Trilineage Potency of Human Nucleus Pulposus Cells before and after Cryo-Preservation

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INTRODUCTION:Low back pain (LBP) is a major cause of disability in many countries, affecting more than half a billion people worldwide. A promising and future-oriented approach to treat LBP is cell therapy using stem or progenitor cells. Over the last decade, cells positive for Tie2 and mesenchymal stromal cell markers have been found within the nucleus pulposus (NP) of human intervertebral discs (IVD). However, little is known about the effect of expansion and cryo-preservation on here called “heterogenic” human NP cells (hNPCs) and their stemness in a context of cell therapy for regeneration of the IVD. Therefore, the aim of our study was to expand hNPCs whilst investigating their differentiation potential before and after cryo-preservation and to find and optimal approach to cryo-preserve them.

METHODS:HNPCs from three human trauma patients (32, 55 and 69 years old) undergoing spinal surgery were isolated with a mild two-step digestion protocol. After subsequent expansion until complete confluency, hNPCs were separated and then differentiated into osteogenic, adipogenic or chondrogenic lineages for 21 days or were cryo-preserved for one week at -150°C with five cryo-preservation media (90% fetal bovine serum and 10% dimethyl sulfoxide (DMSO); 90% low glucose medium + 10% DMSO and three commercially available media) to compare their effect on the cell’s viability and differentiation potential. Cell viability was determined with trypan blue and by cytometry employing propidium iodide. The differentiation potential was assessed using histological analysis and qPCR.

RESULTS:HNPCs cultured in osteogenic medium showed a significant ($p<0.01$) higher expression of calcium deposits (up to 11-fold) vs. controls, indicating osteogenic differentiation. Furthermore, evidence for adipogenic and chondrogenic differentiation was observed using histological analysis and determining genes typical for chondrogenic and adipogenic lineages like collagen type 2 (up to 350-fold) or adiponectin (up to 3’700-fold). In addition, most hNPCs maintained their differentiation potential, even after cryo-preservation and independent of the cryo-preservation medium used. The hNPCs’ cell viability after storing for one week at -150°C was very similar for all conditions (~85% cell viability).

DISCUSSION & CONCLUSIONS:The study showed heterogenic hNPCs have trilineage potential and as such possess stem cell characteristics. Therefore, they can potentially be used for future clinical trials concerning cell therapy for IVD regeneration. Furthermore, commercially available cryo-preservation media seem to perform just as well as homemade media in terms of cell viability and maintaining hNPCs differentiation potential.

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