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Redox-responsive chemosensitive polyspermine delivers ursolic acid targeting to human breast tumor cells: The depletion of intracellular GSH contents arouses chemosensitizing effects

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ABSTRACT

Antitumor efficacy of ursolic acid (UA) is seriously limited due to its low hydrophilicity and needy bioavailability. To overcome these obstacles, chemosensitive polyspermine (CPSP) conjugated with UA and folic acid (FA) as a novel targeted prodrug was designed and successfully synthesized in this investigation. This prodrug not only showed high aqueous solubility, GSH-triggered degradation and good biocompatibility, but also exhibited better inhibition effect on the tumor cells proliferation in comparison with free UA. FA-CPSP-UA could down-regulate the generation of GSH and manifest excellent ability in enhancing antitumor efficacy. In addition, FA-CPSP-UA could inhibit the expression of MMP-9, which led to restricting MCF-7 cells migration. Taken together, the results indicated that FA-CPSP-UA, as a carrier, can efficiently deliver UA to folate receptor positive cancer cells and improve tumor therapy of UA by Chemosensitive effect.

1. Introduction

Ursolic acid (UA), as a pentacyclic triterpenic acid, widespread in a large number of natural medicinal plants with multiple pharmacological properties, including anti-inflammatory, liver-protective, anti-atherosclerotic, anti-epileptic, anti-cancer, anti-epileptic, and anti-diabetic activities [1–6]. Various papers have shown that UA exhibits excellent anti-tumor effect, mainly through the induction of apoptosis, inhibition of tumor cell proliferation, suppression of tumor tissue angiogenesis, repression of tumor invasion and metastasis, interference with tumor micro-environment and other effects to achieve the purpose of anti-tumor [7–10]. Pharmacology research shows that UA can inhibit the proliferation and induce apoptosis in MCF-7 human breast cancer cells [7,11]. However, the potential clinical applications of UA are ultimately obstructed due to its low hydrophilicity, non-specific distribution and poor bioavailability. To address these limitations, the great efforts had been devoted to improving anticancer efficacy of UA by designing intelligent drug delivery systems. Recently, various drug delivery systems such as polymeric nanoparticles, phospholipid nanoparticles, micellar nanosystems and liposomes have attracted increasing attention since their remarkable superiority in enhancing antitumor efficacy in cancer treatment [12–16].

To devise the excellent drug delivery systems, quite a few strategies are proposed: (i) targeting molecules have been introduced to stimulate cellular uptake by targeting nanocarriers to specific receptors out of the cell surface; (ii) combining a sensitizer (drug) with prodrugs to increase chemosensitivity to UA [17–20]. The folate receptors are overexpressed in many cancer cells, including breast, ovary, endometrium, kidney, lung, head and neck, myeloid cancers and so on [21,22]. Meanwhile, they are internalized into cells after ligand binding [23]. Because of absence or low expression of these receptors on normal tissues, folate-linked carrier system does not normally accumulate in healthy tissues. Compared with over other ligands, the advantages of FA are high affinity and specificity towards receptors, good conjugating property, low cost, easy availability and non-immunogenicity [24]. Moreover, glutathione (GSH) acts as antioxidant and protects cells against reactive oxygen species (ROS) and xenobiotics (heavy metals and drug metabolites, *etc.*) [25,26]. GSH-mediated detoxification is one of the most major mechanisms responsible for the cancer drug resistance and the GSH levels in tumor cells are comparatively higher than those in normal

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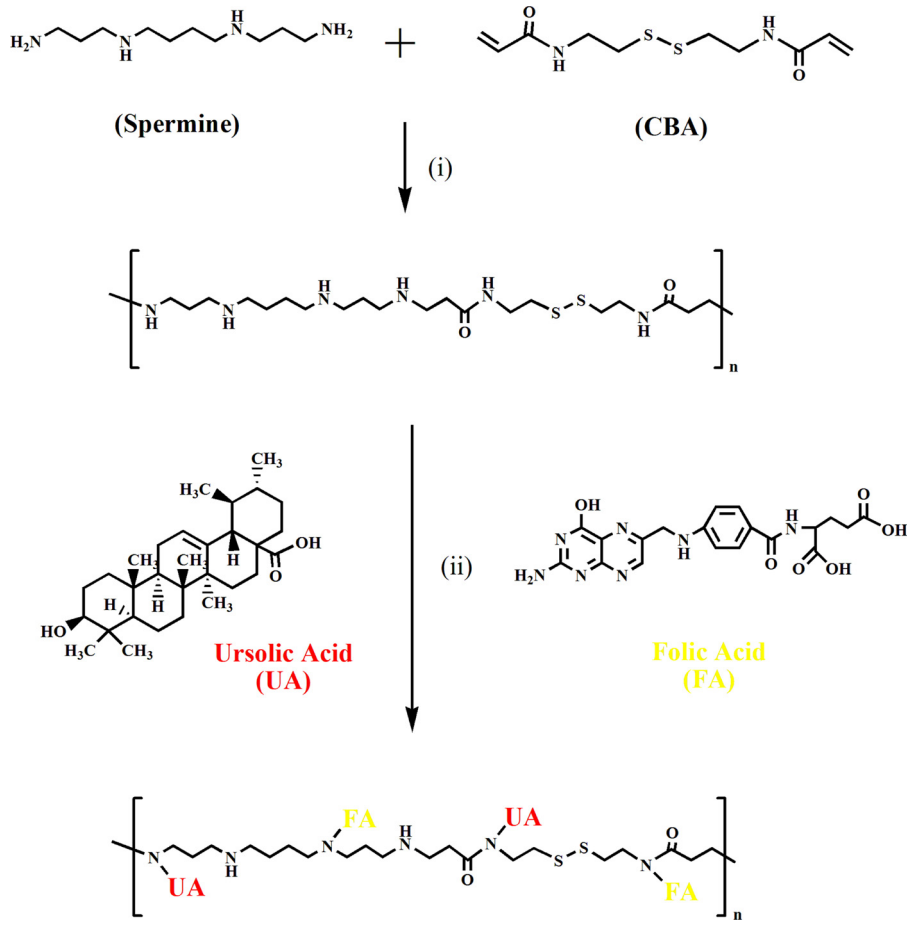

Scheme 1. Synthesis scheme of the FA-CPSP-UA conjugate using folic acid (FA), ursolic acid (UA), and chemosensitive polyspermine (CPSP).

cells [27]. It has been observed that elevated levels of GSH are associated with resistance to chemotherapeutic agents in cancer cells [28]. As a drug sensitizer in combination therapy with chemotherapeutic agent, buthionine sulfoximine (BSO) can selectively inhibit the synthesis of GSH to reduce the GSH-mediated detoxification [29]. In addition, utilizing the concentration difference of the GSH in the bloodstream (< 5 μM) and the cytosol of cancer cells (up to 2–10 mM), the redox-cleavable disulfide bonds were introduced to improve intracellular drug release in response to intracellular levels of GSH [30–33].

In order to effectively treat human breast cancer by combining the sensitizer with prodrug, we designed and synthesized the GSH-triggered degradable FA-targeted chemosensitive polyspermine prodrug (FA-CPSP-UA) for UA delivery in which UA was loaded by chemical conjugation (Scheme 1). The strong hydrophilic properties of CPSP made UA well dispersed in water [34]. The conjugation FA of prodrug could provide active targeting and enhance cellular uptake of the prodrug, which greatly increased tumor specificity and highly improved drug efficacy. The high intracellular GSH concentration facilitated FA-CPSP-UA degradation by segmentation of a large of disulfide bonds, which extremely enhanced intracellular drug release. Moreover, the large intracellular GSH was consumed in the course of the degradation process, which reduced the GSH-mediated detoxification and promoted the chemotherapeutic efficacy. Compared to free UA, the FA-CPSP-UA not only showed better inhibition effect on the MCF-7 cells proliferation, but exhibited excellent ability in inhibiting the migration of MCF-7 cells. Therefore, because of its self-sensibilization effect and prominent anticancer activity, the FA-CPSP-UA provides a promising strategy for treating breast cancer.

2. Materials and methods

2.1. Materials

Spermine, acryloyl chloride, cystamine dihydrochloride, *N,N′*-bis (acryloyl) cystamine (BAC), *N*-hydroxysuccinimide (NHS), *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC·HCl), folic acid (FA), L-buthionine-S,R-sulfoximine (BSO), and ursolic acid (UA) were purchased from Aladdin-reagent Company (Shanghai, China) and used directly. Methanol, dichloromethane and sodium hydroxide were purchased from Guangzhou Chemical Reagent (China). Dulbecco's modified Eagle's medium (DMEM) and Dulbecco's phosphate buffered saline (PBS) were purchased from Life Technologies Corporation. Reduced glutathione assay kit was purchased from Nanjing Jiancheng Bioengineer Institute. Cell counting kit-8 (CCK-8) and Annexin V-FITC Apoptosis Detection Kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China).

2.2. Cell culture

The MCF-7 human breast cancer cells were obtained from Southern Medical University. MCF-7 cells were cultured in complete DMEM (with 10% FBS, 100 U/mL penicillin G sodium and 0.1 mg /mL streptomycin sulfate). Cells were maintained at 37 °C in a humid atmosphere containing 5% CO_2.

2.3. Synthesis of CPSP

The synthetic route of FA-CPSP-UA is showed in Scheme 1. For CPSP synthesis, we first synthesized, *N,N′*-bis(acryloyl) cystamine (BAC) in