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A smart polymeric platform for multistage nucleus-targeted anticancer drug delivery

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ABSTRACT

Tumor cell nucleus-targeted delivery of antitumor agents is of great interest in cancer therapy, since the nucleus is one of the most frequent targets of drug action. Here we report a smart polymeric conjugate platform, which utilizes stimulus-responsive strategies to achieve multistage nuclear drug delivery upon systemic administration. The conjugates composed of a backbone based on N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer and detachable nucleus transport sub-units that sensitive to lysosomal enzyme. The sub-units possess a biforked structure with one end conjugated with the model drug, H1 peptide, and the other end conjugated with a novel pH-responsive targeting peptide (R8NLS) that combining the strength of cell penetrating peptide and nuclear localization sequence. The conjugates exhibited prolonged circulation time and excellent tumor homing ability. And the activation of R8NLS in acidic tumor microenvironment facilitated tissue penetration and cellular internalization. Once internalized into the cell, the sub-units were unleashed for nuclear transport through nuclear pore complex. The unique features resulted in 50-fold increase of nuclear drug accumulation relative to the original polymer–drug conjugates in vitro, and excellent in vivo nuclear drug delivery efficiency. Our report provides a strategy in systemic nuclear drug delivery by combining the microenvironment-responsive structure and detachable sub-units.

1. Introduction

Targeted drug delivery using nanoscale vehicles such as hydrophilic polymeric carriers has achieved tremendous success for anti-cancer treatment in the recent decades [1–3]. However, many therapeutic agents, of which the target localized in specific cell organelles, and their therapeutic efficacy depends on the access of these agents to the sub-cellular target sites, e.g., the nucleus [4,5]. Previous studies by design multistage or programmed drug delivery system have made contribution in organelle-specific targeting [6–8]. However, efficient nuclear delivery of anti-cancer agents upon systemic administration remains a formidable challenge, which requires overcoming of multiple physiological barriers, including escaping rapid elimination through renal excretion and the mononuclear phagocyte system (MPS), enhancing cellular uptake and endosomal escape, and penetrating through the double-layered nuclear membrane [9].

Overcoming of these physiological barriers requires very different physiochemical properties of a delivery vehicle. The overall accumulation of the drug carriers at the tumor site primarily depends on their pharmacokinetic behavior and the enhanced permeability and retention (EPR) effect. Therefore, to facilitate the long-circulating behavior and the EPR effect, systemic administered drug carriers require a certain size (e.g. 5.5–15 nm) to avoid rapid renal filtration, and relative neutral charge to avoid recognition by the MPS [10]. However, the nuclear pore complex, which controls the access to the interior of cell nucleus, possesses an opening of only ~9 nm in functional diameter [11]. Therefore, the size of the cargo largely determines the efficiency of nuclear targeting. Studies proved that small size nanostructures (2 nm gold nanoparticle) are easily enter the nucleus than larger one (6 nm gold nanoparticle) [12]. Moreover, current strategies for nucleus targeting mostly depend on the conjugation of the nuclear localization sequence (NLS), a short peptide that 'tags' a substrate for active intra-nucleus transport through the nuclear pore complex [13,14]. However, the NLS-tagged vehicles need to overcome the cytoplasmic membrane and enter the cytosol before it can bind to their receptor and be

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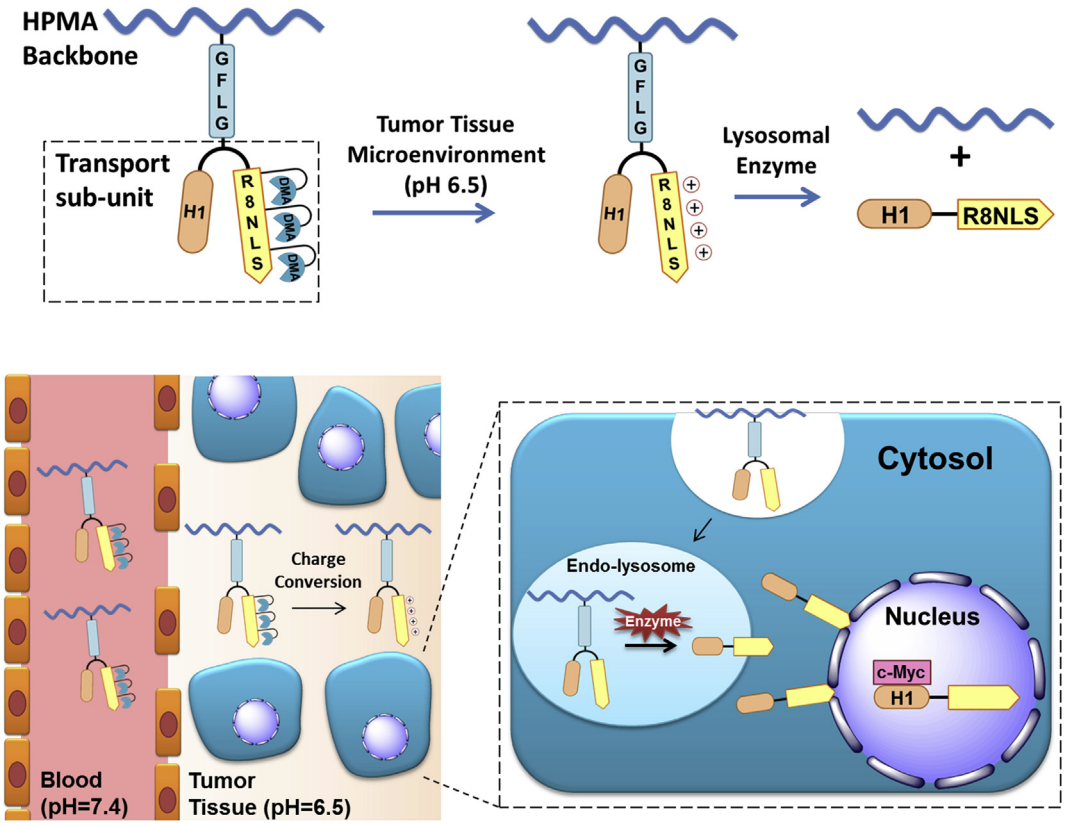
transported into the nucleus [15]. Besides, the NLS modification of delivery vehicles might compromise their pharmacokinetic behavior because the sequence contains sequences of positively charged lysines and/or arginines that increase MPS recognition [16]. Therefore, a multifaceted delivery vehicle using multistep strategy may be required to break down these physiological barriers and achieve efficient nucleus-targeted delivery upon systemic delivery.

Here we report a rationally designed polymeric conjugate platform with stimulus-responsive strategies to achieve multistage tumor cell nucleus targeting upon systemic administration. A 14-amino acid therapeutic H1-S6A, F8A (H1) peptide was used as model drug. H1 peptide, derived from the helix 1 region of nuclear protein c-Myc, can interfere with specific c-Myc DNA binding, and thus generates better therapeutic response in the nuclear region than in the cytoplasm [17,18]. The conjugates are composed of an N-(2-hydroxypropyl) methacrylamide (HPMA) polymer backbone [19–22], and nucleus transport sub-units that are charge-convertible in a pH-responsive way and detachable in an enzyme-responsive way (Scheme 1). The sub-units possess a biforked structure with one end conjugated with H1 peptide, and the other end conjugated with a novel fusion targeting peptide (R8NLS) composed of NLS (PKKKRKV) and cell penetrating peptide (octaarginine or R8). The sub-units were conjugated to HPMA polymers via a glycylphenylalanylleucylglycine (GFLG) linker that is lysosomally enzymatic degradable [23,24]. Thus, once the system is internalized into the cell, the sub-units could be unleashed for nuclear transport of the therapeutic peptides. Moreover, the R8NLS peptide were modified with anionic 2, 3-dimethylmaleic anhydrides (DMA), which could conceal the cationic properties of the peptide in physiological pH, and be rapidly cleaved in the mild acidic tumor tissue (pH 6.5) [25–27]. Therefore, the conjugates with prolonged circulation time could efficiently accumulate in the tumor tissue by EPR effect, and then revealed the cationic R8NLS for tissue penetration and cellular internalization. Once the system internalized into the cell, the sub-units could be unleashed as a relative small molecule for the subsequent endosomal escape via R8 mediation and nuclear drug delivery through nuclear pore complex via the NLS guidance. Our report provides a strategy in systemic nuclear drug delivery by combining the microenvironment-responsive structure and detachable sub-units.

2. Materials and methods

2.1. Materials

The azide-modified H1-S6A, F8A peptide (N3-DNELKRAFAALRDQI) was synthesized by Chinese Peptide Co. Ltd. (Zhejiang, China). The R8NLS peptide (CRRRRRRRRVKRKKKP), octaarginine peptide (R8, CRRRRRRRR), and nuclear localization signal (NLS, CVKRKKKP) were synthesized by Kai jie Biopharm Co. Ltd. (Sichuan, China). N-Boc-ethylenediamine was provided by Chengdu Xi ya Chemical Co. Ltd. (Chengdu, China). Boc-L-propargylglycine was from Han hong Chemical Co. Ltd. (Shanghai, China). Dimethylmaleic anhydride (DMA) was from Acros organics. Lysotracker red was from Invitrogen (Carlsbad, CA). The 4, 6-diamidino-2-phenylindole (DAPI), fluorescein isothiocyanate (FITC), and 3-(4, 5-dimethyl-2-tetrazolyl)-2, 5-diphenyl-2H



Scheme 1. Illustration of the multistage nuclear targeting drug delivery process of dual-responsive HPMA copolymer conjugates with H1 peptide and nuclear targeting peptide (R8NLS) decorated sub-units (P-GFLG-R8NLS-DMA-H1). After passive accumulation of P-GFLG-R8NLS-DMA-H1 at the tumor site through the "EPR" effect, the mild acidic tumor microenvironment (pH 6.5) triggered the charge conversion of R8NLS. Then the activated R8NLS facilitated the tumor cellular uptake of P-GFLG-R8NLS-H1 by endocytosis. Once internalized into the cell, the degradation of GFLG linkage induced by cathepsin B enzyme in lysosomes resulted in the release of sub-units. Finally, R8 mediated endosomal escape and the NLS mediated nuclear membrane translocation of the sub-units.