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Aptamer-functionalized albumin-based nanoparticles for targeted drug delivery

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ABSTRACT

Proteins have been extensively explored as versatile nanocarriers for drug delivery due to their complete biocompatibility, ease of surface modification, and lack of toxicity and immunogenicity. In this study, a facile strategy was used to construct aptamer-functionalized albumin-based nanoparticles for effective drug delivery and targeted cancer therapy. A hydrophobic drug, doxorubicin (DOX) was employed to trigger the self-assembly of bovine serum albumin (BSA) to from stable nanoparticles *via* hydrophobic interaction, and then a tumor targeting aptamer AS1411 was incorporated to the surface of DOX loaded BSA. Due to the specific recognition between AS1411 and its receptor over-expressed on tumor cells, the aptamer-modified nanoparticles show higher cellular uptake and stronger cell inhibitory efficacy against cancerous MCF-7 cells as compared with the nanoparticles without aptamer modification. In addition, DOX loaded aptamer-functionalized nanoparticles can induce more significant down-regulation of Bcl-2 and PCNA as well as up-regulation of pRB, PARP and Bax in MCF-7 cells compared with unmodified nanoparticles, indicating the aptamer modification can induce cell apoptosis more effectively. Besides, aptamer-modified nanoparticles exhibit a significantly improved capability in up-regulating p16, p21 and E-cadherin, and down-regulating EpCAM, vimentin, Snail, MMP-9, CD44 and CD133, implying the favorable effects of drug delivery on the prevention of tumor progression and metastasis.

1. Introduction

Severe toxic and side effects are the key bottlenecks to the development of cancer chemotherapy. In aiming to address these issues, numerous efforts have been devoted to exploring nano-sized drug delivery systems (DDSs), which show potentials to deliver chemotherapeutic drugs specifically into tumor tissues *via* the enhanced permeability and retention (EPR) effect, to reduce toxicity to normal tissues [1].

To date, diverse nano-sized DDSs have been developed [2,3]. Among these DDSs, protein-based materials have been considered as effective building blocks to construct nano-sized DDSs for cancer therapy. Proteins are a class of natural biomacromolecules that show great potentials in biomedical applications. In recent years, proteins have been extensively explored as versatile nanocarriers for drug delivery due to their ideal biocompatibility, convenience of surface modification, and lack of immunogenicity. As the most abundant plasma protein, albumin is particularly attractive for the construction of nano-sized DDSs due to its easy and inexpensive production, and *in vivo* stability [4]. In addition, since albumin is a major protein in the circulating system involved in the maintenance of osmotic pressure, albumin-based DDSs show a long-circulating ability in blood, which is the key principle to achieve effective cancer therapy [4]. More importantly, numerous therapeutic agents with diverse physiochemical properties can be loaded in albumin due to the co-existence of hydrophobic and hydrophilic domains in albumin [5–9]. However, to realize efficient tumor targeting delivery is still a challenge, which significantly limits the clinical outcomes of the albumin-based DDSs.

To address the issue aforementioned, we herein report a facile strategy to construct aptamer-functionalized albumin-based nanoparticles for tumor targeting drug delivery. As ligands, aptamers possess the advantages of high specificity and stability [10–18]. In our study, anticancer drug doxorubicin (DOX) was employed as a typical hydrophobic drug to induce self-assembly with BSA molecules to form DOX@BSA nanoparticles. After that, aptamer AS1411, which has a high affinity and specificity with its receptor, nucleolin, over-expressed on cancer cells [19–24], was decorated onto the surface of DOX@BSA nanoparticles to form DOX@Apt-BSA. The resulting nano-sized aptamer-functionalized delivery system exhibits tumor cell-targeting ability attributed to the surface aptamer modification. In addition, the effects of drug delivery on the expression of diverse proteins involved in tumor development and metastasis were studied.

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2. Experimental

2.1. Materials

Bovine serum albumin (BSA) was purchased from Bovogen. Doxorubicin hydrochloride (DOX·HCl) was provided by Zhejiang Hisun Pharmaceutical Co., Ltd. (Zhejiang, China). Aptamer (5'-COOH-AS1411) was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Hoechst 33342, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), and *N*-hydroxysuccinimide (NHS) were provided by Sigma-Aldrich. Triethylamine (TEA) and dimethyl sulfoxide (DMSO) were purchased from Shanghai Chemical Co. (Shanghai, China) and distilled prior to use. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was provided by Amresco. All other reagents were of analytical grade and used as received.

MCF-7 cells and COS-7 cells obtained from China Center for Typical Culture Collection (Wuhan, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mg/ml $NaHCO_3$, and 100 U/ml penicillin-streptomycin. Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Preparation of drug loaded nanoparticles

DOX·HCl (4 mg) was dissolved in 2 ml of DMSO. TEA was then added and stirred at room temperature for 12 h in dark to neutralize the solution to obtain DOX. Subsequently, 20 ml of BSA aqueous solution (3.35 mg/ml) was added slowly and the mixture was stirred at 4 °C for 12 h. Thereafter, the formed DOX loaded albumin nanoparticles (DOX@BSA) were transferred to a dialysis tube (MWCO 14 kDa) and dialyzed against deionized water at 4 °C for 2 h.

To conjugate aptamer to the nanoparticle surface, 20 μl of EDC solution (0.1 mg/ml), 10 μl of NHS solution (0.1 mg/ml), and 1 ml of 5'-COOH-AS1411 solution (9.37 nmol/ml) were added to the DOX@BSA suspension. After stirring at 4 °C for 12 h to conjugate aptamer to surface of DOX@BSA, the system was dialyzed (MWCO 14 kDa) against deionized water at 4 °C for 2 h. Finally, DOX loaded aptamer-functionalized albumin nanoparticles (DOX@Apt-BSA) were collected after freeze-drying.

2.3. Characterizations of drug loaded nanoparticles

The particle size and zeta potential of nanoparticles in deionized water with a concentration of 1 mg/ml were determined by dynamic light scattering (DLS) in a Nano-ZS ZEN3600 instrument (Malvern). The morphology of nanoparticles was observed using a JEM-2100HR transmission electron microscope (TEM). Before the TEM observation, a droplet of nanoparticle suspension was placed on a copper grid and stained by a 0.2% (w/v) phosphotungstic acid solution.

2.4. Determination of aptamer conjugation

The amount of aptamer AS1411 conjugated to the nanoparticle surface was determined by using Quant-iT™ OliGreen® ssDNA Assay Kit. The concentration of aptamer on the nanoparticles was examined using a fluorescence spectroscopy (Shimadzu RF-5301PC) with an excitation at 520 nm.

2.5. In vitro drug release

1.5 mg of DOX loaded nanoparticles were dispersed in 10 ml of PBS solution (pH 7.4), which was then transferred to a dialysis tube (MWCO 14 kDa). Subsequently, the dialysis tube was immersed in 30 ml of PBS solution and then kept in a constantly shaking water bath at 37 °C. At a predetermined time interval, 10 ml of release medium was taken out and 10 ml of fresh PBS solution was added. The amount of DOX released was determined using fluorescence spectroscopy (Shimadzu RF-5301PC) with an excitation at 488 nm. Data were given as mean ± standard deviation (SD) based on three independent measurements.

2.6. Confocal laser scanning microscopy

Cells (1×10^5 cells in 1 ml of medium) were seeded in a 35 mm glass bottomed culture dish and incubated at 37 °C for 24 h, and then the culture medium was removed. 1 ml of fresh medium (10% FBS) containing a particular agent (free DOX or DOX loaded nanoparticles with a DOX concentration of 5 μg/ml) was added, and the cells were co-incubated with the nanoparticles at 37 °C for 4 h. Then the cells were carefully washed three times by PBS. After the nuclei were stained by Hoechst 33342, the cells were observed by confocal laser scanning microscopy (CLSM) (Nikon C1-si) under magnification of 400.

2.7. Flow cytometry

Cells were seeded in a 6-well plate (2×10^5 cells per well) and incubated in 2 ml of DMEM containing 10% FBS for 24 h. Subsequently, the medium was replaced by the fresh medium (10% FBS) containing a particular agent (free DOX or DOX loaded nanoparticles with a DOX concentration of 5 μg/ml). After 4 h incubation, the medium was removed and the cells were washed with PBS thrice. The cells were then trypsinized, centrifuged, and finally dispersed in PBS for flow cytometry analysis (BD FACS Aria III).

The results are expressed as mean ± SD based on 3 independent measurements. The statistical significance between two sets of data was calculated using Student's t-test. Significant difference was established at $p < 0.05$.

2.8. In vitro cytotoxicity

In vitro cytotoxicity was evaluated using a standard MTT assay. MCF-7 cells were seeded into a 96-well plate (5×10^3 cells per well) and incubated in 100 μl of DMEM containing 10% FBS for 24 h. Subsequently, the medium was replaced by the fresh medium (10% FBS) containing a particular agent (free DOX or DOX loaded nanoparticles). After incubation for 24 h, the medium was removed and 200 μl of fresh DMEM and 20 μl of MTT (5 mg/ml) were added. After 4 h incubation at 37 °C, the medium was cautiously removed and 200 μl of DMSO was added to dissolve the formazan crystals produced by viable cells. The optical density (OD) was measured at 570 nm using a BIO-RAD 550 microplate reader. The average value of eight independent experiments was collected and the cell viability was calculated as

$$\text{cell viability} = (OD_{treated} / OD_{control}) \times 100\%$$

where $OD_{treated}$ was obtained from the cells treated by a particular agent, and $OD_{control}$ was obtained from un-treated cells.

2.9. Western blot analysis

MCF-7 cells were seeded in 6-well plate (5×10^5 cells per well) and then incubated in 2 ml of DMEM containing 10% FBS for 24 h. Subsequently, the medium was replaced by the fresh medium containing a particular agent (free DOX or DOX loaded nanoparticles with a DOX concentration of 5 μg/ml). After 24 h incubation, the medium was removed and the cells were trypsinized, centrifuged, and collected. The proteins were extracted using modified radioimmunoprecipitation assay lysis buffer, supplemented with protease inhibitor cocktail and phenylmethanesulfonyl fluoride (1 mM). Thereafter, equal amounts of proteins were added to SDS-PAGE gels and separated by gel electrophoresis. After transferring the proteins from the gels to polyvinylidene difluoride (PVDF) membrane, the blots were blocked with 5% (w/v) skim milk in PBS solution. The protein expression was detected by