The Role of the Extracellular Calcium-Sensing Receptor in Osteoarthritis

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Disclosures:

Introduction: Osteoarthritis (OA) affects millions of individuals, and, although, the mechanism(s) of OA onset is unclear, the biological outcome is cartilage degradation. The degradation of cartilage is typified by the progressive loss of extracellular matrix components, such as the proteoglycan aggrecan, and type II collagen, resulting from the upregulation of catabolic enzymes aggrecanases a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS-4 and 5) as well as matrix metalloproteinases (MMPs) and type X collagen. There is currently no treatment that will prevent or repair joint damage. The extracellular calcium-sensing receptor (CaSR) is a G protein-coupled receptor (GPCR). It is the principle regulator of parathyroid hormone (PTH) synthesis and secretion, and functions to maintain calcium homeostasis. Recently, tissue-specific and inducible chondrocyte knockouts of CaSR have provided evidence for an important role in chondrogenesis, however, its role in human chondrocytes, and more specifically, its contribution to the pathology of OA remains unclear. We aim to determine the role of CaSR in the pathological development of OA.

Methods: Articular cartilage was isolated from 5 donors undergoing total hip replacement. Cells were recovered from the cartilage of each femoral head or knee by sequential digestion with Pronase followed by Collagenase, and expanded in DMEM supplemented with 10% heat-inactivated FBS. OA and normal articular chondrocytes (PromoCell, Heidelberg, Germany) were transferred to 6-well plates in culture medium containing various concentrations of calcium (0.5, 1.0, 2.5, and 5.0 mM CaCl2), allosteric agonist (cinacalcet, 1 uM) and CaSR antagonist (antagonist, 1 uM), and replenished every third day for a duration of 7 days. Cartilage explants were prepared from the same donors, and included cartilage with the cortical bone approximately 1 cm2 in dimension; bovine articular cartilage (6 months) was used as a control. Explants were cultured in the above mentioned media; however, the incubation period was extended to 21 days. Immunohistochemistry was performed on cartilage explants to measure CaSR expression and markers of OA (ColX, MMP-13, IL-1R). The sulfated glycosaminoglycan (GAG, predominantly aggrecan) content of cartilage was analyzed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay, and aggregan fragmentation was determined by Western blotting using antibody targeted to its G1 domain. Western blotting was also performed on cell lysate from both OA and normal chondrocytes to measure CaSR expression, MAPK and MMP-13 activity.

Results: CaSR expression was markedly increased in superficial cartilage of OA donors when compared to bovine articular cartilage (Fig. 1A). Proteoglycan content of the cartilage explants decreased as a function of calcium, as determined by the DMMB assay and Western blotting of aggregan. The expression of CaSR was also higher in OA versus normal human articular chondrocytes (Fig. 1B), a property that was reflected by the degree of MAPK activity following stimulation with calcium. When normal chondrocytes were cultured in medium supplemented with high calcium (5 mM Ca2+), a modest increase in the activity of MMP-13 was observed; however, this activity was exaggerated in OA chondrocytes (Fig. 1C). When compared to normal cells, OA chondrocytes demonstrated a reduced capacity to synthesize proteoglycan. This property was reversed when OA cells were incubated with the CaSR antagonist.

Discussion: We provide evidence that prolonged activation of CaSR in human chondrocytes decreases proteoglycan synthesis and degradation by increasing the activity of MMP-13.

Significance: Inhibition of CaSR may support a role in cartilage regeneration.

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References:
(A) Immunohistochemistry of CaSR expression in OA and bovine (6 month) cartilage

(B) Western blotting of CaSR in lysate from OA and normal articular chondrocytes

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<th>Norm (Knee)</th>
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