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CD44-engineered mesoporous silica nanoparticles for overcoming multidrug resistance in breast cancer

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ABSTRACT

Multidrug resistance is a major impediment for the successful chemotherapy in breast cancer. CD44 is over-expressed in multidrug resistant human breast cancer cells. CD44 monoclonal antibody exhibits anticancer potential by inhibiting proliferation and regulating P-glycoprotein-mediated drug efflux activity in multidrug resistant cells. Thereby, CD44 monoclonal antibody in combination with chemotherapeutic drug might be result in enhancing chemosensitivity and overcoming multidrug resistance. The purpose of this study is to investigate the effects of the CD44 monoclonal antibody functionalized mesoporous silica nanoparticles containing doxorubicin on human breast resistant cancer MCF-7 cells. The data showed that CD44-modified mesoporous silica nanoparticles increased cytotoxicity and enhanced the downregulation of P-glycoprotein in comparison to CD44 antibody. Moreover, CD44-engineered mesoporous silica nanoparticles provided active target, which promoted more cellular uptake of DOX in the resistant cells and more retention of DOX in tumor tissues than unengineered counterpart. Animal studies of the resistant breast cancer xenografts demonstrated that CD44-engineered drug delivery system remarkably induced apoptosis and inhibited the tumor growth. Our results indicated that the CD44-engineered mesoporous silica nanoparticle-based drug delivery system offers an effective approach to overcome multidrug resistance in human breast cancer.

1. Introduction

Breast cancer is the second leading cause of cancer death among females [1]. A serious impediment to breast cancer therapy is the emergence of multidrug resistance (MDR). From a variety of mechanisms, MDR can make the intracellular drug concentration lower than the effective concentration or vitiate function of drug [2,3]. Among the MDR mechanisms, P-glycoprotein (P-gp) is the best clinically studied as a drug efflux pump [4]. P-gp mediates resistance to a broad spectrum of anticancer drugs, such as doxorubicin (DOX), daunorubicin, epirubicin and paclitaxel, which are commonly used in the treatment of breast cancer [5]. Although the great pharmacologic approach to overcome cancer drug resistance is to use MDR inhibitors, due to the side effects or ineffectiveness of these compounds, not a single inhibitor becomes a clinically applicable agent [2]. There is an urgent need to improve therapeutic effect of breast cancer.

Based on the characters of mesoporous structure, high surface area, large pore volume and good compatibility, mesoporous silica nanoparticles (MSNs) have received much attention in biomaterial field [6–11]. Recently, many great efforts have been made for the reversal of MDR by the MSN-based drug delivery systems [12,13]. Tuning the shell-pore size or nanorod size of MSN may result in more cellular uptake of drug and higher cytotoxicity for MDR cancer cells [14,15]. Stimuli-coupled MSN-based drug delivery system might temporally and spatially regulate the drug-releasing pattern to overcome MDR [2,12,16]. Co-delivering drugs, genes, and other therapeutic agents can improve chemotherapy efficacy [8,17,18]. Among the latter co-delivering nanotechnologies, antibody-mediated delivery systems create a new therapeutic

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approach of selectively delivering drugs to targeted cancer cells by specifically binding to the antigens overexpressed on the cancer cells surface. The antibody-based therapeutics can provide high affinity to cancer cell receptors, facilitate "enhance permeation and retention" (EPR) effect, achieve higher nanoparticle accumulation at the tumor site, significantly enhance delivery efficacy and minimize toxicity [19].

CD44, one of the clusters of differentiation, is related to anti-apoptosis and cancer resistance [20,21]. CD44 is overexpressed in breast cancer stem cells and MDR breast cancer cells [22–24]. There is strong evidence that CD44 is involved in inducing transcriptional up-regulation of the MDR1 gene to promote MDR [25,26]. Misra et al. [27] implicated that soluble CD44 antibody reverse this increase of P-gp expression to sensitize the cells to DOX treatment. Furthermore, it is shown that CD44 plays a role in drug resistance *via* its increase of expression of the antiapoptotic protein Bcl-xL [23]. On the other hand, CD44 monoclonal antibody (McAb) can markedly inhibit proliferation and stimulate apoptosis in different types of cancer [28,29]. In addition, CD44 McAb can not only block hyaluronan binding, but also inhibit MDR1-mediated efflux activity to increase drug retention [25]. Thereby, CD44 McAb in combination with chemotherapeutic drugs might result in enhancing chemosensitivity for the reversal of MDR. Though many drug delivery systems have been developed to target CD44 for exploring approaches to enhance cancer therapies, most nanoparticles are modified by hyaluronan, the ligand of CD44 antigen [30–32]. However, CD44 McAb has not been introduced in drug delivery systems to overcome MDR up to date.

In this report, we synthesize CD44 McAb functionalized MSN containing DOX as a targeted drug delivery system for overcoming the MDR both *in vitro* and *in vivo* for the first time. To facilitate drug bypassing the efflux system, MSN was taken as basic drug delivery platform; to enhance the drug accumulation in MDR cancer cells, targeted CD44 McAb was modified on MSN; to bring higher cytotoxicity, both CD44 McAb and DOX were loaded on MSN. Here, we demonstrate that the CD44 McAb-engineered MSN delivery system loaded with DOX could target the resistant human breast cancer MCF-7 cells, increase drug accumulation, circumvent MDR and magnify the reversal effect of MSN-based drug delivery system.

2. Experimental

2.1. Materials and chemical reagents

Tetraethoxysilane (TEOS, ≥98%), cetyltri-methylammonium chloride (CTAC, ≥98%), (3-aminopropyl) triethoxysilane (APTES, ≥98.0%), 2-(N-Morpholino) ethanesulfonic acid (MES, ≥99.0%), N-hydroxysuccinimidyl (NHS, ≥98%) were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China. Doxorubicin in the form of hydrochloride salt (DOX, ≥99.0%) was purchased from Beijing Huafeng United Technology Company, Beijing, China). 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, ≥98.5%) was purchased from Aladdin Industrial Corporation. Sulforhodamine B sodium salt (SRB) were purchased from Sigma–Aldrich (St. Louis, USA). Sodium hydroxide (NaOH), trichloroacetic acid (TCA), acetic acid, hydrochloric acid (HCl), ethanol and sodium acetate trihydrate ($NaAc{\cdot}3H_2O$) were purchased from Nanjing Chemical Reagent Co., Ltd, Nanjing, China. Anti-Human/Mouse CD44 McAb (MW∼37KD) was purchased from BD Biosciences PharMingen, San Diego, USA. RPMI 1640 and fetal bovine serum (FBS) were obtained from GIBCOL (Grand Island, USA). All well plates and tissue culture flask were purchased from Corning Incorporated (NY, USA).

2.2. Synthesis of MSN

The well monodisperse mesoporous silica nanospheres were synthesized using procedures as reported in the literature [33]. Briefly, 1.56 g of CTAC and 0.3 g of $NaAc{\cdot}3H_2O$ were added to 58.08 ml of water, and the mixture was stirred at 60 °C for 2 h. Then 4.35 ml of TEOS was added dropwise within 10 min under steadily stirring at about 400 rpm. The resulting solution was further stirred for 24 h before cooling to room temperature, and then centrifuged at 16,000 rpm for 10 min to isolate the product from the suspension. After washing with water for 3 times, the final product was obtained by calcination at 630 °C for 5 h.

2.3. Synthesis of CD44 McAb engineered MSN (MSN-CD44)

It is an often applied approach of loading proteins that carboxylic acid of antibody functions into amino-functionalized MSN. Thereby firstly, we prepared the amine-functionalized MSN (denoted MSN-NH_2). 100 mg of MSN were added to the mixture solution of ethanol/APTES (30:1 ml). Then the resulting mixture was stirred at room temperature for 24 h. After that, MSN-NH_2 were obtained by the centrifugation at 15,000 rpm for 5 min to remove supernatant, washed with ethanol, dried under vacuum and dispersed in phosphate buffered saline (PBS). CD44 McAb was covalently attached to the MSN's surface using EDC coupling chemistry. Briefly, to a dispersion of 120 μg CD44 McAb (10 ml, MES buffer (0.1 M), pH 6.0), EDC and NHS were added separately under room temperature, and the activation reaction lasted for 15 min. Then, the MSN-NH_2 dispersed in 100 ml of PBS (pH 8.5) was added to the above solution, and the reaction was kept for 2 h under room temperature. Finally, the resulting MSN-CD44 was washed thrice with PBS (pH 7.4) and collected for detection. In order to intracellularly track DOX-loaded MSN-CD44 with DOX released, the carrier MSN-CD44 was labeled with fluorescein-5-isothiocyanate (FITC) by stirring at 4 °C for 12 h in buffer (0.5 M $NaHCO_3$–Na_2CO_3 and 0.15 M NaCl). Thus the remaining free NH_2 moieties in NH_2-MSNs and amino terminal in MSN-CD44 could conjugate with FITC.

2.4. Characterization

The morphology and size of MSN were analyzed by transmission electron microscopy (TEM, JEOL JEM-200CX, Japan) operated at 200 kV. An X-ray diffraction (XRD) patterns were recorded on an X'Pert Pro powder diffractometer (X'TRA, ARL, Switzerland) equipped with Cu Kα radiation. Data were obtained from 0.6° to 5° (diffraction angle 2θ) at a step size of 0.01°. Nitrogen adsorption–desorption isotherms were measured at 77 K using a Micromeritics ASAP 2020 apparatus. The samples were degassed at 453 K overnight on a vacuum line. Surface area was determined according to the Brunauere Emmere Teller (BET) model. The pore volume was measured from the amount of nitrogen adsorbed at a maximum relative pressure (P/P_0). Fourier transform infrared (FTIR) spectra were collected on a FTIR spectrometer (NEXUS870, NICOLET, USA). For each spectrum, the scans were collected at a resolution of 2 cm^{-1} over the range 500–4000 cm^{-1}. Before FTIR measurements, all the samples were freeze dried. The ratio of the mass of antibody relative to the total mass of MSNs was evaluated based on the Bradford method using the Bio-Rad protein assay as previous report [34].

2.5. Drug loading and accumulation release

Hundred milliliter PBS solution containing 25 mg DOX was mixed with 100 mg MSN or MSN-CD44. After the mixture was shaken for 24 h under dark conditions, the DOX-loaded MSNs (DMSN) or DOX-loaded MSN-CD44 (DMSN-CD44) was centrifuged