

PERSPECTIVES

Phos, Phex and FGF: Mysteries of Phosphate Homeostasis Revealed — or Still Hidden

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The newest member of the calciotropic hormone club is (of all things) a phosphate-regulating hormone. We have long known that in disease, renal phosphate excretion can be affected by a circulating phosphaturic factor, a phosphatonin. The first phosphatonin to be identified was fibroblast growth factor 23 (FGF-23), the cause of several renal phosphate-wasting syndromes. Recent evidence goes much farther than this, however, and establishes that FGF-23 is also part of a hitherto unrecognized (and largely unpredicted) homeostatic system for both phosphate and vitamin D.

Clinical evidence of a phosphatonin first came from the syndrome of tumor-induced osteomalacia (TIO). In this syndrome, small mesenchymal tumors cause renal phosphate wasting and osteomalacia, which can be cured by resection of the tumor, indicating a humoral basis (1). There was more support for the existence of a phosphatonin from studies of the *Hyp* mouse, a faithful animal model of the human disorder X-linked hypophosphatemia (XLH) (1). Renal phosphate wasting could be induced in a normal mouse by parabiosis with a *Hyp* mouse (2,3) and in a normal mouse kidney by transplantation into a *Hyp* mouse (4), indicating that a circulating host factor was critical for the induction of phosphaturia.

The phosphatonin that causes autosomal dominant hypophosphatemic rickets (ADHR), a phosphate-wasting syndrome that clinically resembles XLH, was identified as FGF-23 by positional cloning (5). FGF-23

differs from other FGF family members by having a 73-amino acid extension on the carboxyl-terminal end of the molecule. The mutations that cause ADHR occur in either of two arginine residues in an RXXR motif. This motif, which is a potential site for cleavage of FGF-23 by a protease of the prohormone convertase class, precisely delimits the amino-terminal FGF domain from the unique carboxyl-terminal domain (Fig. 1). It soon became clear that FGF-23 is in fact cleaved at this site by ubiquitous proteases, leading to the idea that FGF-23 accumulates in patients with ADHR because its clearance by proteolytic degradation is impaired.

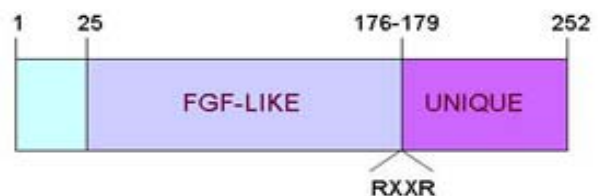


Fig. 1. Schematic of FGF-23, showing the FGF-like domain of approximately 254 amino acids, separated from a large carboxyl-terminal extension by the RXXR processing site that is mutated in ADHR

Almost simultaneously with its identification in ADHR, FGF-23 was identified as one of several gene products that were overexpressed in tumors associated with TIO (6-8). [Two other gene products, MEPE and secreted frizzled related protein-4 (FRP-4) (9-12), were also identified as

overexpressed genes and could also have phosphatonin activity, but their story is outside the scope of this brief review.] FGF-23 was directly shown to have phosphaturic activity in several overexpression models, and as predicted, the ADHR mutation increased its phosphaturic activity (6,13-17). Although it was initially difficult to show that FGF-23 directly inhibited renal phosphate transport (6), several investigators were ultimately able to show that intact FGF-23 inhibited sodium-dependent phosphate transport in cultured opossum kidney cells and reduced expression of the renal sodium-phosphate cotransporter NaPi-2a (8,13,18).

An inappropriately low serum level of $1\alpha,25$ -dihydroxyvitamin D ($1,25[\text{OH}]_2\text{D}$) is another cardinal feature of phosphate-wasting syndromes, both in humans and the *Hyp* mouse model, where renal 1α -hydroxylase activity is impaired. It was thus impressive to see that serum levels of $1,25(\text{OH})_2\text{D}$ were markedly reduced in models of FGF-23 overexpression (6,13,15-17,19) and that the renal enzyme was directly inhibited by recombinant full-length FGF-23 (13).

FGF-23 has subsequently been implicated as a phosphatonin in two additional hypophosphatemic syndromes, fibrous dysplasia and XLH. Hypophosphatemia is relatively common in fibrous dysplasia. Riminucci *et al* (20) have recently shown that FGF-23 is produced in fibrous dysplasia tissue and normal marrow. In some patients with fibrous dysplasia, FGF-23 accumulates to high serum levels that correlate inversely with serum phosphate levels.

FGF-23 is also involved in the pathogenesis of XLH, although it is not clear how. XLH mutations inactivate the cell surface protease Phex, which is expressed in many tissues, but prominently in bone (1,10,21,22). It has been difficult to link Phex mechanistically to phosphate handling: What is the relationship between Phex and FGF-23? FGF-23 levels are increased in the *Hyp* mouse (23), which also carries a mutation that inactivates the *Phex* gene (24,25), and in some (but apparently not all) patients with XLH (26-28). Other unpublished data also support a humoral role of FGF-23 in XLH. As reported at the 2003 Annual Meeting of the American Society for Bone and Mineral

Research (ASBMR), administration of neutralizing antibodies to FGF-23 to either normal or *Hyp* mice causes a marked increase in the serum phosphate level (23). This confirms the role of FGF-23 in the abnormal renal phosphate handling of *Hyp* mice (and by extension in XLH) and indicates that FGF-23 functions as a humoral messenger in both pathological and normal states.

If Phex were a protease that inactivated FGF-23, XLH could be tied together pathogenetically with TIO and ADHR in the following way: Failure to cleave and thereby inactivate FGF-23 as the result of inactive Phex would lead to FGF-23 accumulation and thereby to phosphaturia. But alas, Phex does not seem to target the RXXR site or to cleave full-length FGF-23 (29,30), despite initial reports that it does (8). On the other hand, there is evidence indicating that in some unknown way, Phex acts upstream of FGF-23 to increase the expression of the *Fgf23* gene in the osteoblasts of *Hyp* mice (5,29).

The explanation of the way in which Phex and FGF-23 are related in the pathogenesis of XLH will ultimately have to account for another mystery: Why does the *XLH* mutation act in an X-linked dominant fashion? Dominantly inherited traits rarely result from haploinsufficiency, inactivation of one allele, and are much more likely to result from dominant negative effects. Yet, many of the *XLH* mutations in *PHEX* are deletions that clearly result in a loss of function and cannot behave as dominant negatives. Moreover, haploinsufficiency of FGF-23 is evidently compensated by increased expression of the remaining allele, because serum levels of FGF-23 are normal in *Fgf23* haploinsufficient mice (31). If FGF-23 is downstream of Phex, then why is haploinsufficiency of *PHEX* sufficient to produce a clinical syndrome?

FGF-23 can act as a phosphatonin in various pathological entities, but does it act as a phosphatonin physiologically? Is FGF-23 like ACTH, which regulates cortisol secretion (whether physiologically from the pituitary or ectopically by neuroendocrine tumors), or parathyroid hormone-related protein, which causes hypercalcemia when

secreted in excess by tumors, but does not physiologically regulate serum calcium (except during lactation) (32)?

The recent report of the phenotype of the *Fgf23*-null mouse indicates that FGF-23 has a central role in the regulation of phosphate and vitamin D homeostasis (31). Heterozygous mice are entirely normal, with normal serum levels of FGF-23, but mice in which both alleles of the *Fgf23* gene are disrupted have a remarkable phenotype of extreme hyperphosphatemia and high serum 1,25(OH)₂D levels. They develop hyperphosphatemia because of renal phosphate retention, with increased apical display of the renal sodium-phosphate cotransporter NaPi-2a in the proximal renal tubule, as well as increased phosphate uptake in renal slices. *Fgf23*-deficient mice also have high serum levels of 1,25(OH)₂D from day 10 onward, because of increased activity of the renal 1 α -hydroxylase. *Fgf23*-null mice develop progressive hypercalcemia, presumably because of very high 1,25(OH)₂D levels, and ultimately succumb to renal failure from nephrocalcinosis. The features of hyperphosphatemia and increased 1,25(OH)₂D levels are mirror images of the syndrome of FGF-23 excess and fit the hypothesis that FGF-23 is a physiological regulator of phosphate and vitamin D metabolism.

Other phenotypic features of *Fgf23*-null mice, which could be either primary effects of FGF-23 deficiency or secondary consequences of the impairment of phosphate and vitamin D metabolism, are also striking. The mice have marked growth retardation and growth plate disorganization, with absence of hypertrophic chondrocytes at seven weeks of age and severe osteomalacia. The growth plate phenotype suggests that FGF-23 has a role in bone development. In contrast, the finding of impaired mineralization could be either the direct result of FGF-23 deficiency or a consequence of high 1,25(OH)₂D levels, which are associated with osteomalacia in other circumstances (33,34). The authors (31) suggest that lymphopenia and splenic and thymic atrophy, which occur after the weaning of *Fgf23*-null mice (when 1,25(OH)₂D levels climb), may be a

consequence of an immune suppressive action of high 1,25(OH)₂D levels. It is also striking that *Fgf23*-null mice are hypoglycemic and have markedly reduced triglyceride levels, which suggests an interaction of FGF-23 with intermediary metabolism that was not hitherto apparent. Finally, *Fgf23*-null mice are infertile.

All known members of the FGF family use one of four receptors, each of which can be expressed in multiply spliced forms that have different ligand specificities (35). The identification of FGF-23 as a molecule with a carboxyl-terminal domain that is not shared by other family members and the ability of FGF-23 to cause hypophosphatemia, an effect that is not obviously shared by other FGFs, raised the possibility that FGF-23 has a unique receptor. At the 2003 ASBMR Annual Meeting, however, White *et al.* (36) reported genetic evidence implicating fibroblast growth factor receptor 1 (FGFR1) in a rare phosphate-wasting syndrome. The authors studied a father and two sons who had craniofacial dysplasia with hypophosphatemia (CFDH). The three men had severe skeletal dysplasia, with short stature and craniosynostosis and severe renal phosphate wasting and low 1,25(OH)₂D levels. Sequencing of candidate receptors identified a mutation (Y372C) in FGFR1. The mutation could well induce a gain of function, because orthologous mutations in FGFR2 and FGFR3 both produce gain of function syndromes, the Beare-Stevenson cutis gyrata syndrome and thanatophoric dwarfism type 1, respectively (37). In thanatophoric dwarfism, the mutation to cysteine in a membrane-proximal region of the extracellular domain of FGFR3 activates receptor signaling to produce achondroplasia in its most severe form, because of covalent dimerization of the receptor (38), a mechanism that could well apply to FGFR1 and FGFR2 molecules with orthologous mutations.

The identification of severe phosphate wasting associated with a mutation that putatively activates FGFR1 raises the possibility that FGFR1 is the renal receptor that responds to FGF-23, physiologically and in pathological states of phosphate wasting. FGF-23 has been shown to bind to FGFR3c with reasonably high affinity, but

only in the presence of heparan sulfate, and reportedly does not bind to the extracellular domain of FGFR1c (18); however, binding to other receptor isoforms has not been evaluated. FGFR1 is expressed in the renal tubule (39), but primarily in the distal nephron, whereas the dominant FGFR in the proximal tubule, the site of phosphate reabsorption, is FGFR3.

The likelihood that inhibition of phosphate reabsorption by FGF-23 is mediated by one of the classical FGF receptors raises an interesting hypothesis regarding the function of the unique carboxyl-terminal domain of FGF-23 which, based on modeling studies, would seem not to be required for receptor binding (40). In general, FGFs are heparin-binding molecules associated with the extracellular matrix (35,41). It is possible that the carboxyl-terminal domain of FGF-23 functions to mask a heparin-binding domain of the molecule and thereby permits it to circulate freely, as its humoral role in phosphate handling requires. This hypothesis leads to several predictions. First, the amino-terminal fragment of FGF-23 would not be active when infused systemically, as has been reported (14), but would be active in the presence of heparans in cultured cells or isolated tubules. Second, full-length FGF-23 would have reduced activity at the receptor, unless processed to remove the carboxyl-terminal domain, because the active FGF homodimer binds heparan sulfate. Third, mutations at R176 and R179 that prevent cleavage of FGF-23 would increase its biological activity *in vivo* by preventing cleavage to produce a form that is cleared from the circulation by heparan binding. These predictions are open to experiment. [It is also possible that FGF-23 has reduced affinity for heparin because of the structure of loop 11, which, in the FGF subfamily that contains FGF19, seems to differ from the structure in strongly heparin-binding FGFs (40).]

It is finally worth reviewing which physiological or pathophysiological phenomena need to be explained by a phosphotropic hormone, anyway. Serum phosphate levels vary widely during the day; the phosphate level has a diurnal rhythm on which are superimposed large postprandial fluctuations. The daily variation in phosphate

levels accords with the largely passive absorption of dietary phosphate in the intestine and the marked fluxes of phosphate into cells that occur with postprandial glucose transport. Yet, this loosely captained ship can smartly come about when dietary phosphate is tightly restricted or present in excess, with large shifts in renal phosphate reabsorption that are independent of PTH (42). The phosphate homeostatic system thus allows for large daily fluctuations in the serum phosphate level, while resetting the renal phosphate threshold to deal with unusually high and low dietary phosphate intakes.

The interplay of phosphate handling and vitamin D metabolism is also fascinating (43,44). Changes in postprandial serum phosphate levels are associated with large changes in serum 1,25(OH)₂D levels, even when fasting phosphate levels are not altered. In early renal insufficiency, it was shown long ago that the decline in the level of 1,25(OH)₂D and the appearance of secondary hyperparathyroidism occur at a time when phosphate retention has not yet occurred and the serum phosphate is actually reduced (45). Does FGF-23 play a critical role in the pathogenesis of early renal osteodystrophy by inhibiting renal synthesis of 1,25(OH)₂D in response to changes in phosphate handling? If so, precisely what changes in phosphate handling are sensed?

Prominent among clinically significant hyperphosphatemic states are chronic renal insufficiency, hypoparathyroidism, and familial tumoral calcinosis. Serum levels of FGF-23 are very high in renal insufficiency (26,46), a finding that would be consistent with a response to hyperphosphatemia, reduced renal clearance of FGF-23, or both. In hypoparathyroidism, the persistence of hyperphosphatemia indicates that compensation by FGF-23 does not succeed in correcting for the loss of the hypophosphatemic action of PTH. Hyperphosphatemia is a stimulus for secretion of FGF-23 in hypoparathyroidism (47). If so, why does FGF-23 not compensate for loss of the phosphaturic effect of PTH? FGF-23 levels are reportedly increased in patients with primary hyperparathyroidism as well (48). These

findings suggest a complex relationship between PTH and FGF-23.

Familial tumoral calcinosis (FTC) is a recessively inherited disorder of phosphate metabolism, with hyperphosphatemia and massive subcutaneous deposition of calcium phosphate. In two families, mutations were identified in *GALNT3*, which encodes a glycosyltransferase responsible for initiating mucin-type O-glycosylation (49). Serum FGF-23 levels are markedly increased in subjects with FTC; this could be compensatory for marked hyperphosphatemia. FGF-23 has potential O-linked glycosylation sites, however, raising the possibility that biologically inactivation of FGF-23 because of a failure

of O-linked glycosylation causes FTC. Evidence against this interpretation is that the skeletal phenotype of *Fgf23*-null mice is not present in FTC. Could O-linked glycosylation be required for the phosphaturic effects but not the skeletal developmental effects of FGF-23?

It is still not clear why the body economy requires a hormone to regulate the renal excretion of phosphate, nor do we know the physiological stimulus for secretion of FGF-23, much less the site of the sensor or the site of FGF-23 secretion. FGF-23 has already taught us a remarkable amount about the physiology of phosphate homeostasis, but every lesson taught has revealed another ignorance.

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