REFERENCES:

- [1] Jain A, et al. Diacerein protects against iodoacetate-induced osteoarthritis in the femorotibial joints of rats. *J Biomed Res* 2015;29:405–413.
- [2] Bairwa K, et al. Nanoparticle formulation of 11-keto- β -boswellic acid (KBA): anti-inflammatory activity and in vivo pharmacokinetics. *Pharm Biol* 2016;54:2909–2916.
- [3] Park JW, et al. Ibuprofen-loaded porous microspheres suppressed the progression of monosodium iodoacetate-induced osteoarthritis in a rat model. *Colloids Surf B Biointerfaces* 2016;147:265–273.

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SAT0064 CD14+CD16+ MONOCYTE SUBPOPULATION IS DOMINANT IN THE INFLAMMATION OF OSTEOARTHRITIS SYNOVIAL FLUID

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Background: In osteoarthritis (OA), the activation of inflammation response involving the interaction of cartilage and synovial hyperplasia may contribute to disease progression. However, inflammatory cells in OA synovial fluid (OASF) have been rarely studied.

Objectives: To investigate the phenotype of CD14 +cells and the secretion of proinflammatory cytokines by these cells

Methods: Immunohistochemistry staining in OA synovium was performed using anti-CD14, anti-CD16 and anti-CD56 antibody. The OASF was obtained through arthrocentesis. Mononuclear cells from OASFs were stained with anti-CD3, anti-CD4, anti-CD14, anti-CD16, anti-TLR4 or anti-TLR2 and analysed using flow cytometry. CD14 +CD16+and CD14+CD16 mononuclear cells in OASF were selected with magnetic microbeads. In the supernatant of these cells culture, the concentration of IL-1 β , IL-6, IL-8, TNF α , MMP-1, -3 were measured by Luminex.

Results: In OA synovium, CD14 or CD16 was stained, but CD56 was not expressed. In OASF, there was a substantial number of CD14 +cells (36.6% $\pm$ 25.2%), CD3 +cells (37.4% $\pm$ 12.9%), with a rarity of CD90 +cells (1.7% $\pm$ 1.3%). The proportion of CD14 +cells was increased significantly in recurrent synovial fluid, compared with the proportion in initial synovial fluid. Among CD14 +cells in OASF, CD14 +CD16+monocyte subpopulation (21.2% $\pm$ 21.8%) was more abundant than CD14 +CD16– monocyte subpopulation (10.9% $\pm$ 10.0%). TLR4 and TLR2 expressions were higher in CD14 +CD16+cells than in CD14 +CD16– cells. The concentration of IL-8 and MMP-3 was more increased in the supernatant of CD14 +CD16+cells than in that of CD14 +CD16– cells.

Conclusions: In OASF, the proportion of CD14 +cells was increased in recurrent synovial effusion. Compared to CD14 +CD16– monocyte subpopulation, CD14 +CD16+monocyte subpopulation released more cytokine such as IL-8 and MMP-3, and had higher expressions of TLR4 and TLR2.

Disclosure of Interest: None declared

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SAT0065 CHONDROCYTES UNDER PATHOLOGICAL CONDITIONS PREFERENTIALLY DEGRADES SELECTED tRNAs TO INHIBIT PROTEIN SYNTHESIS

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Background: Oxidative and ER stress play an important role in the pathogenesis of osteoarthritis (OA). These stress conditions can induce the cleavage of transfer RNA (tRNA) via angiogenin. This cleavage at the anticodon loop of the tRNA produces 5'-tRNA and 3'-tRNA halves known as tRNAs.

Objectives: We aim to identify specific tRNA to tRNA isotype fragments that occur during oxidative or ER stress in human OA chondrocytes.

Methods: Primary human articular chondrocytes were enzymatically isolated from OA cartilage. Cells were cultured *in vitro* for 72 hour and then treated with tert-butyl hydroperoxide (TBHP) over a 12 hour time course or with Interleukin-1 β (IL1 β) over a 24 hour time course. RNA was collected for mRNA expression analysis (qRT-PCR). Protein was harvested for western blot analysis. A tRF and tRNA PCR Array that profiles 55 known tRNAs was used to perform an unbiased dissection of the formation of tRNAs in OA chondrocytes. Total RNA was adapted using a First-Strand cDNA Synthesis Kit designed to create cDNA libraries from small RNAs for qPCR detection to identify ten novel tRNAs associated with cell stress in OA chondrocytes.

Results: Using qRT-PCR RNase gene expression was measured. Of these RNases 250 μ M TBHP treatment at 12 hour induced KIAA0430 ($p < 0.05$), RNase7 ($p < 0.0001$) and ZC3H12A ($p < 0.0001$) but not angiogenin or SND1. 10 ng/ml IL1 β treatment at 6 hour induced SND1 ($p < 0.0001$), RNase 7 ($p < 0.01$), ZC3H12A ($p < 0.01$) but not angiogenin or KIAA0430. Chondrocytes undergoing oxidative or ER stress produced tRNA halves in a time dependent manner, tRNA formation was highest at 6 hour of IL1 β treatment and after 12 hour of TBHP treatment. tRNA fragments of tRNAs specific for arginine, glutamic acid, glycine, histidine, lysine and valine were increased after TBHP or IL1 β treatment. Ten tRNAs that were induced by IL1 β and TBHP were selected for further study. Primer sets for these tRNA and the parent tRNAs were designed and screened across a wider time course, and with higher patient numbers.

Conclusions: The expression of several RNases was increased in a time dependent manner in stressed OA chondrocytes and may be involved in the cleavage of tRNA fragments. tRNA halves were grouped together based on their parent tRNA amino acid target. We hypothesised that an increase in fold change in these grouped tRNAs may result in less production of proteins that contain a high percentage of that specific amino acid. These changes in amino acid distribution may account for decreases seen in amino acid rich proteins such as Type II collagen (a glycine rich protein) in stressed chondrocytes. By designing tRNA and parent tRNA primers we will discover if tRNA formation effects total levels of parent tRNAs. The outcomes from this research will provide us with an increased understanding of tRNA to tRNA formation in human chondrocytes and may allow us to identify therapeutic targets for the treatment of OA and provide insights into novel stress signalling pathways.

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SAT0066 THE LONG NONCODING RNA (LNCRNA) HOTTIP IS A MASTER REGULATOR OF CELL CYCLE IN HAND SYNOVIAL FIBROBLASTS IN ARTHRITIS

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Background: Rheumatoid arthritis (RA) and other types of inflammatory arthritis follow a characteristic anatomical pattern of joint involvement. We have recently shown that local synovial stromal cells, specifically synovial fibroblasts, exhibit joint-specific transcriptomes and functions. In particular, hand SF exhibited prominent proliferative and chemotactic activities. Density of stroma and leukocyte infiltration were increased in hand synovium.

Objectives: To explore the role of hand/feet-specific lncRNA HOTTIP in shaping the function of hand synovial fibroblasts in arthritis.

Methods: We studied transcriptomes and epigenomes of hand, shoulder and knee SF from patients with RA or osteoarthritis and from knees of non-arthritic subjects using RNA-sequencing, Illumina HiSeq 2000 ($n=21$), histone ChIP-sequencing (Illumina HiSeq 2500, $n=7$) and Infinium HumanMethylation450 BeadChip ($n=12$). qPCR was used to confirm RNA-sequencing data in a larger cohort of SF from different joints. We silenced the lncRNA HOTTIP in hand SF using LNA GapmeRs, followed by RNA-sequencing, qPCR, protein-protein interaction analysis of RNA-sequencing data (STRING), and *in vitro* assays for proliferation (BrdU assay) and apoptosis (Annexin V/PI staining).

Results: Genome-wide DNA methylation patterns and histone marks at actively transcribed DNA regions (H3K27ac) and enhancers (H3K4me1) defined joint-specific origin of SF. SF from hands and feet specifically expressed the lncRNA HOTTIP. This distal-specific HOTTIP expression coincided with the enrichment of H3K4me3 and H3K27ac and a decrease in repressive marks (H3K27me3, DNA methylation) at the HOTTIP promoter in hand SF. In contrast, the HOTTIP promoter displayed scarce activating, but abundant repressive epigenetic marks in shoulder and knee SF. Silencing of HOTTIP in hand SF altered the expression of 447 protein-coding genes ($\log$ ratio > 2 , FDR < 0.05). These genes were strongly enriched in the mitotic cell cycle protein interaction network ($n=48$ genes, $p=3.3 \times 10^{-7}$). Several of the enriched mitotic cell cycle genes, including NCAPG, CENPO, ZWILCH and BUB1 were confirmed as downregulated by HOTTIP silencing in a larger cohort of hand SF ($n=6$). The basal expression of 36 out of the 48 enriched cell cycle genes correlated with the basal HOTTIP expression in hand SF ($n=6$, RNA-sequencing, $R > 0.06$). We further measured these correlations in a larger cohort of hand SF ($n=21$) for a subset of the 36 genes using qPCR. Among the measured genes, TADA 3 and CDC27 were confirmed to correlate with HOTTIP expression in hand SF ($R=0.5$, $p < 0.05$). Silencing of HOTTIP for 24 hour, 48 hour and 72 hour decreased the incorporation of BrdU into DNA of

hand SF as measured by BrdU proliferation assay ($p < 0.05$, $n = 3$). Apoptosis of hand SF increased at 48 hour of HOTTIP silencing ($p < 0.05$, $n = 3$).

Conclusions: The lncRNA HOTTIP, which is specifically expressed in hand joints via epigenetic mechanisms, is a master regulator of mitotic cell cycle genes and proliferation in hand SF. Distal-specific expression of HOTTIP might imprint hand SF with enhanced proliferative potential, thereby shaping the location-specific joint pathology, e.g. prominent synovial hyperplasia and increased severity of hand arthritis in RA.

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SAT0067 ROLE OF NOX2-DERIVED REACTIVE OXYGEN SPECIES IN S100A8/A9-DRIVEN INFLAMMATORY OSTEOARTHRITIS

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Background: Synovitis in inflammatory osteoarthritis (OA) is driven by locally released DAMPs like S100A8/A9 proteins that have been shown to enhance joint destruction. S100A8/A9 induce ROS (reactive oxygen species) release by phagocytes in OA synovium via the NADPH-oxidase 2 (NOX2)-complex. Assembly of this complex is dependent on the neutrophils cytosolic factor (Ncf1). In this complex, the NOX2 protein is responsible for ROS production.

Objectives: In the present study we investigated whether NOX2-derived ROS are involved in joint pathology during collagenase-induced OA (CiOA).

Methods: CiOA was induced in knee joints of wild type (WT) and Ncf1-deficient (Ncf1^{-/-}) mice. Synovial gene expression of NOX2-subunits was measured with qRT-PCR. Joint pathology was assessed using histology and antibodies against aggrecan neo-epitope VDIPEN. Levels of inflammatory proteins were measured with Luminex or ELISA. Phagocytes present in synovium, blood, bone marrow and spleen were analysed with flow cytometry. Extracellular ROS production by bone marrow-derived phagocytes was measured using an Amplex Red ROS detection assay.

Results: CiOA induction in knee joints of WT mice caused significantly increased synovial gene expression of NOX2 subunits. On day 7 of CiOA, synovial thickening, synovial S100A8/A9 levels and synovial percentages of inflammatory macrophages, PMNs, and monocytes were comparable between WT and Ncf1^{-/-} mice. Cartilage damage and MMP activity, as measured by VDIPEN staining, were comparable, as well as levels of inflammatory mediators in serum and phagocyte percentages in blood, bone marrow and spleen. On day 42 of CiOA, synovitis, cartilage damage, and osteophyte formation in Ncf1^{-/-} mice were unaltered when compared to WT mice. ROS production by Ncf1^{-/-} PMNs was completely absent but Ncf1^{-/-} macrophages, the more predominant phagocyte involved in development of pathology during CiOA, produced ROS in similar amounts as WT macrophages. Ncf1 deficiency thus seems to exclusively affect PMNs, this surprising finding might explain the lack of differences observed between CiOA development in WT and Ncf1^{-/-} mice. ROS production by WT and Ncf1^{-/-} macrophages was strongly upregulated by S100A8 and almost completely inhibited by the pan-NOX inhibitor diphenyleneiodonium chloride (DPI). In order to determine whether NOX1 complexes caused the compensation responsible for Ncf1 independent ROS production, we co-stimulated Ncf1^{-/-} macrophages with NOX1-specific inhibitor ML171. However, no ML171-induced inhibition of ROS production was observed.

Conclusions: Absence of PMN-derived ROS does not alter synovitis and joint pathology in S100A8/A9-driven experimental inflammatory OA. The mechanism that enables Ncf1-independent ROS production by macrophages should be further investigated.

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SAT0068 BILIRUBIN PROMOTES DOWN-REGULATION OF RUNX2 AND UP-REGULATION OF RANKL GENE EXPRESSION IN BONE EXPLANTS AND IN OSTEOBLASTIC AND OSTEOCYTIC CELL LINES

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Background: *In vitro* studies have shown that the retained substances of cholestasis have deleterious effects in human osteoblasts and osteocytic cells. Bilirubin (BIL) and lithocholic acid (LCA) induce alterations in the proliferation, differentiation and apoptosis of osteoblastic and osteocytic cells. However, their effects in human bone tissue and in bone cell lines have not been deeply analysed.

Objectives: To investigate the effects of BIL, LCA and ursodeoxycholic acid (UDCA) in gene expression of human trabecular bone explants as well as in osteoblastic (SAOS2) and osteocytic cells (MLO-Y4/MLO-A5).

Methods: Bone tissue harvested from trabecular bone fragments, SAOS2 and MLO-Y4/MLO-A5 cells were cultured and treated with BIL (50 μ M), LCA (10 μ M) and UDCA (10/100 μ M) for 24 hour. Gene expression of osteocalcin (*BGLAP*), *Cbfa1* (*RUNX2*)/*Osterix* (*OSX*) and *RANKL* (*TNFRSF11B*)/osteoprotegerin (*TNFRSF11B*) were quantified by real time PCR.

Results: BIL diminishes *RUNX2* gene expression in bone tissue (-37%), SAOS2 (-75%), MLO-Y4 (-56%) and MLO-A5 (-77%), and increases *RANKL* expression in 60%, 27%, 72% and 60%, respectively ($p \leq 0.02$). In bone tissue and in osteoblastic and osteocytic cells, LCA increases gene expression of *BGLAP* (NS) and *RANKL* ($p \leq 0.03$). UDCA 100 μ M increases *RUNX2* and *OSX* expression in bone tissue (78% and 82%), MLO-Y4 (72% and 80%) and SAOS2 (75% and 70%) ($p \leq 0.03$). In addition, UDCA 100 μ M significantly increases expression of *BGLAP*, *OPG* and *RANKL* in bone tissue and in osteocytic lines. UDCA 10/100 μ M counteracts the decrease in *RUNX2* induced by BIL in bone tissue, SAOS2, MLO-A5 and MLO-Y4 cells.

Conclusions: The retained substances of cholestasis, particularly bilirubin, cause noxious effects on transcription factors of osteoblast differentiation and on osteoclastic activators in bone tissue and in osteoblastic and osteocytic cells. Ursodeoxycholic acid reverses the harmful effects of bilirubin. These results provide new insights into the low bone formation and at some stages, high resorption, associated with chronic cholestasis.

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SAT0069 ABNORMAL BONE AND CARTILAGE METABOLISM COULD BE ANTAGONISED BY PULSED ELECTROMAGNETIC FIELDS (PEMFs) AND TNF-A AND IL-6 GENE KNOCKOUTS IN A SIMILAR MECHANISM

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Background: Pulsed electromagnetic fields (PEMFs), as a safe and non-invasive method, could positively affect bone and cartilage metabolism. However, the effect and underlying mechanisms of PEMFs on osteoporosis and osteoarthritis remain poorly understood.

Objectives: The present study is designed to investigate the effect of PEMFs on osteoporotic bone and degenerative cartilage together with its potential molecular mechanisms in mice with different gene background.

Methods: Twenty 12 week male and Female wild-type (WT), *TNF α* knockout (*TNF α ^{-/-}*) or *IL6* knockout (*IL6^{-/-}*) mice, respectively, were sham-operated (SHAM) or subjected to destabilisation of the medial meniscus (DMM) and ovariectomy (OVX) surgeries. After surgeries, WT mice were equally assigned to the non-treatment and PEMFs groups. Mice in PEMFs group were subjected to daily 1 hour PEMFs exposure with 8 Hz, 3.8 mT (peak value). Then all mice were euthanized after 4 weeks. Bone mass and subchondral microarchitecture were determined using micro-CT. Bone and cartilage metabolism was assessed by histological analysis, serum analyses, qRT-PCR and Western-Blot.

Results: The surgical models of osteoporosis and osteoarthritis were proved successful evidenced by the analysis of micro-CT data and histological analysis. The bone loss and damaged cartilage were largely repaired by *TNF α* and *IL6* gene knockout and partially inhibited by PEMFs exposure. Interestingly, no difference