Methods Human MSC were isolated from healthy controls. Comparisons were made between podoplanin positive and negative MSC. MSC migration across 8 µm pore filters following treatment with anti-siRNA podoplanin or Rho GTPases inhibitors was assessed. MSC-platelet interactions were assessed by culturing MSC on the basal surface of 3 µm pore filters and perfusing fluorescently labelled platelets in whole blood over the apical surface. In some cases, the apical surface of the filter was pre-coated with EC, forming an EC-MSC co-culture, prior to platelet perfusion.

Results Expression of podoplanin significantly enhanced the migration of MSC compared to MSC lacking podoplanin. Rac-1 inhibition altered the membrane localisation of podoplanin and in turn significantly reduced MSC migration. Blocking Rac-1 activity had no effect on the migration of MSC lacking podoplanin, indicating it was responsible for regulation of migration through podoplanin. When podoplanin-expressing MSC were seeded on the basal surface of a porous filter, they were able to capture platelets perfused over the uncoated apical surface and induce platelet aggregation. Similar microthrombi were observed when EC were co-cultured on the apical surface. Confocal imaging shows podoplanin-expressing MSC extending processes into the EC layer, which could interact with circulating platelets. In both models, platelet aggregation induced by podoplanin-expressing MSC was inhibited by recombinant soluble CLEC-2.

Conclusions Podoplanin enhances the migratory capacity of tissue-resident MSC enabling them to move more rapidly within the rheumatoid joint. Moreover, podoplanin allows MSC to interact with both circulating and tissue platelets to elicit either protective or pathogenic responses.

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Disclosure of Interest None declared.

P119 EPIGENETIC REGULATION OF WNT SIGNALING IN SYSTEMIC SCLEROSIS VIA SFRP1

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Career situation of first and presenting author Young investigator.

Introduction Systemic sclerosis is an autoimmune connective tissue disease. The disease is characterised by inflammation and fibrosis. The main cell type is an activated fibroblast called a myofibroblast that secretes a panopoly of extracellular matrix molecules leading to the skin and lung fibrosis. The mechanism(s) that lead to the myofibroblast generation are unknown. In recent times epigenetic regulation has been shown to play a major role in generation of myofibroblasts in systemic sclerosis and other fibrotic diseases. Epigenetics is defined as a change in gene expression without a change in the DNA bases. This is mainly facilitated by DNA methylated the addition of a methyl group onto cytosine and microRNAs. Wnt is a conserved signaling pathway that is needed for organ formation and is known to be elevated in systemic sclerosis. Enhanced Wnt can lead to fibrosis through beta catenin and extracellular antagonists regulate the threshold of Wnt signalling. sFRP1 is an extracellular

Wnt inhibitor that we have previously demonstrated to be involved in systemic sclerosis.

Objectives To determine the epigenetic regulation of sFRP1 in systemic sclerosis.

Methods Diffuse systemic sclerosis and healthy control serum was collected from 10 patients and controls. sFRP1 was measured with a standard ELISA method using a commercial ELISA. qPCR was performed with SSC fibroblasts or control fibroblasts using Taqman specific kits for miR27a3p and RNU44. Data was normalised to RNU44 and shown as fold change to controls. Transfection of microRNA mimics was performed using 100 nM of miR27 mimics or matched concentration of scramble after 48 hours post transfection media was collected and the cells lysed in RIPA buffer and lysates subjected to western blotting.

Results We found reduced levels of sFRP1 in systemic sclerosis sera compared to healthy controls (n=10). Using taqman PCR we also found elevated levels of microRNA27a3p in systemic sclerosis fibroblasts. Using software to predict targets it was identified that sFRP1 is a direct target of microRNA27a3p. We could demonstrate after transfection of microRNA27a3p into healthy dermal fibroblasts compared to scramble controls that the levels of the target sFRP1 was reduced and elevated levels of collagen1 and beta catenin was present. This suggest that sFRP1 is a direct target leading to upregulation of Wnt signaling. We also found reduced levels of anti-fibrotic PPAR-gamma also after transfection. siRNA knockdown of sFRP1 using small interfering RNA leads to upregulation of collagen and Axin2 and lactate elevation.

Conclusions sFRP1 is regulated by miR27a2p in systemic sclerosis. MiR27a3p can also regulate PPAR gamma. Enhanced Wnt signaling is associated with metabolic alterations.

Disclosure of Interest None declared.

P120 IL-23 RECEPTOR SIGNALING IS IMPORTANT DURING PHYSIOLOGICAL BONE REMODELING AND RADIAL BONE GROWTH THROUGH REGULATION OF OSTEOBLAST DIFFERENTIATION

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Career situation of first and presenting author Student for a master or a PhD.

Introduction In mice, systemic exposure of IL-23 induces chronic arthritis, increased osteoclast differentiation and systemic bone loss. However, the role of IL-23R signaling during physiological bone remodeling is not fully elucidated.

Objectives To examine the role of IL-23R signaling during physiological bone remodeling.

Methods Femurs of naïve 7-, 12- and 26-week-old IL-23R^{GFP/GFP} (IL-23R^{-/-}) and IL-23R^{+/+} (WT) littermate mice were used for micro-CT analysis of the bone and a three-point bending test for bone strength. Bone marrow (BM) cells were either cultured towards osteoclasts with M-CSF and RANKL or were cultured towards osteoblasts with β-glycerophosphate and vitamin C. Osteoclast differentiation and activity were assessed

using tartrate-resistant acid phosphatase (TRAP) staining and bone resorption assay, respectively. Osteoblast differentiation was assessed by alkaline phosphatase staining and activity was determined by calcium measurement in the supernatant.

Results Trabecular bone volume, thickness and number as well as cortical volume and thickness, and femur length were significantly lower in 12-week-old IL-23R^{-/-} mice compared to WT. In addition, three-point bending data revealed reduced maximum force in IL-23R^{-/-} femurs. Surprisingly, bone volume was similar between both groups at the age of 26 weeks. However, similar to 12-week-old mice, endocortical volume and femur perimeter were significantly lower in IL-23R^{-/-} mice compared to WT. To further study the temporal differences in bone phenotype, we studied osteoclasts and osteoblasts from 7- and 12-week-old mice *in vitro*. Osteoclast differentiation and function were similar at both ages between WT and IL-23R^{-/-} mice. Interestingly, BM cells of 7-week-old IL-23R^{-/-} mice had reduced capacity to differentiate towards osteoblasts, compared to WT. In contrast, these cells showed higher differentiation and significantly higher calcium uptake than WT mice at 12 weeks.

Conclusions IL-23R^{-/-} mice have temporally dynamic changes in bone metabolism, which is possibly related to alterations in osteoblasts, however, the exact mechanism still needs to be elucidated.

Disclosure of Interest None declared.

P121/021 MIR-146A AN IMPORTANT KEY PLAYER IN BONE METABOLISM

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Career situation of first and presenting author Post-doctoral fellow.

Introduction 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 remodelling, its influence on bone stability and development of osteoporosis is not known.

Objectives Our aim is to analyse the function of miR-146a in bone metabolism.

Methods Systemic bone, tibiae and femur, of wt and miR-146a deficient animals was assessed histologically and via μ CT analysis, over a period of 3 to 18 months of age. Serum cytokine levels were analysed by Elisa. MRNA expression levels in bone were analysed by qPCR. To induce osteoporosis, ovariectomy (OVX) induced bone loss was performed.

Results When we analysed bone volume of long bones histologically as well as with μ CT analysis we detected significantly increased trabecular bone mass in miR-146a deficient compared to wt animals, starting at an age of 6 months. In addition cortical thickness of systemic bones from miR-146a knock out animals was significantly increased compared to control mice. Analysis of serum in aged miR-146a deficient animals displayed elevated activity

of bone resorbing osteoclasts as amounts of CTX I in miR-146a^{-/-} mice were significantly increased compared to wt animals. Q-PCR analysis of important osteoclast as well as osteoblast marker genes in bones *ex vivo* displayed elevated expression of signature molecules of both cell types in aged miR-146a deficient mice, suggesting a regulatory role of miR-146a in both osteoclasts as well as osteoblasts. When we induced osteoporosis using the OVX disease model, histological analysis of long bones showed significant trabecular bone loss in ovariectomized wt mice. In contrast, we detected no trabecular bone loss in ovariectomized miR-146a knock out animals, suggesting that loss of miR-146a deficiency protects bone loss induced by estrogen deficiency.

Conclusions MiR-146a seems to control bone turnover and miR-146a deficient mice accrue bone over time. Moreover this miRNA has a negative influence on bone loss occurring during oestrogen loss induced osteoporosis. Therefore miR-146a could be possibly used as a therapeutic target in the treatment of osteoporosis.

Disclosure of Interest None declared.

P122 IMPORTANT ROLE OF DENDRITIC CELLS IN INFLAMMATORY ARTHRITIS

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Career situation of first and presenting author Student for a master or a PhD.

Introduction Important role of dendritic cells in inflammatory arthritis.

Objectives Investigation of the role of CD11c⁺ cells in joint inflammation and destruction.

Methods We analyzed histological sections of K/BxN serum transfer arthritis as well as hTNFtg arthritis for the presence of CD11c⁺ cells by immunohistochemistry. We used CD11c-diphtheria toxin receptor (DTR) transgenic mice. K/BxN serum transfer arthritis was induced, and mice were given either DT or PBS or in wt and BARF3 deficient mice. In addition CD11c DTR mice were crossed into hTNFtg animals and also received either DT or PBS. The severity of arthritis was determined clinically and histologically.

Results Both CD8⁺CD11c⁺ and CD11b⁺CD11c⁺, can be found in synovial tissue in TNF driven arthritis. Upon depletion of CD11c⁺ cells clinical signs of K/BxN serum transfer arthritis were significantly reduced. Histological analysis found reduced synovial inflammation after the depletion of CD11c⁺ cells in K/BxN arthritis. In addition, local bone destruction and the number of osteoclasts was also significantly reduced. In addition to K/BxN arthritis, we found that also in TNF-driven arthritis depletion of CD11c⁺ cells led to a striking reduction of synovial inflammation and a complete depletion of osteoclasts.

Conclusions These data show that in addition to initiating an adaptive immune response, CD11c⁺ dendritic cells, are also involved in innate effector mechanisms of inflammatory