IBMS BoneKEy. 2008 November;5(11):449-453
http://www.bonekey-ibms.org/cgi/content/full/ibmske;5/11/449
doi: 10.1138/20080353

MEETING REPORTS

Osteoblasts and Wnt Signaling: Meeting Report from the 30th Annual Meeting of the American Society for Bone and Mineral Research

September 12-16, 2008, Montréal, Québec, Canada

Joseph Caverzasio

Service of Bone Diseases, Department of Rehabilitation and Geriatrics, Faculty of Medicine, University of Geneva, Geneva, Switzerland

Each ASBMR Annual Meeting is rich in new information for osteoblast biology. As expected from recent advances in this field, several laboratories concentrated their efforts on the Wnt signaling pathway and sclerostin, which are the two systems that could provide information for the development of new molecules to stimulate bone formation. Some of the key new findings are summarized below.

The Wnt/LRP5 Signaling Pathway Remains a Potential Drug Target for Osteo-Anabolism

With the identification of Lrp5 mutations in patients with either osteoporosis or osteosclerosis, the Wnt signaling pathway is considered to be of crucial importance for the regulation of osteoblast differentiation and function. The Wnt canonical pathway is triggered by the binding of Wnt family members to a co-receptor complex that includes Frizzled (Fzd, a G protein-coupled receptor (GPCR)-like protein) and Lrp5/6. The canonical signal is transmitted by the β-catenin pathway and the Tcf/Lef1 transcriptional factors. Canonical Wnt signaling is important during embryogenesis and osteoblastogenesis and is tightly regulated by several secreted factors, including members of the Dickkopf (Dkk), Wnt-inhibitor factor (Wif), and secreted frizzled related protein (sFRP) families and sclerostin (*Sost*).

The molecular mechanism by which extracellular factors and intracellular molecules regulate Wnt signaling in osteoblasts and bone formation is not well-understood and these issues were the focus of several interesting studies reported at the meeting.

Whereas genetic evidence has clearly established a prominent role for Lrp5 in bone development, the role for Lrp6 remained unclear. The selective ablation of Lrp6 in osteoblasts is associated with lower bone mass (1). Ablation of both Lrp5 and Lrp6 in osteoblasts induces a severe osteopenia and phenocopied mice lacking β-catenin, suggesting that both Lrp5 and Lrp6 are required to fully activate β-catenin in mature osteoblasts.

Two studies investigated the role of Kremen (Krm1 and Krm2), transmembrane proteins serving as receptors for molecules of the Dkk family that antagonize Wnt signaling through binding to Lrp5 and Lrp6. One study focused on Krm2 (2). Surprisingly, the expression pattern of this molecule was exactly opposite to that of Dkk1. It was mainly observed in non-mineralized bone and not in mineralized cultures. This observation did not suggest that Krm2 is involved in the action of Dkk1 on regulation of Lrp5. Thus, the authors created a Krm2 overexpression mouse model driven by a Col1a1 promoter and found a striking reduction (2/3) in bone mass due to both a marked reduction in bone formation and increased bone resorption. Cell analysis suggested that Krm2 is involved in inactivation of Lrp6. Clearly, further studies

are required to determine the mechanism by which Krm2 influences the differentiation of osteoblasts.

Another study investigated the physiological relevance of Krm1 and Krm2 as Wnt modulators for bone remodeling (3). Double, but not single mutants showed enhanced Wnt signaling and osteoblast differentiation markers and increased trabecular bone volume with small changes in bone resorption parameters, suggesting a major effect on bone formation. The triple knockouts in which Dkk1 was haplo-insufficient failed to show significant changes in BV/TV above and beyond the Krm1/2 double knockouts, suggesting a functional interaction between Kremens and Dkk1 as negative regulators of Wnt/β-catenin signaling and trabecular bone formation.

Because of its prominent role in the regulation of bone mass, the Wnt signaling system is of potential interest for anabolic drug development. One regulatory system of interest is the sFRP family that functions as a Frizzled decoy receptor, antagonizing Wnt activity and therefore, unlike Dkk1 and Sost, suppressing both canonical and non-canonical pathway activation. It was reported that deletion of sFRP4 in growing mice strongly favors trabecular bone formation but has an opposite effect on cortical bones without influencing bone resorption (4). This interesting observation suggests that the Wnt system may differentially influence trabecular and cortical bone.

Another player in the Wnt system investigated and reported at the meeting was Fzd9. Analysis of the skeletal phenotype of Fzd9-deficient mice indicated a 40% decrease in trabecular bone volume at the age of 24 and 72 weeks, with no effects at 6 weeks. Thus, Fzd9 is an interesting target for the treatment of bone diseases in adults.

Wnts are divided into Wnt-1 and Wnt-5a signaling classes, which activate canonical (Wnt/β-catenin) and non-canonical intracellular pathways (Wnt/Ca^{2+} and Wnt/Planar Cell Polarity (PCP)), respectively. Recently, the non-canonical Wnt-4 has been found to be regulated by parathyroid hormone (PTH) and expressed in bone lining cells, osteocytes, and hypertrophic chondrocytes (5). At this year's meeting, the same group reported that the differentiation of primary osteoblastic cells is increased by Wnt-4 (6). This effect is associated with minimal activation of the canonical pathway but a clear activation of the Wnt/Ca^{2+} pathway (a 12x increase in CamkII phosphorylation and a 2x increase in JNK phosphorylation). Using specific inhibitors to CamkII and JNKs, the authors found that Wnt-4 stimulates bone marker gene expression through non-canonical means. Their data support a role for non-canonical Wnts and imply that the non-canonical pathway is also important for regulation of bone.

A new molecule for regulation of β-catenin translocation in the nucleus, FHL2, has been presented (7), and the paper has recently been published online (8). FHL2 is a member of the LIM-only subclass of the LIM protein superfamily. The authors found that FHL2 is a key factor involved in dexamethasone-induced osteogenic cell differentiation in mesenchymal stem cells that are precursors of osteoblasts. Immunocytochemical analysis showed that FHL2 overexpression induces β-catenin translocation to the nucleus where the two proteins co-localize. This study provides good evidence that FHL2 is an important factor for the differentiation of mesenchymal osteoprogenitors into osteoblasts via activation of Wnt/β-catenin signaling.

The Molecular Mechanism by Which Sclerostin Inhibits Osteoblastic Cell Differentiation Remains Unclear

Bone diseases associated with mutations or deletions in *Sost* indicate that sclerostin is a major regulator of bone formation. Sclerostin is a member of the DAN family of glycoproteins that shares the capacity to inhibit BMP activity. Recent reports documented that sclerostin antagonizes

canonical Wnt signaling (9;10) by binding to the Wnt coreceptor Lrp5. These studies have been performed in Xenopus embryos and HEK293 cells. This molecular interaction had not yet been documented in osteogenic cells. At this year's meeting, an antagonistic effect of sclerostin on activation of Lrp5/6 by Wnts was not confirmed in two different osteoblastic cell systems having decreased Wnt3a- and BMP2-induced differentiation responses to sclerostin (11). The absence of a sclerostin antagonizing effect on activation of Lrp5/6 contrasted with the complete blunting action of Dkk1, a well-recognized antagonist of Lrp5 activity. The study also found that sclerostin by itself can stimulate different signaling pathways such as PKC, ERK1/2 and Akt. Data from this study and another report (12) also documented that sclerostin inhibits osteogenic cell differentiation induced by a selective inhibitor of GSK3β acting downstream of the Wnt/Lrp5 receptor. Thus, it remains unclear whether the blunting effect of sclerostin on BMP- and Wnt-induced osteoblastic cell differentiation is mediated by a direct interaction with Lrp5/6. Alternative mechanisms must be considered.

A previous report indicated that continuous administration of PTH suppresses sclerostin expression in osteocytes (13). Most of the *in vivo* effects of PTH are mediated by cyclic-AMP-dependent signaling downstream of the PTH/PTHrP GPCR. In a mouse model lacking Gsα in osteoblasts, sclerostin was markedly increased (14), suggesting that the cAMP pathway also mediates inhibition of PTH-induced sclerostin expression. In these mice, osteoblastic cell differentiation and maturation were markedly impaired with the formation of woven instead of lamellar bone.

Progress in Understanding the Regulation of Osterix Expression and Function

The transcription factor Osterix (Osx) is essential for osteoblast differentiation and bone formation, as mice lacking Osx die at birth with a complete absence of intramembranous and endochondral bone formation. Mice harboring a tamoxifen-inducible cre recombinase under the control of the CMV/βactin promoter to selectively delete Osx after birth displayed disappearance of bone trabeculae, thin and porous cortical bones, and a massive accumulation of mineralized cartilage (15). Calcein incorporation was almost completely absent in lumbar vertebrae and long bones of postnatal Osx-null mutants, indicating that Osx is required for bone formation postnatally. Inactivation of Osx also affected the development of osteocytes, suggesting that Osx plays a key role in regulating and maintaining osteocyte function. Using the time-specific and site-specific Cre/*loxP* system, another study also reported that Osx plays a significant role in regulating osteoblast differentiation and bone formation in adult bone (16).

To better understand the control of transcriptional activation by Osx, Osx-interacting proteins were identified using affinity purification techniques. A Jumonji C (JmjC)-domain containing protein, NO66, that interacts with Osx in osteoblast cells has been discovered (17). NO66 is expressed in all developing bones and exhibits a JmjC-dependent histone demethylase activity. Interaction between NO66 and Osx inhibits gene activation by Osx. Knockdown of NO66 in osteoblast cells triggers accelerated osteoblast differentiation and mineralization as well as a marked stimulation in expression of Osx target genes including *Col1a1*, *osteocalcin* and *bone sialoprotein*. These data provide the first evidence that NO66 serves as a negative regulator of Osx target genes in osteoblasts by modulating histone methylation states.

Dlx5 is a homeodomain-containing transcription factor expressed in developing skeletal elements. Dlx5-null mice have delayed cranial ossification and abnormal osteogenesis. In osteoblasts exposed to BMP2, it was found that Dlx5 precedes Osx expression and binds to a BMP2-responsive homeobox sequence, suggesting that Dlx5 is essential for mediating Osx expression by BMP2 (18). Furthermore, data presented

suggest that Dlx5 is a novel target of p38 MAPK that has been shown to be relevant for the osteogenic effect of BMP2. The phosphorylation of Dlx5 by p38 enhances Dlx5 transcriptional activity, increasing Osx expression and suggesting a p38-induced Dlx5 phosphorylation for regulation of Osx transcriptional activity.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

References

1. Zylstra CR, Wan C, VanKoevering KK, Sanders AK, Lindvall C, Clemens TL, Williams BO. Osteoblast-specific deletion of *Lrp6* reveals distinct roles for *Lrp5* and *Lrp6* in bone development. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S2. [Abstract]

2. Schulze J, Seitz S, Schneebauer M, Amling M, Schinke T. Transgenic over-expression of the Wnt antagonist Kremen-2 in osteoblasts leads to severe impairment of bone formation and increased bone resorption. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S2. [Abstract]

3. Saito H, Ellwanger K, Clément-Lacroix P, Hesse E, Maltry N, Niedermeyer J, Lee RW, Rawadi G, Westphal H, Niehrs C, Baron R. Deletion of the Dkk1 co-receptors Kremen 1 and Kremen 2 in mice leads to increased bone formation and bone mass. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S2. [Abstract]

4. Saito H, Hinkle R, Ebert D, Blanton C, Jaiswal N, Elenich L, Cody D, Baron R, Sabatakos G. Deletion of the Wnt signaling antagonist secreted frizzled related protein 4 (sFRP4) in mice induces opposite bone formation phenotypes in trabecular and cortical bone. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S3. [Abstract]

5. Bergenstock MK, Partridge NC. Parathyroid hormone stimulation of noncanonical Wnt signaling in bone. *Ann N Y Acad Sci*. 2007 Nov;1116:354-9.

6. Bergenstock MK, Tamasi J, Partridge NC. WNT-4 acts through the non-canonical pathways to stimulate osteoblast and bone marrow stromal stell cell differentiation. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S90.

7. Hamidouche Z, Haÿ E, Vaudin P, Charbord P, Marie PJ, Fromigué O. FHL2 mediates dexamethasone-induced mesenchymal stem cell osteogenic differentiation by activating Wnt/beta-catenin signaling and Runx2 expression. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S385. [Abstract]

8. Hamidouche Z, Haÿ E, Vaudin P, Charbord P, Schüle R, Marie PJ, Fromigué O. FHL2 mediates dexamethasone-induced mesenchymal cell differentiation into osteoblasts by activating Wnt/beta-catenin signaling-dependent Runx2 expression. *FASEB J*. 2008 Nov;22(11):3813-22.

9. Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J, Harris SE, Wu D. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J Biol Chem*. 2005 May 20;280(20):19883-7.

10. Semënov M, Tamai K, He X. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J Biol Chem*. 2005 Jul 22;280(29):26770-5.

11. Caverzasio J. Wnt/LRP5-independent inhibition of osteoblastic cell differentiation by sclerostin. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S72. [Abstract]

12. Grabenstaetter T, Sakane Y, Jacobi C, Lu C, Bauer A, Fernandez C, Ramage P, Hartmann L, Leupin O, Kneissel M, Halleux C. SOST blocks GSK3-beta inhibitor-induced alkaline phosphatase activity. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S251. [Abstract]

13. Keller H, Kneissel M. SOST is a target gene for PTH in bone. *Bone*. 2005 Aug;37(2):148-58.

14. Wu JY, Maes C, Chen M, Weinstein LS, Kronenberg HM. Deletion of the G protein subunit Gsα in early osteoblasts leads to accelerated osteoblast maturation and formation of woven bone with abnormal osteocytes, resulting in severe osteoporosis. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S71. [Abstract]

15. Zhou X, Zhang Z, Feng JQ, Darnay BG, Kim J, de Crombrugghe B. Osterix is required for skeletal growth and bone homeostasis after birth. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S22. [Abstract]

16. Baek W, Lee M, Oh J, Kim S, Akiyama H, de Crombrugghe B, Kim J. Osterix, a positive regulator in adult bone formation. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S86.

17. Sinha KM, Yasuda H, Coombes MM, Dent SR, de Crombrugghe B. Regulation of the osteoblast-specific transcription factor Osterix by NO66, a Jumonji family histone demethylase. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S87.

18. Ortuño MJ, Ulsamer A, Ruiz S, Susperregui AG, Osses N, Rosa JL, Ventura F. BMP2 induces Osterix expression through up-regulation of Dlx5 and its phosphorylation by p38 MAPK. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S88.