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Rational design of cancer-targeted selenium nanoparticles to antagonize multidrug resistance in cancer cells

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Abstract

Multidrug resistance is one of the greatest challenges in cancer therapy. Herein we described the design and synthesis of folate (FA)-conjugated selenium nanoparticles (SeNPs) as cancer-targeted nano-drug delivery system for ruthenium polypyridyl (RuPOP), which exhibits strong fluorescence, which allows the direct imaging of the cellular trafficking of the nanosystem inside the cancer cells. This nanosystem could effectively antagonize against multidrug resistance in liver cancer. FA surface conjugation significantly enhanced the cellular uptake of SeNPs by FA receptor-mediated endocytosis through nystain-dependent lipid raft-mediated and clathrin-mediated pathways. The nanomaterials overcame the multidrug resistance in R-HepG2 cells through inhibition of ABC family proteins expression. Internalized nanoparticles triggered ROS overproduction and induced apoptosis by activating p53 and MAPKs pathways. Moreover, FA-SeNPs exhibited low *in vivo* acute toxicity, which verified the safety and application potential of FA-SeNPs as nanodrugs. This study provides an effective strategy for the design of cancer-targeted nanodrugs against multidrug resistant cancers.

Key words: Multidrug resistance; Nanodrug delivery; Cancer targeting; Selenium nanoparticles

Background

Multidrug resistance is becoming one of the most important obstacles for cancer therapy. P-glycoprotein (P-gp or ABCB1), adenosine triphosphate (ATP)-dependent active efflux pump, is often overexpressed in the plasma membrane of most multidrug resistant cancer cells.[1-3] One of the most common malignancies in the world is hepatocellular carcinoma. Importantly, a serious obstacle for the successful treatment of liver cancer is the development of drug resistance. Nowadays, doxorubicin (DOX)-based combination chemotherapy is the main therapeutic strategy for hepatocellular carcinoma, but it failed to treat drug resistance cancers.[4] What is more, some drugs, such as cyclosporin-A (a calcium channel blocker)[5] and verapamil (an immunosuppressive peptide)[6] are the two most studied agents to reverse the drug resistance. However, they are not effective and specific to P-gp overexpressing cancer cells. Until now, no effective treatment is available for end-stage hepatocellular carcinoma.[7] Therefore, developing new therapeutic agents which can overcome drug resistance for hepatocellular carcinoma cancer patient is urgently needed.

In order to overcome the multidrug resistant and reduce the side effect, targeted nanodrug delivery systems were widely used by improving the stimuli-triggered drug release and cancer-targeted drug delivery to minimize the side effects.[8,9] Significantly, the cancer targeting ligands could bind to their receptor on the cancer cell membrane, which could enhance the selective accumulation and uptake of the nanoparticles in the tumor-bearing organs, and reduce the toxicity toward normal cells at the same time. Nanotechnology is now widely used, as a drug carrier for cancer therapy.[8,10] So far, many nanosystems with different functions had been reported for cancer therapy, such as oxides, metals, polymers, mesoporous silica and semiconductors.[10-13] Among them, selenium nanoparticles (SeNPs) receive more and more attention as nanocarriers due to their biocompatibility,

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straightforward synthesis, low-toxicity, degradability *in vivo*, excellent antioxidant activity and chemopreventative effects, such as SeNPs used as 5-fluorouracil (5-FU) and DOX carriers.[12,14] We had also reported cancer-targeted nanoparticles enhanced anticancer effects.[10,14] But there was no report about multidrug resistance in our previous work. The folate receptor (FAR) is frequently overexpressed on cancers cells and has been used for targeted delivery of FA inked liposomes to cancer cells *in vitro*.[15] FAR transports the captured drugs into the cell by receptor-mediated endocytosis and this has found use in cancer therapy by enhancing the concentration of drugs in the cancer cells.[16] Studies also found the FA-targeted drugs could target specific cancer cells and non-targeted the normal cells.[17] Therefore, FA could be linked to SeNPs to target FAR-overexpressing cancers.

The major limitation of cisplatin is the side effects in normal tissues, which include neurotoxicity, ototoxicity, nausea and vomiting, and especially nephrotoxicity.[18] The serious limitations of cisplatin-based treatments have spurred scientists to search for alternative metal-based anticancer drugs.[19,20] Specially, ruthenium (Ru) displays several favorable properties suitable for drug design and medicinal applications. Studies have shown that Ru complexes exhibited low cytotoxicity toward normal cells and high activity against tumor metastasis.[21-23] Till now, a number of Ru complexes have been synthesized and identified as novel anticancer agents.[24] Among them, KP109 and NAMI-A have already entered clinical trials.[21,23,25,26] Previously we found the Ru complex RuPOP exhibited higher anticancer activity and lower toxicity than cisplain.[21] However, the use and development of the RuPOP complex were limited by its poor aqueous solubility. Therefore this study aimed to construct a drug delivery system for hydrophobic Ru complexes conquering their drawbacks. Following our investigation, we found that pluronic F-127 was a good surface modification agent. The pluronic is a group of block copolymers consisting of propylene oxide and ethylene oxide with central hydrophobic poly (propylene oxide) flanked by two hydrophilic chains of poly (ethylene oxide).[27,28] Pluronics could absorb hydrophobic drugs by intermolecular forces and thus increase the loading rate of RuPOP. Our results confirmed that as-synthesized FA-SeNPs nanosystem could be used as a cancer-targeted carrier of RuPOP to enhance anticancer efficacy against multidrug resistant cancer cells. The underlying mechanisms of FA-SeNPs were also elucidated. Taken together, this study may provide an effective strategy for the design and development of nanodrugs against multidrug resistant cancers.

Methods

Preparation of FA-SeNPs

The solution of 20 mM vitamin C, 5 mM of sodium selenite (Na_2SeO_3) solution and 0.8 mg/mL of chitosan (CS) solution was freshly prepared before the experiment. Pluronic F-127 (2.5 g) was activated by 4-nitrophenyl chloroformate (4-NPC) (125 mg) in dichloromethane (10 ml) at room temperature for 12 h.[29] The reaction mixture was dialyzed against distilled water (DW) for 24 h and freeze-dried. Then added thioglycolic acid (150 μL) to the amine terminated pluronic in DW for 12 h with stirring. The resultant solution was dialyzed against DW for 24 h and freeze-dried. The preparation of RuPOP-loaded SeNPs was as described in our previous works.[14] 2.5 g thiolated pluronic dissolved in 1 mL methanol and 500 μL 0.1 M CS-FA were added in stirring for 12 h at room temperature then dialyzed against DW for 24 h.

Characterization of FA-SeNPs

The FA-SeNPs were characterized by different methods including Zetasizer particle size, Fourier transform infrared spectroscopy (FT-IR), transmission electron microscope (TEM), UV-vis spectroscopy, and fluorescence spectroscopy analysis. The TEM images were obtained at an accelerating voltage at 80 kV on Hitachi (H-7650). Zetasizer Nano ZS particle analyzer (Malvern Instruments Limited) was used to measure size distribution and zeta potential of the nanoparticles.

Determination of loading rate of RuPOP in FA-SeNPs

The concentration of Se and RuPOP was determined by ICP-AES analysis.

Hemolysis activity examinations

The hemolysis properties of FA-SeNPs were examined by spectrophotometry as reported.[30,31] For the studies of erythrocyte agglutination, each sample was treated to a hemolysis assay for 1 h (5 μM SeNPs, 5 μM RuPOP and 5 μM FA-SeNPs), placed on a glass slide, covered by a cover slip and analyzed by a phase contrast microscope (Life technologies, EVOS FL auto).

Cell lines and cell culture

HepG2 hepatocellular carcinoma cells, L02 human hepatic cells, and R-HepG2 drug-resistant hepatocellular carcinoma cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). HepG2 and L02 were incubated in DMEM, but R-HepG2 were incubated in 1640 with 100 U/ml penicillin, 50 U/ml streptomycin and 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C with 5% CO_2 atmosphere.

MTT assay

The effects of FA-SeNPs on a series of cancer cells were examined by MTT assay.[32]

In vitro cellular uptake of FA-SeNPs

The *in vitro* cellular uptake of FA-SeNPs was quantitatively determined by measuring the fluorescence intensity of RuPOP-loaded nanoparticles inside the cells by using Spectra Max M5 Microplate reader (Bio-Tek).[14]

Folate competing assay

FA-SeNPs and excess amount of FA competed for binding FARs on R-HepG2 cells. The uptake of FA-SeNPs was then measured by using Spectra Max M5 Microplate reader (Bio-Tek).[14]