Nanoparticle-engineered Adipose-derived Stem Cells Enhanced Migration towards Brain Tumors *in vivo*

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Statement of Purpose. Glioblastoma multiforme (GBM) is malignant brain tumor disease and accounts for 23% of all primary brain tumors. The major challenge in the treatment of GBM is its extremely invasive nature and delicate anatomical location in the brain, which precludes surgical removal. Previous research has shown that transplanted neural stem cells (NSCs) possess remarkable tumor tropic migratory capacity [1], but the use of NSCs in clinics is severely limited by the ethical and technical challenges to obtain these cells in human. Unlike NSCs, adipose-derived stem cells (ADSCs) represent an abundant and easily accessible autologous stem cells source. Hypoxia is a common feature of solid tumor niche including GBM, which leads to upregulation of SDF-1α. SDF-1α/CXCR4 axis has been shown to play an important role in mediating mesenchymal stem cell homing to tumor hypoxic core [2]. *The goals of this study* are: (1) to examine the potential of harnessing human *ADSCs* as drug delivery vehicle for targeting GBM in vivo, and (2) examine the ability of CXCR4-overexpressing hADSCs to migrate towards GBM tumor *in vitro* and *in vivo* using biodegradable nanoparticles.

Methods: End-modified poly(β-amino ester)s (PBAEs) were synthesized as previously described [3]. hADSCs were transfected using biodegradable nanoparticles containing PBAE/CXCR4 plasmids. Transfection efficiency was examined using quantitative gene expression, western blot and flow cytometry. SDF-1α secretion and gene expression by hypoxia-conditioned U87MG tumor cells were determined by ELISA assay and RT-PCR. Migration of ADSCs towards hypoxia conditioned medium form brain tumor cell in vitro was measured using a transwell assay. The migration and penetration of CXCR4-overexpressing hADSCs into GBM were examined using a tumor spheroids model in vitro and an intracranial GBM mouse model in vivo.

Results: Transmission electron microscopy and dynamic light scattering confirmed that PBAE efficiently condensed CXCR4 plasmid into polymeric nanoparticles with a diameter about 208 nm and ζ potential about -7.5 mV (data not shown). Both quantitative PCR (**Fig. 1A**) and western blot (Fig. 1B) confirmed that PBAE led to substantial CXCR4 upregulation in ADSCs compared to cells transfected using control (pCDNA). Hypoxia immunostaining showed tumor spheroid formation with a hypoxic core. Confocal scanning of glioma spheroids after 48 h migration confirmed CXCR4-overexperssion enhanced the penetration of ADSCs into GBM spheroid core. When injected intracranially, GFP-luciferase positive GBM cells formed GBM xenograft, as shown by bioluminescence imaging. We observed long-range migration of ADSCs (red) from the left hemisphere (L) across the corpus callosum (cc) towards the GBM xenograft (R) in vivo. CXCR4-overexpression further enhanced ADSC migration and penetration into GBM xenograft in vivo.

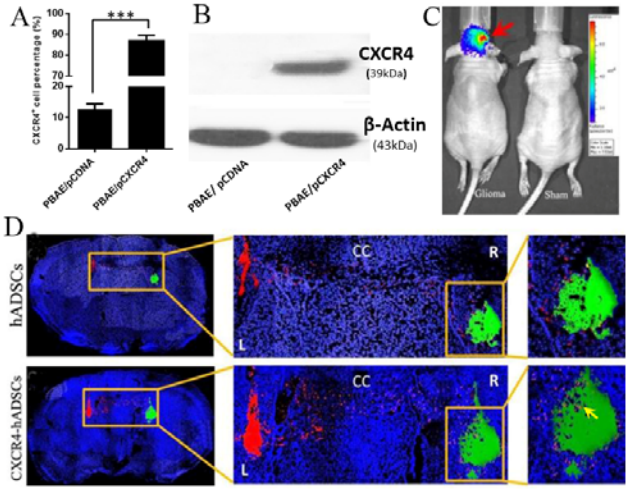

Figure 1. PBAE led to efficient upregulation of CXCR4 in ADSCs, as shown by gene expression (A) and western blot (B). (C) Bioluminescence imaging (BLI) confirmed GBM tumor formation 12 days after inoculation of GFP-Luciferase positive GBM cells. (D) Immunostaining of brain slices confirmed hADSCs exhibited tropism towards GBM *in vivo*, and CXCR4 overexpression further enhanced tumor penetration. hADSCs (red) from the left hemisphere (L) migrated across the corpus callosum (cc) in response to a malignant glioma in the right hemisphere (R) Blue: cell nuclei; Green: GBM tumor cells; Red: migrating ADSCs.

Conclusions: Here we report that ADSCs can migrate towards GBM tumor both in vitro and in vivo. PABE led to efficient transfection of ADSCS and markedly upregulated CXCR4 expression. CXCR4-overexpressing ADSCs further enhanced the tumor tropism and penetration. Our results suggest that non-viral engineered ADSCs could serve as drug delivery vehicles for targeting and eradicating of GBM cells, thereby enhancing the treatment outcomes of this devastating diseases.

References:

[1] Aboody, K. S. PNAS. 2000; 97.23: 12846-12851.
[2] Ehtesham, M. Neoplasia 2004; 6.3: 287-293.
[3] Keeney, M. ACS nano 2013; 7.8: 7241-7250.

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