Enhancing Therapeutic Potential of Human Neural Stem Cells

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Statement of Purpose: The control over cell differentiation following transplantation remains a major challenge for stem cell-based therapy, as stem cells display a tendency to either maintain an undifferentiated phenotype or undergo undesired differentiation. For example, only a small percentage of implanted neural stem cells (NSCs) typically differentiated into neurons [1]. Manipulation of the cell transcriptional network is a more effective approach for regulating fate-specification of stem cells [2, 3]. Transcription factors, such as Neurogenin-2 (N-2), Mash1 and NeuroD, when delivered into human embryonic stem cell-derived NSCs through viral transduction, have been shown to induce rapid and efficient production of functional neurons [4]. From a clinical translation perspective, non-viral transfection methods are favored due to the concern of viral vectors regaining reproductive capability or tumor formation through insertional mutagenesis. The objective of this study is to establish a highly effective, cell compatible, nanoparticle-based transfection method to deliver specific transcriptional factors, such as N-2 and Mash1, to human fetal tissue-derived NSCs, and to investigate the neuronal differentiation of transfected cells.

Methods: We employed a poly (β-amino esters) (PBAEs)-based nanoparticle system and developed a transfection protocol using a buffer containing various concentrations of dimethyl sulfoxide (DMSO) to enhance nanoparticle uptake [5]. *In vitro* transfection of PBAEs/DNA nanoparticles was carried out in human fetal tissue-derived NSCs. Cells were seeded in 96-well plates at a density of 1.5×10^4 cells per well one day before transfection. PBAEs/DNA nanoparticles with various PBAE structures and different concentrations of plasmid DNA were added. Two h later, transfection agents with different concentrations of DMSO (0–35%) were applied for 1 min. The second and third transfections with the same protocol were performed at days 5 and 10, respectively. Flow cytometry and WST-1 assay were used to inspect gene transfection efficiency and cell viability, respectively. Immunocytochemistry and western blot assay were applied to evaluate neuronal differentiation of transfected NSCs.

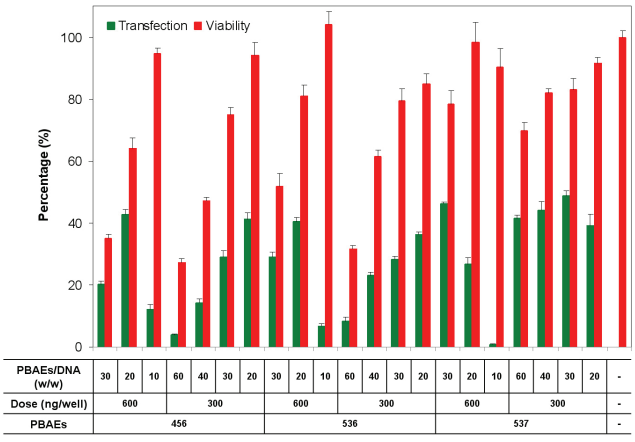

Figure 1. Nanoparticle compositions, including PBAE structures (456, 536, and 537), polymer/EGFP plasmid DNA ratios (10, 20, 30, 40, and 60 w/w), and doses (300 and 600 ng/well) were screened for their transfection efficiencies and cytotoxicities.

Results: Initial screenings of a wide range of nanoparticle conditions were shown in **Figure 1**. The condition for further study was chosen based on high transfection efficiency and low cytotoxicity. For example, PBAE 537 with 20 (w/w) polymer/DNA has obtained transfection efficiency of 35.6 ± 1.9% with high cell viability (91.9 ± 1.4% compared to untreated cells). A 1-min treatment DMSO (25%) brief enhanced the efficiency to 47.7 ± 3.5%. The metabolic activity of the transfected cells was not significantly influenced when DMSO concentration was below 25% (data not shown). Through triple transfections with this protocol, 80% transfection efficiency was achieved at day 10 (**Fig. 2**).

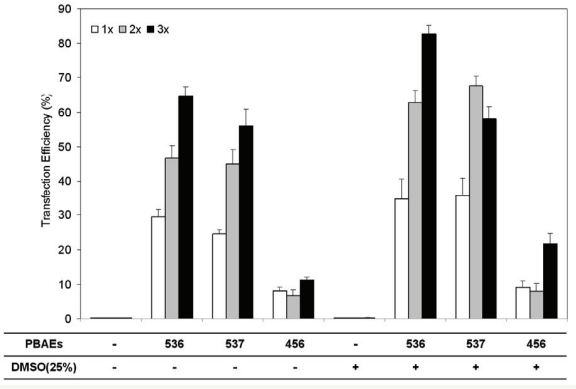

Figure 2. Transfection efficiency of NSCs treated with different PBAE (536, 537, 456) nanoparticles at polymer/DNA ratio of 20 (w/w) and 0.3 μg DNA dose, and a brief treatment with 25% DMSO for 1 min.

Using the optimized transfection protocol, N-2 and Mash1 were successfully transfected into NSCs using PBAE 536 (data not shown). A higher number of βIII tubulin positive immature neurons were differentiated from NSCs transfected with nanoparticles containing plasmids encoding N-2, Mash1, or both at day 10, compared with un-transfected cells (**Fig. 3A**). Western blot analysis confirmed these transcriptional factors not only enhanced neural differentiation with higher level of βIII tubulin expression, but promoted neuron maturation with MAP2 expression (**Fig. 3B**).

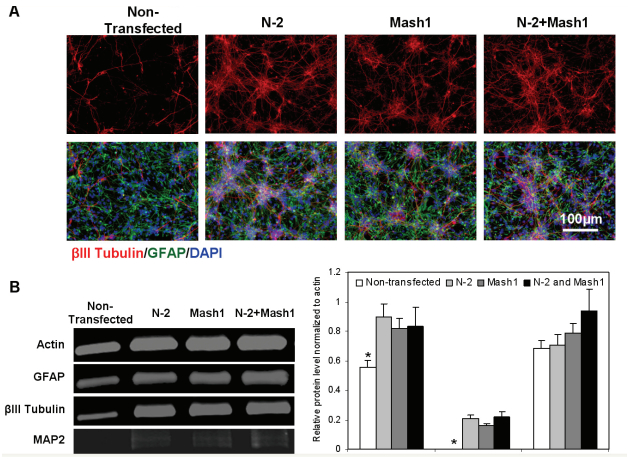

Figure 3. (A) Immunofluorescence staining of NSCs at day 10 after transfection with nanoparticles containing plasmids encoding N-2, Mash1, or their combination. (B) Western blot analysis confirmed transfected NSCs expressed higher levels of βIII tubulin and MAP2 expression compared to un-transfected cells (* $P < 0.05$).

Conclusion: PBAEs-based nanoparticles are a viable strategy to deliver transcriptional factors to NSCs. Transient expression of N-2 and Mash1 can enhance and accelerate the neuronal differentiation for human fetal tissue-derived NSCs.

References: 1. Stem Cell Reviews and Reports, 2012: p. 1-17. 2. Nature, 2011. 476(7359): p. 220-223. 3. Molecular Therapy, 2011. 19(10): p. 1905-1912. 4. Molecular Therapy, 2008. 16(3): p. 450-457. 5. Adv. Healthcare Mater. 2013. 2(3): p. 468–480.