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Cancer stem cell therapy using doxorubicin conjugated to gold nanoparticles via hydrazone bonds

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ABSTRACT

Nanoparticle-mediated delivery of chemotherapies has demonstrated enhanced anti-cancer efficacy, mainly through the mechanisms of both passive and active targeting. Herein, we report other than these well-elucidated mechanisms, rationally designed nanoparticles can efficiently deliver drugs to cancer stem cells (CSCs), which in turn contributes significantly to the improved anti-cancer efficacy. We demonstrate that doxorubicin-tethered gold nanoparticles via a poly(ethylene glycol) spacer and an acid-labile hydrazone bond mediate potent doxorubicin delivery to breast CSCs, which reduces their mammosphere formation capacity and their cancer initiation activity, eliciting marked enhancement in tumor growth inhibition in murine models. The drug delivery mediated by the nanoparticles also markedly attenuates tumor growth during off-therapy stage by reducing breast CSCs in tumors, while the therapy with doxorubicin alone conversely evokes an enrichment of breast CSCs. Our findings suggest that with well-designed drug delivery system, the conventional chemotherapeutic agents are promising for cancer stem cell therapy.

1. Introduction

Nanoparticle-mediated delivery of chemotherapeutic agents has demonstrated enhanced anti-cancer efficacy, while simultaneously reducing side effects in cancer prevention and treatment [1–4]. In the past two decades a number of therapeutics based on nanoparticles are under clinical trials or have been approved for clinical use [5–7]. The key rationale for the success of nanoparticle-mediated delivery lies in its ability to overcome the biological and physiological barriers, known to remove therapeutics from the body or prevent them from reaching tumor tissues and cells [8–10]. These barriers include the degradation of therapeutics in blood, rapid clearance by the immune system, glomerular filtration and excretion, and cellular barriers [9,10]. In particular, nanoparticles with the proper structures, sizes, and surface properties would readily accumulate and retain in solid tumors because of the enhanced permeability and retention (EPR) effect, which is also known as a passive targeting mechanism [11,12]. In addition, active targeting by endowing nanoparticles with ligand can promote enhanced cell binding, further increasing the drug delivery efficacy [13–16].

The discovery of cancer stem cells (CSCs) has changed the view of chemotherapy. CSCs in tumor evade the anti-cancer effects of standard chemotherapy, emerging as an underestimated biological barrier to the success of systemic chemotherapy [17–19]. CSCs are thought to be responsible for the origin, growth, recurrence, and metastasis of cancer [17,18,20–24]. Increasing evidences support the idea that, instead of merely targeting the bulk non-CSCs, successful cancer curing may require both undifferentiated CSCs and differentiated non-CSCs to be efficiently eliminated [18,24–26]. To this end, several promising strategies have been developed for CSC-targeted therapy. These include direct inhibiting CSCs by blocking their essential self-renewal signaling, forcing CSCs to differentiate into bulk tumor cells that are susceptible to standard therapies, as well as screening and identification of drug candidates that can specifically kill CSCs [24–28]. However, CSCs are probably genetically unstable, and their surface marker phenotypes vary from patient to patient and constantly change as the disease progresses, potentially affecting the effect of such therapies [29]. Moreover, there are evidences that non-CSCs in tumors seem to spontaneously and stochastically turn into CSCs de novo [30,31].

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Nevertheless, the standard chemotherapy towards CSCs using conventional chemotherapeutic agents is unfortunately ineffective as it leads to the enrichment of CSCs and further results in tumor relapse [29,32–35].

Therefore, we explored to establish a method that can affect both CSCs cells and their differentiated daughter cells. We have previously demonstrated that DOX-Hyd@AuNPs (Fig. 1a), a rationally designed gold nanoparticle-based drug delivery system tethering with doxorubicin on the surface with a poly(ethylene glycol) spacer via an acid-labile linkage, facilitates intracellular drug delivery and overcomes multidrug resistant in MCF-7/ADR cancer cells [36]. In this study, we address this challenge using DOX-Hyd@AuNPs that can overcome the inherent therapeutic resistance of CSCs and indiscriminately deliver chemotherapeutics to both cell populations (i.e., CSCs and non-CSCs cells). We proposed that with efficient deliver of drug to CSCs, DOX-Hyd@AuNPs can elicit marked enhancement in tumor growth inhibition and efficiently attenuated tumor growth by inhibiting the tumor initiating ability of breast CSCs (Fig. 1b).

2. Materials and methods

2.1. Materials

DOX-Hyd@AuNPs with a diameter of 30 nm and DOX conjugation were synthesized according to the previous report [36]. mPEG@AuNPs with a diameter of 30 nm but without DOX conjugation was synthesized similarly.

2.2. Animals

Female NOD/SCID mice were obtained from Beijing HFK Bioscience Co., Ltd. and used at 4–6 weeks of age. All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The procedures were approved by the University of Science and Technology of China Animal Care and Use Committee.

2.3. Mammosphere culture

Cells (1000 cells/ml) were cultured in suspension in serum-free DMEM-F12, supplemented with B27 (1:50, Invitrogen), 20 ng/ml hEGF (BD Biosciences), 0.4% low-endotoxin bovine serum albumin (BSA, Sangon Biotech), and 5 μg/ml insulin (Sigma–Aldrich). To propagate mammospheres *in vitro*, mammospheres were collected by gentle centrifugation, dissociated into single cells as previously described [37], and then cultured to generate mammospheres of the next generation.

2.4. ABCB1 efflux assay

The mammosphere cells were seeded into ultra low attachment 6-well plate and treated at 37 °C with equivalent DOX, at a final concentration of 1 μg/ml for 2 h. After incubation, the mammosphere cells were collected and washed twice with PBS. One third of the treated cells were kept on ice to provide a control for maximal loading. Another one third of the cells was warmed at 37 °C, and was used as the positive control for ABCB1-mediated DOX efflux. The final third of the cells were warmed to 37 °C in the presence of vinblastine (Millipore), an inhibitor of ABCB1, at a concentration of 22 μM. All of the cells were incubated for 8 h. The MFI of the mammosphere cells were measured and analyzed by flow cytometry.

2.5. Distribution of DOX-Hyd@AuNPs in CSCs

MDA-MB-231, BT-474 and MCF-7 mammosphere cells were treated at 37 °C with equivalent DOX, at a final concentration of 1 μg/ml for 2 h. An anti-ABCB1 antibody (biotin-labeled UIC2, 1 μg/ml, Millipore) was added and the cells were cultured for another 15 min. After removal of the media, cells were washed twice with cold PBS, and treated with fixative solution, containing 1% formaldehyde and 0.25% Triton X-100 in TBS, for 5 min. The cells were then incubated with FITC labeled avidin (Santa Cruz) for 30 min following DAPI staining for 5 min at a concentration of 0.1 μg/ml. The cells were directly observed under a Zeiss LSM 710 confocal microscope using a 63× objective.

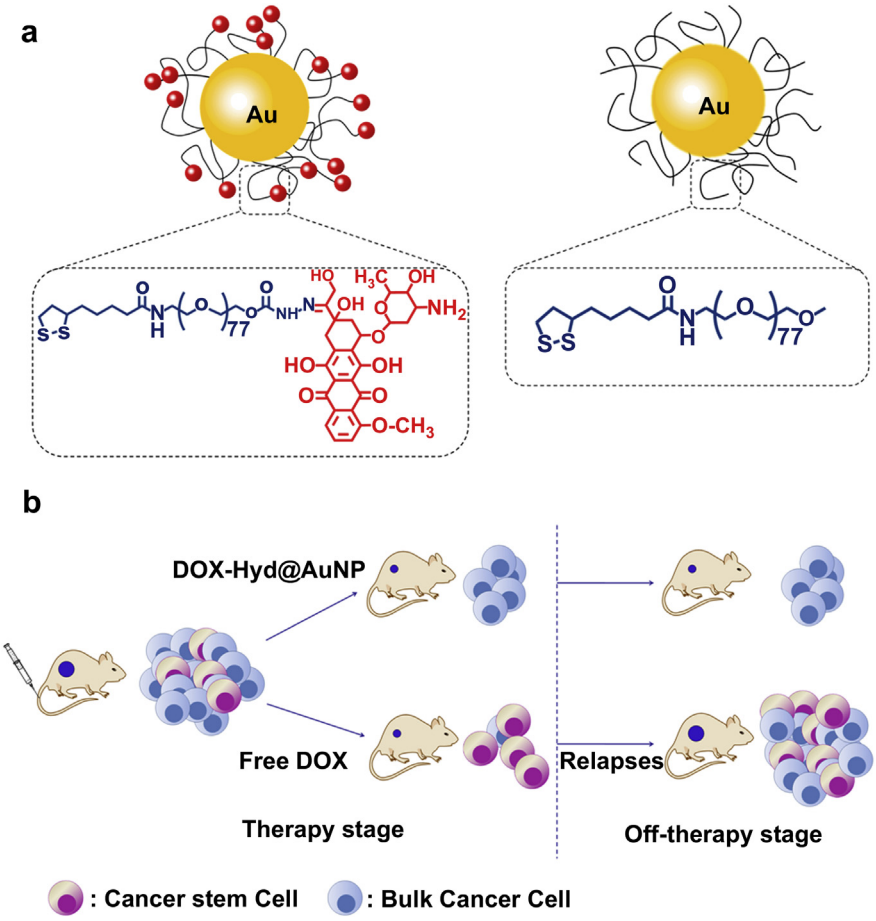

Fig. 1. Schematic illustration of (a) endosomal pH-responsive DOX-Hyd@AuNPs (left) and a gold nanoparticle conjugated with poly(ethylene glycol) monomethyl ether (mPEG@AuNPs) (right); (b) relapses of solid tumors after initial remission by free DOX and DOX-Hyd@AuNP, respectively.