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 results of these functions, chronic Pi deprivation causes several biological alterations, such as bone demineralization with unmineralized osateoid 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; phosphatonins.

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 HYPECALCIURIA (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 HHRT. 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, NEPHROLITIASIS 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 receptors *in vitro*, 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-

tly to multiple FGF receptors, and the Klotho-FGFr complex binds to FGF-23 with much higher affinity than FGFr or 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 , lymphonode and ventro 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-IIa in the proximal tubule epithelium (17-20). Under physiological conditions, FGF-23 may exert a continuous negative pressure on NaPi-IIa expression, as in FGF-23 knockout mice, expression and activity of NaPi-IIa are abnormally elevated (19). Additionally, FGF-23 reduces intestinal absorption of dietary Pi through a VDR-dependent decrease in NaPi-IIb 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-hydroxylase (18). FGF-23 circulates as the intact protein and as a fragment resulting from proteolysis of the full-length protein. FGF-23 is cleaved between arginine 179 ad serine 180 to generate small N-terminal 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 correlation 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 administration of recombinant FGF-23 leads to an increase in parathyroid 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 chromosome) gene characterized by mineralization Pi homeostasis defects. This familial disorder manifests with hypophosphatemia, low circulating [1,25-dihydroxy-vitamin D_3(1-25OH_2D_3)] levels, high serum alkaline phosphatase and osteomalacia, and decreased expression and activity of NaPi-IIa 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 spontaneous Phex knockout model) suggested that it might represent the sought-after phosphatonin (8). Indeed, FGF-23 knockout reversed 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 phopshoglycoprotein (MEPE), which belongs to a group of extracellular matrix protein involved in the regulation of bone mineralization and protects it from proteolytic cleavage by cathepsin-B (also expressed in osteoblasts). This protection is critical in preventing 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 demonstrate increased expression and proteolytic activity of cathepsin D, an upstream activator of cathepsin B, and protease inhibitors 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. Osteoglophonic dysplasia is 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 leading to a down-regulation of NaPi-IIa and IIc . These patients have skeletal abnormalities and low levels of serum Pi with an increase of renal Pi excretion and inappropriately levels of 1-25 diydroxyvitamin D_3.

AUTOSMAL RECESSIVE HYPOPHOSPHATEMIC RICKETS (ARHR)

DMP1 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 dentinogenesis (8). In 2006, Lorentz-Depiereux et al. (27) identified an homozygous mutations in the DMP1 gene of patients diagnosed with autosomal recessive hypophosphatemic rickets (ARHR) characterized by hypophosphatemia and skeletal abnormalities like the phenotypic features of Dmp1-null knockout mice (28). These mice have 8-fold higher circulating level of FGF-23 and the potentially pathogenic role of this growth factor in ARHR was demonstrated by Liu et al. (29). The role of DMP1 in matrix mineralization is indirect and may be mediated by regulating the osteocyte production of FGF-23, which ultimately targets renal phosphate reabsorption 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 minhibin and phosphatonin-like activit (2). Patients with DMP1 gene mutations have short long bones ad osteosclerosis of these long bones. These patients showed either slightly elevated levels of FGF-23 or at least inappropriately normal levels of this phosphaturic 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-Depiereux et al. (31) sequenced 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 mutations and concluded that this is the fourth gene (in addition to PHEX, DMP1, FGF23 itself and FGFr) that, if mutated, causes hypophosphatemic rickets due to elevated FGF23 levels.

TUMORAL CALCINOSIS

It is a rare genetic disease inherited in an autosomal recessive fashion, characterized by ectopic calcifications around large joints. The soft-tissue lesions of TC are typically lobulated, well demarcated calcifications that are most distributed along the extensor 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 abnormalities, ocular involvement with range from angioid streaks to corneal calcification deposits and neuronal calcifications (33). Several genes coding for phosphatonins or proteins controlling their activities are found involved in the pathogenesis of genetic hypo/hyperphosphatemic 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 Fgf23 null mice (42) where the levels of serum intact FGF23 are low, and Klotho/FGFR 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 sever hypephosphatemia 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 Fgf23 and Klotho null mice resembles the one of the patients with TC. Galnt3-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 associates with TC include hyperphosphatemia secondary to increased renal tubular phosphate reabsorption and elevated or inappropriately normal [1-25$(OH)_2D_3$] levels. Initial reports described two forms of TC [Familial TC (hyperphosphatemic familial tumoral calcinosis (HFTC), and Hypeprphosphatemia/Hyperostosis Syndrome (HHS)] due to GALNT3 gene mutations (45). Recently, Ichikawa S et al. indicated that tumoral calcinosis and hyperostosis-hyperphosphatemia syndrome represent a continuous spectrum of the same disease caused by increased phosphate levels, rather than two distinct disorders (45). Table 1 shows the diseases caused by mutations of genes encoding phosphatonins.

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

Hypophosphatemic diseases	Responsible gene
Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH)	NaPi-IIc
Hypophosphatemia Nephrolitiasis ad Osteoporisis	NaPi-IIa
Autosomal Dominant Hypophosphatemic Rickets (ADHR)	FGF23
X-Linked Hypophosphatemic Rickets (XLH)	PHEX
Osteoglophonic Dysplasia	FGFr
Autosomal Recessive Hypophosphatemic Rickets (ARHR)	DMP1
Autosomal Dominant Hypophosphatemic Rickets 2 (ADHR2)	ENPP1
Hyperphosphatemic diseases	**Responsible gene**
Tumoral Calcinosis	GANT3, FGF23, Klotho

NaPi-IIc: NaPi cotransport type IIc
NaPi-IIa: NaPi contransport type IIa
FGF23: fibroblast growth factor 23
PHEX: phosphate-regulating gene with homologies to endopeptidase on the X chromosome
FGFr: FGF receptor
DMP1: dentin matrix protein 1
ENPP1: ectonucleotide pyrophosphate/phosphodiesterase 1
GALNT3: UDP-N-acetyl-alpha-D-galactosamine:polypeptide N- acetylgalactosminyl transferase 3
Klotho: FGF23 cofactor

Other Phosphatonins

SECRETED FRIZZLED RELATED PROTEIN 4 (sFRP-4)
It is a member of secrete frizzled family and the gene is located on chromosome 7p14.1. It is able to inhibit Na/Pi uptake in vitro (46) by inhibiting Npt2a present in the renal proximal tubule and suppressing the synthesis of 1,25-dihydroxy vitamin D_3 (47). Moreover, sFRP-4 also acts as a minihibin to directly affect bone mineralization and similar to other sFRPs, it can block both canonical and noncanonical Wnt signaling (48).

MATRIX EXTRACELLULAR PHOSPHOGLYCOPROTEIN (MEPE)
It is a protein expressed in bone, salivary gland, dental tissue, bone marrow and brain. The administration of MEPE into normal rats caused a rapid dose-dependent increase of Pi excretion that was attributable to a reduction in absolute and fractional Pi reabsorption by the nephron (1, 49). The role of MEPE in the pathogenesis of XLH was suggested to be due to the upregulation of its mRNA producing elevated circulating MEPE levels in some XLH patients, as well as in Hyp mice (50). Under in normal circumstance MEPE is proteolyzed to release a peptide containing an ASARM sequence, which negatively affects mineralization and Pi uptake. In absence of PHEX (Hyp mice), there is an increase of expression of MEPE and osteoblastic protease and the release of MEPE cause hypophosphatemia and osteomalacia /rickets (1, 50).

FGF-7
It is also known as KGF protein and it is expressed in keratinocytes and several epithelial tissues. FGF-7 is able to inhibit Na/Pi transport in animal kidney cells but not influence the synthesis of vitamina D (51).

Conclusions

Phosphaturic peptides indicated as phosphatonins are a group of hormones important in the regulation of Pi homeostasis. Mutations of the gene coding for these hormones are able to give hypo and hyperphosphatemic disorders. Many aspects of the functional role of phosphatonins are not known and further elucidation to the potential pathways involved in the regulation of Pi and vitamin D in bone and kidney could provide therapeutic target for these disorders.

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