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Co-delivery of sorafenib and siVEGF based on mesoporous silica nanoparticles for ASGPR mediated targeted HCC therapy

Guirong Zheng, Ruirui Zhao, Aixiao Xu, Zhichun Shen, Xian Chen, Jingwei Shao*

Cancer Metastasis Alert and Prevention Center, Pharmaceutical Photocatalysis of State Key Laboratory of Photocatalysis on Energy and Environment, Fujian Provincial Key Laboratory of Cancer Metastasis Chemoprevention and Chemotherapy, College of Chemistry, Fuzhou University, Fuzhou 350116, China

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ABSTRACT

Combination with chemotherapeutic drug and gene therapy has been proven highly effective in suppressing tumor progression. Hence, an asialoglycoprotein receptor (ASGPR)-targeting nanodrug delivery system based on mesoporous silica (MSN) nanocarrier for co-delivery of sorafenib (SO) and vascular endothelial growth factor (VEGF) targeted siRNA (siVEGF) to hepatocellular carcinoma (HCC) was successfully designed and synthesized. The structure of nanoparticles was characterized by IR, particle size, zeta potential and N2 adsorption-desorption. The nanoparticles were further evaluated for drug release, cellular uptake, transfection, cell cytotoxicity and cell cycle against HepG2 and Huh7 cells. *In vitro* testing demonstrated that MSN-LA delivery system could not only induce S cell cycle arrest, enhance the cytotoxicity and improve the tumor target of SO and siVEGF, but also enhance the siVEGF transfection efficiency in ASGPR-overexpressing Huh7 cells. Overall, the MSN-LA delivery system can be a promising drug carrier which could further enhance the anti-cancer efficacy of SO and siVEGF *via* the active targeting property of LA.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers, accounting for almost 90% of global cases of primary liver cancer (El-Serag 2012; Zhang et al. 2015). Recently, mesoporous silica nanoparticles (MSN) have drawn attention as promising anti-tumor drug carriers for the treatment of HCC because of their biocompatibility, low cytotoxicity and prominent drug loading property (Jiang et al. 2017; Li et al. 2017a; Zhang et al. 2013; Zhao et al. 2017). Moreover, MSNs can be easily developed as an efficient drug/gene codelivery system to achieve an expected therapeutic effect (Ma et al. 2013; Powell et al. 2016; Shen et al. 2014; Zheng et al. 2017a). In addition, many previous research on MSN-based prodrug delivery system have made great progress, such as UA@MSN-UA (Li et al. 2017a) and DOX@MSN-PLGA (Zheng et al. 2013), confirming the prominent delivery capacity of MSN. However, improving the targeting of the above nano-drug delivery system requires additional studies.

Sorafenib (SO) is a multi-kinase inhibitor that can target many growth factor receptors, including VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-b, c-KIT, FLT-3 and RET, leading to the inhibition of tumor growth and neoangiogenesis (Lin and Chao 2015; Roviello et al. 2016; Zhang et al. 2014). The food and drug administration (FDA) approved SO as an efficient multi-kinase inhibitor for the treatment of advanced renal cell carcinoma (RCC) in 2005, and it remained the only FDA approved systemic therapy for advanced HCC at the current stage (Kim et al. 2017; Motzer et al. 2013). Although sorafenib presented some benefits on clinical studies, such as on the time to progression (TTP) and overall survival (OS), its efficacy for the treatment of HCC remained unsatisfactory (Gao et al. 2015). Additionally, the side effects and resistance profile of SO restrict its further clinical application (Li et al. 2017a; van Malenstein et al. 2013). Thus, finding a novel method to decrease the toxicity of SO and increase its therapeutic efficacy is exceedingly urgent.

Recently, combination of antineoplastic drugs with small interfering RNA (siRNA) has been used as a promising therapeutic modality (Deng et al. 2013; Ganesh et al. 2013). RNA interference (RNAi)-based therapy using small interfering RNA (siRNA) shows great potential to treat diseases (Choi et al. 2015; Hauptman and Glavac 2013). According to the literature, various cancers including the cancers of the breasts, bladder, lungs, ovaries and prostate have been inhibited by siRNA (Chang et al. 2014; Hauptman and Glavac 2013). However, its clinical application was still restricted because of rapid degradation, renal clearance, low intracellular uptake and limited stability (Ballarin-Gonzalez et al. 2013; Ozcan et al. 2015). As a result, additional studies were required to develop safe and effective carriers for siRNA (Sun et al. 2016). To overcome those setbacks, siRNA is often coated on

* Corresponding author at: 2 Xueyuan Road, Sunshine Technology Building, 6FL, #604, Fuzhou University, Minhou District, Fuzhou, Fujian 350116, China.
E-mail address: shaojingwei@fzu.edu.cn (J. Shao).

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nanocarrier that allows effective co-delivery to tumor sites. Therefore, using MSN to co-deliver drugs with siRNA might provide a promising approach for tumor therapy.

Lactobionic acid (4-O-β-D-galactopyranosyl-D-gluconic acid, LA) is a relatively new product derived from lactose oxidation, and can be hydrolyzed to galactose and glucose acid under the catalysis of enzymes (Alonso et al. 2013). It has high potential applications as a bioactive compound or as an ingredient in foods and pharmaceutical products because of its antioxidant, chelating and humectant properties (Goderska et al. 2014). Recent studies have showed that lactobionic acid was able to target human hepatocellular carcinoma effectively (Fu et al. 2014). The surfaces of liver cells and many cancer cells contain a significant number of ASGPR, which can specifically recognize galactose and *N*-acetylgalactosamine-terminated glycoproteins and bind with them (Tsend-Ayush et al. 2017). These receptors have been implicated in facilitating hepatic infection by multiple viruses including hepatitis B, and is also a target for liver-specific drug delivery (Hu et al. 2014). Hence, targeting anticancer drugs coated with LA to ASGPRs may be a good approach for HCC treatment (Thao et al. 2017).

In the present study, ASGPR-targeting nanodrug delivery system based on mesoporous silica co-delivery of SO and VEGF-siVEGF (SO/siVEGF@MSN-LA) to HCC was successfully designed and synthesized (Fig. 1). Firstly, MSN-NH2 was obtained through multiple steps and SO was loaded inside the MSN-NH2 by non-covalent interactions. Then the surface of nanoparticles was conjugated with lactobionic acid (LA). Finally, siVEGF were loaded into the nano-carrier by electrostatic interaction. The characteristic of the nanoparticles was confirmed by transmission electron microscopy (TEM), DLS, FTIR and N2 adsorption-desorption. The drug release profile of SO was then characterized. The targeting of MSN-LA was evaluated by cellular uptake. Further, cell cytotoxicity and cell cycle arrest were used to detect the anti-tumor activity of SO/siVEGF@MSN-LA in Huh7 cells. Finally, *in vitro* transfection of siVEGF@MSN-LA in Huh7 cells were investigated by fluorescence microscopy and western blot assay.

2. Materials and methods

2.1. Materials

Mesoporous silica nanoparticles (MSN) was synthesized and purified in our laboratory. Lactobionic acid (LA) was purchased from Sigma-Aldrich (St Louis, MO, USA). Sorafenib (SO) was purchased from Meilun Biological Technology Co., Ltd. (Dalian China). Cy5-labeled siVEGF (sense: 5′-GGAGUACCCUGAUGAGAUCdTdT-3′, anti-sense: 5′-GAUCUCAUCAGGGUACUCCdTdT-3′) and negative siRNA (Sense primer: 5′- GGAGUACCCUGAUGAGAUCdTdT-3′, antisense primer: 5′-GAUCUCAUCAGGGUACUCCdTdT-3′) were synthesized by Sangon Biotech (Shanghai, China). Hexadecyl trimethyl ammoniumchloride (CTAC), triethanolamine (TEA), (3-aminopropyl) triethoxysilane (APTES), tetraethoxysilane (TEOS), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) were obtained from Aladdin Reagents Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) was obtained from Life Technologies GmbH (Darmstadt, Germany).

2.2. Synthesis of mesoporous silica nanoparticles (MSN)

The MSN was prepared as described previously (Jiang et al. 2017; Li et al. 2017a; Zhao et al. 2017). Firstly, 2 g of CTAC, 0.1 g of TEA and 20 mL deionized water were placed in a round-bottom flask. Then, the mixture was stirred at 95 °C for 1 h. After mixing, 1.5 mL of TEOS was added dropwise to the solution by injector over a period of 20–30 min while stirring. Following the addition, the solution was vigorously stirred at the same temperature for another 1 h. The crude substance was then centrifuged at 12,000 rpm for 15 min and the supernatant was removed. The crude substance was washed with deionized water and pure alcohol twice, respectively. In order to remove the unreacted residuals, the products were scattered in acidic ethanol (concentrated hydrochloric acid:ethanol = 5:1, V:V) and refluxed for 24 h. The dispersion was then centrifuged and washed three times with water to remove the unreacted CTAC. Then the MSN was dried with vacuum dryer at − 50 °C for 24 h.

2.3. Synthesis of MSN-NH2 nanoparticles

100 mg MSN was dissolved in 20 mL pure alcohol, and 400 μg of APTES was added to the mixture. Then the mixture was vigorously stirred at room temperature for 12 h. The products were obtained by centrifugation at 12,000 rpm for 15 min. The products were then washed three times with pure alcohol to remove the unreacted APTES. Finally, the MSN-NH2 was dried under vacuum desiccation at − 50 °C for 24 h.

2.4. Synthesis of SO loaded MSN-NH2 nanoparticles

30 mg of MSN-NH2 was dissolved in 30 mL acetone and the solution was stirred for 1 h. 10 mg of SO was then added to the solution. After stirring at room temperature for 30 min, the mixture was centrifuged at 13,000 rpm for 30 min and the supernatant was removed. SO@MSN was washed with deionized water and dried with a vacuum dryer at − 50 °C for 24 h.

2.5. Synthesis of SO@MSN-LA

40 mg LA, 100 mg EDC, 100 mg NHS were added to a round-bottomed flask containing 40 mL 0.1 mol MES (pH = 6.0). The reaction mixture was then vigorously stirred at room temperature for 24 h.

30 mg of SO@MSN-NH2, 20 mL DMF, 100 mg of EDC, and 40 mg NHS were added to a round-bottomed flask. The mixture was stirred at room temperature for 4 h and 5 mL 2% LA solution was added. The solution was stirred for another 12 h and the mixture was centrifuged at 13,000 rpm for 30 min. The product was collected after washing with deionized water in triplicate and dried with vacuum dryer at − 50 °C for 24 h. To evaluate the loading efficiency of SO in SO @MSN-LA nanoparticles, the supernatant fraction produced by high speed centrifugation was collected and the release amount of SO was measured by UV-1750.

2.6. Preparation of siVEGF loaded SO@MSN-LA

Briefly, 20 mg of SO@MSN-LA was dispersed into 20 mL ddH_2O solution and sonicated 1 h. 6 μL SO@MSN-LA solution and 2 μg siVEGF was stirred gently for 20 min to form the SO/siVEGF@MSN-LA nanoparticles. To evaluate the loading efficiency of siVEGF in SO/siVEGF@MSN-LA nanoparticles, the supernatant fraction produced by high speed centrifugation was collected and the release amount of siVEGF was measured by UV-1750.

2.7. Preparation of FITC labeled MSN and MSN-LA

2 mg FITC and 20 μL APTES were dispersed in 2 mL ethanol in the dark and the mixture was stirred at room temperature for 12 h. Then, FITC was covalently conjugated to APTES. 200 mg MSN or MSN-LA was dissolved in 50 mL ethanol separately, sonicated for 30 min and mixed with 3 mL FITC/APTES ethanol solution. Next, the mixture was stirred in the dark for 24 h. The mixture was then centrifuged and washed with pure ethanol until the supernatants were clear.

2.8. Characterization of MSN, MSN-NH2, MSN-LA and SO@MSN-LA nanoparticles

Fourier transform infrared (FT-IR) spectra was measured on a FT-IR