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 synthesis of folate (FA)-conjugated selenium nanoparticles (SeNPs) as cancer-targeted nano-drug delivery system for ruthenium polypyridyl (RuPOP) exhibits strong fluorescence, which allows the direct imaging of the cellular trafficking of the nanosystem. 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.

From the Clinical Editor:
In the combat against hepatocellular carcinoma, multidrug resistance remains one of the obstacles to be overcome. The authors designed and synthesized folate (FA)-conjugated selenium nanoparticles (SeNPs) with enhanced cancer-targeting capability. This system carried ruthenium polypyridyl (RuPOP), an efficient metal-based anti-cancer drug with strong fluorescence. It was shown that this combination was effective in antagonizing against multidrug resistance in vitro.

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

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 immnosuppressive 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 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,
Among them, selenium nanoparticles (SeNPs) receive more and more attention as nanocarriers due to their biocompatibility, 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 Specifically, 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, KIP109 and NAMI-A have already entered clinical trials.25,26 Previously we found the Ru complex RuPOP exhibited higher anticancer activity and lower toxicity than cisplatin.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 non-ionic surfactant consisting of poly (ethylene oxide) with central hydrophobic poly (propylene oxide) flanked by two hydrophilic chains of poly (ethylene oxide).27,28 Pluronic 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 (Na2SeO3) 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 activated pluronic F-127 solution was dialyzed against distilled water (DW) for 24 h and then the solution was freeze-dried to powder for further use. 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 thiouolic 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% CO2 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
Intracellular colocalization of FA-SeNPs

Based on the strong fluorescence of RuPOP, fluorescence microscopy was used to monitor the intracellular trafficking of the nanosystem. Briefly, 8 × 10⁴ R-HepG2 cells/mL were seeded in 2-cm dish and allowed to incubate for 24 h. Lyso-tracker DND-99 was added to each dish and incubated for 2 h. The cells were then incubated with 1 μg/mL of DAPI H33342 (Sigma-Aldrich) for 30 min. After rinsing by PBS for 3 times, the cells were exposed to 2 μM RuPOP-loaded FA-SeNPs for different times and observed under fluorescence microscopy.

Mechanisms of cellular uptake of FA-SeNPs

The mechanisms of cellular uptake of FA-SeNPs in R-HepG2 cells were investigated by endocytosis inhibitors.¹⁴

In vitro drug release of FA-SeNPs

FA-SeNPs (10 mg) were suspended in 10 ml PBS with pH values at 5.3 and 7.4 respectively, with constantly shaking in dark at 37 °C. After different periods of time, 0.3 ml of solution was taken out from the tubes and centrifuged. The RuPOP concentration in the supernatant was detected by fluorescence microplate.
Table 1
Cytotoxic effects of FA-SeNPs and RuPOP.

<table>
<thead>
<tr>
<th>Material</th>
<th>IC_{50} (μM)</th>
<th>HepG2</th>
<th>R-HepG2</th>
<th>L02</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA-SeNPs</td>
<td>0.33 ± 0.02</td>
<td>0.24 ± 0.02</td>
<td>1.40 ± 0.13</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>RuPOP</td>
<td>0.71 ± 0.03</td>
<td>1.04 ± 0.12</td>
<td>0.90 ± 0.08</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>DOX</td>
<td>0.28 ± 0.02</td>
<td>2.84 ± 0.29</td>
<td>0.36 ± 0.02</td>
<td>10.1</td>
<td></td>
</tr>
</tbody>
</table>

RI (resistance index): the ratio of IC_{50} (R-HepG2) against IC_{50} (HepG2).

reader (Spectra Max M5, Bio-Tek) with excitation and emission wavelength set as 450 nm and 602 nm respectively.

Flow cytometric analysis

The flow cytometry was used to analyze the cell cycle distribution. DNA histogram displays the proportion of cells in G0/G1, S, and G2/M phases, and the sub-G1 peak was quantified in cell cycle pattern to determine the apoptotic cells with hypodiploid DNA content.

Measurement of intracellular ROS generation

Intracellular ROS overproduction induced by FA-SeNPs in R-HepG2 cells was measured by staining cells with DHE as a probe with excitation and emission wavelength set as 300 and 600 nm.

Western blot analysis

Western blot analysis was used to analyze the effects of FA-SeNPs on the expression levels of cellular proteins altered by treatments of the nanoparticles.

Acute toxicity experiments

ICR mice (body weight of 18-22 g) used in this study were provided by Shanghai Super-B&K Laboratory Animal Corp. Ltd. The mice were housed in cages in a room with controlled temperature of 20-25 °C and humidity of 40%-70%. The mice were fed Se-deficient formula feed and allowed ad libitum access to diet and water. One hundred and fifty mice were randomly divided into 15 groups with 10 mice per group. FA-SeNPs, SeMet or selenite was fed orally at the doses indicated in Table 2 for 14 consecutive days. All mice groups were sacrificed at 14 days. Then the livers were taken out for H&E staining. Cumulative mortality within 14 days after the treatment was used for the calculation of LD_{50}. Forty mice were randomly divided into five groups with 8 mice per group. They received saline as control, FA-SeNPs, SeMet or selenite at the dose of 10 mg Se/kg orally respectively. All mice groups were sacrificed at 6, 12, 24, and 48 h. All animal experiments were approved by the Animal Experimentation Ethics Committee of Jinan University.

Statistical and synergistic analysis

All experiments were carried out at least in triplicate and results were expressed as mean ± SD. Difference between two groups was analyzed by two-tailed Student’s t test. Statistical analysis was performed using SPSS statistical program. The difference between three or more groups was analyzed by one-way ANOVA multiple comparisons. Difference with $P < 0.05$ (*) or $P < 0.01$ (**) was considered statistically significant.

Results

Preparation and characterization of FA-SeNPs

In the study, FA-SeNPs were successfully prepared as illustrated in Figure 1, A. As shown in Figure 1, B, the FA-SeNPs were highly uniform and monodisperse spherical particles with a 180 nm diameter. In contrast, SeNPs were easily aggregated and precipitated in aqueous solutions in Figure 1, B. From the Figure 1, C, we know that the FA-SeNPs are more stable than SeNPs. We confirmed the S-Se bond and the successful conjugation of FA-CS and RuPOP-pluronic F-127 to SeNPs by FTIR. As shown in Figure 1, D, the absence of an S-H stretching mode in the IR spectrum of FA-SeNPs indicated the formation of the HS-Se bond between the ligands and the SeNPs surface; the peaks at 1490 and 679 cm$^{-1}$ in FA-SeNPs were assigned to the bending vibration of N-H and the symmetrical bending vibration of amino groups, respectively. The spectrum of FA-SeNPs retained the special peaks of SeNPs 528 cm$^{-1}$. The peak AT 1213 cm$^{-1}$ was assigned to stretching vibration of C-N from RuPOP. The UV-Vis and fluorescence spectra of FA-SeNPs and RuPOP further verified the successful loading of RuPOP into the nanosystem (Figure S1).

Hemocompatibility of FA-SeNPs

Hemocompatibility can serve as a marker for membrane activity and predict potential side effects of exogenous drugs and materials. The hemolysis of red blood cells induced by SeNPs, FA-SeNPs and RuPOP was shown in Figure 1, E, F and G. SeNPs alone induced slight hemolysis both after 10 min and 2 h. In contrast RuPOP induced slight hemolysis after 10-min incubation, but it induced higher hemolysis after 2 h with highest concentration. Compared with SeNPs and RuPOP, FA-SeNPs didn’t induce hemolysis of erythrocyte. Moreover, SeNPs and RuPOP induced prominent agglutination of erythrocytes after incubation for 1 h, while no agglutination was observed in erythrocytes exposed to FA-SeNPs (Figure 1, H).

Antagonizing multidrug resistance in R-HepG2 Cells by FA-SeNPs

MTT assay was used to study the effects of FA-SeNPs on cancer cells. From Table 1, we found that the FA-SeNPs displayed significant anticancer activities against R-HepG2 and HepG2 cancer cells and showed low toxicity toward L02 normal cells. Especially, the IC_{50} of R-HepG2 was 0.24 ± 0.02 μM, while that of HepG2 was 0.33 ± 0.02 μM, which suggests that the FA-SeNPs can antagonize multidrug resistant cancer. FA-SeNPs at the concentrations of 0.2-0.8 μM exhibited higher growth inhibition on R-HepG2 cells than HepG2 cells and L02 cells (Figure 2, A and Figure S2). Generally, the effects of FA-SeNPs on cancer cells were much higher than those on normal cells (Figure 2, A). Therefore, the FA-SeNPs could well antagonize multidrug resistant cancer cells (R-HepG2).
Selective cellular uptake of FA-SeNPs

Cellular uptake was a main factor for the bio-activity of nanoparticles. In Figure 2, B, the cellular uptake of R-HepG2 cells was higher than HepG2 cells after incubation with 20 μM FA-SeNPs for 0.5, 1, 2, 4 and 8 h. The cellular uptake was quantified by measuring the fluorescence intensity of RuPOP. The fluorescent intensity of RuPOP in R-HepG2 cells was significantly stronger than that in HepG2 cells for the same time point (Figure 2, C). It is possible that, the different cellular uptake of R-HepG2 cells and HepG2 cells is regulated by FAR. Therefore, we examined the expression level of FAR in different cells. As shown in Figure 2, D, the expression levels of FAR in the tested cell lines were in the order of R-HepG2 > HepG2 > L02. We also investigated the importance of FAR in cellular uptake of the nanosystem by FA competing assay. As shown in Figure 2, E, FA dramatically inhibited the cellular uptake of FA-SeNPs in cancer cells in a dose-dependent way. These results demonstrate the important role of FAR in the cellular uptake of FA-SeNPs in cancer cells.

Intracellular translocation of FA-SeNPs and pH-mediated drug release

The intracellular translocation of the nanoparticles was monitored by fluorescence microscopy. As shown in Figure 3,
A, FA-SeNPs entered the cytoplasm by endocytosis after 1-h incubation. Under the acid environments, RuPOP was released from FA-SeNPs after 4 h and then entered cytoplasm in a time-dependent manner. These results suggest that endocytosis is the major uptake mechanism of FA-SeNPs in cancer cells.

To further dissect the FA-SeNPs endocytosis mechanism, we used different endocytosis inhibitors to treat the cells before the addition of FA-SeNPs. As shown in Figure 3, B, treatments of NaN3 and DOG markedly inhibited the internalization of FA-SeNPs to 66.4% ± 5.5% (P<0.01) of control. The results showed that sucrose strongly decreased the FA-SeNPs internalization to 59.7% ± 6.5% (P<0.01) of the control. Moreover, nystatin and dynasore induced the uptake of FA-SeNPs to 63.9% ± 1.7% (P<0.01) and 70.8% ± 2.8% (P<0.01) of the control, respectively. Dynamin, a GTP-binding protein, is essential for receptor-mediated endocytosis.35,36

Drug release was studied to simulate the acidic environments of lysosomes and normal body blood environments. As shown in Figure 3, C, the released amount of RuPOP was 17.7% ± 1.6% at pH 5.3 and 8.6% ± 0.9% at pH 7.4 within 2 h, and the final released amount of RuPOP was 72.5% ± 2.7% at pH 5.3 and 16.4% ± 1.2% at pH 7.4 after 48 h treatment. Therefore, RuPOP was released from FA-SeNPs in a pH-dependent manner. It is possible that the SeNPs were expanded to snowflake particles under acidifying stimulus, which led to enhanced drug-release over prolonged periods.37 Moreover, in acidic solution, the proton competes with RuPOP to bind to oxygen atom of pluronic F-127, which would promote the drug release from SeNPs.

Inhibition of ABC family protein expression by FA-SeNPs

First, we analyzed the expression levels of ABC family protein in R-HepG2 cells, HepG2 cells and L02 cells without
FA-SeNPs. As shown in Figure 3, the expression levels of ABCB1, ABCC1 and ABCG2 in R-HepG2 cells were higher than those in HepG2 cells and L02 cells. Then we further studied the expression levels of ABC family proteins in R-HepG2 cells in a dose-dependent manner. As shown in Figure 3, expression levels of ABC family proteins in R-HepG2 cells were decreased when the concentration of FA-SeNPs increased.

FA-SeNPs induces apoptotic cell death

The effects of FA-SeNPs on the cell cycle distribution of cancer cells were examined by flow cytometric analysis. As shown in Figure 4, A, the R-HepG2 cells exposed to FA-SeNPs for 72 h demonstrated a significant dose-dependent increase in sub-G1 cell population from 5.8% to 94.8%. In contrast, treated HepG2 cells demonstrated lower increase in sub-G1 cell population from 0.7% to 26% (Figure 4, B).

As shown in Figure 4, C, the expression levels of caspase-8 (extrinsic death receptor-mediated pathways) and caspase-9 (intrinsic mitochondria-mediated pathways), two initiator caspases, as well as caspase-3, an effector caspase, were decreased by FA-SeNPs dose-dependently in R-HepG2 cells. Moreover, the truncation of caspases triggered the proteolytic cleavage of PARP.

Activation of ROS-mediated signaling pathways

As shown in Figure 5, A, FA-SeNPs trigged the production of ROS after 10 min and reached the maximum value; the amount
of ROS declined to 186% ± 1.62% with 0.96 μM FA-SeNPs after 100 min. RuPOP induced a trace of the generation of ROS in whole process. We could find that the fluorescent intensity of DHE in R-HepG2 cells treated with FA-SeNPs was much stronger than RuPOP in Figure 5, B.

Overproduction of ROS could trigger various downstream signaling to activation cells apoptosis (Figure 5, C-E). As shown in Figure 5, C; FA-SeNPs induced the elevation of total p53 and its phosphorylation at Ser 15 site. Moreover, p-ATM, p-BRCA1 and phosphorylated histone H2A.X (an important biochemical marker of DNA damage) were also increased in cells exposed to FA-SeNPs. Taken together, these results indicate that ROS-mediated p53 phosphorylation plays an essential role in apoptosis induced by FA-SeNPs in cancer cells.

To examine the roles of MAPKs and AKT in FA-SeNPs-induced apoptosis, we examined the level of MAPks and AKT in treated cells. As shown in Figure 5, D, FA-SeNPs exhibited differential effects on the phosphorylation of p38, JNK, ERK, and AKT. The phosphorylation of pro-apoptotic kinases p38 and JNK displayed a trend of up-regulation in a dose-dependent manner. In contrast, the phosphorylation of anti-apoptotic kinases ERK and AKT was effectively suppressed by FA-SeNPs.

**Acute toxicity of FA-SeNPs**

As shown in Table 2, selenomethionine (SeMet) and selenite caused 100% mortality at a dose of 50 mg Se/kg. However, FA-SeNPs caused only 10% mortality at a dose of 373.3 mg Se/kg.
As calculated by Bliss method using NDST software, the LD_{50} values of FA-SeNPs, SeMet and selenite were found to be 1000.3 ± 52.0, 30.5 ± 3.0 and 31.1 ± 4.3 mg Se/kg (Table 3), respectively. Therefore, the acute toxicity of SeMet and selenite was about thirty-two fold larger than that of FA-SeNPs. As shown in Figure 6, A, B and C after 48 h treatment, FA-SeNPs induced lower ALT, AST and LDH enzymatic activities than those of SeMet and selenite, indicating the lower injury of the nanomaterials of the mice liver. Furthermore, H&E staining was also used to verify the toxic effects of FA-SeNPs, SeMet and selenite on the liver. As shown in Figure 6, D, E, F and G compared with normal group, FA-SeNPs caused slight hydropic and fatty degeneration liver cells. In contrast, SeMet induced significant degeneration and apoptosis of liver cells, while selenite caused hypertrophy and fatty degeneration.

**Table 2**

<table>
<thead>
<tr>
<th>FA-SeNPs</th>
<th>SeMet</th>
<th>Selenite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se dose (mg Se/kg)</td>
<td>Mouse Mortality (%)</td>
<td>Mouse Mortality (%)</td>
</tr>
<tr>
<td>1800</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>1440</td>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>1152</td>
<td>70</td>
<td>32</td>
</tr>
<tr>
<td>921.6</td>
<td>40</td>
<td>25.6</td>
</tr>
<tr>
<td>373.3</td>
<td>10</td>
<td>20.48</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>LD_{50} (mg/kg)</th>
<th>95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA-SeNPs</td>
<td>1003.8 ± 52.0</td>
<td>943.7-1047.7</td>
</tr>
<tr>
<td>SeMet</td>
<td>30.5 ± 3.0</td>
<td>27.5-33.5</td>
</tr>
<tr>
<td>Selenite</td>
<td>31.1 ± 4.3</td>
<td>27.0-35.6</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n=3).

**Discussion**

Many kinds of nanomaterials have been synthesized and applied in cancer therapy. Among them, SeNPs have significant anticancer activity and low toxicity to normal cells that attracted a great deal of attention as a novel anticancer agent and drug carrier. However, the poor selectivity between different cancer cells and normal cells and the low drug loading rate have limited its future clinical application. This study described the synthesis of FA-conjugated SeNPs as cancer-targeted drug delivery systems for RuPOP to antagonize multidrug resistance in liver cancer. The introduction of pluronic F-127 greatly increased the loading rate of RuPOP into the nanomaterials and increased the hydrophilic of drugs.

Although the combination of different chemotherapeutic agents is often employed to prevent drug resistance in cancer patients, the ability of the cancer cells to adapt and develop multidrug resistance ultimately leads to the treatment failure in most cancer patients. However, multidrug resistance is a complicated process with the involvement of acquired and multiple multidrug resistant mechanisms as a result of the expression of drug efflux pumps, up-regulation of antiapoptotic proteins, and increase in regulators of drug metabolism. ABC family proteins are often overexpressed in the plasma membrane of most multidrug resistant cancer cells and decreased cellular uptake of drugs. Therefore, we designed a cancer-targeted drug delivery system for RuPOP to antagonize multidrug resistance in R-HepG2 cells by enhancing cellular uptake of drugs. Firstly, the physicochemical properties of the drug are very important. Its high stability enabled the nanoparticles to enter the cells by endocytosis. Secondly, FA-SeNPs were able to target R-HepG2 cells overexpressing FAR, then enter and accumulate in R-HepG2 cells through endocytosis after 1-h incubation. Under the acid environments, RuPOP was released from FA-SeNPs after 4 h and then entered cytoplasm in a time-dependent manner. It is possible that the SeNPs were expanded to snowflake particles under acidifying stimulus, which led to enhanced drug-release over prolonged periods. Moreover, in acidic solution, the proton competes with RuPOP to bind to oxygen atom of pluronic F-127, which would promote the drug release from SeNPs. The high expression levels of FAR in cancer cells effectively increased the cellular uptake of FA-SeNPs in cancer cells, and improved its selectivity between cancer and normal cells, thus finally enhancing the anticancer efficacy.

Further studies were carried out to elucidate the anticancer mechanisms of FA-SeNPs. Many studies have shown that caspases, a family of cysteine proteases, play a vital role in apoptosis. Therefore, Western blot analysis was performed to analyze the expression and roles of caspase-8, caspase-9 and caspase-3 in FA-SeNPs-induced apoptosis. Moreover, activation of caspases triggered the proteolytic cleavage of PARP, an important biochemical marker of apoptosis. These results indicated that FA-SeNPs induced liver cancer cell apoptosis by triggering intrinsic and extrinsic apoptotic pathways. Consistently, our previous studies also showed that 5FU-SeNPs, TF-SeNPs and SeNPs@ATP induced apoptosis also in cancer cells through PARP cleavage and caspases activation.

ROS has been identified as an essential chemical signaling molecule that could regulate the apoptotic signaling pathway triggered by various anticancer agents, including cisplatin and Ru complexes. ROS overproduction can activate various kinases including MAPK and AKT, consequently causing protein modification and DNA damage and inducing cells apoptosis. Our previous work also indicated that modified SeNPs could induce apoptosis by ROS-mediated MAPK and AKT pathways. And various multifunctional SeNPs also develop their anticancer effect by these pathways including 5FU-SeNPs and TF-SeNPs. In this study, the combination of SeNPs and RuPOP significantly increased the intracellular ROS generation, which led to marked improvement in anticancer efficacy. And the MAPKs and AKT pathways played an important role in apoptosis induced by FA-SeNPs with the generation of ROS.

Se is an essential trace element with many biological functions, but exhibits a narrow margin between beneficial and toxic effects. Therefore, toxicity examination is always a crucial concern for development of Se-based anticancer drugs. Many studies have demonstrated that SeNPs have less toxicity, higher bioavailability and stronger biological activities than inorganic...
In this study, we have compared the cytotoxicity of FA-SeNPs against several human cancer and normal cell lines, and our results demonstrate that FA-SeNPs exhibited a broad spectrum of growth inhibition against human cancer cells, but showed the lowest cytotoxicity toward human normal cells. These results suggested that FA-SeNPs possess great selectivity between cancer and normal cells and display potential application in treatment of human cancers. Moreover, the acute toxicity studies were carried out for safety evaluation. The results showed that the acute toxicity of SeMet and selenite was about 2-3 folds larger than that of FA-SeNPs and the lower injury of the nanomaterials of the mice liver. Consistently, previous studies also showed that SeNPs were less toxic than SeMet and selenite. Therefore, the FA-SeNPs could reduce the acute toxicity of SeNPs. Taken together, these results demonstrate that FA-SeNPs exhibit much lower acute toxicity than the common Se positive controls SeMet and selenite, which indicate that FA-SeNPs is an efficient and low toxicity drug delivery system for anticancer drugs.

In summary, herein we have demonstrated the design of cancer-targeted nanoparticles loaded with RuPOP complex. Importantly, the FA-SeNPs increase the sensibility to cancer cells overexpressing FAR and against the multidrug resistant of R-HepG2 cells by inhibition of ABC family proteins expression. Internalized FA-SeNPs induce cells apoptosis through up-regulating the level of ROS in cells to trigger MAPKs and AKT pathways. Moreover, FA-SeNPs exhibit lower in vivo acute toxicity than selenomethionine and selenite, which verify the safety and application potential of FA-SeNPs as nanodrug system. We would expect that this study may provide an effective strategy for the design and development of nanodrugs against multidrug resistant cancers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nano.2015.01.009.

References


