Sclerostin is a master regulator of mineralization in human osteoblasts

+1Findlay DM; 1Lim HP; 1Wijenayaka AR; 1Welldon KJ; 2Rowe PS; 1Atkins GJ
1Bone Cell Biology Group, Discipline of Orthopaedics and Trauma, University of Adelaide, and the Hanson Institute, Adelaide, SA 5000, Australia.
2Department of Internal Medicine, The Kidney Institute & Division of Nephrology, MS 3018, University of Kansas Medical Center, 3901 Rainbow Boulevard 6020(B) Wahl Hall East, Kansas City, KS 66160, USA
gerald.atkins@adelaide.edu.au

INTRODUCTION:
Sclerostin (SCL/SOST) is almost exclusively produced by osteocytes in steady-state bone. Loss of SOST function causes high bone mass disease in humans and mice. Transgenic over-expression of SOST results in low bone mass and decreased bone strength as the result of a significant reduction in osteoblast activity and bone formation. These findings together indicate that SCL has a key role in the regulation of bone mass. Anabolic responses to PTH and mechanical loading appear to be in part mediated by the suppression of SOST expression, while mechanical unloading results in elevated SOST expression. Neutralising antibodies to SCL increase bone strength and formation parameters in ovariectomized rats.

The mechanism of action of SCL remains to be elucidated, with reports that it acts via binding to LRP5/6 and LRP4. Importantly, almost nothing is known regarding either the cellular targets of SCL or its functional effects. The identity of the cells that respond to sclerostin has not been identified, although the localized expression of sclerostin implies that it may have local paracrine or autocrine activities. We tested the hypothesis that sclerostin would regulate the behaviour of the cells actively involved in mineralisation, the late osteoblast or pre-osteocyte.

METHODS:
Cultures of human primary osteoblastic cells (HO) were obtained from human trabecular bone obtained at joint replacement surgery. HO were promoted to differentiate by exposing them to mineralizing culture media for up to 5 weeks. Cells were treated with recombinant human sclerostin (rhSCL), in the dose range 1-50 ng/ml, according to two protocols. Cells were exposed to SCL throughout the culture or were first allowed to differentiate for 5 weeks (until the cultures were mineralized) before initiation of SCL treatment. Parallel cultures of MLO-Y4 osteocyte-like cells were also investigated. Cultures were examined for mineralisation by measuring the calcium in the cell layer. Cultures were extracted for RNA and mRNA species encoding genes expressed through osteoblast differentiation (eg RUNX2, osterix) and into the osteoblast-osteocyte transition (eg osteocalcin [OCN], SOST, DMP-1, MEPE, PHEx) were measured by real time RT-PCR.

RESULTS:
Treatment of cultures with rhSCL caused significant inhibition of in vitro mineralization in a dose-dependent manner, with 1 ng/ml having a significant effect (Fig 1). Analysis of gene expression in cultures exposed continuously to rhSCL showed little effect of the treatment until late in the cultures, when SCL decreased the expression of mature osteocyte markers, DMP-1 and that of SOST itself. The expression of the pre-osteocyte marker E11 was increased. E11 expression was also increased by SCL in the MLO-Y4 osteocyte cell line. To enable comparison of effects of SCL on immature and mature osteoblasts, primary HO were cultured for 5 weeks, a process that served to differentiate the cells to a post-proliferative and more uniformly mature pre-osteocyte stage. Cultures were then exposed acutely to sclerostin and gene expression analysed. These cells were exquisitely sensitive to sclerostin. and concentrations of 1 – 50 ng/ml consistently increased the expression of E11, while decreasing the expression of the mature markers DMP-1 and SOST. Concomitantly, the expression of MEPE was increased by sclerostin, at both the mRNA and protein levels and PHEx mRNA was decreased (Fig. 2). This implies that mature osteocytes, by virtue of their expression of sclerostin, are able to impede differentiation of pre-osteocytes as well as alter levels of key regulators of bone mineralization. Consistent with this, MEPE protein levels were increased by sclerostin, assessed by western blot, and immunostaining revealed that sclerostin promoted an increase in the levels of the MEPE-ASARM peptide, which has been shown previously to bind to and inhibit the growth of nascent bone mineral.

DISCUSSION:
Our results suggest that sclerostin acts through regulation of the PHEx/MEPE axis and behaves as a master regulator of physiological bone formation, in a local paracrine fashion. The regulation by sclerostin that we have identified on both pre-osteocyte and osteocyte activity are consistent with its localization in the bone and its established role in the inhibition of bone formation.

Fig 1: Effect of SCL on in vitro mineralization. HO were cultured for 35 days in the presence of rhSCL (0-50 ng/ml). Cell layer-associated calcium was then determined. Data shown are means of triplicate real-time RT-PCR reactions normalised to GAPDH mRNA expression.

Fig 2: Effect of rhSCL on gene expression, increasing the expression of MEPE and decreasing PHEx mRNA expression. Data shown are means of triplicate real-time RT-PCR reactions normalised to GAPDH mRNA expression.

REFERENCES:
4. Martin A, David V, Laurence JS, Schwarz PM, Lafer EM, Hedge AM, Rowe PS 2008 Degradation of MEPE, DMP1, and release of SIBLING ASARM-peptides (minhibins): ASARM-peptide(s) are directly responsible for defective mineralization in HYP. Endocrinology 149:1757-1772