Differentiation of Mesenchymal Stem Cells Towards a Nucleus Pulposus-like Phenotype In Vitro: Implications for Cell-Based Transplantation Therapy

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Basic Science

Abstract

In Brief

Author Information

Objective. Because mesenchymal stem cells can differentiate into chondrocyte-like cells, we ask the question, can mesenchymal stem cells commit to the nucleus pulposus phenotype?

Background. Back pain, a significant source of morbidity in our society, is linked to degenerative changes of the intervertebral disc. Absence of suitable graft tissue limits therapeutic approaches for repair of disc tissue. For this reason, there is considerable interest in developing “tissue engineering” strategies for the regeneration of the nucleus pulposus.

Methods. Rat mesenchymal stem cells were immobilized in 3-dimensional alginate hydrogels and cultured in a medium containing transforming growth factor-β1 under hypoxia (2% O2) and normoxia (20% O2). Mesenchymal stem cells were examined by confocal microscopy to evaluate their viability and metabolic status after labeling with Celltracker green, a thiol sensitive dye, and Mitotracker red, a dye sensitive to the mitochondrial membrane potential. Flow cytometry, semiquantitative reverse transcription polymerase chain reaction and Western blot analysis were carried out to evaluate phenotypic and biosynthetic activities and the signaling pathways involved in the differentiation process.

Results. Under hypoxic conditions, mesenchymal stem cells formed large aggregates and exhibited positive Celltracker and Mitotracker signals. Glucose transporter-3, matrix metalloproteinase-2, collagen type II and type XI, and aggrecan mRNA and protein expression was upregulated, whereas there was no change in the levels of decorin, biglycan, fibromodulin, and lumican. Hypoxia maintained the expression of CD44 (hyaluronan receptor), ALCAM (CD166), and endoglin (transforming growth factor-β receptor). Likewise, expression of β3 and α2 integrin was upregulated. Transforming growth factor-β treatment increased MAPK activity and Sox-9, aggrecan, and collagen type II gene expression. Basal levels of the phosphorylated MAPK isoform ERK1/2, but not p38, were higher under hypoxic conditions than normoxia, and its activation was further augmented by treatment of cells with transforming growth factor-β. In hypoxia, transforming growth factor-β sustained phosphorylated p38 expression for an extended time period. Pharmacological inhibition of ERK1/2 and p38 enzymatic activity resulted in a decrease in Sox-9, aggrecan, and collagen type II mRNA levels.

Conclusions. Our results indicate that hypoxia and transforming growth factor-β drive mesenchymal stem cell differentiation towards a phenotype consistent with that of the nucleus pulposus. Measurement of selected signaling molecules and response to specific inhibitors suggest involvement of MAPK signaling pathways. It is concluded that mesenchymal stem cells could be used to repopulate the damaged or degenerate intervertebral disc.

When cultured in hypoxia and in the presence of transforming growth factor-β, mesenchymal stem cells commit to a nucleus pulposus-like lineage. During this differentiation process, these environmental factors modulate the MAPK signaling pathways. It is suggested that mesenchymal stem cells could be used to repopulate the damaged or degenerate intervertebral disc.

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