activation of canonical and noncanonical NF-κB signalling, and increased the expression of inflammatory cytokines and adhesion molecules, which could be blocked by targeting NIK.
Conclusions These findings suggest that in addition to regulating noncanonical signalling, NIK can serve as an amplifier of canonical NF-κB signalling and associated inflammatory responses in EC, which may play a role in development and maintenance of chronic inflammation. Consequently, NIK may be an attractive therapeutic target.
Disclosure of interest P. Kucharzewska Employee of: Employed by AstraZeneca, K. Jeucken: None declared, C. Maracle: None declared, J. P. van Hamburg: None declared, H. Olsson Employee of: Employed by AstraZeneca, S. Tas: None declared

0005 SMOC2 HAS DIFFERENTIAL EFFECTS ON CARTILAGE AND BONE FORMATION

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Introduction SMOC2, a secreted calcium-binding protein of the BM-40/SPARC family was identified from a chondrogenic extract of articular cartilage and is increased in osteoarthritic cartilage. Alterations in bone and cartilage resulting from dysregulated signalling pathways are a hallmark of osteoarthritis.
Objectives To evaluate the role of SMOC2 in *in vitro* osteogenesis and chondrogenesis and its interaction with Wnt signalling.
Methods Wild-type Smoc2 (Smoc2+) or Smoc2 lacking the calcium binding domain (?CaBD) were stably overexpressed and wild-type Smoc2 was silenced (Smoc2-). Alkaline phosphatase (ALP) activity assay and Alizarin Red staining were performed in MC3T3 cells and human periosteal derived cells (hPDCs). Proteoglycan content and mineralization were assessed in ATDC5 micromasses by Alcian blue and Alizarin Red staining. Mice femoral head caps (FHC) were stimulated with recombinant IL-1b and SMOC2. Cartilage loss was evaluated by DMMB assay and Safranin O staining. TCF1 levels were analysed by immunohistochemistry. Human articular chondrocytes (hACs) were stimulated with recombinant SMOC2. Gene expression of osteoblast markers (*Opn, Osx, Col1a2*), chondrogenic markers (*Acan, Col2a1, Col10a1, Mmp13*) and canonical Wnt target genes (*Axin2, Lef1*) were analysed by qPCR and Wnt signalling by Western blot.
Results Alizarin red staining and ALP activity were reduced in Smoc2 +MC3 T3 cells. Osteoblast gene expression levels were altered in Smoc2 +cells. ?CaBD cells and calcium supplementation to Smoc2 +showed a partial rescue of the effects of Smoc2 +cells. We validated these results in hPDCs. We could not observe an effect on osteogenesis when silencing Smoc2. Smoc2 +ATDC5 micromasses showed reduced chondrogenic gene expression and Alcian blue and Alizarin Red staining intensity. We observed a reverse pattern with an increased chondrogenic gene expression in Smoc2- ATDC5 micromasses. Smoc2 +and Smoc2- ATDC5 cells altered canonical Wnt signalling in opposite directions. SMOC2 also alters protein levels of CamKII. IL-1b triggered cartilage loss was increased and TCF1 levels were reduced in mice FHC stimulated with recombinant SMOC2. In hACs, we observed a negative effect of SMOC2 on chondrocyte markers and canonical Wnt target genes.
Conclusions Our results suggest an inhibitory role of Smoc2 on mineralization. The inhibitory effects on mineralization are at least partially regulated through interaction with calcium. Moreover, Smoc2 is a regulator of chondrogenic differentiation and cartilage homeostasis by regulating canonical and non-canonical Wnt signalling.
Disclosure of interest None declared

0006 LUMICAN: A NOVEL GYCOPROTEIN MEDIATING INFLAMMATION IN OSTEOARTHRITIS

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Introduction Lumican (LUM) is major extracellular matrix protein which is present in glycoprotein format in adult articular cartilage. Recently, LUM was show to augment pathogen-associated molecular patterns (PAMPs) activation of the TLR4 signalling cascade. Given that TLR4 is highly associated with inflammation in rheumatic disease, we aim to decipher the LUM role in Osteoarthritis and TLR4 associated inflammation.
Objectives To measure LUM in synovial fluid from patients with arthritic conditions and to study the role of LUM on TLR4 activation in osteoarthritis.
Methods Synovial fluid (SF) was obtained from knee meniscus tear (n=11), first carpometacarpal (CMC-I) OA (n=11) and knee OA (n=40) patients. LUM glycoprotein levels were analysed by enzyme-linked immunosorbent assay (ELISA). Human monocytes were isolated from healthy individuals and differentiated into M1-like and M2-like macrophages by lipopolysaccharide (LPS) and interleukin-4, respectively. LUM (1 ug/ml) was added either together with macrophage polarisation inducers or alone. Primary chondrocytes and OA cartilage explants were stimulated for 24 hour with LUM (1 µg/ml), LPS (10 ng/ml), or a combination of both. Conditioned media was analysed for selected secreted molecules by xMAP technology. Macrophages surface expression markers CD197 and CD206 were analysed by Fluorescence-Activated Cell Sorting (FACS). Cartilage explants were immunofluorescently double stained for collagen type II and X.
Results LUM glycoprotein levels were significantly upregulated in knee OA SF vs. controls. In normal articular chondrocytes, LUM combined with LPS caused an upregulated secretion of catabolic markers characteristic of OA such as IL-6, MMP-1, and MMP-13 in comparison to LPS stimulation. LUM alone had no observable effects. A similar response occurred in cartilage explant cultures, with increased MMP-1 secretion levels and marked cartilage degradation with the LPS/LUM combination. Interestingly, LUM stimulation with the polarisation inducer, LPS for M1, and IL-4 for M2 macrophages, upregulated M1 macrophage secretion of TNF-alpha and downregulated IL-10 secretion in M2 macrophages relative to control and LUM alone. FACS results followed the same trend, with increased CD197 expression in LUM/LPS combination vs. LUM alone or control, and downregulation of CD206 expression when LUM/IL-4 combination vs. LUM alone or control.
Conclusions The results presented here show that LUM is highly upregulated in SF of OA and RA patients. In addition, we observed that TLR-expressing chondrocytes do not respond to LUM, however LUM augmented the LPS-induced TLR4 signalling cascade and consequently the catabolic response.