The Extracellular Calcium-Sensing Receptor (CaSR) as a Regulator of Cartilaginous End-plate Intervertebral Disc Calcification

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Introduction: Intervertebral discs (IVDs) are virtually avascular tissue relying on the diffusion of nutrients and metabolites from blood vessels in the vertebral bodies that partially penetrate the subcondral plate, and terminate at the cartilaginous end-plate (CEP). Calcification of CEP is viewed as a catalyst to IVD degeneration, as it impedes nutrient diffusion to the disc. Currently, biological repair strategies of the degenerative disc involving supplementation of growth factors are being pursued. However, improving disc nutrition is essential for their success and it is possible that optimal disc repair may require both suppression of calcification and growth factor supplementation.

The extracellular calcium-sensing receptor (CaSR) is a G protein-coupled receptor (GPCR). CaSR is the principle regulator of parathyroid hormone (PTH) synthesis and secretion, and functions to maintain calcium homeostasis. Recent evidence suggests that CaSR plays a direct role in modulating vascular calcification, however, its function in the calcification of other tissues, such as the IVD, remain unknown. We have previously shown that human Link N (DHLSDNYTLDHDRAlH) can act as a growth factor and stimulate the synthesis of proteoglycans and collagen by IVD cells in vitro and improve disc height and proteoglycan levels in vivo in a rabbit model of IVD degeneration. We also showed recently that Link N can downregulate hypertrophic and osteogenic differentiation of human mesenchymal stem cells. However the role of Link N in IVD calcification remains unclear. We propose that CaSR is a regulator of calcification in CEP, and modulation of its expression and function by Link N may modulate the process.

Methods: Human CEP were obtained from donor lumbar spines of Thompson grade 2, 3 and 4 through organ donations within 24 hs after death. CEP cells were isolated from tissue by sequential digestion with Pronase followed by Collagenase. Cells were expanded for 7 days under standard cell culture conditions. Immunohistochemistry was performed on CEP tissue to validate the grade and expression of CaSR. Free calcium levels were also measured and compared between grades. Immunocytochemistry, Western blotting and MAPK signaling were performed on cultured CEP chondrocytes of various grades to demonstrate expression of CaSR and calcification markers, including the function of the receptor. CEP cells of various grades were cultured in either high calcium (5 mM Ca^{2+}), CaSR allosteric agonist (cinacalcet, 1 uM), CaSR allosteric antagonist (antagonist, 1 uM), Link-N (1 ug/uL), or regular medium (1 mM Ca^{2+}) for 7 days, and monitored for changes in calcification potential and CaSR expression.

Results: Immunohistochemistry on CEP tissue revealed an increase in the expression of CaSR that paralleled the degree of IVD degeneration (Fig. A). Immunocytochemistry on isolated CEP cells demonstrated an upregulation of CaSR and type X collagen in Thompson grades 2 to 4 discs. The expression of matrix metalloproteinase (MMP)-13 and CaSR (Fig. B) were significantly higher in CEP cells from degenerated discs, as determined by Western blotting. The magnitude of CaSR signaling was also significantly enhanced in grade 4 versus grade 2 CEP cells. The expression (Fig. C) and function of CaSR was significantly decreased when cells were incubated for 7 days with Link-N. When CEP cells were incubated for 7 days in either high calcium or cinacalcet, MMP-13 expression was increased.

Discussion: CaSR has been described as a mediator in ectopic calcification, and its expression is upregulated in CEP of degenerative discs. Moreover, CaSR expression could be modulated following administration of agonist, antagonist, or Link-N. We provide evidence that CaSR upregulation may be a hallmark of CEP calcification.

Significance: Inhibition of CaSR or modulation of its expression by Link-N may be used as potential therapeutics in CEP calcification and hence IVD degeneration. Therefore, an understanding on the mechanism(s) of CEP calcification is key to designing specific therapies to prevent pathological calcification.

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References:
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(A) CEP cartilage from Thompson grade 2 and 4 discs demarcating CaSR expression and alizarin red staining for calcified tissue.

(B) Western blotting for CaSR expression in CEP cells isolated from grades 2, 3 and 4 discs.

(C) CaSR expression is modulated following 7 day treatment in growth media containing 1 mM Ca²⁺, 5 mM Ca²⁺, agonist (1 µM), antagonist (1 µM) or Lnk-4 [1 µg/ml].