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Co-delivery of all-trans-retinoic acid and doxorubicin for cancer therapy with synergistic inhibition of cancer stem cells

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ABSTRACT

Combination treatment through simultaneous delivery of two or more drugs with nanoparticles has been demonstrated to be an elegant and efficient approach for cancer therapy. Herein, we employ a combination therapy for eliminating both the bulk tumor cells and the rare cancer stem cells (CSCs) that have a high self-renewal capacity and play a critical role in cancer treatment failure. All-trans-retinoic acid (ATRA), a powerful differentiation agent of cancer stem cells and the clinically widely used chemotherapy agent doxorubicin (DOX) are simultaneously encapsulated in the same nanoparticle by a single emulsion method. It is demonstrated that ATRA and DOX simultaneous delivery-based therapy can efficiently deliver the drugs to both non-CSCs and CSCs to differentiate and kill the cancer cells. Differentiation of CSCs into non-CSCs can reduce their self-renewal capacity and increase their sensitivity to chemotherapy; with the combined therapy, a significantly improved anti-cancer effect is demonstrated. Administration of this combinational drug delivery system can markedly augment the enrichment of drugs both in tumor tissues and cancer stem cells, prodigiously enhancing the suppression of tumor growth while reduce the incidence of CSC in a synergistic manner.

1. Introduction

Nanomedicine has been delivering significant benefits to the treatment of cancer over recent decades [1–3] that may overcome the limitations of therapeutic agents *via* prolonging the circulation half-life, improving pharmacokinetics and increasing their uptake by tumor cells [4,5]. Drug delivery systems with nanoparticles have been demonstrated to increase therapeutic efficacy against the most difficult cancer challenges, including drug resistance and tumor metastasis by incorporating targeting strategies and multifunctional capabilities [6–9]. Cancer stem cells (CSCs), also called tumor-initiating cells or stem-like cancer cells, are known to be resistant to chemotherapy and radiotherapy and are associated with tumor metastasis and recurrence after treatments [10–12]; as such, they have attracted increasing attention in recent years with regard to the development of advanced therapeutic methods. The possible therapeutic strategies that can eliminate CSCs generally include inhibiting their self-renewal pathway, differentiating the CSCs, or targeting the CSCs' niche [13–17]. In practice, delivering drugs into the rare population of CSCs in tumor tissue is still challenging. Recently, studies of nanoparticle delivery system-based approaches to tackle the CSC problem have been reported [18,19]. The superior performance of nanoparticles show some therapeutic advantages for CSCs therapy as well, such as enrichment of therapeutic agents within CSCs and delivery of more than one functional agent to CSCs [20,21]. Several studies have successfully targeted and eliminated CSCs using a nanoparticle mediated delivery system, showing good results in overcoming tumor drug resistance and relapse [19,22]. Our previous studies also demonstrated that dual pH-sensitive polymer–drug conjugate nanoparticles showed enhanced inhibition to the progression of drug-resistant SK-3rd cancer stem cells [23], and gold nanoparticles conjugated with doxorubicin *via* hydrazone bonds can deliver more doxorubicin (DOX) to CSCs by overcoming drug resistance through

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evading the efflux of P-gp, resulting in the removal of all tumor cell sub-populations and averting the potential repopulation of the tumor mass by CSCs [24]. Therefore, nanoparticle-based drug delivery systems offer a potential approach for effectively targeting CSC therapies.

However, evidence exists indicating that non-CSCs in the tumor can spontaneously and stochastically turn into CSCs de novo [25,26], undermining the efficacy of therapeutic strategies that only target CSCs [27,28]. Hence, it is crucial to eliminate CSCs and non-CSCs simultaneously for effective cancer therapy. Recently, combination therapy has been developed for efficient therapy [29–31]. Co-delivery (or simultaneous delivery) is an approach that delivers two or more different functional agents in one nanoparticle. Studies have demonstrated that simultaneous delivery systems show more significant therapeutic efficiency than administration of single drug loaded nanoparticles [32–34]. More importantly, there is evidence demonstrating that co-delivery of two agents may have a synergistic effect on cancer therapy, which is not observed with a simple physical mixture of two individual drug loaded nanoparticles [35,36]. The co-delivery systems used possess some unique features, such as the similar pharmacokinetics of the two drugs and simultaneous delivery of two agents into the same cell by one nanoparticle [29,35]. The application of a co-delivery system for targeting CSC and non-CSC therapy in a synergistic manner has not been reported yet.

All-trans-retinoic acid (ATRA) is a powerful differentiating agent that acts through obstructing multiple signaling pathways involved in stem cell maintenance [37–39]. However, no effective cytotoxicity and tumor inhibition can be obtained with ATRA treatment [19,40]. Therefore, combination with other therapeutics would be requisite for efficient cancer therapy, killing both CSCs and normal cancer cells. DOX has been employed as a traditional chemotherapeutic drug for multiple cancer therapies but may be resisted by CSCs in many solid tumors, which may also further enrich their CSCs after treatment, resulting in chemoresistance, tumor relapse and metastasis [41–43]. In this study, we address a promising strategy of co-delivery of an ATRA and DOX based-therapy for both CSCs and non-CSCs (Scheme 1). Nanoparticles simultaneously encapsulating two drugs (ATRA and DOX) were prepared by a single emulsion method, and we demonstrate that drug loaded nanoparticles effectively increase drug uptake by breast CSCs. Treatment with these nanoparticles induces breast CSC differentiation by ATRA, which attenuates their tumor initiating ability and eventually enhances the cytotoxicity of DOX. Furthermore, intravenous administration of the nanoparticles effectively increases the enrichment of the drugs both in tumor tissue and cancer stem cells, and co-delivery of ATRA and DOX remarkably enhances the suppression of tumor growth while decreasing breast CSCs in the tumor in a synergistic manner.

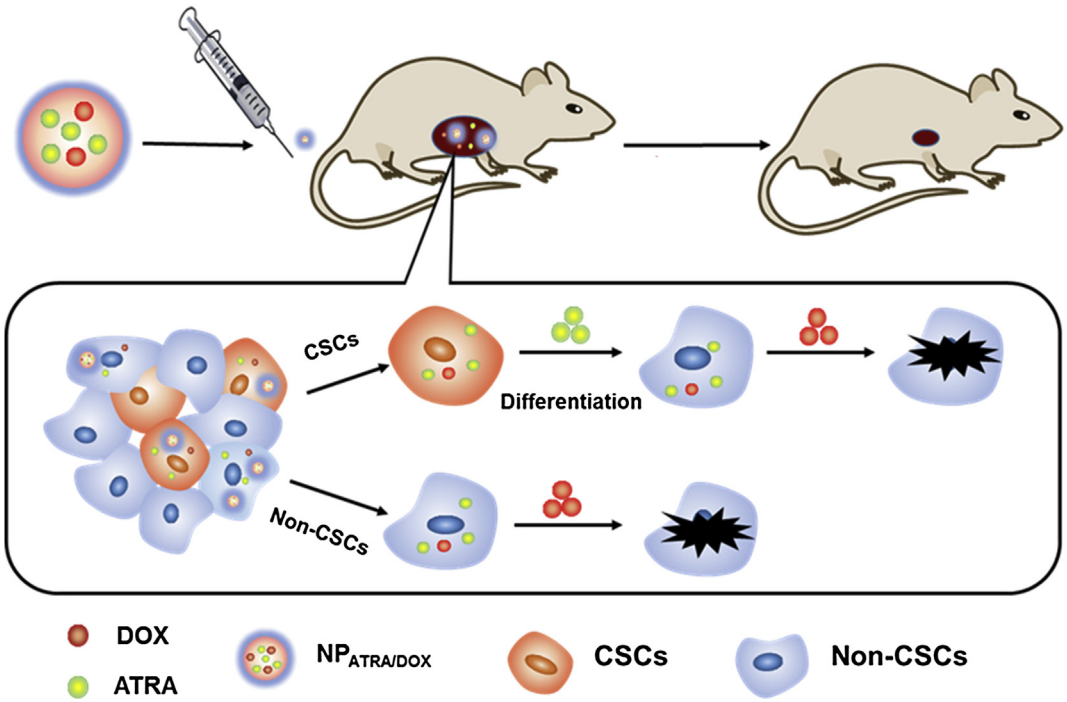
2. Materials and methods

2.1. Materials

Methoxy polyethylene glycol (MW = 5000) was purchased from Sigma–Aldrich (St. Louis, MO). DL-Lactide was purchased from Sigma–Aldrich (St. Louis, MO). The block copolymer poly(ethylene glycol)-block-polylactide (PEG-*b*-PLA) was synthesized by ring-opening polymerization of D,L-lactide with methoxy polyethylene glycol as the initiator according to a previously reported method [44]. The average number of repeated polymerization units of D,L-lactide was 76, equal to a molecular weight of 11,000 for the PLA block. ATRA was purchased from Sigma–Aldrich (St. Louis, MO). Doxorubicin hydrochloride was a product of Hisun Pharmaceutical Co (Hangzhou, China). The hydrophobic DOX was made according to the method previously reported [5]. The ALDEFLUOR™ KIT was purchased from STEMCELL Technologies (Vancouver, Canada). Ultra-purified water was prepared using a Milli-Q Synthesis System (Millipore, Bedford, MA). All other solvents and reagents were used as received.

2.2. Preparation of ATRA- and DOX-loaded nanoparticles

DOX- and ATRA-loaded nanoparticles were prepared by a single-emulsion technique. As a typical example, a solution of ATRA (8 mg), DOX (2 mg) and PEG-*b*-PLA (100 mg) in a mixed solvent of 2 mL of chloroform and dimethyl sulfoxide (DMSO) (3:1, v/v) was emulsified in 8 mL of ultra-purified water by probe ultrasonication at 450 W (Sonics & Materials, Newtown, CT) for 2 min over an ice bath to form an oil-in-water emulsion. The organic solvent chloroform was evaporated by using a rotary vacuum evaporator and the resulting product was further dialyzed against water for 12 h to remove DMSO with membrane dialysis tubing (molecular weight cutoff of 3.5 kDa, Spectrum Laboratories, Rancho Dominguez, CA). The free



Scheme 1. Schematic illustration of tumor suppression by eliminating both CSCs and non-CSCs by co-delivery of the ATRA and DOX nanoparticle system.

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