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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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OSTEOCYTES EMERGING FROM OBSCURITY

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Of the three major bone cell types, osteocytes have remained the most elusive and least understood due to their location within the mineralized bone matrix. At this year's ASBMR meeting, the osteocyte truly emerged from its obscurity, with a number of research abstracts and symposium presentations highlighting the key role these cells play in diverse skeletal functions, such as the regulation of bone formation, mechanosensation, glucocorticoid-induced bone loss, and phosphate homeostasis. It was exciting to see that the 2007 ASBMR meeting included for the first time a concurrent oral session devoted to osteocyte biology. The main themes emerging from the osteocyte-related abstracts are summarized below.

Sclerostin – A Potential Approach for the Treatment of Osteoporosis

SOST, and its protein product sclerostin, is highly expressed in osteocytes (1) and acts as an inhibitor of Lrp5-Wnt-β-catenin signaling (2-4). One of the hottest topics at this year's meeting was the use of antibodies to sclerostin to increase bone formation and prevent bone loss. Treatment with sclerostin antibodies increased markers of bone formation in postmenopausal women and increased bone formation in ovariectomized, aged female or male rats (5-8). This approach looks promising as an anabolic treatment for osteoporosis, which could be particularly useful for the treatment of patients in whom significant bone loss has already occurred. As sclerostin regulates Wnt-β-catenin signaling, which is important in mechanosensation, an advantage of targeting sclerostin therapeutically is that the new bone formed will presumably be laid down in mechanically appropriate locations. However, this remains to be confirmed.

Further insight into the molecular mechanism by which sclerostin inhibits Lrp5-Wnt-β-catenin signaling came from a study showing that mutations in the β-propeller 1 region of Lrp5, including the G171V high bone mass mutation, render Lrp5 resistant to sclerostin binding (9). Mutations in β-propeller 2, 3 or 4 regions had no effect. This identifies β-propeller 1 as the critical region for sclerostin binding. This study further showed that sclerostin inhibits signaling by Wnt1 and Wnt10b but not Wnt3a.

Interactions of PTH with sclerostin may explain, in part, some of the ability of intermittent PTH to enhance bone formation. PTH suppresses *SOST* expression in UMR-106 cells and in adult bone *in vivo*. This was confirmed in a study where primary osteocytes from transgenic mice with targeted GFP expression in osteocytes were used (10). Another study further elucidated the mechanism for inhibition of *SOST* expression by PTH in UMR-106 cells (11). This appears to be mediated via down-regulation of MEF2 transcription factors, which are essential for the activity of the *SOST* enhancer. Together, these studies provide additional potential targets for therapeutics to prevent bone loss.

Mechanosensation in Osteocytes – The Role of β-Catenin and Hemichannels

Osteocytes are widely viewed as the main cell type responsible for sensing and coordinating adaptive responses to mechanical loading. This was elegantly demonstrated in studies showing that osteocytes are much more sensitive to mechanical loading compared to osteoblasts (12;13). These investigations revealed that, following *in vivo* loading, osteocytes are the first cells to respond with a rapid increase in β-catenin signaling. This appears to be mediated via crosstalk of prostaglandin signaling with the β-catenin pathway through GSK phosphorylation. The study authors further proposed that this initial prostaglandin-mediated activation of β-catenin in osteocytes is followed by an amplification of β-catenin signaling, mediated by up-regulation of Wnt/Lrp5 and down-regulation of inhibitors of Lrp5 signaling, such as sclerostin. The authors proposed a unifying model for load-related bone formation that integrates prostaglandin signaling with the Wnt-β-catenin pathway and provides an intriguing molecular explanation for strain signal amplification.

Studies also provided new insight into the mechanism of prostaglandin release in response to shear stress, which appears to occur through connexin 43 hemichannels (14;15). These investigations demonstrated that shear stress causes intracellular assembly of Cx43 channels. Antibodies targeted to hemichannels (not gap junctions) had no effect on ATP release from P2X7 channels but inhibited PGE_2 release. In contrast, an inhibitor of the P2X7 channel blocked ATP, but not PGE_2 release in response to shear stress. The opening of hemichannels may be mediated by α5 integrins. This provides an intriguing new function for these integrin subunits in osteocytes, which will be very exciting, if confirmed.

Glucocorticoid-Induced Bone Fragility and Osteocyte Apoptosis – Role of Hemichannels and β-catenin

The importance of osteocyte apoptosis in glucocorticoid-induced bone fragility continues to receive support from research presented at this year's meeting. One interesting study used a bisphosphonate analog, IG9402, which has no effect on osteoclasts but protects osteoblasts and osteocytes from apoptosis (16). Treatment with this reagent maintained bone strength in glucocorticoid-treated mice, without inhibiting resorption, suggesting that preservation of osteocyte/osteoblast viability is an important mechanism for the beneficial effects of bisphosphonates on bone. Investigators from the study had proposed previously that bisphosphonates promote osteocyte viability by interacting with hemichannels. Here they showed that IG9402 had no effect on glucocorticoid-induced bone fragility in mice with targeted deletion of connexin 43 in osteoblasts/osteocytes, supporting a role for Cx43 hemichannels in the protective effects of bisphosphonates.

Mechanical loading has a protective effect on glucocorticoid-induced osteocyte apoptosis and one study proposed a potential molecular mechanism (17). This work revealed that the protective effects of loading on dexamethasone-induced apoptosis of the osteocyte-like cell line MLO-Y4 occur through the rapid production of prostaglandins. This signal enhances cell viability both through the classical cAMP/PKC pathway as well as through crosstalk with the β-catenin pathway via phosphorylation of GSKα and β. Agents that preserve osteocyte viability may therefore be good targets for therapeutics to preserve bone strength.

Osteocytes as Regulators of Phosphate and Calcium Homeostasis

The key role that osteocytes play in the regulation of phosphate and potentially calcium homeostasis was highlighted this year in a number of oral presentations, as well as in a symposium entitled "Osteocytes and the Regulation of Phosphate Homeostasis." Several genes that play key roles in phosphate homeostasis, including *PHEX*, *Dmp1* and *FGF23*, are highly expressed in osteocytes. Two studies reported novel mutations in *PHEX* associated with X-linked hypophosphatemic rickets (18;19).

The 2006 ASMBR meeting saw the first reports that mutations in *Dmp1* are associated with autosomal recessive hypophosphatemic rickets. Follow-up work presented at this year's meeting showed that the MIV (A1G) Dmp1 mutant protein that lacks the signal sequence fails to be secreted (20). The 1484-1490del mutant, which lacks the C-terminal 18 amino acids and contains 33 novel amino acids, is secreted more rapidly than wild type Dmp1, but is non-functional. The importance of the C-terminus of Dmp1 was elegantly demonstrated in a study in which a 57kDa C-terminal fragment of Dmp1 was re-expressed in *Dmp1*-null mice (21). This fragment rescued the skeletal abnormalities and hypophosphatemia just as efficiently as the full length Dmp1 protein and restored circulating FGF23 levels to normal.

In another study, the 10kb *DMP1* promoter was used to drive a tamoxifen-inducible Cre transgene to selectively and inducibly delete the PTH/PTHrP receptor in osteocytes (22). Inducible expression was confirmed using Rosa26 for newborns and Z/AP for adults. When tamoxifen was administered at 6 weeks of age, low calcium, increased PTH and an improper homeostatic response of serum calcium and phosphate were observed, especially with a low calcium diet. These effects were not observed when tamoxifen was administered at 12 weeks.

Overall, osteocytes are emerging as major regulators of phosphate metabolism and appear to be the main source of elevated serum FGF23 in various types of osteomalacia, suggesting that they may function as an endocrine organ. Similar to phosphate homeostasis, the osteocyte network may also function as an endocrine gland to regulate calcium homeostasis but through other unique mechanisms. It will be exciting to follow developments in this field at future ASBMR meetings.

Dynamic Properties of Osteocytes

The first study in which live osteocytes were imaged within their lacunae was reported at the 2006 ASBMR meeting. Two abstracts presented this year extended these observations to show the dynamic properties of both osteocytes and osteoblasts (23;24). These investigators used transgenic mice expressing GFP targeted to osteocytes via the 8kb-*Dmp1* promoter and/or expressing DsRed targeted to osteoblasts via the 3.6kb *col1a1* promoter. Time-lapse imaging of calvarial explants from these mice showed that both osteoblasts on the bone surface and osteocytes within their lacunae are more motile than previously thought and showed that dendritic connections between adjacent osteocytes and between osteocytes and cells on the bone surface may be transient. Imaging of double transgenic mice showed the heterogeneity of cells on the bone surface and identified a Dmp1-GFP-positive, E11-positive surface motile cell that may represent an osteocyte precursor. Dynamic imaging of mineralization in primary bone cell cultures isolated from these transgenic mice integrated mineralization with the transition from osteoblast to osteocyte and suggested that the embedding Dmp1-positive cells are responsible for mineralization. After viewing these movies showing the dynamic nature of cells in bone, we may never view static histological sections in the same way again.

Conclusions

The recent explosion in research on osteocytes has been fueled by the availability of new research tools, such as cell lines, reporters targeted to osteocytes, and targeted and timed deletion of genes in osteocytes *in vivo*. With the exciting research that is emerging in this field, it is clear that we can no longer ignore the osteocyte and that it fully deserves to share the limelight with the osteoblast and osteoclast.

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