IS THE NOTOCHORD CELL THE KEY TO INTERVERTEBRAL DISC HOMEOSTASIS?

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Introduction:
There is no biological explanation for the disparity in people who do and do not develop degenerative disc disease in the absence of trauma. The canine species is a case in point with respect to factors that may have a genetic link. Nonchondrodystrophic dogs maintain their notochord cells for many years and are not known to develop degenerative disc disease, whereas other species of purebred dogs such as beagles (the chondrodystrophic breeds) do develop degenerative disc disease. The disparity in clinical and pathological change in the disc when comparing human disc disease to the disc of species with notochord cell preservation is a significant finding which suggests a critical physiological difference in the cellular configuration and cell signaling within the disc nucleus.

Intervertebral disc integrity may be dependent upon factors produced by notochord cells present within the nucleus of mongrel dogs that interact with the disc matrix.

Methods: Canine notochord cells (obtained from mongrel dogs) were cultured within alginate beads in serum deficient conditions to produce notochord-enriched media (NCEM). NCEM was used to culture bovine disc chondrocytes, from which we evaluated proteoglycan production (PG), cell proliferation and gene expression of proteoglycan species of interest. We extracted proteoglycans with Guanidine HCl after first culturing the bovine disc chondrocytes with NCEM. Next, we used column chromatography and scintillation counting to assay PG production. ³H Thymidine incorporation, cell harvesting and scintillation counting were used to assay cell proliferation. The protein content of NCEM was evaluated via SDS-PAGE. Aggrecan, versican, hyaluronan synthase and CD44 receptor gene expression was evaluated with RT-PCR. Peptide sequencing of NCEM was performed by MS/MS mass spectroscopy.

Results: Proteoglycan production via column chromatography and scintillation counting yielded a dose dependent relationship between proteoglycan production and NCEM concentration (figure 1).

³H thymidine labeling resulted in a four-fold increase in cell proliferation under all NCEM concentrations, but did not reveal a dose dependent relationship (figure 2).

RT-PCR has demonstrated increased gene expression for aggrecan, CD44, and hyaluronan synthase in chondrocytes cultured with NCEM as compared with DMEM only.

Discussion: Canine NCEM contains soluble factors that up regulate gene expression for important proteoglycans as well as the proteoglycans themselves in bovine chondrocytes. This up regulation is dose dependent, though cell proliferation does not appear to share such a relationship: suggesting a specific anabolic role for the NCEM that appears to regulate some element of chondrocyte homeostasis. A clear difference in gene expression of the important aggregating proteoglycan ‘aggrecan’ plus other essential molecules such as hyaluronan synthase and the CD44 receptor were evident in chondrocytes cultured with DMEM only vs NCEM.

Conclusions: Notochord cells and their products offer the opportunity for future novel therapeutic options in the treatment of degenerative disc disease. Identification of the nature and identity of the factors that seem to offer ‘rescue’ of chondrocyte proteoglycan production may lead to new approaches in the treatment of the inexorable progression of chondrocyte matrix degeneration.

Acknowledgements: The authors gratefully acknowledge The Canadian Arthritis Network.

Figure 1: ³⁵S sulfate incorporation vs NCEM ‘dose’

Figure 2: ³H thymidine incorporation vs ‘dose’ of NCEM