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 IncRNA 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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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.

Methods: 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.

Conclusions: Twenty 12 week male and Female wild-type (WT), TNFγ knockout (TNFγ−/−) 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 TNFγ and IL6 gene knockout and partially inhibited by PEMFs exposure. Interestingly, no difference

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 μM) and UDCA (10/100 μM) 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<0.02). In bone tissue and in osteoblastic and osteocytic cells, LCA increases gene expression of BGLAP (NS) and RANKL (p<0.03). UDCA 100 μM increases RUNX2 and OSX expression in bone tissue (78% and 80%), MLO-Y4 (72% and 80%) and SAOS2 (75% and 70%) (p<0.03). In addition, UDCA 100 μM significantly increases expression of BGLAP, OPG and RANKL in bone tissue and in osteocytic cells. UDCA 100/100 μM 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 osteoblastic 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.

Disclosure of Interest: None declared

in Micro-CT data analysis was found between PEMFs group and gene knockouts, although a slight increase could be observed in TNFα−/− mice when compared to the PEMFs group. Negative effects on bone and cartilage were proved by testing key cytokines in analysis and catabolism. PEMFs treatment and gene knockouts corrected the negative effects by targeting mediators in molecular pathways like Wnt and RANK. The differences in mRNA and protein level changes between PEMFs and gene knockouts were minor.

Conclusions: PEMFs alleviated surgeries induced bone loss and cartilage degeneration by promoting anabolism and inhibiting catabolism possibly in a similar manner to TNF-α and IL-6 gene knockouts, which imply that TNF-α and IL-6 may become new potential targets for PEMFs in treating degenerative bone diseases.

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SAT0070

ROLE OF C/EBPB IN 1,25D-INDUCED ACTIVATION OF RANKL EXPRESSION
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Background: 1 alpha 25-dihydroxyvitamin D3 (1,25D) is the active form of vitamin D3, which is responsible for osteoblast activation, subsequently bone formation. Although recent studies have shown that 1,25D stimulates RANKL expression in osteoblast differentiation[2], its molecular mechanism of action is not fully understood.

Objectives: The aims of this study were to evaluate cellular response of human bone-derived cells to 1,25D treatment by observing expression during osteoblast differentiation

Methods: In this study, MG63, SaOS2, and primary bone-derived cells (BdCs) were cultured and isolated to further elucidate the effect of 1,25D on osteoblasts. Those were incubated in osteogenic medium (ascorbic acid, beta-glycerol phosphate, and dexamethasone) for 1, 3, and 7 days with or without 20 μM 1,25D. The osteoblast activity and differentiation status were evaluated by intercellular Alkaline Phosphatase (ALP) activity, ALP staining, Alizarin Red S (ARS) staining, and histomorphometric staining. In this situation, C/EBPb gene manipulation with siRNA or overexpression system were subjected to report assay of human RANKL promoter, quantitative PCR(qPCR), immunoblotting and immunostaining of osteoblastic gene expression (alkaline phosphatase, osteocalcin, vitamin D3 receptor, RANKL, and C/EBPb etc.)

Results: 1,25D promotes osteoblast differentiation and expression of osteogenic markers in three different cells. Intriguingly, treatment of 1,25D to those cells are accompanied by stabilising C/EBPb proteins and stimulating RANKL expression. Moreover, Overexpression of C/EBPb significantly increases RANKL mRNA and protein. In contrast, suppression of C/EBPb decreases RANKL expression. Thus, C/EBPb is a key mediator involved in 1,25D induced RANKL expression.

Conclusions: our preliminary data indicated that human bone-derived cells response to vitamin D3 promoted RANKL expression via activation of C/EBPb and enhanced osteoblast activity and differentiation. This study provides insight into the molecular mechanism of RANKL expression and osteoblast activation in human bone-derived cells response to 1,25D.

REFERENCE:

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SAT0071

SUBCHONDRAL OSTEOPATHY BUT NOT CAR蒂LAGE DAMAGE IS PREVAlent IN KNEE JOINTS OF PREMATURELY AGEING MITOCHONDRIAL DNA MUTATOR MICE
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Background: Mitochondrial dysfunction has been demonstrated in ageing and osteoarthritic tissues. However it remains unclear whether dysfunctional mitochondria are directly implied in the pathogenesis of osteoarthritis.

Objectives: We investigated knee joints of prematurely ageing mitochondrial DNA mutator mice (Polyg(2000)a) to evaluate a causal relationship between mitochondrial dysfunction and different features of osteoarthritis.

Methods: Bone structural parameters and chondropathy were evaluated in knee joints of mice displaying increased mtDNA mutations rates and accelerated ageing, due to expression of a proofreading-deficient mtDNA polymerase, using micro-computed tomography and histopathological analysis.

Results: Homozygous mutants displayed osteopenia of the epiphyseal trabecular bone and subchondral cortical plate in comparison to wild type controls and heterozygous mutants. Osteopenia was associated with a strong increase of osteoblast number (0.88±0.30/mm bone perimeter) compared to heterozygous (0.25±0.03/mm) and wild type mice (0.12±0.04/mm). New bone formation was not observed. Wild type mice displayed only low grade cartilage degeneration (OARSI grade ≤1) due to loss of cartilage proteoglycans. Increased tbio-femoral chondropathy was not apparent in hetero- and homzygous mitochondrial DNA mutator mice.

Conclusions: Mitochondrial dysfunction and premature ageing in mice with somatically acquired mtDNA mutations predisposes to enhanced subchondral bone resorption as potential early step of osteoarthritis, but not to cartilage damage or new bone formation. This phenotype potentially corresponds to an osteoporotic osteoarthritis phenotype in humans.

Disclosure of Interest: None declared


SAT0072

MIRNA-146A IS A KEY PLAYER IN BONE METABOLISM AND OSTEOPOROSIS
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Background: Micro RNAs (miRNAs) play a crucial role in the regulation of bone metabolism. MiR-146a, an important anti-inflammatory miRNA, was found to negatively impact osteogenesis and bone regeneration in vitro, by controlling the differentiation of mesenchymal stem cells. But to date the role of miR-146a in bone

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