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MEETING REPORT

Meeting Report from the 29th Annual Meeting of the American Society for Bone and Mineral Research

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OSTEOBLASTS: STAYING THE COURSE

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This year's meeting catalogued the steady progress in our understanding of osteoblast biology. Now that many of the key factors controlling this cell lineage are known, studies are increasingly focused on the control of osteoblast activity by a wide range of signal transduction pathways and factors.

They're Alive!

Early in the meeting, we were treated to beautiful video images showing the dynamic nature of osteoblasts and their osteocyte descendants – a far cry from the static histology we're used to seeing. Different colored GFPs driven by the 3.6kb *Col1a1* and *DMP1* promoters were used to visualize calvarial osteoblasts and osteocytes, respectively, in real time (1;2). The continuum between these two cell types was emphasized as we watched an individual osteoblast gradually become surrounded by extracellular matrix, take on the DMP1 marker and mineralize. Interesting studies from the same group also suggest that a function we normally attribute to osteoblasts, induction of mineralization, may, in fact, be a role of the osteocytes that secrete matrix vesicle-like structures associated with hydroxyapatite crystallite nucleation (3).

Signaling and More Signaling

A number of presentations continued the recent trend of focusing on osteoblast regulatory mechanisms. The essential roles of a number of key signal transduction pathways in osteoblast function were revealed using a combination of *in vitro* biochemical analysis and *in vivo* gene deletion/transgenic studies.

G protein-coupled receptors, also known as GPCRs, regulate many pathways, including those required for PTH signaling in osteoblasts. However, the functional roles of specific G proteins in bone are poorly understood. Designer GPCRs provide powerful tools for dissecting the roles of these G proteins in osteoblasts. A transgenic approach was used to selectively express in osteoblasts an engineered GPCR (Rs1) that signals through the Gs/cAMP/PKA pathway. These mice displayed a massive and progressive increase in bone formation and bone mass (4). Likewise, mice lacking Gsα have impaired bone formation, resulting in marked bone fragility (5). Transgenic expression of Gi in osteoblasts using the *Col I* 2.3 kb promoter negatively affected bone formation (6). Lastly, transgenic mice expressing the Gαq subunit in osteoblasts, the major mediator of PTH-dependent phosphoinoside/PKC signaling, have osteopenia due to impaired osteoblast differentiation and reduced matrix formation (7). Taken together, these data suggest opposing roles of Gs vs. Gi or Gq signaling in osteoblasts. The knowledge obtained from these studies will help define new potential therapeutic targets for improved

treatment of metabolic bone diseases such as osteoporosis.

It is well established that canonical Wnt signaling increases osteoblast activity and bone formation. Wnts signal by blocking GSK3 activity and stabilizing β-catenin, thereby increasing osteoblast proliferation/differentiation and osteogenesis (8). However, much less is known about interactions between Wnt and other signaling pathways. Studies showed the Wnt pathway to be firmly intertwined with other important signals. Notably, Wnt3a stimulated phosphorylation of S6K in osteoblasts, one of the two major downstream targets of mTOR signaling. Furthermore, mTOR activity is markedly increased in Wnt10b transgenic mice. Interestingly, Wnt activated mTOR in a β-catenin-independent manner (9). The non-canonical Wnt signaling pathway component, Wnt5a, also increased osteoblast differentiation of human mesenchymal stem cells *in vitro* (10). PTH, a major regulator of calcium homeostasis and osteoblast activity, significantly increased levels of β-catenin protein and β-catenin-dependent transcriptional activity in cultured osteoblasts by recruiting LRP5/6, coreceptors of Wnt canonical signaling, to the PTH1R and stabilizing β-catenin. Furthermore, intermittent PTH rapidly increased levels of LRP5/6 phosphorylation and β-catenin protein *in vivo* (11). Osterix (Osx), a critical osteoblast differentiation factor, was shown to inhibit cell proliferation by antagonizing Wnt signaling. This was accomplished by induction of the Wnt inhibitor, Dkk1. *Osx*(-/-) calvarial osteoblasts failed to express Dkk1 while Osx activated *Dkk1* gene expression. Consistent with this finding, Osx inhibited β-catenin-dependent TOPFLASH reporter activity and β-catenin-induced secondary axis formation in Xenopus embryos, supporting the notion that Osx inhibits osteoblast proliferation by blocking Wnt signaling, thereby allowing differentiation to proceed (12).

Because type I diabetics exhibit defects in bone formation, it has long been suspected that insulin may have direct actions on bone. However, insulin can cross-react with the IGF-1 receptor, also active in bone, so it has not previously been possible to establish direct actions for insulin. A clever use of IGF-1 receptor (IGF-1R) and insulin receptor (IR)-deficient mice allowed investigators to clearly establish that insulin can stimulate osteoblast proliferation and differentiation in the absence of IGF-1 signaling (13). Effects of insulin, as in other systems, were mediated by Akt and GSK3β and resulted in up-regulation of Runx2. These workers also showed that mice lacking the IR in osteoblasts have decreased bone volume. A related study further explored the basis for Akt actions in osteoblasts. *Akt1*(-/-) mice have a low-turnover osteopenia associated with increased osteoblast apoptosis. Akt1 was shown to protect osteoblasts from apoptosis by stimulating phosphorylation of cytoplasmic FoxO3a, thereby blocking its nuclear translocation and up-regulation of Bim, a pro-apoptotic factor whose expression is largely dependent upon the active FoxO3a in the nucleus (14).

The central position of GSK3β as a kinase involved in both insulin/Akt and Wnt signaling was further emphasized by a presentation describing the results of GSK3β haploinsufficiency (15). These mice have greater bone mass and formation rates and their osteoblasts show accelerated rates of differentiation *in vitro* when compared with wild-type littermates. Although levels of the Runx2 transcription factor were not affected by GSK status, transcriptional activity was severely attenuated by GSK over-expression. This regulation may be the consequence of direct phosphorylation of Runx2 by GSK, in that mutation of 3 consensus GSK phosphorylation sites rendered Runx2 no longer sensitive to GSK3β inhibition.

You Gotta Carry That Load

Polycystin-1 (PC1) and connexin 43 (Cx43) have both been implicated in the response of osteoblasts/osteocytes to mechanical signals. PC1 is a component of the primary cilia thought to form a mechanosensing complex in bone, while Cx43 mediates the gap junctional communication linking

osteocytes to each other and to surface osteoblasts. Two presentations described the consequences of PC1 (16) and Cx43 (17) deficiency. PC1-deficient mice have osteopenia and decreased expression of the bone-related type II Runx2 isoform. Furthermore, a genetic link was established between Runx2 and PC1 by showing that double heterozygous *PC-1/Runx2* mice have a more severe osteopenia than was seen with haploinsufficiency of either gene. Furthermore, PC1 overexpression stimulated the promoter controlling type II Runx2 via a mechanism involving both PI3K and Akt. Disruption of gap junctional communication via osteoblast-specific deletion of Cx43 also caused an osteopenia that the authors suggest may be due to an inability of knockout animals to properly adapt to ambulatory loads. The canonical MAP kinase pathway is also a component of the response of osteoblasts to mechanical loads/matrix signals and transgenic manipulation of this pathway can alter bone development and osteoblast differentiation (18). This requirement for canonical MAPK signaling in osteoblast differentiation was confirmed by analysis of ERK1/2 inactivation in mesenchymal precursors using a *Prx1-Cre* (19). ERK-deficient mice showed delays in the formation of primary ossification centers in long bones while *Prx1*-driven MEK1 over-expression led to accelerated osteogenesis, synostoses of long bones and premature lambdoid suture closure.

I Can't Breathe

As one of the most metabolically active cells in the body, osteoblasts require an adequate blood supply whose formation precedes overt bone formation. This year, a number of studies emphasized the intimate relationship between angiogenesis and bone formation. The pericyte, a cell in intimate contact with the vasculature, has long been suspected of being an osteogenic progenitor (20). A smooth muscle actin-GFP mouse was used to track pericytes into osteoblast and adipocyte lineages *in vitro* and *in vivo*, thereby confirming that this cell type is an osteoprogenitor (21). The hypoxia inducible factor-1α transcriptional regulator (HIF-1α) induces angiogenesis genes under conditions of low oxygen tension. Several presentations established the important function of this factor in osteoblasts. HIF-1α-deficient osteoblasts expressed reduced levels of VEGF and osteoblast differentiation markers *in vitro*. Introduction of a HIF-1α mutation into osteoblasts *in vivo* reduced bone volume and bone formation parameters and increased osteoclast numbers, effects that were exacerbated by ovariectomy (22). Interestingly, stimulation of HIF-1α activity was also shown to stimulate bone regeneration in a distraction osteogenesis model (23).

Other Players

Calcium receptors (CaRs) play a key role in sensing circulating ionized calcium concentrations. It is now clear that they are also critical for bone development. Mice lacking the CaR in osteoblasts had smaller, undermineralized skeletons and, depending on the stage of osteoblast differentiation at which gene excision took place, either reduced or increased levels of osteoblast differentiation markers (24;25). ATF4, an osteoblast-enriched transcription factor required for normal differentiation and bone formation, was shown to mediate the anabolic actions of PTH in long bones and calvaria in studies using an ATF4-deficient mouse model. This study also demonstrated that ATF4 is a novel downstream target of PTH in osteoblasts; PTH increased *Atf4* gene expression and ATF4-dependent transcriptional activity through multiple signaling pathways mainly involving PKA and C (26). Ephrin B2, produced by osteoclast precursors, was recently shown to enhance osteoblast differentiation and bone formation via interactions with its osteoblastic receptor, EphB4 (27). Interestingly, mice lacking Ephrin B1 in osteoblasts displayed reduced peak bone mass. Conversely, overexpression of Ephrin B1 enhanced osteoblast proliferation *in vitro* (28). These results suggest that factors coupling osteoblast-osteoclast communication play important roles in bone formation, an underexplored area of bone biology. A number of new factors interacting with the Runx2 transcription factor were also identified. Among these are Nell-1, a

downstream Runx2 target that can partially compensate for Runx2 haploinsufficiency (29), Zfp521, a FosB interacting protein that antagonizes Runx2 transcriptional activity (30) and TGFβ Inducible Early Gene (TIEG) that directly stimulates expression of Runx2 and its downstream targets (31).

Conflict of Interest: None reported.

References

1. Dallas SL, Veno PA, Bonewald LF, Rowe DW, Kalajzic I. Dynamic imaging of fluorescently tagged osteoblast and osteocyte populations integrates mineralization dynamics with osteoblast to osteocyte transition. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S13. [Abstract]

2. Veno PA, Nicolella DP, Kalajzic I, Rowe DW, Bonewald LF, Dallas SL. Dynamic imaging in living calvaria reveals the motile properties of osteoblasts and osteocytes and suggests heterogeneity of osteoblasts in bone. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S13. [Abstract]

3. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet*. 2006 Nov;38(11):1310-5.

4. Hsiao EC, Boudignon B, Chang PW, Benscik M, Peng J, Manalac C, Halloran B, Conklin B, Nissenson R. A stone mouse: transgenic expression of an engineered Gs-coupled receptor in osteoblasts produces increased bone mass. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S6. [Abstract]

5. Wu JY, Rodda S, Sims NA, Poulton IJ, Chen M, Weinstein LS, McMahon AP, Kronenberg HM. Impaired bone formation in mice lacking the G protein subunit Gsα in early osteoblasts leads to marked bone fragility. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S7. [Abstract]

6. Millard SM, Peng J, Bencsik M, Storer A, Lu W, Wronski TJ, Conklin B, Nissenson RA. Designer G protein coupled receptors suggest opposing roles for Gs and Gi signaling in osteoblasts. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S7. [Abstract]

7. Ogata N, Shinoda Y, Yano F, Wettschureck N, Offermanns S, Segre GV, Nakamura K, Kawaguchi H. Gαq signaling in osteoblasts inhibits bone formation. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S36. [Abstract]

8. Hartmann C. A Wnt canon orchestrating osteoblastogenesis. *Trends Cell Biol*. 2006 Mar;16(3):151-8.

9. Ouyang H, Inoki K, Zhang X, Zhu T, Bennett C, Lindvall C, Williams BO, MacDougald OA, Guan K. mTOR signaling: A novel molecular mechanism underlying Wnt's anabolic effects on osteogenesis. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S32. [Abstract]

10. Spencer GJ, Genever PG. Evidence for lineage dependent non-canonical Wnt signal transduction by bone cells. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S40. [Abstract]

11. Wan M, Yang C, Yuan H, Wu X, Lu C, Chang C, Cao X. Parathyroid hormone activates β-catenin signaling through LRP5/6. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S83. [Abstract]

12. Zhang C, Lyons J, Sinha K, McCrea P, Zhou X, de Crombrugghe B. Inhibition of Wnt signaling by Osterix. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S70. [Abstract]

13. Fulzele K, Liu Z, Wan C, Zhang W, Bruening J, Clemens TL. Insulin promotes osteoblast differentiation independent from IGF-1 signaling and its receptor is required for normal post natal bone acquisition. *J Bone Miner*

Res. 2007 Sep;22(Suppl 1):S20. [Abstract]

14. Kawamura N, Kugimiya F, Kadowaki T, Nakamura K, Chung U, Kawaguchi H. Akt1 contributes to maintenance of bone mass and turnover by inhibiting apoptosis of osteoblasts through FoxO3a/Bim axis. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S60. [Abstract]

15. Hirata M, Kugimiya F, Kawamura N, Ohba S, Nakamura K, Kawaguchi H, Chung U. GSK-3β inhibits osteoblastic bone formation through suppression of Runx2 transcriptional activity by the phosphorylation at a specific site. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S69. [Abstract]

16. Xiao ZS, Zhang SQ, Magenheimer BS, Calvet JP, Quarles LD. Polycystin-1 selective activation of Runx2-II isoform transcription is mediated through the calcium-PI3K-Akt pathway. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S41. [Abstract]

17. Zhang Y, Taylor AF, Paul EM, Davison A, Bronson SK, Donahue HJ. Inadequate bone adaptation in bone cell specific connexin43 deficient mice. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S80. [Abstract]

18. Ge C, Xiao G, Jiang D, Franceschi RT. Critical role of the extracellular signal-regulated kinase-MAPK pathway in osteoblast differentiation and skeletal development. *J Cell Biol*. 2007 Feb 26;176(5):709-18.

19. Matsushita T, Landreth G, Murakami S. ERK1 and ERK2 are essential for osteoblast differentiation. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S41. [Abstract]

20. Towler DA. Vascular biology and bone formation: hints from HIF. *J Clin Invest*. 2007 Jun;117(6):1477-80.

21. Kalajzic I, Wang L, Jiang X, Lamothe K, Aguila LH, Rowe DW. Pericytes derived from adipose tissue; characterization and evaluation of their osteogenic potential. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S61. [Abstract]

22. Deng L, Liu X, Wan C, Wang J, Qi J, Luo T, Wang J, Zhou Q, Zhu Y, Wei L, Clemens TL. Disruption of hypoxia inducible factor-1α in osteoblasts exacerbates ovariectomy induced bone loss in mice. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S5. [Abstract]

23. Wan C, Gilbert SR, Wang Y, Cao X, Shen X, Ramaswamy G, Jacobsen KA, Alaql ZS, Gerstenfeld LC, Einhorn TA, Eberhardt AW, Deng L, Clemens TL. Genetic and pharmacologic activation of the hypoxia inducible factor pathway promotes angiogenesis and accelerates bone regeneration. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S19. [Abstract]

24. Dvorak MM, Tu C, Elalieh H, Chen T, Liu B, Kream BE, Bikle DD, Chang W, Shoback DM. Conditional ablation of the osteoblast calcium-sensing receptor causes abnormalities in skeletal development and mineralization. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S86.

25. Chang W, Tu C, Chen T, Liu B, Elalieh H, Dvorak M, Clemens T, Kream B, Halloran B, Bikle D, Shoback D. Conditional knockouts in early and mature osteoblasts reveal a critical role for Ca^{2+} receptors in bone development. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S79. [Abstract]

26. Yu S, Luo M, Franceschi RT, Jiang D, Zhang J, Patrene K, Hankenson KD, Roodman GD, Xiao G. ATF4 Is required for the anabolic actions of PTH on bone in vivo. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S3. [Abstract]

27. Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T, Suda T, Matsuo K. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab*. 2006 Aug;4(2):111-21.

28. Xing W, Govoni K, Kapoor A, Mohan S. Targeted disruption of ephrin B1 in osteoblasts reduces bone size in mice. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S32. [Abstract]

29. Zhang X, Ting K, Cowan CM, Lee H, Wong J, Hsu T, Pathmanathan D, Kuroda S, Soo C. Nell-1, a key functional mediator of Runx2, partially rescues craniofacial defects in Runx2 haploinsufficient mice. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S31. [Abstract]

30. Hesse E, Wu M, Rowe GC, Neff L, Horne WC, Baron R. Zfp521, a D2D FosB-interacting protein, is a novel inhibitor of Runx2 activity with opposite effects on osteoblasts and bone formation in vitro and in vivo. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S70. [Abstract]

31. Hawse JR, Subramaniam M, Monroe DG, Lian JB, Stein GS, van Wijnen AJ, Oursler MJ, Spelsberg TC. TIEG: a novel mediator of Runx2 expression and activity in osteoblasts. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S41. [Abstract]