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.

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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 osteoarthritis 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 (SmCaBD) 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 (hPDGs). Proteoglycan content and mineralization were assessed in ATDC5 micromasses by Alcian blue and Alizarin Red staining. Mice femoral head caps (FHC) were stimulated with arthritis inducers or alone. Primary chondrocytes and OA cartilage explants were incubated 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 Alizarin Red staining and ALP activity were reduced in Smoc2+ or Smoc2 cells. Calcium supplementation to Smoc2+ cells showed a partial rescue of the effects of Smoc2- cells. We validated these results in hPDGs. 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+ alters protein levels of CamKII. IL-1β 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 noncanonical Wnt signalling.

Disclosure of interest None declared

LUMICAN: A NOVEL GYCOPROTEIN MEDIATING INFLAMMATION IN OSTEOARTHRITIS

Introduction LUMican (LUM) is a major extracellular matrix protein which is present in glycoprotein form in adult articular cartilage. Recently, LUM was shown 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.

Conclusions The results presented here show that LUM is highly upregulated in SF of OA and RA patients. In addition, we observed that TLR4-expressing chondrocytes do not respond to LUM, however LUM augmented the LPS-induced TLR4 signalling cascade and consequently the catabolic response.
Moreover, we also demonstrate the ability of LUM to upregulate M1 macrophage and downregulate M2 macrophage polarisation. Our findings strongly support a pathogenic role of LUM, as mediator of PAMP-induced TLR-4 activation of inflammation, cartilage degradation and macrophage polarisation in OA and other rheumatic diseases.

Disclosure of interest None declared

0007 ACPA-INDUCED MOBILITY OF PRIMED SYNOVIAL FIBROBLASTS: THE MISSING LINK BETWEEN ACPA-INDUCED BONE LOSS AND SYNOVIAL CHANGES

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Introduction Anti-citrullinated proteins antibodies (ACPAs) injected in mice induce IL-8 dependent bone loss and arthralgia, but no synovial changes. We hypothesised that additional stimuli, sensitising the synovial compartment to ACPA effects, might be needed for the transition from bone to synovial pathology.

Methods Fibroblast like synoviocytes (FLSs) were isolated from synovial tissue biopsies obtained from RA patients. Polyclonal ACPA and other non-ACPA IgGs were separated from peripheral blood of RA patients by affinity purification on protein G and cyclic citrullinated peptide (CCP)~2 columns. Monoclonal ACPAs were cloned from synovial fluid B-cells. FLS migration was tested by scratch-assays using IncuCyte (Essen bioscience). SF adhesion was analysed by xCELLigence System Real-Time Cell Analyzer (ACEA bioscience). Peptidylarginine deiminases (PAD) expression and protein citrullination were evaluated by immunohistochemistry and immunofluorescent stainings.

Results FLS mobility was not affected by polyclonal ACPA stimulation. However, exposing the cells to IL-8 or to a transient serum starvation increased PAD expression and the amount of citrullinated proteins in the cells. In line with these findings, starved FLSs obtained sensitivity to ACPAs and responded with an increased mobility to antibody stimulation. Similar effects were observed in the presence of three out of ten monoclonal ACPAs, suggesting that only ACPAs with certain fine specificity could target FLSs. The ACPA-induced migration was abolished by pre-incubating the cells with PAD inhibitor or by combining the ACPAs with citrullinated but not native fibrinogen. IL-8 alone could not influence fibroblast migration but it synergistically increased the response to ACPAs. The role for inflammatory stimuli in sensitising FLSs to ACPA binding was further supported by the labelling of synovial tissues of RA patient, but not of healthy controls, using monoclonal ACPAs.

Conclusions We demonstrated that FLS response to ACPA stimulation was enabled by stress- or cytokine-induced citrullination in the cells. These results suggest an important role for transient synovial insults in setting the stage for the ACPA-mediated transition towards chronic synovitis.

Disclosure of interest None declared