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Delivery of bortezomib with nanoparticles for basal-like triple-negative breast cancer therapy

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ABSTRACT

Basal-like triple negative breast cancer (TNBC) has received particular clinical interest due to its high frequency, poor baseline prognosis and lack of effective clinical therapy. Bortezomib, which was the first proteasome inhibitor approved for the treatment of multiple myeloma, has been proven to be worth investigating for this subtype of breast cancer. In our study, the amphiphilic copolymer poly(ethylene glycol)-block-poly(*D,L*-lactide) (PEG-*b*-PLA) was utilized as an excellent delivery carrier of bortezomib (BTZ) to overcome its clinical limitations including low water solubility and unstable properties. Bortezomib encapsulated nanoparticles (NP_{BTZ}) can efficiently deliver the drug into both CSCs (cancer stem cells) and non-CSCs, resulting in proliferation inhibition and apoptosis induction. Remarkably, NP_{BTZ} can more effectively affect the stemness of CSCs compared with free BTZ. Administration of this drug delivery system can markedly prolong the bortezomib circulation half-life and augment the enrichment of drugs in tumor tissue, then enhance the suppression of tumor growth, suggesting the therapeutic promise of NP_{BTZ} delivery in basal-like TNBC therapy.

1. Introduction

Breast cancer is a worldwide health issue, as it is the most frequently diagnosed cancer and the leading cause of cancer-related mortality in females [1,2]. It is a heterogeneous disease consisting of distinct entities characterized by clinical morphology, molecular profile and response to therapy [3]. Triple-negative breast cancers (TNBCs), defined by their lack of estrogen receptors, progesterone receptors, and Her2 receptors, are an especially aggressive group (with a frequency of 12–20%) [4,5]. Based upon sophisticated microarray analysis [6], breast cancer can be further divided into five distinct molecular subgroups: 'luminal A', 'luminal B', 'Her2-positive', 'normal breast-like' and 'basal-like'. Consistent studies have shown that up to 85% of TNBC correspond to 'basal-like breast cancer', derived from the breast epithelium cells [7–10]. Basal-like TNBC has received particular clinical interest due to its high frequency, poor baseline prognosis and its tendency to affect younger and premenopausal women.

Clinically relevant biomarkers have been used to guide the application of targeted agents for breast cancer therapy. For example, in regard to estrogen receptor-positive breast cancer, tamoxifen is an effective targeted therapy for hormone receptors [11]. Lack of standard molecular targets makes TNBC an extremely challenging and frustrating condition for both medical oncologists and patients. The core systemic treatment option available for these patients is chemotherapy with standard cytotoxic agents, such as platinum or epirubicin and paclitaxel alone or together [12]. Studies have shown an initially marked chemosensitivity for patients with TNBCs, but the coming drug-resistance and recurrence resulted in unfavorable overall survival [13].

An increasing body of evidence suggests that the specific survival of a rare population of cells displaying stem-like properties may be responsible for the emergence of drug-resistance after an initial response to chemotherapy and could provide an explanation for therapeutic failure [14,15]. This population, interchangeably called cancer stem-like cells (CSCs) or tumor-initiating cells (TICs), retains the capacity to self-renew and regenerate a heterogeneous tumor comprised mostly of non-CSCs, and is currently considered as a major obstacle for cancer therapy [16,17]. Effectively incapacitating CSCs has the potential to significantly improve outcome for women with basal TNBC [18].

It is necessary to effectively eliminate both the CSCs and their more differentiated daughter cells that constitute the bulk of the tumor in order to achieve durable treatment remissions in TNBC patients following therapy [19]. Several strategies including inhibiting the self-renewal pathway, differentiating CSCs or targeting the CSCs niche, have been developed based on the increased understanding of the features of CSCs [20]. It has been demonstrated that targeting Notch and Hedgehog, two key pathways of prostate cancer stem cell, can effectively overcome the chemoresistance in the docetaxel treatment [21]. With the help of chemical compound libraries and whole-genome RNA interference

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screening platforms, researchers have tried to identify inhibitors that specifically target CSCs [22]. Mickie Bhatia and colleagues showed that dopamine receptor antagonist has specific toxicity to CSCs [23]. Recently, Lieberman and colleagues reported work that shed new light on treatment for patient with basal-like TNBC, they found that both CSCs and non-CSCs within this breast cancer subtype were commonly addicted to the proteasome and sensitive to proteasome inhibitors using a genome-wide siRNA lethality screen [24].

Inhibition of the proteasome disrupts protein homeostasis and attenuates multiple signaling pathways that promote tumor formation has become a very attractive anticancer therapy [25]. At least 14 novel proteasome inhibitors are currently in advanced stages of clinical development, and one, bortezomib (BTZ), which reversibly binds to the chymotrypsin-like activity of proteasome β5 subunit, is the first proteasome inhibitor to be clinically approved for treating relapsed myeloma and multiple other hematological malignancies [26,27]. The in vitro experiments showed that BTZ demonstrates cytotoxicity against various other tumor cell types, including lung, colorectal, lymphoma, prostate and breast [28–30]. However, its in vivo anti-tumor efficacy in solid tumors appears limited [31,32]. The low water solubility, unstable property and poor penetration into the tumor are the well-known drawbacks of BTZ that might explain its lack of efficacy in solid tumors [33,34].

Inspirationally, nanoscale carriers have the potential to partly circumvent these challenges that currently limit the successful translation of small-molecule agents into the clinical arena [35,36]. The advantages of using nanoparticles for drug delivery include enhanced water solubility, tumor accumulation and improved antitumor efficacy, while simultaneously reducing nonspecific toxicity [37]. The nanoparticle system-based approaches offer a promising prospect for CSC-based therapy [38,39], and simultaneous delivery of dual functional agent for inhibition of both non-CSCs and CSCs was rarely reported.

In this study, we used poly (ethylene glycol)-*block*-poly(*D,L*-lactide) (PEG-*b*-PLA), an amphiphilic polymer with the capacity to encapsulate hydrophobic agents that has employed in a variety of clinical applications [40,41], to prepare BTZ encapsulated nanoparticles (NP_{BTZ}) through a single emulsion method [42]. We demonstrated that thus nanoparticle system can effectively deliver BTZ to both CSCs and non-CSCs in vitro (Scheme 1). More importantly, NP_{BTZ} increase drug availability by improving pharmacokinetics and biodistribution in tumor tissue and remarkably enhanced the suppression of tumor growth in the MDA-MB-468 orthotopic tumor murine model, indicating that NP_{BTZ} is promising for the treatment of basal-like TNBC.

2. Materials and method

2.1. Materials

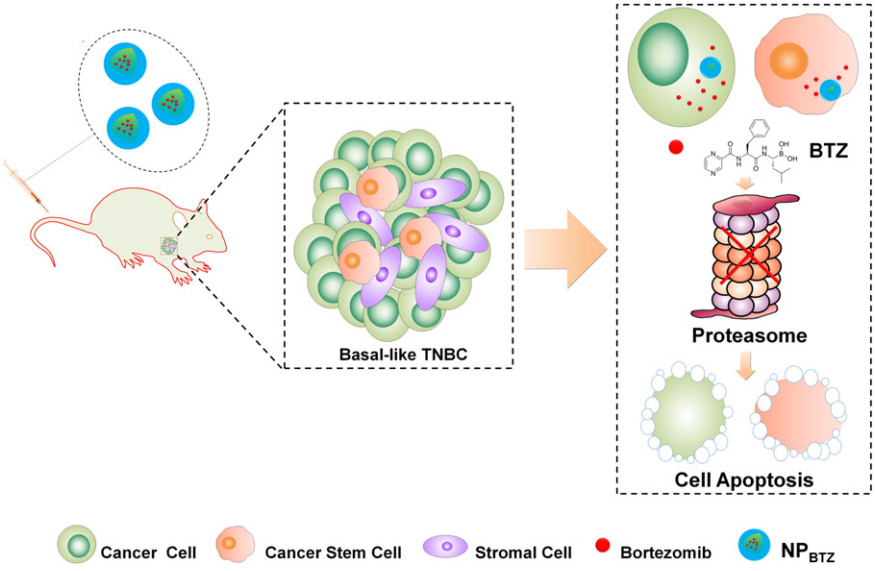
The diblock copolymer of poly (ethylene glycol) (molecular weight [MW] = 5000) with poly(*D,L*-lactide) (MW = 11,000) (PEG_{5K}-PLA_{11K}) was synthesized as previously reported [43]. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and bortezomib (BTZ) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island) and L-glutamine were purchased from Gibco BRL (Eggenstein, Germany). ALDEFLUOR™ KIT was purchased from STEMCELL Technologies (Vancouver, Canada).

2.2. Cell culture

The human basal-like triple-negative breast cancer cell lines MDA-MB-468 and HCC1937 from American Type Culture Collection were cultured in DMEM supplied with 10% fetal bovine serum (FBS, ExCell Bio, Shanghai, China) and antibiotics (Gibco, Grand Island) at 37 °C with 5% CO_2. For mammosphere culture, cancer cells (1000 cells/mL) were cultured in suspension with serum-free DMEM/F12 (Invitrogen, Carlsbad, CA), supplemented with B27 (Invitrogen, Carlsbad, CA), 20 ng/mL hEGF (BD Biosciences, Franklin Lakes, NJ), 0.4% low-endotoxin bovine serum albumin (Sangon Biotech, Shanghai, China) and 4 mg/mL insulin (Sigma-Aldrich, St. Louis, MO). To propagate in vitro, the mammospheres were collected by gentle centrifugation (800 g, 5 min), dissociated into single cells and cultured to generate mammospheres.

2.3. Animals

Female NOD/SCID mice and ICR mice were obtained from Vital River Laboratories (Beijing, China) and used at 4–6 weeks of age (initially weighing 20–22 g). All animals received care in compliance with the principles 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.



Scheme 1. A schematic view of basal-like triple negative breast cancer (TNBC) therapy via bortezomib-loaded nanoparticles. A polymeric nanoparticle system that is capable of encapsulating bortezomib and forming NPBTZ is used to specifically inhibit the function of proteasome and treat the basal-like TNBC.