Phosphatonins: new hormones involved in numerous inherited bone disorders

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Summary
Phosphate (Pi) homeostasis is under control of several endocrine factors that play effects on bone, kidney and intestine. The control of Pi homeostasis has a significant biological importance, as it relates to numerous cellular mechanisms involved in energy metabolism, cell signaling, nucleic acid synthesis, membrane function, as well as skeletal health and integrity. Pi is essential for diverse biological processes, and negative Pi balance resulting from improperly regulated intestinal absorption, systemic utilization, and renal excretion. As a result of these functions, chronic Pi deprivation causes several biological alterations, such as bone demineralization with unmineralized osteoid typical of osteomalacia in adults and rickets in developing animals and humans (1). Phosphatonins are new hormones playing an important role in the control of Pi homeostasis together with parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D$_3$. Most insight into the underlying mechanisms was established by defining the molecular basis of different inherited disorders that are characterized by an abnormal regulation of Pi homeostasis.

KEY WORDS: phosphate homeostasis; bone metabolism; genetic disorders; phosphatonin.

Phosphate Homeostasis in Humans

The normal plasma Pi concentration in an adult body is 2.5-4.5 mg/dl and men have slightly higher Pi concentration than women, with the majority of Pi being present in bone tissue as a component of hydroxyapatite. Classically, it was well accepted that PTH, 1,25-dihydroxy vitamin D$_3$, and calcium-sensing receptors (CaSR) coordinately regulate calcium homeostasis (2). The type II family of transporters that include NaPi-IIa, IIb and IIc mediates most of the transepithelial Pi transport in the intestine and kidney. The type IIa and IIc are expressed mostly in the apical membrane of the renal proximal tubule, and type IIb exhibits a broader tissue distribution and it play a role in transcellular flux of Pi in the small intestine (3). PTH and 1,25-dihydroxy vitamin D$_3$ are major known regulators of Pi homeostasis. 1,25-dihydroxy vitamin D$_3$ increase the intestinal and proximal convolute and proximal straight tubule (4). PTH inhibits renal Pi reabsorption and current evidence suggest that elevated PTH rapidly reduces the number of NaPi-IIa by inducing surface retrieval and subsequent lysosomal degradation (1, 5).

Phosphatonins

Recent studies have shed new light on the mechanisms that control Pi balance in normal and in various pathological states. In particular, new understanding of X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), and tumor-induced osteomalacia (TIO) has suggested the existence of a novel phosphate regulatory pathway that is independent of the classic mechanisms. This group of factors, collectively termed phosphatonins, have emerged as major regulators of Pi homeostasis and suggest the existence of a network of humoral interactions and feedback loops involving intestine, kidney, parathyroid gland, and bone (6). Phosphatonins have demonstrated the capability to reduce serum Pi via direct inhibition of renal Pi absorption in the proximal tubule and also indirectly via the decrease of 1,25-dihydroxy vitamin D$_3$ synthesis (7). The identification of the key molecules involved in the regulation of Pi homeostasis was largely possible through the molecular definition of rare human disorders (8-11). Human disorders have also been associated with mutation of NaPi-IIa and IIc, FGF23, FGF-receptor (FGFR), Klotho, a co-receptor for FGF23 as well as in dentin matrix protein 1 (DMP1) (12).

HEREDITARY HYPOPHOSPHATEMIC RICKETS WITH HYPERCALCIURIA (HHRH)
The gene involved in the pathogenesis of HHRH is localized on chromosome 9q34 encoding NaPi-IIc. A number of homozygous point mutations, deletion or compound heterozygous mutations of this gene show inactivating mutations in NaPi-IIc causes HHRH. Patients affected by HHRH show bowing of the lower extremities and rickets due to hypophosphatemia as the result of urinary phosphate wasting. In addition, these patients show high levels of 1,25-dihydroxy vitamin D3 with hypercalciuria (12, 13).

HYPOPHOSPHATEMIA, NEPHROLITHIASIS AND OSTEOPOROSIS
Patients with this disease present low level blood Pi due to urinary Pi wasting, and increase levels of 1.25 dihydroxy vitamin D$_3$ and urinary calcium excretion. These patients have mutations in NaPi-IIa gene (14).

DISORDERS LINKED TO FGF-23 ACTIVITY

Autosomal Dominant Hypophosphatemic Rickets
Fibroblast growth factor-23 (FGF-23) appears to be the primary molecule responsible for the regulation of phosphate homeostasis. FGF-23 belongs to the FGF-19 family of growth factors. One common feature of these proteins is their very weak affinity to FGF receptor, suggesting the need for a cofactor to enhance their receptor binding and to allow the initiation of downstream signaling. Recent studies suggest that Klotho protein binds direc-
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tly to multiple FGF receptors, and the Klotho-FGFR complex binds to FGF-23 with much higher affinity than Klotho alone. Thus, Klotho is an essential cofactor for FGF-23 signaling, and the lack of it renders renal cells incompetent for FGF-23 signaling (15). It is highly expressed in bone, with predominant localization in osteocytes, in endothelial cells, thymus, lymphonome and ventral lateral thalamic nuclei (2). In 1997, Dr. Econs described the clinical and radiographic findings for an autosomal dominant form of hypophosphatemic rickets (ADHR) (12, 16). ADHR is caused by FGF-23 gene mutations resulting in high serum levels of protease resistant FGF-23. The FGF-23 gene is localized on chromosome 12p13.5. The hypophosphatemic effects of FGF-23 are exerted through several mechanisms. FGF-23 inhibits renal Pi reabsorption by reducing the apical expression and activity of NaPi-ll in the proximal tubule epithelium (17-20). Under physiological conditions, FGF-23 may exert a continuous negative pressure on NaPi-ll expression, as in FGF-23 knockout mouse, expression and activity of NaPi-ll are abnormally elevated (19). Additionally, FGF-23 reduces intestinal absorption of dietary Pi through a VDR-dependent decrease in NaPi-llb activity. This phenomenon is most likely secondary to FGF-23-mediated reduction of circulating 1,25 dihydroxyvitamin D$_3$ synthesis through suppression of 1a(OH)hydroxylase expression and stimulation of catabolic 24-hydroxyla-

se (18). FGF-23 circulates as the intact protein and as a fragment resulting from proteolysis of the full-length protein. FGF-23 is clea-
ved between arginine 179 ad serine 180 to generate small N-ter-

cinal and C-terminal fragments. The activity of intact FGF-23 is well documented but recent study indicated that also C-terminal fragments of FGF-23 are phosphaturic (1, 21). In addition, a cor-

relation of FGF-23 and PTH has been demonstrated and FGF-23 exerts a negative control on PTH synthesis and secretion and it is a physiologically relevant regulator of PTH (22). The admini-

stration of recombinant FGF-23 leads to an increase in parathy-

roid Klotho levels, allowing the activation of the MAPK pathway (22).

Genetic Disorders Associated with Pi Homeostasis Exchange

X-LINKED HYPOPHOSPHATEMIC RICKETS (XLH)

XLH rickets is a disease due to a mutation in PHEX (phosphate-

regulating gene homologies to endopeptidases on the X chro-

mosome) gene characterized by mineralization Pi homeostasis de-
fects. This familial disorder manifests with hypophosphatemia, low circulating [1,25-dihydroxy-vitamin D$_3$]$_3$ levels, high se-

rum alkaline phosphatase and osteomalacia, and decreased ex-

pression and activity of NaPi-ll in renal proximal tubules (2). PHEX is a membrane-anchored endopeptidase but it substrate is not yet known. Elevated circulating levels of FGF-23 in Hyp mice (a spontaneuous PheX knockout model) suggested that it might re-

present the ‘sought after phosphaturin’ (8). Indeed, FGF-23 knockou

t reveresd hypophosphatemia in Hyp mice, implying that increased plasma FGF-23 levels in Hyp mice and in XLH patients may be at least partially responsible for the phosphate imbalance (19). However PHEX is able to bind to matrix extracellular pho-

psiglycoprotein (MEPE), which belongs to a group of extracel-

lular matrix protein involved in the regulation of bone mineraliza-

tion and protects it from proteolytic cleavage by cathepsin-B (also expressed in osteoblasts). This protection is critical in preven-

nting the proteolytic release of a small, acidic, protease-resistant ASARM peptide (acidic serine-aspartate-rich-MEPE-associated motif), (23) (24) a factor inhibiting bone mineralization in vivo and in vitro, which also affects renal phosphate handling that causes phosphaturia. The role of PheX in protecting the enzymatic release of ASARM peptide goes beyond MEPE binding. Hyp mice de-


mulate increased expression and proteolytic activity of cathe-

psin D, an upstream activator of cathepsin B, and protease inhibi-

tors improve bone mineralization defects in Hyp mice (25). It has been speculated that a continued proteolytic degradation of the extracellular protein matrix, release of small integrin-binding ligand, N-linked glycoprotein (SIBLING) ASARM peptides, and persistent FGF-23-mediated hypophosphatemia (2, 25).

OSTEOGLOPHONIC DYSPLASIA

The most important receptor for FGF-23 is most likely FGF receptor type 1C. This receptor mediates the actions of FGF-23. Osteoglyphonic dysplasia is a rare genetic disorder in humans due to mutations of the gene encoding FGF receptor type 1C (26). These mutations render the receptor constitutively active therefore lea-

ing to a down-regulation of NaPi-ll and ilc. These patients have skeletal abnormalities and low levels of serum Pi with an increa-

se of renal Pi excretion and inappropriately levels of 1-25 di-

hydroxyvitamin D$_3$.

AUTOSOMAL RECESSIVE HYPOPHOSPHATEMIC RICKETS (ARHR)

DM1P is an acidic phosphorylated extracellular matrix protein that was originally identified from a rat incisor cDNA library and thought to have a primary function in the regulation of dentinogen-

esis (8). In 2006, Lorenz-Degiereux et al. (27) identified an ho-

mologous mutator in the DMP1 gene of patientsdiagnosed with autosomal recessive hypophosphatemic rickets (ARHR) charac-

terized by hypophosphatemia and skeletal abnormalities like the phenotypic features of Dmp1-null knockout mice (28). These mice have 8-fold higher circulating level of FGF23 and the potential-

ly pathogenic role of this growth factor in ARHR was demonstrated by Liu et al. (29). The role of DMP1 in matrix mineralization is in-

direct and may be mediated by regulating the osteocyte produc-

tion of FGF-23, which ultimately targets renal phosphate reab-

sorption and vitamin D metabolism. Moreover, DMP1 belongs to the same family of SIBLING proteins as MEPE and is similar to MEPE. Proteolytic degradation of DMP1 leads to the release of the ASARM peptide with potent mineralization and phosphatosis-like activit (2). Patients with DMP1 gene mutations have short long bo-

nes ad osteosclerosis of these long bones. These patients showed either slightly elevated levels of FGF-23 or at least inap-

propriately normal levels of this phosphatogenic hormone (30).

AUTOSOMAL RECESSIVE HYPOPHOSPHATEMIC RICKETS 2

In a cohort of 60 probands with autosomal recessive hypophosphatemic rickets (ARHR2) who were negative for mutation in known hypophosphatemia genes, Lorenz-Degiereux et al. (31) se-

quenced the candidate gene ENPP1 and identified homozygosity for a deletion, missense, and frameshift mutations in 4 families that were not found in 355 controls. The authors found inappropriately elevated plasma FGF23 levels in all 6 patients with ENPP1 mu-

tations and concluded that this is the fourth gene (in addition to PHEX, DMP1, FGF23 itself and FGF1) that, it mutated, causes hypo-

phosphatemic rickets due to elevated FGF23 levels.

TUMORCALCINOSIS

It is a rare genetic disease inherited in an autosomal recessive fa-

shion, characterized by ectopic calcifications around large joints. The soft-tissue lesions of TC are typically lobulated, well demar-

cated calcifications that are most distributed along the external surfaces of large joints (32). Mineral depositions manifest as soft tissue masses especially around hips, shoulders and elbow. The occurrence of the disease is often associated with dental abnor-

malities, ocular involvement with range from angioid streaks to cor-

neal calcification deposits and neuronal calcifications (33). Several genes coding for phosphonins or proteins controlling their ac-

tivities are found involved in the pathogenesis of genetic hypo/hy-

perphosphatemic disorders. In particular genes encoding FGF23 (34-36) or GALNT3 (37-40) and Klotho (41). Animal models with
a deficit in the FGF23 activity are represented by Fgfr23 null mice (42) where the levels of serum intact FGF23 are low, and Klotho/Fgfr23 null mice (43) where serum intact FGF23 is up-regulated in response to the absence of the Klotho/FGFR complex activity (43). These models develop severe hyperphosphatemia starting in early life, due to increased renal Pi tubular resorption; such abnormal Pi homeostasis in the mutant mice affects skeletal mineralization and produces extensive soft tissue calcifications, and the result effect going shortened lifespan. The phenotype of Fgfr23 and Klotho null mice resembles the one of the patients with TC. Gain3-deficient mice have a biochemical phenotype of TC and provide in vivo evidence that Galnt3 plays an essential role in proper secretion of FGF23 in mice. In this model is present a reduction of intact FGF23 secretion, leading to decreased circulating FGF23 and hyperphosphatemia, despite increased FGF23 expression (44). Biochemical abnormalities associated with TC include hyperphosphatemia secondary to increased renal tubular phosphate reabsorption and elevated or inappropriately normal [1-25(OH)]D3 levels. Initial reports described two forms of TC (Familial TC (hyperphosphatemic familial tumoral calcinosis (HFTC), and Hyperphosphatemia/Hyperostosis Syndrome (HHS)) due to GALNT3 gene mutations (45). Recently, Ichikawa S et al. indicated that tumoral calcinosis and hyperostosis-hyperphosphate-

Table 1 - Lists the diseases caused by genetic aberrant function of phosphatonin.

<table>
<thead>
<tr>
<th>Phosphatonin diseases</th>
<th>Responsible gene</th>
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<tr>
<td>Tumoral Calcinosis</td>
<td>GALNT3, FGF23, Klotho</td>
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References

2. Keita PR and Ghishan FK. Recent advances in the renal-skeletal-gut axis that controls phosphorus homeostasis. Laboratory Investigation 2009; 89; 7-14.


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