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MEETING REPORTS

Osteocytes Play to Standing Room Only: Meeting Report from the 30th Annual Meeting of the American Society for Bone and Mineral Research

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Sarah L. Dallas and Lynda F. Bonewald

University of Missouri at Kansas City, Kansas City, Missouri, USA

The osteocyte session at the 2008 ASBMR meeting opened to standing room only with dozens of meeting attendees turned away. One of the ground-breaking papers presented at this session, emphasizing the importance of β-catenin in osteocytes, received the ASBMR award for the most outstanding abstract (1), underscoring the increased interest in the osteocyte as an important regulatory cell in the skeleton.

The Osteocyte as a Regulator of Bone Mass and Calcium Metabolism Through the Actions of PTH

A concept emerging from last year's meeting is that the osteocyte is a key regulator of bone mass and may be a major target for the anabolic actions of PTH in the skeleton. Several abstracts at this year's meeting built upon this exciting theme. Deletion of the β-catenin gene in the osteocyte lineage using Cre recombinase driven by the dentin matrix protein-1 (Dmp1) promoter resulted in bone loss as high as 60-75% (1). This "moth-eaten" bone phenotype was more severe in females than males. Though the pups appear normal at birth, premature lethality occurs at 2-3 months of age, most likely due to bone fragility due to increased osteoclast activity. As β-catenin is a cell viability factor, these studies support previous observations that osteocyte cell death signals osteoclast activation (2). Targeted deletion of myocyte enhancer 2 (*Mef2*), a controller of *Sost* expression in osteocytes, resulted in increased bone mass, greater in male than in female mice (3). As *Sost*/sclerostin is an osteocyte-selective negative regulator of Wnt/β-catenin signaling, these studies suggest that this pathway may play an important role in osteocytes to regulate gender differences in bone mass.

Several abstracts provided new insight into the role of the osteocyte in regulation of PTH anabolic responses. Mice in which a constitutively active PTH receptor 1 was expressed in osteocytes using the Dmp1 promoter showed a two-fold increase in bone, accompanied by increased osteoblast and osteoclast number (4). This was associated with decreased sclerostin levels in osteocytes and upregulation of Wnt/β-catenin signaling. However, crossing these mice with Lrp5-null mice only partially attenuated the bone phenotype, suggesting involvement of another pathway independent of Lrp5/Wnt/*Sost*. In studies taking a loss-of-function approach, mice with an osteocyte-specific deletion of the PTH/PTHrP receptor were shown to have a low bone mass phenotype with delayed secondary ossification and a lack of downregulation of sclerostin in response to PTH (5). PTH-induced bone gain was shown to be blunted but not abolished in mice overexpressing sclerostin (6). PTH effects were also blunted in *Sost*-null mice, suggesting that other pathway(s) exist to mediate the anabolic actions of PTH. Together, these studies mechanistically link the PTH and Wnt signaling pathways and suggest that the osteocyte is a key regulator of bone mass and may be a major target for PTH anabolic actions. There is a remarkable degree of convergence of findings from different laboratories using complimentary approaches, leading to a paradigm shift with regard to the role of the PTH/Wnt signaling pathway in bone.

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Role of Osteocytes in Calcium and Phosphate Metabolism

Recent studies have suggested that the osteocyte may be a key regulator of phosphate metabolism and potentially of calcium homeostasis. Several papers at this year's meeting provided new insight into this emerging area. Inducible deletion of the PTH/PTHrP receptor in postnatal or young adult mice using a DMP1-CreERT2 promoter resulted in smaller, hypocalcemic mice, suggesting that the PTH receptor in osteocytes plays a role in calcium homeostasis (5). Another study showed that osteocyte lacunae become enlarged during lactation, when PTHrP is elevated (7). This is accompanied by induction of TRAP expression in osteocytes. After weaning, lacunar size and TRAP expression returned to normal. These data suggest that osteocytes can modify their lacunae by removing and replacing their perilacunar matrix. One function of the lacuno-canalicular network may therefore be to provide a large surface area that can be used to mobilize calcium from the skeleton to regulate calcium homeostasis. These findings support theories, proposed by Talmage decades ago, that the osteocyte-lining cell complex is the source of calcium in response to PTH (8). Conversely, osteocytes may lose their capacity to remodel with age, as perilacunar hypermineralization occurs around osteocytes in aged ovariectomized female rats, also potentially compromising mechanotransduction (9).

Deletion of Dmp1 or Phex results in hypophosphatemic rickets, characterized by elevated levels of the phosphaturic factor, FGF23, in osteocytes (10). One study showed that Phex deletion leads to reduced production of 7B2, a helper protein for subtilisin-like proprotein convertase (SPC) (11). The SPC/7B2 complex appears to be important in cleavage of Dmp1 into N- and C-terminal fragments that normally inhibit FGF23 expression. Therefore this study positions Phex → SPC/7B2→Dmp1→FGF23 in a common pathway and further supports the notion that the osteocyte functions as an endocrine regulator of phosphate homeostasis. The effects of Dmp1 fragments on FGF23 expression in osteocytes appear to be mediated indirectly, as Dmp1 did not directly regulate FGF23 promoter activity (12). Interestingly, suppression of FGF23 expression was observed in osteocytes in neonatal Dmp1-null bones that were transplanted into Dmp1-null adults, suggesting a compensatory mechanism developing with age (12).

Mechanisms for Mechanosensation and Transduction in Osteocytes

Osteocytes are widely viewed as the cells that sense and control responses to mechanical strain in the skeleton. It was elegantly shown at this year's meeting that osteocytes are the first to respond to mechanical loading with an increase in β-catenin signaling by 1 hour and that it is not until 24 hours later that the signal propagates to surface osteoblasts (13). Reciprocal downregulation of sclerostin and Dkk1, two inhibitors of Lrp5-mediated Wnt signaling, was observed by 24 hours after loading. All markers returned to normal within 48 hours. Some osteocytes activated β-catenin signaling in response to loading, even though they were expressing sclerostin and Dkk1, suggesting a Wnt/Lrp5-independent mechanism. A likely mechanism for this is through prostaglandin-mediated activation of β-catenin, through actions on GSK3β and Akt (14).

Mechanical perturbation of α5β1 integrins may provide the mechanism for opening of connexin-43 hemichannels in osteocytes, leading to prostaglandin release in response to loading (15). The primary cilium may also play a role in osteocyte mechanosensation (16). Gene silencing of either Polaris, a protein required for cilia formation, or the adenyl cyclase isoform, AC6, in MLO-Y4 osteocyte-like cells prevented the rapid decrease in cAMP that occurs in response to fluid flow shear stress. AC6 localizes to primary cilia and the inhibition of cAMP may be mediated via calcium binding to AC6. As evidence accumulates that osteocytes are the mechanoresponsive cells in bone and the molecular mechanisms are further unraveled, this will have important

implications for potential treatments to maintain and increase bone mass.

Identification of Osteocyte-Specific Genes to Study Osteocyte Differentiation

Gene expression profiling studies identified myosin-related genes, the transcription factors myogenin, Mef2c and Myf5, as well as contractile-related and cytoskeletal/cell motility-related proteins as being higher in osteocytes compared to osteoblasts (17;18). Some of these gene products may play a role in contractility and dynamic motions of osteocytes. The receptor-like protein tyrosine phosphatase-m (RPTPm), expressed in cells that form networks, was shown in bone to be exclusively expressed in osteocytes (19). Deletion of RPTPm resulted in low bone mass, supporting a role for the osteocyte network in maintenance of bone mass.

Several abstracts addressed osteoblast to osteocyte differentiation. A new transgenic mouse line has been generated expressing multiple fluorescent reporters, engineered using a bacterial artificial chromosome (BAC) (20). The mouse line expresses GFP driven by the bone sialoprotein-1 promoter, mCherry (red) driven by the Dmp1 promoter and ECFP (blue) driven by the TRAP promoter. This will be a valuable new tool to address questions concerning differentiation of osteoblasts into osteocytes. In the meantime, live cell imaging of primary osteoblasts from mice expressing DsRed driven by the type I collagen promoter and GFP driven by the Dmp1 promoter showed that mineralization was associated exclusively with clusters of cells expressing both GFP and the osteocyte marker, E11/gp38 (21). A wave of GFP expression preceded mineralization and during mineralization the GFP-positive cells change from polygonal, motile cells to stationary, dendritic cells. The data suggest that the cell responsible for mineral deposition is already transitioning towards being an osteocyte.

Perspective

It is an exciting time for osteocytes, with more and more discoveries showing that these cells can no longer be viewed as the inactive, “placeholder” cells of bone. Now that sophisticated tools are available for manipulating gene expression in these cells, osteocytes are emerging as the “control freaks” of the skeleton. They appear to be major regulators of bone mass by integrating hormonal and mechanical loading signals. One can hardly wait to see what new and exciting discoveries concerning osteocyte function will be presented at the 2009 meeting.

Conflict of Interest: The authors report receiving grant support from Procter & Gamble and holding patents on MLO-Y4 and MLO-A5 cell lines.

Peer Review: This article has been peer-reviewed.

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