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Research Paper

Dual targeting mesoporous silica nanoparticles for inhibiting tumour cell invasion and metastasis

Wenqing Li, Zhaoming Guo*, Kun Zheng, Kun Ma, Changhao Cui, Li Wang, Yue Yuan, Yu Tang

School of Life Science and Medicine, Dalian University of Technology, Panjin, Liaoning 124221, China

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ABSTRACT

The invasion and metastasis of tumour cells are closely correlated with poor prognosis of cancer patients. In this study, a CD44 and N-cadherin dual targeting drug delivery system based on mesoporous silica nanoparticles (MSNs) has been successfully constructed for inhibiting tumour cell invasion and metastasis. Amino modified MSN (MSN/NH_2) was first synthesized and then functionalized with hyaluronic acid (HA) and ADH-1, constructing the carrier ADH-1-HA-MSN. Doxorubicin hydrochloride (DOX) was selected as a model anticancer drug. The prepared vector had a spherical shape with a narrow distribution of particle size. Flow cytometry and confocal microscopy studies showed that the modification with HA significantly enhanced CD44-mediated cellular uptake of this nanocarrier. ADH-1-HA-MSN/DOX exhibited higher cytotoxicity compared to non-ADH-1 modified counterparts. Of note, a transwell chamber assay demonstrated that the migration and invasion of tumour cells were markedly inhibited by ADH-1-HA-MSN/DOX. Furthermore, Western blotting analysis revealed that ADH-1-HA-MSN/DOX inhibited tumour cell invasion and metastasis by down-regulating N-cadherin expression. Taken together, these results indicated that ADH-1-HA-MSN might be a promising targeted drug delivery system for inhibiting cancer invasion and metastasis.

1. Introduction

Tumour metastasis is a major obstacle to the success of cancer therapy. How to inhibit cancer metastasis, particularly the initiation of metastasis, is key to improving the survival rates of cancer patients. Multiple cell types and various signalling pathways are involved in the mechanism of cancer metastasis (Qian et al., 2017). Epithelial-mesenchymal transition (EMT) is a critical step in tumour metastasis (Cheng et al., 2007; Thiery et al., 2009; Wang et al., 2015). Primary tumour cells will obtain the ability of motility and invasiveness when they undergo EMT (Hugo et al., 2007; Voulgari and Pintzas, 2009). Therefore, treatment targeting EMT offers one potential avenue for preventing tumour metastasis.

EMT is a cell biological process engaged in embryonic development, tissue repair, organ fibrosis and cancer progression (Ko et al., 2015; Thiery, 2009; Thiery et al., 2009). When EMT occurs in tumour cells, epithelial cells lose their characteristics and instead take on mesenchymal properties including down-regulation of the epithelial cell marker E-cadherin and up-regulation of the mesenchymal cell markers such as N-cadherin, Snail, and vimentin (Qian et al., 2017; Thiery, 2009). N-cadherin is a transmembrane glycoprotein and plays a crucial role in the early stage of invasion and metastasis of cancer cells (Hazan et al., 2000; Islam et al., 1996; Wu et al., 2017; Yang et al., 2016). Many studies have shown that up-regulation of N-cadherin promoted the migration and invasion of cancer cells (Shintani et al., 2008; Wu et al., 2017; Yang et al., 2016). Hence, down-regulation or inhibition of N-cadherin expression is a promising anti-metastatic strategy.

ADH-1 (N-AC-*CHAVC*-NH_2), a cyclic pentapeptide, is derived from the His-Ala-Val (HAV) site of N-cadherin and is an effective antagonist of N-cadherin-mediated adhesion and migration. It is reported that ADH-1 could inhibit N-cadherin dependent cancer progression in vitro and in vivo (Augustine et al., 2008; Perotti et al., 2009; Shintani et al., 2008; Turley et al., 2015; Yarom et al., 2011). In addition, ADH-1 has no toxic effects and has been studied in clinical trials.

In recent years, nanoscale drug delivery systems (NDDS) have attracted great interest due to their superior properties for cancer therapy including improved therapeutic effects, increased stability of anti-tumour drugs in vivo and enhanced drug delivery specific to tumour cells. Among the various NDDS, mesoporous silica nanoparticles (MSNs) are widely studied as drug delivery carriers due to their high drug loading capacity, ease of surface modification, excellent biocompatibility and low toxicity (Cheng et al., 2016; Zhang et al., 2016a, 2016b; Zhao et al., 2015). Additionally, drug-loaded MSNs modified with targeting ligands increased cellular uptake and showed higher cytotoxicity (Cheng et al.,

* Corresponding author.
E-mail address: guozm@dlut.edu.cn (Z. Guo).

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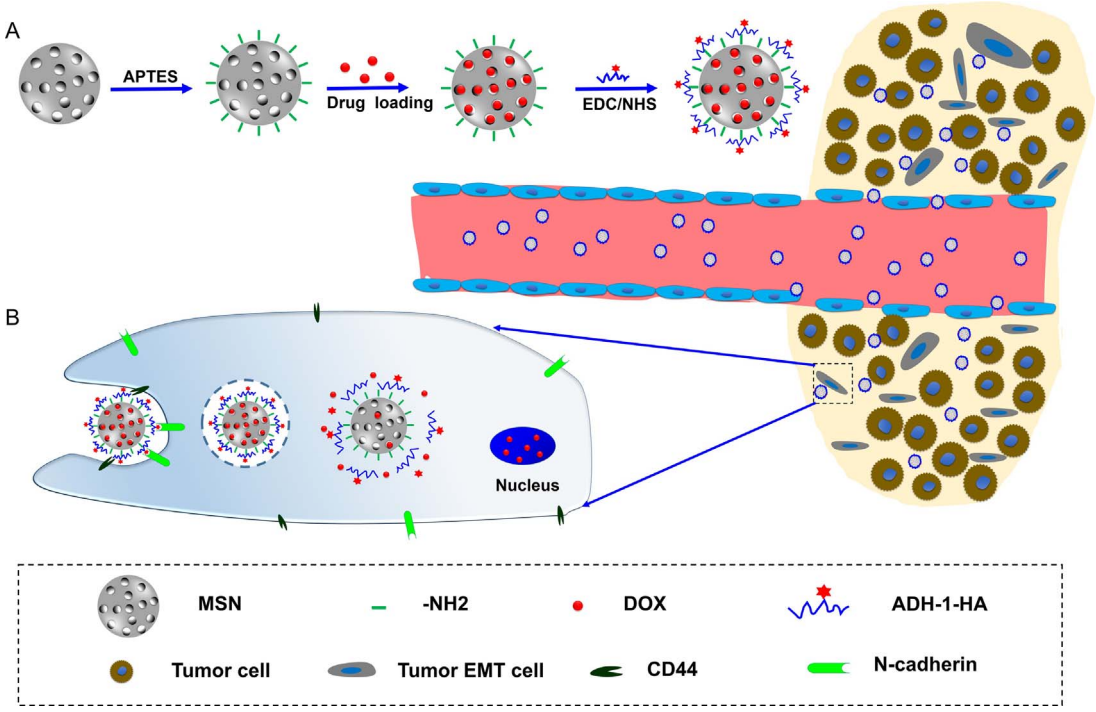

Scheme 1. (A) Schematic illustration of the preparation of ADH-1-HA-MSN/DOX. (B) Schematic illustration of the targeted delivery process of ADH-1-HA-MSN/DOX for CD44-mediated endocytosis, the interaction with N-cadherin and drug release in A549/EMT cells.

2015; Xiao et al., 2015; Yu et al., 2013).

Hyaluronic acid (HA), a natural muco-polysaccharide, has many excellent properties including hydrophily, biocompatibility and specific recognition of the CD44 receptor, which is overexpressed on many tumour cells such as human lung cancer cells (A549, H69, H69/ADR) (Cho et al., 2011; Zhong et al., 2015). Therefore, HA functionalized MSNs could be effectively uptaken by CD44 overexpressing cancer cells via the CD44-mediated endocytosis pathway, thus improving therapeutic efficacy (Han et al., 2015; Quan et al., 2015; Ramzy et al., 2017; Yang et al., 2015). Additionally, the multiple hydroxyl and carboxyl groups of the HA molecule contribute to their ability to be grafted and modified.

Based on the background above, we proposed a strategy that a CD44 and N-cadherin dual targeting drug delivery system (ADH-1-HA-MSN) could be able to inhibit tumour cell invasion and metastasis by down-regulating N-cadherin expression. As shown in Scheme 1, MSNs were used as the drug delivery carriers and the classical anticancer drug doxorubicin (DOX) was chosen as the model drug. The MSNs and ADH-1 were conjugated by HA. HA also acted as an active targeting ligand to achieve targeted drug delivery as well as a cross-linking molecule to conjugate ADH-1 on the surface of MSNs. ADH-1 acted as an antagonist to block the function of N-cadherin, which normally promotes tumour cell motility, invasion and metastasis. We hypothesized that ADH-1-HA-MSN/DOX could inhibit cancer cell invasion and metastasis through the following steps: first, this drug delivery system can be specifically recognized by tumour cells via the specific interaction between HA and CD44 receptors. Second, ADH-1 inhibits the invasion and metastasis of tumour cells undergoing EMT by blocking the function of N-cadherin. Finally, the encapsulated DOX further kills the tumour cells.

The morphology, size and drug release profiles of ADH-1-HA-MSN were characterized. A549 lung cancer cells were induced by TGF-β1 to establish the EMT cell model (A549/EMT). The cellular uptake and cytotoxicity of ADH-1-HA-MSN/DOX against A549 and A549/EMT cells were evaluated in vitro. The inhibition effect of ADH-1-HA-MSN/DOX on tumour invasion and metastasis was investigated by a transwell chamber assay. The mechanism involved was further studied by Western blotting.

2. Materials and methods

2.1. Materials

Cetyltrimethylammonium bromide (CTAB, 98%), tetraethyl orthosilicate (TEOS, 99%), 3-aminopropyltriethoxysilane (APTES), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride (EDC·HCl) and doxorubicin hydrochloride (DOX) were purchased from Aladdin (Shanghai, China). HA (MW = 37 kDa) was purchased from Freda (Shandong, People's Republic of China). 4′, 6-diamidino-2-phenylindole (DAPI) was obtained from Solarbio (Beijing, China). Cell Counting Kit-8 (CCK-8) was purchased from Beyotime. Foetal bovine serum (FBS), DMEM, trypsin-EDTA and penicillin-streptomycin were obtained from HyClone. The ADH-1 peptide (N-AC-*CHAVC*-NH_2) was obtained from GL Biochem Peptide Ltd. (Shanghai, China). RIPA Lysis Buffer, PMSF and the enhanced chemiluminescence kit were purchased from Coolaber Science & Technology Co., Ltd. The BCA Kit was obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China). All antibodies were obtained from Biodragon-immunotech (Beijing, China). Transwell filter and matrigel were purchased from BD Biosciences (USA). All other chemicals were of analytical grade and used without further purification.

2.2. Characterizations

The particle sizes, polydispersity index (PDI) and zeta potentials of MSN/NH_2 and ADH-1-HA-MSN were measured by Malvern Zetasizer Nano-ZS at 25 °C. Prior to the measurement, 1 mg of lyophilized nanoparticles was dissolved in 2 mL of ethanol. The testing angle of incident light is 173°. All samples were measured in triplicate and the data were calculated using the Malvern software package. The morphology of the functionalized MSNs was observed by an ultrahigh resolution field emission scanning electron microscope (FE-SEM, Nova NanoSEM 450). Nitrogen adsorption-desorption measurements were carried out in liquid nitrogen atmosphere and samples were out-gassed for 6 h before the measurements were taken. The surface area and pore size distribution of the samples were measured by Brunauer Emmett