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Co-delivery of paclitaxel and anti-survivin siRNA via redox-sensitive oligopeptide liposomes for the synergistic treatment of breast cancer and metastasis

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ABSTRACT

The overexpression of survivin in breast cancer cells is an important factor of paclitaxel (PTX) resistance in breast cancer. To overcome PTX resistance and improve the antitumor effect of PTX, we developed a novel liposome-based nanosystem (PTX/siRNA/SS-L), composed of a redox-sensitive cationic oligopeptide lipid ($LHSSG2C_{14}$) with a proton sponge effect, natural soybean phosphatidylcholine (SPC), and cholesterol for co-delivery of PTX and anti-survivin siRNA, which could specifically downregulate survivin overexpression. PTX/siRNA/SS-L exhibited high encapsulation efficiency and rapid redox-responsive release of both PTX and siRNA. Moreover, *in vitro* studies on the 4T1 breast cancer cells revealed that PTX/siRNA/SS-L offered significant advantages over other experimental groups, such as higher cellular uptake, successful endolysosomal escape, reduced survivin expression, the lowest cell viability and wound healing rate, as well as the highest apoptosis rate. In particular, *in vivo* evaluation of 4T1 tumor-bearing mice showed that PTX/siRNA/SS-L had lower toxicity and induced a synergistic inhibitory effect on tumor growth and pulmonary metastasis. Collectively, the collaboration of anti-survivin siRNA and PTX via redox-sensitive oligopeptide liposomes provides a promising strategy for the treatment of breast cancer and metastasis.

1. Introduction

Breast cancer remains a major threat to women's health around the world. In 2014, more than 200,000 women in the United States developed breast cancer and about 40,000 women died from this disease (Siegel et al., 2014). Although chemotherapy is commonly used in breast cancer treatment, it often causes drug resistance, which in turn restricts the efficacy of chemotherapy (Holohan et al., 2013). Gene therapy combined with chemotherapy has recently been considered as an alternative strategy to circumvent drug resistance that involves restoration of the sensitivity of cancer cells to chemotherapeutic drugs, thereby improving therapeutic efficacy (Lee et al., 2015; Li et al., 2016; Qu et al., 2014).

Paclitaxel (PTX), a microtubule stabilizing agent or mitotic inhibitor, is the first-line anticancer drug for the treatment of breast cancer (Jordan and Wilson, 2004). However, drug resistance in clinical practice is the maintain drawback for PTX application (Murray et al., 2012), which is associated with the upregulation of drug resistance proteins and mutations in the β-tubulin gene (Verma and Ramanathan, 2015). Recent studies have shown that PTX resistance is also related to the upregulation of anti-apoptotic proteins such as survivin, which is overexpressed in various breast cancer cells (Khan et al., 2014; Promkan et al., 2011; Wang et al., 2015). Notably, the expression level of survivin can be induced after PTX therapy (Ho et al., 2009; Hu et al., 2012), thereby resulting in

Abbreviations: ATCC, American Type Culture Collection; CLSM, confocal laser scanning microscopy; TEM, transmission electron microscopy; EE, entrapment efficiency; EPR, enhanced permeability and retention; GSH, glutathione; nS-L, non-reduction-sensitive blank liposome; PTX, Paclitaxel; SPC, soybean phosphatidylcholine; SS-L, reduction-sensitive blank liposome.

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drug resistance (Singh et al., 2015), tumor recurrence (Jha et al., 2012), poor prognosis, low survival rate, and tumor metastasis (Chu et al., 2012; Parvani et al., 2015). Consequently, survivin gene knockout or downregulating survivin expression has become an attractive target for novel cancer treatment regimens (Coumar et al., 2013). Anti-survivin siRNA has emerged as a promising therapeutic agent due to its safety, tolerability, and the specificity of survivin gene silencing (Montazeri Aliabadi et al., 2011; Salzano et al., 2014, 2015). Therefore, the combination of PTX and anti-survivin siRNA might restore the sensitivity of breast tumor cells to PTX, thereby obtaining the synergistic anti-tumor effect and ultimately promoting patient compliance.

To accomplish the synergistic effect of PTX and anti-survivin siRNA on the treatment of breast cancer and metastasis, we constructed the redox-sensitive oligopeptide liposomes for co-delivering PTX and anti-survivin siRNA (PTX/siRNA/SS-L) based on a redox-sensitive cationic lipid (LHSSG2C$_{14}$), natural soybean phosphatidylcholine (SPC), and cholesterol, which would be more significant than sequentially delivering these in two separate carriers (Sun et al., 2011). Due to the different physiochemical properties of PTX and siRNA (Gandhi et al., 2014), PTX is encapsulated in the bilayer of the liposomes via hydrophobic effect, whereas siRNA is bound to the positively charged liposomes through electrostatic interaction (Fig. 1). The prepared redox-sensitive liposomes were expected to successfully escape endo-lysosomes via the proton sponge effect of histidine in LHSSG2C$_{14}$ after simultaneously delivering two drugs into the cells, and rapidly release two drugs through the breakdown of the disulfide bond in LHSSG2C$_{14}$, which is triggered by the reducing environment of the cytoplasm. The released anti-survivin siRNA can specifically downregulate survivin expression and increase PTX sensitization in the tumor cells, thereby resulting in the synergistic inhibition of growth and metastasis of breast cancer.

To prove the effectiveness of our design, the co-delivery liposomes were optimized by measuring the particle size, zeta potential, morphology, and encapsulated efficiency. Furthermore, the intracellular transport and the reductive-responsiveness of the co-delivery liposomes were evaluated. *In vitro* and *in vivo* antitumor efficacy studies were performed to assess the synergistic effect of co-delivery of survivin-targeted siRNA and PTX on the growth and metastasis of 4T1 murine breast cancer.

2. Materials and methods

2.1. Materials

Natural soybean phosphatidylcholine (SPC) was supported by Tai-Wei Pharmaceutical Co., Ltd. (Shanghai, China). Ditetradecyl 2-(4-(2-(2-(2-(2-(2,6-diaminohexanamido)-3-(1H-imidazol-4-yl) propanamido) ethyl) disulfanyl) ethylamino)-4-oxobutanamido) pentanedioate (LHSSG2C$_{14}$, Fig. A.1, Supplementary material) and ditetradecyl 2-(2-(2,6-diaminohexanamido)-3-(1H-imidazol-4-yl) propan-amido) pentanedioate (LHG2C$_{14}$, Fig. A.2, Supplementary material) as a control were synthesized by our group as previously described (Sun et al., 2015). Cholesterol and Nile red were obtained from Sigma-Aldrich (China). Paclitaxel was procured from

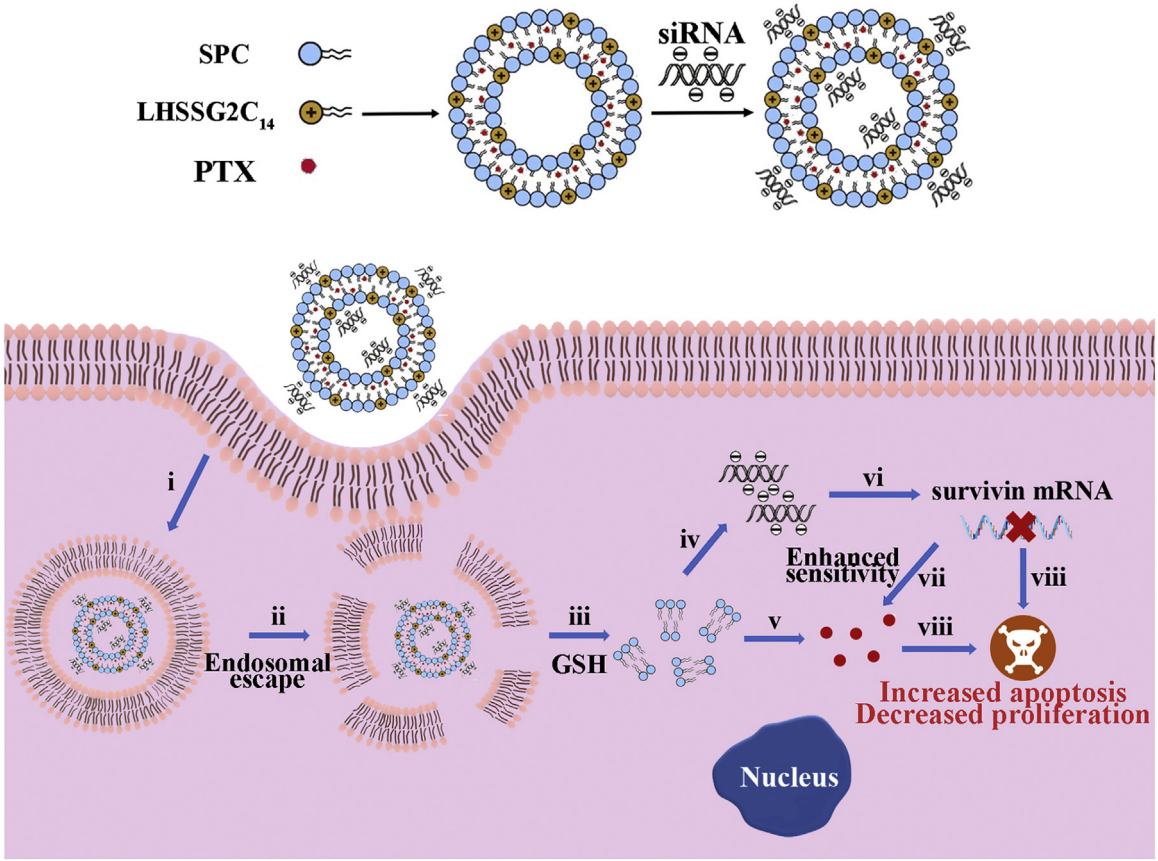

Fig. 1. Schematic illustration of the structure of redox-sensitive oligopeptide liposomes encapsulating PTX and siRNA, as well as the intracellular transport of the co-delivery system including endocytosis into endosomes (i), endosomal escape (ii), reduction-responsive liposomes disassembly (iii), siRNA release (iv), PTX release (v), downregulation of survivin mRNA expression (vi), enhanced sensitivity of tumor cells to PTX (vii), increased apoptosis and decreased proliferation induced by the combination of PTX and anti-survivin siRNA (viii).