Introduction: The anabolic effect of high impact mechanical loading on skeletal architecture has been repeatedly demonstrated, but the cellular and molecular events occurring between load and ultimate bone formation remain obscure. The discovery of sclerostin, an inhibitor of bone formation produced by mature osteogenic cells, and sclerosing bone dysplasias that result from its mutation, suggest it is a pivotal protein in modulating bone formation. We examined expression of SOST mRNA across a variety of clonal cell lines that span the osteogenic phenotype from immature osteoblast to mature osteocyte. No sclerostin expression was detected in MC3T3-E1 immature osteoblasts or, surprisingly, MLO-Y4 mature osteocytes, whereas MLO-A5 immature osteocytic cells expressed low levels of SOST. Highest expression was observed in a model of mature osteoblasts, UMR 106.01. We examined the influence of bone morphogenetic proteins on SOST expression. Treatment with BMPs-2, -4, or -6 were without effect on SOST in MLO-Y4 mature osteoblasts but elicited a robust increase in SOST expression in immature MLO-A5 osteocytes. Oscillatory fluid flow applied to UMR 106.01 mature osteoblasts transiently decreased expression of sclerostin at the RNA and protein level. Overall, our results indicate that BMP treatment and in vitro mechanical loading demonstrate opposite effects upon sclerostin expression.

Materials and Methods: Cell culture: Four clonal cell lines spanning the osteogenic phenotype were used: MC3T3-E1 cells, clone 14, representing an immature osteoblast; UMR 106.01 cells, representing a mature osteoblast; MLO-A5 cells, representing an immature osteocyte; and MLO-Y4 cells, which possess phenotypic characteristics of a mature osteocyte.

Oscillatory fluid flow: On the day of experimentation, slides were placed into a parallel plate flow chamber in series with rigid wall tubing and a Hamilton glass syringe. The syringe was displaced by an electromagnetic loading device. Flow rate was selected to induce a peak shear stress of 20 dynes/cm² at 1Hz. Static controls were placed into flow chambers, but not exposed to flow fluid. Upon termination of flow, slides were placed into fresh flow media for an additional 0-24 hours.

Results: Effect of BMPs on SOST expression in osteoblastic cells: BMP-2 (100ng/mL), BMP-4 (50ng/mL), or BMP-6 (250ng/mL) were added to MLO-Y4 mature osteoblastic cells, and total RNA was collected 24-96 hours later for quantitative PCR analysis. We observed a modest trend, albeit not statistically significant, toward increased SOST expression. A similar trend, again not statistically significant, was observed for gremlin, another BMP antagonist. In contrast to weak induction by BMPs in mature osteocytic cells, strong SOST induction was observed in immature osteocytic cells. Maximal SOST induction was observed with BMP-6 treatment after 24 hour BMP treatment; BMP-2 and BMP-4 induced SOST expression but to a smaller magnitude than BMP-6, and did not reach statistical significance. Because the observed effect of BMP treatment on SOST expression was maximal at 24 hours and declined thereafter, we repeated these experiments in immature osteocytic MLO-A5 cells and examined SOST expression at earlier time points. We observed increased SOST expression that achieved statistical significance after 6 hours of treatment that continued to rise until 24 hours. Cells treated with BMP-6 for 36 hours expressed less SOST than cells treated with BMP-6 for 24 hours. Again, BMP-6 demonstrated greater effect on SOST expression than did BMP-2 or BMP-4.

Effect of OFF on SOST expression in osteocytic and osteoblastic cells: We sought whether oscillatory fluid flow (OFF) regulated sclerostin expression in vitro. OFF was applied at a flow rate designed to induce a peak shear stress of 20 dynes/cm² at a frequency of 1Hz, and was maintained for two hours. Immediately upon cessation of OFF, total RNA was collected and analyzed for SOST expression by qPCR. SOST mRNA was not regulated by OFF in the absence of BMP induction, whereas BMP-6-induced SOST expression was reduced by OFF. In contrast to BMP-free experiments, MLO-A5 cells that had been pre-treated with BMP-6 for 22 hours demonstrated a modest, but significant, reduction in SOST expression compared to BMP-6-treated static cells. We next tested whether the differential effect of OFF on basal versus BMP-stimulated SOST expression occurred in mature osteoblasts, or whether this finding was a differentiation-dependent result. In contrast to MLO-A5 immature osteocytes, UMR 106.01 mature osteoblasts demonstrated a significant reduction in SOST mRNA after 2 hours of OFF. Shorter durations of OFF of 30 minutes or 1 hour were without significant effect on SOST expression. We next examined how long the suppressive effect of OFF on SOST mRNA was maintained. Upon cessation of OFF, UMR 106.01 cells were immediately lysed for RNA collection, or returned to fresh flow media for an additional 4 hours, after which time total RNA was collected. We found that a post-flow culture period of 4 hours eliminated the suppressive effect of OFF on SOST, thereby indicating the transient effect of OFF on SOST expression.

Discussion: The discovery of sclerostin as a negative regulator of bone formation, and sclerosing bone dysplasias resulting from its mutation, suggest its importance as a key regulator of skeletal homeostasis and as a potential target for future anabolic therapies. Its mechanism of action has been attributed to antagonism of BMP and Wnt-Lrp5 signaling. We report similar effects of BMPs-2, -4, or -6 on sclerostin expression in an immature osteocytic MLO-A5 cell line, with maximal induction elicited by BMP-6 after 24 hours of exposure. We observed minor increases in SOST or Gremlin1 expression in mature osteocytic MLO-Y4 cells in response to BMPs. These differential responses to BMP treatment suggest that the ability of BMPs to induce expression of their own antagonists is dependent upon the degree of osteogenic differentiation. Further studies are required to delineate the mechanism for these differential responses between immature and mature osteocytic cells, but may involve altered expression of BMP receptors, intracellular Smad adapters, or histone acetylation.

Constitutive expression of SOST was unaffected by OFF in immature osteocytes, whereas BMP-6-induced SOST was reduced by OFF. The lack of an effect of OFF on basal SOST expression in immature osteocytes may result from the low levels of SOST initially expressed, thereby making accurate analysis of any reduction technically challenging. We did observe significant reduction in SOST expression in mature osteoblastic UMR 106.01 cells when exposed to OFF for 2 hours. Sclerostin protein was also suppressed (near significance at p .07). That the reductions in SOST mRNA or sclerostin protein were modest (approximately 50% and 20%, respectively) and less than those reported in vivo suggests that our in vitro model does not faithfully replicate in vivo conditions that enable load-induced sclerostin expression.

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