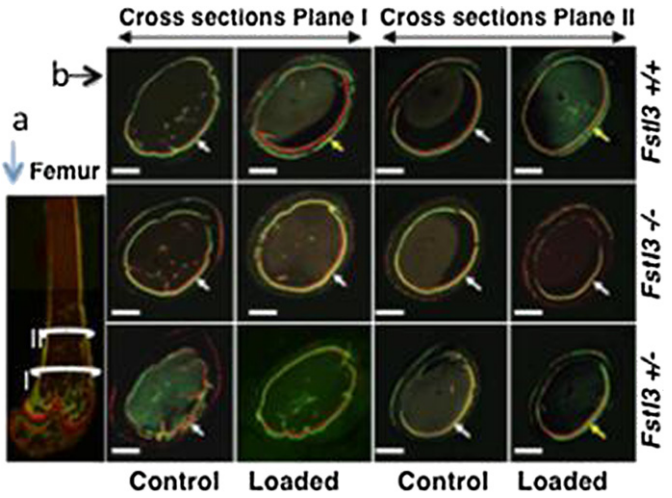

Figure 1. Fstl3 gene deletion results in the loss of load dependent of bone growth (a) Sagittal section of rat femur showing the position of cross sections. (a-b) Double fluorochrome-labeled sections of femurs at the distal diaphyseal end at planes I and II showing minimal bone Deposition in non-loaded control and loaded Fstl3+/+, Fstl3-/-, and Fstl3+/- mice. Note the bone deposition (i) at the medial (arrow) endosteal surface in Fstl3+/+ femur as compared to minimal bone formation at all sides of the Fstl3-/- femur, and (ii) regain of mechanosensitivity in heterozygous mice.

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SCLEROSTIN REGULATION OF WISP1 AND MMPS IN CHONDROCYTES IS DEPENDENT ON THE LRP5/6 BINDING DOMAIN BUT NOT SECRETION FROM THE CELL

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Purpose: Sclerostin (SOST) is a secreted protein that inhibits Wnt signaling by binding the LRP5/6 cell-surface Wnt co-receptor. SOST has also been reported to bind intra-cellularly and target BMPs for proteosomal degradation in cells in which it is co-expressed. While previously considered an osteocyte-specific protein, it has recently been demonstrated that chondrocytes in non-calcified articular cartilage express SOST mRNA. Chondrocyte SOST expression is upregulated in osteoarthritis and by inflammatory cytokines such as intereukin-1 (IL-1). Exogenous soluble SOST protects cartilage from IL-1-mediated proteoglycan loss and inhibits metalloproteinase expression and activity in vitro, but it is unclear if SOST expressed by chondrocytes functions in the same manner. The present study aimed to investigate the function of chondrocyte SOST, and explore the molecular mechanism whereby it may function in this cell type and have chondroprotective effects.
Methods: Expression constructs for full length human SOST (WT), non-secretable SOST (M1), secretable SOST with inactive LRP5/6 binding domain (M2), and non-secretable SOST with inactive LRP5/6 binding domain (M3) were generated using a PCR approach. In M2 and M3 the LRP5/6 binding domain was replaced by the artemin sequence from the same cysteine-knot family which does not bind LRP5/6. SW1353 human chondrosarcoma cells were stably transfected with the different constructs or the empty vector. SOST expression was determined by Western blotting of cell lysates and ELISA quantitation of culture media. Cells were cultured ± IL-1 for 24 hours and cell viability (trypan blue exclusion), cell metabolism (MTT assay), MMP1, MMP3 and MMP13 gene expression (qRT-PCR), MMP13 protein secretion (Western blot) and MMP13 activity (fluorogenic assay) were measured.
Results: Transfected cell lines synthesized SOST, with similar levels of intracellular protein detected in all cells but secreted protein in the media only in WT and M2 cells as expected. There was no effect of any of the SOST expression constructs on cell viability or metabolism compared with the vector control. M1 and M2 constructs decreased basal (un-stimulated) MMP13 expression. IL-1 stimulated Wnt-β-catenin activity as measured by significantly increased WISP1 expression ($p < 0.05$). WT and M1 SOST, significantly ($p<0.05$) suppressed IL-1 induced WISP1, MMP1, MMP3 and MMP13 mRNA expression. However only WT SOST decreased MMP13 protein secretion and activity. In contrast, M2 and M3 constructs had no effect on WISP1 or MMP gene expression, nor MMP13 activity in the presence or absence of IL-1.
Conclusion: Our data demonstrate for the first time that chondrocytes can secrete soluble SOST. Furthermore we show that the LRP5/6 binding domain is critical for SOSTs inhibition of IL-1 induced Wnt-β-catenin activation and MMP expression and activity in chondrocytes. Surprisingly however, secretion from the cell was not necessary for SOST to inhibit IL-1-stimulated WISP1 expression, or for SOSTs anti-MMP activity. This suggests a hitherto unrecognized intra-cellular role for the LRP5/6 binding domain of SOST, potentially through cross-talk between BMP and Wnt pathways. Improved understanding of the regulation of these molecular pathways in chondrocytes, may lead to novel therapeutic approaches in diseases such as osteoarthritis.

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THE SCLEROSTIN AND MEPE AXIS IN THE DEVELOPMENT OF OSTEOARTHRITIS

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Purpose: Sclerostin (SCL) is a natural inhibitor of Wnt signaling with emerging compelling links with the subchondral bone (SB) changes observed in osteoarthritis (OA). Moreover, it has recently been established that SCL regulates bone and cartilage mineralization through the recognised mineralization inhibitor matrix extracellular phosphoglycoprotein (MEPE). To establish the expression and function of both SCL and MEPE in OA, we determined the spatial relationship between their expression and hallmarks of OA in a spontaneous model of OA, the Str/ort mouse. Furthermore, as the re-initiation of developmental processes in OA is well established, we sought to examine the role of SCL in endochondral ossification.
Methods: Knee joints from male Str/ort mice, were analysed at 8, 18 and 40+ weeks of age (before, early and late OA). Age-matched 'OA protected' CBA male mice knees were also analysed. SB-thickness and epiphyseal mass/architecture parameters were measured by microCT analysis. Multiple sections from regular intervals across joints were stained with Toluidine blue and scored for OA hallmarks. SCL and MEPE expressions were then determined by immunohistochemistry (IHC). We have previously established the role of MEPE in endochondral bone growth however the role of SCL has yet to be examined. Here embryonic and postnatal growth plates were analysed by IHC for SCL expression to establish its temporal and spatial expression in endochondral bone growth. The chondrogenic cell line ATDC5, a model of endochondral ossification, was cultured in mineralizing conditions for up to 34-days and protein lysates examined for SCL expression by western blotting.
Results: Our results revealed that SCL expression is enhanced at the osteochondral interface at unaffected regions of the joint. Similarly, MEPE expression is enhanced in the articular cartilage (AC) of the lateral aspect of the Str/ort mouse joint, where SB thickening is not observed. In OA joints, marked suppression of SCL expression at the osteochondral interface in Str/ort mice at advanced stages of OA; in regions of the OA joint where AC integrity is compromised and SB thickening is observed, as shown by microCT scanning. MEPE expression is lost in the diseased aspect of the joint in both the AC and the underlying osteocytes of the SB. Moreover, strong SCL and MEPE expression is found close to ossified ligament insertions, menisci, and in emerging osteophytes, which are increased with disease severity. Osteophytes form through endochondral ossification and as such, we established the localisation of SCL in endochondral bone growth. Interestingly, whilst we observe an embryonic expression of MEPE by both proliferating, and more relevantly mineralizing hypertrophic chondrocytes, this is lost in postnatal chondrocytes. Our results also show increased SCL expression in ATDC5 cells throughout the 34-day differentiation period, suggesting a role for SCL in the endochondral processes observed in osteophyte formation.
Conclusions: Our data suggest that SCL and MEPE are pivotal in restricting pathological ossification in OA. Further investigation into the precise mechanisms by which they function in OA will identify whether their targeted delivery can protect against OA bone pathology and will therefore provide a basis for research towards clinical benefit.