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# Choline transporter-targeting and co-delivery system for glioma therapy

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ABSTRACT

Combination of gene therapy and chemotherapy is a promising approach for glioma therapy. In this study, a co-delivery system of plasmid encoding human tumor necrosis factor-related apoptosis-inducing ligand (pORF-hTRAIL, Trail) and doxorubicin (DOX) has been simply constructed in two steps. Firstly, DOX was intercalated into Trail to form a stable complex. Secondly, DOX-Trail complex was condensed by Dendrigraft poly-L-lysine (DGL) to form a nanoscaled co-delivery system. Choline transporters are both expressed on blood–brain barrier (BBB) and glioma, Herein, a choline derivate with high choline transporter affinity was chosen as BBB and glioma dual targeting ligand. Choline-derivate modified co-delivery system showed higher cellular uptake efficiency and cytotoxicity than unmodified co-delivery system in U87 MG cells. In comparison with single medication or unmodified delivery system, Choline-derivate modified co-delivery system induced more apoptosis both *in vitro* and *in vivo*. The therapeutic efficacy on U87 MG bearing xenografts further confirmed the predominance of this dual targeting and co-delivery system.



## 1. Introduction

Current treatments for glioblastoma multiforme (GBM) are insufficient with a nearly universal recurrence after surgery, radiation therapy, and chemotherapy [1]. Poor chemotherapy outcome after surgery contributes to this high recurrence. The blood–brain barrier (BBB) existing at the early stage or the tumor margin of glioma limits the penetration of most therapeutic agents [2]. Even though some chemotherapeutics could permeate across the BBB, single medication could not obtain optimal efficacy due to the drug resistance. Based on the above consideration, two strategies including glioma specific targeting and combination therapy were employed for better outcomes.

For glioma targeting, two-order targeting strategy was commonly employed in designing drug delivery or diagnosis systems to overcome the BBB and accumulate into tumor. In brief, one ligand with binding affinity to the receptors or transporters expressed on the vasculature endothelial cells could facilitate the transcytosis across the BBB. Another ligand with high affinity to the receptors overexpressed on glioma cells could enhance the accumulation into tumor [3–5]. Dual targeting to BBB and glioma with one ligand was another choice. For example, Angiopep-2, one of the family of Kunitz domain-derived peptides, could target to the low-density lipoprotein receptor-related protein-1 (LRP1) expressed on brain capillary endothelial cells (BCECs) and glial cells [6,7]. In our previous study, a choline derivate with high BBB choline transporter affinity was challenged to mediate the gene delivery into brain [8]. Because of high malignancy, choline transporters are overexpressed on glioma cells. This indicates that choline transporters may facilitate the glioma targeting [9]. In another work, we developed a choline-derivate modified nanoprobe for glioma MRI imaging and gained notable signal contrast between tumor and normal brain region [10]. Herein, choline derivate was selected as a BBB and glioma dual targeting ligand.

Combination therapy in glioma has attracted wide attention due to the inefficiency of single medication. Attempts have been made on the co-delivery of gene drug and chemotherapeutics. The synergistic effect arisen from the co-delivery of human tumor necrosis factor-related apoptosis-inducing ligand (pORF-hTRAIL, Trail) and paclitaxel or doxorubicin (DOX) has been proved to gain enhanced anti-tumor effect [11,12]. In this study, Trail and DOX were chosen as model drugs.

A co-delivery system of DOX and nucleic acid was reported by Bagalkot et al. [13]. DOX is known to intercalate within the double helix of DNA strand due to the presence of flat aromatic rings in this molecule. At present, aptamer and plasmid DNA were developed as

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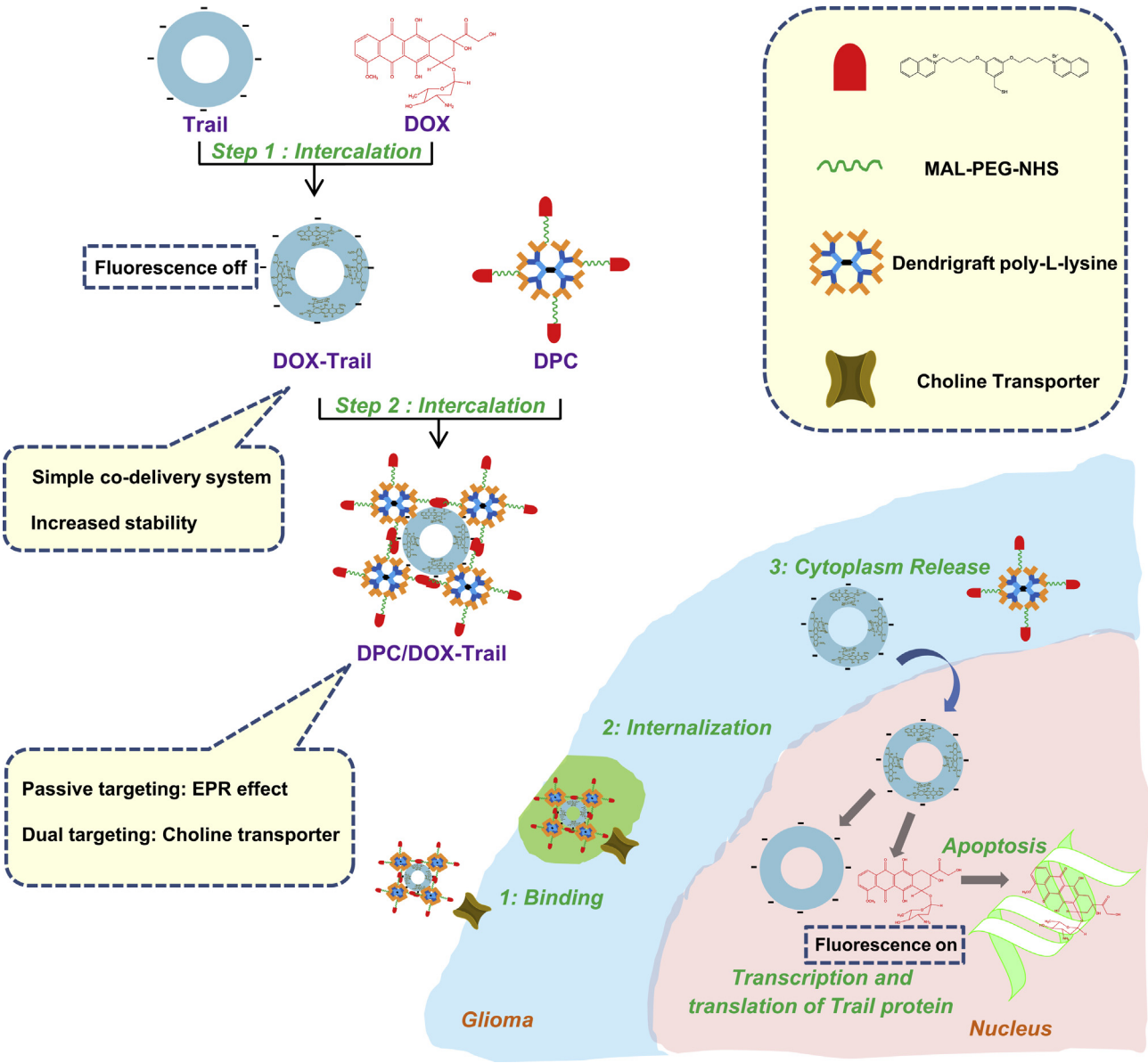
vectors which could form stable complex upon DOX intercalation. Combined chemoimmunotherapy using plasmid-DOX complex achieved comparable therapeutic effect and reduced cardiotoxicities to DOX in two different tumor models. The therapeutic benefits came from the improved pharmacokinetics of DOX by increasing blood circulation. In turn, intercalation (complexation) can also protect the plasmid against nucleases [14]. While, a flaw that mars perfection may be the small size of complex resulting in rather fast renal clearance. To take the advantage of enhanced permeability and retention (EPR) effect, plasmid-DOX complex was further condensed by dendrimer to form nanoparticles (NPs). This nanosized co-delivery system preferentially accumulated in tumor and significantly inhibited its growth in a subcutaneous tumor model [15].

In this study, a co-delivery system for glioma therapy was established. Firstly, DOX was intercalated into Trail to form a stable complex. Trail served both as a DOX carrier and a gene drug. Secondly, DOX-Trail complex was condensed by Dendrigraft poly-L-lysine (DGL) to form a nanoscaled co-delivery system (Scheme 1). Choline derivate was further conjugated to this system to realize the glioma specific drug delivery. Targeting efficiency and anti-tumor effect of the system were evaluated both *in vitro* and *in vivo*.

## 2. Materials and method

### 2.1. Materials

DGL generation 3 with 123 lysine groups were purchased from Colcom, France. α-Malemidyl-ω-N-hydrox-ysuccinimidyl polyethyleneglycol (NHS-PEG-MAL, MW 3500) was obtained from Jenkem Technology (Beijing, China). Doxorubicin hydrochloride (DOX·HCl) was purchased from Beijing Huafeng United Technology Corp. The plasmid Trail and pGL2-control vector (InvivoGen, San Diego, CA, USA) were purified using QIAGEN Plasmid Mega Kit (Qiagen GmbH, Hilden, Germany). Annexin V-FITC TUNEL Apoptosis Assay Kit and TdT *In Situ* Apoptosis Detection Kit were purchased from KeyGEN (Nanjing, China). Near-infrared probe (NIR783) was kindly




**Scheme 1.** Construction of dual targeting and co-delivery system. DOX was intercalated into Trail plasmid to form a stable complex which was further condensed by choline-derivate modified DGL. This co-delivery system could accumulate into glioma cells by EPR and dual targeting effect. After cytoplasm releasing, Trail and DOX educe combination therapy on glioma.