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Study and evaluation of mechanisms of dual targeting drug delivery system with tumor microenvironment assays compared with normal assays

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ABSTRACT

A dual targeting delivery system was developed to completely conquer the two barriers that glioma treatment faces: the blood–brain barrier (BBB) and the brain–glioma barrier. Recently, a system comprising AS1411 aptamer (for glioma targeting) and TGN peptide (for BBB targeting) modified nanoparticles (AsTNPs) was developed, which can effectively target brain glioma and improve the survival of glioma-bearing mice. However, the in vitro models currently used are far too different from the in vivo tumor microenvironment that the glioma targeting delivery system actually faces. In this study, the pharmacology mechanisms of AsTNPs were explored in several models that imitated the tumor microenvironment. AsTNPs can be selectively taken up by endothelial and glioma cells, effectively penetrating the BBB and brain–glioma barriers to reach glioma cells and display their anti-glioma effect. The cell monolayers, tumor spheroids and coculture systems were more suitable in vitro models for the pharmacology evaluation of targeted drug delivery systems.

1. Introduction

Noninvasive treatment to improve glioma-bearing survival is a promising direction of glioma therapy. However, most chemotherapy fails because of the limited blood–brain barrier (BBB) penetration and poor glioma targeting of the chemotherapeutics [1–3]. As a disease of the whole brain, effective treatment for brain glioma needs to conquer two barriers: the BBB and the brain–glioma barrier [3,4]. Dual targeting delivery systems were developed to completely conquer these two barriers and presented promising results [5–7]. These systems utilized glioma targeting ligands and BBB targeting ligands to precisely target glioma. Previously, we prepared AS1411 aptamer and TGN peptide modified nanoparticles (AsTNPs) [5]. It was demonstrated that AsTNPs could effectively target brain glioma and improve survival of glioma-bearing mice.

Tumor is a complex pathology that involves not only tumor cells, but also the tumor-associated parenchymal cells including vascular cells, macrophages, immune cells, etc. [8]. The characteristics and interaction among all these cells construct the microenvironment of tumor. The physical conditions of glioma are also extremely different from normal cells, and include leaky vasculature, low pH, low pO_2 and high interstitial fluid pressure [9–13]. The complex microenvironment is a double-edged sword for tumor management. Leaky vasculature makes nanosized systems extremely valuable in drug delivery to tumor through enhanced permeability and the retention effect [14–16]. The low pH of tumor has also been exploited for controlled release drugs or functional moieties from drug delivery systems [17–19]. On the other hand, the complex microenvironment is also a big hurdle for drug delivery. For example, the elevated tumor interstitial fluid pressure restricts drugs or drug delivery systems by withdrawing the flow-driven penetration into tumors [20]. Thus, a good understanding of the effect of the tumor microenvironment on drug delivery systems is useful for predicting the performance of these systems and designing and optimizing these systems.

Three-dimensional (3-D) tumor spheroids have been proposed to targeted delivery and therapy evaluation [6,21–23]. These tumor spheroids are increasingly used as models of intermediate complexity between in vitro cultured cells and in vivo tumors because of several advantages, among which are poor drug penetration, drug resistance, altered protein expression and enzyme activity, viable rim with gradients of oxygen tension, nutrients, catabolites and cell proliferation [24–27]. These characters are approximately the same as the in vivo microenvironment of tumors. Studies performed on these spheroids can more accurately reflect in vivo performance.

Docetaxel (DTX) is a taxane derivative that is structurally similar to paclitaxel [28]. As an inhibitor of microtubule depolymerization, DTX has been widely used for the treatment of various

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cancers clinically [29–31]. Thus DTX was used as model drug in this study.

In this study, we established several models that mimic the tumor microenvironment to characterize the dual glioma targeting delivery system: AsTNPs. To elucidate the targeting properties, we implement a range of experiments for evaluating the targeting effect. We performed not only cellular uptake but also penetration through monolayers of C6 cells (glioma cell line) and bEnd.3 cells (cell lines that were widely used for the replacement of endothelial cells [32]) (Fig. 1), because the penetration was more important for drug delivery systems reaching distant cells. Cytotoxicity and cell tubulin staining were both carried out to more precisely determine the anti-proliferation effect, which was caused by the internalization of docetaxel (DTX) or DTX-loaded formulations rather than polymers. On the other hand, quantitative apoptosis was performed on C6 cell layers and cocultured systems constructed by the bEnd.3 cell monolayer and C6 cells, which could more effectively predict the in vivo antitumor effect. Further, 3-D C6 spheroids were established and cocultured with bEnd.3 monolayers to evaluate the penetration effect of AsTNPs. The anti-proliferation effect of DTX-AsTNPs was also performed on this model.

2. Materials and methods

2.1. Materials

AS1411 aptamer and TGN peptide were synthesized by Sangon Biotech Co. Ltd (Shanghai, China). DTX was purchased from Knowshine (Shanghai, China). Methoxy poly(ethyleneglycol)-poly(ε-caprolactone) (MPEG-PCL; Mw: 3 k-15 k) and carboxyl poly(ethylene glycol)-poly(ε-caprolactone) (HOOC-PEG-PCL; Mw: 3.4 k-15 k) were synthesized as previously described [33]. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxy-succinimide (NHS) and Coumarin-6 were purchased from Sigma (St Louis, MO, USA). Low melting point agarose was purchased from Amresco (Solon, OH, USA). Plastic cell culture dishes and plates were purchased from Wuxi NEST Biotechnology Co. Ltd (Wuxi, China). Transwell® was purchased from Corning Incorporation (Corning, NY, USA). Dulbecco's modified Eagle medium (high glucose) cell culture medium (DMEM) and fetal bovine serum (FBS) were purchased from Life technologies (Grand Island, NY, USA). An Annexin V-FITC cell apoptosis detection kit and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Beyotime (Haimen, China). The C6 cell line was obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The bEnd.3 cell line (the immortalized mouse brain endothelial cell line) was purchased from American type culture collection (ATCC) (Manassas, VA, USA). A cell counting kit-8 (CCK-8) was purchased from Dojindo (Minato-ku, Japan). Goat anti β tubulin (N-20) was purchased from Santa Cruz (CA, USA). Rabbit anti CD31 polyclonal antibody was purchased from Abcam Ltd (Hong Kong, China). Alexa Fluor 488 conjugated donkey anti-goat IgG and Dylight 647 conjugated donkey anti-rabbit IgG were purchased from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA, USA). All the other chemicals were analytical reagent grades and purchased from Sinopharm Chemical Reagent (Shanghai, China).

BALB/c mice (male, 4–5 weeks, 18–22 g) were obtained from the Shanghai Slac Laboratory Animal Co. Ltd (Shanghai, China) and maintained under standard housing conditions. All animal experiments were carried out in accordance with protocols evaluated and approved by the ethics committee of Fudan University.

2.2. Preparation nanoparticles

PEG-PCL nanoparticles (NPs) were prepared by the emulsion/solvent evaporation method described previously [34]. Briefly, 28 mg of MPEG-PCL, 1 mg of HOOC-PEG-PCL and 1 mg of MAL-PEG-PCL were dissolved in 1 ml dichloromethane, and then added to 5 ml of 0.6% sodium cholate hydrate solution. The mixture was pulse-sonicated for 75 s at 200 W on ice using a probe sonicator (Scientz Biotechnology Co. Ltd, China). Then the emulsion was applied to a rotary evaporator to remove the dichloromethane

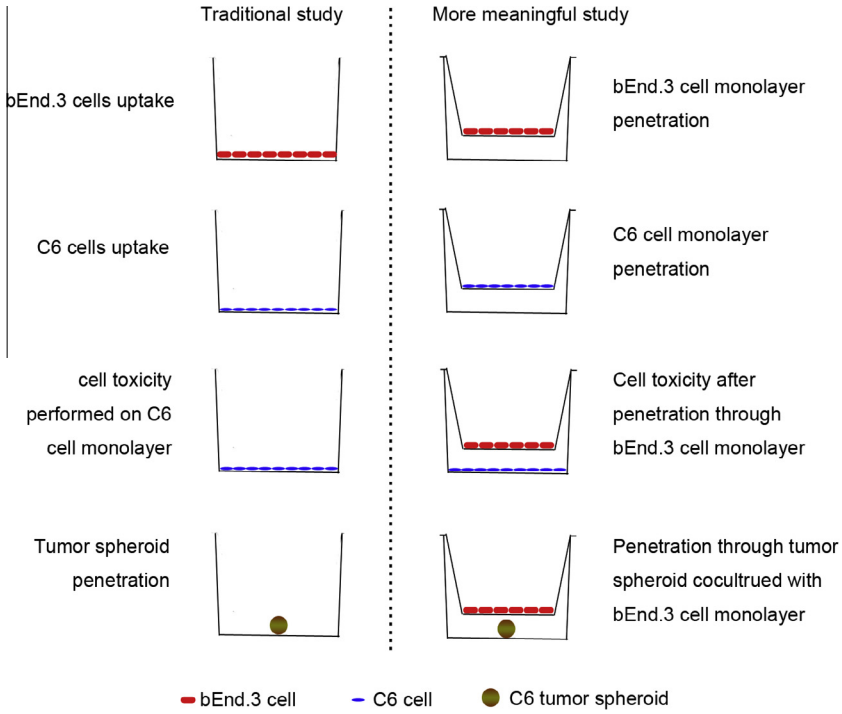

Fig. 1. The elucidation of characterization performed in this study.