36.7±8.7%, $p<0.05$). Also in OA, ES/BS was higher in severe lesions than in moderate lesions (16.4±7.4% vs. 9.7±5.3% $p<0.001$), but not significantly higher in moderate than in normal-mild lesions (7.8±3.0% vs. 9.7±5.3%, $p = 0.17$). The OS/BS was higher in moderate lesions than in normal-mild lesions (18.0±11.5% vs. 10.5±4.1%, $p<0.01$), and was also higher in severe than in moderate lesions (29.7±13.6% vs. 18.0±11.5%, $p<0.001$).

Conclusions: In this cross-sectional study of human hip OA using stereology-based quantitative histology on entire femoral heads, OA was associated with increased bone mass and increased bone remodelling in the subchondral region. In the subchondral region with normal or mildly affected cartilage and in the central region, bone remodelling was higher in OA patients than in controls, although the bone mass did not differ. Furthermore, subchondral bone volume and remodelling was higher, the more severe the osteoarthritic lesions. Therefore, we conclude, that subchondral bone remodelling was affected already at an early stage of OA, where changes in bone mass were not present.

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FIBRONECTIN FRAGMENT MEDIATES CARTILAGE CATABOLIC PROCESS THROUGH TLR-2 SIGNALING PATHWAY IN HUMAN ARTICULAR CHONDROCYTES

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Purpose (the aim of the study): Fibronectin (FN), one of ECM protein comprising cartilage, contributes to attachment, migration, and proliferation of the cells. Also, increased level of fibronectin fragments (FN-fs), which are found in the synovial fluid (SF) of osteoarthritis (OA) patients, produces a potent catabolic effect in cartilage. However, little is known about the cellular receptors and signaling mechanisms that are mediated by FN-fs. Here we investigated whether the 29-kDa amino-terminal FN-f (29-kDa FN-f) regulates cartilage metabolism via Toll-like receptor-2 (TLR-2) signaling pathway in human articular chondrocytes.

Methods: Human articular chondrocytes were enzymatically isolated from articular cartilage obtained at the time of joint replacement surgery of subjects with knee osteoarthritis, and cultured in monolayer. In order to investigate whether 29-kDa FN-f induces MMPs production through TLRs, human chondrocytes were transfected with TLR-2 expression plasmid or small interfering RNAs (siRNAs) targeting TLR-2 or TLR-4 and Myeloid differentiation factor 88 (MyD88). In 29-kDa FN-f-stimulated chondrocytes, the relative levels of mRNA for matrix metalloproteinase 1 (MMP-1), MMP-3, and MMP-13 were analyzed by real-time quantitative reverse transcription-polymerase chain reaction. Protein expression levels of MMP-1 and MMP-3 and the regulatory effect of TLR-2 on 29-kDa FN-f-mediated signaling pathways were assessed by immunoblotting. MMP-13 production was measured by ELISA. Association of 29-kDa FN-f with human chondrocytes through TLR-2 was evaluated by fluorescence microscopic analysis.

Results: Analysis of the expression of TLR family members in cartilage tissues showed that TLR-2, 3, 4, and 5, except for TLR-1, were highly expressed in OA cartilage compared to normal cartilage. Among FN and various FN-fs including 29-, 45-, and 120-kDa FN-fs, in particular, 29-kDa FN-f significantly increased the expression of TLR-2 in human chondrocytes in a dose- and time-dependent manner. To further confirm whether TLR-2 signaling pathway is involved in 29-kDa FN-f-induced catabolic processes, the effect of 29-kDa FN-f on MMPs production was investigated in TLR-2 knocked-down or overexpressed chondrocytes. Our findings demonstrate that TLR-2 knockdown significantly inhibited 29-kDa FN-f-induced MMPs production at both mRNA and protein levels but TLR-4 knockdown failed to do it. Conversely, TLR-2 overexpression led to enhanced production of MMPs by 29-kDa FN-f. In addition, knockdown of MyD88, a downstream adaptor in TLR-2 signaling pathways, markedly reduced MMPs production induced by 29-kDa FN-f. Also, knockdown of TLR-2 apparently inhibited 29-kDa FN-f-mediated activation of IκBα and p38, but not activation of JNK and ERK. Exposure to SF from affected joints of OA patients highly elevated the expression of MMP-1, -3, and -13 in primary chondrocytes without reducing the cell viability. However, TLR-2 knockdown of chondrocytes significantly suppressed SF-induced MMP induction.

Conclusions: 29-kDa FN-f mediates catabolic responses in articular chondrocytes through MyD88-dependent TLR-2 signaling pathway. Modulation of TLR-2-mediated signaling may be as a potential therapeutic strategy for the prevention of cartilage degradation in OA.

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FSTL3: A NOVEL REGULATOR OF GROWTH PLATE DYNAMICS?

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Purpose: The TGFb ligand superfamily have long been implicated in development and maintenance of bone and cartilage. We have previously shown that mice deficient in the activin/TGFb signalling antagonist, Follistatin-like 3 (Fstl3-/-), develop spontaneous osteoarthritis with age, and exhibit premature growth plate closure. Herein, we examine the hypothesis that Fstl3-/- mice have an altered endochondral growth phenotype which reflects their predisposition to developing osteoarthritis.

Methods: 4- and 8-week-old male Fstl3-/- and age-matched wild-type mice were culled after treating for 1 week with 1mg/ml BrdU in drinking water. Tibiae were analysed for standard cortical and trabecular parameters by microCT analysis. The endochondral growth phenotype of these mice was assessed by standard histology of growth plate structure; immunohistochemical labelling for Col10a1 and Mmp13, (known markers of chondrocyte hypertrophy); Safranin O staining and analysis of BrdU incorporation to examine cellular proliferation (Invitrogen, CA, USA). Metatarsal rudiments were dissected from E14.5 Fstl3-/- and wild-type (WT) embryos and cultured for up to 10 days. Mineralisation zone and metatarsal length was measured digitally at several time points during culture.

Results: At 4-weeks Fstl3-/- mice displayed widened growth plates, corresponding to an increase in the size of the hypertrophic zone of chondrocytes, in comparison to age matched WT mice. No striking differences were seen in the growth plate measurements at 8 weeks. Fstl3-/- mice showed decreased Safranin-O staining in the hypertrophic zone at both 4 and 8 weeks of age, indicating an alteration in the composition of sulphated proteoglycans, and disordered columnar organisation of growth plate chondrocytes. Moreover, there was also an increased proportion and intensity of BrdU positive labelling in 8, but not 4-week old, Fstl3-/- mice when compared to WT mice. Immunolabelling for markers of chondrocyte hypertrophy at 4 wks of age revealed increases in MMP13 expression in Fstl3-/- growth plates, but a decreased expression of Col10a1, which supports an alteration of the chondrocyte phentoype. MicroCT analysis revealed increased trabecular bone volume/total volume (BV/TV), number and pattern factor in 4-week-old FSTL3 null mice compared to WT mice. At 8-weeks of age, Fstl3-/- mice have increased tibia length and microCT analysis reveals their increased trabecular thickness. In E14.5 embryonic metatarsal organ cultures deletion of FSTL3 resulted in accelerated mineralisation and increased growth compared to WT controls.

Conclusions: Taken together, our initial data suggest that Fstl3-/- mice display an accelerated bone and endochondral growth phenotype. This may either contribute indirectly to their development of osteoarthritis or may point to a fundamental chondrocyte deficit that underpins osteoarthritic initiation. Further studies into the precise growth plate phenotype of Fstl3-/- mice may elucidate the molecular mechanisms responsible for initiating osteoarthritis in these mice.

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A NEW ANIMAL MODEL FOR DETERMINING THE EFFECTS OF DIABETES ON OSTEOARTHRITIS

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Purpose: The prevalence of both type 2 diabetes and that of osteoarthritis (OA) are high and are increasing rapidly worldwide. A recent publication indicates that within the US Veteran population, rates of total joint replacement (likely due to severe OA) are 1) higher among those with diabetes and 2) occur at younger ages in those with diabetes. These two points together suggest that diabetes may have the effect of either worsening or accelerating the progression of OA. However, due to frequent co-morbid conditions associated with diabetes, testing the effect of diabetes is challenging in human subjects. Although there are several rodent models for type 2 diabetes, most require induction using a high fat diet, a confounding factor expected to also affect OA. The