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Overcoming tumor resistance to cisplatin by cationic lipid-assisted prodrug nanoparticles

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ABSTRACT

Chemotherapy resistance has become a major challenge in the clinical treatment of lung cancer which is the leading cancer type for the estimated deaths. Recent studies have shown that nanoparticles as drug carriers can raise intracellular drug concentration by achieving effectively cellular uptake and rapid drug release, and therefore reverse the acquired chemoresistance of tumors. In this context, nanoparticles-based chemotherapy represents a promising strategy for treating malignancies with chemoresistance. In the present study, we developed cationic lipid assisted nanoparticles (CLAN) to deliver polylactide-cisplatin prodrugs to drug resistant lung cancer cells. The nanoparticles were formulated through self-assembly of a biodegradable poly(ethylene glycol)-*block*-poly(lactide) (PEG-PLA), a hydrophobic polylactide-cisplatin prodrug, and a cationic lipid. The cationic nanoparticles were proven to significantly improve cell uptake of cisplatin, leading to an increased DNA-Pt adduct and significantly promoted DNA damage *in vitro*. Moreover, our study reveals that cationic nanoparticles, although are slightly inferior in blood circulation and tumor accumulation, are more effective in blood vessel extravasation. The CLANs ultimately enhances the cellular drug availability and leads to the reversal of cisplatin resistance.

1. Introduction

Cancer remains one of the most causes of death worldwide [1]. Cisplatin is among the most widely used chemotherapeutics for cancer therapy. However, the extension of cisplatin-based therapy has been significantly hindered by acquired chemotherapy resistance [2] and poor prognosis [3] of tumor cells which are caused by the reduced intracellular drug accumulation, increased inactivation of drug molecules, and enhanced DNA damage repair and tolerance [4–6]. Although much attention has been paid to develop cisplatin derivatives (such as carboplatin, oxaliplatin, satraplatin, oxoplatin and tetraplatin) to improve its activity for drug-resistant cancers [7–10], the unsatisfied tumor cell uptake and severe side effects are further believed as major clinical barriers [11,12].

Nanoscale drug delivery vehicles could preferentially deliver Pt agents to solid tumors by altering the biodistribution of associated drugs and thus reduce their side effects [13–18]. For instance, NC-6004, the cisplatin-incorporating polymeric micelles based on poly(ethylene glycol)-poly(glutamic acid), has been studied in Phase III clinical trial in Asia [19,20]. NC-4016 whereas cisplatin is substituted by oxaliplatin is also conducting Phase I clinical research [21]. Furthermore, nanoparticles as drug carriers have been reported to raise intracellular drug concentration by bypassing copper transport protein (Ctr1 protein) [22–24] through endocytosis of drug resistance cells and could facilitate intracellular drug concentration within the therapeutic window, therefore reverse the acquired chemoresistance of tumors [25–28].

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Liberation of active drug from the carrier is another crucial step to improve the drug concentration level to overcome the cisplatin resistance of lung cancer [14]. With rational design, it is believed that the active Pt(IV) conjugated polymeric prodrug could be stable during the blood circulation and exhibit rapid release according to the reduction environment after cellular internalization [29]. For example, a PEGylated gold nanorods prodrug Pt-PEG-GNRs was developed and confirmed its promoted drug internalization and decreased propensity toward cellular deactivation which can effectively reverse cisplatin resistance [30]. Meanwhile, a *m*PEG-*b*-PCL-*b*-PLL conjugated Pt(IV) micelles displayed higher cytotoxicity against SKOV-3 tumor cells mainly due to the effective internalization by the cells [31]. Thus, nanoparticles with conjugated Pt(IV) prodrug are promising to effectively increase intracellular drug concentration which is necessary to overcome cisplatin resistance.

Furthermore, the ability of nanoparticles to extravasate into tumors and ultimately enter tumor cells is also necessary to successful cancer treatment [32–35]. It is reported that cationic liposomes can selectively target tumor vasculature, leading to preferential accumulation of cationic liposomes in the solid tumor, a phenomenon not noted with anionic (negatively charged) or electroneutral liposomes [36,37]. Moreover, it is documented that non-phagocytic cells could ingest cationic nanoparticles at a higher extent [38,39], and the uptake degree will be promoted dependent on the increased positive charge of nanoparticles [40]. Interestingly, a coarse-grained model for gold nanoparticles was also developed to simulate the interaction with lipid membranes, the results showed that the level of penetration increases as the charge density increases [41]. We had ever shown that the transformation of nanoparticles from anionic to cationic surface after entering the tumor site will contribute to enhanced cellular uptake and sequentially conquer the drug resistance of tumor cells [24]. In these contexts, we hypothesized that the integration of cisplatin prodrug and cationic component into nanoparticles would represent a more promising strategy for reversing tumor resistance to cisplatin *in vivo*.

As a proof-of-concept, we have developed an integrated nanoparticles platform through self-assembly of a biodegradable poly(ethylene glycol)-*block*-poly(lactide) (PEG-PLA), a hydrophobic polylactide-cisplatin prodrug, and a cationic lipid. To construct the Pt(IV) prodrug, a polylactide derivative was synthesized and coupled with carboxyl-functionalized Pt(IV) species (shown in Scheme 1). Next, the cationic lipid-assisted nanoparticles (CLAN) system was prepared with cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) lipid and clinically validated PEG-PLA copolymer through a single emulsification method. Thus, this delivery system with high biocompatibility can not only stabilize by PEG protection to extend its life-time *in vivo*, but also improve tumor uptake by positive charge mediated extravasation. Additionally, the Pt(IV) prodrug was reduced by cellular reducing agents and liberated from the polymer to recover its activity, resulting in increased intracellular drug concentration and reversal of cisplatin resistance.

2. Materials and methods

2.1. Materials and characterizations

The block copolymer PEG-PLA was synthesized by ring-opening polymerization of lactide (LA, Dai Gang Biomaterial, China) using methoxy polyethylene glycol (PEG, M_n = 5000, Sigma-Aldrich, Saint Louis, MO) as the initiator according to a previously reported method [42]. The degree of polymerization of LA was 56, corresponding to a molecular weight of 8060 for the PLA block. The LA derived monomer 3,6-benzyloxymethyl-1,4-dioxane-2,5-dione (LA-OBn) was synthesized referring to the literature [43]. LA was purified by sublimation twice under reduced pressure at 100 °C. Ethyl lactate was purchased from Sinopharm Group Co., Ltd. (China) and purified *via* vacuum distillation. *N*,*N*-Diisopropylcarbodiimide (DIC), 4-dimethylaminopyridine (DMAP), glutathione (GSH) and rhodamine B (RhoB) were purchased from Aladdin (China). Stannous octoate ($Sn(Oct)_2$) was purchased from Sigma-Aldrich. $Pd(OH)_2$/C was purchased from Alfa Aesar (Haverhill, MA). Cisplatin was purchased from Shandong Boyuan Pharmaceuticals (China). DOTAP was purchased from Avanti Polar Lipids (Alabaster, AL). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) and sodium ascorbate were purchased from Sangon Biotech (China). The Annexin-V-FLUOS Staining Kit was purchased from Roche Diagnostics (Indianapolis, IN). Ultra-purified water was prepared using a Milli-Q System (Millipore, Bedford, MA).

The size and zeta potential measurements were carried out in aqueous solution using a Malvern ZS90 (Malvern Instruments Ltd., England) dynamic light scattering (DLS) instrument with a He-Ne laser (633 nm) and 90° collecting optics. The data was analyzed using a Malvern Dispersion Technology Software 5.10. Morphology of nanoparticles was examined by JEOL-2010 (JEOL, Tokyo, Japan) transmission electron microscopy (TEM) at an accelerating voltage of 200 kV. The amount of Pt was determined by XSeries 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Thermo Fisher Scientific, Wilmington, DE).

2.2. Cell culture

The lung cancer cell line A549 (ATCC code CCL-185) and the cisplatin-resistant cell line A549R (purchased from Shanghai Fumengjiyin Biotechnology (FMGbio) Co., Ltd.) were cultured in Roswell Park Memorial Institute medium (RPMI 1640, Invitrogen, Carlsbad, CA) supplied with 10% fetal bovine serum (FBS, ExCell Bio, China), 1% penicillin/streptomycin (Sigma-Aldrich) at 37 °C with 5% CO_2. The A549R cell line with stable expression of green fluorescent protein (A549R-GFP) was obtained by transfection with a retrovirus according to a standard protocol [44]. The cisplatin resistance of A549R and A549R-GFP cells was maintained with 2 μg/mL cisplatin. The cisplatin-resistant cells were cultured without cisplatin for 3 weeks before experiments.

2.3. Animals and tumor model

Female BALB/c nude mice and ICR mice at 6–8 weeks of age were obtained from Beijing Hfk Bioscience (China). All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the University of Science and Technology of China Animal Care and Use Committee.

The xenograft tumor model was generated by injection of 5×10^6 A549R or A549R-GFP cells (100 μL) with 20% Matrigel (BD Bioscience, Franklin Lakes, NJ) into the right flank of BALB/c nude mice.

2.4. Synthesis of PLA-Pt and RhoB-conjugated PLA (PLA-RhoB)

Benzyl-substituted copolymer (PLA-OBn) was synthesized by ring opening polymerization. In a flame-dried and nitrogen-purged round-bottom flask, LA-OBn (356.4 mg, 1.0 eqv) and LA (1297.17 mg, 9.0 eqv) were heated to 135 °C in a glove box (H_2O and O_2 contents < 0.1 ppm) for 0.5 h, $Sn(Oct)_2$ (10.0 mg, 0.025 eqv) and ethyl lactate (43.4 mg, 0.37 eqv) was then added as the catalyst and initiator, respectively. The mixture was stirred at 135 °C for 2 h. The resultant polymer was dissolved in chloroform, followed by the precipitation into a cold diethyl ether/methanol mixture (100/3, v/