

## COMMENTARY

# Gs- $\alpha$ in osteoblasts regulates bone formation and osteoblast differentiation

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**Commentary on:** Wu JY, Aarnisalo P, Bastepe M, Sinha P, Fulzele K, Selig MK, Chen M, Poulton IJ, Purton LE, Sims NA, Weinstein LS, Kronenberg HM. Gs $\alpha$  enhances commitment of mesenchymal progenitors to the osteoblast lineage but restrains osteoblast differentiation in mice. J Clin Invest. 2011; 121:3492-504.

The anabolic effect of intermittent parathyroid hormone (PTH) is thought to result largely from activation of Gs signaling in osteoblasts, but the precise role of Gs signaling in regulating osteoblast differentiation is not clear. Wu *et al.*<sup>1</sup> have recently examined this issue by targeted deletion of Gs- $\alpha$  in osteoblast lineage cells. Mice lacking osteoblast Gs- $\alpha$  display severe skeletal defects at birth with fractures of the ribs and long bones. The lamellar structure of bone is disrupted, and this is associated with a loss of osteoprogenitors and an accelerated differentiation of osteoblasts. These findings highlight the importance of Gs signaling at multiple stages of osteoblast differentiation, and suggest the possibility that this pathway can be further exploited in the development of novel anabolic therapies.

Gs is a heterotrimeric G protein that couples signaling from a subset of activated G protein-coupled receptors (GPCRs) to downstream effectors, most notably adenylyl cyclase, leading to increased accumulation of cyclic AMP and increased activity of protein kinase A. Interest in the role of Gs signaling in regulating osteogenesis has derived from two major lines of investigation. First, it is well appreciated that activation of Gs-coupled GPCRs in osteoblasts has a profound effect on bone formation and bone resorption. This has been most extensively studied for the PTH1 receptor the activation of which is linked to enhanced bone formation and bone resorption.<sup>2</sup> Other Gs-coupled GPCRs in osteoblasts such as E-series prostaglandin receptors<sup>3</sup> and  $\beta$ -adrenergic receptors<sup>4</sup> likewise are linked to alterations in skeletal homeostasis. In addition, patients with genetic alteration in Gs- $\alpha$  function display bony abnormalities that are likely to be due at least in part to changes in Gs signaling in osteoblasts. Gain of function mutations in Gs- $\alpha$  (as in McCune Albright's disease) result in fibrous dysplasia of bone characterized by increased bone turnover, formation of woven bone and defects in osteoblast differentiation.<sup>5</sup> Loss of function mutations (as in Albright's hereditary osteodystrophy and progressive osseous heteroplasia) result in abnormal bone development and/or heterotopic ossification.<sup>6</sup>

There are a number of plausible mechanisms by which Gs signaling in osteoblasts may alter bone development and skeletal homeostasis. These include possible effects on osteoprogenitor pools, altered rates of osteoblast differentiation and effects on the function and disposition of mature osteoblasts. A few years ago, Sakamoto *et al.*<sup>7</sup> examined the skeletal effects of deleting Gs- $\alpha$  from relatively mature osteoblasts in mice using 2.3-kb Col I-cre. These mice died shortly after birth and displayed a reduction in bone formation in the primary spongiosa of the long bones; cortical thickness was increased due presumably to suppression of endocortical bone resorption. Wu *et al.*<sup>1</sup> have now examined in detail the impact of deletion of Gs- $\alpha$  from early, committed osteoblasts using *osx*-cre. The results provide a number of new insights into the control of bone formation and osteoblast differentiation by Gs signaling. Deletion of Gs- $\alpha$  early in course of osteoblast differentiation resulted in profound osteopenia associated with a marked reduction in bone formation and production of woven bone. There was a striking decrease in the number of osteoprogenitor cells likely due to an effect on the commitment of mesenchymal precursors to the osteoblast lineage. There was also an acceleration of osteoblast differentiation evidenced by increased osteocyte density *in vivo* and increased mineralized colony formation *in vitro*. The combination of these effects resulted in a marked decrease in the number of osteoblasts on active bone-forming surfaces *in vivo*. Bone resorption was not extensively investigated, but did not appear to be altered in a major way. The reduction in bone mass and quality in the newborn pups were associated with extensive fractures of the ribs and long bones.

The mechanism(s) underlying the effect of Gs- $\alpha$  deficiency on osteoprogenitor number is not entirely clear. The mice displayed increased skeletal expression of *Sost* and *Dkk1*, as might be expected from the known effect of cyclic AMP signaling to downregulate expression of these genes in osteocytes.<sup>8,9</sup> Correspondingly, these mice displayed a decrease in the activity of a canonical *wnt* reporter gene in bone *in vivo*. However, it is not clear whether the effect of Gs- $\alpha$  deficiency on the commitment

of mesenchymal progenitors is a cell autonomous effect (perhaps mediated by downregulation of canonical wnt signaling) or is due to increased production of sclerostin and/or dkk1 by mature osteoblasts/osteocytes (perhaps downregulating canonical wnt signaling in mesenchymal progenitors by a paracrine mechanism). Indeed, previous studies have shown that constitutive activation of Gs-GPCR signaling in relatively mature osteoblast lineage cells is sufficient to produce a phenotype resembling fibrous dysplasia and to augment canonical wnt signaling in presumed osteoprogenitor cells.<sup>10,11</sup>

The effect of Gs- $\alpha$  deletion to accelerate osteoblast differentiation was replicated in cultured bone marrow stromal cells. Strikingly, this was seen even when Gs- $\alpha$  deletion was initiated at a very early stage of osteoblast differentiation. Late-stage osteoblast differentiation was also enhanced by Gs- $\alpha$  deficiency as evidenced by increased expression of dentin matrix protein 1 *in vivo* and *in vitro*. These findings suggest that Gs- $\alpha$  signaling constrains osteoblast differentiation from the time of early osteoblast commitment through the osteoblast-osteocyte transition. This is consistent with studies demonstrating that sustained treatment with PTH to activate Gs signaling blocks osteoblast differentiation *in vitro*.<sup>12,13</sup> We have seen a similar effect of forskolin that bypasses Gs to activate adenylyl cyclase directly (Kao, R. and Nissenson, R., unpublished data). Thus, Gs signaling in osteoblasts appears to serve as a timing mechanism that allows bone formation to occur at a pace that permits optimal formation of lamellar bone. Excessive Gs signaling, as in fibrous dysplasia or hyperparathyroidism, results in increased bone turnover, delayed osteoblast differentiation and the formation of woven bone. Insufficient Gs signaling results in the production of woven bone associated with accelerated osteoblast maturation, abnormal osteocytes and reduced numbers osteoblast progenitors.

These findings have implications for the development of anabolic therapies for osteoporosis. Treatments based on activation of Gs signaling would ideally need to be tailored to provide the optimal level of signaling in the appropriate subpopulations of osteoblast lineage cells. It is in many ways remarkable that

intermittent treatment with PTH is effective in this regard, producing enhancement of normal osteogenesis and production of lamellar bone. As Gs signaling can be initiated by a diverse array of GPCRs in cells at various stages of osteoblast differentiation, it may be possible to develop more efficacious and/or more conveniently administered anabolic agents that exploit the Gs signaling pathway in osteoblasts.

### Conflict of Interest

The author declares no conflict of interest.

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