systems (Minneapolis, MN, USA). PE conjugated anti-IL-24 and BD Perm II solution for intracellular staining were obtained from BD Pharmingen (Shanghai, China). The other materials and solvents were purchased from the local commercial providers and used as received without further purification.