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MEETING REPORTS

Phosphate Metabolism and Its Regulators: Meeting Report from the 30th Annual Meeting of the American Society for Bone and Mineral Research

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The discovery of FGF23 has unified the pathogenesis of phosphate disorders: FGF23 is a phosphatonin which increases renal phosphate excretion; high FGF23 levels cause nearly all chronic hypophosphatemic disorders and low FGF23 levels cause hyperphosphatemia and tumoral calcinosis.

The paradigm of heritable hypophosphatemic rickets is human X-linked hypophosphatemia or its mouse equivalent, the *Hyp* mouse. These disorders are caused by mutations that inactivate a protease known as Phex, but how does the Phex mutation increase expression of the FGF23 gene and thereby cause renal phosphate wasting? The answer may lie in a proteolytic cascade: Phex somehow increases expression of a chaperone called 7B2 which is required for activity of the subtilisin-like protease PC-2. This protease cleaves proBMP-1 (which is also a protease) to active BMP-1, which in turn cleaves the matrix protein DMP-1 to fragments which, in an unspecified manner, inhibit expression of FGF23 (1). The model ties the *Hyp* mutation in Phex to DMP-1 mutations which are also associated with hypophosphatemic rickets and has the additional advantage of making several testable predictions. DMP-1 is an osteocyte protein, but osteoblasts could also contribute to osteomalacia or hypophosphatemia. Deletion of Phex specifically from osteocytes reproduces the hypophosphatemia of the *Hyp* phenotype but with much milder osteomalacia (2). This suggests that osteocytes regulate phosphate excretion via FGF23, but local effects of osteoblast Phex such as production of ASARM peptides as "minhibins" may contribute importantly to osteomalacia.

The production of FGF23 is regulated by concerted effects of glycosylation and proteolysis. O-glycosylation mediated by Galnt3 largely protects FGF23 from cleavage by subtilisin-like proteases, and impairment of glycosylation shunts FGF23 into a degradative pathway, preventing secretion of the biologically active molecule and thus producing hyperphosphatemia and tumoral calcinosis. A mouse model of the Galnt3 mutation features hyperphosphatemia but not tumoral calcinosis (3). Galnt3 is co-expressed with FGF23 in tumors associated with hypophosphatemic rickets (4) and multiple O-glycosylation sites play a role in processing, as well as in the biological activity of the secreted full-length molecule (5).

The osteocyte is not only the site of FGF23 production but also a master regulator of bone turnover and the response to mechanical loading. Unloading the hindlimbs of *DMP-1*(-/-) mice by tail suspension increased serum phosphate levels (6); much more work remains to unravel the possible relationship between mechanical load and phosphate metabolism. Further evidence that DMP-1 null mice have a local defect in production of FGF23 by osteoblasts/osteocytes comes from transplantation experiments in which FGF23 expression is determined by the genotype of the bone cells rather than the environment (7).

A cross-sectional study of the differential diagnosis of hypophosphatemic patients concluded that a serum FGF23 level >30 ng/ml was consistent with FGF23-induced hypophosphatemia (8). FGF23 can be rapidly detected in tumor extracts for diagnosis of tumor-induced osteomalacia (9). Serum FGF23 levels rise with treatment of vitamin D deficiency (10); $1,25(OH)_2D$ levels in treated patients were not measured but could explain this increase, but no relationship between $1,25(OH)_2D$ and FGF23 levels was observed in another study (11). Both PTH and FGF23 levels are correlated with hypophosphatemia early after renal transplant (12). Hypophosphatemia in another acute setting, after liver transplantation, was correlated with increased renal phosphate excretion and with high PTH levels, but the FGF23 level was suppressed (13).

FGF23 action requires the membrane protein Klotho as a cofactor with FGF receptors. As predicted from this model, introducing the Klotho null mutation into *Hyp* mice gives an FGF23-negative phenotype (14). The cFos gene is downstream of FGF23/Klotho (15). High local concentrations of stanniocalcin-2 are associated with renal calcifications in Klotho mice (16). Membrane expression of Klotho is markedly diminished in parathyroid adenomas (17).

Phosphate itself is an important cellular messenger. Phosphate is required for terminal differentiation and subsequent apoptosis of growth plate chondrocytes. Culture of day 15.5 mouse metatarsals recapitulates many of these findings, demonstrating a role of extracellular phosphate in growth, mineralization and caspase-dependent apoptosis of limb bud chondrocytes (18). A decrease in mitochondrial membrane potential is associated with caspase-dependent apoptosis in hypertrophic chondrocytes in the RCJ3.1C5.18 model (19). Phosphate induced apoptosis in resting zone chondrocytes via nitric oxide/JNK signaling (20). Extracellular phosphate can signal in the same pathway as FGF23 in HEK293 cells transfected with Klotho (21).

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References

1. Yuan B, Meudt J, Feng JQ, Drezner MK. 7B2 protein mediated inhibition of DMP1 cleavage in osteoblasts enhances FGF-23 production in *hyp*-mice. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S16. [Abstract]

2. Yuan B, Feng JQ, Xing Y, Meudt J, Rowe PS, Drezner MK. Aberrant *Phex* function in osteocytes has limited effects on bone mineralization in X-linked hypophosphatemia (XLH). *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S16. [Abstract]

3. Ichikawa S, Sorenson AH, Fritz TA, Moh A, Mackenzie DS, Hui SL, Econs MJ. Ablation of the Galnt3 gene in mice leads to low circulating Fgf23 concentrations and hyperphosphatemia despite increased Fgf23 gene expression. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S6. [Abstract]

4. Gafni RI, Bhattacharyya N, Brahim JS, Dumitrescu CE, Theman TA, Kelly MH, Molinolo AA, Collins MT. Evidence supporting the necessity of UDP-N-acetyl-alpha-D-galactosamine poly-peptide N-acetylgalactosaminyl-transferase 3 (GalNac-T3) in the processing of fibroblast growth factor 23 (FGF23) in humans. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S173. [Abstract]

5. Suzuki H, Ito N, Fukumoto S, Fujita T. Significance of O-linked glycosylation of FGF23 protein. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S174. [Abstract]

6. Ma D, Yu S, Zhang R, Yuan B, Xie Y, Drezner M, Dallas S, Bonewald L, Feng J. Mechanical unloading partially rescues hypophosphatemic rickets in *Dmp1*-null mice. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S46. [Abstract]

7. Liu S, Zhou J, Tang W, Quarles LD. Local osteocyte defect in *Dmp1* null mice causes overproduction of Fgf23. *J*

Bone Miner Res. 2008 Sep;23(Suppl 1):S63. [Abstract]

8. Endo I, Fukumoto S, Ozono K, Namba N, Tanaka H, Inoue D, Minagawa M, Sugimoto T, Yamauchi M, Michigami T, Matsumoto T. Clinical usefulness of measurement of fibroblast growth factor 23 (FGF23) in hypophosphatemic patients-proposal of diagnostic criteria using FGF23 measurement. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S222. [Abstract]

9. Mannstadt M, Lorente C, Jüppner H. Rapid detection of intact FGF-23 in tumor tissue from patients with oncogenic osteomalacia. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S173. [Abstract]

10. Burnett-Bowie SM, Leder BZ, Henao MP, Mendoza NT, Finkelstein JS. The effect of treating vitamin D deficiency on FGF23 levels in humans. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S174. [Abstract]

11. Lips P, Dijkstra JE, van Schoor N, Lomecky M, Chel V, Vervloet M, Dijstelbloem H. Fibroblast growth factor 23 (FGF-23) in vitamin D deficient older persons. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S173. [Abstract]

12. Trombetti A, Richert L, Hadaya K, Graf JD, Martin PY, Rizzoli R. FGF-23 and post-transplant hypophosphatemia: evidence for a causative link. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S282. [Abstract]

13. Nafidi O, Lapointe RW, Lepage R, Brossard JH, Kumar R, D'Amour P. Phosphatonins and liver resection-related hypophosphatemia. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S284. [Abstract]

14. Brownstein C, Gundberg C, Lifton R, Carpenter T. Increased bone volume and correction of HYP mouse hypophosphatemia in the Klotho/HYP Mouse. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S6. [Abstract]

15. Farrow E, Summers L, Davis S, Schiavi S, White K. c-Fos is associated with renal FGF23-mediated signaling: effect of the Hyp mutation. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S283. [Abstract]

16. Takei Y, Yamamoto H, Masuda M, Fukaya M, Sato T, Taketani Y, Takeda E. High expression of the calcium/phosphate-regulating hormone stanniocalcin 2 is associated with renal calcification in the Klotho mutant mice. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S173. [Abstract]

17. Krajisnik T, Bjorklund P, Akerstrom G, Westin G, Larsson T. A possible pathogenic role of aberrant Klotho expression in primary and secondary hyperparathyroidism. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S177. [Abstract]

18. Zalutskaya AA, Demay MB. Phosphate regulates chondrocyte differentiation, proliferation and apoptosis in a model of embryonic endochondral bone formation. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S272. [Abstract]

19. Miedlich SU, Demay MB. Regulation of hypertrophic chondrocyte apoptosis. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S412. [Abstract]

20. Zhong M, Schwartz Z, Boyan BD. Phosphate induced apoptosis in growth plate chondrocytes via a nitric oxide and JNK-dependent pathway. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S156. [Abstract]

21. Yamazaki M, Okada T, Tachikawa K, Ozono K, Michigami T. Signaling of extracellular inorganic phosphate mediated via sodium-phosphate co-transporters influences FGF23 signaling in renal tubular cells. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S283. [Abstract]