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PERSPECTIVES IN BASIC SCIENCE

PHEX gene and hypophosphatemia

MARC K. DREZNER

Departments of Medicine and Cellular Biology, Duke University Medical Center, Durham, North Carolina, USA

PHEX gene and hypophosphatemia. X-linked hypophosphatemia (XLH) and tumor-induced osteomalacia (TIO) are diseases that have in common abnormal proximal renal tubular function resulting in increased renal clearance of inorganic phosphorus and hypophosphatemia. The recent discovery of the PHEX gene has provided new insights to these disorders. In this regard, identification of the PHEX gene product as a membrane-bound endopeptidase suggests that the pathophysiologic cascade underlying XLH likely involves inactivation mutations of the gene causing a failure to clear an active hormone, phosphatonin, from the circulation. The presence of this hormone through unknown mechanisms decreases the sodium-dependent phosphate cotransporter in the kidney, resulting in impaired phosphate transport. In contrast, TIO likely evolves secondary to tumor overproduction of the putative phosphatonin, which exerts physiologic function despite efforts to counteract the resultant hypophosphatemia with overproduction of PHEX transcripts that are insufficient to accommodate the enhanced substrate load. These potential pathophysiologic mechanisms for XLH and TIO provide valuable inroads to understanding phosphate homeostasis, as well as vitamin D metabolism, bone mineralization, and calcium metabolism.

X-linked hypophosphatemia (XLH) and tumor-induced osteomalacia (TIO) are examples of rachitic and osteomalacic disorders in which phosphate depletion predominates. Both of these diseases have abnormal proximal renal tubular function in common, which results in an increased renal clearance of inorganic phosphorus and hypophosphatemia. In addition, the disorders are characterized by low or inappropriately normal serum 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] levels and defective bone mineralization [1, 2]. Until recently, the pathophysiologic mechanisms underlying these diseases have remained poorly understood. However, the recent discovery of the PHEX gene [3] has provided new insight into these disorders and revealed possible alternative regulatory mechanisms for phosphate homeostasis, bone mineralization, and vitamin D metabolism. This review focuses on describing the impact that the discovery of the PHEX gene has had on the understanding of the pathophysiology of XLH and TIO and the modulation of fundamental processes operative in kidney and bone.

Key words: rickets, osteomalacia, vitamin D, phosphatonin, bone mineralization.

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PHENOTYPIC CHARACTERISTICS OF X-LINKED HYPOPHOSPHATEMIA AND TUMOR-INDUCED OSTEOMALACIA

X-linked hypophosphatemia occurs as an X-linked dominant disorder with complete penetrance of a renal tubular abnormality resulting in phosphate wasting and consequent hypophosphatemia. It is the prototypic renal phosphate wasting disorder, characterized in general by progressively severe skeletal abnormalities and growth retardation. The *hyp*-mouse model harbors a homologous mutation and is an excellent mimic of the human disease. Indeed, much of our understanding of the pathophysiology of XLH derives from studies of the murine homologue.

In contrast, TIO is a sporadic condition characterized by remission of the unexplained bone disease after resection of a coexisting tumor. The tumors have been of mesenchymal origin in the large majority of patients. However, the recent observation of TIO concurrent with breast carcinoma, prostate carcinoma, oat cell carcinoma, small cell carcinoma, multiple myeloma, and chronic lymphoctytic leukemia indicates that the disease is likely secondary to a variety of tumors, including those of epidermal and endodermal derivation.

Quite remarkably, these diseases of notably different genesis have amazingly similar clinical presentations. This has led to much speculation about links between the pathophysiologic basis for the disorders.

Clinical features

The clinical expression of the XLH is widely variable, ranging from a mild abnormality, the apparent isolated occurrence of hypophosphatemia, to severe rickets and/or osteomalacia [4]. In children, the most common clinically evident manifestations include short stature and limb deformities. The majority of children with the dis-

ease exhibit enlargement of the wrists and/or knees secondary to rickets, as well as bowing of the lower extremities. Additional signs of the disease may include late dentition, tooth abscesses secondary to poor mineralization of the interglobular dentine, and premature cranial synostosis. Many of these features do not become apparent until the age of 6 to 12 months or older [5]. In spite of marked variability in the clinical presentation, bone biopsies in affected children and adults invariably reveal low turnover osteomalacia without osteopenia. The severity of the bone disorder has no relationship to gender, the extent of the biochemical abnormalities, or the severity of the clinical disability [6]. In untreated youths and adults, the serum 25(OH)D levels are normal and the concentration of $1,25(OH)_2D$ is in the low to normal range [7–9]. The paradoxical occurrence of hypophosphatemia and normal serum calcitriol levels is due to aberrant regulation of renal 25(OH)D-1α-hydroxylase activity. Studies in *hyp*- and *gy*-mice, the murine homologues of the human disease, have established that defective regulation is confined to the enzyme localized in the proximal convoluted tubule, the site of abnormal phosphate transport [10–13].

Patients with TIO usually present with bone and muscle pain, muscle weakness, and, occasionally, recurrent fractures of long bones. Additional symptoms common to younger patients are fatigue, gait disturbances, slow growth, and skeletal abnormalities, including bowing of the lower extremities. The duration of symptoms before diagnosis ranges from 2.5 months to 19 years, with an average of >2.5 years. The age at diagnosis is generally the sixth decade, with a range of 7 to 74 years. Approximately 20% of the patients are younger than 20 years at presentation [2]. The biochemical abnormalities of the disorder include hypophosphatemia and an abnormally low renal tubular maximum for the reabsorption of phosphorus per liter of glomerular filtrate (TmP/GFR), indicative of renal phosphate wasting. The serum phosphorus values range from 0.7 to 2.4 mg/dL. Additional abnormalities include gastrointestinal malabsorption of phosphorus, which, coupled with renal phosphorus wasting, results in a negative phosphorus balance. Serum 25(OH)D is normal and serum $1,25(OH)_2D$ inappropriately normal relative to the hypophosphatemia [2]. Aminoaciduria, most frequently glycinuria, and glucosuria are occasionally present. Radiographic abnormalities include generalized osteopenia, pseudofractures, and coarsened trabeculae, as well as widened epiphyseal plates in children.

Physiology

Investigators generally agree that the primary inborn error in XLH results in an expressed abnormality of the renal proximal tubule that impairs phosphate reabsorption. This defect has been indirectly identified in affected patients and directly demonstrated in the brush border membranes of the proximal nephron in *hyp*-mice. Until recently, whether this renal abnormality is primary or secondary to the elaboration of a humoral factor has been controversial. In this regard, the presence of a primary renal abnormality is supported by the observations that primary cultures of renal tubule cells from *hyp*-mice exhibit a persistent defect in renal phosphate transport [abstract; Gutteridge et al, *J Bone Miner Res* 5(Suppl 1): S205, 1990] [14], likely caused by decreased expression of the Na^+-phosphate cotransporter (NPT-2) mRNA and immunoreactive protein [15–17]. In contrast, transfer of the defect in renal phosphate transport to normal and/or parathyroidectomized normal mice parabiosed to *hyp*-mice implicated a humoral factor in the pathogenesis of the disease [18, 19]. Current studies, however, have provided compelling evidence that the defect in renal phosphate transport in XLH is secondary to the effects of a circulating hormone or metabolic factor. Thus, immortalized cell cultures from the renal tubules of *hyp* and *gy* -mice exhibit normal Na^+-phosphate transport, suggesting that the paradoxical effects observed in primary cultures may represent the effects of impressed memory and not an intrinsic abnormality [20, 21]. Moreover, the report that cross-transplantation of kidneys in normal and *hyp* -mice results in neither transfer of the mutant phenotype nor its correction unequivocally established the humoral basis for XLH [22]. Subsequent efforts, which resulted in localization of the gene encoding the Na^+-phosphate cotransporter to chromosome 5, further substantiated the conclusion that the renal defect in brush-border membrane phosphate transport is not intrinsic to the kidney in XLH [23]. Although these data establish the presence of a humoral abnormality in XLH, the identity of the putative factor, the spectrum of its activity, and the mechanism by which it functions have not been definitively elucidated. Regardless, preliminary reports suggest the production of a phosphaturic factor by *hyp*-mouse osteoblasts and marrow mesenchymal cells maintained in culture (abstract; Nesbitt et al, *J Bone Miner Res* 12:S113, 1997) [24]. These studies argue that a circulating factor, phosphatonin, may play an important role in the pathophysiologic cascade responsible for XLH.

There is strong evidence that a humoral factor produced by the tumor is likewise responsible for TIO. This possibility has been supported by: (*a*) the presence of phosphaturic activity in tumor extracts from three of four patients with TIO (abstract; Lau et al, *Clin Res* 27: 421A, 1979) [25, 26]; (*b*) the occurrence of hypophosphatemia and increased urinary phosphate excretion in heterotransplanted tumor-bearing athymic nude mice [27]; (*c*) the inhibition of phosphate uptake in opossum kidney cells by conditioned medium collected from cultured tumor cells obtained from affected patients [28–31]; (*d*) the demonstration that extracts of the heterotransplanted

tumor inhibited renal 25-hydroxyvitamin D-1α-hydroxylase activity in cultured kidney cells [29]; and (*e*) the coincidence of aminoaciduria and glycosuria with renal phosphate wasting in some affected subjects, indicative of complex alterations in proximal renal tubular function [32]. Indeed, partial purification of "phosphatonin" from a cell culture of a sclerosing hemangioma causing TIO has reaffirmed this possibility [30]. These studies reveal that the putative phosphatonin may be a peptide with molecular weight of 8 to 25 kd, which does not alter glucose or alanine transport but inhibits sodium-dependent phosphate transport. However, recent studies that document the presence in various disease states of additional phosphate transport inhibitors [33] indicate that the TIO syndrome is heterogeneous and "phosphatonin" may be a family of hormones. In this regard, Rowe et al have reported that screening of conditioned medium from the tumor cells of an affected patient, using an antiserum raised preoperatively, and subsequent Western analysis revealed the presence of two proteins of 56 and 58 kd [28]. More recently, they have successfully extracted mRNA from a tumor in an affected patient and cloned a novel gene that codes for a protein of over 430 residues with N-glycosylation motifs and glycosaminoglycan attachment sites. Characteristics of the predicted protein are consistent with coding of a hydrophilic circulating protein with two small localized regions of hydrophobicity (abstract; Rowe, *Bone* 23:S653, 1998). Similar to XLH, the mechanism of action by which phosphatonin functions in TIO remains unknown. Although there is no evidence that the factor is parathyroid hormone, parathyroid hormone-related protein, or the recently described phosphate regulator, human stanniocalcin, recent observations suggest that parathyroid hormone/parathyroid hormone-related protein receptors, in some cases, may modulate the activity of the factor.

CLONING OF THE PHEX GENE

Efforts to better understand XLH have more recently included attempts to identify the genetic defect underlying this disease. In 1986, Read et al [34] and Machler et al [35] reported linkage of the DNA probes DXS41 and DXS43, which had been previously mapped to Xp22.31-p21.3, to the *HYP* gene locus. In subsequent studies, Thakker et al [36] and Albersten et al (abstract; Ninth Workshop on Human Gene Mapping #401, p 317, 1987) reported linkage to the *HYP* locus of additional polymorphic DNA, DXS197, and DXS207 and, using multipoint mapping techniques, determined the most likely order of the markers as Xpter-DXS85-(DXS43/DXS197)-*HYP*-DXS41-Xcen and Xpter-DXS43-*HYP*-(DXS207/DXS41)-Xcen, respectively. The relatively small number of informative pedigrees available for these studies prevented a definitive determination of the genetic map along the Xp22-p21 region of the X-chromosome and only allowed the identification of flanking markers for the *HYP* locus 20 centimorgans (cM) apart. More recently, the independent and collaborative efforts of the HYP consortium resulted in the study of some 13 multigenerational pedigrees and consequent refined mapping of the Xp22.1-p21 region of the X chromosome, identification of tightly linked flanking markers for the *HYP* locus, construction of a YAC contig spanning the *HYP* gene region, and eventual cloning and identification of the disease gene as PHEX, a PHosphate-regulating gene with homologies to Endopeptidases located on the X chromosome. In brief, these studies ascertained a locus order on Xp22.1 of: Xcen-DXS451-(DXS41/DXS92)-DXS274-DXS1052-DXS1683-*HYP*-DXS7474-DXS365-(DXS443/DXS3424)-DXS257-(GLR/DXS43)-DXS315-Xtel.

Moreover, the physical distance between the flanking markers, DXS1683 and DXS7474, was determined as 350 kb, and their location on a single YAC was ascertained. Subsequently, a cosmid contig spanning the *HYP* gene region was constructed, and efforts were directed at discovering deletions within the *HYP* region. Identification of several such deletions permitted characterization of cDNA clones that mapped to cosmid fragments in the vicinity of the deletions. Database searches with these cDNAs detected homologies at the peptide level to a family of endopeptidase genes that includes neutral endopeptidase (NEP), endothelin-converting enzyme-1 (ECE-1), and the Kell antigen. These efforts clearly established PHEX as the candidate gene responsible for XLH [37–41].

Subsequent studies determined that the human PHEX gene consists of 22 exons that encode a 749 amino acid protein. However, PHEX gene expression as a 6.6 kb transcript in bone, adult ovary, and fetal lung, as well as adult lung and fetal liver [42–45] indicate that only 35% of the PHEX mRNA contains the 2247 base pairs coding sequence, with the remaining 65% representing untranslated regions. Further investigations have unequivocally established that deactivating mutations of the PHEX gene underlie the phenotypic expression of XLH. In this regard, extensive mutational analysis of some 170 families with XLH reveal a range of defects in the PHEX gene that include nonsense, deletions, duplications, insertions, deletional insertions, splice site, and missense mutations. Moreover, the mutations involve almost the entire length of the gene, and the majority, if translated, will result in a functional loss of the PHEX protein activity [46–49]. Interestingly, of all the PHEX abnormalities reported to date, only one has involved the putative intracellular domain, and none has involved the putative transmembrane region. Somewhat unexpectedly, PHEX mutations have not been found in a significant percentage of affected probands. This may result from study of only a fraction of the mRNA transcript (approximately

33%), limited investigation of the 5′-untranslated region and gene promoter or from misdiagnosis, and confusion of X-linked recessive hypophosphatemia or autosomal dominant hypophosphatemia for XLH.

POSSIBLE ROLES FOR PHEX AND PHOSPHATONIN IN THE PATHOGENESIS OF X-LINKED HYPOPHOSPHATEMIA AND TUMOR-INDUCED OSTEOMALACIA

Although mutations in the PHEX gene clearly underlie XLH, the pathogenesis of the disorder is not immediately apparent. Nevertheless, several observations suggest the likely cascade of events that result in the primary abnormalities characteristic of the syndrome.

First, the role of PHEX in XLH must explain the X-linked dominant expression of the disorder with little, if any, gene dosage effect. In this regard, it is likely that mutations in the PHEX gene result in an haploinsufficiency defect, in which one half the normal gene product in females (or null amounts in males) results in the phenotype. The alternative possibility that the PHEX gene results in a dominant negative effect is unlikely because, inconsistent with this prospect, several mutations reported in affected humans [49] and the murine *Gy* mutation almost certainly result in the lack of message production [50].

Second, as noted earlier in this article, despite the absence of PHEX expression in kidneys, in *hyp*- and *gy*-mice, evidence suggests that the proximate cause of renal phosphate wasting is a decreased concentration of the renal sodium-dependent phosphate cotransporter. Hence, the PHEX mutation must serve to regulate the expression of this transporter indirectly.

Third, the parabiosis data and renal cross-transplantation studies in *hyp*-mice discussed previously support the possibility that the pathophysiology of XLH involves elaboration of a humoral phosphate-wasting factor, phosphatonin. Because PHEX codes for a membrane-bound enzyme, it is clear that the PHEX protein is not phosphatonin. However, it is possible that the inactivating PHEX mutations play a role in regulating the concentration of phosphatonin, which, in turn, controls the expression of the renal sodium-dependent phosphate cotransporter.

With these considerations in mind, Figure 1 illustrates the most plausible pathophysiologic basis for XLH. In this cascade of events, an inactivating mutation of PHEX produces inadequate amounts of the PHEX endopeptidase, resulting in ineffective or inadequate degradation/inactivation of phosphatonin and circulation of excessive amounts of this protein, consequent repressed expression of the sodium-dependent phosphate cotransporter, renal phosphate wasting, and hypophosphatemia. Although this postulate is consistent with most available data, it does conflict with the failure of parabiosis of normal and *hyp*-mice to rescue the mutant phenotype. Although several explanations for this discrepancy are possible, further data are necessary to resolve the incongruity. Alternatively, it is possible that PHEX functions in a different fashion to regulate phosphatonin activity. In this regard, the gene may function indirectly to inhibit the expression of phosphatonin, and an inactivating mutation would result in phosphatonin overexpression, leading to renal phosphate wasting. In any case, further work by several groups indicates that the osteoblast is the functional locus for the abnormalities underlying XLH (abstract; Nesbit et al, *J Bone Miner Res* 12:S113, 1997) [51, 52]. To this end, these data illustrate that the osteoblast is a unique source of phosphatonin (Fig. 1) produced in a developmentally dependent fashion. Moreover, differential phosphatonin activity in *hyp*-mouse osteoblasts is contingent on PHEX expression in normal cells. Thus, with the appearance of significant PHEX production in normal cells, decreased phosphatonin activity is observed. In contrast, with loss of PHEX function in *hyp*-mouse osteoblasts, stable phosphaturic activity is maintained.

In TIO, the interplay between phosphatonin and PHEX is remarkably different in spite of the notably similar phenotypic expression of this tumor-associated syndrome and XLH. As noted previously in this article, in patients with TIO, the hyperphosphaturia that characterizes the syndrome is most likely the consequence of unregulated and excessive elaboration of a phosphaturic factor by the tumor, likely phosphatonin. However, wild-type PHEX transcripts are expressed in relative overabundance in tumors from affected patients in contrast to the loss of gene function manifest in subjects with XLH [53]. This probably reflects a response of PHEX expression to the hypophosphatemia or the primary phosphatonin overabundance. Recent studies favor the latter because phosphate depletion in animal models fails to alter PHEX expression (abstract; Meyer and Meyer, *Bone* 23:S545, 1998). In any case, the increased PHEX transcripts may be insufficient to accommodate the enhanced substrate load, resulting in abnormally high circulating levels of the active phosphaturic hormone (Fig. 2). Regardless, the presence of high levels of PHEX expression in tumors of osteoblast lineage is consistent with the intrinsic osteoblast defect postulated to exist in XLH.

ROLE OF PHEX IN THE PHYSIOLOGIC REGULATION OF KIDNEY AND BONE FUNCTION

Although the studies related previously firmly define the role of PHEX in the pathogenesis of XLH and TIO, little information is available regarding the possible function of this gene in regulating physiologic activities. However, several recent studies suggest that PHEX may play

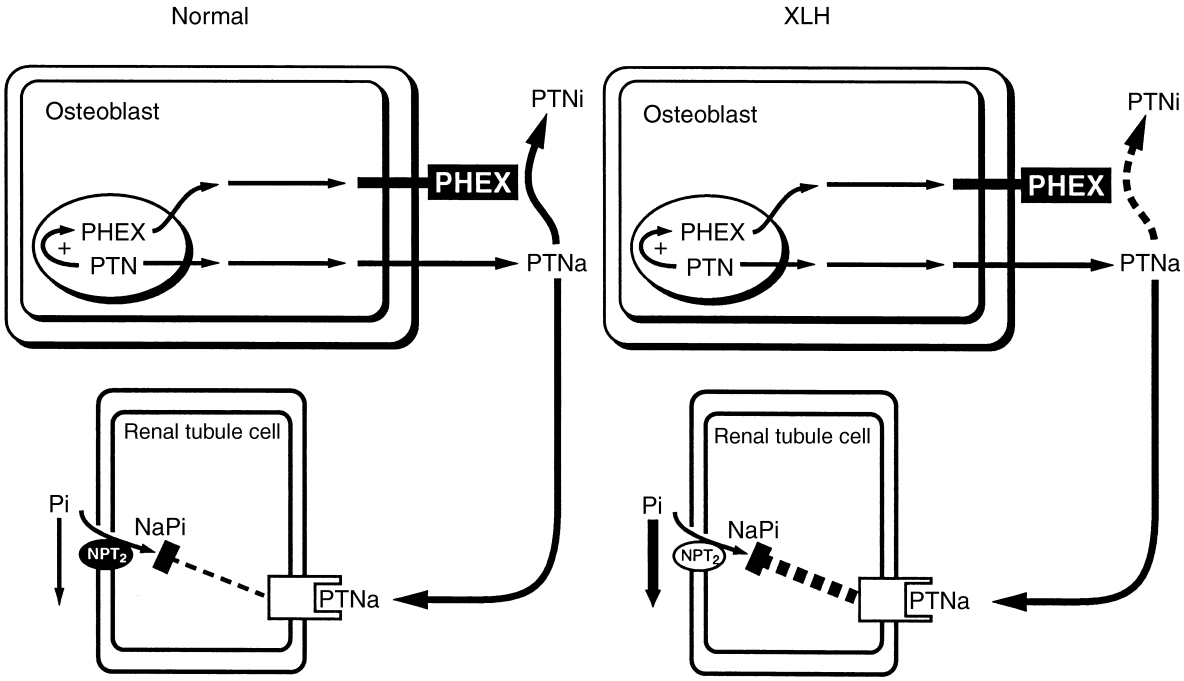

Fig. 1. Pathophysiologic basis for X-linked hypophosphatemia (XLH). Under normal conditions, the osteoblast produces PHEX and phosphatonin (PTN), a circulating phosphaturic hormone. The PHEX protein, a membrane-bound endopeptidase, degrades (solid arrow) a substantial quantity of the active phosphatonin (PTNa) to an inactive metabolite (PTNi). The remaining circulating active hormone interacts with a renal tubule cell receptor that, by unknown mechanisms and to a small degree downregulates (dotted arrow) the sodium-dependent phosphate cotransporter (NPT2), thereby minimally compromising the transport of phosphate. The inactivation of PTN by PHEX is crucial to limiting the effects of PTNa on the transport of phosphate and consequently urinary phosphate (Pi) excretion. In XLH-defective PHEX fails to inactivate (dotted arrow) the majority of PTNa. Thus, excessive PTNa interacts with the renal receptor and markedly decreases (dotted arrow) NPT2 mRNA and protein content (the marked decrease indicated by the open circle compared with the closed circle under normal conditions). The resultant limited phosphate reabsorption is reflected by substantial urinary phosphate wasting.

a role in physiologic regulation of bone mineralization and vitamin D metabolism, as well as phosphate homeostasis.

Bone mineralization

Recent studies of Xiao et al suggest that PHEX may contribute to the regulation of bone mineralization [52]. They reported that the abnormal mineralization in *hyp*-mice is due, at least in part, to an intrinsic osteoblastic defect associated with abnormal PHEX function. In this regard, they found that mutant osteoblasts fail to mineralize under culture conditions supporting mineralization in normal osteoblasts. More important, they discovered that the *hyp*-mouse osteoblasts produce a factor(s) that is capable of regulating the mineralization of the extracellular matrix. Hence, the mineralization defect observed in mutant cells is transferable to normal osteoblasts in coculture experiments. Because a physiologically relevant site of PHEX expression is the osteoblast, it is likely that production of this mineralization inhibitor is the result of the inactivating mutations of PHEX. Indeed, dysfunction of the gene product may result in failure to degrade an endogenously synthesized but undefined inhibitor of mineralization that is a substrate of PHEX. Of course, this substrate may be phosphatonin, but further studies are necessary to characterize the mineralization inhibitor.

These observations provide new insights into novel factors that regulate bone mineralization. By extrapolation, it is reasonable to believe that under normal conditions, PHEX regulates an osteoblast-derived factor, which, in turn, modulates mineralization of the extracellular matrix. Conceivably, upregulation of PHEX in the setting of (phosphatonin-mediated) hypophosphatemia may reduce the concentration of such a factor and facilitate mineralization despite inadequate circulating phosphate. Alternatively downregulation of PHEX secondary to vitamin D or hyperphosphatemia may increase the mineralization inhibitor and appropriately limit bone mineralization. Of course, much additional investigation is necessary to validate these events. However, current studies clearly indicate that PHEX may play an important, but poorly appreciated, role in the regulation of bone mineralization and may serve to protect the integrity of mineralization during states of mineral deprivation or excess.

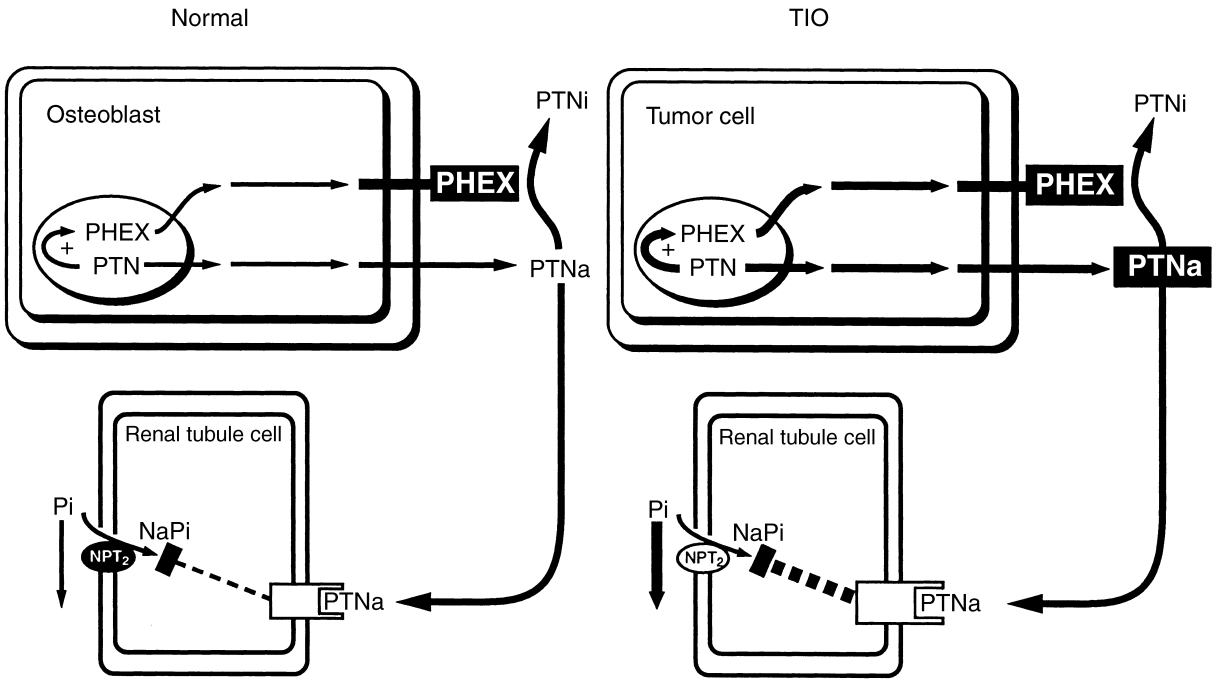

Fig. 2. Pathophysiologic basis for tumor-induced osteomalacia (TIO). TIO tumor cells, generally of mesenchymal origin, produce active phosphatonin (PTNa) in excess (bold arrows). The increased PTN production, through a presumed feedback mechanism (bold arrow), enhances PHEX production. However, the overproduction of active phosphatonin (PTNa) exceeds the capability of PHEX to degrade sufficient amounts of the product to an inactive product (PTNi). Hence, in spite of enhanced PHEX, an overabundance of PTNa circulates to the kidney where interaction with the receptor decreases (dotted arrow) the sodium-dependent phosphate cotransporter (NPT2) mRNA and protein production, thereby limiting phosphate (Pi) transport and resulting in phosphate wasting.

Vitamin D metabolism

For several decades, investigators have considered the serum phosphorus concentration an important factor that regulates $1,25(OH)_2D$ production. In this regard, oral phosphate depletion and hypophosphatemia and oral phosphate loading and hyperphosphatemia stimulate and suppress renal 25(OH)D-1α-hydroxylase activity, respectively. However, over the past several years, a growing body of evidence suggests that the effects of an altered serum phosphorus concentration on enzyme function are linked to alterations in renal phosphate transport. Perhaps the most compelling evidence in this regard is the recognition that altered serum phosphorus levels secondary to abnormalities of renal phosphate transport paradoxically influence renal enzyme activity. Among the data favoring this possibility are the following:

- Patients with XLH and TIO, with phosphatonin-dependent renal phosphate wasting, exhibit impaired enzyme activity and circulating $1,25(OH)_2D$ levels inappropriately low for the prevailing serum phosphorus concentration [7–9].
- Murine homologues of XLH manifest abnormally regulated 25-hydroxyvitamin D-1α-hydroxylase activity in the proximal convoluted tubule [10, 11], the site of abnormal phosphate transport, but normal enzyme activity in the proximal straight tubule [12], in which normal phosphate transport prevails.
- Additional human diseases marked by defective renal phosphate transport, including Fanconi's syndrome, autosomal dominant hypophosphatemia and adult-onset hypophosphatemia, likewise display impaired enzyme activity and circulating $1,25(OH)_2D$ levels inappropriately low for the prevailing serum phosphorus concentration [54].
- Patients with tumoral calcinosis and enhanced renal phosphate transport exhibit apparent increased 25-hydroxyvitamin D-1α-hydroxylase activity and elevated circulating $1,25(OH)_2D$ levels inappropriately high for the prevailing hyperphosphatemia [54].

Although these data clearly favor a role for altered renal phosphate transport in the regulation of $1,25(OH)_2D$ production, recent observations challenge this possibility. In this regard, investigators have reported normally regulated 25-hydroxyvitamin D-1α-hydroxylase activity in NPT2 knockout mice (abstract; Portale et al, *Bone* 23:S364, 1998). These animal models exhibit hypophos-

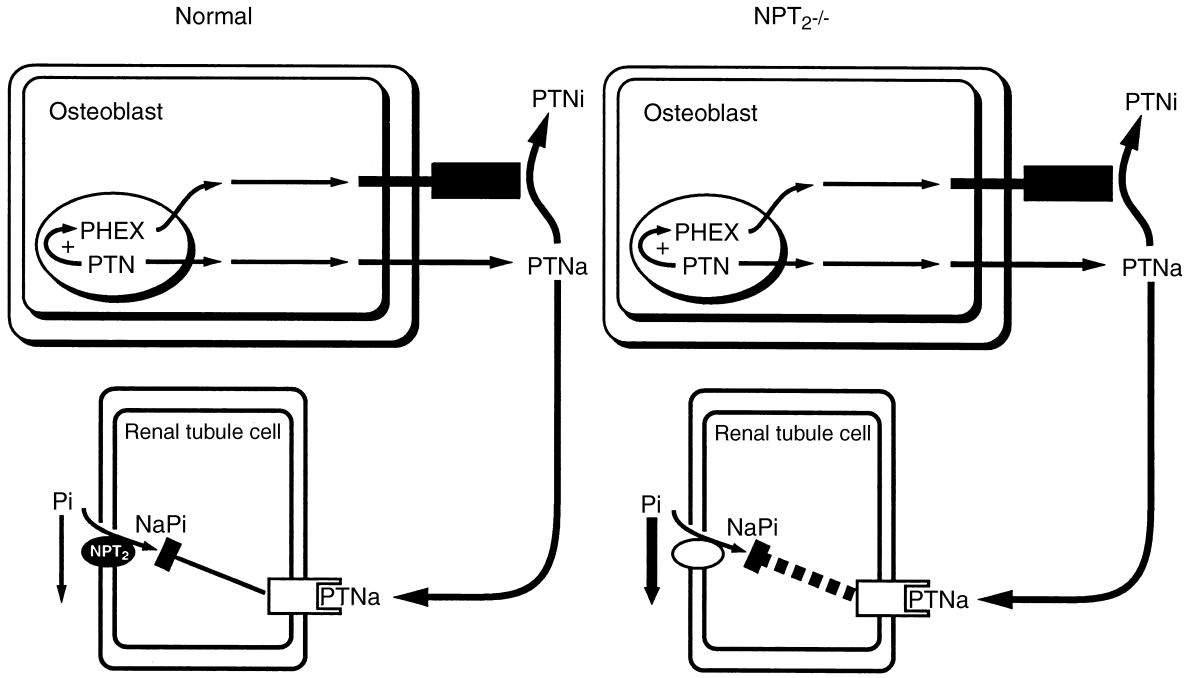

Fig. 3. Pathophysiologic basis underlying abnormal phosphate homeostasis in the Na^+-phosphate cotransporter (NPT2) knockout mouse (NPT2−/−). In the NPT2−/− mouse, the production of PHEX and phosphatonin (PTN) proceed normally and a limited amount of active phosphatonin (PTNa) escapes degradation to an inactive metabolite (PTNi). However, the genetic defect in this mouse results in an obliteration (empty circle) of the sodium-dependent phosphate cotransporter (NPT2) in the absence of an enhanced PTNa load. Thus, phosphate (Pi) transport is compromised, and urinary phosphate wasting ensues.

phatemia secondary to the NPT2 protein deficiency and consequent renal phosphate wasting. This defect is remarkably similar to the abnormality underlying hypophosphatemia in XLH (Fig. 3). However, in XLH and in the *hyp*-mouse, abnormally regulated enzyme activity prevails. Although the reason for this evident disparity is unclear, it seems plausible that the defective enzyme function in XLH and the murine homologue results from a PHEX-dependent event. To this end, the presumptive PHEX-dependent phosphatonin excess underlying the disease may negatively impact renal $1,25(OH)_2D$ production directly and independent of effects on NPT2, renal phosphate reabsorption, and circulating phosphate levels. Clearly, further investigations are essential to test this possibility. However, the plausibility of the hypothesis is enhanced by the recent observation that $1,25(OH)_2D$ decreases PHEX expression in osteoblasts (abstract; Escarot and Desbarats, *Bone* 23:S181, 1998). Such an effect may be part of a feedback loop in which excess $1,25(OH)_2D$ suppresses PHEX expression, which consequently results in enhanced phosphatonin levels, decreased 25(OH)D-1α-hydroxylase activity, and diminished $1,25(OH)_2D$ production, correcting the primary abnormality. Currently, several laboratories are investigating this possibility.

Hormonal regulation

Although most studies to date link PHEX with only regulation of the putative phosphate-regulating hormone, phosphatonin, recent data suggest that the PHEX gene may have a more profound impact on hormonal function. In this regard, important observations to date include the following:

- The deduced structure of PHEX, including the preservation of the catalytic glutamate and histidine residues (equivalent to Glu^{648} and His^{711} of the related membrane-bound endopeptidase, neprilysin), argues strongly that the protein has protease activity [54].
- In addition, the wide range of PHEX mutations in patients with XLH, which aligns with regions required for peptidase activity in neprilysin, suggests that PHEX indeed functions as a protease [54].
- Lipman et al have reported the unexpected finding that PHEX effectively degrades parathyroid hormone, indicating that the enzyme is rather promiscuous in its substrate specificity [54].

Such information raises the possibility that under physiologic conditions, PHEX functions to modulate parathyroid hormone bioavailability and bioactivity, particularly at the level of the osteoblast. In addition, the

protein may enzymatically influence other hormonal and paracrine/autocrine effects produced by osteoblasts and involved in regulating osteoblast maturation, as well as mineralization and phosphate reabsorption (discussed previously in this article). Clearly, further work is necessary to clarify these issues, but current trends suggest that PHEX may modulate calcium and phosphorus metabolism through hormonal and metabolic pathways previously poorly appreciated.

Phosphate homeostasis

The recognition that PHEX plays a central role in the pathogenesis of XLH, a disease in which aberrant phosphate homeostasis is primary, has led to a study of the possibility that PHEX is important in the physiological regulation of phosphate balance. Although a host of conflicting data have emerged, several potentially important preliminary observations continue to fuel the speculation that PHEX does regulate phosphate homeostasis. These include the following:

- An age-dependent decline in PHEX may underlie a similar decrease in NPT2 expression and the consequent renal phosphate loss, as well as the long-recognized decline of the serum phosphorus concentration with age in mammals (abstract; Morita et al, *Bone* 23:S652, 1998).
- Phosphate depletion, unlike phosphatonin (discussed previously), has no apparent effect on PHEX expression (abstract; Meyer and Meyer, *Bone* 23:S545, 1998) but appears to regulate NPT2 expression (abstract; Morita et al, *Bone* 23:S328, 1998), suggesting that a series of complex short feedback loops interact in the regulation of phosphate homeostasis.

Many additional studies are currently underway to confirm these findings and to elucidate further a possible role for PHEX in the regulation of phosphate balance. Currently, it seems plausible to assume that such a purpose for PHEX will be identified in the near future.

CONCLUSION

Over the last several years, study of the genetic disease, XLH, and the acquired disorder TIO has provided new insight into regulation of calcium and phosphorus balance. With the discovery of PHEX and the recognition of a putative substrate, phosphatonin, the pathogenesis of these diseases has become more certain. Perhaps more important, however, a series of important observations has set the stage for integrating PHEX function into the physiologic regulation of many processes that regulate calcium and phosphorus metabolism at the bone and kidney. The next decade promises exciting advances in this most important area of study.

Reprint requests to Marc K. Drezner, M.D., Department of Medicine, Box 3285, Duke University Medical Center, Durham, North Carolina 27719, USA.
E-mail: drezn@mc.duke.edu

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