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**MIR-146A AN IMPORTANT KEY PLAYER IN BONE METABOLISM**

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**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** The objective of this project is the analysis of the role of miR-146a in bone metabolism.

**Methods** Systemic bone, tibiae and femur, of wt and miR-146a deficient animals was assessed histologically and via  $\mu$ 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  $\mu$ CT analysis we detected significantly increased trabecular bone mass in miR-146a deficient compared to wt animals, starting at an age of 6 months. However, cortical thickness of systemic bones from miR-146a knock out animals was significantly reduced 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<sup>-/-</sup> 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 oestrogen 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

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**INCREASED GPR22 ACTIVATION TRIGGERS OSTEOARTHRITIS**

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**Introduction** G protein-coupled receptors (GPCRs) are considered interesting drug targets. GPR22, an orphan receptor, was

previously associated to osteoarthritis (OA) in a genome wide association study.

**Objectives** To investigate GPR22 expression in human healthy and OA cartilage and assess the functional role of GPR22 with *in vitro* and *in vivo* models.

**Methods** GPR22 protein levels were examined by immunohistochemistry. Chondrogenic ATDC5 cells stably overexpressing *Gpr22* (GPR22+) and embryonic limb bud cells from mutant *Gpr22*<sup>V385A/V385A</sup> mice were cultured as micromasses, and read-outs included gene expression and evaluation of the extracellular matrix. Downstream GPR22 signalling was determined with a PKA activity fluorescence-based assay. Cholecystokinin receptor antagonist AG-041R was tested as GPR22 antagonist. PKA concentrations and kinetics were measured by fluorescence resonance energy transfer (FRET) imaging by PKA sensors in ATDC5 cells. Destabilisation or removal of the medial meniscus (DMM or TMM), for 12 or 8 weeks respectively, was surgically induced in mutant 8 week-old *Gpr22*<sup>V385A/V385A</sup> and wild-type C57/Bl6 mice. Vehicle or AG-041R was injected in the knee joint every 5 days, starting 1 week post-DMM until the mice were sacrificed. Severity was analysed according to OARSI scoring and immunohistochemistry.

**Results** In healthy human articular cartilage, GPR22 protein was absent, while it is present in OA cartilage. GPR22 +cells showed increased mineralization and decreased proteoglycan content compared to controls after 21 days. Gene expression demonstrated a decrease in Aggrecan and Collagen 2. In contrast, levels of hypertrophic markers Col10 and Mmp13 were increased, suggesting a shift of the cells' differentiation status. PKA activity was reduced in GPR22 +cells compared to controls. The effects on markers, proteoglycan content and mineralization were antagonised by AG-041R. FRET imaging confirmed the neutralising effect of AG-041R on intracellular PKA concentration in GPR22 +cells. *In vivo*, DMM- and TMM- operated knees of GPR22<sup>V385A/V385A</sup> mice showed increased cartilage damage, and increased GPR22 and COL10 expression compared to controls. Intra-articular injections of AG-041R prevented cartilage damage in DMM-induced GPR22<sup>V385A/V385A</sup> mice.

**Conclusions** GPR22 is present in human OA cartilage and not in healthy tissue. Overexpression of GPR22 accelerates chondrocyte hypertrophy. GPR22 can be antagonised by AG-041R. Our preliminary *in vivo* data show a therapeutic effect of AG-041R in the DMM model. Thus, GPR22 is genetically and functionally linked to OA and may be a potential therapeutic target.

**Disclosure of interest** None declared

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**WHY JOINT LOCATION MATTERS IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS**

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**Introduction** Inflammatory arthritis, such as rheumatoid arthritis (RA) and spondyloarthropathies, follows a characteristic anatomical pattern of joint involvement. We suggest that the local cell types, systemic triggers and site-specific exogenous factors that activate these local cells synergistically contribute to the site-specific occurrence of arthritis.