GENE EXPRESSION PROFILING OF EARLY IVD DEGENERATION POINTS TO DOWN-REGULATION OF CANONICAL WNT SIGNALING AND CAVEOLIN-1 EXPRESSION

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INTRODUCTION

Current regenerative medicine (RM) strategies for intervertebral disc (IVD) degeneration aim at restoring the physiological function of the IVD, using adult stem or progenitor cells, growth factors, and/or gene therapy. However, the biomolecular events involved in IVD degeneration remain largely unexplored. Aging and early degeneration of the IVD involves chondroid metaplasia of the nucleus pulposus (NP), which is characterized by the replacement of notochordal cells (NCs) by chondrocyte-like cells (CLCs).

AIM

The aim of the study was to investigate the biomolecular signaling pathways involved in the process of early IVD degeneration with a view to develop new regenerative strategies.

THE UNIQUE ADVANTAGES OF THE CANINE MODEL

Like humans, dogs suffer from spontaneous IVD degeneration characterized by similar macroscopic, histopathological, and biochemical changes. Chondrodystrophic and non-chondrodystrophic dog breeds with specific differ with regard to IVD onset of degeneration and IVD disease.

Chondrodystrophic (CD)

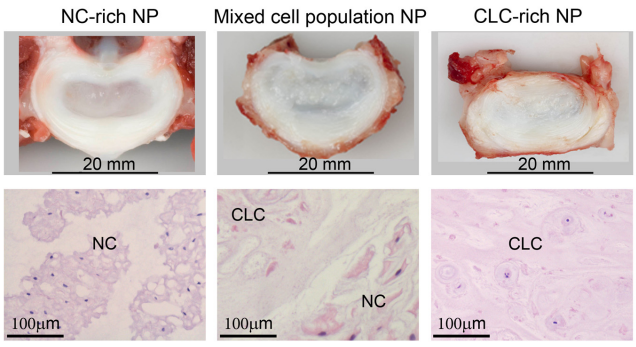
- Early onset
- NCs are replaced by CLCs by 1 year of age
- All spinal levels

Non-chondrodystrophic (NCD)

- Late onset
- NCs remain predominant cell type until late in life
- Selected spinal levels ("wear and tear")

MATERIALS AND METHODS

Cadaveric spines were collected from five NCD dogs and six CD dogs and the IVDs were histopathologically classified prior to assignment of the samples in one of the following groups:
1) healthy NP tissue containing only NCs (NC-rich),
2) NP tissue with a mixed population of NCs and CLCs (Mixed),
3) NP tissue containing solely CLCs (CLC-rich).

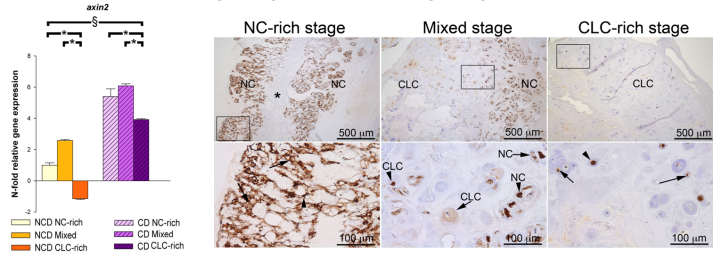


A two-color DNA microarray with a reference experiment design was performed on 44 k Canine Gene Expression Microarrays V1 (G2519F, Agilent Technologies) for both CD and NCD dogs. Microarray data was validated by qPCR, and mechanistically explored by immuno-histochemistry, primary NC culture, and specific KO mice.

KEYPOINTS: RESULTS & DISCUSSION

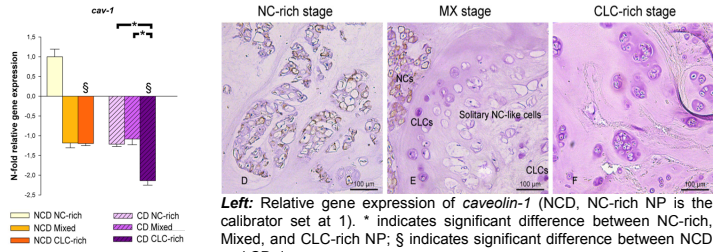
❑ Early IVD degeneration involves several signaling pathways, including Wnt signaling/cytoskeletal remodeling.

❑ Canonical Wnt signaling activity is significantly decreased in early IVD degeneration. Chondrodystrophic and non-chondrodystrophic dogs, which show loss of NCs and IVD degeneration early and late in life, respectively, exhibit clear intracrine differences regarding canonical Wnt signaling.



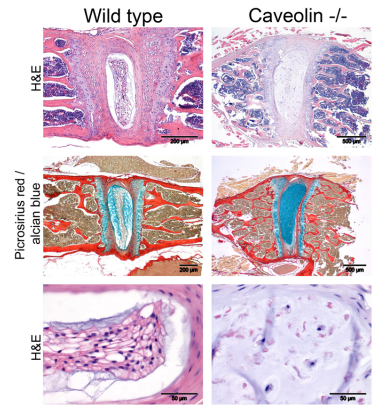
Left: Relative gene expression of *axin2* (NCD, NC-rich NP is the calibrator set at 1)
* indicates significant difference between NC-rich, Mixed, and CLC-rich NP;
§ indicates significant difference between NCD and CD dogs.
Right: NP sections stained for β-catenin, showing nuclear (arrowhead) and cytoplasmic (arrow) staining.

❑ Caveolin-1 was investigated further, given its role in the regulation of canonical Wnt signaling and the marked down-regulation of *caveolin-1* expression at the onset of degeneration. In line with qPCR analysis, caveolin-1 protein is predominantly located in the cell membranes of NCs, and occasionally in their cytoplasm; caveolin-1 protein is seldom observed in CLCs.



Left: Relative gene expression of *caveolin-1* (NCD, NC-rich NP is the calibrator set at 1). * indicates significant difference between NC-rich, Mixed, and CLC-rich NP; § indicates significant difference between NCD and CD dogs.
Right: NP sections stained for caveolin-1.

❑ The essential physiological role of caveolin-1 in the preservation of NCs is illustrated by the IVD phenotype of caveolin-1 KO mice. The absence of caveolin-1 coincides with loss of NCs and early IVD degeneration in caveolin-1 KO mice.



Unlike NP from wild-type mice, NP from caveolin-1 KO mice show relatively few healthy NC clusters; most NP cells lack the morphological characteristics of NCs and show signs of apoptosis, the NP contains an abundance of intercellular chondroid matrix, similar to the CLC-rich NP.

TRANSLATIONAL RM IMPLICATIONS

Caveolin-1 replacement therapy, employing caveolin mimetic peptides may be a new RM strategy to be implemented in the regeneration of the degenerated IVD.

CONCLUSION

Early IVD degeneration involves significant down-regulation of canonical Wnt signaling and Caveolin-1 expression, which appears to be essential to the physiology and preservation of Notochordal Cells.