NON-CHONDRODYSTROPHIC CANINE NOTOCHORD CELLS SEGRETE CONNECTIVE TISSUE GROWTH FACTOR AND UP REGULATE PROTEOGLYCAN GENE EXPRESSION IN INTERVERTEBRAL DISC CHONDOCYTES

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INTRODUCTION: Non-chondrodystrophic (NCD) dogs maintain large populations of notochord cells within their intervertebral discs for many years and are not known to develop degenerative disc disease until much later in life. Chondrodystrophic breeds develop disc disease much earlier and they have a paucity of such notochord cells. Upregulated proteoglycan production has been demonstrated in chondrocytes cultured with conditioned medium obtained from non-chondrodystrophic canine notochord cells (NCCM)\textsuperscript{1,2}. We have previously reported upregulation of aggrecan, versican and hyaluronic acid synthase-2 genes as a consequence of treatment of chondrocytes with NCCM\textsuperscript{1}. We have also previously reported that NCCM contains connective tissue growth factor (CTGF or CCN-2)\textsuperscript{3}. CCN-2 is a recently discovered growth factor that is ubiquitous and atypical. The receptor for CCN-2 has never been identified and it’s signaling and mode of signal transduction is unknown apart from suggestions that CCN-2 signals via cell surface integrins. In some tissues CCN-2 is induced by TGF-β, however CCN-2 is also an autoinducer. CCN-2 has been shown to stimulate healing of defects in articular cartilage and has been hypothesized to be of potential utility in the treatment of damage to articular cartilage\textsuperscript{4}. Since we have identified the presence of CCN-2 in NCCM, we chose to examine aggrecan gene expression as a function of culturing bovine intervertebral disc-derived chondrocytes with known doses of recombinant human CTGF as well as NCCM and minimal media devoid of anabolic substances (DMEM).

METHODS: Canine notochord cells (obtained from NCD dogs) were cultured within alginate beads in serum-deficient conditions (DMEM) to produce notochord cell conditioned medium (NCCM). Bovine disc-derived chondrocytes were obtained and cultured for three days in totally serum-free medium and then cultured for 24 hours with DMEM, NCCM and DMEM + doses of 50, 100, and 200 ng/mL as well as NCCM + 200 ng/mL of recombinant human CTGF (rCTGF or rCCN-2). After 24 hours, total RNA was extracted (Trizol) from the chondrocytes, the RNA was quantified at OD\textsubscript{260/280} and then 1 ug total RNA was reverse transcribed. The resulting cDNA obtained was subjected to semi-quantitative RT-PCR using aggrecan specific primers (Table 1). Gene expression was normalized using the house-keeping enzyme HPRT (Table 1).

RESULTS: Aggrecan gene expression was modestly produced in the chondrocytes treated with DMEM only, however in a dose-dependent relationship, aggrecan gene expression was robustly increased by rCTGF and NCCM as compared to DMEM. NCCM induced chondrocyte aggrecan gene expression at a similar level to between 100 and 200ng/mL rCTGF. NCCM +200ng/mL CTGF resulted in similar upregulation of aggrecan as 200ng/mL alone.

Table 1

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<th>Gene</th>
<th>Primer sequence</th>
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<tr>
<td>Aggrecan</td>
<td>5' GAC AGA TGA TTC AGA GGC AAC</td>
</tr>
<tr>
<td></td>
<td>3' CAG GCA ATT GAT CTC GTA TC</td>
</tr>
<tr>
<td>HPRT</td>
<td>5' CTC ATG GAC TAA TTA TGG ACA</td>
</tr>
<tr>
<td></td>
<td>3' TAC GTC TGA AAC GAA CGG AAC</td>
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DISCUSSION & CONCLUSIONS: Notochord cells secrete connective tissue growth factor (CTGF). CTGF (CCN2) is a recently discovered multifunctional growth factor known to induce proteoglycan and type II collagen production, cell proliferation and differentiation in chondrocytes. There is no difference between CTGF gene expression in non-chondrodystrophic or chondrodystrophic canine notochord cells suggesting a possible role for CTGF as an anabolic factor within the disc nucleus that is to at least some degree, dependent upon the population of notochord cells within the disc nucleus.

REFERENCES:

3 Erwin, W.M., Inman, R.D., Spine (In press)

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