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Bioorthogonal, two-component delivery systems based on antibody and drug-loaded nanocarriers for enhanced internalization of nanotherapeutics

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ABSTRACT

Nanocarriers play an important role in targeted cancer chemotherapy. The optimal nanocarrier delivery system should provide efficient and highly specific recognition of the target cells and rapid internalization of the therapeutic cargo to reduce systemic toxicity as well as to increase the cytotoxicity to cancer cells. To this end, we developed a two-step, two-component targeted delivery system based on antibody and drug-loaded nanocarrier that uses bioorthogonal click reactions for specific internalization of nanotherapeutics. The pretargeting component, anti-HER2 humanized monoclonal antibody, trastuzumab, functionalized with azide groups labels cancer cells that overexpress HER2 surface receptors. The drug carrier component, dibenzylcyclooctyne substituted albumin conjugated with paclitaxel, reacts specifically with the pretargeting component. These two components form cross-linked clusters on the cell surface, which facilitates the internalization of the complex. This strategy demonstrated substantial cellular internalization of clusters consisted of HER2 receptors, modified trastuzumab and paclitaxel-loaded albumin nanocarriers, and subsequent significant cytotoxicity in HER2-positive BT-474 breast cancer cells. Our results show high efficacy of this strategy for targeted nanotherapeutics. We foresee to broaden the applications of this strategy using agents such as radionuclides, toxins, and interfering RNA.

1. Introduction

Targeted delivery of nanocarriers offers great promise to improve the efficacy of chemotherapy while reducing its systemic toxicity [1–5]. Important features of targeted nanocarriers include their biocompatibility, high drug loading capacity, possibility of loading both hydrophobic and hydrophilic compounds, and favorable pharmacokinetics [6,7]. Antibody–drug conjugates (ADCs), a new class of nanoscale therapeutic agents, where drug molecules are chemically linked to the target-specific antibody have recently been approved for targeted therapy of cancers that overexpress a specific cell surface receptor [8]. Approximately 20–30% of breast cancers overexpress HER2/*neu* due to the gene amplification that results in high aggressiveness and generally poor prognosis [9,10]. The HER2 receptor regulates multiple physiological pathways, including cell proliferation and differentiation [11]. The humanized anti-HER2 monoclonal antibody (mAb), trastuzumab (Herceptin®), is used as a first-line treatment for HER2/*neu*-positive breast cancers [12]. The cytotoxic mechanism of trastuzumab includes the inhibition of the P13K/Akt and Ras/MAPK signaling pathways, leading to cell cycle arrest [13]. Unfortunately, approximately 50% of patients with HER2-positive disease do not benefit from trastuzumab or the disease becomes refractory to the drug [14], even though the HER2 level remains high [15]. The HER2 receptor is also characterized by poor internalization capability [16], even after antibody binding and subsequent heterodimerization, perhaps due to localization of the receptor in a membrane protrusion and/or in lipid raft areas where the receptor has poor contact with the lipid bilayer. An ADC, trastuzumab emtansine conjugate (T-DM1) [17], was recently reported to have significantly improved efficacy compared to standard monotherapeutics for trastuzumab-refractory disease [18,19]. However, the direct conjugation of chemotherapeutics to mAb may reduce its therapeutic index [20,21] due to the decreased binding affinity [22]. In addition, antibody conjugation does not enhance the ADC internalization. Long

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circulation half-life of ADCs can also result in systemic toxicity. In fact, thrombocytopenia has been observed in a small number of patients treated with ADCs [23,24].

Here, we report a new strategy for a two-step, target-specific drug delivery that utilizes enhanced internalization of therapeutic conjugates by *in situ* complexation, driven by bioorthogonal click chemistry. Use of click chemistry for the synthesis of targeted nanotherapeutics was recently reported [25]. However, our two-step, two-component system for the intracellular delivery of therapeutics is based on prelabeling of target receptors with azide functionalized mAbs, followed by the delivery of dibenzylcyclooctyne (DBCO) functionalized nanocarriers. The *in situ* click reactions between components induce the formation of cross-linked clusters on the cell surface, leading to their rapid internalization (Fig. 1). Both components can be modified with appropriate imaging agents for tracking their delivery, internalization, and accumulation in target cells. Optimal synthetic strategy and substitution ratios for pretargeting and delivery components are of key importance for drug solubility, efficiency of delivery, and binding affinity with the targets. For this study, we conjugated paclitaxel with albumin by covalent bonding. Paclitaxel is an antineoplastic taxane drug widely used for treating advanced breast cancer and metastasis [26]. Since paclitaxel is a highly hydrophobic compound, it is typically administered as micelles made in Cremophor EL (CrEL) and dehydrated ethanol, or as encapsulated vehicles made by nanoparticle albumin-bound (*nab*) technology. Excipients, such as Cremophor EL, are relatively toxic and can induce severe hypersensitive reactions [27] including dyspnea, flushing, rash, chest pain, and tachycardia [28]. The *nab*-technology involves high-pressure homogenization of albumins in the presence of drugs [29,30], and the particle size of clinically used *nab*-paclitaxels, Abraxane® is in the range of 140–160 nm. This drug uses physical entrapment of paclitaxel and starts to release drug molecules before reaching the target [31]. Hence, in this study we substituted paclitaxel molecules with albumin by covalent bonds via a succinyl spacer. Unlike physical entrapment of drug molecules [32] this approach enables to control the degree of substitutions and prevent the dissociation and release of drug *en route* to targets. Albumin was chosen as a model platform for the delivery component because albumin is a highly soluble, chemically and thermally stable, and biodegradable plasma protein, which make it a suitable carrier for drug delivery [33].

In our study, *in situ* complexation was achieved by multiple bioorthogonal click reactions between azido-trastuzumab and a model nanocarrier, DBCO-functionalized albumin–paclitaxel conjugate. Copper-free, strain-promoted click chemistry [34–36], or alternative bioorthogonal strategies [37,38], have received considerable attention for imaging applications [33–37] but, to the best of our knowledge, this is the first study to employ bioorthogonal click chemistry for chemotherapy. This strategy was evaluated in HER2-positive and HER2-negative breast cancer cell lines. We have demonstrated that this delivery system provides high efficacy and highly efficient intracellular drug accumulation in HER2-overexpressing cells.

2. Materials and methods

2.1. Cell lines

BT-474 and MDA-MB-231 cells were purchased from the American Type Culture Collection (ATCC) and cultured according to the manufacturer's direction using ATCC® 46-X and DMEM (Cellgro) media, respectively. Both media were supplemented with 1% Penicillin–Streptomycin, and 10% FBS. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 unless otherwise mentioned. Third or fourth passages of cells with 70–80% confluency were used for imaging experiments.

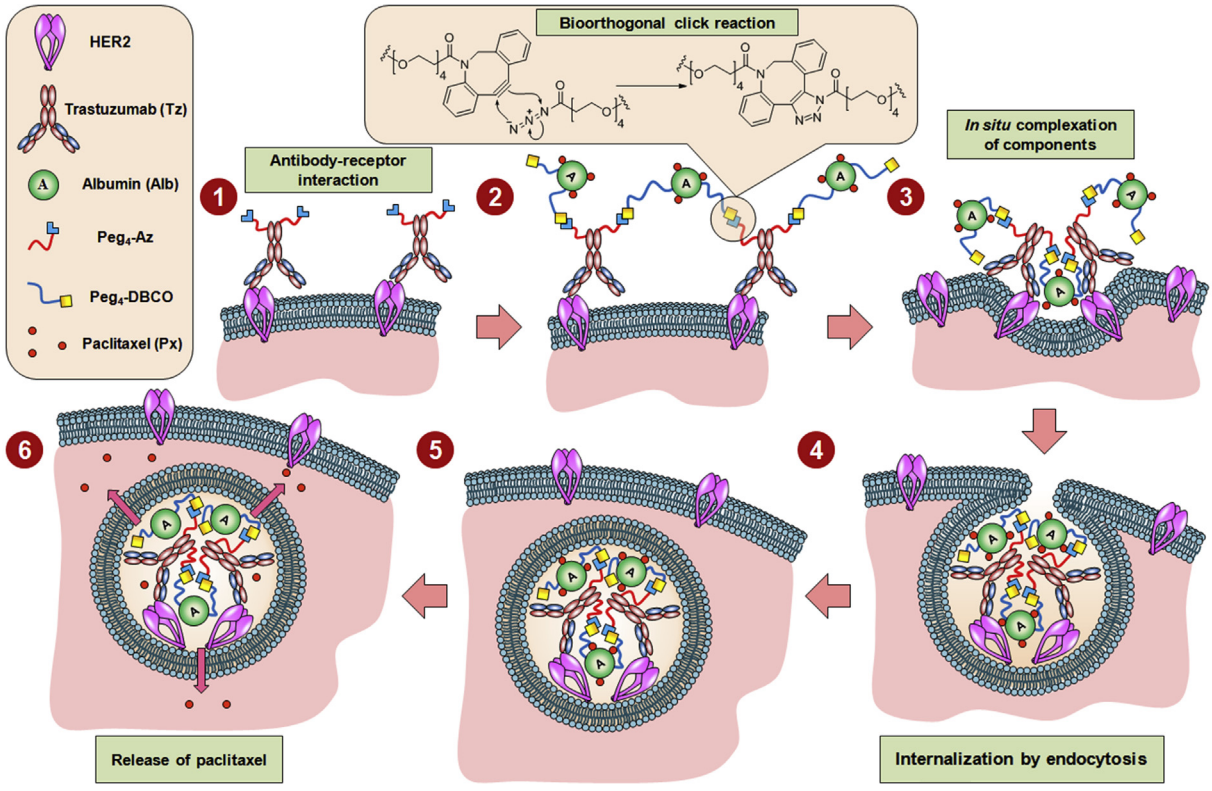

Fig. 1. Schematics of the therapeutic strategy based on *in situ* bioorthogonal click chemistry. The strategy proceeds via interaction between functionalized trastuzumab and HER2 receptors on the cell surface, and bioorthogonal multiple click reactions between azide functional groups in trastuzumab and DBCO groups in albumin, followed by cluster formation, internalization, and release of drug molecules.