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MMP3 CLEAVES AGRIN IN CARTILAGE AND REDUCES ITS CHONDROGENIC POTENCY

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Purpose: Osteoarthritis is a chronic disabling disease characterized by cartilage breakdown for which there is no cure. We recently discovered that the heparan sulphate proteoglycan Agrin is expressed in cartilage, where it is essential for the maintenance of the chondrocytic phenotype and for the production of cartilage extracellular matrix whilst exogenous Agrin enhances chondrocyte differentiation and cartilage formation in vitro and in vivo. Here we study the mechanism that leads to loss of Agrin in OA and the functional consequences of Agrin cleavage by metalloproteinase 3 (MMP3).
Methods: Agrin expression was determined by immunohistochemistry. Osteoarthritis was induced in 8 week old 129sv mice by destabilisation of the medial meniscus (DMM) and N-Agrin and C-Agrin expression was evaluated by immunofluorescence at 7 days and 8 weeks post-surgery. Ex vivo human cartilage samples were treated with MMP3 and the cleavage of C-Agrin was compared by immunofluorescence. The effect of MMP3 on Agrin transfected chondrocytes was analysed by qPCR.
Results: We discovered that detection of Agrin in osteoarthritic samples is lost in earlier stages of disease progression when using an antibody detecting the C-terminal portion of Agrin compared to when using an antibody recognizing the N-terminal domain. This could be explained by the well-described MMP3 mediated Agrin cleavage which separates the N-terminal domain (predicted to adhere to the basement membrane through interaction with laminin) from the C-terminal domain. In keeping with this hypothesis, treatment of chondrocyte cultures or cartilage explants with recombinant MMP3 resulted in the loss of Agrin detection when using the C-terminal, but not the N-terminal antibody. Using antibodies specific to the N and C-terminal portions of Agrin, we found that C-Agrin is lost in the articular cartilage as early as 2days post surgical-induction of OA (destabilization of the medial meniscus), whereas N-Agrin remains highly detectable for at least 7days post surgical-induction of OA. In functional terms, treatment with recombinant MMP3 reduced the capacity of Agrin to upregulate SOX9 mRNA. Conversely, overexpression of Agrin in chondrocytes strongly downregulated MMP3 mRNA expression, thereby suggesting a negative feedback loop.
Conclusions: The cleavage of Agrin by MMP-3 in chondrocytes may contribute to the progression of OA by reducing SOX9 transcription.

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A NOVEL METHOD FOR THE ASSESSMENT OF KNEE JOINT SPACE WIDTH AND SUBCHONDRAL BONE MICRO ARCHITECTURE

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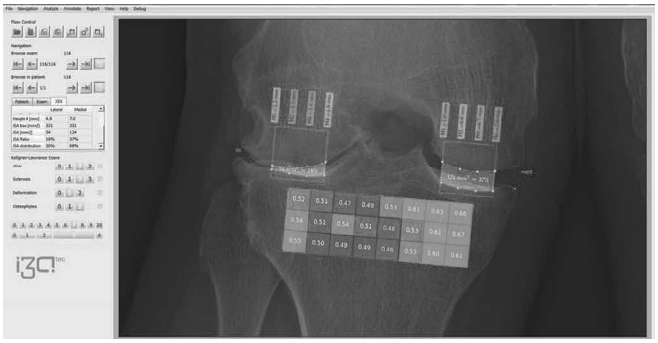
Purpose: Assessment of osteoarthritis (OA) of the knee usually involves AP and lateral radiographs to visually evaluate medial and lateral joint spaces, but perspective errors and low reproducibility are limiting factors. In addition to joint space width, subchondral bone area may provide important information on the status of OA. However, no adequate standard has been developed so far to quantify subchondral changes. The method described here combines an objective assessment of joint space width/area (JSW/A) and texture analysis of the adjacent subchondral bone micro architecture (BMA) to discriminate between patients with and without OA.
Methods: The study included 274 standardized knee radiographs from 110 patients with OA, and 164 controls. Knee joint space analysis was performed at the medial and lateral compartment, applying an entropy based algorithm for automated detection of tibial landmarks and joint space contours. Furthermore, subchondral bone texture was assessed by using fractal analysis at predefined regions of the proximal tibia. A matrix of 3x8 ROIs was used to gain sufficient textural information (FIG.). Self-similarity of the texture, reflecting 2D projection of the 3D trabecular structure, has been used to calculate the Bone Structure Value (BSV) which provides indirect information on the trabecular structure.
Results: Comparing mean BSVs of the medial compartment of selected 226 female patients, a statistical significant deviation of 7.64% ($p<0.01$) in values was determined between case and controls. The odds-ratio for a high BSV was found to be 3.08 (95% CI, 1.78-5.30) with a sensitivity of 64% and a specificity of 63.4%, respectively. A combination of JSW/A & BSV showed a further increase in discriminative power between the controls and OA patients. Differences in BSV were found between left/right knee and male/female. Furthermore, a rising BMI was identified to be linked to lower BSV values.
Conclusions: The novel method described here is sufficient to significantly discriminate between subjects with and without OA. Furthermore, fractal analysis alone may provide important information on bone quality aspects. Future work should therefore focus on the potential role of bone micro architecture assessments for early prediction and follow up of OA. Moreover, such algorithms could serve not just as early disease predictor for OA but also additional degenerative bone diseases like osteoporosis.



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ELF3 CONTRIBUTES TO CARTILAGE DEGRADATION BY CONTROLLING THE EXPRESSION OF MMP13 IN VIVO, IN A SURGICAL MODEL OF OSTEOARTHRITIS IN MICE

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Purpose: The E74-like factor 3 (Elf3) is a member of the ETS family of transcription factors. In previous work we showed that ELF3 levels are elevated in osteoarthritic (OA) cartilage, and that ELF3 contributes to the IL1β-induced expression of matrix metalloproteinase 13 (Mmp13), Nos2, and Ptgs2/Cox2 in chondrocytes *in vitro*. Here, we aimed to investigate the contribution of Elf3 to cartilage degradation *in vivo*, using mouse models of gain- and loss-of-function of Elf3 subjected to the destabilization of the medial meniscus (DMM) model of OA.
Methods: We performed DMM surgeries in 12-week-old male mice with Col2a1Cre-driven cartilage-specific Elf3 knockout (cKO) or control Elf3ff (WT) littermates. We generated mice overexpressing Elf3 in cartilage and synovium (TRE-Elf3:Comp-tTA; Tg) by crossing transgenic mice that express Elf3 under the control of the tetracycline-responsive element (TRE) with Comp-tTA mice expressing tTA under the control of the Cartilage Oligomeric Matrix Protein (Comp) promoter. We evaluated whether Elf3 overexpression impacts DMM-induced cartilage degradation using 6 month-old Ctrl (Comp-tTA) and Tg mice. At 4, 8 and 12 weeks (WT and cKO mice) or at 8 weeks (Ctrl and Tg mice) post-DMM, knees were processed for histological assessment of OA, conducted following OARSI guidelines. Total RNA was isolated from the articular cartilage of non-operated and DMM-operated mice at 8-wks post-DMM. The total RNA was reverse-transcribed and amplified using SYBR Green I-based qPCR and specific primers for Elf3 and Mmp13. Data were normalized using Eef1a1, Gapdh and Hprt1 as housekeeping genes.
Results: Histological assessment of cartilage degradation showed attenuation of cartilage loss at 8 and 12 weeks after surgery in cKO mice compared to WT controls. The decreased cartilage degradation in cKO mice correlated with reduced Mmp13 mRNA, assessed by RTqPCR, and reduced collagenase activity, assessed by C12C immunostaning, at 8