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PERSPECTIVES

FGF23: Phosphate Metabolism and Beyond

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Abstract

FGF23 was identified as a humoral factor involved in the development of several hypophosphatemic diseases about 10 years ago. Since then, it has been shown that FGF23 is produced by bone and works as a hormone to reduce phosphate and 1,25-dihydroxyvitamin D levels by binding to a Klotho-FGF receptor complex. Therefore, deficient actions of FGF23 result in a disease with hyperphosphatemia and high 1,25-dihydroxyvitamin D. In addition, FGF23 is one of the key players in the development of chronic kidney disease-mineral and bone disorder (CKD-MBD). Recent studies also suggest that high circulatory FGF23 is associated with an increased risk of cardiovascular events and mortality. However, there still remain many unanswered questions about FGF23. This *Perspective* summarizes both the current state of knowledge of FGF23, as well as gaps in our understanding. *IBMS BoneKEy*. 2010 August;7(8):268-278.

Keywords: Hypophosphatemia; hyperphosphatemia; Klotho; chronic kidney disease

Introduction

Bone contains about 99% of the body's calcium (Ca) and 85% of the body's phosphate, and these ions are in dynamic equilibrium with those present in extracellular fluid. In the case of calcium, two calciotropic hormones, parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] increase serum calcium levels by enhancing osteoclastic bone resorption, renal calcium reabsorption and intestinal Ca absorption. PTH also works to reduce serum phosphate levels by inhibiting proximal tubular phosphate reabsorption and 1,25$(OH)_2$D increases serum phosphate by enhancing intestinal phosphate absorption. However, because changes in serum Ca rapidly modify PTH levels through the Ca-sensing receptor on parathyroid cells and PTH regulates circulatory 1,25$(OH)_2$D, these calciotropic hormones have been considered to regulate primarily serum Ca levels. On the other hand, there are several diseases, as shown below, with abnormal phosphate metabolism without alterations in serum Ca levels. In addition, serum phosphate levels are maintained within a certain range in healthy people, and both hyperphosphatemia and hypophosphatemia cause ectopic calcification and rickets/osteomalacia, respectively. These results have suggested that there is a regulatory mechanism of serum phosphate other than that of PTH and 1,25$(OH)_2$D.

The Identification and Biological Function of FGF23

FGF23 was cloned in 2000 by homology to FGF15 in mice and also as a gene responsible for autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR) by positional cloning (1;2). Furthermore, FGF23 was identified as a humoral factor responsible for tumor-induced rickets/osteomalacia (TIO) (3). Patients with ADHR and TIO show similar clinical features; both are characterized by impaired proximal tubular phosphate reabsorption. In addition, hypophosphatemia usually enhances the production of 1,25$(OH)_2$D and increases 1,25$(OH)_2$D levels. However, 1,25$(OH)_2$D remains low to low-normal in patients with ADHR and TIO, indicating that both phosphate reabsorption

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and vitamin D metabolism are deranged in these patients. The similar clinical features had suggested that these diseases are caused by the same mechanism.

The FGF23 gene encodes a protein with 251 amino acids (1;3). The N-terminal 24 amino acids consist of a signal peptide and secreted FGF23 is thought to consist of 227 amino acids. The biological activity of FGF23 has been investigated using recombinant FGF23 with 227 amino acids. When recombinant FGF23 was injected into mice, FGF23 reduced both serum phosphate and $1,25(OH)_2D$ (4). Serum phosphate during a chronic state is believed to be determined mainly by renal handling of phosphate. More than 90% of phosphate filtered from glomeruli is reabsorbed in renal tubules and most of this phosphate reabsorption occurs in proximal tubules. Type 2a and 2c sodium-dependent phosphate co-transporters (NaPi-2a, 2c) mediate this proximal tubular phosphate reabsorption. The reduction of serum phosphate by FGF23 was associated with decreased expression of NaPi-2a and NaPi-2c (4). In addition, $1,25(OH)_2D$ is produced from 25-hydroxyvitamin D [25(OH)D] by 25(OH)D-1α-hydroxylase. 25(OH)D can also be converted to 24,25-dihydroxyvitamin D by 25(OH)D-24-hydroxylase. The same 24-hydroxylase hydroxylates $1,25(OH)_2D$ to 1,24,25-trihydroxyvitamin D with less activity. Therefore, 25(OH)D-1α-hydroxylase increases circulatory $1,25(OH)_2D$ levels while 24-hydroxylase works to reduce $1,25(OH)_2D$. FGF23 has also been shown to reduce serum $1,25(OH)_2D$ both by inhibiting the expression of 1α-hydroxylase and also by enhancing the expression of 24-hydroxylase in the kidney (4). Because $1,25(OH)_2D$ enhances intestinal phosphate absorption, FGF23 reduces serum phosphate by inhibiting proximal tubular phosphate reabsorption and intestinal phosphate absorption (Fig. 1).

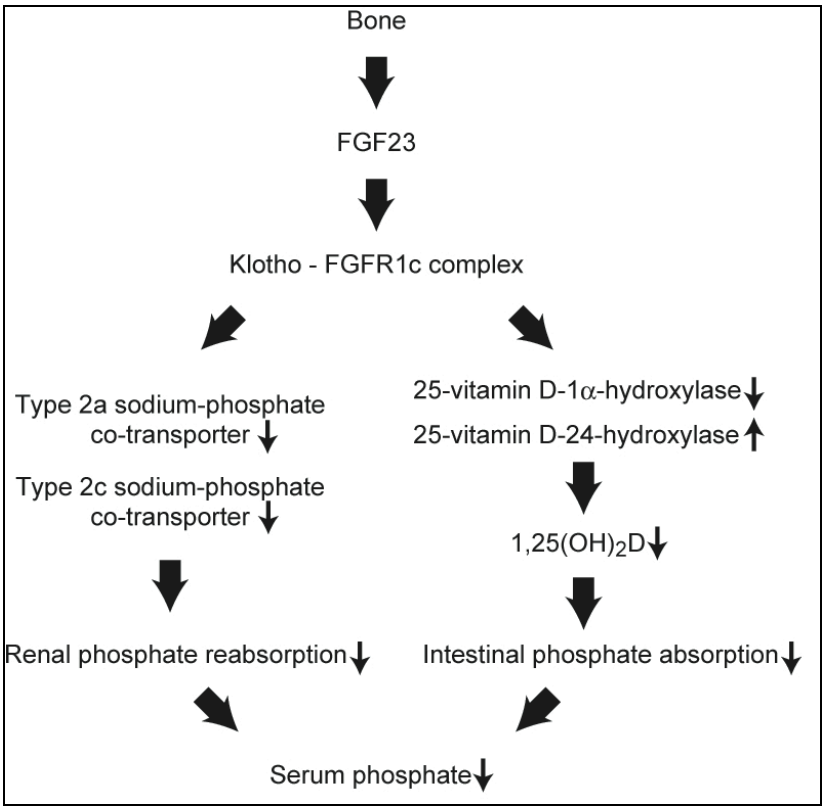

Fig. 1. The actions of FGF23. FGF23 is produced by bone and acts by binding to a Klotho-FGF receptor 1c (FGFR1c) complex. FGF23 reduces serum phosphate by inhibiting proximal tubular phosphate reabsorption and intestinal phosphate absorption through lowering 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] levels.

FGF23 as a Pathological Humoral Factor in Hypophosphatemic Diseases

A part of the FGF23 protein is proteolytically cleaved between 179Arg and 180Ser by enzymes that recognize a 176Arg–X–X–179Arg motif (5). While uncleaved FGF23 has biological activities as shown above, processed N-terminal and C-terminal fragments do not (6). Mutations found in patients with ADHR change either 176Arg or 179Arg, thus destroying the Arg–X–X-Arg motif (1). Actually, the mutant FGF23 protein in patients with ADHR was shown to be resistant to the processing, suggesting that these mutations cause excess FGF23 activity by increasing full-length FGF23 (6;7). This hypothesis was tested by measuring circulatory full-length FGF23 and the results indicated that FGF23 was high when patients were hypophosphatemic (8). However, hypophosphatemia disappeared in one patient during her course of the disease and FGF23 was not high when she was normophosphatemic (8). These results indicate that mutations in the FGF23 gene that destroy the 176R–X–X–179R motif do not always result in high FGF23, thus suggesting that these mutations somehow cause abnormal regulation of FGF23 production. Still, hypophosphatemic rickets/osteomalacia in patients with ADHR seems to be caused by excess actions of FGF23.

TIO is a paraneoplastic syndrome usually caused by phosphaturic mesenchymal tumor, mixed connective tissue variant (PMTMCT) (9). Tumors causing TIO were shown to abundantly express FGF23 while the mechanism of this overproduction remains to be clarified (3;10). In addition to ADHR and TIO, several hypophosphatemic diseases have been shown to be associated with high FGF23 levels. The *Hyp* mouse is a model of X-linked dominant hypophosphatemic rickets (XLH) (11). FGF23 was shown to be overexpressed in bone from *Hyp* mice (12). Similarly, the overexpression of FGF23 in bone was also observed in *DMP1* knockout mice, the model of autosomal recessive hypophosphatemic rickets 1 (ARHR1) (13). In addition, the expression of FGF23 in bone, including regions affected by fibrous dysplasia, was reported in patients with McCune-Albright syndrome (14). Furthermore, hypophosphatemic disease caused by intravenous administration of iron polymaltose was shown to be associated with high FGF23 (15-17). In one report, treatment with octreotide and excision of the nevus were followed by reduction and normalization of FGF23 in a patient with linear nevus sebaceous syndrome (18). While the precise sources of FGF23 have not been reported in several diseases, these results suggest that bone is the principal organ that produces excess FGF23 in these hypophosphatemic diseases other than TIO and possibly linear nevus sebaceous syndrome.

Recent identification of *ENPP1* as a gene responsible for ARHR2 was a surprise (19;20). *ENPP1* works to produce pyrophosphate that has a strong inhibitory activity on mineralization. Therefore, inactivating mutations in *ENPP1* have been reported in patients with generalized arterial calcification of infancy (GACI), sometimes a fatal disease in infancy (21;22). Some of the patients who survived GACI were reported to develop hypophosphatemia with reduced renal tubular phosphate reabsorption during childhood (23). In addition, recent papers reported patients with FGF23-related hypophosphatemic rickets without features of GACI in patients with *ENPP1* mutations. It is currently unknown how mutations in *ENPP1* cause high FGF23 levels and why the mutations in the same gene cause clearly different, somewhat opposing phenotypes. While FGF23 levels in patients with GACI have not been reported, it is possible that reduced pyrophosphate or enhanced calcification increases FGF23 production, and patients with less severe phenotypes of GACI develop hypophosphatemic rickets after surviving the critical period of infancy. Table 1 lists the diseases caused by aberrant function of FGF23, along with the responsible genes.

FGF23 as a Physiological Humoral Factor

FGF23 knockout mice show hyperphosphatemia with enhanced renal

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Table 1. Diseases caused by aberrant function of FGF23

Hypophosphatemic diseases	Responsible gene
X-linked dominant hypophosphatemic rickets (XLH)	*PHEX*
Autosomal dominant hypophosphatemic rickets (ADHR)	*FGF23*
Autosomal recessive hypophosphatemic rickets 1 (ARHR1)	*DMP1*
Autosomal recessive hypophosphatemic rickets 2 (ARHR2)	*ENPP1*
McCune-Albright syndrome/fibrous dysplasia	*GNAS1*
Linear nevus sebaceous syndrome	
Tumor-induced rickets/osteomalacia (TIO)	
Hypophosphatemic disease caused by iron polymaltose	
Hyperphosphatemic diseases	
Familial hyperphosphatemic tumoral calcinosis	*GALNT3, FGF23, Klotho*

PHEX: phosphate-regulating gene with homologies to endopeptidases on the X chromosome
FGF23: fibroblast growth factor 23
DMP1: dentin matrix protein 1
ENPP1: ectonucleotide pyrophosphatase/phosphodiesterase 1
GNAS1: guanine nucleotide-binding protein, alpha-stimulating activity polypeptide 1
GALNT3: UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3

tubular phosphate reabsorption and high $1,25(OH)_2D$ levels, indicating that FGF23 is working physiologically to reduce serum phosphate and $1,25(OH)_2D$ levels, at least in mice (24;25). FGF23 is produced by bone and works in the kidney, indicating that FGF23 is a systemic factor and there is a specific receptor for FGF23 in the kidney. *Klotho* was originally identified as a gene whose expression is severely reduced in a transgenic mouse model mimicking senescence (26). Klotho was also found to be a binding protein to FGF23 in the kidney (27). Subsequent studies indicated that FGF23 binds to the Klotho-FGF receptor complex (27;28). While there are several reports indicating that different FGF receptor subtypes can be involved in FGF23 signaling (27;28), mainly the FGF receptor 1c seems to be involved *in vivo* (29) (Fig. 1).

Familial hyperphosphatemic tumoral calcinosis is a rare inherited disease characterized by hyperphosphatemia and high $1,25(OH)_2D$ (30). These features are identical to the phenotypes of FGF23 knockout mice and just mirror images of those in patients with hypophosphatemic diseases caused by excess actions of FGF23, suggesting that this disease is caused by impaired actions of FGF23. Currently, three genes, *GALNT3*, *FGF23* and *Klotho* are known to be responsible for familial hyperphosphatemic tumoral calcinosis (31-35) (Table 1). The *GALNT3* gene product mediates the O-glycosylation of 178Thr and thereby prevents the processing of FGF23 protein (36;37). In patients with homozygous mutations in *GALNT3*, this O-linked glycosylation seems to be impaired and FGF23 protein is susceptible to the processing as Western blotting of the plasma of affected patients showed increased amounts of processed fragments and decreased full-length FGF23 (36). Similarly, Western blotting of mutant

FGF23 protein found in patients with hyperphosphatemic tumoral calcinosis caused by a mutation in *FGF23* indicated increased processed fragments and decreased full-length FGF23 (34). In contrast, a mutation in *Klotho* seems to cause resistance to FGF23 because Klotho and the FGF receptor form a receptor for FGF23 (33). These results also indicate that FGF23 is physiologically decreasing serum phosphate and $1,25(OH)_2D$ in humans.

FGF23 and Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)

The regulatory mechanisms of FGF23 production are not clear enough. Acute changes of serum phosphate do not alter FGF23 levels (38). To the contrary, a high phosphate diet and $1,25(OH)_2D$ increase and a low phosphate diet decreases FGF23 levels (39-43). While $1,25(OH)_2D$ was reported to regulate FGF23 production through its effect on the *FGF23* promoter (44), it is unknown how phosphate content in the diet modulates FGF23 levels. In contrast to diseases mentioned above in which aberrant actions of FGF23 are primary causes of diseases, FGF23 levels can change secondarily and modify the disease course. FGF23 levels were shown to be high in patients with CKD while the precise mechanism of this high FGF23 is unknown (45-47). This increased FGF23 seems to contribute to the abnormal mineral metabolism in patients with CKD. During the progression of CKD, FGF23 starts to increase well before the elevation of PTH or phosphate (48). This rise of FGF23 reduces $1,25(OH)_2D$ and inhibits phosphate reabsorption in renal tubules. Clinical studies indicate that FGF23 levels are associated with increased fractional excretion of phosphate (FEpi) and low $1,25(OH)_2D$ (49). Recent animal work using a rat model of CKD also indicated that the inhibition of FGF23 activity by anti-FGF23 antibodies reduces FEpi and increases $1,25(OH)_2D$ (50). Thus, this increased FGF23 seems to protect against hyperphosphatemia, but at the same time works to increase PTH by lowering $1,25(OH)_2D$. However, FGF23 was also reported to decrease PTH production and secretion probably through the Klotho-FGF receptor complex in parathyroid glands (51;52). This is complicated further by the fact that the expression of Klotho and FGF receptor is reported to be reduced in the parathyroid of patients with CKD (53). Overall, it is possible that the increase of FGF23 works to prevent the development of hyperphosphatemia and secondary hyperparathyroidism in the early stages of CKD. However, apparently FGF23 cannot compensate for the abnormal mineral metabolism in patients with advanced CKD.

FGF23 and Cardiovascular Risk and Mortality

There are several reports indicating that high FGF23 levels are associated with increased cardiovascular events or mortality. The initial report indicated that high FGF23 levels at the time of the initiation of hemodialysis predicted high mortality during the first year of dialysis (54). This relationship was also reported in patients undergoing hemodialysis for 70 months on average (55). High FGF23 was also associated with an increased risk for progression of CKD, aortic calcification and left ventricular mass index in patients with CKD (56-58). Even in subjects with preserved renal function, high FGF23 was reported to be associated with mortality in coronary artery disease, endothelial dysfunction and vascular stiffness (59-62). There are several possibilities to explain these results. One is that FGF23 modifies vascular or cardiac function particularly in patients with exceptionally high FGF23 levels. Because Klotho is not expressed in vascular tissue, this effect of FGF23 must be Klotho-independent. However, Klotho-independent FGF23 action has not been clearly shown and it is questionable whether FGF23 in subjects with preserved renal function and with FGF23 levels that are not so high has Klotho-independent action. Another possibility is that FGF23 sensitively reflects some adverse events that also affect vascular or cardiac function. Because the range of FGF23 values in subjects with various diseases is quite wide, FGF23 can be a good marker for subtle changes of modifiers of FGF23 levels. However, this

wide variation of FGF23 levels again does not explain the relationship between FGF23 and cardiovascular events in subjects with preserved renal function.

Future Research Areas

Certainly there are several issues regarding FGF23 that are still unclear. First, the regulatory mechanisms of FGF23 production and circulatory FGF23 levels are cloudy. This is important not only in the interpretation of FGF23 levels in their relationship to phosphate and vitamin D metabolism but also in the explanation of the relevance to use of FGF23 as a predictor of cardiovascular events. In addition, the physiological significance of *PHEX*, *DMP1* and *ENPP1* gene products in the regulation of FGF23 production needs to be clarified. The phosphaturic humoral factor called phosphatonin has been considered to be involved in the development of TIO and XLH. Several factors including FGF23, matrix extracellular phosphoglycoprotein (MEPE), secreted frizzled-related protein 4 (sFRP4) and FGF7 were shown to reduce serum phosphate and/or inhibit phosphate uptake of cells. However, clear elevation of circulating levels in patients with TIO and XLH was only demonstrated for FGF23. Therefore, the significance of MEPE, sFRP4 and FGF7 in phosphate metabolism and hypophosphatemic diseases needs further study. Furthermore, the detailed mechanisms of FGF23 action need to be explored. While Klotho is expressed mainly in distal convoluted tubules and the initial signal by FGF23 is observed in this segment *in vivo* (26;66), the regulation of phosphate reabsorption and vitamin D metabolism by FGF23 occurs in proximal tubules. Clinically, it is important to examine if there is any measure to lower FGF23 and if this reduction of FGF23 causes decreased cardiovascular events, especially in patients with CKD. It has been shown that phosphate binder can reduce FGF23 in rodents (67). However, it is necessary to prove that the reduction of FGF23 has any beneficial effects in humans. Finally, it is necessary to clarify whether the modulation of FGF23 activity can be a novel therapeutic approach for patients with FGF23-related hypophosphatemic diseases (68). Despite these questions, the discovery of FGF23 has certainly deepened our understanding of the regulation of mineral metabolism and will stimulate further research in the future.

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