CXCR4-overexpressing adipose-derived stem cells enhanced brain tumor tropism *in vitro*

Xinyi Jiang[1], Christine Wang[2], Fan Yang[1, 2]
[1] Department of Orthopaedic Surgery, Stanford University, Stanford, CA 94305, USA
[2] Department of Bioengineering, Stanford University, Stanford, CA 94305, USA

Statement of Purpose Glioblastomas are the most common and aggressive primary brain tumors in humans. Despite technical advances in neurosurgery, radiotherapy, and chemotherapy, the prognosis of most patients with glioblastomas remains very poor. Residual tumor is characterized by its diffuse and highly infiltrative nature which is inaccessible to surgery and relatively resistant to radiation and chemotherapy, and consequently elicits recurrence of the tumor. Previous research has shown that transplanted neural stem cells (NSC) 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. Recent research suggests that ADSCs possess a homing capacity that allows them to migrate towards disseminated infiltrating brain tumor cells in vivo. SDF-1/CXCR4 axis has been well characterized as one of the major signaling pathways which mediate the hADSCs homing to tumor tissues. Although CXCR4 is highly expressed in mesenchymal stem cells in vivo, their expression is markedly decreased during ex vivo expansion. To enhance hADSCs tropism for glioma cells, in this study we created CXCR4-overexpressing hADSCs using our optimized non-viral gene delivery protocols.

Methods: Human ADSCs (hADSCs) were isolated from fat tissue which obtained from the abdominal fat of a female patient who had undergone a free flap breast reconstruction surgery at Stanford University according the procedures approved and guided under the Stanford Institutional Review Board protocol. End-modified poly(β-amino ester)s (PBAEs) were synthesized as previously described [2]. The developed and optimized biodegradable PBAE nanoparticles encapsulated with plasmid gene were characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) respectively. CXCR4 overexpression in hADSCs after transfection with PBAEs nanocomplex was confirmed with immunostaining and quantified by flow cytometry. To demonstrate CXCR4-engineered hADSCs tropism for adult brain tumor cells in vitro, both of cell monolayer and 3D tumor spheroid migration assay were performed using ChemoTX system.

Results: We confirmed formation of polymeric nanoparticles after mixing PBAE solution and DNA solution. TEM showed the as-formed nanoparticles with diameter of ~ 200nm and ζ potential of -7.5 mV. PBAE nanoparticles resulted in over 63-fold higher CXCR expression in hADSCs than the control (Fig. 1A). Immuostaining confirmed CXCR4 expression on the surface of hADSCs transfected with polymeric nanoparticles, but not in untransfected control (Fig. 1B). When encapsulated in 3D collagen hydrogels containing GBM tumor spheroids, CXCR4-overexpressin ghADSCs demonstrated higher migratory capacity towards GBM tumor spheroids than untransfected ADSCS (Fig. 1C). Pre-incubation of ADSCs with CXCR4 antagonist (AMD3100) significantly reduced such tumor tropism (data not shown), confirming the enhanced migration was due to CXCR4-overexpression.

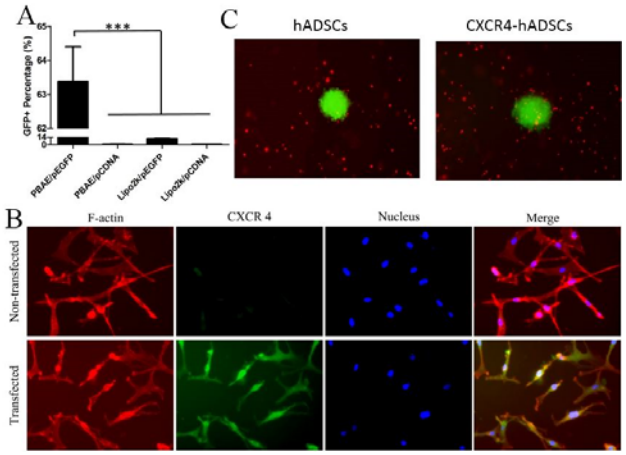

Figure 1. (A) Flow cytometry quantification of transfection efficiency of ADSCs using PBAE nanoparticles. (B) Immunofluorescent staining confirmed PBAE nanoparticles led to extensive CXCR4 expression in hADSCs (bottom row), whereas minimal CXCR4 signals were detected in untransfected ADSCs (upper row). Green: FITC-labeled CXCR4, Red: F-actin, Blue: cell nuclei. Scale bars=20 μm. (C) CXCR4-overexpressing hADSCs showed enhanced migration and penetration toward GBM tumor spheroids in collagen gels in vitro. Red: PKH 26 labeled migrating cells. Green: GFP-positive U87MG tumor cells. Scale bar = 500 μm.

Conclusions: Here we report that PBAE-based polymeric nanoparticles led to efficient non-viral gene delivery to ADSCs, which allowed substantial up-regulation of CXCR4. CXCR4-overexpressing ADSCs exhibited markedly enhanced migration and penetration into GBM tumor spheroids in vitro, and such tropism would be largely abolished using CXCR4 antogonist. These results suggest that non-viral engineered ADSCs could serve as potential drug delivery vehicles to enhance targeting and eradication of GBM.

References:

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Acknowledgments

The authors would like to thank Alliance in Cancer and Gene Therapy for funding this project. X. J. would like to thanks Stanford Child Health Research Institute for postdoctoral fellowship support.