Results: Loss of α5 integrin expression was related to chondrocyte dedifferentiation in the osteoarthritic articular cartilage, while α1, α2 and α3 integrin expression increased in fibrous areas in osteoarthritic cartilage. The αV integrin was expressed in hyperthrophic cartilage and increased in the articular cartilage of osteoarthritic rats, where bone was developed and neovascularization was observed. The effect of GDF-5 (growth differentiation factor 5) and BMP-7 (bone morphogenetic protein 7) on integrins expression during chondrocytes differentiation was also analyzed. A micromasses culture system of mouse embryo mesenchymal cells, were treated with GDF-5 or BMP-7 for 4 and 6 days. The expression of integrins was analyzed by immunohistochemistry. GDF-5 induced the expression of α5 sub-unit while BMP-7 induced the expression of αV sub-unit. This suggests a switch in the signal for chondral differentiation towards hypertrophy, where GDF-5 could keep the state of the articular chondrocyte and BMP-7 would induce hypertrophy. The typical chondrocyte phenotype in the articular cartilage is preserved due to the presence of Indian hedgehog (Ihh), which is dependent on α5 integrin and GDF-5 to keep the articular cartilage and prevent hypertrophy. This was supported by decrease of Ihh expression in articular cartilage during OA. Treating mouse knees organ cultures with Cyclopamine an Ihh signaling inhibitor, articular cartilage ossification was observed similarly with OA rat knees, probably by a process apparently similar to endochondral ossification.
Conclusions: The typical chondrocyte phenotype in the articular cartilage is preserved due to the presence of Indian hedgehog (Ihh), which is dependent on α5 integrin and GDF-5 to keep the articular cartilage phenotype and prevent hypertrophy and OA.

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ALARMINS S100A8 AND S100A9 ELICIT AN ENHANCED CATABOLIC RESPONSE IN CHONDROCYTES FROM OSTEOARTHRITIS PATIENTS THAT IS TOLL LIKE RECEPTOR 4 DEPENDENT

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Purpose: S100A8 and S100A9 are two Ca^{2+}-binding proteins classified as damage associated molecular patterns (DAMPs) or alarmins that are found in high amounts in the synovial fluid of osteoarthritis (OA) patients. Previously, we found that S100A8 and S100A9 are associated with cartilage degradation in murine collagenase-induced OA. We also showed that S100A8 and S100A9 stimulate expression and activity of matrix metalloproteinases (MMPs) and pro-inflammatory cytokines in murine chondrocytes. In the current study, we investigated whether and via which receptor S100A8 and/or S100A9 can have a catabolic effect on chondrocytes from OA patients.
Methods: Using immunohistochemistry, we stained for S100A8 and S100A9 protein, MMPs and a cartilage breakdown epitope specific for MMPs (VDIPEN) in cartilage from OA donors. Isolated chondrocytes or explants from OA and non-OA donors were stimulated with S100A8 and/or S100A9. mRNA and protein levels of MMPs, cytokines and cartilage matrix molecules were determined with RT-qPCR and Luminex. For receptor blocking studies, specific inhibitors for Toll-like receptor 4 (TLR4) (intracellular TAK242) and RAGE and carboxylated glycans (blocking antibodies) were used.
Results: In cartilage of OA patients, localisation of S100A8 and S100A9 protein was found close to chondrocytes and was associated with proteoglycan (PG) depletion, MMP1 and -3 and VDIPEN expression.
Stimulation of chondrocytes with S100A8 and S100A9 caused a significant upregulation of MMP1, -3, -9 and -13 at the mRNA (4.7-fold mean increase) and the protein level (3.0-fold mean increase). Moreover, S100A8 and S100A9 caused a huge increase in cytokine and chemokine expression. IL-6, IL-8 and MCP-1 were all greatly increased by S100A8 and S100A9 at both the mRNA (19.7-fold mean increase) as well as the protein level (28.8-fold mean increase). Thereby, the expression of anabolic markers (aggrecan and collagen type II) was significantly reduced at the mRNA level (2.7-fold mean decrease). Together, upregulation of MMPs and cytokines and downregulation of production of cartilage matrix molecules favor cartilage loss by the OA chondrocyte.
Blocking TLR4 inhibited the upregulation of MMPs, IL-6, IL-8, MCP-1 and collagen type II by S100A9 in OA chondrocytes. In contrast, the blocking of carboxylated glycans and RAGE did not alter the S100-effects. Finally, the catabolic effect of S100A8 and S100A9 was significantly more pronounced in chondrocytes from OA patients when compared to non-OA. TLR4 mRNA expression was enhanced in OA chondrocytes, which might be the underlying mechanism of increased sensitivity.
Conclusions: S100A8 and S100A9 have a catabolic effect on human chondrocytes that is dependent on TLR4. OA chondrocytes are more sensitive for S100-stimulation than normal chondrocytes.
This study underlines the potential of S100A8 and S100A9 as mediators of cartilage damage during OA.

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F-SPONDIN MEDIATES CATABOLIC EFFECTS ON ARTICULAR CHONDROCYTES VIA ITS THROMBOSPONDIN REPEAT (TSR) DOMAIN

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Purpose: We have previously shown that the thrombospondin-related, extracellular matrix protein, F-spondin, is upregulated in osteoarthritis (OA), and induces the production of catabolic mediators, PGE2 and MMP-13 in cartilage explants in a TGF-β dependent manner. In this study, we characterize the role of individual protein domains of F-spondin in modulating its catabolic effects in chondrocytes.
Methods: OA chondrocytes were harvested from tibial cartilage obtained from OA patients with end-stage disease undergoing knee replacement surgery. Transient transfections were performed using TransIT-LT1 reagent (Mirus) and cDNAs encoding full length (FS1) or a truncated C-terminal (FS7) portion of F-spondin coding sequence. Levels of TGF-β1 (R&D systems) and PGE2 (Cayman Chemical) were determined by ELISA, MMP-13 expression was measured by qRT-PCR (Applied Biosystems). TGF-β activity was also measured in conditioned media supernatants by incubation with mink lung epithelial cells (MLEC) expressing a TGF-β-responsive luciferase reporter.
Results: Consistent with our previous observations of the effects of intact F-spondin in cartilage explants, overexpression of FS1 in cultured OA chondrocytes from 3 patients increased expression levels of MMP-13 ~100% compared to mock-transfected controls. Similarly, FS1 also increased PGE2 levels by 25% ($p < 0.05$). Both effects could be mimicked by transfection with a construct encoding only the c-terminal TSR repeat domain FS7; MMP-13 and PGE2 levels were elevated by FS7 above control vector transfected cultures, ~50% ($p < 0.005$) and 71% ($p < 0.05$), respectively. Since the F-spondin TSR domain harbors consensus sequences for latent TGF-β activation (WxxW and KRFK), we tested whether F-spondin overexpression increases TGF-β levels and activity in chondrocyte cultures. In OA chondrocytes, TGF-β levels in culture supernatants were increased 30% by FS1 and 70% by FS7. Similarly, measurement of active TGF-β using MLEC reporter cells showed that FS1 and FS7-transfected culture media supernatants stimulated luciferase activity 30% and 100%, respectively ($p < 0.05$). Evidence of enhanced activation of TGF-β was also demonstrated by increased SMAD signaling. Immunoblot of FS treated chondrocytes showed increased phosphorylation of SMAD 1, 5, 8 relative to mock transfected cells. Similarly, both FS1 and FS7 stimulated expression of a SMAD-luciferase reporter in OA chondrocytes.
Conclusions: Our data provide evidence that F-spondin, via its TSR domain, can act as a latent TGF-β-activating protein and enhance the catabolic activity of chondrocytes via induction of MMP-13 and PGE2. The TSR domain of F-spondin may therefore represent a novel therapeutic target for slowing cartilage breakdown in OA.

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INHIBITING CALCINEURIN ACTIVITY UNDER PHYSIOLOGICAL CONDITIONS SELECTIVELY PREVENTS ARTICULAR CHONDROCYTE HYPERTROPHY

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Purpose: Proliferation and hypertrophic differentiation of chondrocytes as well as extracellular matrix (ECM) mineralization and chondrocytes apoptosis occur during the course of osteoarthritis (OA). OA is further characterized by an imbalanced production of ECM-degrading enzymes such as matrix metalloproteinases (*MMPs*) and aggreganases (*ADAMTS'*), which deplete major structural components like collagens (*COLs*) and proteoglycans (PGs, e.g. aggrecan) from the ECM. Both chondrogenic markers are crucial to biomechanical strength and