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Tumortropic adipose-derived stem cells carrying smart nanotherapeutics for targeted delivery and dual-modality therapy of orthotopic glioblastoma

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ABSTRACT

Chemotherapy is typically used to treat malignant brain tumors, especially for the tumors in surgically inaccessible areas. However, owing to the existence of blood-brain barrier (BBB), the tumor accumulation and therapeutic efficacy of clinical therapeutics is still of great concerns. To this end, we present herein a prominent therapeutic strategy adopting adipose-derived stem cells (ADSCs) capable of carrying nanotherapeutic payloads selectively toward brain tumors for thermo/chemotherapy. The nanoparticle (NP) payload was obtained from *co*-assembly of poly(γ-glutamic acid-*co*-distearyl γ-glutamate) with poly(lactic-*co*-glycolic acid), paclitaxel (PTX), and oleic acid-coated superparamagnetic iron oxide NPs in aqueous solution. The particle size and drug loading content were ca 110 nm and 8.4 wt%, respectively. After being engulfed by ADSCs, the nanotherapeutics was found rather harmless to cellular hosts at a PTX concentration of 30 μM over 48 h in the absence of pertinent stimulus. Nevertheless, the ADSC-based approach combined with high frequency magnetic field exhibits a sound therapeutic performance with a 4-fold increase in therapeutic index on brain astrocytoma (ALTS1C1)-bearing mice (C57BL/6 J) as compared to the typical chemotherapy using a current first-line chemodrug, temozolomide. Immunohistochemical examination of brain tumor sections confirms the successful cellular transport and pronounced cytotoxic action of therapeutics against tumor cells *in vivo*. This work demonstrates the promise of ADSC-mediated chemo/thermal therapy against brain tumors.

1. Introduction

Astrocytoma arising from star-shaped glial cells is the most common glioma, accounting for > 50% of neuroepithelial tumors in young adults [1]. Owing to the location of malignant gliomas within the central nervous system, the precise treatment is strictly required for reducing the risk of neurological damage from the medical treatment. Despite contemporary surgical techniques allowing to safely remove cerebral tumors in certain areas, the neoplasm embedded deep inside brain tissue may not be adequate for surgical options. Unfortunately, chemo- and radiation therapies frequently utilized as alternatives for therapeutic treatment of inoperable brain tumors have shown deficiency in clinical studies due to the prohibition of paracellular therapeutic transport across cerebral endothelium (i.e., the blood-brain barrier, BBB) [2,3] and the low radiation tolerance of the normal brain tissues [4,5]. Although several findings pointed out that the BBB is disrupted during the rapid progression of brain tumors, which allows nanotherapeutics to accumulate at tumor sites via the enhanced permeability and retention (EPR) effect, [6,7] other studies have reported that the BBB disruption can be only found in the core of the high-grade gliomas [8,9]. This means that the BBB may still essentially function in the remnant tumor regions except the core. To this end, a reliable approach to the effective delivery of therapeutics across BBB into brain tumor is urgently required.

Mesenchymal stem cells isolated from adipose tissue exhibit a sound capability of being chemotactically recruited into brain tumors through stromal-derived factor-1/C-X-C chemokine receptor type 4 (SDF-1/CXCR4) axis [10,11]. This is mainly because these adipose-derived stem cells (ADSCs) play a crucial role in promoting tumor growth by secreting a broad variety of cytokines, chemokines, and growth factors, such as vascular endothelial growth factor, hepatocyte growth factor and interleukin-8 for tumor angiogenesis and tissue development [12–14]. The innate tumor-homing ability allows ADSCs to serve as a potential therapeutic vehicle for targeted delivery to brain tumors.

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Indeed, adopting ADSCs as a cellular vehicle into brain tumors has shown the promise in gene delivery and therapy. For instance, Josiah et al. have developed a new therapeutic strategy using myxoma virus-infected ADSCs as a cellular Trojan to deliver the oncolytic virus to brain tumors [15]. Their data suggest that the therapeutic payloads can be effectively transported into the tumors, thus resulting in a pronounced increase in survival of the brain tumor-bearing mice. Choi et al. have found that the ADSCs exhibit an excellent capability of migrating toward the brain tumor-initiating cells (i.e., brainstem gliomas) [16]. For this reason, they genetically engineered ADSCs to encode the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) for targeted gene therapy against brainstem gliomas [17]. Their results provide a clear evidence of improved short- and long-term therapeutic effects *in vivo* with the ADSC-based gene therapy. Although above studies demonstrate the potential of ADSCs in gene therapy, the applications are somewhat limited due to the high cost and complicated procedure in attaining genetically engineered ADSCs.

By contrast, many cells without additional genetic modification were adopted to serve as the cell-based carriers of therapeutic nanoparticles (NPs) in a cellular hitchhiking manner for enhanced targeted delivery to a particular organ or tumor (tumor hypoxia) or even cancer cells in the circulation [18–22]. For instance, leukocytes after selective pickup of the E-selectin/TRAIL-conjugated liposomes in blood circulation became capable by mimicking the cytotoxic activity of natural killer cells to effectively induce the apoptosis of circulating tumor cells and thus prevent the cancer metastasis [20–22]. In this study, the ADSCs isolated from C57BL/6 J mice were employed as a cellular Trojan system for targeted delivery of therapeutic NPs to brain tumors. To endow the NPs with stimulus-responsive property for hyperthermia and chemotherapy against tumors, the superparamagnetic iron oxide nanoparticles (SPIONs) were entrapped into the chemotherapy (paclitaxel, PTX)-laden NPs which were subsequently taken up by ADSCs via endocytosis. Upon chemotactic recruitment of the ADSC-based therapy to tumor sites, hyperthermia and drug liberation can then be activated by high frequency magnetic field (HFMF). Characterizations of cell-mediated therapeutics with respect to cellular uptake of SPION/PTX-loaded NPs (SPNPs) by ADSCs, HFMF-mediated hyperthermia and intercellular drug transport from host to tumor cells, cytotoxic effect against cancer cells, and chemotactic migration of therapeutic payload-laden ADSCs were performed. A murine glioblastoma model of ALTS1C1 cancer cells, derived from primary astrocytes transformed by SV40 large T antigen and serial *in vivo* passage was adopted for evaluating *in vivo* chemotactic recruitment of SPNP-loaded ADSCs and therapeutic efficacy of the dual-modality treatment, involving the use of multiple immunohistochemical (IHC) staining of tumor tissue sections [23]. Fig. 1 depicts the strategy developed in this work for selective delivery of SPNPs by ADSCs across BBB into brain tumors for dual-modality treatment.

2. Materials and methods

2.1. Materials

Poly(lactic-*co*-glycolic acid) (PLGA; LA/GA 75/25; Mn 10,000 g/mol) was purchased from Green Square (Taiwan). PTX was obtained from Seedchem (Australia). Preparations and characterizations of oleic acid-coated SPIONs and poly(γ-glutamic acid-*co*-distearyl γ-glutamate) (poly(γ-GA-*co*-DSGA)) were performed according to the previously published protocols [24,25] and described in Supplementary data. Isolation and multiple identifications of ADSCs were conducted according to the method of Luna et al. [26] and described in brief in Supplementary data. The synthesis and characterization of ALTS1C1 cells were derived from primary astrocytes transformed by SV40 large T antigen and serial *in vivo* passage [23] and were deposited in Bioresource Collection and Research Center (BCRC-60582), Taiwan. Dulbecco's modified Eagle's medium (DMEM) and Minimum Essential Medium Eagle-Alpha Modification (α-MEM) medium were purchased from Gibco (USA) and HyClone (USA), respectively. 3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) and hematoxylin and eosin (H & E) were purchased from Sigma-Aldrich (St. Louis, MO). Six- to 8-week-old C57BL/6JNarl male mice were purchased from National Laboratory Animal Center, Taiwan. The approved guides for the care and use of laboratory animals by the Institutional Animal Care and Use Committee (IACUC) of National Tsing Hua University, Taiwan (approved number: IACUC: 10,129) were followed at all time. All surgeries were performed under Zoletil/Rompun anesthesia, and all efforts were made to minimize suffering.

2.2. Preparation of SPION/PTX-loaded NPs (SPNPs)

The SPNPs were prepared using an oil-in-water emulsion technique. PLGA (10.0 mg), oleic acid-coated SPIONs (4.0 mg) and PTX (2.55 mg)

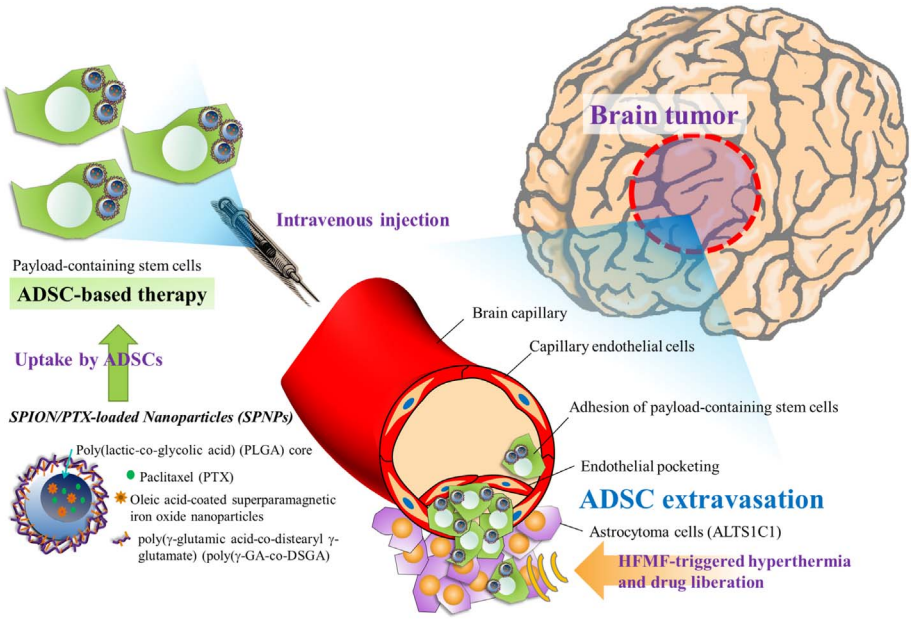

Fig. 1. Schematic description of the ADSC-mediated delivery of SPNPs toward brain tumors for dual-modality treatment of orthotopic astrocytoma.