Transient Expression of Neurogenin-2 through Nanoparticles Enhances Neuronal Differentiation of Human Embryonic Stem Cell-Derived Neural Progenitors

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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 (~20%) of implanted neural stem cells (NSCs) typically differentiated into neurons [1]. In general, stem cell fate decision can be regulated by intrinsic and extrinsic factors. Intrinsic regulation involves manipulation of the cell transcriptional network. Several such approaches have been successfully developed for cellular re-programming through resetting the transcriptional network using small molecules and delivery of mRNAs, transcription factors, plasmids or viral vectors encoding key transcription factors [2, 3]. This approach, however, has not yet been widely adopted for directing the reverse process–differentiation of stem and progenitor cells. When compared to extrinsic factors (biophysical and biochemical cues), fate specification of stem cells can be more effectively regulated by the intrinsic factors through manipulation of the cell transcriptional network. It has recently been shown that transfecting human embryonic stem cell (hESC)-derived NSCs with Neurogenin-2, Mash1 or NeuroD using viral vectors, induced 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. In this project, we will establish a highly effective nanoparticle transfection method to regulate the expression level and dynamics of transcription factor Neurogenin-2 in hESC-derived NSCs, and to investigate the effect of Neurogenin-2 expression level and duration on neuronal differentiation.

Methods: We developed an *in vitro* transfection protocol using biocompatible nanoparticles as a transfection agent and brief treatment with a transfection buffer containing 20–35% of dimethyl sulfoxide (DMSO) to promote the nuclear translocation. In *vitro* transfection of linear polyethylenimine (PEI)/DNA micelles was carried out in hESC-derived NSCs. Cells were seeded in 24-well plates at a density of 8×10^4 cells per well one day before transfection. PEI/DNA nanoparticles containing different concentration of plasmid DNA were added to each well. Six hours later, transfection agents with different concentrations of DMSO (0–35%) were applied for brief treatment. Flow cytometry and WST assay was used to check gene transfection. Immnocytochemistry and RT-PCR were used to evaluate Neurogenin-2 expression and cell differentiation.

Results: Using DMSO brief treatment protocol, we have achieved 60%-90% transfection efficiency in several types of stem cells. For example, transgene expression in hESC-derived NSCs can be tuned by adjusting DMSO concentrations (**Fig. 1**). About 3%, 32%, 59%, 68%, 53%, and 46% cells were transfected with nanoparticles followed by 1 min treatment with 0%, 15%, 20%, 25%, 30%, and 35% DMSO, respectively (**Fig. 2A**). The metabolic activity of the transfected cells was not significantly influenced when DMSO concentration was below 25% (**Fig. 2B**). In addition, nanoparticle composition and dose, and transfection time can be tuned to vary transfection efficiency. Using the optimized transfection protocol, Neurogenin-2 was successfully transfected into fetal-tissue-derived hNSCs (52%, **Fig. 3A**). A higher number of βIII tubulin positive immature neurons were differentiated from Neurogenin-2-transfected NSCs at 1 week, compared to the negative control (without transfection) (**Fig. 3B**). Quantitative real time PCR (RT-PCR) also confirmed Neurogenin-2 transfected into hNSCs enhanced βIII tubulin expression (>3-fold change), but inhibited glial fibrillary acidic protein (GFAP) expression (~60 fold change), compared to the negative control (**Fig. 3C**).

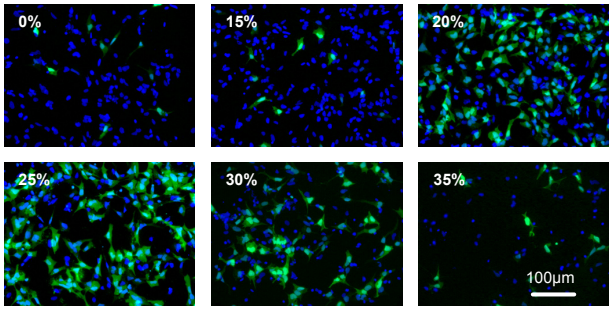

Figure 1. Transgene expression in hESC-derived NSCs mediated by EGFP plasmid containing nanoparticles at 0.1 μg/cm^2 DNA dose with treatment of DMSO of different concentrations: 0%, 15%, 20%, 25%, 30%, and 35%.

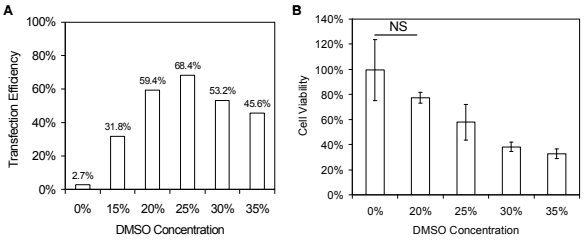

Figure 2. (A) Percentage of cells transfected quantified by FACScan analysis as a function of buffer concentration at 24 h after transfection. (B) Cell viability examined by WST-1 assay as a function of buffer concentration at 24 h after transfection.

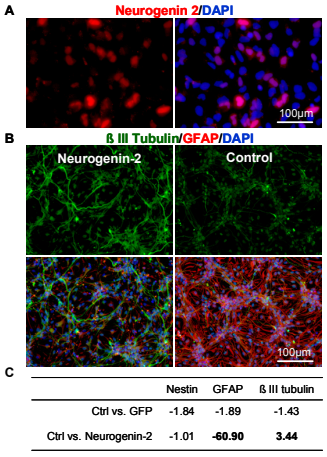

Figure 3. (A) Neurogenin-2 expression in fetal-tissue-derived hNSCs mediated by DNA nanoparticles at 0.1 μg/cm^2 plasmid dose with 1 min, 20% DMSO treatment. At day 1, about 52% cells expressed Neurogenin-2. (B) Differentiation of Neurogenin-2-transfected hNSCs at 1 week. Scale bar = 100 μm. (C) Quantitative RT-PCR also confirmed Neurogenin-2 transfected hNSCs showed enhanced βIII tubulin expression and inhibited GFAP expression, when compared to negative control.

Conclusion: Brief treatment with buffer containing 25% of DMSO can significantly promote gene transfection. Transient expression of Neurogenin-2 can enhance and accelerate the neuronal differentiation for fetal-tissue-derived hNSCs.

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.