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The eradication of breast cancer cells and stem cells by 8-hydroxyquinoline-loaded hyaluronan modified mesoporous silica nanoparticle-supported lipid bilayers containing docetaxel

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ABSTRACT

Breast cancer stem cells (BCSCs), which can fully recapitulate the tumor origin and are often resistant to chemotherapy and radiotherapy, are currently considered as a major obstacle for breast cancer treatment. To achieve the goal of both targeting BCSCs and bulk breast cancer cells, we developed 8-hydroxyquinoline-loaded hyaluronan modified mesoporous silica nanoparticles (MSN)-supported lipid bilayers (HA-MSS) and docetaxel-loaded MSS. The results showed that the size of all the nanoparticles was smaller than 200 nm. BCSCs were enriched from MCF-7 cells by a sphere formation method and identified with the $CD44^+/CD24^-$ phenotype. Quantitative and qualitative analysis demonstrated that HA promotes the uptake of HA-MSS in CD44-overexpressing MCF-7 mammospheres, revealing the mechanism of receptor-mediated endocytosis. DTX or DTX-loaded MSS showed much enhanced cytotoxicity against MCF-7 cells compared with MCF-7 mammospheres, whereas 8-HQ or 8-HQ-loaded HA-MSS showed much enhanced cytotoxicity against MCF-7 mammospheres compared with MCF-7 cells. In the MCF-7 xenografts in mice, the combination therapy with DTX-loaded MSS plus 8-HQ-loaded HA-MSS produced the strongest antitumor efficacy, with little systemic toxicity (reflecting by loss of body weight) in mice. Thus, this combination therapy may provide a potential strategy to improve the therapy of breast cancer by eradication of breast cancer cells together with BCSCs.

1. Introduction

Breast cancer is the most commonly diagnosed cancer among women and a leading cause of cancer-related death [1]. Despite great advances have been achieved in the diagnosis and treatment of breast cancer, breast cancer is still a major cause of cancer-related deaths among women [2]. Although conventional chemotherapy or radiation therapy could eliminate most of bulk breast cancer cells, breast cancer stem cells (BCSCs) are resistant to these conventional therapies, and are responsible for the resistance and relapse of breast cancer [3].

The combination of conventional chemotherapy drugs and anti-CSCs drugs is a promising strategy for complete eradication of cancer [4–7]. Zhang et al. developed octreotide (Oct)-modified paclitaxel (PTX)-loaded PEG-b-PCL polymeric micelles (Oct-M-PTX) and salinomycin (SAL)-loaded PEG-b-PCL polymeric micelles (M-SAL), and the combination of Oct-M-PTX and M-SAL produced the striking antitumor activity towards MCF-7 breast cancer xenograft [8]. 8-Hydroxyquinoline (8-HQ) is an organic compound, and was identified as having preferential activity against CSCs-like sphere cells by a small molecule drug compound library screening [9]. When combined with paclitaxel, 8-HQ showed much enhanced

Abbreviations: DTX, docetaxel; NPs, nanoparticles; PEG, polyethylene glycol; 8-HQ, 8-hydroxyquinoline; HA, hyaluronan or hyaluronic acid; EDC, ethyl-dimethyl-aminopropyl-carbodiimide; CSCs, cancer stem cells; BCSCs, breast cancer stem cells; MSN, mesoporous silica nanoparticle; SLB, supported lipid bilayers; MSS, mesoporous silica nanoparticle-supported lipid bilayers; HA-MSS, hyaluronan modified mesoporous silica nanoparticle-supported lipid bilayers.

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therapeutic effect and relapse prevention effect against both MCF-7 and MDA-MB-435 breast cancer xenograft, compare with a single 8-HQ or paclitaxel [10]. Thus, the combination of 8-HQ and docetaxel (DTX) has great potential in complete eradiation of breast cancer, both targeting BCSCs and bulk breast cancer cells.

However, conventional free drugs showed a series of problems such as poor solubility, limited stability, poor biodistribution and severe nonspecific toxicity [11]. To address this challenge, targeted delivery of drugs-loaded in nanoparticles can overcome these problems [12]. Ashley et al. developed a nanoparticle, mesoporous silica nanoparticle core coated with supported lipid bilayer (SLB) (MSS), which synergistically combines favorable features of mesoporous silica particles and liposomes [13]. MSS showed many features of an ideal therapeutic delivery platform, which potentially improves drug loading capacity, stability and enables controlled release of high concentrations of multicomponent cargos within the cytosol of cancer cells to enhance therapeutic outcomes.

CD44 has been investigated as a potential target because it is overexpressed in BCSCs as well as in other tumors [14]. Hyaluronic acid (HA), a naturally occurring polysaccharide composed of N-acetyl-D-glucosamine and D-glucuronic acid, could specifically bind to its receptors CD44 and be internalized via receptor-mediated endocytosis [15]. Thus, it may be possible to take HA as a potential ligand in receptor-mediated nanoparticles. We hypothesize that MSS coated with HA will offer selective targeting strategies and promote MSS binding and internalization in CD44-overexpressing BCSCs [16].

In this study, we developed DTX-loaded MSS and 8-HQ-loaded MSS coated with HA, to achieve the goal of both targeting CD44-overexpressing BCSCs and bulk breast cancer cells. We hypothesize that the combination therapy with DTX-loaded MSS plus 8-HQ-loaded MSS coated with HA could kill the breast cancer cells together with eliminating BCSCs Human breast cancer MCF-7 cells were chosen as the model of cancer cells. The targeting activity of 8-HQ-loaded MSS coated with HA was investigated, and the *in vitro* and *in vivo* antitumor activity of the combination therapy was investigated.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS, ≥98%), 3-[2-(2-aminoethylamino)ethylamino] propyltrimethoxysilane (AEPTMS, technical grade), hexadecyltrimethylammonium bromide (CTAB, ≥99%), dimethyl sulfoxide (DMSO, ≥99.9%), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT, ≥99%), EGF, bFGF, bovine serum albumin (BSA), insulin, collagenase from Clostridium histolyticum (Type I), 8-hydroxyquinoline and coumarin-6 (488/523 nm) were purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile (chromatographic grade) and methanol (chromatographic grade) were purchased from Merck (Darmstadt, Germany). Trifluoroacetric acid (TFA, chromatographic grade) was purchased from TEDIA (Fairfield, OH). Hyaluronic Acid (HA, pH 6.8, M_W = 370,000) was purchased from Furuida Co., Ltd. (Shandong, China). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) was purchased from KaYon Biological Technology Co., Ltd. (Shanghai, China). 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine(DOPE), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (18:0PEG-2000 PE) and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL). Fetal bovine serum (FBS, Gibco®), StemPro® Accutase® Cell Dissociation Reagent, trypsin-EDTA solution (0.25% trypsin with 0.53 mM EDTA) and B27 supplement (Invitrogen™) were purchased from Life Technologies Corporation (Foster City, CA). Dulbecco's Modified Eagle's Medium with or without phenol red (DMEM, Hyclone®), DMEM/F12 (Hyclone®) and Dulbecco's phosphate-buffered saline (D-PBS, Hyclone®) were purchased from Thermo Fisher Scientific (Waltham, MA). FITC labeled mouse anti-human CD44 antibody, PE labeled mouse anti-human CD24 antibody, FITC labeled mouse lgG2b κ isotype control antibody, PE labeled mouse lgG2a κ isotype control antibody and matrigel were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ). Hoechst 33342 (350/461 nm) was purchased from Dojindo Laboratories (Kumamoto, Japan). β-estradiol was purchased from General Pharmaceutical Co., Ltd. (Shanghai, China). All other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China). All well plates and tissue culture flask were purchased from Corning Incorporated (NY, USA).

2.2. Synthesis of mesoporous silica nanoparticles (MSN)

Mesoporous silica nanoparticles were prepared according to the method described by Huh with modifications [17]. Briefly, the mixture of CTAB (1.0 g, 2.745 mmol), 2.0 M of NaOH (3.5 mL, 7.0 mmol) and H_2O (480 g, 26.637 mol) was heated at 80 °C for 30 min. To the mixture, TEOS (4.67 g, 22.4 mmol) was added rapidly via injection. Following the injection, a white precipitation was observed after stirring for 3 min. The reaction was maintained at 80 °C for 2 h. The products were then centrifuged at 3500 rpm for 15 min, and the supernatant was removed. Then they were washed with deionized water and pure ethanol, and dried under vacuum. Finally, the particles were calcined at 550 °C for 5 h to remove surfactants and other excess organic matter.

MSN was modified with amine-containing silane 3-[2-(2-aminoethylamino) ethylamino] propyltrimethoxysilane (AEPTMS) as described previously [18]. Briefly, 25 mg of calcined particles were added to 1 mL of 20% AEPTMS in pure ethanol. The particles were incubated in AEPTMS for 24 h at room temperature, centrifuged at 10,000 rpm for 3 min to remove unreacted AEPTMS, and resuspended in 1 mL of 0.5× Dulbecco's Phosphate Buffered Saline (D-PBS). The resultant nanoparticles were Ap-MSN.

2.3. Loading of MSN with DTX, 8-HQ or Coumarin-6

Prior to liposome fusion, Ap-MSN (50 mg) was incubated with in 2.0 mL DTX (5 mg/mL), 8-HQ (5 mg/mL), or coumarin-6 (2.5 mg/mL) for 24 h at room temperature, respectively. Excess reagent was removed by centrifugation at 10,000 rpm for 10 min, and liposomes were immediately fused with the prepared Ap-MSN as described below.

2.4. Development of liposomes

The liposomes were prepared by a lipid-film based method. Briefly, 15 mg DOPC, 1.25 mg DOPE, 7.5 mg cholesterol and 1.25 mg PEG-2000 PE were dissolved in 3 mL chloroform, and evaporated to form a thin lipid film with a rotary evaporator (Shensheng, Shanghai, China). Then, the formed lipid film was re-hydrated in 0.5× D-PBS at a concentration of 10 mg/mL and were extruded for 15 times, each through two stacks each of progressively decreasing pore-sized polycarbonate membranes (100, 200 nm) (Nucleopore, Whatman), using a Mini-Extruder set (Avanti Polar Lipids, Inc., USA). Resultant liposomes were stored at 4 °C, and would be used in less than one week.

2.5. Modification of the liposomes with HA

HA was conjugated with the liposomes based on an EDC-activation method, by grafting the carboxyl group of HA with the primary amines of PE in the liposomes in the presence of EDC. Briefly, HA (2 mg/mL, dissolved in deionized water) was pre-activated by EDC (pH 4.0) for 2 h at 37 °C. The activated HA was added to the liposomes in PBS (10 mg lipid/mL) at the ratio of 1:1 (v/v), and the pH was adjusted to 8.6 by NaOH. The mixture was incubated for 24 h at 37 °C in a shaker bath under a nitrogen atmosphere. At the end of incubation, the liposomes were purified by centrifugation (15,000 *g*) for 30 min at 4 °C. The liposomes were suspended in 1× D-PBS (pH 7.4). The suspended liposomes were lyophilized and stored at −20 °C for use.

2.6. Development of mesoporous silica nanoparticle-supported lipid bilayers (MSS)

The mesoporous silica nanoparticle-supported lipid bilayers (MSS) were prepared by fusing the liposomes with MSN as described by Sengupta and Liu [19–22]. Briefly, DTX-loaded Ap-MSN were dissolved in 0.5× D-PBS (25 mg/mL) and exposed to excessive liposomes for 60 min at room temperature. The resultant DTX-loaded MSS were stored in the presence of excessive liposomes for up to 3 months at 4 °C. To remove excessive liposomes, DTX-loaded MSS were centrifuged at 10,000 rpm for 5 min, washed twice, and re-suspended in 0.5× D-PBS. Similarly, 8-HQ-loaded Ap-MSN were dissolved in 0.5× D-PBS (25 mg/mL) and exposed to excessive HA-liposomes for 60 min at room temperature. The resultant 8-HQ-loaded HA-MSS were stored in the presence of excessive liposomes for up to 3 months at 4 °C. To remove excessive liposomes, 8-HQ-loaded HA-MSS were centrifuged at 10,000 rpm for 5 min, washed twice, and re-suspended in 0.5× D-PBS.

2.7. Characterization of the nanoparticles

The ultrastructure of drug-free MSN, Ap-MSN, MSS and HA-MSS was investigated by field-emission transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For TEM imaging, the nanoparticles were dispersed in deionized water at a concentration of 5 mg/mL, and 5 μL of this solution was transferred onto a holey carbon-coated copper TEM grid. After air-drying, the