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A4.9 HOW OSTEOBLAST REGULATES ENERGY METABOLISM AND SYSTEMIC INFLAMMATION DEPENDENT OF FRA-2 EXPRESSION

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Background and Objectives The transcription factor Fra-2 (Fosl2) is a member of the AP-1 complex and an important regulator of bone homeostasis. We have previously shown that Fra-2 controls bone development, osteoclast size [1] and osteoblast differentiation through direct regulation of Collagen 1a2 and Osteocalcin (Ocn) [2]. Recent studies have established that the skeleton functions as an endocrine organ affecting metabolism through Ocn [3], although only few transcription factors and only one osteoblast-derived hormone are known to affect the crosstalk between bone and metabolism.

Materials and Methods We have generated mice with specific deletion of Fra-2 (*Fosl2*) or ectopic expression of Fra-2 in osteoblast to study the role of Fra-2 beyond the bone e.g. in metabolism.

Results Here we show that mice with osteoblast specific deletion of Fra-2 (*Fosl2*) have despite a low bone mass, an increased body weight. In contrast, ectopic expression of Fra-2 in osteoblasts display increased bone mass and decreased body weight accompanied with reduced serum glucose and insulin levels, improved glucose tolerance and insulin sensitivity. In addition, these Fra-2 mutant mice are protected from metabolic impairment, when challenged with high fat diet (HFD). Surprisingly a systemic inflammation and macrophage infiltration in liver, spleen and lung was observed in Fra-2 osteoblast specific mice. Mechanistically, we showed that in osteoblasts Fra-2 transcriptionally represses an important adipocytokine Adiponectin (Adipoq), while it induces Ocn, both responsible for the glucose and insulin metabolism alteration. Whereas, the systemic inflammation was likely due to the transcriptional increased of Osteopontin (OPN) expression by Fra-2, which is known as a potent inductor of macrophage activation.

Conclusions Taking together these results show that Fra-2 expression in osteoblast transcriptionally modulates Adipoq, Ocn and OPN expression and secretion representing a novel mechanism for the endocrine function of the skeleton on systemic metabolism and inflammation.

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A4.10 HYDROGEN SULFIDE ATTENUATES STORE-OPERATED Ca^{2+} ENTRY IN ENDOTHELIAL AND SMOOTH MUSCLE CELLS

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Background and Objectives Endothelial cells are active participants in inflammatory processes. They are involved in diverse activities including the regulation of leucocyte extravasation, angiogenesis, cytokine production, protease and extracellular matrix synthesis, vasodilation, etc. The small gaseous molecule hydrogen sulphide (H_2S) is involved in a variety of physiological processes like vascular relaxation, angiogenesis, neurotransmission and inflammation. In the vascular system, ATP-sensitive K^+-channels are a major target for H_2S but over the last few years evidence has accumulated that several Na^+- and Ca^{2+}-permeable channels are also sensitive to H_2S. In the present study we investigated the effect of H_2S on Ca^{2+} signalling in cultured endothelial and smooth muscle cells with special emphasis given to the role of H_2S in modulating store-operated Ca^{2+} channels.

Materials and Methods Experiments were performed with human microvascular endothelial cells (HMEC-1), endothelial cells isolated from porcine aorta, and smooth muscle cells isolated from rat aorta and rat trachea. Mobilisation of intracellular Ca^{2+} and Ca^{2+} entry was monitored by measuring the intracellular free Ca^{2+} concentration with FURA-2 in the absence and presence extracellular Ca^{2+}, respectively. Activity of endothelial nitric oxide synthase (eNOS) in intact cells was determined as conversion of incorporated L-[^{3}H]-arginine into L-[^{3}H]-citrulline.

Results Incubation of human and porcine endothelial cells with the H_2S-donor NaHS (100 μM, 10–45 min) evoked a release of Ca^{2+} from intracellular stores that was not accompanied by Ca^{2+} influx from the extracellular space. In accordance with these data suggesting that H_2S may inhibit store-operated Ca^{2+} entry, incubation of cells with NaHS attenuated Ca^{2+} influx induced by depletion of Ca^{2+} stores with receptor agonists (ATP, histamine) or the endoplasmatic reticulase ATPase inhibitor, thapsigargin. As a consequence, the stimulatory effect of these agonists on endothelial NO formation was strongly diminished, whereas the response to the Ca^{2+} ionophore A23187 was barely affected. Similar to the results obtained with endothelial cells, depletion of intracellular Ca^{2+} stores in smooth cells isolated from rat aorta or rat trachea also resulted in a pronounced Ca^{2+} entry that was completely blocked upon pretreatment of cells with NaHS.

Conclusions H_2S inhibits the stimulatory effect of Ca^{2+} of mobilising agonists on endothelial NO formation by attenuating store-operated Ca^{2+} entry. Inhibition of store-operated Ca^{2+} channels by H_2S is not peculiarity of endothelial cells but also occurs in vascular and tracheal smooth muscle cells. These hitherto undescribed effects may be in part possible for the beneficial effects of H_2S in sulphur bath therapy.

A4.11 BASELINE ELEVATED SERUM LEVELS OF CALPROTECTIN AS INDEPENDENT MARKER FOR RADIOGRAPHIC SPINAL PROGRESSION IN ANKYLOSING SPONDYLITIS

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Background and Objective Syndesmophytes formation and complete fusion of the total spine are common characteristics leading to functional impairment and disability in ankylosing spondylitis (AS) patients. Predictors for progression of structural damage are smoking, elevated levels of acute phase reactants and the presence of syndesmophytes at baseline. These predictors identify increased risk for progression at group level but their specificity is not strong enough to be used as biomarkers in individual patients. We recently

demonstrated that calprotectin, a heterodimer of S100A8 and S100A9, expressed and secreted during monocyte infiltration into inflamed tissues, is a good biomarker of treatment responses in AS. Here, we aimed to determine if calprotectin levels are predictive for radiographic spinal progression in AS patients.

Materials and Methods 76 AS patients were selected from German Spondyloarthritis Inception Cohort (GESPIC). Spinal radiographs were scored by two readers using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) system. Subsequently, anteroposterior views of the lumbar spine were scored for presence of syndesmophytes. Radiographic spinal progression was defined as 1) mSASSS worsening by ≥2 units after 2 years, and 2) development of a new syndesmophyte or progression of existing syndesmophytes after 2 years. Serum calprotectin levels were determined by ELISA.

Results High calprotectin levels were associated with mSASSS worsening over two years in AS, with an Area Under the Curve (AUC) of 0.740 (95% CI 0.614–0.866; $P = 0.004$). The odds ratio (OR) for radiographic spinal progression (mSASSS worsening by ≥2 units) in patients with calprotectin serum level >0.5 µg/ml was 6.2 (95% CI 1.6–24.2, $P = 0.009$). The association between calprotectin levels and mSASSS progression remained significant after adjusting for other independent risk factors (syndesmophytes at baseline, elevated C-reactive protein (CRP), and smoking): OR = 5.5 (95% CI 1.2–25.8; $P = 0.030$). Analysis of syndesmophyte formation and/or progression as outcome for structural damage yielded similar results: calprotectin levels were significantly associated with progression of syndesmophytes. The AUC was 0.670 (95% CI 0.520–0.819; $P = 0.031$). The predictive value of calprotectin was independent but similar to that of CRP.

Conclusions Calprotectin is an independent predictor for radiographic spinal progression in AS.

A4.12 ENDOTHELIN-1 PROMOTES THE ENDOTHELIAL-TO-MESENCHYMAL TRANSITION IN CULTURES OF HUMAN ENDOTHELIAL CELLS

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Background and Objectives Endothelial/microvascular injury and myofibroblast activation are crucial events that seem to contribute to the development of fibrosis in connective tissue diseases such as systemic sclerosis (SSc) [1]. Endothelin-1 (ET-1) contributes to the fibrotic process by inducing myofibroblast activation and increased extracellular matrix (ECM) synthesis [2]. Recently, it has been shown that myofibroblast activation from altered microvasculature may arise from the transition of endothelial-to-mesenchymal cell process (EndoMT), thus expressing α-smooth muscle actin (α-SMA), vimentin and fibrillar collagens [3]. The study investigated the possible involvement of ET-1 in the EndoMT in cultures of human endothelial cells.

Material and Methods Human dermal microvascular endothelial cells (HMVEC, Lonza Clonetic, Switzerland) and human umbilical vein endothelial cells (HUVEC, Lonza), were treated with or without ET-1 (100 nM, Enzo Life Science, UK) for 3 and 6 days. The expression of α-SMA, a marker of myofibroblast phenotype, and platelet endothelial cell adhesion molecule (PECAM-1 or CD31), a marker of endothelial phenotype, were evaluated by immunofluorescence (IF) and western blot analysis (WB) using primary antibodies to human α-SMA (dilution 1:50 for IF and 1:100 for WB, Dako Cytomation, Denmark) and to human CD31 (dilution 1:200 for IF and 1:1000 for WB, CellSignalling Technology, Denver, USA), in accordance with recent evidences [4, 5]. Data were obtained from four different experiments.

Results After 6 days of treatment ET-1 induced the expression of α-SMA in cultures of HUVEC, which maintained their ability to express CD31. Interestingly, ET-1 induced the α-SMA expression also in cultures of HMVEC after 6 days of treatment, without modulating the expression of CD31 when compared to untreated cells and confirming the data obtained in HUVEC cultures. The results were obtained by IF and confirmed by WB.

Conclusions These preliminary results show that ET-1 seems to induce the α-SMA expression in human endothelial cells thus supporting a possible direct involvement in promoting the EndoMT [2, 4–6]. The implications in the fibrotic process that characterise SSc are under evaluation [7].

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A4.13 EVALUATION OF HISTOLOGY AND ADIPOKINE EXPRESSION OF SYNOVIAL TISSUE FROM PSORIATIC ARTHRITIS PATIENTS

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Background The pathophysiology of psoriatic arthritis (PsA) has still not been examined in minute detail as has rheumatoid arthritis (RA). As it is often difficult to differentiate between both diseases and there are numerous publications that synovial inflammation and cytokine pattern are different in PsA and RA, we analysed the synovial histological differences with respect to immunomodulatory adipokines resistin, visfatin and adiponectin.

Methods First, synovial tissues from affected joints with PsA were stained with hematoxylin/eosin to visualise the histology of the synovial membrane. Thereafter, macrophages were detected by immunohistochemistry using an anti-CD16 antibody, fibroblasts using an anti-vimentin antibody. Collagen type IV-antibodies were used to visualise vascular endothelial cells and anti-CD20 antibodies to identify B-lymphocytes. In addition, serial tissue sections were stained for resistin-, visfatin, and adiponectin.

Results In the majority of patients, the synovial tissue from PsA showed no hyperplasia of the lining layer in comparison to RA (lining thickness in % of PsA patients: 1 cell layer, 64%; 2–3 cell layers, 21%; >4 cell layers, 24%. Vice versa, an increased cellularity in the sublining could be detected. In PsA, a lower number of immune-cell follicles were observed although the number of synovial vessels was increased. In all samples, cellular adipokine signals were detectable. Adiponectin was mainly located in vessels showing a strong expression of this molecule. In contrast to RA, the expression of adiponectin in the lining layer was completely absent in most PsA patients. Resistin was detectable in the sublining but also in the lining layer. A strong expression of visfatin could be found in the sublining, and to a lower extent in the lining layer. There was no association between the localisation of the adipokines and the quantity or of compact inflammatory infiltrates.

Conclusions The synovial membranes from PsA show clear differences to those of RA-affected joints. Adipokine expression is -in part- also different between RA and PsA patients. Here, specifically adiponectin is more prominent in the RA than in the PsA lining layer. Adipokines may therefore play different roles in inflammatory processes operative in these chronic rheumatic diseases.

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