Objective The heparan sulphate proteoglycan syndecan-4 (Sdc4) has been associated strongly with osteoarthritis, a disease that mimics key aspects of early cartilage remodelling during endochondral ossification, but its role in embryonic and adult bone formation remains unclear. Therefore, we used *Sdc4*$^{-/-}$ mice to analyse the distribution and functional role of Scd4 in endochondral ossification of mouse embryos and in adult fracture repair, which recapitulates endochondral ossification, but like osteoarthritis, involves an inflammatory component.

Methods *Sdc4* promoter activity was analysed in *Sdc4*$^{-/-}$*/LacZ knock-in* animals using β-galactosidase stainings. E16.5 embyros were used for histological (alcian blue/alizarin red) and immunohistological (PCNA, Col10a1, ADAMTS-4, BC-3, Sdc2) staining and the calcified bone area was quantified using whole mount staining of these embryos. Histological (Masson-Goldner, alcian blue) and immunohistological (Col10a1, Sdc2, PCNA) staining at day 7, 14 and 28 fracture calli were performed. These experiments were repeated with anti-TNF treatment during fracture healing. Callus size and cartilage area were quantified using image J Chondrocytes were isolated from neonatal knee joints and embyronal cartilage. Proliferation was investigated using MTT assay. Gene expression analysis for Sdc-2, Sdc-4 with and without stimulation using TNFα and WNT3a was performed using quantitative RT-PCR.

Results In *Sdc4*$^{-/-}$*/LacZ knock-in* animals, *Sdc4* promoter activity was detectable in all stages of chondrocyte differentiation during embryogenesis. *Sdc4* deficiency inhibited chondrocyte proliferation both in vivo and in vitro, but this did not lead to a growth phenotype at birth. In contrast to embryogenesis, fracture healing in adult mice was markedly delayed in *Sdc4*$^{-/-}$ animals and accompanied by increased callus formation. Analysing the discrepancy between the mild embryonic and the severe adult phenotype, we found a compensatory up-regulation of *Sdc2* in the developing cartilage of *Sdc4*$^{-/-}$ mice that was absent in adult tissue. Stimulation of chondrocytes with Wnt3a in vitro, led to an increased expression of *Sdc2*, while stimulation with TNFα resulted in an up-regulation of *Sdc4* but a decreased expression of *Sdc2*. In consequence treatment with a blocking anti-TNF antibody during fracture healing abolished the difference in callus size between wildtype and *sdc4*$^{-/-}$ mice.

Conclusions We conclude that Sdc4 is functionally involved in endochondral ossification and that the loss of Sdc4 impairs adult fracture healing due to the inhibition of compensatory mechanisms under inflammatory conditions.

A8.14 THE ANTI-PROLIFERATIVE FUNCTION OF RSK2 IN SYNOVIAL FIBROBLASTS PROTECTS AGAINST TNF-α-INDUCED JOINT DESTRUCTION IN INFLAMMATORY ARTHRITIS

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Background/Objectives The pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNF-α) directly activates the ribosomal S6 kinase RSK2 in vitro. We recently demonstrated the protective effect of RSK2 against TNF-induced bone loss. Interestingly, we found an increased activation of RSK2 in the joints of arthritis patients as well as in the inflamed joints of mice overexpressing the human TNF-α (*hTNF*tg). These observations prompted us to investigate the function of RSK2 in the development of TNF-α-induced inflammatory arthritis.

Materials and Methods *hTNF*tg mice were crossed with RSK2-deficient (*Rsk2*$^{y/-}$) mice. Clinical scoring and histomorphometry of the joints were assessed. We compared the levels of circulating pro-inflammatory cytokines as well as the cellularity of myeloid lineages in the spleen. The expression of cytokines and mesenchymal markers in the joints was determined via QPCR. Bone marrow transfer of *Rsk2*$^{y/-}$ and wild-type littermates into *hTNF*tg mice was performed and clinical scoring as well as histomorphometry of the joints was assessed. Primary fibroblast-like synoviocytes (FLS) from *hTNF*tg and *hTNF*tg; *Rsk2*$^{y/}$ mice were isolated to analyse their expression of inflammatory cytokines and metalloproteinases as well as their proliferation and apoptosis in vitro.

Results RSK2 deficiency in *hTNF*tg mice resulted in an early onset of clinical signs of arthritis as well as a drastic exacerbation of inflammation, increased cartilage destruction and increased local bone destruction. Increased levels of circulating pro-inflammatory cytokines and the increased proportion of all myeloid lineages in the spleen confirmed the enhanced inflammation in the *hTNF*tg mice lacking RSK2. Increased activation of synovial fibroblasts and macrophages in the joints of *hTNF*tg*; Rsk2*$^{y/-}$ mice was demonstrated by the locally increased expression of pro-inflammatory cytokines and matrix metalloproteinases (MMPs). Importantly, the phenotype could not be transmitted by the transfer of *Rsk2*$^{y/-}$ bone marrow into *hTNF*tg mice that demonstrated the essential role for RSK2 expression in mesenchymal cells driving the pathogenesis. In agreement, although no difference in the expression of pro-inflammatory cytokines or MMPs nor a change in apoptosis was detected in synovial fibroblasts isolated from *hTNF*tg*; Rsk2*$^{y/-}$, these cells displayed an increased proliferation rate.

Conclusions The anti-proliferative function of RSK2 controls a cell autonomous negative feed-back against the activation of synovial fibroblasts by TNF-α, therefore limiting joint destruction in arthritis. Thus, activation of RSK2 is a potential target for the treatment of both local and systemic bone destruction in RA.

A8.15 THE FOCAL CONTACT PROTEIN LASP-1 MODULATES THE MIGRATION CAPACITY OF SYNOVIAL FIBROBLASTS

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Background and Objectives RA synovial fibroblasts (SF) have been suggested to contribute to the spreading of disease through their ability to leave cartilage destruction sites, migrate via the bloodstream and re-initiate the destructive process at distant articular cartilage surfaces. In this context, the actin-crosslinking protein Lasp-1 is of interest, because it is localised at leading edges of migrating cells and regulates metastatic dissemination of different tumours. Therefore, it is particularly important to investigate the role of Lasp-1 in SF migration and its effects on RA.

Materials and Methods To identify different Lasp-1 expression levels in the hind paws of wt and hTNFtg mice, an established model for human RA, Western- blot analyses were performed. In parallel, Lasp-1 expression and its sub-cellular distribution was investigated in SF from wt and hTNFtg mice by Western-blot analyses and immunofluorescence. The migratory capacity of SFs derived from wild-type, Lasp-1$^{-/-}$, hTNFtg and Lasp1$^{-/-}$/hTNFtg mice was studied in a modified scratch assay as well as in live cell imaging

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