Intervertebral disc regeneration

Studies on stem cell niches and cell transplantation

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Göteborgs universitet kommer att offentligen förvaras i lokal Hjärtat, Sahlgrenska universitets sjukhuset, Göteborg, fredagen den 26 november 2010 kl. 0900.

av

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Fakultetsopponent: Professor Jeremy Fairbank, University of Oxford, UK

Avhandlingen baseras på följande delarbeten:

I. Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region. A study in four species.
Henriksson HB, Thornemo M, Karlsson C, Hägg O, Junevik K, Lindahl A, Brisby H. Spine (Phila Pa 1976). 2009 (21): 2278-87.

II. Migrating prechondrocytic cells from stem cell niches supports growth and regeneration of the mammal intervertebral disc. A study in three species.
Henriksson HB, Svala E, Skioldebrand E, Junevik K, Lindahl A, Brisby H. Submitted.

III. Human disc cells from degenerated discs and mesenchymal stem cells in co-culture result in increased matrix production.
Svanvik T, **Henriksson HB**, Karlsson C, Hagman M, Lindahl A, Brisby H. Cells Tissues and Organs. 2010; 191(1): 2-11

IV. Transplantation of human mesenchymal stems cells into intervertebral discs in a xenogeneic porcine model.
Henriksson HB, Svanvik T, Jonsson M, Hagman M, Horn M, Lindahl A, Brisby H. Spine (Phila Pa 1976). 2009 (2):141-8.

V. Investigations of different cell types and gel carriers for cell based intervertebral disc therapy - *in vitro* and *in vivo* studies.
Henriksson HB, Hagman M, Horn M, Lindahl A and Brisby H. Submitted.

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Low back pain is a common condition in the Western world and disc degeneration (DD) is considered a major cause. DD is characterized by dysfunctional cells and decreased matrix production. The aim of this thesis was to explore normal growth and regeneration in the intervertebral disc (IVD). Further, to test possibilities of cell therapy treatment for DDs.
The methods used include *in vitro*- and *in vivo* experiments. *In vitro* methods were: monolayer, 3D cell cultures and explants models with human mesenchymal stem cells (hMSCs), articular chondrocytes and IVD cells. Cells/ tissues were analyzed for cell proliferation markers; BrdU, KI67, migration markers: β1-INTEGRIN, SNAIL-homolog-1 (SNAI1), SNAIL-homolog-2 (SLUG), progenitor/stem cell markers: STRO1, C-KIT, Notch1, CD105 and chondrogenic lineage markers: GDF5 and SOX9, matrix markers: COLLAGEN I and II, glycosaminoglycans, AGGRECAN by biochemical methods, flowcytometry, Real-time PCR and microscopy. Disc height was measured with MRI. Results: a potential stem cell niche was identified in the IVD region lateral to the epihyseal plate and in the annulus fibrosus outer region, based on findings of label-retaining cells and presence of cells expressing stem cell/progenitor markers, in young and mature animals. Migrating cells expressing SNAI1, SLUG, β1-integrin and GDF5 and SOX9 around niches were observed. Results from the cell therapy experiments; *In vitro* analyses; 3D co-culture system of hMSC and IVD cells showed an increased COLLAGEN II production. Xenotransplanted cells survived *in vivo* 6 months (porcine IVDs) and produced matrix in hydrogel/MSCs injected IVDs. Taken together, these findings illustrate a normal slow regeneration of the IVD, and that growth and regeneration is presumably supported by progenitor cells deriving from niches adjacent to the IVD. Further, that human IVD cells and MSCs interact positively on matrix production when co-cultured and the survival of transplanted cells *in vivo* support the possibility for cell therapy treatment of DD. These results encourage further studies to arrest IVD degeneration, by stimulation of regenerative mechanisms *in situ* or by cell therapy.

Key words: intervertebral disc, disc degeneration, mesenchymal stem cells, stem cell niche, xenotransplantation, cell therapy

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