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pH triggered re-assembly of nanosphere to nanofiber: The role of peptide conformational change for enhanced cancer therapy

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ABSTRACT

pH-triggered conformational change and subsequent re-assembly of nanostructures provide a new strategy in nanomedicine for controlled drug release and enhanced therapy. Here, we reported the development of a novel pH-responsive nano-assembly as a drug carrier from peptide amphiphile (PA) consisting of mimicking peptide and stearic acid moieties. The mimicking peptide is a basic 17-amino acid peptide derived from antennapedia homeodomain, and undergoes a conformational transition of the secondary structure from β-sheet at pH 7.4 to α-helix at pH 5.0. Such transition therefore leads to simultaneous evolution of the self-assembled structure of PA from nanosphere to nanofiber, promotes assemblies retention and then release drugs in the cytoplasm of tumor cell. *In vitro* studies showed that the doxorubicin (Dox)-loaded PA nanoparticle (PA@Dox) could be uptaken efficiently by the cell due to the membrane penetrating capability of the mimicking peptide and subsequently the released Dox further induce apoptosis of murine colon carcinoma CT26 (MCCC) cell. In a mouse xenograft model of MCCC, administration of PA@Dox *via* lateral tail vein injection could remarkably retard the tumor growth. The overall results suggested that the PA-based nanocarriers adopting the novel strategy of pH-triggered secondary structural change could enhance therapeutic efficacy and be used as a promising platform for potential development of new generation of drug carriers for cancer therapy.

1. Introduction

In recent years, the rapid development of nanomedicine has shown high potential for cancer therapy [1–5]. However, the practical application and clinical translation of nanomedicine have been hampered by the off-target effect and low therapeutic efficacy which are caused by the lack of selectivity toward tumor cell/tissue, insufficient drug release at the tumor site, poor cell membrane penetrating performance, and severe endosomal entrapment of nanocarriers [1,6–10]. Specifically, unsatisfied drug release profile is regarded as one of the key challenges. To address this issue, efforts have been explored with particular interests on the fabrication of environmental responsive nanocarriers, including redox-, pH-, light- and other stimuli-responsive delivery systems [11,12]. Although tremendous progress has been made in this research field, very few investigations, to the best of our knowledge, have been focused on the function and potential application of environmental factor-triggered conformational alteration.

pH-responsive materials-based nanocarriers have been utilized intensively to escape the cellular endosome *via* the so-called "proton sponge effect". Many pH-sensitive drug carriers, such as liposomes [13], polymer-drug conjugates [14], and polymeric nanoparticles (*e.g.* micelles and polymersomes) [15], have been developed to improve drug delivery and anti-tumor efficacy. As one of the most important drug carriers, peptide amphiphile (PA), which is composed of alkyl chain as hydrophobic "tail" and amino acid residues as hydrophilic "head group" [16,17], can integrate not only biological function with precisely defined sequence of amino acids [18], but also favorable conformation for cell membrane penetration to target cell [19]. For certain pH-sensitive peptides, the pH evolution may cause secondary structure transition and subsequent structural re-assembly in nanoscale. This unique phenomenon inspires new strategy for construction of smart vehicles for controlled drug release.

To this end, herein a novel pH-sensitive PA based on mimicking peptide and stearic acid was designed. As a derivative from a third helix of the antennapedia homeodomain, the mimicking peptide bears 17-

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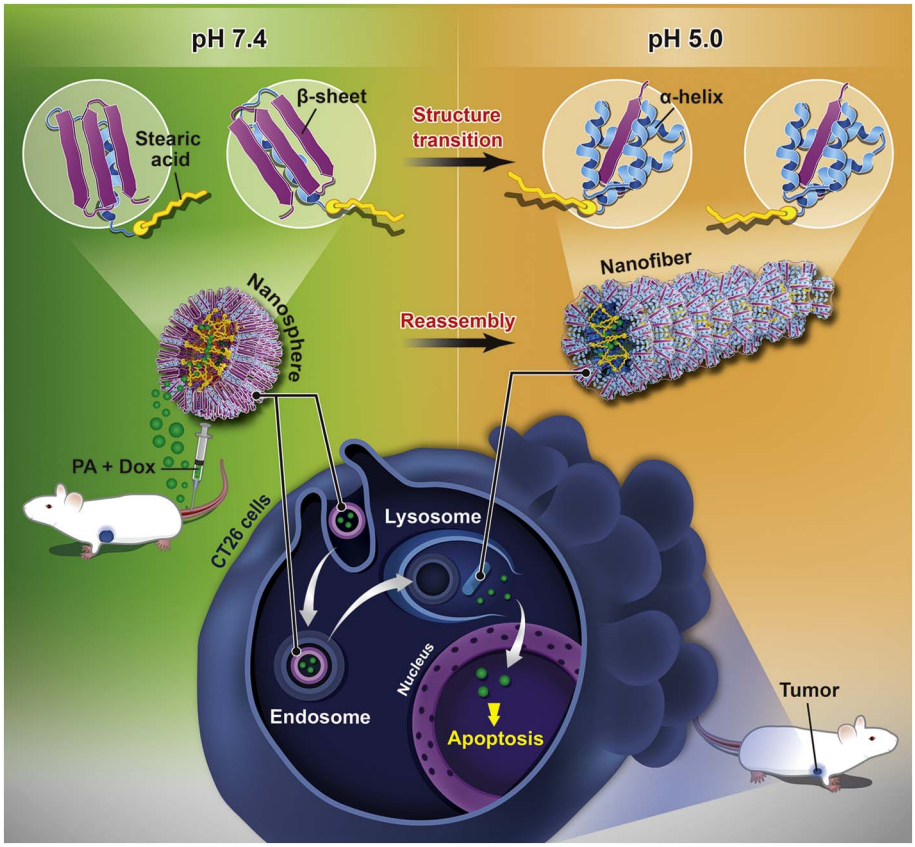

Fig. 1. Schematic illustration of pH-induced secondary structure transition of mimicking peptide amphiphile in acidic microenviroment of tumor cell for controlled release and cell apoptosis.

amino acid residues and is rich in arginine and lysine (DRQI KIWF QNRR MKWKK) [20], which endows the peptide with pH-sensitivity, *i.e.*, under acidic microenvironment (such as pH 5.0), this peptide could be protonated accompanied by the shift of its secondary structure from β-sheet to α-helix. More importantly, with the contribution of the two critical tryptophan residues, which precisely mimicked the Trp48 and Trp56 in the third helix of the antennapedia homeodomain, the PA can facilitate cellular attachment and enhance cell membrane penetration efficiency [21] *via* clathrin-mediated endocytosis. The alkyl chain of stearic acid not only acts as hydrophobic "tail" for specific biological function, but assists the peptides self-assembly into supramolecular nanostructures such as nanosphere, nanofibre, and nanotapes [22], *etc.*

Besides, the enhanced cellular uptake can be achieved from the shift of secondary structure of the PA from β-sheet to α-helix in a weakly acidic microenvironment, such as endosomes (pH 5.0). Such conformational change could also lead to an evolution of the PA self-assemblies from nanosphere to nanofiber (Fig. 1), which is believed to promote drug release into the cytosol far from the transmembrane efflux pumps. The effect of pH-induced conformational/structural transition of PA on its antitumor efficacy was investigated in detail both *in vitro* and *in vivo* to demonstrate that the peptide-based materials developed herein provide a novel platform to deliver and release antitumor drug specifically into tumor cell for enhanced therapeutic efficacy.

2. Materials and methods

2.1. Materials

The mimicking peptide (DRQI KIWF QNRR MKWKK) was purchased from GL Biochem Co., (China). Stearic acid, trifluoroacetic acid (TFA), dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and acetone were purchased from Shanghai Chemical Reagent Co. Ltd. (China) and purified according to standard protocols prior to use. All other reagents and solvents were of analytical grade from Guangzhou Chemical Reagent Co. Ltd. (China) and used without further purification. Pyrene (Sigma-Aldrich, USA), doxorubicin (Dox, Zhejiang Hisun Pharmaceutical Co. Ltd., China), 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide (MTT, GBCBIO Technologies Inc., China) and triisopropylsilane (TIS, Acros), transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL)kit (Roche, Switzerland), hematoxylin-eosin dye (H & E, Goodbio Technology Co. Ltd., China), trypsin (Gibco, USA), and 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, USA) were used as received.

The CT26 cell and Male mice (BALB/c mice) were purchased from the Animal Laboratory of the Sun Yat-sen University (Guangzhou, China). The cell was cultured in RPMI 1640 medium (HyClone, USA) containing 10% fetal bovine serum (FBS) (LONSA, USA). All procedures followed the guidelines outlined in the "Principles of Laboratory Animal Care" (NIH) and were approved by the local Animal Care and Use Committee.

2.2. Synthesis of PA

Stearic acid (142.3 mg, 0.5 mmol) and DCC (113.3 mg, 0.55 mmol) were dissolved in 15 mL of anhydrous DMF in a 100 mL round-bottomed flask and stirred for 24 h at 0 °C and then filtered. Then the mimicking peptide (1187 mg, 0.5 mmol) and DMAP (67.3 mg, 0.55 mmol) were added to the above mixture solution and stirred for additional 24 h at room temperature and concentrated under reduced pressure. The concentrated solution was added dropwise into excessive acetone and the resulting precipitate was collected by centrifugation. Removal of the side chain protective groups was performed using a mixture of TFA, deionized water, and TIS with the volume ratio of