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Robust, active tumor-targeting and fast bioresponsive anticancer nanotherapeutics based on natural endogenous materials

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ABSTRACT

The clinical success of cancer nanomedicines critically depends on availability of simple, safe and highly efficient nanocarriers. Here, we report that robust and multifunctional nanoparticles self-assembled from hyaluronic acid-g-poly(γ-benzyl-L-glutamate)-lipoic acid conjugates achieve a remarkably high loading (up to 25.8 wt.%) and active targeted delivery of doxorubicin (DOX) to human breast tumor xenograft *in vivo*. DOX-loaded nanoparticles following auto-crosslinking (DOX-CLNPs) are highly stable with little drug leakage under physiological conditions while quickly release ca. 92% DOX in 30 h under a cytoplasmic-mimicking reductive environment. The *in vitro* assays reveal that DOX-CLNPs possess a superior selectivity and antitumor activity to clinically used pegylated liposomal doxorubicin hydrochloride (DOX-LPs) in CD44 receptor overexpressing MCF-7 human breast cancer cells. Strikingly, DOX-CLNPs exhibit a superb tolerated dose of over 100 mg DOX equiv./kg, which is more than 5 times higher than DOX-LPs, and an extraordinary breast tumor accumulation of 8.6%ID/g in mice. The *in vivo* therapeutic studies in MCF-7 human breast tumor-bearing nude mice show that DOX-CLNPs effectively inhibit tumor growth, improve survival rate, and significantly decrease adverse effects as compared to DOX-LPs. DOX-CLNPs based on natural endogenous materials with high drug loading, great stability and CD44-targetability are highly promising for precision cancer chemotherapy.

Statement of Significance

We demonstrate that with rational design, simple and multifunctional anticancer nanotherapeutics can be developed to achieve highly efficient and targeted cancer chemotherapy. Doxorubicin-loaded multifunctional nanoparticles based on hyaluronic acid-g-poly(γ-benzyl-L-glutamate)-lipoic acid conjugates exhibit a high drug loading, superior stability, fast bioresponsivity, high tolerability, and obvious selectivity toward CD44-overexpressing tumors *in vivo*. These nanotherapeutics achieve effective tumor suppression, drastically improved survival rate and reduced side effects as compared to clinically used pegylated liposomal doxorubicin in MCF-7 human breast tumor-bearing nude mice. Unlike previously reported multifunctional nanomedicines, the present nanotherapeutics primarily based on natural endogenous materials are simple and straightforward to fabricate, which makes them potentially interesting for clinical translation.

1. Introduction

Nanomedicines present a most promising treatment for various cancers that are incurable nowadays [1,2]. It should be noted, however, that though significant scientific progress has been made in the past decade, few nanomedicines have been approved by the authorities for use in the clinics [3]. The clinical results reveal that current nanomedicines can indeed improve the pharmacokinetics and biodistribution of chemotherapeutic agents leading to a better tolerability and broader therapeutic window [3–6]. The therapeutic efficacy has, however, not much improved likely due to several existing challenges including drug leakage in circulation, poor tumor accumulation and cellular uptake, and inefficient drug release at the target site [7–9]. In order to achieve efficacious cancer treatments, a number of multifunctional nanocarriers have recently been developed and explored [10,11]. These studies

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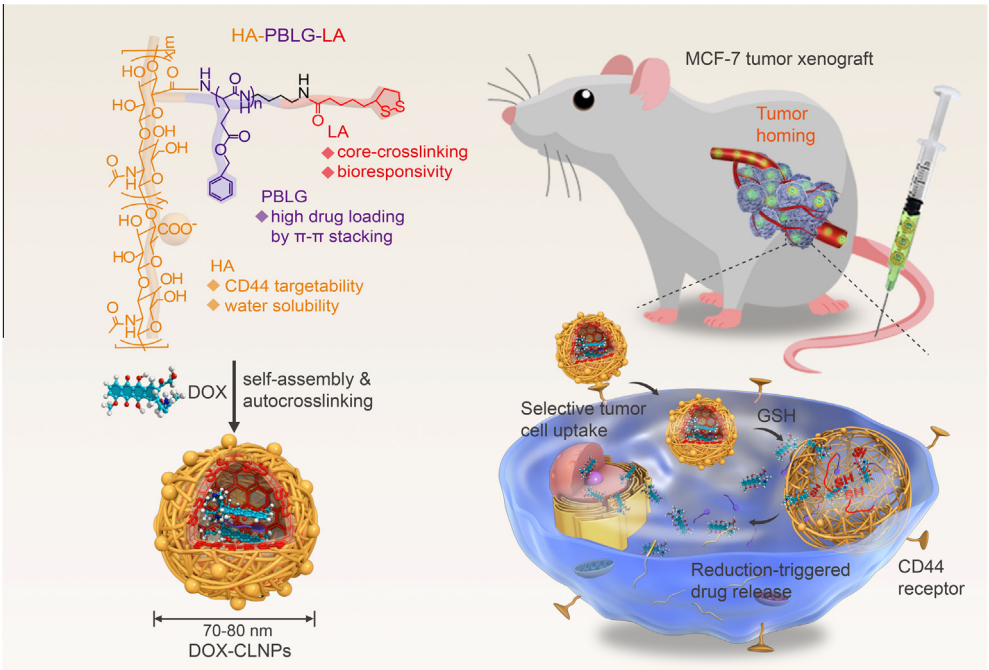
provide a proof-of-concept that multifunctional nanomedicines through solving these systemic delivery barriers instigate better therapeutic outcomes in different tumor models [12–14]. Nevertheless, multifunctional nanocarriers that are typically made from novel and complex synthetic materials encounter involved preparation and potential safety concerns [10,15]. In addition, many reported multifunctional nanomedicines still suffer from a low drug loading level, poor stability, and insufficient tumor accumulation [9].

In this paper, we report robust, bioresponsive and highly specific anticancer nanotherapeutics based on fully natural endogenous materials i.e. hyaluronic acid (HA), L-glutamate and lipoic acid (LA) for high loading and targeted delivery of doxorubicin (DOX) to human breast tumor xenograft *in vivo* (Scheme 1). These multifunctional nanotherapeutics are readily assembled from hyaluronic acid-g-poly(γ-benzyl-L-glutamate)-lipoic acid (HA-PBLG-LA) graft copolymer. We recently reported that DOX-loaded reversibly crosslinked nanoparticles based on HA-LA conjugates (i.e. without PBLG block) exhibit potent antitumor effect toward CD44-positive drug-resistant human breast cancers *in vitro* and *in vivo* [16]. However, HA-LA nanoparticles revealed a moderate drug loading content (lower than 15.1 wt.%) and a relatively large particle size (154–225 nm). HA is a biocompatible and biodegradable natural polysaccharide, and has been approved by the FDA as intra-articular injection [17,18]. Notably, taking advantages of its effective targeting ability to CD44 receptor overexpressing cancer cells [16,19,20], HA-drug conjugates with paclitaxel, irinotecan, doxorubicin, and 5-fluorouracil have been developed and advanced to Phase I–III clinical trials for the treatment of various cancers such as bladder, colorectal, breast, lung, prostate, sarcoma [21]. PBLG as other polypeptides possesses excellent biocompatibility and biodegradability [22,23]. Notably, several nanomedicines based on polypeptides (NK105, NK012, NK911, NC-6004, NC-4016, NC-6300, CT-2103, CT-2106, etc.) have entered different phases of clinical trials [4,24–26]. Lipoic acid (LA) is a natural antioxidant produced by human body and has been employed to treat diseases like diabetes and Alzheimer's disease [27,28]. Remarkably, our results showed that the present multifunctional nanoparticulate doxorubicin possesses an ultrahigh drug loading level (up to 25.8 wt.%), small particle size (72–80 nm), superior stability, triggered cytoplasmic drug release, high tolerability and therapeutic index, and excellent tumor accumulation and selectivity toward CD44-overexpressing tumors *in vivo*, resulting in effective tumor suppression, drastically improved survival rate, and markedly decreased side effects as compared to clinically used pegylated liposomal doxorubicin hydrochloride (DOX-LPs) in MCF-7 human breast tumor-bearing nude mice.

2. Materials and methods

2.1. Materials

Unless stated otherwise, reactions were conducted in oven-dried glassware under an atmosphere of nitrogen using anhydrous solvents. Sodium hyaluronic acid (HA, molecular weight: 35 kDa, Shandong Freda Biopharm Co., Ltd.), lipoic acid (LA, 98%, J&K), N^{ε}-benzyloxycarbonyl-L-glutamic acid (H-Glu(OBzl)-OH, GL Biochem (Shanghai) Ltd.), 1,4-butanediamine (98%, J&K), N,N′-carbonyldiimidazole (CDI, 98%, J&K), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98%, Alfa Aesar), N-hydroxysuccinimide (NHS, 98%, J&K), 1,4-dithio-D,L-threitol (DTT, 99%, Merck), glutathione (GSH, 99%, Roche), doxorubicin hydrochloride (DOX·HCl, >99%, Beijing Zhongshuo Pharmaceutical Technology Development Co., Ltd.), and doxorubicin hydrochloride pegylated liposome injection (DOX-LPs, 20 mg/mL, Shanghai Fudanzhangjiang Biomedical Co., Ltd) were used as received. N,N-dimethyl formamide (DMF) was dried by refluxing over anhydrous magnesium sulfate and distilled under reduced pressure before use. Dichloromethane (DCM), ethyl acetate, and petroleum ether (b.p. 60–90 °C) were refluxed over CaH_2 and distilled before use. All the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received. BLG-NCA was prepared according to the Fuchs-Farthing method using triphosgene [29].



Scheme 1. Illustration of robust, active tumor-targeting and fast bioresponsive anticancer nanotherapeutics based on hyaluronic acid-g-poly(γ-benzyl-L-glutamate)-lipoic acid (HA-PBLG-LA) conjugates for high efficient and targeted breast tumor chemotherapy *in vivo*.