IBMS BoneKEy. 2010 February;7(2):88-92
http://www.bonekey-ibms.org/cgi/content/full/ibmske;7/2/88
doi: 10.1138/20100431

MEETING REPORTS

Unraveling Osteocyte Signaling Networks: Meeting Report from the 31st Annual Meeting of the American Society for Bone and Mineral Research

September 11-15, 2009 in Denver, Colorado

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Previous ASBMR meetings in 2007 and 2008 have heralded the emergence of the osteocyte as a major controller of bone cell function that integrates hormonal, mechanical and growth factor signals to regulate bone mass. Building on this groundbreaking work, ASBMR 2009 was the year in which many of the major signaling pathways in osteocytes that control these important functions began to be identified and crosstalk between these pathways was discovered. Much of this was accomplished through targeted expression or deletion of genes in osteocytes using the 10- or 8-kb dentin matrix protein 1 promoter.

PTH Signaling in Osteocytes and Crosstalk with Other Signaling Pathways

Several key presentations at this year's meeting further defined the role of the osteocyte in the regulation of skeletal responses to PTH. Mice with an osteocyte-targeted deletion of the PTH/PTHrP receptor failed to show a bone anabolic response to intermittent PTH treatment (1). The PTH/PTHrP receptor is a G-protein coupled receptor and new research shows that osteocyte-specific deletion of $G_s\alpha$ resulted in osteopenia and increased osteocyte density (2), confirming a key role for osteocyte G-protein coupled receptor signaling in the regulation of bone mass.

PTH signaling in osteocytes may crosstalk with other signaling pathways, such as the Wnt/β-catenin signaling pathway. One study showed that mice expressing a constitutively active PTH/PTHrP receptor targeted to osteocytes displayed a dramatic increase in bone cortical thickness due to increased bone formation and intracortical remodeling (3). However, crossing these mice with mice null for the Wnt co-receptor, low density lipoprotein receptor-related protein 5 (Lrp5), rescued the bone formation phenotype, suggesting interplay between these pathways. Interestingly, the abnormalities in remodeling were not rescued, suggesting that although PTHR1 activation in osteocytes has effects on both bone formation and bone resorption, they are controlled by distinct mechanisms.

PTH also crosstalks with the Wnt/β-catenin signaling pathway by altering expression of the *SOST* gene and its protein product, sclerostin, an inhibitor of Wnt signaling that binds to the Wnt co-receptors Lrp5 and Lrp6. The central role of the osteocyte in the regulation of bone mass via Wnt/β-catenin signaling was highlighted in several presentations at this year's meeting. Using an affinity purification/proteomics approach, Lrp4 was identified as a novel sclerostin-interacting partner that is expressed in osteoblasts and osteocytes (4). Knockdown of Lrp4 enhances osteoblast differentiation and blocks sclerostin inhibitory action on mineralization *in vitro*. These exciting findings suggest a novel role for Lrp4 in bone. It was previously known that *SOST*-deficient mice display increased bone mass and Lrp5-deficient mice show low bone mass. New research shows that in double transgenics in which both genes are deleted there is a full or partial rescue of the high and low bone mass phenotypes seen in the single knockout animals, confirming the interdependence of the pathways (5). Interestingly, osteocyte-specific deletion of the *SOST* gene failed to rescue the severe

osteopenia observed in mice with an osteocyte-targeted deletion of β-catenin (6). This suggests a key role for β-catenin-dependent Wnt signaling in osteocytes in the regulation of bone mass that is downstream of sclerostin. These studies are of high significance because sclerostin remains a key target for the development of new therapeutics aimed at preventing bone loss in osteoporosis.

Research at this year's meeting also showed that PTH is a powerful regulator of FGF23 expression in osteocytes (7), demonstrating the interplay between these pathways in the control of phosphate homeostasis. Circulating FGF23 was elevated in mice expressing a constitutively active PTH/PTHrP receptor in osteocytes. Treatment with PTH or PTHrP also stimulated FGF23 expression in osteocytes, suggesting that inhibition of phosphate reabsorption by PTH is due to its actions not only on the kidney, but also on osteocytes to increase production of circulating FGF23.

Taken together, the new findings highlighted above underscore the key role of the osteocyte in regulating bone mass and controlling bone anabolic responses to PTH and emphasize that diverse signaling pathways are not only active in osteocytes but also crosstalk with one another. These findings also highlight the important function of osteocytes as an endocrine gland in the regulation of calcium and phosphate homeostasis.

Signaling Pathways Mediating Mechanotransduction in Osteocytes

The signaling pathways that control skeletal responses to mechanical loading have been somewhat of a holy grail for researchers in the field over the past few decades. The 2009 ASBMR meeting was no exception in that there were many abstracts addressing this theme. The opening and closing of connexin 43 (Cx43) hemichannels has been proposed as a mechanism by which osteocytes release signaling factors, such as prostaglandins, in response to mechanical loading. Exciting new research at this year's meeting suggests that AKT and MAPK phosphorylation may reciprocally regulate the opening and closing of Cx43 hemichannels in response to shear stress (8). The role of Cx43 in osteocyte function was further explored in studies showing that conditional deletion of Cx43 in late osteoblasts and osteocytes results in osteopenia and that Cx43 is differentially required for maintaining osteocyte viability in cortical but not cancellous bone (9;10). Interestingly, the osteopenic phenotype in Cx43 conditional knockout mice appears to be due to altered osteoclast activity rather than to decreased bone formation.

Crosstalk between signaling pathways in response to loading was also a prominent theme at this year's meeting. One study in osteocyte-like cells showed that in response to fluid flow shear stress, the PGE_2 signaling pathway crosstalks with β-catenin signaling via PI3 kinase/AKT (11). This may be a mechanism by which PGE_2, released in response to loading, can activate β-catenin signaling to promote new bone formation. In contrast, load-related activation of β-catenin signaling appears to be independent of ERK1/2 activation (12). Recently it has been shown that PGE_2 activation of PI3K/Akt is independent of PKA activation, whereas both PI3K/Akt and PKA signaling activate β-catenin binding to the Cx43 promoter, stimulating its expression (13). New research now shows that osteocyte-targeted expression of constitutively active PKA results in increased bone volume, suggesting that PKA activation in osteocytes plays a key role in bone formation (14). Another intriguing study showed that the inflammatory cytokines TNFα and Il-1β can attenuate the responses of osteocytes to mechanical loading (15), again suggesting that there is crosstalk between multiple signaling pathways in osteocytes.

Polycystin 1, encoded by the *Pkd1* gene, is a component of the mechanosensing complex in primary cilia. Echoing findings from the field of nephrology, which have established the primary cilium as playing a key role in mechanosensing in the kidney, new work at this year's ASBMR meeting showed that osteocyte-targeted deletion of *Pkd1* results in a dramatic decrease in bone formation in response to anabolic loading (16). This was associated with reduced Wnt

signaling, underscoring the important role of the osteocyte in controlling bone mass in response to loading and suggesting crosstalk between signaling pathways.

Signaling Pathways in Dying or Stressed Osteocytes

Much attention has been focused previously on osteocyte cell death as both necrosis and apoptosis lead to activation of osteoclastic bone resorption. Death signaling pathways are activated in osteocytes during apoptosis that induce signals of bone resorption (17;18). At this year's meeting autophagy was shown to be a major means by which osteocytes respond to glucocorticoid stress to maintain their viability (19). Autophagosomes and expression of LC3II was shown in MLO-Y4 cells and primary osteocytes, respectively, in response to glucocorticoid treatment and the mTOR signaling pathway appeared to be responsible. It will be important to determine if autophagic osteocytes send signals to other bone cells.

Osteoblast to Osteocyte Transition

The mechanisms controlling the transition from osteoblast to osteocyte are still not fully understood and new research at ASBMR 2009 raised provocative questions about this process that warrant further investigation (20). In this study, MLO-Y4 and MLO-A5 osteocyte-like cells were grown in 3D culture in collagen gels, with osteoblasts layered on top to mimic their *in vivo* organization. The presence of osteocytes in the gel prevented invasion of osteoblasts into the gel. In contrast, osteoblasts penetrated into gels that did not contain osteocytes. These observations raise the intriguing possibility that, rather than embedded osteocytes sending a positive signal to induce certain osteoblasts to embed, the embedding process might actually be regulated by a negative signal from the osteocytes that prevents osteoblasts from embedding. This could potentially explain the staggered spacing of osteocytes within bone matrix, as osteoblasts would theoretically only embed at positions where the inhibitory signal is weakest (*i.e.*, in the spaces between osteocytes). It will be interesting to see how this research develops and/or whether the inhibitory signals can be identified.

Conclusions

In summary, far from being the quiescent cell as it is often referred to in textbooks, the osteocyte is a highly active cell type that regulates functions as diverse as mechanosensation, phosphate homeostasis, and skeletal responses to hormonal signals. The molecular signaling pathways that regulate these diverse functions of the osteocytes are beginning to be defined and the complex patterns of crosstalk between these different signaling pathways revealed. These may hold promise for the development of novel therapeutics for the treatment of metabolic bone diseases.

Conflict of Interest: Dr. Bonewald reports holding patents on the MLO-Y4 and MLO-A5 cell lines. Dr. Dallas: none reported.

Peer Review: This article has been peer-reviewed.

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