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## COMMENTARY

# Differential effects of hedeghog signaling on postnatal bone remodeling

**Kingston King-Lun Mak**[1,2,3]

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China. [2]Ministry of Education Key Laboratory for Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong, China. [3]The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, China.

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A tight coordination between osteoblasts and osteoclasts are required to maintain proper bone mass. Dysregulation of this highly coordinated process will lead to many common bone diseases such as osteoporosis. It is therefore critical to understand fully how signaling networks control the overall bone mass during remodeling and homeostasis. Hedghog (Hh) signaling, one of the important pathways that regulates endochondral ossification, has been shown to have very different effects in postnatal remodeling, depending on its signaling strength as well as its temporal and spatial activation.[1,2] Interestingly, a recent publication by Joeng and Long[3] further delineates the complexity of the precise effect of Hh signaling in osteoblasts, prompting a more dynamic outcome of downstream effectors that integrates probably other signaling pathways as well as effects from different cell lineages within the bone microenvironment. They investigated the activated role of Hh signaling mediated by Gli2 in early osteoblasts by generating an Osx-ΔNGli2 transgenic mouse model with a constitutively active form of Gli2 specifically expressed in Osx-lineage cells. They surprisingly found that the overall bone mass of the transgenic mice was reduced, which was independent of osteoclastogenesis. More interestingly, osteoblasts isolated from the transgenic mice indicated that Hh signaling intrinsically stimulates osteoblast differentiation similar to its positive role during endochondral ossification. However, this result was in contrast to the phenotypes of the Osx-ΔNGli2 transgenic mice, in which osteoblast number was reduced and osteoblast differentiation was inhibited *in vivo* and resulted in osteopenia. Therefore, they raised a possibility that a yet unknown indirect mechanism may be triggered by Gli2 overexpression within the microenvironment to inhibit osteoblast differentiation.

Previous studies addressed similar roles of activated Hh signaling in postnatal bone remodeling using two different genetic mouse models with deletion of the Patched (Ptch), a membrane-bound inhibitory receptor upstream of Gli transcription factors. Interestingly, the two mutant mice showed contrasting phenotypes on overall bone mass.[1,2] Ptch$^{+/-}$ mice showed an increase of bone mass primarily because of enhanced osteoblast differentiation, albeit osteoclast differentiation was also increased. Bone formation was dominated over bone resorption in the Ptch$^{+/-}$ mice likely because of the reduced truncated repressor form of Gli3, which in turn reduced repression of Runx2 and osteoblast differentiation.[2] However, another study using conditional knock-out mouse model, with Ptch specifically deleted only in mature osteoblasts (osteocalcin$^{+}$ cells), showed an opposite phenotype in which overall bone mass was significantly reduced.[1] Although both studies demonstrated that osteoblastogenesis and osteoclastogenesis were enhanced by the removal of the Ptch, bone resorption was dominated over bone formation in the Ptch conditional knock-out mice. It was further showed that the excessive bone resorption was an indirect stimulation of osteoclast differentiation by parathyroid hormone related peptide from the mature osteoblasts to induce receptor activator of nuclear factor κ-B ligand (RANKL) expression. This effect of Hh signaling is likely through both Gli2 and Gli3 actions.

To account for the discrepancies of these three studies, it is obviously directed to the differences in the timing and location of Hh signaling activation and the strength of Hh activities. It appears that sustained (Ptch conditional knock-out mice) or constitutive active Hh signaling (Osx-ΔNGli2) resulted in overall reduction of bone mass, although via different mechanisms, whereas partial activation of Hh signaling (Ptch$^{+/-}$ mice) enhanced bone formation. However, there is no simple correlation whether early or late activation (Osx$^{+}$ cells vs Oscal$^{+}$ cells) of Hh signaling within the osteoblast lineage will result in a consistent phenotype in total bone mass *in vivo*. The only consensus from all these studies is that when osteoblasts are isolated and cultured *in vitro*, Hh signaling autonomously stimulates osteoblast differentiation disregard to the ways of activation and the differentiation status of osteoblasts. This strongly confers the existence of crosstalks with other cell lineages within the bone microenvironment. Osteoclast lineage

has been proven to be closely interacted with osteoblasts and possibly other cell lineages are also involved as well, which require further investigation.

The other possibility that explains the phenotypic variations among different mouse models can be due to the different hierarchy of genetic manipulation. For the Ptch mutants, Hh signaling was manipulated more upstream along the pathway in which removal of Ptch may affect the activities of Gli2 and Gli3 and some other Hh effectors that impact the overall signaling cascades, depending on the strength of Hh activation that selectively triggers subsets of downstream signaling. For example, partial upregulation of Hh signaling in the Ptch$^{+/-}$ mice predominantly regulates Gli3 repressor only, with little or no alteration of Gli2 expression, localization or processing in osteoblast precursors. However, with increasing signaling strength with ubiquitous upregulation of Hh signaling in osteoblasts in the Ptch conditional knock-out mice, the processing of both Gli2 and Gli3 existed. It seems that higher level of Hh activation stimulates the activation of Gli2. On the other hand, for the Osx-ΔNGli2 transgenic mice, the outcomes of the bone phenotype should be interpreted as mediated solely from the Gli2 only, without affecting Gli3 or other Hh effectors. Therefore, it seems that the ratio of Gli2 to Gli3 (both activated forms and repressor forms, respectively) is critical to initiate particular subsets of signaling cascades, which ultimately lead to very different outcomes in terms of overall bone mass.

It is also conceivable to consider that the phenotypes observed from the Osx-ΔNGli2 transgenic mice may be a consequence of activation of other signaling pathways as well. For instance, Wnt acts as a mediator of Hh signaling in osteoblast differentiation during skeletal development.[4–6] Postnatally, Wnt from osteoblasts inhibits osteoclast differentiation primarily by upregulating osteoprotegerin expression.[7] It has been also shown that Wnt/β-catenin is able to enhance expression of Gli2 as well.[8] Thus, constitutive Gli2 expression may trigger a vicious cycle of Wnt activation, which inhibits bone resorption through osteoprotegerin expression. This counterbalances the Hh-induced parathyroid hormone related peptide/RANKL expression (probably through Gli2) that promotes bone resorption. Consequently, the ratio of RANKL/osteoprotegerin remains constant, and therefore no net change of osteoclast differentiation was observed in the Osx-ΔNGli2 transgenic mice. It is important to further investigate whether the expression of parathyroid hormone related peptide, RANKL and osteoprotegerin are upregulated to confirm this hypothesis. Apart from Wnt signaling, Bmp signaling is also a candidate that needed to be investigated. Bmp signaling has been shown to promote bone formation and stimulates osteoclast differentiation through a RANKL-independent pathway.[9] It is interesting to examine the expression level of Bmp2, 4, 7 in the Osx-ΔNGli2 transgenic mice and compare the results with the Ptch conditional knock-out mice where Bmps were upregulated.

Still, the inhibitory effect of osteoblast differentiation by Gli2 activation in the Osx-ΔNGli2 transgenic mice cannot be fully reconciled. The existence of indirect mechanisms may be partially accounted by integrative bone physiology. Recent findings demonstrated that bone secretes osteocalcin as a novel bone-derived hormone to regulate glucose metabolism and male fertility, indicating that the skeleton actively interacts with other organs.[10–12] It is possible that other organs also exert feedback mechanisms in response to the overtly activated Gli2 activities to inhibit osteoblast differentiation. It is unclear whether circulating osteocalcin level has been perturbed in the Osx-ΔNGli2 transgenic mice or yet other unknown bone-derived hormones were released, which results in the overall change of energy metabolism and therefore indirectly impact overall bone mass through unknown mechanism. These areas are definitely worth further interrogation to dissect the detail mechanism of Hh signaling in regulating bone mass and energy expenditure.

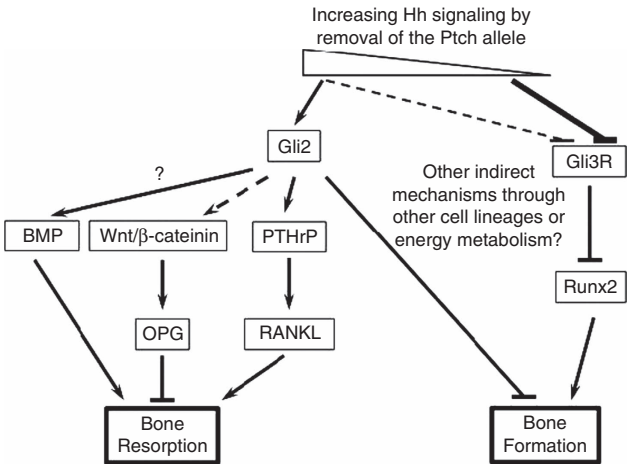


**Figure 1** Differential regulation of postnatal bone mass in response to the strength of Hh signaling activities. Low Hh signaling activation predominantly acts through Gli3 to relieve inhibition of osteoblast differentiation. High level of Hh signaling shifts to Gli2 activation and induces a dynamic signaling cascade to regulate bone resorption. Gli2 may also trigger the differentiation of other cell lineages within the microenvironment and/or induce indirect feedback mechanisms through endocrinology regulation.

After all, it is clear that Hh signaling not only regulates osteoblast differentiation, but it also has an indirect role in regulating osteoclast differentiation and possibly the differentiation of other cell lineages (**Figure 1**). The bone microenvironment possesses many other cell lineages, including endothelial cells, macrophages, stromal cells or hematopoietic stem cells, which may also contribute to the balance of overall bone mass. The phenotypes from the transgenic and mutant mice so far cannot be fully explained by the current findings. Collectively, it seems that minimal Hh signaling activity is required for proper bone remodeling as supported by the fact that postnatal osteoblasts show Hh activities, and it gradually reduces as osteoblasts mature.[1] Ohba *et al* [2] also showed that inhibition of Hh signaling with cyclopamine treatment reduced the overall bone mass in mice. However, mouse models with genetic removal of Smoothened in mature osteoblasts displayed a gain of bone mass only in aged mice, which remain an unresolved question. In conclusion, there are still many hurdles to overcome before we can translate the effects of Hh signaling in postnatal bone remodeling for therapeutic treatment that may be beneficial to human patients suffering osteoporosis.

## Conflict of Interest

The author declares no conflict of interest.

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