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REVIEW ARTICLE

# The Roles of Fibroblast Growth Factor (FGF)-23, α-Klotho and Furin Protease in Calcium and Phosphate Homeostasis : A Mini-Review

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**Abstract** The roles of calcitonin, parathormone and calcitriol in the regulation of plasma calcium and phosphate are well-established. However, in autosomal-dominant hypophosphatemic rickety patients, studies have revealed normal plasma levels of calcium, associated with normal thyroid and parathyroid functions, but decreased levels of phosphate and calcitriol despite adequate reserves of vitamin D. Also, in tumoral calcinosis, persistent hyperphosphatemia with increased levels of $1,25(OH)_2D_3$ have been observed. These studies indicate the involvement of factors other than the ones already known. The first decade of this century/millennium has led to the discovery of the involvement of fibroblast growth factor-23, furin protease and α-klotho in the homeostasis of calcium and phosphate, which is the subject of this mini-review.

**Keywords** Calcitriol · Fibroblast growth factor-23 · Furin · α-Klotho · Sclerostin · Transient receptor potential vanilloid type 5 (TRPV5) protein

## Basic Concepts

The roles of three hormones, namely, thyrocalcitonin, parathyroid hormone (PTH/parathormone) and calcitriol (1, 25-dihydroxy cholecalciferol/$1,25(OH)_2D_3$) in the regulation of plasma calcium and phosphate are well established. For instance, post in-take of meal, dietary $Ca^{2+}$ and $PO_4^{3-}$ uptake/absorption in the intestines takes place; while $Ca^{2+}$ uptake is promoted because of the increased in vivo population of calcium transporting and binding proteins caused by increased de novo synthesis induced by calcitriol, the phosphate uptake occurs due to the increased activity of the $Na^+/PO_4^{3-}$ co-transporter-2b; both these processes being brought about by calcitriol hormone derived from its pre-pro-precursor, vitamin D. This increased uptake of these two minerals in the intestine leads to a tide in plasma which needs to be dissipated. Thyrocalcitonin is released by thyroid glands in response to this tide, which promotes mineralization of the soft bones by inhibiting osteoclastic activities on the one hand and promoting osteoblastic activity on the other hand, thereby normalizing plasma levels of $Ca^{2+}$ and $PO_4^{3-}$. Whenever plasma $Ca^{2+}$ decreases (hypocalcemia), parathormone (PTH) is released by parathyroid glands. This hormone acts in conjunction with calcitriol in two ways, (a) by promoting osteocytes to secrete sclerostin, which binds bone morphogenetic receptor, leading to inhibition of osteogenesis, while promoting osteoclastic activity [1], i.e. (i) active release of proteases, glycosidases and phosphatases which cause breakdown of proteoglycan mesh and release of covalently-bound phosphate (organic phosphate) from proteins (such as, osteogenin), (ii) increased release of $H^+$ due to increased osteoclastic carbonic anhydrase and (iii) consequential release of hydroxyapatite from γ-carboxy-glutamate residues of peptides/proteins and its dissociation under acidic conditions, leading to demineralization and release into plasma of large amounts of $Ca^{2+}$ and $PO_4^{3-}$ from soft regions of bones, thereby reversing the osteogenic processes induced by thyrocalcitonin, and (b) by selective reabsorption, in conjunction with calcitriol, of $Ca^{2+}$ on the

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one hand and promotion of phosphaturia by inhibiting $Na^+/PO_4^{3-}$ 'co-transporter-a' on the other hand in the renal proximal tubules, thereby restoring normo-calcemic levels. Note must, however, be taken that calcitonin and PTH being proteins, get degraded by proteolytic enzymes and have, thus, short half-lives (<4 min.), thereby confining their roles to rapid correction/regulation of plasma $Ca^{2+}$, whereas calcitriol being a steroidal hormone, has a long half-life (>6 h.), acts intracellularly over a long period (even days) of time, whose synthesis is, however, under strict control at the α-1 hydroxylation of $25(OH)D_2$ step that takes place in the kidney. The active hormone, calcitriol, can, however, also be deactivated by further hydroxylation at C-24 position into $1,24,25(OH)_3$ cholecalciferol, called calcitetrol. Furthermore, the two proteinic hormones act via signal transducing pathways, involving second messenger/protein kinases.

Nutritional studies have revealed that the dietary restriction of phosphate intake results in decreased plasma levels of PTH by direct or indirect effect of plasma $PO_4^{3-}$ levels [2] and that there occurs a delayed or improved secondary hyper-parathyroidism independent of plasma levels of $Ca^{2+}$ or calcitriol.

Beside its major participation in the mineralization of skeletal tissues, $Ca^{2+}$ plays vital roles as cofactor of a variety of enzymes, including blood clotting cascade, second messenger, in plasma membrane functions, metabolic regulations through calmodulin, etc.

Phosphate also plays vital roles in cellular activity. For instance, it is required to generate energy-rich compounds, such as, nucleoside triphosphates, which in turn generate other energy-rich compound, such as, coenzyme-A derivatives, creatine-phosphate, $FAD^+$, $NAD^+$, etc. and also serves as the linker through phosphodiester bonds between nucleosides, the building blocks of DNA and RNA. Nucleoside phosphates are also the starting donor molecules for the synthesis of proteoglycans, a key component in the extracellular-matrix, and glycosylation of proteins. The energy-rich compounds in turn lead to the survival and proliferation of cells. In addition, $PO_4^{3-}$ participates in the mineralization of skeletal tissues, formation of intracellular inorganic polyphosphates and also in the regulation and function of many proteins and enzymes through phosphorylation/de-phosphorylation processes.

Normal human plasma levels of $Ca^{2+}$ and $PO_4^{3-}$ are ~2.5 and ~1.3 mmol/l, respectively. Ordinarily, there exists an inverse relationship between the two in the plasma. However, notwithstanding the interrelationship between the three hormones, discussed above, in the regulation of plasma $Ca^{2+}$ and $PO_4^{3-}$, it has been observed that despite normo-calcemic status, some patients exhibited acute and persistent hypo-phosphataemia with associated symptoms. These diseases were investigated and classified as (i) autosomal dominant hypophosphatemic rickets (ADHR) (ii) X-linked hypophosphataemia (XLH) [3] and (iii) oncogenic osteomalacia (OOM) [4]. Thus, in these conditions, no inverse relationship existed between plasma $Ca^{2+}$ and $PO_4^{3-}$.

Research studies reported during the early years of this century on these diseases, and related calcium and phosphate homeostasis, reveal the involvement of at least three additional major proteins that have been identified as fibroblast growth factor-23 (FGF-23), furin protease (FP) and α-klotho.

Since hypo-phosphatemia has almost always been associated with phosphaturia, renal excretion [5] rather than intestinal absorption has been identified as the site of disorder. Such a condition has been attributed to FGF-23 [4]. FGF-23 has so far been implicated in (1) ADHR (2) OOM (3) XLH (4) chronic renal failure (CRF) and (5) familial tumoral calcinosis (FTC) [6], a disease associated with hyper-phosphatemia, an autosomal recessive disorder (reverse of ADHR) in which secretion process of this hormone is affected due to mutation at $S^{129}$ residue, the site of GalNAc-osylation [7]; this last disease is also associated with ectopic calcification.

## Fibroblast Growth Factors (FGFs)

FGFs are a family of structurally-related protein molecules, 22 of which are found in humans, while FGF-15 is found exclusively in mouse. These molecules are involved in a variety of cellular functions, such as, angiogenesis, wound-healing, embryonic development and cellular-proliferation and differentiation. FGFs also bind heparin and interact with cells via cell-surface associated heparan sulfate-proteoglycans causing signal transduction. FGF-1 and FGF-2 are also known, respectively, as acidic and basic FGFs. FGF-1 to FGF-10 bind their corresponding receptors (FGFRs) on cell surfaces. FGF-11 is involved in intracellular processes unrelated to other FGFs and is, therefore, also known as iFGF. FGF-12 does not bind FGF-receptors and is so known because of its sequence homology with other FGF molecules. FGF-13 and FGF-14 are also FGF homologous factors but have distinct functional differences with other FGFs. Mouse FGF-15 is an ortholog of human FGF-19; therefore, there is no human FGF-15. FGF-16 through FGF-23 are newer molecules and not all are well understood. FGF-19 to FGF-23 are hormone-like growth factors that have more systemic effects not found in other FGFs [8]. FGF-19, produced by intestinal cells, binds FGF-receptor-4-expressing hepatocytes, leading to the down regulation of bile acid synthetic pathway. FGF-20 is homologous to Xenopus FGF-20 (XFGF-20). FGF-21 has

been shown to possess anti-hyperglycemic property as well as plasma triacylglycerol lowering effects along with the body weight.

## FGF-23

The gene for this protein is located on chromosome-12 that possesses three exons and is expressed in bone (osteocytes) and connective tissues, the synthesis of which appears to be regulated by dietary phosphorus intake in healthy men [9]. The translated product, FGF-23, is a 251 amino acids, 30 kDa protein, which is O-glycosylated (GalNAc) on $S^{129}$ residue by specific ppGalNAcase T3 prior to secretion [7]. To blood, however, osteocytes are the major contributor of this hormone [10] which acts in an endocrine manner in far away tissues, e.g. intestines, liver, kidney, bone and adipose. This growth factor binds less-tightly to heparin-sulfate but specifically binds FGF-receptor-1-expressing kidney cells [11], leading to the decreased synthesis of active hormone of vitamin D, the $1,25(OH)_2D_3$, by causing repression of the gene for α-1 hydroxylase [12], thereby regulating calcium homeostasis. Ordinarily, FGF-23 also inhibits renal tubular re-absorption of phosphate [13] by decreasing the gene expression of $Na^+$-dependent phosphate transporters (Na/Pi-2a and Na/Pi-2c) in the proximal convoluted tubules [14, 15], thereby promoting phosphaturia. As such, this hormone was initially (before the year 2000) called "Phosphatonin".

The FGF-23 gene-product is a hormone that possesses a high sequence homology with FGF-19 and FGF-21 but is the only member that contains pro-convertase processing site, a proteolytic cleavage site RXXR, which when cleaved (by a specific Furin protease) results in the generation of inactive 18 kDa amino-terminal- and a 12 kDa carboxy-terminal-fragments. Activity of the intact FGF-23 is regulated by the glycosylation and Furin protease. Glycosylation site is flank by RXXR site. Upon glycosylation at $S^{129}$ by ppGalNAc T3, FGF-23 is protected against the proteolysis by furin. Also, any mutation in RXXR site is bound to make it resistant to cleavage, thereby increase the half-life as well as the plasma concentration of this protein, leading to persistent phosphaturia due to the inhibition of $Na^+$-dependent phosphate transporters and decreased *de novo* synthesis of $1,25(OH)_2D_3$. The result is hypophosphatemic rickets, as in ADHR. Similarly, over-production of this hormone, as under hyperplasic conditions, leads to 'Tumor-induced Osteomalacia'. On the contrary, loss of FGF-23 activity, such as, by intracellular proteolysis, leads to increased plasma levels of phosphate, resulting in 'calcinosis'; such a condition has been elucidated in tumoral calcinosis [16], wherein mutations in FGF-23, such as, $S^{71}G$, $S^{129}F$ and $R^{176}Q$, appear to alter the conformation of FGF-23 such as to make it more susceptible to proteolytic cleavage by specific furin Protease, leading to increased intracellular breakdown of FGF-23 and release of C-terminal fragment rather than intact FGF-23 into circulation, thereby preventing phosphaturia.

## α-Klotho

α-Klotho is a 130 kDa protein that has been reported to bind FGF-23 [8, 17], its receptor (FGF-R) [18] and plasma membrane $Na^+/K^+$-ATPase. Its binding to $Na^+/K^+$-ATPase causes increased $Na^+$ gradient, which in turn increases trans-epithelial influx of $Ca^{2+}$ in kidney and choroid plexus, leading to secretion of α-Klotho.

α-Klotho also possesses β-glucuronidase activity and thereby modifies sugar chains of transient receptor potential vanilloid type 5 (TRPV5) in distal tubules; this prevents internalization of $Ca^{2+}$ channel and, therefore, its inactivation [19]. The result is increased $Ca^{2+}$ absorption. In other words, α-klotho also directly participates in $Ca^{2+}$ and $PO_4^{3-}$ homeostasis.

## FGF-Receptor-1

This receptor (FGF-R-1) is expressed in only three organs, kidney, parathyroid and pituitary and consists of three extra-cellular immunoglobulin-like domains, $D_1$–$D_3$, a single-span trans-membrane domain and an intra-cellular split tyrosine kinase domain. In this molecule, there is a short stretch of acidic amino acids that resides between $D_1$ and $D_2$ domains, which in the absence of the ligand FGF-23, folds in such a manner that it binds heparansulfate proteoglycan-binding site on the $D_3$ domain, thereby serves auto-inhibitory function, preventing activation of the receptor. FGFs interact with $D_2$ and $D_3$ domains of FGF-R, $D_3$ being highly ligand-specific especially when α-klotho is bound to it. In fact, binding of α-klotho to FGF-R makes it highly specific for the binding of FGF-23. Heparan sulfate proteoglycan (HSPG) is a cofactor in ternary complex formation between FGF-23 and its receptor (FGF-R). Thus, it is the $D_3$ domain of the receptor-1 which imparts specificity for the binding of FGF-23 [20], $D_1$ and $D_2$ being only specific for the structurally homologous regions of the FGFs. The ultimate complex at the cell surface comprises 2 identical FGF-23 ligands, 2 identical FGF-R subunits, 1 or 2 heparansulfate proteoglycan chains and 2 molecule of α-klotho; the consequential intra-cellular events, such as repression of α-1 hydroxylase and $Na^+/PO_4^{3-}$ cotransporters *a* and *c*, are as a result of the activation of the tyrosine kinase, which resides in the split domains of the

FGF-R exposed on the cytosolic side of the plasma membrane.

## Furin Protease

Furin, also called as paired basic amino acid cleavage enzyme (PACE), is a product of the gene that resides upstream of an oncogene, FES [21], and hence given the name (furin, F-upstream region). It is a subtilisin-like apo-/pro-protein convertase, endo-peptidase in nature, that requires $Ca^{2+}$ and in which a serine residue participates in catalytic activity. This serine protease is enriched in trans-golgi network, where it cleaves other proteins into their mature active forms [22]. Although ubiquitously expressed at very low levels, furin is found in highest concentrations in liver and kidney, whereas brain, spleen and thymus possess much lower levels.

Furin family of proteins exist as pro-enzymes in which ~10 kDa heterogeneous amino-terminal, called the pro-region, is cleaved prior to its activation. This is followed by a highly conserved ~55 kDa catalytic domain and a C-terminal tail that has variable length as well as amino acid sequence. Furin is the only pro-convertase that possesses a trans-membrane domain; it cleaves the target proteins down-stream of basic amino acid consensus sequence of 'RX(R/K)R'. While the acidic amino acid sequence $^{771}$CPSDSEEDEG$^{780}$ appears to be responsible for its trans-Golgi-network localization, the second domain, $^{759}$YKGL$^{762}$, directs the internalization from cell surface. It is the phosphorylation of $^{775}$Serine residue that modulates intracellular routing.

In summary, it is the activity of the furin protease that regulates the half-life of FGF-23; the latter in turn is responsible, after proper docking on its receptor (FGF-R), made possible by the participation of α-klotho, for the regulation of renal α-1 hydroxylase of vitamin $D_2$ and repression of $Na^+$/Pi-2a and 2c transporters.

**Conflict of Interest** Since this is a single author review article and involving no experimental work, research funding or support, I certify that there is no conflict with anyone in this regard.

## References


1. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staehling-Hampton K, Appleby M, Brunkow ME, Latham JA. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J. 2003;22:6267–76.
2. Perwad F, Azam N, Zhang MYH, Yamashita T, Tenenhouse HS, Portale AA. Dietary and serum phosphorus regulate fibroblast growth factor-23 expression and 1,25-dihydroxyvitamin D metabolism in mice. Endocrinology. 2005;146:5358–64.
3. Kida Y, Fukumoto S, Yamashita T, Jonsson K, Econs M, Juppner H. Fibroblast growth factor-23 in oncogenic osteomalacia and X-linked hypophosphatemia. New Engl J Med. 2003;349:505–6.
4. Jonsson KB, Zahradnik R, Larsson T, White KE, Sugimoto T, Imanishi Y, Yamamoto T, Hampson G, Koshiyama H, Lunggren O, Oba K, Yang IM, Miyauchi A, Econs MJ, Juppner H. Fibroblast growth factor-23 in oncogenic osteomalacia and X-linked hypophosphatemia. New Engl J Med. 2003;348:1656–63.
5. Rodriguez M, Lopez I, Munoz J, Aguilera-Tejero E, Almaden Y. FGF23 and mineral metabolism, implications in CKD-MBD. Nefroligia. 2012;32(3):275–8.
6. Larson T, Davis SI, Garringer HJ, Mooney SD, Draman MS, Cullen MJ, White KE. Fibroblast growth factor-23 mutants causing familial tumoral calcinosis are differentially processed. Endocrinology. 2005;146:3883–91.
7. Bergwitz C, Banerjee S, Abu-Zahra H, Kaji H, Miyauchi A, Sugimoto T, Juppner H. Defective O-glycosylation due to a novel homozygous S129P mutation is associated with lack of fibroblast growth factor-23 secretion and tumoral calcinosis. J Clin Endocrinol Metab. 2009;94(11):4267–74.
8. Nabeshima Y. Regulation of calcium homeostasis. Clin Calcium. 2010;20(11):1677–85.
9. Antonuicci DM, Yamashita T, Portale AA. Dietary phosphorus regulates serum fibroblast growth factor-23 concentrations in healthy men. J Clin Endocrinol Metab. 2006;91:3144–9.
10. Ubaidus S, Li M, Sultana S, de Freitas PHL, Oda K, Maeda T, Takagi R, Amizuka N. FGF-23 is mainly synthesized by osteocytes in the regularly distributed osteocytic lacunar canalicular system established after physiological bone remodeling. J Electron Microsc (Tokyo). 2009;58:381–92.
11. Liu S, Quarles LD. How fibroblast growth factor-23 works. J Am Soc Nephrol. 2007;18(6):1637–47.
12. Perward F, Zhang MYH, Tenehouse HS, Portale. Fibroblast growth factor-23 impairs phosphorus and vitamin D metabolism in vivo and suppresses 25-hydroxy vitamin D 1-α hydroxylase expression in vitro. Am J Renal Physiol. 2007;293:1577–83.
13. White KE, Larsson TE, Econs MJ. The roles of specific genes implicated as circulating factors involved in normal and disordered phosphate homeostasis: frizzled related protein-4, matrix extracellular phosphoglyco- protein and fibroblast growth factor-23. Endocrinol Rev. 2006;27:221–41.
14. Gattineni J, Bates C, Twombley K, Dwarkanath V, Robinson ML, Goetz R, Mohammadi M, Baum M. FGF-23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia in vivo predominantly via FGF-receptor-1. Am J Physiol Renal Physiol. 2009;297:F282–91.
15. Ramon I, Kleynen P, Body JJ, Karmali R. Fibroblast growth factor-23 and its role in phosphate homeostasis. Eur J Endocrinol. 2010;162:1–10.
16. Masi L, Gozzini A, Franchi A, Companacci D, Amedei A, Falchetti A, Franceschelli F, Marcucci G, Tanini A, Capanna R, Brandi ML. A novel recessive mutation of fibroblast growth factor-23 in tumoral calcinosis. J Bone Joint Surg. 2009;91:1190–8.
17. Nabeshima Y. Discovery of α-klotho and FGF-23 unveiled new insight into calcium and phosphate homeostasis. Clin Calcium. 2008;18(7):923–34.
18. Razzaque MS, Lanske B. The emerging role of the fibroblast growth factor-23-klotho axis in renal regulation of phosphate homeostasis. J Endocrinol. 2007;194:1–10.
19. de Groot T, van der Hagen EA, Verkaart S, Te Boekhorst VA, Bindels RJ, Hoenderop JG. The role of the TRPV5 N-terminus in channel activity, tetramerization and trafficking. J Biol Chem. 2011;286(37):32132–9.
20. Urakawa L, Yamazak Y, Shimada T, Lijima K, Haseqawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts

FGF receptor into a specific receptor for FGF23. Nature. 2006;444:770–4.
21. Wise RJ, Barr PJ, Wong PA, Kiefer MC, Brake AJ, Kaufman RJ. Expression of a human proprotein processing enzyme: correct cleavage of the von Willebrand factor precursor at a paired basic amino acid site. Proc Natl Acad Sci USA. 1991;87(23): 9378–82.
22. Thomas G. Furin at the cutting edge: from protein traffic to embryogenesis and disease. Nat Rev Mol Cell Biol. 2002; 3(10):753–66.