

## MEETING REPORTS

### Phosphate Metabolism: Meeting Report from the 32nd Annual Meeting of the American Society for Bone and Mineral Research

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Seiji Fukumoto

University of Tokyo Hospital, Tokyo, Japan

At the 2010 ASBMR Annual Meeting, many important papers concerning phosphate metabolism were presented. Since the discovery of fibroblast growth factor 23 (FGF23) about 10 years ago, the FGF23-Klotho pathway has been shown to play essential physiological and pathophysiological roles in phosphate metabolism. New findings concerning the production and actions of FGF23, and Klotho, were presented at this year's meeting too.

#### Regulation of FGF23 Production

FGF23 is produced by bone and decreases serum phosphate and 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] levels by binding to a Klotho-FGF receptor (FGFR) complex in the kidney (1). While 1,25(OH)<sub>2</sub>D and a high phosphate diet have been shown to increase circulating FGF23, the precise regulatory mechanisms of FGF23 production remain to be clarified. Using a rat osteoblast-like cell line, UMR106, and mouse calvarial cells, FGF23 production was shown to be enhanced through activation of FGFR1 (2). Recently, it had been shown that transgenic mice overexpressing nuclear high molecular weight isoforms of FGF2 in osteoblasts exhibit hypophosphatemia and high FGF23 (3). Overexpression of high molecular weight isoforms of FGF2 in ROS17/2.8 cells was now shown to enhance FGF23 promoter activity, and CREB was involved in this enhanced FGF23 production (4). These results suggest that FGF23 production is regulated by local as well as systemic factors. Further studies are necessary to clarify the relationship between these systemic and local factors.

#### Mechanism of Action of FGF23

FGF23 was shown to activate Erk1/2 and the MAP kinase inhibitor PD98059 abolished the inhibitory effect of FGF23 on the expression of *Cyp27b1* that encodes a protein responsible for the production of 1,25(OH)<sub>2</sub>D *in vitro* (5). However, it was unclear whether all *in vivo* actions of FGF23 are mediated by MAP kinase. The *Hyp* mouse is a model of the human disease X-linked hypophosphatemic rickets/osteomalacia (XLH), the most common cause of vitamin D-resistant rickets/osteomalacia. The gene responsible for *Hyp* and XLH is phosphate-regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*). Overexpression of FGF23 in bone is believed to underline the pathogenesis of *Hyp* and XLH. The expression of early growth response 1 (*egr-1*), a downstream transcription factor of MAP kinase, was shown to be increased in *Hyp* mice. In addition, the MAP kinase inhibitor PD0325901 increased serum phosphate and 1,25(OH)<sub>2</sub>D levels and enhanced the expression of *Cyp27b1* and type 2a sodium-phosphate cotransporter (*Npt2a*) in *Hyp* mice (6). These results indicate that most actions of FGF23 on phosphate and vitamin D metabolism are mediated by the MAP kinase pathway, at least in the setting of excess actions of FGF23.

#### Treatment of FGF23-Related Hypophosphatemic Diseases

Several kinds of hypophosphatemic rickets/osteomalacia including XLH have been shown to be associated with high FGF23 levels and considered to be caused by excess FGF23 activity. Therefore, the inhibition of FGF23 activity may be useful for

treating these diseases (7). Another approach would be the inhibition of FGF23 production. 7B2 is a helper protein for subtilisin-like proprotein convertase 2 (SPC2). It was shown that the production of 7B2 was reduced in *Hyp* mice, and decreased 7B2/SPC2 activity not only inhibited the degradation of FGF23 but also enhanced FGF23 production through the inhibition of proteolysis of dentin matrix protein 1 (DMP1) (8;9). These results suggest that the enhancement of 7B2/SPC2 activity might be useful for correcting phenotypes of *Hyp*. In fact, treatment with hexa-D-arginine, a stimulator of 7B2/SPC2 activity, for 5 weeks resulted in increased phosphate and decreased FGF23 levels, enhanced *Npt2a* expression and corrected impaired mineralization by histological examination (10). Therefore, 7B2/SPC2 appears as another drug target for hypophosphatemic diseases.

While targeted deletion of *PHEX* in osteoblasts using the *osteocalcin* promoter causes the typical *Hyp* phenotype, deletion of *PHEX* in osteocytes using the *DMP1* promoter results in less severe osteomalacia despite comparable hypophosphatemia and high FGF23 in these mice (11). These results suggest that *PHEX* in osteoblasts has some other role than regulating FGF23 production. It was found that *SOST* expression was enhanced in *Hyp* mice and in mice with targeted deletion of *PHEX* in osteoblasts, but not in mice without *PHEX* in osteocytes. In addition, sclerostin was shown to inhibit mineralization *in vitro* (11). Therefore, it is possible that *PHEX* in osteoblasts regulates mineralization through its suppressive effect on *SOST* expression.

Part of the FGF23 protein is proteolytically cleaved and only full-length FGF23 has biological activity to reduce serum phosphate. A C-terminal fragment of FGF23 was recently shown to impair the action of full-length FGF23 (12). This inhibition of FGF23 action by a C-terminal fragment was suggested to play a role in the impaired action of FGF23 in patients with tumoral calcinosis in whom the processing of FGF23 was enhanced (13). In patients with XLH, calcitonin was shown to reduce FGF23 to 77% of baseline levels by 4 hours and

increase serum phosphate and 1,25(OH)<sub>2</sub>D while FGF23 did not change in control subjects (14).

Thus, there appear to be several candidate molecules for the treatment of FGF23-related hypophosphatemic diseases. 7B2/SPC2 and calcitonin were shown to reduce FGF23 levels. In addition, anti-FGF23 antibodies, a C-terminal fragment of FGF23 and the inhibitor of MAP kinase (6) seem to inhibit FGF23 actions. Furthermore, the modulation of sclerostin activity may influence mineralization. Additional studies are necessary to examine whether these approaches can be applied to humans.

### **Klotho and PTH**

The *Klotho* mouse was created by a transgenic method and the expression of *Klotho* was severely reduced. The *Klotho* mouse was reported to be a model of early senescence and exhibits several phenotypes resembling aging (15). *Klotho* and FGF23-null mice show similar phenotypes including hyperphosphatemia, high 1,25(OH)<sub>2</sub>D and low PTH (16). Deletion of *PTH* from *Klotho* mice (17) or *Klotho* knockout mice (18) produced similar results. Phenotypes of *Klotho* or *Klotho* knockout mice, including impaired growth, short life-span, hypercalcemia, high 1,25(OH)<sub>2</sub>D and high FGF23, were corrected by deletion of the *PTH* gene. In contrast, deletion of the *PTH* gene produced subtle difference in the phenotypes of *Klotho* mice and *Klotho* knockout mice. While *Klotho* knockout mice without *PTH* still showed ectopic calcification, this phenotype was not observed in *Klotho* mice with *PTH* deletion. Overall, the results indicate that low PTH in *Klotho* or *Klotho* knockout mice still has FGF23-independent roles.

### **New Actions of FGF23**

*Klotho* is expressed in several restricted tissues including the kidney and parathyroid glands. This limited distribution of *Klotho* is considered to determine the tissue-specific actions of FGF23. Several tumor cell lines that cause osteoblastic metastases were shown to express *Klotho* (19). In addition,

FGF23 enhanced the expression of *egr-1* and *Cyp24A1* in MCF7 human breast cancer cells. *Cyp24A1* encodes an enzyme that degrades 1,25(OH)<sub>2</sub>D. Furthermore, FGF23 impaired the inhibitory effect of 1,25(OH)<sub>2</sub>D on cell proliferation (19). Therefore, it is possible that FGF23 acts on tumor cells in bone and modulates tumor progression. Klotho was also shown to be expressed in syncytiotrophoblasts in the placenta suggesting a role of FGF23 in the regulation of mineral homeostasis in the fetus (20).

### Conclusion

The discovery of FGF23 has expanded our knowledge of phosphate metabolism. In the past 10 years, the physiological actions of FGF23 and the consequences of aberrant FGF23 function have been delineated. The focus of research on phosphate metabolism now seems to be shifting to the modulatory methods of FGF23 action, the interaction of FGF23 with other humoral factors, and new targets of FGF23. It is hoped that this research activity will result in better management of disorders of phosphate metabolism and additional clinical benefit for patients in the future.

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