Molecular Interaction Between Human Cartilaginous Endplates and Nucleus Pulposus Cells

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Introduction / Scientific Background

Impaired endplate permeability
a possible key contributor to intervertebral disc degeneration
(Horner 2001, Benneker 2004)

Signs of degeneration:
- enhanced matrix degradation:
- increased expression of matrix degrading enzymes (MMPs) and inflammatory cytokines and mediators (Weiler 2002, Roberts 2005 …)

Hypothesis:
Disc matrix degradation is regulated via molecular interactions between the cartilaginous endplate (CEP) and the disc.
Objectives:

(1) Determination of molecular interactions between cartilaginous endplates (CEP) and disc cells (NP)
   - bioassay with CEP samples and nucleus pulposus (NP) cells of the same donor patients (n=6)
   - influence on matrix degradation and inflammation

(2) Characterisation of degenerative changes of endplate biopsies and CEP-cells

Analysis of samples from disc degeneration patients (n=28):

   - histology (n=15) / ultra struktural investigation by TEM (n=8)
   - metabolic activity and differentiation capacity of isolated CEP cells from enplate tissue (n=5)
Methods:

Bioassay

disc donor patients

Cartilaginous endplate (CEP)

Nucleus pulposus (NP) cell isolation

5 mm punches serum-free medium

24 h

CEP-conditioned medium (CEP-CM)

Alginate beads culture chondrogenic medium

serum-free medium

(Immu-no-)Histology

Microarray

Gene expression

0% 25% 50% 75% 100%

IL-1β (10 ng/ml)
Evaluation:

- gene expression
  - after differentiation (day 0 – 21)
  - with CEP-conditioned media
  - with IL-1β (positive control)

Results – gene expression of nucleus pulposus cells

(example of one representative donor)
Results – gene expression of nucleus pulposus cells

- increased expression of matrix metalloproteinases (MMPs) under influence of CEP-conditioned media and with positive-control (IL-1β).
Results – gene expression of nucleus pulposus cells

- increased expression of interleukins (IL-8 and IL-6) under influence of CEP-conditioned media (CEP-CM) and with positive-control (IL-1β).
Results – Summary of gene expression results of all donors

- Decreased expression of aggrecan and collagen type 2 (* p=0.02) (n=6)

- Increased expression of IL-6, MMP3, MMP13 (* p=0.02) and IL-8 (* p=0.04) (n=6)

(#= 100% CEP-CM vs. control medium)
Results – Analysis of conditioned media by Cytokine Array

Analysis of CEP-conditioned media (CM):

• strong signals for MIF and Serpin E1
• lower expression of CD154, IFN-γ, IL-23
• identification of further cytokines (C5/C5a, IL-6, RANTES)

➢ all cytokines are regulators in inflammatory pathways
  (macrophage activation, involved in acute and chronic inflammation processes)
Results – Histology / ultrastructural investigation

paraffin histology (Alcian-blue staining)

weak degeneration

stronger degeneration

¬ CEP samples show varying intensity of proteoglycan staining and signs for cellular degeneration and matrix degradation
Summary / Discussion

Signs for molecular interactions between endplate and nucleus pulposus cells:

- conditioned media of endplate tissue have pro-inflammatory influence on nucleus pulposus cells increased expression of MMP3, MMP13, IL-6, and IL-8 identification of pro-inflammatory cytokines in CEP-CM

- endplate tissue shows donor specific degenerative changes

- cells from different endplate biopsies vary with regard to metabolic activity and differentiation capacity possible correlation with degeneration?

Further investigations of functional regulation mechanism is subject of ongoing investigations!
Thank you for your attention!

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