studies. Furthermore, a transmigration assay using SF from all four genotypes and murine endothelioma cells (bEnd.5) as an endothelial barrier was carried out. For more detailed information, SF transmigration was evaluated when endothelial cells were also pre-treated with TNF-alpha, mimicking inflammatory conditions.

Results Lasp-1 expression is upregulated in SF from hTNFtg mice and localises to structures of cell adhesion and invasion. In the scratch assay, a significantly reduced migration rate was detected in Lasp-1$^{-/-}$ SFs after 24 hrs (–43.7% versus wt, $p < 0.05$) and in Lasp1$^{-/-}$/hTNFtg, respectively (–69.11% versus hTNFtg, $p < 0.05$). Live cell imaging studies showed a slower migration and striking differences in migration morphology of Lasp1$^{-/-}$/hTNFtg compared to hTNFtg SF. Furthermore, analyses showed a significant reduction of transmigration of Lasp1$^{-/-}$/hTNFtg compared to hTNFtg SF that was even enhanced by TNF-alpha stimulation of the endothelial cells.

Interestingly, interbred Lasp1$^{-/-}$/hTNFtg mice presented milder clinical symptoms and analyses of histopathology revealed less cartilage degradation and less attachment of synovial tissue to the cartilage than hTNFtg mice at an age of 14 weeks.

Conclusions Our data provide that the migratory capacity of SF is regulated by Lasp-1 and influences the severity of arthritis in hTNFtg mice. SF – when activated – migrate through the formation of invasive and adhesive membrane structures such as invadopodia, where Lasp-1 is prominently localised. Thus, targeting Lasp-1 may be a promising strategy to reduce the invasive and migratory behaviour of synovial fibroblasts in RA.

A8.16 THE ROLE OF ADIPOCYTOKINES IN OSTEOARTHRITIS OSTEOPHYTE FORMATION

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Background and Objectives Although obesity is an established risk factor in osteoarthritis (OA), there is limited information about the role of adipose tissue derived factors in bone formation. Adipocytokines such as adiponectin, resistin, and visfatin, are known to be associated with the pathogenesis of rheumatoid arthritis (RA) and OA. Adipocytokines are locally produced in RA and OA joints by osteoblasts, osteoclasts, and chondrocytes. In contrast to their joint-destructive effects in RA, the role of adipocytokines in OA bone remodelling and osteophyte formation is unclear. Therefore, the adipocytokine expression during osteophyte development and in cells of bone formation was analysed as well as their effect on these cells.

Methods Osteophytes, cartilage, and osteoblasts were obtained from OA patients during joint replacement surgery. Serial sections of bone tissue were stained (Masson trichrome, TRAP) and scored from grade one (no ossification, mainly connective tissue and cartilage) to five (ossified mineralised osteophytes, <10% connective tissue, ossified remodelling zones). Immunohistochemistry against alkaline phosphatase, collagen-type II, adiponectin, resistin, and visfatin was performed. OA osteoblasts were stimulated with adiponectin or resistin and immunoassays for IL-6, IL-8, and MCP-1 were performed.

Results All adipocytokines were detectable in cultured osteoblasts and all osteophyte grades. In non ossified osteophytes (grade 1), especially adiponectin and to a lower extent resistin and visfatin were detectable in connective tissue fibroblasts. In ossified osteophytes (grade 2–5), resistin and visfatin and to a lower extend adiponectin were expressed by osteoblasts and resistin and visfatin by osteoclasts. In all osteophyte grades adiponectin was detectable in blood vessels and visfatin was found in about 50% of the chondrocytes.

Osteoblast stimulation with adiponectin increased the release of the inflammatory mediators IL-6 (2.6-fold), IL-8 (4.9-fold), and MCP-1 (2.1-fold). In contrast, resistin led to a non-significant decrease of these factors. The osteoblast populations showed individual differences in the baseline expression of the analysed factors and in their responsiveness to adipocytokines.

Conclusions The adiponectin and visfatin expression in osteophyte connective tissue and cartilage suggests their involvement in early osteophyte development. Resistin and visfatin in osteoblasts and osteoclasts in ossified osteophytes indicates a role in osteophyte formation at later stages. The stimulation of osteoblasts with adiponectin induces the release of inflammatory mediators. Therefore, the analysed adipocytokines most likely are involved in osteophyte formation at different stages and correspondingly affect cells of cartilage and bone formation to a different extent.

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A8.17 THE ROLE OF CXCR2 SIGNALLING IN ARTICULAR CARTILAGE HOMEOSTASIS

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Background and Objectives The production of ELR+ CXC chemokines is widely studied in arthritis and is thought to contribute to the inflammatory phenomena that lead to cartilage breakdown. Healthy articular chondrocytes however, also express their own chemokine receptors and ligands, however their function in these cells is puzzling because chondrocytes are encased in a dense extracellular matrix and are not known to migrate in vivo. This study aims to identify the function of this signalling mechanism in articular cartilage.

Materials and Methods Adult human articular chondrocytes were expanded in monolayer culture under standard conditions. CXCR1 and CXCR2 expression was confirmed using semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) and Western blotting. Chemokine receptors and ligands were detected in human articular cartilage from healthy and osteoarthritis patients and in mouse articular cartilage using immunohistochemistry. CXCR1/2 signalling was blocked at specific receptor level in human chondrocytes using validated blocking antibodies and siRNA. Chondrocyte phenotypic gene expression was assessed using real time RT-PCR. The content of highly sulphated proteoglycans in chondrocyte micromasses was analysed using Alcian blue staining, guanidine HCl extraction and spectrophotometric quantification. Surgical destabilisation of the medial meniscus (DMM) was used to induce instability into the left knees of 8 week old CXCR2$^{-/-}$ mice and wild type BALB/C controls (N = 10 per group). Right knees were sham operated as control. 8 weeks following surgery, mice were culled, knee joints were paraffin embedded and sectioned. Representative sections were stained using Safranin orange and osteoarthritis severity was assessed by Chambers scoring.

Results ELR+ CXC chemokines and their receptors, CXCR1 and CXCR2 were expressed in normal human articular cartilage. CXCR1 and CXCR2 were expressed in articular cartilage from osteoarthritis