REVIEW ARTICLE

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New data in the control of phosphate balance

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ABSTRACT

Inappropriate renal phosphate transport may alter serum phosphate concentration, bone mineralisation and increase the risk of renal lithiasis or soft tissue calcifications. The molecular identification of renal phosphate transporters and of proteins that regulate their activities have improved our knowledge of the mechanisms that control phosphate balance. In this review we present recent findings on the consequences of mutations affecting several human genes encoding for renal phosphate transporters or for proteins regulating phosphate transport activity. We also describe the role played by the fibroblast growth factor 23 (FGF23)-Klotho axis in phosphate homeostasis and its involvement in the pathophysiology of phosphate disturbances in chronic kidney diseases.

Key-Words:
Phosphate transport; NPT2a; NPT2c; NHERF1; FGF23; Klotho.

INTRODUCTION

Several lines of evidence indicate that inappropriate control of serum phosphate concentration is responsible for severe disorders. Hyperphosphataemia decreases life expectancy[1-3] and hypophosphataemia is associated with bone demineralisation and an increased risk of renal stone occurrence[4]. The identification of genes encoding for renal phosphate transporters or associated proteins, and the discoveries of a new hormone, the fibroblast growth factor 23 (FGF23), and the multifunction protein Klotho have greatly improved our knowledge of the mechanisms that govern phosphate homeostasis. The disruption or the overexpression of the genes encoding these proteins in mice and the identification of mutations in human have emphasised their central role in human phosphate physiology and in the pathophysiology of phosphate disorders, in particular in chronic kidney disease.

THE CENTRAL ROLE OF THE KIDNEY IN PHOSPHATE HOMEOSTASIS

Phosphate is filtered at the glomerulus then reabsorbed almost exclusively in the proximal tubule. The amount of phosphate reabsorbed by the proximal tubule is hormonally regulated and determines, in subjects with normal renal function or moderately reduced glomerular filtration rate (GFR), serum phosphate levels. Two type 2 sodium-phosphate co-transporters, NPT2a (SLC34A1) and NPT2c (SLC34A3), are expressed at the apical domain of renal proximal tubular cells and reabsorb phosphate from the glomerulus filtrate[5,6]. The targeted disruption of the NPT2a gene in mice and the loss of function mutations in the human NPT2a gene

increase urinary phosphate excretion, induce hypophosphataemia and are both associated with renal stone occurrence and / or bone demineralisation, confirming the key role played by this carrier in phosphate homeostasis[7-9].

Although the phenotype of mice with NPT2c gene disruption has not yet been published, mutations in the human NPT2c gene are responsible for hereditary hypophosphataemic rickets with hypercalciuria (HHRH), a disorder close to that observed in patients with NPT2a mutations[10-13].

NPT2a and NPT2c have similar affinities for phosphate but differ in several features. First, their stoichiometry for sodium ion: NPT2a carries three sodium ions with phosphate while NPT2c carries only two[5,14,15]. Second, the hormonal regulation of these two sodium-phosphate co-transporters is not identical (see below). Third, studies carried out in rats suggest that NPT2c is preferentially expressed before weaning age, its expression decreasing thereafter[14]. These differences may explain why the defect in NPT2a function cannot be compensated by NPT2c in later life, although the renal expression of NPT2c is increased in NPT2a-/– mice they still exhibit a profound renal phosphate transport defect[16].

The expression of a third type 2 sodium-phosphate co-transporter mRNA, NPT2b (SLC34A2), has been reported in the kidney[17,18], but this transporter is mainly expressed in the lung and small intestine[18]. The tubular location of NPT2b in the kidney and its role in renal phosphate reabsorption is unknown. Intestinal NPT2b expression is up-regulated by calcitriol[18,19], which may account for the stimulation of intestinal phosphate absorption induced by calcitriol treatment. In the lung, NPT2b seems to play a central role in the reabsorption of phosphate released from phospholipid cleavage. Indeed, mutations of this transporter in humans lead to lung calcifications[20]. Serum phosphate concentration and renal phosphate transport do not seem to be altered in these patients under normal phosphate diet.

Two other types of sodium phosphate co-transporters, type 1 and type 3, are expressed in the kidney. NPT1 (SLC17A1) is expressed at the apical membrane of proximal tubular cells and in the liver. It is a non-specific anionic carrier whose physiological role in phosphate homeostasis is still unknown[21,22].

Type 3 phosphate transporter family is composed of PiT1 (SLC20A1) and PiT2 (SLC20A2). These proteins, initially identified as retrovirus receptors, transport phosphate with a high affinity[23,24]. These transporters are widely expressed, which suggests that they may play an important role in supplying cells with phosphate rather than playing a key role in the regulation of phosphate balance at the body level[23]. Overexpression of PiT1 in cultured vascular smooth muscle cells grown in a high phosphate-rich medium increases cellular calcifications, suggesting that PiTs could be involved in the mechanisms leading to pathological vascular and soft tissue calcifications such as those observed in uraemic patients[25].

The molecules and the mechanisms leading to the reabsorption of phosphate from the lumen to the proximal tubule cells have been almost completely elucidated. Scarce information exists on phosphate transport at the basolateral side of the proximal tubular cell, however.

HORMONAL CONTROL OF RENAL PHOSPHATE TRANSPORT

Parathyroid hormone and its signalling pathway

Parathyroid hormone (PTH) binds to type 1 PTH receptor (PTH1R) in proximal tubular cells, stimulates cAMP synthesis and phospholipase C pathway and decreases renal phosphate transport. PTH induces the retrieval of NPT2a from proximal tubular cell brush border membrane[26]. The effect of PTH on NPT2c is more uncertain. Infusion of PTH in NPT2a-/– mice fails to further decrease renal phosphate transport although NPT2c is expressed in the renal brush border membrane of the proximal tubular cells[16] in these animals,[27,28] suggesting that PTH cannot lower NPT2c expression in this model. By contrast, administration of PTH in thyroparathyroidectomised rats markedly decreases NPT2c expression in renal brush border membrane vesicles[29].

NPT2a needs to be correctly located at the cellular membrane to exert its physiological role. Sev-

eral data indicate that the correct targeting of NPT2a to the apical membrane and the control of its retrieval by PTH require the presence of the sodium-proton exchanger regulatory factor 1 (NHERF1). NHERF1 belongs to the PDZ domain protein family. It contains two PDZ domains that bind to the carboxy-terminal end of NPT2a and PTH1R[30,31]. The targeted disruption of NHERF1 gene in mouse results in a phenotype similar to that observed in NPT2a$^{-/-}$ mice, due to the decrease in NPT2a expression in the renal brush border membranes of proximal tubular cells[32]. The mechanism underlying the decrease in NPT2a in NHERF1$^{-/-}$ mice is complex and may associate abnormal targeting and increased PTH-induced retrieval from the brush border membrane. Sodium-phosphate transport is decreased in NHERF1$^{-/-}$ renal proximal tubule cells in primary culture by comparison with their wild type counterpart, this may be associated with a lower NPT2a membrane abundance[33] suggesting that NHERF1 is mandatory for proper sorting of NPT2a. In contrast, experiments performed on kidney slices showed no difference of phosphate transport between wild type and NHERF1-/– mice[34]. These latter results suggest that the defect in renal phosphate transport of NHERF1-/– mice requires the presence of an extra-renal factor. This factor may be PTH itself. Indeed, in the presence of NHERF protein, the synthesis of cAMP in response to PTH is inhibited in PS120 cells and in opossum kidney cells[31,35]. Interestingly, it has been known for many years that the truncation of the carboxy-terminal region of PTH1R, which is the site of NHERF-PTH1R interaction, enhanced cAMP synthesis but not phospholipase C in response to PTH[36]. The levels of urinary cAMP excretion in NHERF1$^{-/-}$ mice has not been reported, so it is unknown if an increase in cAMP synthesis in response to PTH in the proximal tubule may account for the decrease in NPT2a apical expression. However, we have very recently identified mutations in the PDZ2 and the inter region domain of NHERF1 in humans with renal phosphate loss and nephrolithiasis or bone demineralisation[37]. Urinary cAMP excretion was increased in these patients, contrasting with normal serum PTH concentration and undetectable PTH-related peptide levels. Experiments performed in cultured renal cells showed that these mutations increased PTH-induced cAMP synthesis resulting in a specific inhibition of renal phosphate transport. These mutations in NHERF1 increase the sensitivity of proximal tubule to PTH.

Fibroblast growth factor 23

PTH can increase urinary phosphate excretion but its main role after birth is to maintain serum ionised calcium concentration constant, not serum phosphate concentration, however. PTH releases calcium and phosphate from bone, stimulates calcium reabsorption in the kidney and decreases phosphate reabsoprtion. The dissociation of calcium and phosphate reabsorption allows the increase in plasma ionised calcium concentration.

Hypophosphataemia with inappropriate urinary phosphate excretion can occur in the absence of hyperparathyroidism, suggesting the existence of non-PTH phosphaturic factors. These factors have been identified. The fibroblast growth factor 23 (FGF23) is the better characterised of these factors and we begin to understand its physiological role. FGF23 is a 251 amino-acid peptide synthesised by osteocytes and osteoblasts[38-40], in response to high phosphate intake, hyperphosphataemia or increased serum calcitriol concentration[41-47]. Injection of recombinant FGF23 in animals induces a rapid and marked inhibition of renal phosphate reabsorption resulting in severe hypophosphataemia, bone demineralisation and low serum calcitriol concentration. FGF23 decreases NPT2a and NPT2c mRNA and protein expression in the kidney. It also inhibits 1-alpha hydroxylase expression in the renal proximal tubule and stimulates the 24 hydroxylase, the enzyme that converts calcitriol and 25-OH vitamin D into inactive metabolites[48-56]. Infusion of FGF23 decreases intestinal absorption of phosphate by inhibiting NPT2b expression, which further lowers serum phosphate concentration[53]. This effect on NPT2b is mediated by the reduction of calcitriol levels since it is abolished in mice with disrupted vitamin D receptor gene[57]. Recent findings suggest that FGF23 may control PTH synthesis and secretion. Injection of FGF23 in animals rapidly decreases PTH secretion within 10 minutes through the MAPK pathway[58]. It also inhibits PTH gene expression in parathyroid glands[58]. Furthermore, at variance with its effect in renal proximal tubule, FGF23 dose-dependently increases 1 alpha hydroxylase expression in bovine parathyroid cells[59], which may contribute to reduce PTH gene transcription.

The disruption of FGF23 gene in mouse is associated with hyperphosphataemia, elevated renal

phosphate reabsorption, hypercalcaemia, low serum PTH levels, high concentrations of circulating calcitriol, soft tissue calcifications, accelerated senescence and pulmonary emphysema[60-62]. Similarly, administration of inactivating monoclonal antibodies anti-FGF23 results in hyperphosphataemia and high serum calcitriol levels[63].

Intact FGF23 circulates in the plasma of normal subjects as a 32 kd peptide, which is thought to be the active form. A still unidentified enzyme inactivates FGF23 by cleavage between amino acids 176-179, which results in two peptides that can be detected in the plasma. The site of this cleavage in the body is unknown. A correct glycosylation of FGF23 is important for intact its stability. Indeed, mutations in the glycosylation sites of FGF23 or inactivating mutations of UDP-N-acetyl-alpha-D-galactosamine/polypeptide N acetylgalactosaminyltransferase 3 gene (*GALNT3*), the enzyme responsible of FGF23 O-glycosylation, increase intact FGF23 degradation and result in tumoural calcinosis or hyperostosis–hyperphosphataemia syndrome[64-70]. In these disorders, intact FGF23 plasma concentration is low, contrasting with elevated levels of the c-terminal peptide.

The proper role of hyperphosphataemia and the elevated serum calcitriol concentration in the development of soft tissue calcifications in FGF23-deficient disorders has been considered in several studies. The double knock outs of FGF23 and 1alpha hydroxylase genes in mice or the double disruption of FGF23 and vitamin D receptor genes result in a normal phenotype with normal survival, suggesting that the overproduction of calcitriol is harmful in the absence of FGF23[61,62,71]. However, in these mice, serum phosphate concentration is respectively low or normal which can contribute to the normalisation of the phenotype. Selective normalisations of serum phosphate or calcitriol concentrations by diet show that normal serum phosphate concentration fully rescues the phenotype of $FGF23^{-/-}$ mice, including mortality and soft tissue calcifications while, in hyperphosphataemic animals with normal calcitriol levels, vascular calcifications and survival were improved but not normalised[72]. Similarly, normalisation of serum phosphate concentration in patients with tumoural calcinosis has a marked beneficial effect on soft tissue calcifications[67]. These data suggest a dominant role for phosphate in calcification formation.

Klotho and FGF receptors

The observations that FGF23 can bind with low affinity to multiple FGF receptors, and that inactivation or overexpression of FGF23 results in disorders that alter calcium phosphate homeostasis led researchers to look for an FGF23-specific receptor. Indeed, dysfunctions of fibroblast growth factors or their receptors are associated with abnormal foetal development or cancer occurrence without modification of calcium or phosphate balance. Interestingly, mice with an insertional disruption of the Klotho gene by a transgene resulting in a hypomorphic allele exhibit a phenotype similar to that of FGF23-null mice[60,73]. The complete targeted disruption of the Klotho gene led to an identical phenotype[74]. The Klotho gene encodes a 1014-amino acid long protein with a long extracellular NH2 extremity, a single pass transmembrane domain and a short intracellular carboxy-terminal region. The extracellular domain is composed of two homologous regions named KL1 and KL2. Klotho is expressed at the cell surface but is also present in the plasma as two secreted forms. One of the secreted forms of Klotho results from the shedding of Klotho from the cell surface. This form is made of the KL1 and KL2 domains. The second secreted form of Klotho is due to an alternative RNA splicing in exon 3 that gives a protein of 549 amino acids containing only the KL1 domain. Several data converge to show that Klotho is important for FGF23 function. The transmembrane and the KL1-KL2 secreted forms of Klotho binds to FGF23[75,76]. Injection of an anti-Kotho antibody that abrogates Klotho-FGF23 interaction in mice reproduces the disorders of Klotho and FG23-null mice[76]. Klotho binds to multiple FGF receptors increasing the affinity of FGF receptors for FGF23[75,76]. The Klotho-FGF receptor-FGF23 complex activates the phosphorylation of ERK1/2 and FGF receptor substrate[75,76]. In Klotho deficient or inactivated mice, serum intact FGF23 concentration is increased but is ineffective in controlling serum phosphate levels[77]. In summary, Klotho is a co-receptor that specifically increases the sensitivity of FGF receptors (FGFR) to FGF23.

Klotho is expressed in a limited number of organs: kidney, brain, the pituitary gland, the parathyroid gland, ovary, testis, skeletal muscle, duodenum and pancreas[58,73]. Surprisingly, in the kidney Klotho is not expressed in the proximal tubule but, instead, in the distal tubule[78]. To date, the mechanism by

which FGF23 decreases renal phosphate transporter expression and 1-alpha-hydroxylase and 24-hydroxylase expression in the renal proximal tubule is unknown. The FGFR that mediates FGF23 action in the renal proximal tubule is uncertain. Two types of FGFRs are expressed in proximal tubular cell: type 1 and type 3. Disruption of FGFR1 is lethal in utero. Gain of functions FGFR1 mutations are associated with hypophosphataemia and inappropriate serum calcitriol concentration, but FGF23 levels are elevated, making conclusions difficult[79]. Disruption of FGFR3 gene does not modify phosphate, calcium or calcitriol concentration and does not reverse hypophosphataemia in hyp mice, a model of hypophosphataemia with increased FGF23 concentration[80]. However in this model FGFR3 is associated with an increase in FGF23 production.

The role of Klotho in the renal distal tubule seems to be independent of FGF23.

While co-immunoprecipitation studies indicate that soluble Klotho can bind FGF23[63,76], the function of the circulating forms of Klotho remains to be established.

Klotho isoform that contains KL1 and KL2 has a weak beta-glucuronidase activity[81]. Addition of the extracellular domain of Klotho on cells expressing the calcium ion channel TRPV5 increases calcium entry. This effect is reproduced by a beta-glucuronidase and is due to the retention of TRPV5 in the plasma membrane[82]. The physiological signification of these findings is not completely understood.

Overexpression of Klotho in mice significantly extends life span, represses insulin and insulin like growth factor signalling and increases manganese-superoxide dismutase expression which reduces oxidative stress[83,84]. The calcium-phosphate balance in these mice has not been reported.

ALTERATION OF THE FGF23-KLOTHO AXIS IN HUMAN DISEASES

Role of FGF23 in chronic kidney disease

Serum intact FGF23 concentration increases early when glomerular filtration rate declines[85,86]. In chronic kidney disease, serum FGF23 concentration is correlated with serum phosphate concentration, urinary fractional excretion of phosphate and inversely correlated with serum calcitriol and PTH concentrations[85,87,88]. The early increase in FGF23 levels in chronic kidney disease prevents hyperphosphataemia, by decreasing phosphate absorption in the renal proximal tubule and in the intestine, however by inhibiting the 1-alpha-hydroxylase activity, FGF23 may also generates a secondary hyperparathyroidism[85]. The increase in serum intact FGF23 concentration in chronic kidney disease may also be partially due to impaired FGF23 degradation. The role of the kidney in FGF23 cleavage has not been established, however. High levels of FGF23 concentrations are associated with accelerated degradation of glomerular filtration rate in non-diabetic patients with chronic kidney disease independently of other factors[89]. In dialysis patients, serum FGF23 concentration is markedly elevated and predicts the future development of refractory hyperparathyroidism[90,91]. PTH-induced phosphate release from bone may stimulate FGF23 production in dialysis patients, which in turn controls PTH secretion. Higher levels of serum FGF23 may be necessary to control serum PTH and phosphate concentration in patients who will develop refractory hyperparathyroidism. It is unknown if Klotho expression decreases in the parathyroid glands as observed in the kidney, and if this mechanism might also be implicated in the genesis of refractory hyperparathyroidism.

In the absence of refractory hyperparathyroidism, we have found no correlation between serum FGF23 concentration and bone mineralisation density at several skeletal sites in a population of haemodialysis patients, suggesting the lack of direct effect of FGF23 on bone[88].

Increased serum FGF23 concentrations in dialysis patient are also associated with increased mortality within the first year of haemodialysis[92].

The increased production of FGF23 during the dialysis period may result in an autonomous secretion of FGF23 in some patients. This phenomenon may explain persistent high serum FGF23 levels and the hypophosphataemia observed in many patients following successful renal transplant[93].

Table I

Human genetic disorders associated with inappropriate renal phosphate reabsorption: mechanisms and serum FGF23 concentrations

Disorder	Serum phosphate concentration	Mutated gene	Mechanism	FGF23 concentration
Autosomal Dominant Hypophosphataemic Rickets	low	FGF23	increased stability of FGF23	increased
X-linked hypophosphataemia	low	PHEX	unknown	increased
Autosomal Recessive Hypophosphataemia	low	DMP1	unknown	increased
Mc Cune Albright syndrome	low	GNAS	hypersecretion of FGF23 by bone cells	increased
Familial tumoural calcinosis	high	FGF23	glycosylation defect, instability of FGF23	intact: low c-terminal: increased
hyperostosis–hyperphosphataemia syndrome	high	GALNT3	glycosylation defect, instability of FGF23	intact: low c-terminal: increased
	high	Klotho	resistance to FGF23	intact: increased
Hypophosphataemia with hyperparathyroidism	low	translocation t(9,13) (q21.13;q13.1)	increased Klotho abundance in plasma	increased
Hypophosphataemia with renal lithiasis or bone demineralisation	low	NPT2a	defect of phosphate transport	normal
	low	NPT2c	defect of phosphate transport	normal
	low	NHERF1	hyperreponsiveness of renal proximal tubule to PTH	normal

Various genetic disorders with abnormal serum FGF23 concentrations responsible for hypo or hyperphosphataemia are shown in Table I.

Involvement of Klotho in pathology

The expression of the membrane and KL1 forms of Klotho is decreased in the kidney in patients with chronic renal failure but has not been reported in other organs[94]. The consequences of this decrease on FGF23 action in the kidney are uncertain.

In human, Klotho polymorphisms have been associated with longevity and the risk of cardiovascular calcifications and with bone mineral density in postmenopausal women[95-101].

Klotho is expressed in ovary; recently the levels of mRNA KL1 form of Klotho in epithelial ovarian cancer have been associated with poor survival prognosis in this context[102]. The role of KL1 Klotho in cancer requires further elucidation since KL1 has also anti-tumoural properties. It can suppress IGF type 1 receptor autophosphorylation[83], but can also facilitate tumour by stimulating angiogenesis and inhibiting apoptosis[103-106].

Other bone-derived phosphaturic factors

The matrix extracellular phosphoglycoprotein (MEPE) is 525-amino-acid protein expressed in bone. Its cleavage releases an acid-rich motif peptide (ASARM) located in the C-terminal part of MEPE. ASARM peptide is an inhibitor of bone mineralisation. Administration or overexpression of MEPE induces renal phosphate leak, hypophosphataemia and bone demineralisation[107-109]. Increased levels of ASARM and MEPE peptides and of FGF23 have been reported in humans with X-linked hypophosphataemia (XLH) and in Hyp mice, two disorders due to mutations in Phex gene (phosphate regulating gene with homologies to endopeptidases on the X chromosome)[52,110-112]. The phosphaturic effect of MEPE may be mediated by FGF23 questioning the role of MEPE as a phosphaturic factor. The release of ASARM from MEPE would decrease PHEX expression and activity[113-115], which would inhibit bone mineralisation and increase FGF23 secretion by a still unidentified mechanism[52,87,116]. This view is consistent with the inability of MEPE gene disruption to reverse the phenotype of Hyp mice[116].

Like MEPE, dentin matrix protein 1 (DMP1) belongs to the SIBLING (small integrin-binding ligandN-linked glycoproteins) protein family. Humans with mutation in DMP1 gene and DMP1 null mice exhibit hypophosphataemia, increased excretion of phosphate in urine and elevated FGF23 plasma concentration[117,118]. The mechanism by which inactivation of DMP1 increases FGF23 expression remains to be determined.

CONCLUSION

Our knowledge of the mechanisms that participate in maintaining serum phosphate concentration within the normal range has greatly improved during the last few years. The pathophysiology and consequences of disorders with inadequate renal phosphate reabsorption have been elucidated. The understanding of the genesis of secondary hyperparathyroidism in chronic kidney disease has been modified. Its treatment and prevention will probably benefit from the development of new drugs, interfering with phosphate transporters, hormonal receptors or associated proteins.

Conflict of interest statement. None declared.

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