IBMS BoneKEy. 2009 November;6(11):455-458
http://www.bonekey-ibms.org/cgi/content/full/ibmske;6/11/455
doi: 10.1138/20090413

MEETING REPORTS

The Actors in Phosphate Metabolism and Bone Mineral Homeostasis: Meeting Report from the 31st Annual Meeting of the American Society for Bone and Mineral Research

September 11-15, 2009 in Denver, Colorado

Aline Martin and L. Darryl Quarles

Kansas University Medical Center, Kansas City, Kansas, USA

The skeleton is a mineral reservoir that plays a key metabolic role in the maintenance of systemic phosphorus/calcium homeostasis as well as an endocrine organ. Coordination of mineral homeostasis relies on the coupled mechanisms of absorption by the intestine, bone remodeling and kidney excretion that are traditionally viewed from the perspective of the PTH/vitamin D axis. We are beginning to recognize the importance of a novel hormonal axis involving FGF23, which is produced by osteocytes in bone. FGF23 targets the kidney to inhibit renal phosphate reabsorption and serves as a counter-regulatory hormone for $1,25(OH)_2D$. There is growing evidence to suggest that FGF23 functions to coordinate bone mineralization with renal phosphate handling through pathways involving Phex, a transmembrane endopeptidase and Dmp1, an extracellular matrix SIBLING protein. However, many aspects of FGF23 local and systemic regulation and functions remain to be elucidated. There were several reports at the 31st ASBMR Annual Meeting that advance our understanding of this bone-kidney axis regulating vitamin D and phosphate metabolism.

Mutant Phex in *Hyp* mice (murine homologous for X-linked hypophosphatemic rickets) induces an intrinsic bone mineralization defect associated with the increased production of FGF23 by bone and hypophosphatemia. Interestingly, blocking $1,25(OH)_2D$ inactivation in *Hyp* mice by ablation of Cyp24 improves skeletal growth and endochondral bone formation (1). However, the mechanisms underlying the increased production of FGF23 when Phex is mutated remain to be defined. Phex expression in bone is critical, since the osteoblast-specific deletion of Phex is sufficient to induce a *Hyp*-like phenotype (2). The SIBLINGs-derived ASARM peptides, together with hydroxyapatite crystals, were shown to cause Phex to undergo autolytic cleavage and thus generate an extracellular 60 kDa Phex isoform (3). It is proposed that this new 60 kDa Phex isoform may participate in the mineralization of extracellular matrix in calcified biomineralization foci. Other findings expand the skeletal abnormalities associated with inactivation of Phex to include abnormalities in the Wnt-β-catenin pathway. Indeed, it was shown that activation of the PI3-kinase/Akt pathway in Phex mutant *Hyp* osteoblasts leads to increased β-catenin activation (4). However, conflicting data regarding the role of sclerostin (Sost) in *Hyp* were reported. Bone and osteoblasts derived from *Hyp* mice displayed decreased Sost expression (4), whereas other work showed increased Sost in *Oc-Cre*-mediated conditional deletion of Phex and suggested a role of Sost in the intrinsic mineralization defect in *Hyp* bone (2).

Production of FGF23 by osteocytes is regulated by both local (*e.g.*, Phex and Dmp1) as well as by systemic (*e.g.*, $1,25(OH)_2D$) factors. Studies confirmed that FGF23 is not regulated by extracellular Pi in osteocytes (5). There is controversy, however, regarding the role of FGF23 in regulating parathyroid gland function and the role of PTH produced by the parathyroid gland in regulating FGF23 production. It is well established that FGF23 and PTH serum levels are positively correlated in several

hyperparathyroid disorders, but PTH has not been shown to directly stimulate FGF23 promoter activity in osteoblasts *in vitro*. New data were presented showing that activation of PTHR1 in osteocytes *in vivo* and *in vitro* results in an increased expression of FGF23 (6). Since PTH effects might be mediated through a cAMP-dependent mechanism, these findings are consistent with earlier reports of increased FGF23 in patients with McCune Albright's syndrome caused by activating mutations in *GNAS1*. On the other hand, other investigations found that reduction in bone turnover by administration of OPG or alendronate to mice is accompanied by increased FGF23 levels (7). In these studies, PTH administration decreased FGF23 in association with increased bone turnover. Interestingly, the activation in osteocytes of PTHR1 and elevations of FGF23 expression (6) are also associated with periosteal bone formation through activation of Wnt signaling and suppression of Sost (8). A similar concomitant increase in FGF23 expression, activation of the Wnt-β-catenin pathway and decreased expression of Sost is also observed in Phex-deficient hypomineralized cortical bone (4). Finally, the complete deletion of PTH in *Fgf23*(-/-) mice, which already display low PTH levels, partially restores their bone phenotype (9). These unexpected results, however, might be explained by the reduction in elevated $1,25(OH)_2D$ levels after deletion of PTH in *Fgf23*(-/-) mice. Thus, the role of PTH in regulating FGF23 is uncertain, and may reflect both indirect effects on $1,25(OH)_2D$ or other pathways as well as differences in bone remodeling derived from acute and chronic administration of PTH.

Circulating levels of biologically active full-length FGF23 are also regulated by proteolytic processing by a furin-like convertase and the glycosyl transferase, GALNT3. Loss of GALNT3, which is seen in the disease familial tumoral calcinosis, results in only inactive C-terminal FGF23 in the circulation, and indicates that O-glycosylation may be important in preventing FGF23 degradation by furin (10). In fact, the O-glycosylation level of FGF23 can also be modulated by cAMP which inhibits GALNT3 (11).

The end-organ effects of FGF23 are restricted due to the limited expression of the transmembrane co-receptor αKlotho, which determines its specificity for the FGF receptor (FGFR). FGF23 induces phosphate wasting by down-regulating Napi2a cotransporters in the proximal tubule of the kidney, whereas αKlotho is predominately expressed in the renal distal tubules, suggesting the possibility of a paracrine cross-talk between the distal and proximal tubules. Klotho also exists as a circulating form. The presence of a soluble secreted Klotho that can impart an FGF23 effect on FGFR-expressing tissues is discordant with the apparent tissue-restricted function of FGF23. For example, bone that does not express Klotho has been proposed to be a target for FGF23. Whether physiological concentrations of soluble Klotho might permit FGF23 to affect bone is unknown. *In vitro* studies of cultured osteoblasts supplemented with FGF23 and αKlotho reveal a dose-dependent response of increased proliferation and lower mineralization (12). Besides, soluble Klotho permitted activation of the canonical Wnt pathway following FGF23 signaling *in vitro* (13).

In the presence of normal renal function, three Na-dependent Pi co-transporters, Npt2a, Npt2b and Npt2c, are involved in the control of systemic phosphate balance. The creation of Npt2b knockout mice revealed the importance of Npt2b in the intestinal absorption of Pi (14). The loss of this transporter resulted in an unchanged phosphatemia with increased fecal Pi excretion combined with reduced urinary Pi, elevation of $1,25(OH)_2D3$ and lower FGF23 serum levels. These changes were not associated with any skeletal disorders. On the other hand, Npt2a and Npt2c are implicated in the regulation of phosphate reabsorption in the kidney. Different FGFR signaling pathways might be activated in response to FGF23 to result in internalization of Napi2a/Napi2c from the renal brush border membrane. Possibilities include the MAPK, AKT, PKC, PLC-γ and Src pathways (15). It has been shown that FGF23 injection in mice induces pERK1/2 activation in the distal tubule but not in the proximal tubule shortly after injection and

that Napi2a downregulation occurs in the proximal tubule only 20 to 50 minutes after pERK1/2 activation (16). This suggests again the presence of a factor communicating between the distal and the proximal tubules to inhibit phosphate reabsorption following FGF23 signaling. On the other hand, the existing idea that FGFR1c is the primary target for FGF23 was challenged by studies showing that upon stimulation with FGF23, FGFR3, but not FGFR1, was activated in proximal tubular cells (15). Additional work is needed to clarify the tubular segments, molecular targets, and function of FGF23 in the kidney.

Conflict of Interest: Dr. Quarles reports that he is a consultant and on the Speakers' Bureau for Amgen; a consultant for Cytochroma and Novartis; and an advisory board member for Shire Pharmaceuticals. Dr. Martin: none reported.

Peer Review: This article has been peer-reviewed.

References

1. Xiao S, Bai X, Qiu D, Goltzman D, Karaplis A. Deletion of 25-hydroxyvitamin D-24-hydroxylase gene restores normal skeletal growth in Hyp mice. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

2. Yuan B, Meudt J, Zhang R, Xing Y, Feng J, Drezner M. Increased SOST production: a factor underlying abnormal bone mineralization in X-linked hypophosphatemia. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

3. Gorski J, Huffman N, Liu S, Quarles L, Rowe P. Exposure to hydroxyapatite crystals induces Phex endoprotease to undergo autolytic processing. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

4. Ren J, Xiao Z, Martin A, Quarles D. Coordinated increase in FGF23 and Wnt/β-catenin signaling in Hyp mice. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

5. Miyagawa K, Ozono K, Tachikawa K, Mikuni-Takagaki Y, Michigami T. Significance of osteocyte as a regulator of phosphate metabolism. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

6. Rhee Y, Farrow E, Lee R, Bivi N, Lezcano V, Plotkin L, White K, Bellido T. FGF23 gene expression is upregulated by PTH receptor activation in osteocytes in vitro and in vivo: a parathyroid-bone link influencing the endocrine function of osteocytes. *J Bone Miner Res*. 2009; 24(Suppl 1). [Abstract]

7. Samadfam R, Richard C, Nguyen-Yamamoto L, Bolivar I, Goltzman D. Bone formation regulates circulating concentrations of FGF23 and involves the action of DMP1. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

8. Rhee Y, Allen M, Condon K, Plotkin L, Lezcano V, Vyas K, O'Brien C, Burr D, Bellido T. PTH receptor signaling in osteocytes governs periosteal bone formation and intra-cortical remodeling: divergent role of Sost and the Wnt pathway. *J Bone Miner Res*. 2009; 24(Suppl 1). [Abstract]

9. Yuan Q, Sitara D, Rowald K, Densmore M, Erben R, Lanske B. Deletion of PTH rescues phenotype of Fgf23 null mice. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

10. Masi L, Beltrami G, Gozzini A, Ottanelli S, Amedei A, Falchetti A, Casentini C, Fossi C, Brandi M. A case of tumoral calcinosis-hyperphosphatemia due to a novel mutation in GALNT3 gene. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

11. Bhattacharyya N, Andreopoulou P, Dumitrescu C, Miwa H, Gafni R, Collins M. Cyclic AMP-mediated processing of FGF23: Regulation by O-glycosylation. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

12. Shalhoub V, Ward S, Stevens J, Renshaw-Gegg L, Hawkins N, Richards W. Elevated levels of fibroblast growth factor 23 (FGF23) and alpha-klotho stimulate MC3T3.E1 cell proliferation and inhibit mineralization. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

13. Ohata Y, Yamazaki M, Okada T, Ozono K, Michigami T. Soluble form of Klotho

functions as a humoral modulator in FGF23- and canonical Wnt-induced signaling. *J Bone Miner Res*. 2009; 24(Suppl 1). [Abstract]

14. Sabbagh Y, O'Brien S, Song W, Boulanger J, Schiavi S. Intestinal Npt2b transporter is an active component of systemic phosphate regulation in mice. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

15. Khan M, Jan De Beur S. Fibroblast growth factor 23 (FGF23) with klotho differentially activates FGFR signaling to regulate sodium phosphate co-transporter trafficking. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

16. Farrow E, Summers L, Schiavi S, White K. Temporal refinement of the renal FGF23 signaling axis. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]