type-II collagen antibody (Abcam) and F4/80 (AbD Serotec) were used. Cell death inside articular cartilage was examined by TUNEL assay. Expression of catabolic, inflammatory, and anabolic factors such as MMP3, MMP13, cathepsin F, IL1b, IL6, type II collagen and type X collagen mRNA in cartilaginous tissues of :7CKO and Cont mice was quantified by Q-PCR.

Results: In this study, we found that the absence of locally produced Bmp7 reduced proteoglycan contents in articular cartilage matrix in the adult mice (at 8 and 24 weeks of age). This may not be due to the defect in cartilage formation since there was no significant alteration in both articular structure and proteoglycan contents in the juvenile :7CKO mice (at 4 weeks of age). Structural analyses of articular cartilage indicated that cartilage thickness and chondrocyte density were not significantly altered between the genotypes at 8 and 24 weeks of age. TUNEL assay revealed that elimination of Bmp7 expression did not affect chondrocyte survival at 8 weeks of age. Histological evaluation revealed that extensive numbers of macrophages were infiltrated in the synovial tissues of :7CKO mice at these stages suggesting that severe synovitis occurred in the absence of Bmp7. In contrast, appearance of synovial tissues of :7CKO mice at these stages suggesting that severe synovitis was quite similar between the genotypes at 4 weeks of age.

Conclusion: Our data showed that the absence of endogenous Bmp7, although is does not affect articular cartilage formation and survival of articular chondrocyte, but enhance articular cartilage degeneration and synovial inflammation in adult mice.

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EPIGENETIC MODIFYING COMPOUNDS ALTER ACTIVITY OF PRIMARY HUMAN ARTICULAR CHONDROCYTES AND MESENCHYMAL STEM CELLS UNDERGOING CHONDROGENESIS


Purpose: A major therapeutic challenge in osteoarthritis is to identify disease modifying therapeutic strategies. Current therapies cause cessation or reversal of cartilage loss. Suitable therapeutics may target the chondrocytes or stem cells resident within the diseased cartilage or expanded in vitro prior to implantation. Epigenetic regulation of cell behavior is essential for both correct development and homeostasis of cartilage (1). This epigenetic control does not alter the DNA sequence and allows modifiable changes to gene expression that are heritable. Thus allowing a cell to respond to environmental and biological cues. Targeting the epigenetic modifying machinery can therefore provide important clues to the biology of chondrocyte differentiation and homeostasis and to potential therapeutic interventions.

Our library of epigenetic modifying compounds includes those targeting histone deacetylases, histone deacetylase inhibitors, protein kinases and bromodomains. In this preliminary study we have screened this library for impact on cell phenotype in chondrogenesis and in primary human articular chondrocytes (HAC).

Methods: Primary human articular chondrocytes (HAC) and human mesencymal stem cells (MSCs) undergoing chondrogenesis were treated with epigenetic modifying compounds for 72 hours. Metabolic activity was assessed after 72h using PrestoBlue before cells were fixed and assessed for cell number (DAPI count), ECM production (Alcian blue) and nodulus formation (Nile Red). HAC were treated with GSK-J4 and GSK-J5 for 4h and TIMP3 and COL2A1 expression assessed by qPCR. HAC were treated with GSK-J4 and GSK-J5 for 3h in the presence and absence of 2ng/ml TGFb and expression JMJD3 and the TGFb target gene TIMP3 was assayed by qPCR.

Results: Compounds including Methylstat, an inhibitor of the Jumonji C methyltrasnferases, histone deacetylases, histone protein kinases and bromodomains. In this preliminary study we have screened this library for impact on cell phenotype in chondrogenesis and in primary human articular chondrocytes (HAC).

Conclusion: Our preliminary study shows that Fibrillin-1 protein expression is increased during OA development in a well known model of spontaneous OA, and in control aged-matched non-OA CBA mice, in knee joints with different degrees of OA severity. The knees of Tsk and littermate control male mice of 60–80wks of age were fixed and microCT scanned. Abnormal ectopic calcified regions were analysed. After scanning, joints were decalcified and processed for paraffin embedding; serial coronal sections were cut at 6μm and sections at regular intervals stained with Toluidine Blue.

Results: Fibrillin-1 was localised in the pericellular matrix of articular chondrocytes in normal joints. During the development of OA in Str/ort mice, however, Fibrillin-1 immunolabelling was decreased, in particular during the development of OA during the Str/ort period. In addition, mutations in Fibrillin-1 in the TSK mouse lead to abnormal ossification in the knee joint as well as severe OA development. These data suggest that Fibrillin-1 plays an important role in joint homeostasis and that abnormal expression or mutations in its gene can lead to OA development.

Conclusion: Our preliminary study shows that Fibrillin-1 protein expression is increased during OA development in a well known model of spontaneous OA, suggesting TGFb bioavailability may be modified during this period. In addition, mutations in Fibrillin-1 in the TSK mouse lead to abnormal ossification in the knee joint as well as severe OA development. These data suggest that Fibrillin-1 plays an important role in joint homeostasis and that abnormal expression or mutations in its gene can lead to OA development.

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DYNAMIC CHANGES OF EXTRA CELLULAR MATRIX PROTEINS IN EQUINE ARTICULAR CARTILAGE TREATED WITH INTERLEUKIN-1B IN VITRO

Purpose: Osteoarthritis (OA) is a chronic low-grade inflammatory disease for which the diagnosis currently relies on clinical and radiological means whereas sensitive serum biomarkers specific for an early stage of the disease are lacking. The aim of the study was to identify specific proteomic alterations of articular cartilage in vivo and in vitro inflammation model. The pro-inflammatory cytokine interleukin (IL-1β) was used to induce degradation of articular cartilage explants cultured in vitro with the purpose of mimicking OA cartilage in early and later developmental stages. The specific objective was to longitudinally characterize and quantify the release of cartilage matrix components to the media by using a quantitative proteomic approach.

Methods: Full thickness equine articular cartilage explants were harvested from the weight bearing part of the distal metacarpal bone III in the metacarpal-phalangeal joint of three horses. The explants were cultured in the presence or absence of interleukin IL-1β in-vitro and cell media was changed and sampled at day 3, 6, 9, 12, 15, 18 and 22. Proteins were isolated from the harvested media and following trypic digestion the peptides were labeled with isobaric tandem mass tags (TMT) and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). The TMT reporter ion intensities in MS1/MS spectra were divided by reference reporter ion intensities, giving a relative amount for each quantified peptide. The relative amount of released components in media from IL-1β stimulated and unstimulated explants were compared over time.

Results: IL-1β stimulation resulted in an abundance of proteins related to the extra cellular matrix and the proteins were released at different time points during culturing time. Release of several cartilage matrix components e.g. aggrecan, cartilage oligomeric matrix protein (COMP), chondroadherin, thrombospondin-1 and proteoglycan 4 displayed high amounts at days 3 and 6, similar to the early stage of the disease process of OA in vivo. Collagen type XII release was identified at day 9 and 12 and the release of collagen type VI increased continually from day 18-22. Several small leucine-rich proteoglycans (SLRP) such as biglycan and lumican had the highest release at day 22, together with a high amount of procollagen type II C-propeptide and C endopeptidase showing signs of new synthesis of collagen type II, by the presence of procollagen type II C-propeptide and C endopeptidase enhancers, and this collagen synthesis was not at all prominent in the high amount of procollagen type II C-propeptide and C endopeptidase enhancers, and this collagen synthesis was not at all prominent in the IL-1β stimulated media.

Conclusions: This inflammatory in vitro model display structural changes seen at distinct developmental stages of OA in vivo. Novel findings presented are the release, at different time points after IL-1β stimulation, of cartilage components in a specific time pattern in the order as follows; COMP, collagen type XII, collagen type VI, SLRPs and finally collagen type II, indicating a degradation of the collagen network in a distinct pattern, without evidence for new synthesis of collagen type II. IL-1β resulted in the unstimulated explants release capacity with an increase of collagen synthesis. The identification of early biochemical changes in cartilage would be of great importance in both finding disease modifying pharmaceutical compounds as well as in the development of biomarkers for early OA.

229 IMMUNOHISTOCHEMICAL LOCALIZATION OF NOTCH SIGNALING COMPONENTS, MATRIX COMPONENTS AND STRO-1 IN PREGNATAL, POSTNATAL AND OSTEARTHRITIC EQUINE CARTILAGE


Purpose: The growth plate and articular cartilage of developing synovial joints include specific zones of chondrocyces with different phenotypes. The chondrocyces in the growth plate change from resting to proliferative and hypertrophic phenotypes. Phenotypical changes also occur in the chondrocyces of the articular cartilage during the progression of osteoarthritis (OA). The expression of proteins on the chondrocyces as well as in the surrounding matrix affects the phenotype and can be used to characterize cells of specific phenotypes. The aim of the study was to localize components with potential effects on chondrocyces such as NF-kB and the Notch signaling components including the receptor Notch1, the ligand Delta4 and the target gene Hes1. Additionally the stem cell indicating marker STRO-1 and matrix molecules were localized to further characterize cells and matrix in developing prenatal and postnatal equine cartilage as well as in OA cartilage.

Methods: Immature equine osteochondral tissues, representing different developmental stages of the articular and growth cartilage of the distal radius were collected post mortem from two fetuses (6.5 and 10 months gestational age) and from two young horses (2-day-old and 1-year-old). Articular cartilage from fetlock joints of adult horses was collected and articular cartilage with superficial chondronecrosis, fibrillation and adjacent cluster formations, compatible with OA cartilage was identified by histology and included in the study. The localization of Notch signaling components, STRO-1 and the matrix molecules cartilage oligomeric matrix protein (COMP), fibromodulin, chondroadherin and matrilin-1 was examined by immunohistochemistry.

Results: Notch1, Delta4 and Hes1 indicating active Notch signaling was localized in hypertrophic cells in growth plates of the distal radius in all horses, as well as in resting cells of prenatal horses and in proliferative cells of the youngest fetus. The same proteins were present in all zones of the radial articular cartilage of the postnatal horses, whereas only a few cells were stained in the youngest fetus.

Stro-1 was present in the resting zone of the youngest fetus, with weak immunolabeling in the resting zone of the 10-month-old fetus and only detected as weak immunolabeling in the proliferative zone of the 2-day-old horse and not detected in the growth plate of the 1-year-old horse. In articular cartilage the chondrocyces stained positive for STRO-1 in all zones of the youngest fetus, whereas the staining became restricted to the superficial and middle zones in the postnatal horses. Most matrix molecules gradually changed in location with increasing age. Most interterritorial localization was found in the growth plate. The most prominent changes were observed in the hypertrophic zone where COMP, fibromodulin and chondroadherin were localized interterritorially in the prenatal horses but shifted to a pericellular location in the postnatal horses. Matrilin-1 was present in the hypertrophic zone of the prenatal horses but absent in the postnatal horses. In the articular cartilage the matrix molecules were present in all zones with an age related shift from interterritorial to pericellular location in all zones but the superficial zone, which displayed interterritorial staining in all horses. The Notch1 receptor and the Delta4 ligand were detected in all zones of OA cartilage with the strongest staining in the superficial zone and in cell clusters. Intense Stro-1 and weak Hes1 immunolabeling was detected in the superficial zone and in cell clusters. All matrix components were present in OA cartilage.

Conclusions: This study demonstrates presence of Notch signaling components in equine cartilage of different developmental stages which support previous findings of a role for Notch signaling in endochondral ossification and differentiation of chondrocyces. The studied matrix molecules known to be involved in the formation and organization of the collagen network showed age related differences in localization of the molecules, which may indicate increased mechanical load or altered expression related to different developmental stages. The weak staining of Hes1 in OA cartilage suggests low activity of the Notch1 signaling, and the strong staining of STRO-1 in the superficial zone and OA cell clusters indicates stem cell like characteristics of the chondrocyces. This is the first study demonstrating the localization of Notch signaling components, STRO-1 and matrix components in equine growth cartilage of different developmental stages and OA cartilage.

230 OSTEOCLASTS REDUCE THE ACTIVATION OF WNT CANONICAL PATHWAY IN CHONDROCYTES

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Purpose: Osteoclastogenesis is enhanced in early osteoarthritis (OA). We have previously demonstrated that cartilage degradation is reduced in vivo when osteoclastogenesis is inhibited in murine models with high expression of known bone-osteoclast receptor. We therefore hypothesized that inhibition of osteoclast cell function, might contribute to the mechanisms that promote cartilage breakdown. Our purpose was to evaluate whether osteoclast-secretion molecules affect directly the metabolism of chondrocyte and the contribution of Wnt pathway.

Methods: Osteoclasts were obtained after differentiation of RAW cells cultured with RANKL. Primary murine chondrocytes (Ch) were then cultured in the presence of osteoclast conditioned medium (OC-CM) for 48h. The gene and protein expressions of catabolism and anabolism