micro computed tomography (µCT, Skyscan 1172, 4.5 um resolution) of the knee at 3, 7, 14, 28, 56, 84, 112 days (n < 8 per group). Changes in subchondral bone at 28 days post DMM (n = 6) were measured separating the subchondral plate from the subchondral trabecular bone at 28 days post DMM and in all mice by day 14. At day 7 most samples had 1 or 2 osteophytes, with some having up to 4 osteophytes by day 14. From day 28 onwards the osteophytes fused and formed into one or two large osteophytes. Osteophytes increased in size and bone content with time, plateauing by day 112. The trabeculae of the subchondral bone at day 28 showed a significant increase (p < 0.03) in trabecular number on the medial side of both the DMM and sham operated mice when compared to the respective contralateral leg. In the sham samples, the reduction of trabecular number translated to a significant increase in trabecular space, whereas in the DMM mice an increase in trabecular thickness occurred, eliminating the increase in trabecular space. Although bone changes occurred within a week of DMM induction, cartilage damage scores were not significant until day 56 (p = 0.05), compared to earlier time points.

Conclusions: We herein characterised differential bone changes in the DMM murine model of OA: there was accelerated osteophyte development within the first week, whereas subchondral bone changes were not evident until day 28. Interestingly sham operated animals showed subchondral trabecular bone loss in the ipsilateral knee, highlighting the need to use the contralateral as an internal control. The early bone changes in this OA model significantly preceded cartilage degradation, consistent with altered bone biology being an important driver of OA pathogenesis. Supported By Arthritis Research UK (20199).

651 PPARDELTA PROMOTES THE PROGRESSION OF POST-TRAUMATIC OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a degenerative joint disorder, characterized by the breakdown of articular cartilage, subchondral bone thickening, osteophyte formation and synovial inflammation. The human burden of this disease influences both the independence and quality of life of those afflicted. Currently, there are no pharmaceutical treatments to stop, slow or reverse disease progression, resulting in greatly reduced quality of life for OA patients and the need for joint replacement surgeries in many cases. The lack of available treatments for OA is partially due to our incomplete understanding of the molecular mechanisms promoting disease initiation and progression. Recent findings from our laboratory indicate that activation of the transcription factor PPARdelta induces the expression of enzymes involved in proteoglycan breakdown and can lead to cartilage degeneration in OA, prompting us to speculate whether inhibition of PPARdelta can protect from cartilage breakdown in OA.

Purpose: We aimed to characterize the role of PPARdelta in OA through in-vitro, ex-vivo, and in-vivo studies to determine whether it is a feasible target for OA therapy.

Hypothesis: We hypothesize that inhibition of PPARdelta will slow the progression of OA in animal models.

Methods: Primary mouse chondrocytes and cartilage explants were treated with a pharmacological PPARdelta agonist (GW501516) to evaluate changes in gene expression (qPCR), and histology (Safranin-O, immunohistochemistry) consistent with OA development. In order to examine the role of PPARdelta in vitro, cartilage-specific knockout mice and wild-type littermate controls were subjected to destabilization of medial meniscus surgery (DMM) at 20 weeks of age. 28 weeks post-surgery mice were compared through classical histological and biochemical measures of OA progression including Safranin-O staining with OARSI scoring, immunohistochemistry for cartilage matrix breakdown products, and picrosirius red staining for collagen fiber structure and orientation.

Results: In vitro, PPARdelta agonism (by GW501516) results in the upregulation of expression of proteases implicated in cartilage degeneration (including MMPs, and ADAMTS genes). Activation of PPARdelta also results in proteoglycan breakdown in the cartilage matrix of an explant culture model with cartilage-specific deletion of PPARdelta. In vivo, PPARdelta do not demonstrate any abnormalities in skeletal growth or development, but do show significant chondroprotection in comparison to wild-type littermate controls after surgical induction of OA. OARSI scoring and immunohistochemistry confirm strong protection of mutant mice against cartilage matrix breakdown, and decreased breakdown products in the cartilage of PPARdelta knockout mice.

Conclusion: This study provides strong evidence for catalytic roles of endogenous PPARdelta in post-traumatic OA and suggests that pharmacological inhibition of PPARdelta is a promising therapeutic strategy.

652 DKK3 PREVENTS MATRIX LOSS INDUCED BY PRO-INFLAMMATORY CYTOKINES IN HUMAN CARTILAGE EXPLANTS

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Purpose: Osteoarthritis (OA) is a prevalent disease with high socio-economic impact characterized by loss of articular cartilage. Highlighted by a number of genetic and cell biology studies, the interception of cell signaling pathways imply an important mechanism in OA development and pathogenesis. Dkk3 is a member of the Dickkopf family of WNT antagonists which is recently recognised to be upregulated in OA cartilage. In the pathogenesis of OA the WNT signaling pathway plays a role but the function of DKK3 in inflammation of joints is still poorly described. According to recent reports the activation of WNT signaling leads to an upregulation of TNF-α. In our study we investigated the response of DKK3 in human OA cartilage explants to the pro-inflammatory cytokines IL1-β and TNF-α.

Methods: Osteochondral explants (5mm) were created from human femoral condyles (n = 3 donors) of OA patients. The explants were treated in duplicates with 10ng/ml IL1-β, 10ng/ml TNF-α and 10ng/ml IL1-β + 10ng/ml TNF-α for 21 days. Controls were left untreated. The medium was collected for GAG analysis. Safranin-O stained paraffin sections (2.5 µm) of the explants were assessed by Mankin score. The release of glycosaminoglycans (GAG) from explants into the culture medium was measured based on 1,9-dimethylmethylen blue binding. For immunolabeling, explants were incubated with monoclonal antibody Dkk3 to determine whether an elevation in Dkk3 presence was associated with the activation of TNF-α and IL1-β. Paraaffin embedded 2.5µm sections were deparaffinized and blocked with 10% donkey serum for 30 min, and incubated incubated overnight at 4°C with antibodies against Dkk3 in a dilution of 1:100 in 0.05 M Tris Buffer. After washing the slides with TBS, the secondary antibody alexa fluor 488 was applied for one hour at room temperature containing 10µM 4',6-diamidino-2-phenylindole (DAPI) for nuclear staining. The slides were observed under a confocal laser scanning microscope connected to a digital imaging system. Negative controls leaving the primary antibody were prepared. To identify the ability of Dkk3 to reduce the activity of pro-inflammatory cytokines on matrix loss, we incubated cartilage explants with 10ng/ml recombinant human Dkk3 prior to IL1-β and TNF-α.

Results: Osteochondral explants show an increased loss of GAG and differences in the Mankin score by the use of pro-inflammatory cytokines IL1-β and TNF-α. Immunofluorescence analysis using Dkk3 antibody showed that TNF-α/IL1-β treatment (10ng/ml, 21 days) promoted an increased occurrence of Dkk3+ chondrocytes mainly in the surface and superficial zone of the cartilage fraction of the explants and lacking positive staining in the deep zone compared to control. Treatment with Dkk3 prior to IL1-β and TNF-α indicate a decreased GAG loss in explants.

Conclusions: The regulation of signaling in the pathogenesis of OA is subjected to several parameters which influence also the balance and activity of pro-inflammatory factors in joints. The appearance of Dkk3+ chondrocytes mainly in the surface and superficial zone of the cartilage fraction of the explants and lacking positive staining in the deep zone compared to control. Treatment with Dkk3 prior to IL1-β and TNF-α indicate a decreased GAG loss in explants.

653 RELATIONSHIP BETWEEN SUBCHONDRAL BONE CYSTS, THE SEVERITY OF KNEE OSTEOARTHRITIS, AND ALIGNMENTS OF LOWER EXTREMITIES

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Purpose: To evaluate the characteristics of the subchondral bone cysts (SBCs) in osteoarthritis (OA) of the knee.

Methods: Subjects were 29 patients (53 knees) with knee OA (mean age 65.3 ± 9.8, range 50 – 80 years, all female, Kelgren-Lawrence (KL) grades II: 23, III: 12, IV: 18). The anteroposterior radiographs of lower extremities in standing position and the CT images of the knee joints were all prepared. Femorotibial (FT) joint was divided into 5 regions: medial femoral condyle (MFC), medial tibial plateaux (MTP), lateral femoral condyle (LFC), lateral tibial plateaux (LTP) and subspinous (SS). The location, number, and size of SBCs were measured in CT. SBCs were defined as the cysts greater than 2 mm in diameter, occurring in the region within 10 mm from the joint surface. The alignments of lower extremity were measured in radiographs: percentage of mechanical axis (%MA), femorotibial angle (FTA), lateral distal femoral angle (LDFA), medial proximal tibial angle (MPTA), and joint line convergence angle (JLCA). Relationship between the number of SBCs and the severity of OA or alignments of lower extremity was analyzed.

Results: SBCs were detected in 88.5% of knees, 5.8% in MFC; 51.9% in MTP; 5.8% in LFC; 13.5% in LTP; and 17.2% in SS. The mean number of SBCs was 2.5 in MFC; 2.1 in MTP; 3.0 in LFC; 1.2 in LTP; 1.6 in SS. The mean size of SBCs resulted respectively as follows: 2.9 mm in MFC; 3.6 mm in MTP; 3.8 mm in LFC; 3.0 mm in LTP, 5.2 mm in SS. The number of SBCs in MFC and MTP had a significantly positive correlation with KL grades, FTA and JLCA, and had a significantly negative correlation with % MA and MPTA. However, there was no correlation in LFC, LTP and SS.

Conclusions: SBCs were frequently detected in patients with knee OA, and more prevalent in medial FT joint. The incidence in medial FT joint was affected by the severity of knee OA or varus knee deformity, although the mean size was small and the number was few. However, SBCs were present in lateral FT joint and SS, and these were not affected by the severity of knee OA or varus knee deformity.

654 THE ASSOCIATION OF BODY COMPOSITION AND METABOLIC SYNDROME WITH KNEE OA AND KNEE PAIN IN A KOREAN COMMUNITY RESIDENTS

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Objectives: Obesity, a major concern of contemporary health care system is closely related to both musculoskeletal pain and OA. Recently, proinflammatory effect of obesity, independent of its biomechanical effect has also been gaining interest in the pathogenesis of OA. In this study, we sought to delineate the relationship between body composition parameters and knee OA/knee pain in a Korean community residents. In addition, we tried to examine the influence of metabolic syndrome on knee OA/knee pain independent of BMI by assessing the risk stratified by body mass index (BMI) and the presence or absence of metabolic syndrome (MetS).

Methods: Participants were from the population-based Hallym Aging Study. The presence of knee OA or knee pain, demographic data was obtained by questionnaire. Knee pain was assessed using the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index and a 100-mm visual analog scale (VAS). Radiographic evaluations consisted of weight-bearing knee anteroposterior radiographs. Total body fat mass and total lean mass were measured with Dual x-ray absorptiometry. Each subject underwent MRI of the more symptomatic knee or the dominant knee depending on the symptom presence. MRI scans of the knee were obtained with a 1.5-T scanner with a phase-array knee coil and were read according to WORMS grading system.

Result: The mean age of participants (230 men, 274 women) was 70.2 years. Radiographic knee OA was present in 37.3% of subjects. BMI, total fat mass, and fat/muscle ratio were all significantly and positively correlated with knee OA while total lean mass was significantly and negatively correlated with it. After adjusting for age, sex, and manual work, the association remained significant only for fat/muscle ratio. The association between fat/muscle ratio and pain VAS score was significant after adjustment. To analyze the association between metabolic syndrome features independent of body mass index, we divided the population into 4 groups, (metabolically non-obese, normal weight (MNNW), metabolically obese, normal weight (MONW), metabolically non-obese, obese (MNOB), and metabolically obese, obese (MOOB)). ORs for knee OA was significantly higher only among MOOB compared to MNNW after adjustment. The severity of knee pain was not significantly associated with any of the 3 subgroups compared to MNNW. Cartilage degeneration as measured with MRI was significantly associated only for BMI after adjustment, and was not significantly associated with any of the 3 subgroups compared to MNNW. Neither any component of the metabolic syndrome, nor the number of metabolic component was significantly associated with radiographic knee OA, knee pain, or cartilage degeneration measured with MR.

Conclusion: These results suggest that aside from BMI, body composition, specifically fat/muscle mass ratio may have important implications for the pathogenesis of knee OA and knee pain, while metabolic syndrome may not.

655 ANCIENT CHINESE MEDICINE BASED ON A LECTIN (MASL), THAT TARGETS GLYCOPROTEINS CONTAINING ALPHA-2-3-SIALIC ACID RESIDUES, DECREASES PROINFLAMMATORY MEDIATORS PRODUCTION AND EXTRACELLULAR MATRIX DEGRADATION RESPONSE IN ARTICULAR CARTILAGE

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Purpose: Glycosylated proteins are essential components of the extracellular matrix (ECM) of cartilage and contribute to the maintenance of its function. A shift from α-2,6- to α-2,3-linked sialic acids of glycoproteins modifies the binding ability of proteins to substrates influencing cellular anchoring and affecting signal transduction. Intriguingly, the predominance of α-2,3-sialylation of chondrocytes glycoproteins was associated with the pathophysiology of rheumatic diseases including rheumatoid arthritis (RA) and osteoarthritis (OA). A highly O-glycosylated protein with α-2,3-sialic acid, involved in the induction of inflammation and tissue repair, is the transmembrane mucin receptor named Podoplanin (PDIPN). The present study aimed to assess the effect of specifically targets α-2,3-sialic acid residues with a lectin-based drug (MASL) on chondrocyte dedifferentiation and cartilage breakdown processes.

Methods: For immunofluorescence and immunohistochemistry assays, in situ cartilage was fixed and frozen immediately using Tissue-Tek O.C.T. and isopentanol in liquid nitrogen. Primary cells in monolayer culture were fixed with formaldehyde for optical microscopy assays. 4 mm cartilage punches were prepared from cartilage explants that were cut in the operating room immediately after surgery and cultured in DMEM with 1% FCS. Chondrocytes were isolated from articular cartilage and cultured in DMEM with 15% FCS. Cell viability was evaluated by the colorimetric MTT assay. Cell adhesion and growth was assessed with fibronectin-coated well plates and Wound Healing Assay Kit. Reactive oxygen species levels were measured by DCHF-DA and by Flow Cytometry. RNA was isolated with TRIZOL® Reagent and analyzed by Real-Time RT-PCR.

Results: The treatment of chondrocytes with 400 and 720 nM of MASL did not affect cell viability, adhesion or growth. To mimic pathological conditions, cells and cartilage explants were treated with 5 µg/ml oligomycin. Treatment of chondrocytes with oligomycin did not affect cell viability but increased ROS levels over 10 fold and MMP3, IL-6 and COX2 mRNA levels over 3–10 folds. The treatment of cells with MASL effectively protected chondrocytes from ROS production when incubated in the presence of oligomycin. Moreover, oligomycin induced the expression of inflammatory cytokines including IL-6 and COX2, and this induction was reverted by treatment with nanomolar concentrations of MASL. 5 µg/ml of oligomycin for 7 days decreased safranin uptake and disrupted the ECM structure of cartilage punches as evidenced by ulceration increasing lacunae space. However, the presence of 400 nM of MASL prevented the cartilage destruction and inhibited COX2 and MMP3 induction by oligomycin treatment. Immunohistochemistry assays revealed that OA cartilage contained significantly higher levels of PDIEN, a protein involved in cartilage destruction.

Conclusions: This study demonstrates that physiologically relevant concentrations of MASL protect chondrocytes from detrimental effects of ROS, inflammatory cytokines and MMPs and preserve chondrocyte phenotype and articular cartilage structure under pathological conditions.

4 These authors contributed equally to this work.