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The potential use of lapatinib-loaded human serum albumin nanoparticles in the treatment of triple-negative breast cancer

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ABSTRACT

Triple-negative breast cancer (TNBC) is an aggressive cancer with limited treatment options. However, the shared feature of epidermal growth factor receptor (EGFR) expression in TNBC offers the opportunity for targeted molecular therapy for this breast cancer subtype. Previous studies have indicated that lapatinib, a selective small-molecular dual-tyrosine kinase inhibitor of HER2 and EGFR, is effective in reducing cancer progression and metastasis, indicating that it might be a candidate for TNBC treatment. However, its poor water solubility, low and variable oral absorption, and large daily dose all limit the clinical use of lapatinib. In this study, we developed human serum albumin (HSA) nanoparticles loaded with lapatinib for intravenous administration to overcome these disadvantages and enhance its efficacy against TNBC. 4T1 cells (a murine TNBC cells) were selected as the cell model because their growth and metastatic spread are very close to those of human breast cancer cells. Lapatinib-loaded HSA nanoparticles (LHNPs) were prepared by Nab technology. LHNPs displayed cytotoxicity similar to the free drug but exhibited superior capacity to induce early apoptosis in 4T1 monolayer cells. Importantly, LHNPs showed improved penetration and inhibition effects in tumor spheroids compared to lapatinib solution (LS). Pharmacokinetic investigations revealed that HSA nanoparticles (i.v.) effectively increased the accumulation of lapatinib in tumor tissue at 2.38 and 16.6 times the level of LS (i.v.) and Tykerb (p.o.), respectively. Consequently, it had markedly better suppression effects both on primary breast cancer and lung metastasis in tumor-bearing mice compared to the commercial drug Tykerb. The improved anti-tumor efficacy of LHNPs may be partly attributed to its close binding to SPARC, which is widely present in the extracellular matrix of tumor tissue. These results demonstrated that LHNPs might be a promising anti-tumor agent for TNBC.

1. Introduction

Breast cancer is the second most common cancer, leading to high mortality worldwide. The International Agency for Research on Cancer (IARC) estimated that over 1.7 million new breast cancer cases were diagnosed, and the number of deaths rose to 5.22 million in 2012 (Stewart and Wild, 2014). Among all breast cancers, triple-negative breast cancer (TNBC) has an unfavorable prognosis and high mortality (Haffty et al., 2006). Accounting for approximately 15% of all breast cancers, TNBC is characterized by the absence of the estrogen receptor (ER), the progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) (Bauer et al., 2007). The lack of receptors results in the lack of effective therapeutic targets, for example, patients with TNBC derive no benefit from HER2-targeted monoclonal antibodies such as Trastuzumab or Pertuzumab. Until now, no standard-of-care therapy has been recommended for patients with TNBC, and therefore there is an urgent need for new therapeutic agents.

In the last few years, many studies have proven that the epidermal growth factor receptor (EGFR) is frequently overexpressed in TNBC (Nakajima et al., 2014; Gumuskaya et al., 2010; Reis-Filho et al., 2006). EGFR expression ranges from 45% to 76% of cases with TNBC (Nielsen et al., 2004; Collins et al., 2009; Tan et al., 2008; Martin et al., 2012; Reis-Filho et al., 2006; Gumuskaya et al.,

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2010) in different reports and is positively associated with the poor clinical outcome (Park et al., 2014). Accordingly, EGFR may potentially be used in diagnosis and molecular targeting therapy for this breast cancer subtype. Clinical benefits of the novel EGFR inhibitor Cetuximab (Imclone Systems Inc., New York, NY and BristolMyers Squibb, Princeton, NJ) have been reported in TNBC patients (Cunningham et al., 2004). It is tempting to speculate that patients with TNBC may derive optimal benefit from other EGFR-targeted therapies.

Lapatinib, a small-molecule tyrosine kinase inhibitor that acts on both HER2 and EGFR, was approved in combination with capecitabine for use in HER2-positive patients with advanced metastatic breast cancer (Tevaarwerk and Kolesar, 2009). The selective EGFR-targeting and clinical benefit of lapatinib in cancer progression and metastasis indicate that lapatinib might be a candidate therapeutic drug for TNBC. Unfortunately, few studies have been conducted to demonstrate its clinical efficacy against TNBC, primarily due to the poor solubility of lapatinib in water (only 7 μg/mL), which restricts its clinical use (Budha et al., 2012). Lapatinib was approved as a tablet (Tykerb, unique commercial tablets, GlaxoSmithKline) that must be taken at a large daily dose (1250 mg/day) due to its low oral bioavailability, which results in many side effects such as diarrhea, nausea and rash (Burris et al., 2009). Accordingly, it is necessary to develop an injectable delivery system for systemic delivery that effectively elevates bioavailability of lapatinib and precisely targets the tumor.

Currently, nanoparticle-based drug delivery has been shown to improve the solubility of drugs and to generate enhanced permeability and retention (EPR) to deliver drugs to tumors. Among the existing polymer biomaterials used for the preparation of nanoparticles, human serum albumin (HSA) has obvious advantages such as high stability during storage and *in vivo* and lack of toxicity and antigenicity. Moreover, albumin is the most abundant plasma protein and can be preferentially taken up by tumors as nutrition ingredient (Kratz, 2008). In addition, 60-kDa glycoprotein (gp60) receptor on vascular endothelia cells can specifically bind to HSA nanoparticles and transport the nanoparticles across the endothelial barrier to tumor. Furthermore, SPARC (secreted protein acidic and rich in cysteine), which is widely present in the extracellular matrix (ECM) of tumor tissue, can attract nanoparticles to the inner tumor areas (Elzoghby et al., 2012; Matsumura and Maeda, 1986). Abraxane is a successful commercial product using HSA nanoparticles with paclitaxel and showed increased therapeutic efficacy over Taxol (Paclitaxel injection) (Desai et al., 2006). Therefore, HSA nanoparticles could be a promising carrier for anti-tumor drugs.

Considering its high binding efficacy (>99%) to albumin (Medina and Goodin, 2008), lapatinib was designed to be incorporated in HSA-based nanoparticles (LHNPs) by Nab technology for intravenous administration. The morphology, structure, particle size, encapsulation efficiency and *in vitro* release of LHNPs were characterized, and its anti-tumor effects against TNBC *in vitro* and *in vivo* were also investigated in this study. To our knowledge, it has been the first time to apply HSA nanoparticles for encapsulation of lapatinib and use for TNBC treatment. 4T1 cell line, which was characterized as ER-/PR-/HER-2 negative and EGFR-expressing, closely mimics human TNBC not only in terms of tumor growth but also in metastasis to many distant organs including the lungs, liver, bone and brain (Aslakson and Miller, 1992; Pulaski and Ostrand-Rosenberg, 1998; Mi et al., 2004). 4T1 is also poorly immunogenic and easily implanted into mice, so 4T1 cells were selected as the model system in the present study.

2. Materials and methods

2.1. Materials and animals

Lapatinib ditosylate and Gefitinib were purchased from Melonepharma (Dalian, China). Tykerb was purchased from GlaxoSmithKline. Egg phosphatidylcholine (EPC) was purchased from Shanghai Advanced Vehicle Technology Pharmaceutical Ltd. (Shanghai, China). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetra-zoplium bromide (MTT), coumarin-6, cell cycle detection kit, and cy3-labeled donkey anti-rabbit IgG were ordered from Sigma (USA). Hoechst 33342 dye was bought from Beyotime (Haimen, China). Anti-SPARC was purchased from Santa Cruz (USA). The cell apoptosis kit and TUNEL detection kit were obtained from Roche (Stockholm, Sweden). The 4T1 cell line was obtained from the Chinese Academy of Sciences Cell Bank. RMPI medium, fetal bovine serum (FBS) and trypsin-EDTA solutions were obtained from Gibco (CA). All of the other chemicals were analytical or reagent grade.

Female nude Balb/c mice (18–20 g), ICR mice (20–25 g) and SD rats (180–200 g) were obtained from Shanghai Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China) and maintained at a constant temperature (25 ± 1 °C). The animal studies were carried out according to protocols approved by the ethical committee of Fudan University.

2.2. Preparation of nanoparticles

Lapatinib-loaded HSA nanoparticles (LHNPs) were prepared using Nab technology. Briefly, lapatinib and egg phosphatidylcholine (EPC) at a weight ratio of 1:4 were dissolved in a chloroform and ethanol mixture. The obtained lapatinib solution was added dropwise to 30 mL of HSA aqueous solution (pH 4–6.5), and the mixture was subjected to high shear forces for 5 min to form a coarse emulsion. Final emulsification was carried out by passing the coarse emulsion through a Micro fluidizer (Nano DeBEE, USA) 6–14 times at 100–170 MPa. After evaporation by rotary vacuum to remove the organic solvent, the nanoparticles suspension were further filtered through filter membranes (Millipore, 220 nm pore diameter), and the obtained nanoparticles were frozen at −80 °C, lyophilized (Virtis Model Benchtop K, USA) and stored at −20 °C in airtight vials.

Coumarin-6-loaded nanoparticles were prepared using the same method except that coumarin-6 was added to the chloroform and ethanol solvent before emulsification, and the nanoparticles were run over a sepharose CL-4B column to remove the free coumarin-6.

2.3. Characterization of LHNPs

The particle sizes, polydispersity index (PDI) and zeta potential of the nanoparticles were determined by Malvern Zetasizer (Malvern, nanoZS, UK). Morphological examination was performed using transmission electron microscopy (TEOL2010, JEM) after negative staining with 1% uranyl acetate or 2% phosphotungstic acid solution.

The drug-loading capacity (LC) and encapsulation efficiency (EE) of LHNPs were measured using HPLC. In brief, 100 μL LHNPs were dissolved in 400 μL acetonitrile and sonicated for 5 min, then centrifuged at 12,400 × *g* for 30 min. The lapatinib in the supernatant was analyzed by HPLC. The mobile phase of the lapatinib assay contained acetonitrile and 0.01 M KH_2PO_4 solution (pH 6.2) (65:35, v/v) and the lapatinib was monitored at 253 nm. The LC and EE were calculated as follows: