studies such as these may suggest different therapeutic targets for treatment of later-stage OA.

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AML1 IS HIGHLY EXPRESSED IN HUMAN OSTEOARTHRITIC CARTILAGE AND RESPONDS TO MECHANICAL LOADING

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Purpose: AML1 is known to be essential for the normal development of hematopoietic stem cells. Pathologically, AML1 is one of the most frequent targets of chromosome translocations associated with leukemia. Although its role in chondroprogenitor cells was established early on, few papers have since emerged more clearly defining its role in articular cartilage and more importantly, osteoarthritis (OA). In this study, we have undertaken to examine the functional expression patterns of AML1 in normal vs. OA articular cartilage and its role in cartilage mechanotransduction, a feature shared by other genes involved in cartilage homeostasis and OA.
Methods: Medial vs. lateral human OA cartilage samples were analyzed and compared through immunoblot analysis for the relative expression levels of AML1 on a knee-by-knee basis. Bovine articular cartilage discs were harvested from the knees of newborn male calves and compressed *ex vivo* in compression chambers, to test whether AML1 is a mechanically-regulated gene. Samples were then subjected to AML1 immunoblot analysis. Normal knee articular cartilage from animal sources (bovine and mouse) as well as human knee OA samples (comparing medial vs. lateral distal femoral compartments) were analyzed IHC localization of AML1.
Results: Fig. 1 shows a significant increase in AML1 protein in the medial compartment of human OA varus knees. Similar results were obtained in n=4 human OA knees. These initial results suggested that AML1 is a potentially mechanically regulable gene product. Therefore, we tested the responsiveness of AML1 expression under increasing strains in bovine articular cartilage (Fig. 2). Results indicated that AML1 is extremely sensitive to load,

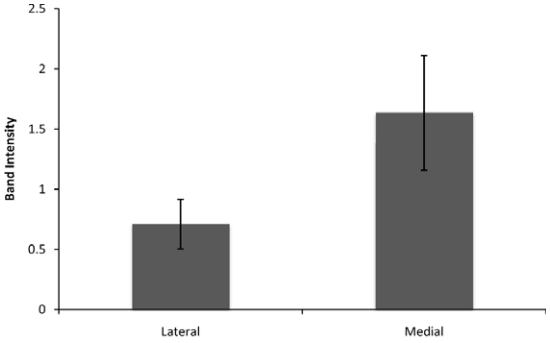

Figure 1. Increased expression of AML-1 in varus human knee OA is restricted to the medial compartment.

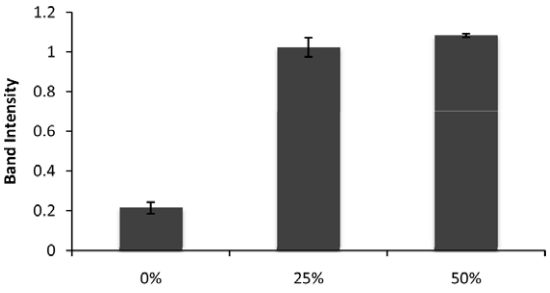

Figure 2. AML-1 is a mechanico-responsive gene in normal bovine articular cartilage.

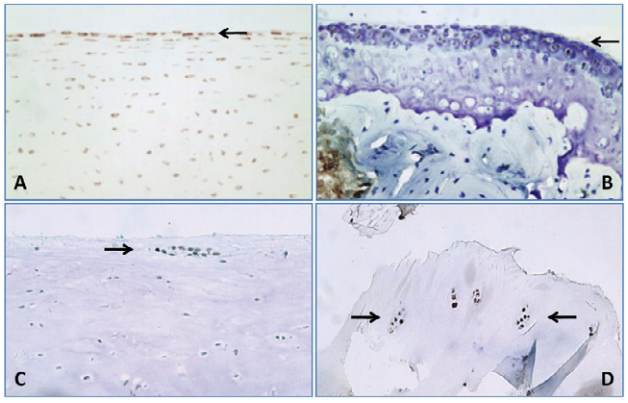

Figure 3. IHC localization of articular cartilage AML-1 expression: bovine (A), mouse (B), human OA: varus-lateral (C), varus-medial (D).

demonstrating a 5-fold increase in AML1 protein expression at 25 and 50% strain values. IHC data (Fig. 3) showed that in normal bovine and mouse cartilage, the highest expression of AML1 is localized in the most superficial zone of articular cartilage. It is interesting to note that it is the superficial zone which experiences the highest degree of tissue strain under mechanical load. Human lateral (varus) OA cartilage showed patchy expression of AML1, but was limited to the superficial zone. Medial OA cartilage showed that AML1 expression was limited to osteoarthritic 'clones' or aggregates of chondrocytes in areas immediately adjacent to focal areas of superficial fibrillation.
Conclusions: Here we show that AML1 protein is normally expressed in a narrow margin of cells at the weight-bearing surfaces of articular cartilage in both human and animal samples. Recent studies have shown that stem cell markers (CD155+/CD166+), indicative of the presence of mesenchymal progenitor cells, also co-exist in the same narrow zone. Given these findings and AML1's known role in cell proliferation, it is interesting to speculate that AML1 may be marking a progenitor cell population localized in surfaces of articular cartilage. In osteoarthritic tissue, AML1 is overexpressed, particularly in the 'non-spared' compartments of malaligned knees. Within this compartmentally-restricted expression pattern another sub-pattern of enhanced expression in chondrocyte clones is seen which could be interpreted as an indication that AML1 plays a role in phenotypic plasticity of chondrocytes in sub-populations of cells in both normal articular and osteoarthritic cartilage.

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POSITIVE REGULATION OF HUMAN OSTEOARTHRITIC CHONDROCYTES UPON ACTIVATION OF THE EphB4 RECEPTOR BY ITS SPECIFIC LIGAND EPHRIN B2

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Purpose: Osteoarthritis (OA) is characterized by cartilage degradation resulting from an increase in catabolic factors and decreased anabolic properties. It has recently been demonstrated that ephrin B2 and its specific receptor EphB4 are involved in the control of bone homeostasis. Our group further revealed that this ephrin system inhibits the pro-resorptive activity of human OA subchondral bone.
To our knowledge, there has been no study on the implication of ephrins in OA cartilage tissue. Thus, we investigated the possible

involvement of these ephrin members on the catabolic/anabolic activities of human OA chondrocytes. More specifically, we looked at the expression level and production of EphB4 receptor and ephrin B2, the activation of EphB4 receptor by ephrin B2 on the modulation of cartilage catabolic/anabolic mediators in the presence/absence of IL-1β, as well as the intra-cellular signaling pathways employed following EphB4 receptor activation. We further investigated factors that could modulate these ephrin members on OA chondrocytes.

Methods: EphB4 receptor and ephrin B2 expression levels and modulation were analyzed by quantitative-PCR and their production by immunohistochemistry. The functional consequences of the ephrin B2-mediated EphB4 receptor activation were investigated by determining the production of some factors involved in the OA process, including IL-1β, IL-6, MMP-1, MMP-2, MMP-9, MMP-13, collagen type II and the receptor PAR-2.

Results: The EphB4 receptor and ephrin B2 ligand are expressed by human normal and OA chondrocytes. Ephrin B2 is produced at similar levels and localized at the superficial zones in both normal and OA cartilage. In contrast, although in normal cartilage the EphB4 receptor is also only localized at the superficial zone, during OA it is observed throughout the cartilage layers with significant ($p<0.01$) increase in both the superficial and deep zones. This coincides with a significant increase in EphB4 receptor gene expression level in OA compared to normal ($p<0.0001$). Modulation experiments performed on OA chondrocytes showed that the receptor is inhibited by IL-1β and TNF-α. Interestingly, EphB4 activation significantly inhibited the gene expression levels of IL-1β, IL-6, MMP-1, MMP-9, MMP-13 and PAR-2, whereas MMP-2 was unaffected. It significantly increased collagen type II α1 expression and also inhibited the IL-1β-stimulated effect on the protein production of IL-6, MMP-1 and MMP-13. EphB4 receptor activation significantly reduced the PI3K/Akt pathway.

Conclusions: Our study is the first to show the presence and role of ephrin B2/EphB4 receptor in human OA chondrocytes. The data support the notion that activation of the EphB4 receptor by ephrin B2 positively impacts the abnormal metabolism of OA cartilage by inhibiting important catabolic factors involved in this disease and at the same time as increasing the anabolic activity. Thus, ephrin B2 could act as a specific therapeutic approach in OA by acting both on cartilage and subchondral bone.

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MODULATION OF OSTEOPROTEGERIN, RANK, RANKL BY HUMAN CHONDROCYTES AND THEIR IMPLICATION IN OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is the most common human joint disease. Recent studies suggest the involvement of osteoprotegerin (OPG), receptor activator of nuclear factor-κB (RANK) and RANK ligand (RANKL) in the abnormal OA subchondral bone metabolism; however, very few studies have looked at these factors in the articular chondrocytes or their functional consequences on these cells. We investigated the expression of OPG, RANK and RANKL on human chondrocytes comparing normal to OA, and the modulation of their production by some catabolic factors. Further, we evaluated on human OA chondrocytes the roles of OPG and RANKL on the production of catabolic/anabolic factors involved in this disease.

Methods: Gene expression level was determined using real-time PCR. The protein production of RANK and RANKL were determined by flow cytometry, and that of OPG by a specific ELISA. Modulation of OPG, RANK and RANKL was determined upon treatment with IL-1β, TNF-α, and PGE_2. The functional consequences were examined following treatment with either soluble RANKL or OPG-Fc (OPG without the heparin binding domain) at 50 and 100 ng/ml, and the production of catabolic factors including cytokines, MMPs and PAR-2, and the anabolic factor collagen type II evaluated.

Results: OPG, RANK and RANKL were all expressed and produced by human chondrocytes. Interestingly, data showed that membranous RANK was produced only by an OA chondrocyte subpopulation (29%) which was localized throughout the cartilage. Compared to normal, the OPG/RANKL ratio was significantly ($p=0.05$) reduced on the OA chondrocytes, whereas the RANK/RANKL ratio was significantly ($p<0.03$) increased. Treatment with catabolic factors showed that OPG production and membranous RANKL levels were significantly enhanced by IL-1β, TNF-α, and PGE_2, whereas membranous RANK was significantly increased only with IL-1β. Administration of soluble RANKL was without effect on the OA chondrocytes. However, addition of OPG-Fc significantly stimulated MMP-13 ($p=0.05$) and PAR-2 ($p<0.04$) production.

Conclusions: Our findings showed that human normal and OA chondrocytes express and produce OPG, RANK, and RANKL. OA chondrocyte treatment with catabolic factors had variable effects on each member of the triad, but pointed to an increased biological effect of OPG. Interestingly, OPG, but not RANK or RANKL, appears to be involved in the progression of OA by significantly increasing two catabolic factors involved in cartilage pathophysiology, MMP-13 and PAR-2.

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LOSS OF PITX1 TRANSCRIPTION FACTOR EXPRESSION IN OSTEOARTHRITIS OCCURS THROUGHT NUCLEAR ACCUMULATION OF PROHIBITIN

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Purpose: Pitx1 is an essential transcription factor for development and maintenance of bone and cartilage specifically in hindlimbs and mandible. Knockout of Pitx1 gene in mouse led to a sever abnormalities in morphogenesis of bone and cartilage tissues whereas Pitx1+/- mice progressively developed with ageing degenerative changes of the knee resembling human OA. These observations prompted us to investigate the role of this transcription factor in OA patients. We have discovered that Pitx1 mRNA and proteins were hardly expressed in human OA articular chondrocytes and cartilage sections when compared to matched control subjects. Recent results from our lab have also shown loss of Pitx1 expression in osteoarthritic (OA) chondrocytes. To understand the mechanism that leads to loss of Pitx1 expression in OA chondrocytes we have investigated the transcriptional regulation of this transcription factor in normal and OA chondrocytes.

Methods: Human Pitx1 promoter gene was cloned upstream of a luciferase reporter gene. Using C28/I2 chondrocyte cell line, we have identified important regions for Pitx1 gene regulation using different complementary approaches (sequencing, mass spectrometry, DNA pull-downs method, ChIP assay and transient