Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Charge-conversional zwitterionic copolymer as pH-sensitive shielding system for effective tumor treatment

Jie Chen [a], Xuan Dong [a,b], Tianshi Feng [a,c], Lin Lin [a], Zhaopei Guo [a], Jialiang Xia [d], Huayu Tian [a,*], Xuesi Chen [a,*]

[a] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[b] Xiamen Institute of Measurement and Testing, Xiamen 361000, China
[c] Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China
[d] School of Chemistry and Chemical Engineering, Lingnan Normal University, 29 Cunjin Road, Zhanjiang, Guangdong 524048, China

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 22 July 2015
Accepted 15 August 2015
Available online xxxx

Keywords:
Cell apoptosis
Charge conversion
pH-responsive
Rev-caspase-3
Tumor therapy

ABSTRACT

A novel pH-responsive gene delivery system for tumor acidity-targeted *p*DNA delivery is prepared by introducing a rapid charge-conversional zwitterionic copolymer to the positive surface of PEI/*p*DNA complexes through electrostatic interaction. The shielding system (OEAL) consists of oligoethylenimine (OEI), poly(L-aspartate) (PBLA), and poly(L-lysine) (PLL). The charge-conversional behavior of the OEAL/PEI/DNA ternary complex is evaluated by zeta potential assay. The surface charges of the complexes can change from negative to positive in the pH range of 7.4–6.8. Under a simulative *in vivo* environment, OEAL/PEI/DNA exhibits promotion of cellular uptake by tumor cells and enhanced gene transfection efficiency because of its good charge-conversional properties. Antitumor experiments further show that the pH-responsive charge-conversional system can mediate a therapeutic gene that can induce tumor apoptosis (*p*KH3-rev-casp-3) to achieve effective tumor inhibition. Accordingly, OEAL can be regarded as a promising tumor microenvironment-sensitive gene delivery shielding system for antitumor therapy.

Statement of Significance

This manuscript focused on the novel pH-responsive gene delivery system for tumor acidity-targeted *p*DNA delivery. The novel system is prepared by introducing a rapid charge-conversional zwitterionic copolymer, consisting of oligoethylenimine, poly(L-aspartate) and poly(L-lysine), to the positive surface of PEI/*p*DNA complexes. The surface charges of the complexes can change from negative to positive from pH 7.4 to 6.8. OEAL/PEI/DNA shows promoting cellular uptake by tumor cells and enhanced gene transfection efficiency. The antitumor experiments further show that the pH responsive charge conversional system can mediate *p*KH3-rev-casp-3 to achieve effective tumor inhibition. Accordingly, OEAL can be regarded as a promising tumor microenvironment sensitive gene delivery shielding system for antitumor therapy.

1. Introduction

Gene therapy has emerged as a promising strategy for treating numerous human diseases, especially cancer [1–4]. However, a gene itself can hardly be applied directly because of its rapid degradation in plasma and poor intracellular uptake [5,6]. Therefore, various vehicles, such as viruses [7], cationic compounds [8–10], and inorganic nanoparticles [11], have been developed to deliver therapeutic genes to target cells.

Among these vehicles, cationic polymers have attracted considerable attention because of their high water solubility and strong ability to package DNA by electrostatic interaction [12]. Gene delivery systems with cationic components are effective because they can stick to cell anionic surfaces in large amounts. This property allows a further good entry into vacuolar compartments [13]. However, these cationic nanocomplexes usually fail to achieve satisfactory effect in anticancer therapy by systemic administration because of nonspecific phagocytosis by non-target tissues and cells, as well as nonspecific interactions with negative blood components, thus leading to rapid blood clearance from the circulation [14,15]. PEGylation [16] or shielding of positive charges with

* Corresponding authors.
E-mail addresses: thy@ciac.ac.cn (H. Tian), xschen@ciac.ac.cn (X. Chen).

http://dx.doi.org/10.1016/j.actbio.2015.08.018
1742-7061/

polyanions [17] of cationic polymers can stabilize the complexes, minimize nonspecific interactions, and prolong circulation time *in vivo*, thereby increasing tumoral accumulation via enhanced permeation and retention effect. However, intracellular uptake in tumors is reduced significantly after PEGylation or shielding with polyanions, leading to a marked reduction in both gene transfection and antitumor efficiency. Ideal gene delivery systems should be capable of prolonging circulation life in the blood, improving efficient delivery at the tumor site, and promoting intracellular uptake of tumor cells.

The pH of tumor microenvironment is estimated to be 6.5, which is significantly lower than that of blood and normal tissues [18,19]. On the basis of the pH difference, substantial research involving pH-sensitive gene carriers has been designed extensively [20]. Among these carriers, gene delivery systems with pH-responsive charge-conversional ability have garnered increasing research focus because of their potential application *in vivo* [19,21]. All the designed gene delivery systems with charge-conversional ability have demonstrated enhanced gene transfection efficiency, prolonged circulation time, and increased antitumor effect. Among these charge-conversional systems, amide bond based polymers, such as citraconic acid amide or *cis*-aconitic acid amide modified amines of the polymers, have attracted considerable attention because of their cleavage when pH changes [22]. However, several limitations prevent the clinical application of the charge-conversional system based amide bond cleavage [23]. On the one hand, precise control of the pH conversional behavior by the amide bond cleavage is difficult. On the other hand, rapid charge conversion also performs an important function in the charge-conversional gene delivery system *in vivo*, whereas the amide bond cleavage is a gradual process in an acidic environment.

In the present study, a novel pH-responsive gene delivery system for tumor acidity-targeted *p*DNA delivery was prepared by introducing a rapid charge-conversional zwitterionic copolymer to the positive surface of PEI/*p*DNA complexes through electrostatic interaction (Scheme 1). The shielding system (OEAL) consisted of oligoethylenimine (OEI), poly(L-aspartate) (PBLA), and poly(L-lysine) (PLL). To achieve effective tumor treatment, rev-caspase-3 (rev-casp-3) gene was applied to promote the constitutive apoptosis of tumor cells [24]. Negatively charged OEAL/PEI/*p*DNA polyplexes form at physiological pH. When these polyplexes come into contact with the acidic microenvironment of tumor tissues, the zwitterionic copolymers rapidly undergo charge-conversion and change into positive charges. These positive charges lead to electrostatic repulsion with positively charged PEI/*p*DNA complexes and de-shielding of the shielding layer from gene delivery systems, thereby enhancing accumulation at tumor tissues, promoting intracellular uptake, improving gene transfection efficiency, and achieving effective tumor treatment.

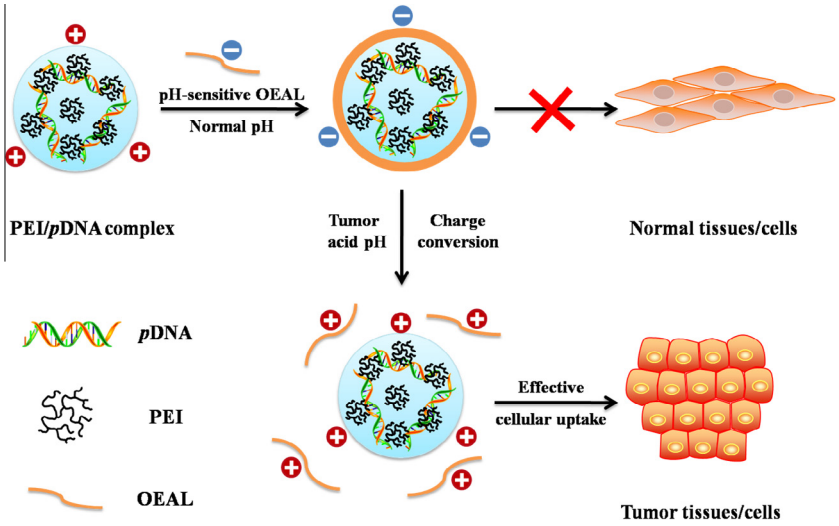
2. Materials and methods

2.1. Materials

Branched PEIs with weight-average molecular weight of 1.8 kDa (OEI) and 25 kDa (PEI-25k) were purchased from Alfa Aesar and Aldrich, respectively. N-carboxyanhydride of β-benzyl-L-aspartate (BLA-NCA) and N-carboxyanhydride of ε-benzyloxycarbonyl-L-lysine (Lys(Z)-NCA) were synthesized according to our previously reported method [25]. Poly(L-glutamic acid) (PLG) was prepared according to our previously reported method [26]. Dichloromethane (DCM) was refluxed with sodium and distilled under nitrogen prior to use. N,N-dimethylformamide (DMF) was stored over calcium hydride (CaH_2) and purified by vacuum distillation with CaH_2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco (Solon, Ohio, USA). Cell culture media, antibiotics, Opti-MEM and fetal bovine serum (FBS) were purchased from Invitrogen (USA). Luciferase plasmid (*p*GL3-control), cell lysates, and luciferase reporter gene assay kit were purchased from Promega (Wisconsin, USA). Rev-casp-3 gene was subcloned into a *p*KH3 vector and designed according to our previously reported method [24]. All other chemicals were purchased from Sinopharm Chemical reagent Co. Ltd. (China) without further treatment.

2.2. Preparation of OEI-PBLA-PLys(Z)

OEI-poly(benzyl-L-aspartate)-poly(benzyloxycarbonyl-L-lysine) (OEI-PBLA-PLys(Z)) was prepared according ring opening polymerization and OEI was used as the macro-initiator. Briefly, branched OEI (0.54 g, 0.3 mmol) and Lys(Z)-NCA (1.79 g, 6.5 mol) were dissolved in 30 mL dried dichloromethane and 20 mL dried DMF, respectively, while BLA-NCA (2.5 g, 10 mmol) was added to a mixture of 1:10 dry 250 mL DMF and DCM (1 g/100 mL) and stirred vigorously to dissolve. After complete dissolution, branched OEI solution was used as the macro-initiator and mixed with BLA-NCA solution at a monomer/initiator molar ratio of 100:1.



Scheme 1. Charge-conversional shielding system for gene carriers.

Please cite this article in press as: J. Chen et al., Charge-conversional zwitterionic copolymer as pH-sensitive shielding system for effective tumor treatment, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.08.018