

Epigenetic alterations in bone cells as the mechanistic basis of high bone mass in sclerosteosis

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INTRODUCTION:

Epigenetic changes confer heritable modifications in gene expression without alterations in DNA sequences. It is well established that epigenetic remodelling is associated with disease and aging, but it is now emerging that epigenetics may also mediate memory of tissue forming responses. Indeed, it has recently been shown that modification of DNA methylation following a single anabolic stimulus is responsible for muscle hypertrophy (growth) and that *memory* of this event is maintained for a long period thereafter (1). We report here, for the first time, that cells extracted from a skeleton under constant anabolic signalling (sclerostin deficiency) display distinct behaviours that reflect a memory of the *in vivo* environment, and speculate that this is epigenetically-governed. Sclerostin, encoded by the SOST gene and secreted by osteocytes, is a negative regulator of bone formation through Wnt/ β -catenin antagonism and mediator of bone mechanotransduction. Mutations in the SOST gene, or in regulatory elements, cause an increase in bone mass as seen in conditions such as sclerosteosis and Van Buchem disease (2). We hypothesise that anabolic signalling within the skeleton results in epigenetic imprinting on cells, and a memory which mediates *in vitro* potency analogous to the *in vivo* environment.

METHODS:

Bone marrow progenitors were isolated from 6-week-old age/sex matched C57BL/6 and *Sost*^{-/-} (knockout) mice; this age was chosen due to rapid gains in bone mass observed between 1-2 months of age. One million cells from each mouse strain were cultured on dentine discs (5mm) for 7 days in the presence of RANKL and MCSF, with 2 days of acidification to promote resorption. TRAP staining was utilised to enable the assessment of osteoclast resorption and number. Osteoclasts, preosteoclasts and resorption area were quantified via image analysis. Murine periosteum derived cells (mPDCs) were isolated from 6-week-old C57BL/6 and *Sost*^{-/-} mice and cultured in control or osteogenic media for 7, 14 or 21 days. Alizarin red was used to stain the monolayer for visualisation of bone nodules. Gene expression of 14 epigenetic enzymes (supported by the literature) were explored by qPCR.

RESULTS SECTION:

Marrow from *Sost*^{-/-} mice yielded 70.1% fewer osteoclasts than C57BL/6 WT (P<0.0001, N=12). Furthermore, the area of pre-osteoclasts and total resorption area were reduced by 14.3% (P<0.05) and 52.7% (P<0.0001), respectively in the *Sost*^{-/-} compared to C57BL/6 (Fig 1A). Conversely, mPDCs isolated from *Sost*^{-/-} mice display enhanced *in vitro* bone formation (2590% increase in matrix mineralisation at 14 days; P<0.0001, N=3), compared age/sex matched C57BL/6 controls (Fig 1B). Further support for divergent behaviour in cells isolated from skeletons of mice with high bone mass was obtained from mRNA analysis of 14 epigenetic enzymes, in which expression levels were positively linked to modification of cell function (Fig 1C).

DISCUSSION:

These data reveal a clear reduction of *in vitro* osteoclastogenesis and an increase in mineralisation/bone formation from mPDC cultures isolated from *Sost*^{-/-} mice compared to controls, despite the absence of osteocyte paracrine signals. Collectively, these data suggest an inherent memory of *in vivo* events. Importantly, neither of these cell types secrete sclerostin protein, indicating the changes seen *in vitro* are derived from the *in vivo* environment. Moreover, the positive association between cell functionality and epigenetic enzyme expression suggests that this memory is epigenetically governed. Although the exact mechanism involved in this phenomenon is not known, this study begins to clarify modified bone homeostasis effector cell functionality, which may contribute to high bone mass with sclerostin deficiency. This has implications for the control of bone cell function in disease and regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE:

These data provide a clearer understanding of the molecular effect of sclerostin deficiency and may help uncover modulators of bone anabolism. It is predicted that this will deliver novel targets to control cell activity for regenerative strategies.

REFERENCES:

(1). R. A. Seaborne et al., Sci Rep 8, 1898 (2018); (2). T. Dreyer et al., J Endocrinol 257, e220209 (2023)

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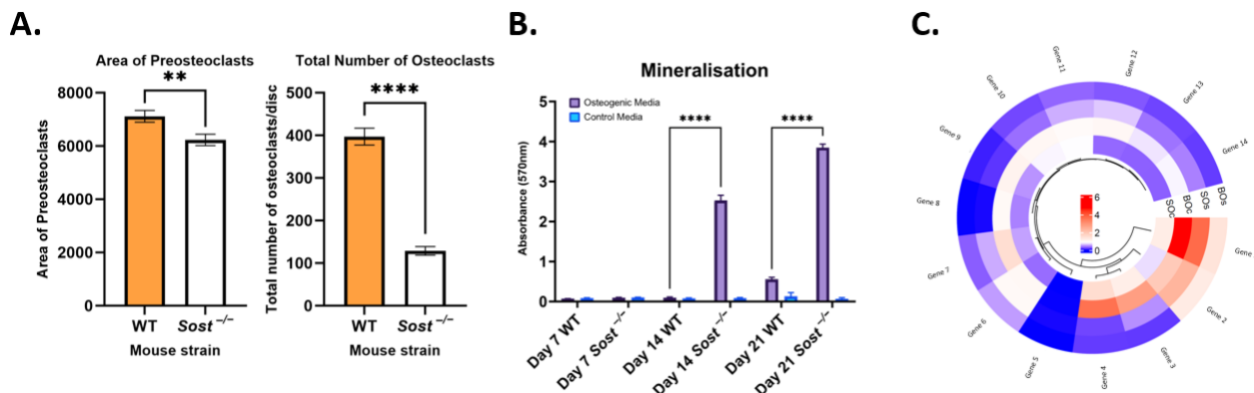


Figure 1: Changes in skeletal cell potency due to *SOST* deficiency (A) Reduction of preosteoclasts and mature osteoclasts observed from *Sost*^{-/-} marrow compared to C57BL/6 (WT) when cultured on dentine discs and quantified by image analysis. (B) Quantification of Alizarin Red stain showing increased mineralisation with *Sost*^{-/-} mPDCs. (C) Expression level of 14 enzymes spanning divergent epigenetic mechanisms in WT osteogenic mPDCs (BOs) and osteoclasts (BOc), and *Sost*^{-/-} osteogenic mPDCs (SOs) and osteoclasts (SOc).