FGF23-mediated regulation of systemic phosphate homeostasis: is Klotho an essential player?

M. Shawkat Razzaque

Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts and Department of Pathology, Nagasaki University School of Biomedical Science, Nagasaki, Japan

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Razzaque MS. FGF23-mediated regulation of systemic phosphate homeostasis: is Klotho an essential player? Am J Physiol Renal Physiol 296: F470–F476, 2009. First published November 19, 2008; doi:10.1152/ajprenal.90538.2008.—Understanding the physiological regulation of mineral ion metabolism is essential for determining the pathomechanisms of skeletal, vascular, and renal diseases associated with an abnormal regulation of calcium and phosphate homeostasis. Normal calcium and phosphate balance is delicately maintained by endocrine factors that coordinate to influence the functions of the intestine, bone, parathyroid gland, and kidney. Under physiological conditions, the kidneys play an important role in maintaining normal mineral ion balance by fine-tuning the amount of urinary excretion of calcium and phosphate according to the body’s needs. Fibroblast growth factor (FGF)23 regulates urinary phosphate excretion to maintain systemic phosphate homeostasis. The exact mode of action of the phosphaturic effects of FGF23 is not fully understood and is an intense area of research. Studies suggest, however, that FGF23, by interacting with FGF receptors, can initiate downstream signaling events and that Klotho, a transmembrane protein, facilitates the interaction of FGF23 with its receptor. FGF23 can inhibit the activities of 1-α-hydroxylase and sodium-phosphate cotransporter in the kidney to influence the overall systemic phosphate balance. This article briefly summarizes how FGF23 might coordinately regulate systemic phosphate homeostasis and how Klotho is involved in such regulation.

vitamin D; mineral metabolism; kidney; calcification
Table 1. Approximate distribution of phosphorus in a healthy 70-kg adult

<table>
<thead>
<tr>
<th></th>
<th>Phosphorus</th>
</tr>
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<tbody>
<tr>
<td>Total body content</td>
<td>~700 g</td>
</tr>
<tr>
<td>In bone</td>
<td>~80%</td>
</tr>
<tr>
<td>In viscera</td>
<td>~10.9%</td>
</tr>
<tr>
<td>In skeletal muscle</td>
<td>~9%</td>
</tr>
<tr>
<td>In extracellular fluid</td>
<td>~0.1%</td>
</tr>
<tr>
<td>Intracellular-to-extracellular ratio</td>
<td>~100:1</td>
</tr>
</tbody>
</table>

From Reference 14.

Fibroblast Growth Factor 23

FGF23 is a protein of ~30 kDa that is proteolytically processed to generate smaller NH2-terminal (18 kDa) and COOH-terminal (12 kDa) fragments. The NH2-terminal fragment of FGF23 contains the FGF receptor (FGFR)-binding domain. In vivo studies using synthetic peptides have indicated that neither of the processed NH2-terminal or COOH-terminal fragments of FGF23 can influence serum phosphate levels (58). FGF23 can decrease the activity of NaPi-2a and NaPi-2c cotransporters to reduce renal phosphate reabsorption and thereby increase urinary phosphate excretion (55). Similarly, FGF23 can suppress the expression of 1-α-hydroxylase to reduce the production of the active vitamin D metabolite 1,25-dihydroxyvitamin D [1,25(OH)2D], which can enhance intestinal phosphorus absorption. A major breakthrough in the active regulation of phosphate homeostasis was achieved with the identification of a potent phosphatonin, named fibroblast growth factor (FGF)23 (1, 67).

FGF23 and Human Diseases

Human FGF23 was identified in patients as a gene associated with autosomal dominant hypophosphatemic rickets (ADHR). Gain-of-function mutations of the FGF23 gene in patients with ADHR are associated with urinary phosphate wasting, which causes rickets in the bone (1). The excessive amount of FGF23 that is produced by the tumor cells is responsible for the clinical symptoms of hypophosphatemia that are seen in patients with tumor-induced osteomalacia (TIO) (57). FGF23 has also been speculated to have a pathogenic role in patients with McCune-Albright syndrome and is believed to be responsible for hypophosphatemia in these patients (53). X-linked hypophosphatemia (XLH) is a common cause of vitamin D-resistant rickets/osteomalacia, which is caused by inactivating mutations in PHEX (a phosphate-regulating gene that is homologous to endopeptidases on the X chromosome) (19). The high level of circulating FGF23 in most patients with XLH induces excessive urinary phosphate wasting, thereby producing clinical symptoms associated with hypophosphatemia (23). Recently, mutations in dentin matrix protein-1 (DMP-1) were found in patients with autosomal recessive hypophosphatemic rickets/osteomalacia (ARHR) (33). The clinical symptoms of hypophosphatemia in patients with ARHR are mostly caused by high circulating levels of FGF23.

Patients in advanced stages of CKD have high serum levels of FGF23, partly due to decreased renal clearance of FGF23. The additional cause of increased levels of FGF23 in CKD patients may be a compensatory response to the hyperphosphatemia. Of relevance, human studies have shown that a dietary phosphorus load can increase serum FGF23 levels (2, 10). CKD patients are unable to excrete urinary phosphate, despite high serum FGF23 levels (13), and develop hyperphosphatemia, although they tend to have a lower level of 1,25(OH)2D, and suffer from hyperparathyroidism. Whether the low levels of 1,25(OH)2D and secondary hyperparathyroidism that are observed in patients with CKD are influenced by increased levels of circulating FGF23 is a complex issue. Since FGF23 has a counterregulatory effect on vitamin D (30), the elevated level of FGF23 in patients with CKD has the potential to reduce vitamin D activity; this effect may eventually facilitate the development of compensatory secondary hyperparathyroidism. Furthermore, calcitriol therapy in patients with CKD may also contribute to increased serum levels of FGF23 (42), because studies have shown that elevated serum 1,25(OH)2D can induce an increase in the serum level of FGF23 (54). The exact pathological significance of elevated serum levels of FGF23 in patients with CKD is not clear, and the question of whether increased levels of FGF23 can exert additional toxic effects, independent of other known risk factors of CKD patients (e.g., race, diabetes, hypertension, and advanced age) will need additional clinical studies.

In addition to the several hypophosphatemic diseases that are caused by an increased activity of FGF23, there are also human diseases that are caused by a reduced activity of FGF23. For instance, patients with familial tumoral calcinosis (FTC) usually develop hyperphosphatemia and ectopic calcification due to loss-of-function mutations in the FGF23 gene (4). Similarly, mutations in the GALNT3 (UDP-N-acetyl-α-b-galactosamine:polypeptide N-acetyl-galactosaminyl transferase 3) gene are also found in patients with FTC (12). GALNT3, a Golgi-associated enzyme, can selectively O-glycosylate a furinlike convertase recognition site in FGF23, and thereby can allow secretion of intact FGF23. Mutations in the GALNT3 gene can impair O-glycosylation of the FGF23 protein; defects in glycosylation may result in rapid processing of the FGF23 protein, which may be the cause of the lower levels of the intact FGF23 protein found in patients with FTC (15). The abnormally high serum levels of phosphate in patients with FTC, due to the reduced activity of the human FGF23, resemble the significantly high serum levels of phosphate that are found in mice without Fgf23 activity [Fgf23−/− mice] (8, 32). Moreover, there are strikingly similar physical, morphological, biochemical, and molecular phenotypes in Fgf23−/− and klotho-knockout
(klotho<sup>−/−</sup>) mice; these similarities led to the identification of Klotho as a cofactor for FGF23 and its receptor interactions (26, 28, 48, 50).

Klotho

The Klotho protein contains a putative signal sequence at its NH<sub>2</sub> terminus and a single transmembrane domain near its COOH terminus that is believed to anchor the protein to the membrane (39). The klotho gene encodes a type 1 membrane protein, which is predicted to be present on the cell surface of Klotho-expressing cells. The human Klotho gene has five exons and can generate two transcripts. The full-length transcript is 5.2 kb and encodes a 130-kDa membrane protein; once the short transmembrane domain is removed, this membrane form can be released into circulation. A recent study has suggested that A Disintegrin and Metalloproteinases (ADAM)-10 and ADAM-17 are capable of cleavage of Klotho from the plasma membrane, and that insulin can stimulate Klotho shedding (5). An alternative mRNA splicing event can generate another transcript, which codes for the NH<sub>2</sub>-terminal region of Klotho and creates a protein with a molecular mass of ~65–70 kDa. Human Klotho has ~86% homology to mouse klotho and has been mapped to chromosome 13q12 (34). Klotho expression has been detected mostly in the distal convoluted tubules of the kidney, the parathyroid gland, and the epithelium of the choroid plexus in the brain (34). The exact role of Klotho in systemic regulation of mineral ion metabolism is not clear, other than that it gives the tissue specificity for FGF23 action. The question of whether there is a functional difference between the membrane form and the secreted form of Klotho needs additional study.

The inactivation of Klotho function in the mouse results in hyperphosphatemia and hypervitaminosis D (Fig. 1), which leads to the development of phenotypes resembling extensive premature aging-like features, including infertility and a shortened life span, in the mutant mouse (26). Interestingly, the phenotype of klotho-ablated mice resembles that of Fgf23-ablated mice (41, 48, 50); this observation led to the identification of the FGF23 signaling pathways.

FGF23 Signaling

FGFs are circulatory factors that can be grouped into several subfamilies (Table 2). Most of the FGFs are able to bind to cell surface FGFRs to activate downstream signaling events with diverse biological consequences. FGF11 to FGF14, however, are unable to bind and activate FGFRs (22); these FGFs are also termed “FGF homologous factors” (43). FGF23 is a member of the FGF19 subfamily. Studies have shown that FGF23 needs Klotho as a cofactor to interact with the FGFRs (Fig. 2). FGF23 has been shown to bind to multiple FGFRs, including FGFR1c, FGFR3c, and FGFR4 (Table 3) (9, 27, 38, 64). A recent study, however, claimed that neither FGFR3 nor FGFR4 is the principal mediator of the FGF23 effects in vivo (31). Klotho appears to allow FGF23 to bind to its receptor complex with much higher affinity than to FGFR alone. Furthermore, FGF23, in the presence of Klotho, can activate downstream signaling events, as indicated by the activation of early growth response element-1 (Egr-1) and the phosphorylation of FGF receptor substrate-2a, extracellular signal-regulated kinase (ERK), p38, c-Jun NH<sub>2</sub>-terminal kinase (JNK), and AKT proteins (16, 27, 64, 68). None of these signaling phosphoproteins was detected in human proximal tubule epithelial cells treated with FGF23 without Klotho. These observations suggest that the FGF23-FGFRs interaction and subsequent signaling activities need Klotho as a cofactor (16, 35).

Cellular, intracellular, transcellular, and pericellular mineral ion transport is achieved through a series of complex events that utilize both active and passive translocation processes. An important question that remains to be solved is how Klotho, produced in the distal tubules, can facilitate the FGF23-mediated phosphate transport process in the proximal tubules. Whether a secretory form of Klotho, released from the distal part of the tubules, can affect the functionality of the proximal part of the tubules is an important unresolved issue.

Genetic mutations in the FGF23 signaling components, such as Klotho or the FGFs, are also linked to diseases associated with altered mineral ion homeostasis and/or dysregulation of vitamin D metabolism. For example, osteoglophonic dysplasia is caused by missense mutations in the ligand-binding and
is Klotho Essential for FGF23 Signaling?

Although Klotho-mediated FGF23 signaling is well documented, it is not yet clear whether FGF23 may also have Klotho-independent effects. This possibility was raised by the observation that FGF23 can have effects on cells that do not express Klotho. For instance, FGF23 has been shown to suppress osteoblast differentiation and bone mineralization in fetal rat calvaria cells (65). Similarly, FGF23 was shown to exhibit weak proliferative effects on a murine bone marrow-derived pro-B cell line that was overexpressing FGFRs in the absence of Klotho (69).

Moreover, a recent study has found that Fgf23−/− mice and Fgf23−/−/NaPi-2a−/− double-knockout mice have a similar osteomalacic phenotype (less mineralization of the bone) despite opposing serum phosphate levels (high serum phosphate in Fgf23−/− mice, but low serum phosphate in Fgf23−/−/NaPi-2a−/− mice). This result suggests that there may exist a serum phosphate-independent intrinsic effect of FGF23 on the bone (60). In fact, in vitro studies have suggested that FGF23 treatment can inhibit the mineralization of calvarial osteoblasts. Since Klotho is not expressed in osteoblasts, any effect of FGF23 on these cells would indicate that Klotho-independent effects. This possibility was raised by the observation that FGF23 can have effects on cells that do not express Klotho. For instance, FGF23 has been shown to suppress osteoblast differentiation and bone mineralization in fetal rat calvaria cells (65). Similarly, FGF23 was shown to exhibit weak proliferative effects on a murine bone marrow-derived pro-B cell line that was overexpressing FGFRs in the absence of Klotho (69).

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It is, however, necessary to mention that the injection of bioactive FGF23 protein into either klotho−/− mice or Fgf23−/−;klotho−/− double-knockout mice does not produce any obvious changes in the serum levels of phosphate (41), implying that Klotho is essential for the in vivo systemic regulation of phosphate homeostasis. Interestingly, when bioactive FGF23 protein was injected into either wild-type or Fgf23−/− mice, the serum phosphate level was significantly reduced by 12 h after the injection (41). Of course, both wild-type and Fgf23−/− mice have endogenous Klotho therefore, the exogenous injection of FGF23 can influence systemic phosphate homeostasis. On the other hand, in klotho−/− or Fgf23−/−;klotho−/− mice, in the absence of Klotho, FGF23 is unable to exert its phosphaturic effects. It is therefore likely that Klotho is essential for the FGF23-mediated systemic regulation of phosphate homeostasis. As mentioned above, the homozygous loss-of-function mutation in Klotho found in a patient with tumoral calcinosis has provided additional evidence for the
dependence of FGF23 on Klotho in the regulation of phosphate and vitamin D homeostasis in humans (20).

Nonetheless, under pathological conditions in which there is a higher concentration of FGF23, it may be possible that FGF23 can exert nonspecific effects without Klotho; for example, FGF23 may be able to bind to its receptors with low affinity without Klotho (64). In this regard, the toxic effects of an extremely high serum level of FGF23 in patients with CKD, who have a reduced level of Klotho, need to be studied carefully (21, 46). Of relevance, the expression of Klotho mRNA and protein is significantly reduced in kidneys obtained from patients with CKD (24). How elevated serum levels of FGF23 might exert toxic effects in CKD patients whose tissue expression of Klotho is low is an important question, yet to be resolved.

Can FGF23 Be a Therapeutic Target?

Is anti-FGF23 therapy a feasible approach to fine-tune existing treatments for diseases associated with abnormal mineral ion metabolism? The clinical application of a controlled therapeutic inactivation of FGF23 might be of therapeutic benefit for patients with excessive urinary phosphate-wasting diseases, including ADHR, ARHR, and XLH. It is worth mentioning that the current treatment for hypophosphatemic diseases that are caused by genetic defects is mostly symptomatic, such as oral phosphate replacement. The prolonged use of such replacement can lead to the development of secondary hyperparathyroidism. Patients with CKD might benefit from the therapeutic lowering of FGF23, particularly if such an intervention could be provided in the early stages of the disease. Additional studies are needed to determine whether normalizing extremely high serum FGF23 levels in patients with late stages of CKD will reduce the various complications, including renal osteodystrophy. Elevated FGF23 levels in patients with CKD may reduce vitamin D activity by suppressing the expression of 1α-hydroxylase. Since Klotho is an important component of FGF23 signaling, targeted manipulation of Klotho activity may also reduce the pathological effects of FGF23 and restore mineral balance. It is, however, worth mentioning that despite a number of studies proposing various roles for FGF23 in CKD patients, these studies have generated as many questions as they have answered. Whether a compensatory increased level of FGF23 in CKD patients is a protective response (in the earlier stage of the disease) or a harmful response (in the later stage of the disease) remains an unsettled issue, and any clinical interventions that target FGF23 therapeutically will need thoughtful consideration. In contrast to anti-FGF23 therapy, providing exogenous bioactive FGF23 protein might help restore the phosphate balance and reduce abnormal calcifications in patients with FTC, which is usually caused by the reduced activity of FGF23.

Concluding Remarks

Future studies will determine not only the therapeutic benefit of the manipulation of FGF23 signaling components but also its diagnostic and prognostic significance. The detection of circulatory FGF23 has become an important tool for determining the underlying causes of diseases associated with abnormal mineral ion metabolism. For instance, serum FGF23 levels can be used to differentially diagnose suspected TIO. Studies have suggested that the pretreatment serum level of FGF23 is a good predictor of the effectiveness of a vitamin D therapy in dialysis patients and might also be a useful marker for predicting the development of refractory hyperparathyroidism (40). FGF23 has been proposed to be an important biomarker of mortality in patients with early renal diseases, particularly in patients in whom the serum FGF23 level increased before the development of hyperphosphatemia (17). In light of these findings, it appears likely that FGF23 has diagnostic, prognostic, and therapeutic value.

Recent studies using mouse genetics have significantly enhanced our understanding of the in vivo regulation of phosphate homeostasis (49). The strikingly similar biochemical phenotypes of hypervitaminosis D, reduced serum PTH levels, and hyperphosphatemia in Fgf23−/− and klotho−/− single-knockout mice and Fgf23−/−/klotho−/− double-knockout mice suggest that these factors constitute a common signaling pathway (Fig. 2). These results provide compelling genetic evidence of the in vivo importance of Klotho in regulating systemic phosphate homeostasis with FGF23. Finally, it is necessary to mention that FGF23-mediated functions are mostly Klotho dependent; Klotho, however, also has numerous FGF23-independent functions, including adipogenesis (7). Our challenge will be to use these experimental observations to fine-tune the existing therapeutic options by manipulating the FGF23-Klotho axis to treat patients suffering from the complications of abnormal mineral ion metabolism (44).

REFERENCES


