differences were also apparent. In mature articular cartilage, the gene-expression level of Sox 9 was lower in the metacarpal than in either the shoulder or the knee joint; that of type X collagen was lower in the metacarpal than in the shoulder joint, but higher in the metacarpal than in the knee joint; that of type IX collagen was lower in the metacarpal and the knee joints than in that of the shoulder.

Conclusions: In the immature articular cartilage of the three synovial joint types, the activity profiles of the key genes were almost identical, with but one exception (type I collagen). Hence, during the early phase of growth, a common type of cartilage is formed in the different joints. During its maturation, articular cartilage undergoes a process of complete structural reorganization (Hunziker et al., Osteoarthritis Cartilage 15:403, 2007). The gene-activity profiles also change, and in a joint-specific manner. This finger-printing may reflect the differing mechanical needs of the three joint types. The terminal structure of mature articular cartilage is achieved via the activity of committed chondrocytes within the superficial zone, whereas immature articular cartilage is formed from a "less" committed pool of chondroprogenitor cells.

156 THE ROLE OF THE VOLUME-SENSITIVE Cl^- CURRENT IN THE PROCESS OF REGULATORY VOLUME DECREASE (RVD) IN FRESHLY ISOLATED RABBIT ARTICULAR CHONDROCYTES

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Purpose: Articular chondrocytes play a crucial role in the production and maintenance of extracellular matrix, which is influenced by mechanical and osmotic environment. Previous studies have demonstrated the presence of the stretch-activated cation channels, mechanosensitive K^+ channels and volume-sensitive Cl^- channels ($I_{Cl,vol}$) in chondrocytes, suggesting that these mechanosensitive ion channels contribute to the cell volume regulation. The present study was designed to elucidate the role of $I_{Cl,vol}$ in the process of regulatory volume decrease (RVD) in rabbit articular chondrocytes.

Methods: Rabbit cartilages were collected from bilateral knee, hip and glenohumeral joints of male animals weighing 2.0 to 3.0 kg. The cartilage was dissected into slices and cultured in DMEM for 1−3 days. On the day of experiments, chondrocytes were isolated by enzymatic digestion and transferred onto a recording chamber mounted on an inverted microscope. Current recording from single chondrocytes was performed using the whole-cell configuration of patch-clamp technique. $I_{Cl,vol}$ was evoked by replacing the isosmotic (300 mOsm) external solution with the hyposmotic (210 mOsm) solutions under conditions where Na^+, K^+ and Ca^{2+} currents were minimized. To monitor the changes in the cell volume, light microscopic images were captured with a CCD digital camera equipped with DS-L2 control unit at 2560×1592 resolution every 1 min, and the cross-sectional area of the cell image was measured using Image-J public domain software.

Results: Exposure of chondrocytes to the hyposmotic solution resulted in a cell swelling, which was accompanied by the activation of an outward rectifying current. The current was reversed at the membrane potential close to the equilibrium potential of Cl^- and was almost completely abolished by the bath application of the conventional Cl^- channel blocker NPPB (5-nitro-2-(3-phenylpropylamino) benzoic acid) at the concentration of 100 μM (99.4±1.3% inhibition measured at +40 mV, n = 4), suggesting that $I_{Cl,vol}$ is functionally expressed in rabbit articular chondrocytes. To investigate the role of $I_{Cl,vol}$ in the cell volume regulation, volume changes of (intact) chondrocytes during the hyposmotic challenge were evaluated by measuring the cross-sectional area of the cell images. When chondrocytes were maintained in the isosmotic condition, their cell volumes were relatively constant. On the other hand, after the introduction of the hyposomotic solution, the cell volume was immediately increased and reached a maximum within a few minutes (122±5.1% increase in the cross-sectional area, n = 17), followed by a gradual decrease in the cell volume even in the hyposmotic solution, namely RVD (46.7±7.8% recovery during a 30-min hyposmotic challenge, n = 17). In the presence of NPPB (100 μM), RVD was significantly attenuated (12.5±5.7% recovery, n = 19, $p < 0.05$), although the maximum increase in the cell volume after exposure to the hyposmotic solution was not significantly affected (125±4.8% increase, $p = 0.07$).

Conclusions: The present study suggests that cell swelling-induced elicitation of $I_{Cl,vol}$ is an essential mechanism underlying RVD in rabbit articular chondrocytes.

157 INHIBITION OF Il-1b-INDUCED MATRIX METALLOPROTEINASE (MMP)-3 AND MMP-13 SYNTHESIS IN ARTICULAR CHONDROCYTES FROM MICE LACKING MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 (mPGES-1)

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Purpose: Osteoarthritis (OA) is characterized, by cartilage degradation. The destructive OA process is due, at least in part, to the induction of matrix metalloproteinases (MMP) expression, especially MMP-13 and MMP-3, which are able to directly degrade the components of the cartilage matrix. Although Il-1β is considered as the main catabolic factor involved in MMP-3 and -13 expressions, the role of prostaglandin E_2 remains controversial. Prostaglandin E synthase (PGES) is the final enzymatic step of the arachidonic cascade leading to PGE_2 synthesis. The microsomal PGES-1 (mPGES-1) is considered as an inducible enzyme whereas cytosolic PGES is not. In OA, the link between PGE_2 and MMP synthesis is unclear.

The goal of this study was to determine and elucidate the role of PGE_2 on MMP synthesis in articular chondrocytes using mice lacking mPGES-1.

Methods: Experiments were performed from mice of the DBA/1lac J strain with a deletion of the Ptges gene which encodes mPGES-1 (Pfizer, Groton, USA). From primary cultures of murine articular chondrocytes (mPGES−/−, +/− and +/+ mice), mRNA and protein expressions were assessed by real-time RT-PCR and immunoblotting respectively. Prostaglandin E_2 (PGE_2) was measured by EIA.

Results: Il-1 β-induced PGE_2 synthesis was significantly reduced in chondrocytes from mPGES-$1^{-/-}$ (n = 3) and mPGES-$1^{+/-}$ (n = 6) compared to mPGES-$1^{+/+}$ (n = 6) (24 h Il-1β, 10 ng/ml, 96%-inhibition for KO mice and 64% inhibition for heterozygotes mice compared to wild-tpe mice; mPGES-1 +/+ Il-1 β stimulated chondrocytes PGE_2 release +/− 3500 pg/ml, $p < 0.01$). 10 ng/ml Il-1β increased MMP-3 mRNA (a 43-fold induction peaking at 8 hours, n = 3) and protein expression and release (peak at 18 hours and sustained up to 24 hours, n = 2). Moreover, 10 ng/ml Il-1β triggered MMP-13 mRNA (a 49-fold induction peaking at 6 hours, n = 4, $p < 0.05$) and protein expression and release (started at 4 hours and increased up to 24 hours, n = 2). Il-1β-induced MMP-3 and MMP-13 mRNA expressions decreased up to 24 h in mPGES-1−/− (n = 3) and +/− (n = 3) chondrocytes compared to mPGES-1 +/+ chondrocytes (n = 3) (at 24 h, for MMP-3, respectively a 75% – $p < 0.05$ – and a 80% – $p < 0.01$ – inhibition; for MMP-13, respectively a 58% – $p < 0.05$ – and a 65% decrease, $p < 0.05$). Il-1β-induced MMP-13 and MMP-3 protein release decreased in mPGES-1 +/− (n = 3) and −/− (n = 2) chondrocytes compared to mPGES-1 +/+ (n = 3) chondrocytes since 8 hours up to 24 hours.

Conclusions: Taken together, these findings clearly demonstrate for the first time that PGE_2 plays a crucial role in the induction of MMP-3 and MMP-13 in an inflammatory context. Therefore, these results show that mPGES-1 could be considered as a critical target to counteract cartilage degradation in OA.

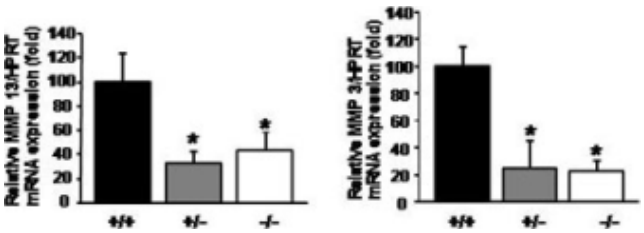

Figure 1. Inhibition of IL-1b-induced MMP-3 and MMP-3 mRNA expression in articular chondrocytes from mice lacking microsomal prostaglandin E synthase-1 (mPGES-1). Articular chondrocytes from mPGES-1 wild-type (+/+). Heterozygote (+/−) and knockout (−/−) mice were incubated during 24 h with 10 ng/ml IL-1b. RNA were extracted and assayed for MMP-3 and MMP-13. The levels of MMP-3 and MMP-13 mRNA were assayed by real-time RT-PCR. Values are the means and SD of 3 independent experiments with n = 3/group/experiment, analyzed in duplicate.