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A facile approach to functionalizing cell membrane-coated nanoparticles with neurotoxin-derived peptide for brain-targeted drug delivery

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ABSTRACT

The blood brain barrier separates the circulating blood from the extracellular fluid in the central nervous system and thus presents an essential obstacle to brain transport of therapeutics. Herein, we report on an effective brain-targeted drug delivery system that combines a robust red blood cell membrane-coated nanoparticle (RBCNP) with a unique neurotoxin-derived targeting moiety. The RBCNPs retain the complex biological functions of natural cell membranes while exhibiting physicochemical properties that are suitable for effective drug delivery. CDX peptide is derived from candoxin and shows high binding affinity with nicotinic acetylcholine receptors (nAChRs) expressed on the surface of brain endothelial cells. Through a facile yet robust approach, we successfully incorporate [D]CDX peptides onto the surface of RBCNPs without compromising the peptide's brain targeting ability. The resulting [D]CDX-RBCNPs show promising brain targeting efficiency both *in vitro* and *in vivo*. Using a glioma mouse model, we demonstrate that doxorubicin-loaded [D]CDX-RBCNPs have superior therapeutic efficacy and markedly reduced toxicity as compared to the nontargeted drug formulations. While RBCNPs are used as a model system to evaluate the surface modification approach, the reported method can be readily generalized to various types of cell membrane-derived nanocarriers for broad medical applications.

1. Introduction

Primary brain and central nervous system (CNS) tumors severely threaten human health due to their fast development, poor diagnosis and rapid recurrence [1,2]. The blood brain barrier (BBB), which mainly consists of capillary endothelial cells and tight junctions, is a highly selective permeability barrier that separates the circulating blood from the extracellular fluid in the CNS [3–5]. However, the BBB also presents an essential obstacle to brain transport of therapeutics. At the early stage of brain tumors, the BBB remains intact. With tumor progression and angiogenesis, the BBB still exists in the infiltrative tumor region, which is intractable to surgical resection and mainly responsible for the rapid recurrence of brain tumors. Thus, conventional chemotherapeutics cannot be delivered to the brain effectively [6]. The development of nanomedicines has the potential to improve the prognosis of CNS diseases, especially in glioma diagnosis and treatment [7–11]. By modifying the surface of nanocarriers such as nanoparticles, liposomes, and micelles with targeting ligands that can specifically penetrate the BBB, it is possible to achieve effective brain-targeted drug delivery [12–16].

As a new class of biomimetic nanoparticles, red blood cell membrane-coated nanoparticles (RBCNPs) retains the complex biological functions of natural cell membranes while exhibiting physicochemical properties that are suitable for effective drug delivery. RBCNPs can achieve prolonged blood circulation and display low immunogenicity in comparison with traditional synthetic nanoscale drug delivery systems

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[17–19]. In previous studies, drug-loaded RBCNPs have exhibited outstanding tumor suppression effect and low systemic toxicity [20,21]. A recent study showed that RBCNPs could deliver doxorubicin (Dox) to solid tumors and inhibit their growth in a lymphoma model with excellent *in vivo* safety and immunocompatibility [22]. By modifying RBCNPs with a targeting ligand, it has been demonstrated that it is possible to further improve their utility for specific applications [23].

CDX peptide (FKESWREARGTRIERG) derived from candoxin show high binding affinity with nicotinic acetylcholine receptors (nAChRs) and has been proven to traverse the BBB [24]. ^{D}CDX peptides are the retro-inverso isomer of ^{L}CDX and are fully resistant to proteolysis. They have higher binding affinity to nAChRs than ^{L}CDX. ^{D}CDX-modified liposomes have demonstrated promising ability to traverse the BBB, resulting in remarkable therapeutic efficacy in glioma treatment when loaded with Dox [25,26]. However, hydrophilic polymers, such as polyethylene glycol (PEG), were widely utilized to the surface modification of nano-sized drug delivery systems including liposomes to prolong the circulation thus enhance the targeting efficiency. Recent studies have suggested that repeated administration of PEGylated liposomes may induce immunogenic response of B cells and accelerate the clearance of drug delivery systems [27,28]. Given that RBCNPs, as a class of bio-inspired nano-sized drug delivery systems, have proven to be effective drug delivery vehicles with prolonged blood circulation, good biocompatibility and low immunogenicity, we hypothesize that by surface modification of RBCNPs with the ^{D}CDX peptide, the resulting ^{D}CDX-RBCNPs would likewise enable brain targeted drug delivery (Fig. 1).

To test this hypothesis, an immediate challenge is to effectively introduce ^{D}CDX peptide onto the surface of RBCNPs. In a previous study, modification of RBCNPs with an active targeting ligand onto the surface was achieved by a lipid insertion method [23]. However, the surface electrical property of cell membrane limited the application of many positively charged targeting ligands such as ^{D}CDX peptide, because these positively charged ligands are prone to adhere to the negatively charged cell membrane and thus their targeting ability is compromised. Herein, we report on a facile yet robust approach to modifying cell membrane-coated nanoparticles with positively charged targeting ligands using a simple avidin-biotin chemistry. The molecular binding affinity of streptavidin to biotin has been widely utilized in biological research and in the development of drug delivery systems [29–35]. Herein, we pre-insert streptavidin onto the surface of RBC membrane, followed by incorporating a biotinylated form of ^{D}CDX to the nanoparticle surface with high efficiency while avoiding the ionic interaction between the peptide and RBC membrane. We demonstrate successful preparation of ^{D}CDX-RBCNPs. Both *in vitro* and *in vivo* targeting efficiency studies verify that ^{D}CDX-RBCNPs possess the capability to traverse the BBB and display exceptional brain targeting effect. When loaded with Dox, ^{D}CDX-RBCNPs/Dox significantly improves the median survival of glioma-bearing mice.

2. Materials and methods

2.1. Synthesis of streptavidin-PEG$_{3400}$-DSPE and biotin-PEG$_{3500}$-^{D}CDX

The synthesis procedure of streptavidin-PEG$_{3400}$-DSPE was as follows: (1) 20 mg of streptavidin (YeSen Biotech Co. LTD.) was dissolved in 2 mL of borate buffer (pH 7.6). Then a 10-fold excess of Traut's reagent (Aladdin) in aqueous solution was added into the reaction system. After stirring for 2 h, the excess Traut's reagent was removed by ultrafiltration at 4 °C. The residue was washed with distilled water three times and the resulting product streptavidin-SH was diluted with water to 1 mL for the next reaction. (2) 5 mg mal-PEG$_{3400}$-DSPE (Laysan Bio Inc.) was dissolved in 1 mL dichloromethane (DCM). The DCM solvent was then removed by rotary evaporation to form a film. After removing the organic solvent completely, the streptavidin-SH solution and phosphate was added into the resulting film and hydrated for 1 h at 37 °C. Subsequently, the residual mal-PEG$_{3400}$-DSPE and phosphate was removed by dialysis in distilled water for 72 h. The targeting product was obtained by freeze-drying (20.3 mg, yielding 95%).

The detailed synthesis method of biotin-PEG$_{3500}$-^{D}CDX was as follows: (1) 0.41 mmoL of biotin (J & K Scientific LTD.) was dissolved in 1 mL of DCM. Then 0.615 mmoL of NHS (Aladdin) and 0.615 mmoL of

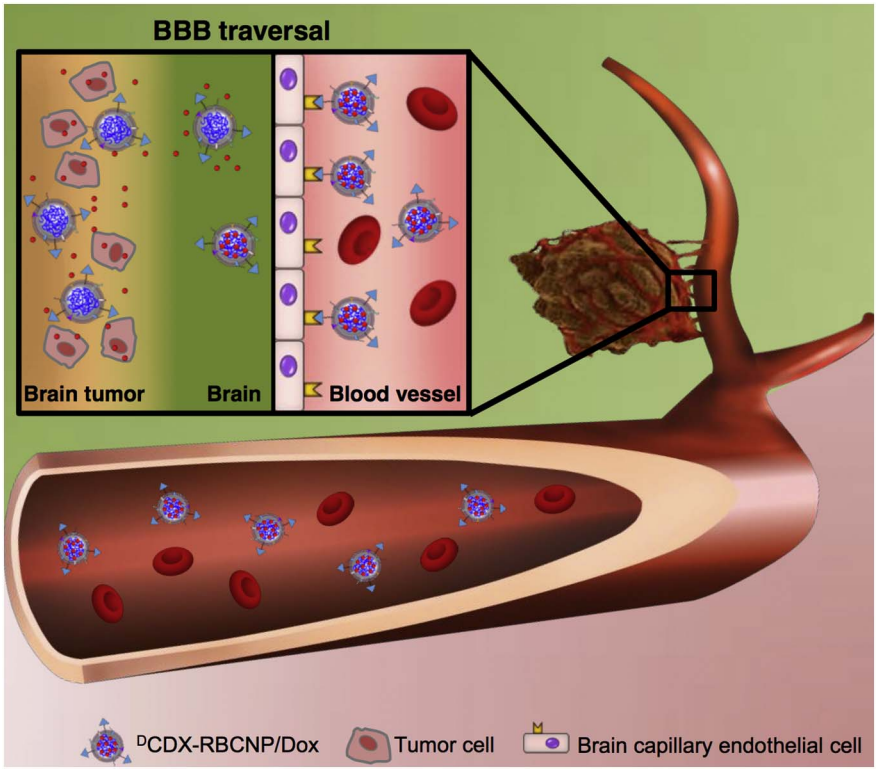

Fig. 1. Schematic of ^{D}CDX-RBCNPs crossing the BBB for brain-targeted drug delivery.