

MEETING REPORTS

Osteoblasts and Wnt Signaling: Meeting Report from the 31st Annual Meeting of the American Society for Bone and Mineral Research

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Wnt Signaling, Serotonin and Bone

Just after the 2008 ASBMR Annual Meeting in Montreal, a group led by Gerard Karsenty reported that Lrp5 controls bone formation by regulating serotonin in the duodenum (1). This information markedly changed the concept that the low bone mass osteoporosis pseudoglioma (OPPG) and high bone mass (HBM) phenotypes were due to cell autonomous alteration in Wnt signaling in osteoblasts. Thus, whereas the role of Wnt signaling in osteoblastogenesis from precursor mesenchymal cells is well-accepted, the importance of this pathway in the activation of osteoblasts for controlling bone formation remains unclear and needs reconsideration.

From the information mentioned above, one group investigated the relationship between circulating serotonin and bone mass measured by high-resolution pQCT in a population-based sample of 215 women that had no medication that could interfere with serum serotonin levels (2). After correction for the effect of serotonin on body mass index, the serum serotonin level was a significant negative predictor of total femoral neck and vertebral BMD, and of trabecular thickness of the radius. Thus, these data provide support for a physiological role of circulating serotonin in regulating bone density and structure.

Wnt Signaling and Osteoblasts

In order to better understand the cell autonomous role of the Wnt pathway for regulation of osteoblasts and bone

metabolism, a group investigated the phenotype of transgenic mice expressing *lefΔN* from the 2.3-*Col1a1* promoter (3). Lymphoid enhancer factor 1 (Lef1) is a Wnt-responsive transcription factor that associates with the nuclear co-regulator β -catenin. Lef1 and the N-terminal truncated isoform of Lef1, *lefΔN* (expressed mainly in mature osteoblasts), are capable of binding to DNA and regulating gene expression. A significant increase in osteoblast activity and in trabecular bone density of the proximal tibia was detected in transgenic mice. There was also a significant increase in mRNA of OPG and in the OPG/RANKL ratio mRNA. However, osteocalcin mRNA in the cortex was decreased 4-fold in *lefΔN* transgenic mice. From these observations, the study concluded that the constitutive expression of *lefΔN* promotes osteoblast activity in trabecular bone and bone development and inhibits end-stage osteoblast maturation in cortical bone and normal bone homeostasis. These data further support a cell autonomous effect of the Wnt pathway in osteoblasts with different effects on trabecular and cortical bone, probably through differences in either the cell autonomous effect of Wnt proteins in osteoblasts of trabecular and cortical bone or in Wnt antagonists present at each bone site. Data also confirm an important effect of this pathway in regulating bone resorption through the OPG/RANKL system.

A recent report indicated that osteoblast-targeted disruption of glucocorticoid signaling is associated with a reduction of Wnt7b and Wnt10b mRNA and β -catenin protein levels in transgenic (*Col2.3-*

11 β HSD2) versus control osteoblasts (4). This change in Wnt expression and signaling was associated with alteration in osteoblastogenesis, suggesting that Wnt signaling by osteoblasts is essential for mesenchymal cell differentiation towards the osteoblastic lineage. To define the individual roles of Wnt7b and Wnt10b in the control of osteoblast differentiation, the same group knocked down Wnt7b or Wnt10b in MC3T3-E1 cells and investigated the phenotype of these cells (5). Knockdown of Wnt7b resulted in complete failure of mineralized formation whereas alteration of cell differentiation was less severe when Wnt10b was knocked down. Further investigation indicated that Wnt7b could compensate for the absence of Wnt10b since its expression was increased several-fold with time incubation. Associated with blunted mineralization in the absence of Wnt7b, osteocalcin expression was markedly reduced, suggesting an alteration in osteoblast differentiation. From these observations, the study concluded that Wnt7b is probably an important Wnt protein for increasing the differentiation of osteoblastic cells. This conclusion is also supported by a recent study showing that Wnt7b, expressed by osteogenic cells *in vivo*, induces osteoblast differentiation *in vitro* (6).

Interaction Between the Wnt Signaling Pathway and Sclerostin

To investigate whether the Wnt pathway is an important target of *Sost* for controlling bone formation, a group studied whether *Sost* deletion could rescue the osteoporotic phenotype in mice lacking β -catenin (Ctnnb1) in osteocytes (*Ctnnb1*^{loxP/loxP}; *Dmp1-Cre* mice) (7). Osteocyte-specific loss of Ctnnb1 resulted in osteoporosis, while *Sost* deficiency induced a high bone mass phenotype. The increased bone mass in response to *Sost* deficiency was markedly decreased in mice lacking β -catenin. The reason for this blunting effect remains unclear. This blunting effect was also observed with heterozygous Ctnnb1 deletion from osteocytes, which by itself did not cause a low bone mass phenotype. From these observations, this study concluded that β -catenin-dependent Wnt signaling in

osteocytes downstream of *Sost* plays a crucial role. A detailed analysis of the *Ctnnb1*^{loxP/loxP}; *Dmp1-Cre* phenotype is needed to better understand the type of interaction between *Sost* and β -catenin-dependent Wnt signaling in osteocytes for controlling bone metabolism.

Previous findings suggested that *Sost* may interact with Lrp5 in osteoblasts to control bone formation. As mentioned above, this concept has recently been challenged by work suggesting that Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum (1). To further investigate this relationship, *Sost*; *Lrp5* double knockout mice were generated (8). As expected, *Sost*-deficient mice displayed high bone mass and Lrp5-deficient mice displayed low bone mass phenotypes. Compared with wild type controls, *Sost* deficiency rescued the Lrp5 mutant osteopenic phenotype. However, the net bone gain in Lrp5 mutants due to *Sost* deficiency was much smaller compared to mice possessing a normal Lrp5 gene. This lower bone gain was also observed in mice lacking just one Lrp5 wild-type allele. The authors concluded from their study that *Sost* targets Lrp5-dependent and -independent pathways to control bone formation *in vivo*. Further studies will be required to assess the cellular and molecular mechanisms by which *Sost* interacts with either Lrp5 or the serotonin pathways to control osteoblastic cell activity and bone formation.

Recent genetic studies in humans and mice have clearly shown that sclerostin is a potent secreted inhibitor of bone formation. *In vitro* studies suggested that sclerostin may inhibit osteoblastic activity by binding to the Wnt co-receptor low density lipoprotein-related (LRP) 5/6. To assess whether sclerostin binds to these molecules at the cellular level, a series of tandem affinity purification screens using HEK293 and UMR106 cells were performed (9). Results identified LRP4 and glypicans as novel sclerostin binding partners. Interaction with LRP6 and, with lower confidence, LRP5, was confirmed. Sclerostin-mediated inhibition of Wnt-induced signaling was increased in the presence of LRP4 in HEK293 and UMR106 cells and diminished in HEK293 cells with knockdown of LRP4.

DKK1 had no effect in these experiments, suggesting that LRP5 was not involved. Finally, silencing of LRP4 by lentiviral-mediated shRNA increased osteoblastic cell differentiation in MC3T3-E1 cells and completely blocked sclerostin-inhibitory action on *in vitro* bone mineralization. From these observations, the authors concluded that the interaction between sclerostin and LRP4 is required for the inhibitory effect of sclerostin on bone formation, and that LRP4 is a potent target for controlling bone formation.

The Wnt Signaling Pathway, Mechanical Loading and Bone Formation

Mechanical loading is an important stimulus for bone formation but the molecular events involved in mechanical signal transduction are not well understood. The role of LRP5 in mechanical loading-related stimulation of cortical and cancellous bone in mice was investigated (10) using either transgenic LRP5G171V, *Lrp5(-/-)* or wild type mice. Mechanical stimulation was performed on the right tibia whereas decreased loading was obtained with ablation of the right sciatic nerve. Essentially, the authors found that two weeks of loading engendered an osteogenic response in all groups with a greater response in cortical and cancellous bone in LRP5G171V mice. From these observations, they concluded that the absence of the LRP5 gene has no effect on bones' osteogenic response, an observation that does not confirm data from a previous report indicating that the Wnt coreceptor LRP5 is essential for skeletal mechanotransduction (11). The presence of the HBM G171V mutation, however, is associated with a higher osteogenic response to loading and some protection from bone loss due to disuse.

Another group also investigated the effect of mechanical loading on bone formation in wild type (WT) and LRP5G171V (HBM) mice in similar experimental conditions (12). Surprisingly, the authors found a smaller stimulation of bone formation in loaded bone from HBM (+26%) compared with WT (+48%) mice, a result that is not consistent with data presented in the above paper. These authors also investigated the role of

LRP5 in PTH-induced changes in bone formation with mechanical loading. Whereas administration of PTH nearly doubled the bone formation response to loading in WT mice (+48% to +90%), it was reduced in HBM mice (from +26% to 12%). The authors concluded that there is probably an overlap in signaling between the HBM LRP5 receptor and PTH that acts to suppress the formation response when both are stimulated simultaneously.

The Non-Canonical Wnt Signaling Pathway and Cytokine Interaction with Wnt Signaling in Bone Pathophysiology

ROR2 is a recently described alternative receptor for Wnt proteins that has been shown to influence bone development and metabolism via non-canonical Wnt signaling with a knockout phenotype resembling that of *Wnt5a* (13). It was reported that ROR2 is one of the genes highly expressed in osteosarcoma (14). Knockdown of ROR2 in the osteosarcoma cell lines U2OS and Saos-2 caused suppression of cell proliferation and invasive ability. Increased expression of ROR2 in osteosarcoma was associated with overexpression of WNT5B. The authors concluded that ROR2 and WNT5B are promising therapeutic targets for the treatment of osteosarcoma.

Proinflammatory cytokines such as tumor necrosis factor (TNF) are known to reduce bone mass in periarticular bone by increasing bone resorption. Whether TNF also influences bone formation *in vivo* is not well understood and was investigated at the meeting (15). For this analysis, the authors used transgenic mice expressing human TNF and investigated changes in bone mass compared with WT mice at different time intervals. They observed that BMD was similar until 4 weeks, after which an accelerated accrual of bone mass due to increased osteoblast number in WT mice was blunted in TgTNF mice with lower BV/TV, TbN, TbTh and Tb connectivity and no difference in osteoclast number and CTx. Associated with this *in vivo* effect of TNF on bone development, mesenchymal stem cells (MSCs) from 12-week-old TgTNF mice cultured in differentiating medium had a reduced expression of *Osx*, *RUNX2*,

AlkPhos, BSP, osteocalcin and delayed mineralization. TgTNF cells also showed reduced Wnts 10b, 5a, and 5b, β -catenin mRNA and DKK1, suggesting alteration in Wnt signaling. To determine if TNF suppressed Wnt signaling *in vivo*, TgTNF mice were crossed with a β -catenin-activated transgene driving expression of nuclear β -galactosidase reporter (BAT-gal) transgenic mice expressing the lacZ transgene under the control of β -catenin/T cell factor responsive elements. LacZ activity was assessed at 4 weeks when osteoblast differentiation was expected to accelerate. TgTNF/BAT-Gal mice showed a 90% reduction in Wnt signaling compared with WT/BAT-Gal mice as measured in trabecular bone sections. From these observations, the authors concluded that proinflammatory cytokines such as TNF can blunt osteoblast proliferation and accelerate accrual of bone in young mice by blocking Wnt signaling.

Wnt Inhibitors and the Regulation of Bone Mass

As part of Lexicon's Genome5000 program to characterize mouse knockout phenotypes of pharmaceutically relevant genes, secreted frizzled related protein-4 (sFRP4) KO mice were generated (16). sFRPs are known to antagonize Wnt signaling either by functioning as decoy receptors for Wnts or by forming non-functional complexes with frizzled (17). A previous study reported that osteoblast-targeted overexpression of sFRP4 in mice resulted in low trabecular bone mass compared to wild type littermates (18). In the current study's sFRP4 KO analysis, the authors found an increase in trabecular bone mass, which is concordant with the previous observation (18). In addition to the increased trabecular bone, ablation of sFRP4 was associated with an increased cortical bone diameter but a reduced cortical bone thickness. Associated with these changes in bone mass and structure, bone breaking strength (maximal load) was elevated by 42% in vertebral bodies but reduced 23% in the femoral shaft. These data, together with previous observations, clearly indicate that sFRP4 influences both cortical and trabecular bone

architecture, probably by interfering with the action of Wnt protein(s) in bone cells.

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References

1. Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schütz G, Glorieux FH, Chiang CY, Zajac JD, Insogna KL, Mann JJ, Hen R, Ducy P, Karsenty G. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell*. 2008 Nov 28;135(5):825-37.
2. Moedder U, Achenbach S, Amin S, Riggs B, Melton L, Khosla S. Serum serotonin levels are related to bone density and structural parameters in women. *J Bone Miner Res*. 2009;24(Suppl 1). [[Abstract](#)]
3. Secreto F, Hoepfner L, Whitney T, Razidlo D, Stensgard B, Li X, Evans G, Hefferan T, Yaszemski M, Westendorf J. Lef1 Δ N dependent increases in bone mass result from enhanced osteoblast activity. *J Bone Miner Res*. 2009;24(Suppl 1). [[Abstract](#)]
4. Zhou H, Mak W, Zheng Y, Dunstan CR, Seibel MJ. Osteoblasts directly control lineage commitment of mesenchymal progenitor cells through Wnt signaling. *J Biol Chem*. 2008 Jan 25;283(4):1936-45.
5. Shao X, Fong-Yee C, Dunstan C, Jia W, Chen D, Seibel M, Zhou H. Differential regulation of osteoblast differentiation by Wnt7b and Wnt10b. *J Bone Miner Res*. 2009;24(Suppl 1). [[Abstract](#)]
6. Tu X, Joeng KS, Nakayama KI, Nakayama K, Rajagopal J, Carroll TJ, McMahon AP, Long F. Noncanonical Wnt signaling through G protein-linked PKCdelta activation promotes bone formation. *Dev Cell*. 2007 Jan;12(1):113-27.
7. Kramer I, Merdes M, Jeker H, Keller H, Kneissel M. Sost deficiency dependent

- bone gain is blunted in osteocyte specific beta-catenin mutant mice. *J Bone Miner Res.* 2009;24(Suppl 1). [\[Abstract\]](#)
8. Kramer I, Merdes M, Keller H, Kneissel M. Sost exerts its action via Lrp5 dependent and independent pathways to control bone formation in vivo. *J Bone Miner Res.* 2009;24(Suppl 1). [\[Abstract\]](#)
 9. Oliver L, Halleux C, Morvan F, Hu S, Lu C, Bauer A, Kneissel M. LRP4 is a novel osteoblast and osteocyte expressed specific facilitator of SOST-mediated inhibition of *in vitro* bone formation. *J Bone Miner Res.* 2009;24(Suppl 1). [\[Abstract\]](#)
 10. Saxon L, Jackson B, Lanyon L, Price J. Absence of LRP5 in mice has no effect on bone's adaptive response to loading or disuse but the high bone mass G171V LRP5 gain of function mutation is associated with an increase in loading's osteogenic effect and protection from disuse. *J Bone Miner Res.* 2009;24(Suppl 1). [\[Abstract\]](#)
 11. Sawakami K, Robling AG, Ai M, Pitner ND, Liu D, Warden SJ, Li J, Maye P, Rowe DW, Duncan RL, Warman ML, Turner CH. The Wnt co-receptor LRP5 is essential for skeletal mechanotransduction but not for the anabolic bone response to parathyroid hormone treatment. *J Biol Chem.* 2006 Aug 18;281(33):23698-711.
 12. Hackfort B, Alvarez G, Akhter M, Cullen D. Differential effects of parathyroid hormone (PTH) and tibial compression on bone formation with variation in Lrp5 expression. *J Bone Miner Res.* 2009; 24(Suppl 1). [\[Abstract\]](#)
 13. Caverzasio J. Non-canonical Wnt signaling: what is its role in bone? *IBMS BoneKEy.* 2009 Mar;6(3):107-15.
 14. Morioka K, Matsuda K, Kawano H, Nakamura K, Kawaguchi H, Nakamura Y. Identification of receptor tyrosine kinase-like orphan receptor 2 (ROR2)/WNT5B signaling as a therapeutic target against osteosarcoma. *J Bone Miner Res.* 2009;24(Suppl 1). [\[Abstract\]](#)
 15. Gilbert L, Lu X, Chen H, Weitzmann M, Nanes M. Systemic bone loss in TNF expressing mice is due to suppression of Wnt signaling and osteoblast differentiation independent of osteoclast activity. *J Bone Miner Res.* 2009;24(Suppl 1). [\[Abstract\]](#)
 16. Brommage R, Liu J, Lee E. Elevated trabecular bone mass with reduced cortical bone thickness in sFRP4 knockout mice. *J Bone Miner Res.* 2009;24(Suppl 1). [\[Abstract\]](#)
 17. Kawano Y, Kypta R. Secreted antagonists of the Wnt signalling pathway. *J Cell Sci.* 2003 Jul 1;116(Pt 13):2627-34.
 18. Nakanishi R, Akiyama H, Kimura H, Otsuki B, Shimizu M, Tsuboyama T, Nakamura T. Osteoblast-targeted expression of Sfrp4 in mice results in low bone mass. *J Bone Miner Res.* 2008 Feb;23(2):271-7.