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


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 interleukin-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 artemisin sequence from the same cytomegano-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.

Results: Transfected cells 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.