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Differentiation Potential of Human Induced Pluripotent Stem Cells (iPSCs) to Nucleus Pulposus-Like Cells in Vitro

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Introduction

Autologous or allogeneic cell delivery to the herniated or degenerated intervertebral disk may promote tissue regeneration and arrest degeneration[1–4]. Induced pluripotent stem cells (iPSCs) derived from the patient's somatic cells represent an attractive cell source, having the potential to differentiate into various cell types[5]. To date, no studies have demonstrated that human iPSCs can differentiate into nucleus pulposus (NP) cells. We have previously shown that both human umbilical cord mesenchymal stromal cells and mouse iPSCs were able to display many NP-like cell markers after cultured in a laminin-rich 3D culture system for inducing differentiation[6,7]. The goal of this study is to evaluate whether human iPSCs cultured in this similar laminin-rich environment can also express a unique NP-like cell phenotype.

Materials and Methods

Cell Generation and Characterization iPSCs were generated from human embryonic dermal fibroblasts through transient inducible over-expression of transcription factors (OCT4, SOX2, KLF4 and MYC) by a lentiviral-based gene delivery system (a poly-cystronic vector with the Reverse Tetracycline Transactivator (M2rtTA) and doxycycline). For the derived colonies, immuno-cytochemistry was performed using a pluripotent markers kit (OCT4, SOX2, SSEA-4, TRA1-60, TRA1-81 and alkaline phosphatase, Applied StemCell). Selected iPSC colonies were maintained in culture upon a PMEF feeder layer.

Cell Differentiation Undifferentiated iPSCs were seeded (10^6/well, Transwell) on wells precoated with Matrigel to generate a soft laminin-rich matrix[6,7]. Cells were cultured in conditioned medium collected from porcine NP tissues (DMEM + 1% ITS)[8] for up to 28 days.

Flow Cytometry Analysis Undifferentiated iPSCs were incubated with the antibodies against CD24, CD31, CD90, integrin subunits a3 (CD49c), a6 (CD49f), and b1 (CD29). For intracellular expression of transcription factors (Brachyury, OCT4), cells were permeabilized before incubation with primary antibodies. Cells were analyzed for fluorescence (Accuri C6) to quantify the percent of positively labeled cells.

Immunohistochemistry Differentiated cells were harvested for cryo-sectioning. Cell morphology and proteoglycan synthesis was assessed by histological staining (H&E and Safranin O). Expression of NP markers was evaluated by immunostaining for matrix proteins (type II collagen (COL II), laminin 10 (LM511)), laminin-related receptors (CD239, integrin subunits a3, a6, b1, b4), and other markers (vimentin (VIM), cytokeratin 8 (KRT8), N-cadherin, CD24), as well as non-NP-markers (type I collagen, E-cadherin).

Results

iPSCs proliferated and formed colonies on PMEF feeder layers similar to embryonic stem cells (Fig. 1A). During proliferation, iPSC colonies expressed many typical human pluripotent markers (i.e. SOX2, TRA1-60 in Fig. 1A), and expressed integrin (α3, α6 and β1 subunit) proteins, other NP markers (CD24 and Brachyury), as well as MSC markers (CD29, CD90). Once cultured under soft laminin-rich matrix conditions, iPSCs were shown to adopt a cell clustering morphology. At day 28 of differentiation, iPSCs expressed NP-related matrix proteins (laminin 10, LM511; type II collagen and proteoglycans as indicated by Saf O, Fig. 1B), LM511 specific receptors (CD239, integrin subunit a3, a6 and b4, Fig. 1 B), as well as many NP markers (CD24, vimentin, cytokeratin 8 and N-cadherin, Fig. 1B); it is noteworthy that type I collagen and E-cadherin were not expressed.

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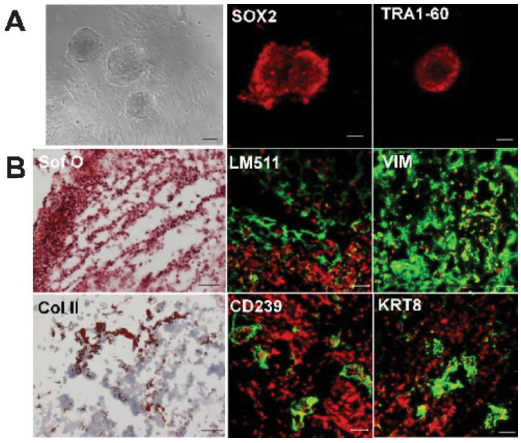
Figure 1 (A) Undifferentiated iPSC colonies cultured on a feeder layer and expressed pluripotency markers (SOX2, TRA1-60, bar = 100 μm). (B) Immunostaining for NP markers, proteoglycans (Saf O), Col II, LM511, CD239, VIM, KRT8 in differentiated iPSCs cultured for 28 days in a Matrigel system (bar = 50 μm).



Conclusion

Our study evaluated a novel cell source that has potential use for cellular therapy in the IVD. Results demonstrate that native NP environment factors (soft laminin-rich matrix and secreted soluble factors) are able to promote human iPSC differentiation into a cell type exhibiting many NP-like markers and morphology. Future studies will focus on selection of NP-genic progenitor cells from these hiPSCs for IVD cellular therapy.

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I confirm having declared any potential conflict of interest for all authors listed on this abstract
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Disclosure of Interest
None declared

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