Evidence for a bone-kidney axis regulating phosphate homeostasis

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A novel circulation phosphaturic hormone is postulated to regulate systemic phosphate homeostasis. Two new studies (see the related articles beginning on pages 683 and 785) reveal that the phosphaturic factor FGF-23 is increased in hypophosphatemic subjects with McCune-Albright syndrome and that secreted frizzled-related protein-4 (sFRP-4), a factor produced by tumors derived from subjects with tumor-induced osteomalacia, also has phosphaturic activity. It remains to be established whether FGF-23 and sFRP-4 represent two distinct phosphatonin or are somehow integrated in a novel phosphate-regulating bone-kidney axis.

Evidence for multiple hormonal systems controlling phosphorus homeostasis

The control of systemic phosphate homeostasis is not completely understood. Increased circulating levels of the calcemic parathyroid hormone (PTH) results in phosphaturia due to inhibition of sodium-dependent phosphate transport in the renal proximal tubule (1). Reductions in serum phosphate concentrations stimulate renal proximal tubule production of 1,25(OH)2D3, which in turn increases the gastrointestinal absorption of calcium and phosphorus (2). The resulting increase in calcium leads to the secondary suppression of parathyroid hormone (PTH) and increases in sodium-dependent phosphate transport in the renal proximal tubule. The PTH–vitamin D axis, however, is not sufficient to explain the physiological complexity of systemic phosphate homeostasis. Indeed, phosphate loading has a marked effect to reduce the net rate of proximal tubule phosphate reabsorption that is independent of PTH and vitamin D (3, 4). In addition, a novel circulating phosphaturic factor, called phosphatonin, is postulated to be primarily responsible for modulating urinary phosphate excretion in a variety of hypophosphatemic disorders.

Will the real phosphatonin please stand up?

Evidence for the existence of phosphatonin. FGF-23 appears to have all of the predicted biological properties of phosphatonin. FGF-23 is an approximately 26-kDa circulating protein consisting of an N-terminal FGF homology domain and a novel 71-amino acid C-terminus of uncertain function. In vivo studies establish that full-length FGF-23 is a phosphaturic hormone (12–15). In addition, FGF-23–induced phosphaturia does not lead to upregulation of 1,25(OH)2D3 production as occurs with PTH-induced phosphaturia. Treatment with FGF-23 causes a more severe form of rickets and osteomalacia (TIO; also called oncogenic osteomalacia, or OOH) (9–11). X-linked hypophosphatemic rickets (XLH) and antosomal dominant hypophosphatemic rickets (ADHR) characterized by hypophosphatemia due to impaired renal tubular reabsorption of phosphate, inappropriately normal or decreased 1,25(OH)2D3 production, and defective calcification of cartilage and bone. TIO is a tumour-mediated hypophosphatemic disorder with phenotypic features similar to those of the hereditary phosphate wasting disorders. MAS is a hereditary fibrous bone dysplasia caused by activating mutations of Gsα that is sometimes associated with hypophosphatemic rickets. Based on the shared phenotype of these disorders, phosphatonin is predicted to be a circulating protein that inhibits sodium-dependent phosphate reabsorption by the renal proximal tubule through mechanisms distinct from PTH, impairs mineralization of bone and cartilage to a degree greater than expected from the accompanying hypophosphatemia, and counters hypophosphatemia-mediated increases in the renal production of 1,25(OH)2D3. These disorders may share a common pathogenesis mediated by a single phosphatonin or represent distinct entities with common features caused by different phosphatoinis. At present there are at least three factors with the predicted characteristics of phosphatonin.
endopeptidase (PHEX) (6, 18), also is a metalloprotease phosphate-regulating tumor suppressor (MST) (17) that prevents the hydrolysis and inactivation of the 176-RXXR-179 motif by a furin-type convertase sensitive to inhibition by decanoyl-Arg-Val-Lys-Arg-chloro-methyl-ketone (17) is reported to generate biologically inactive N- and C-terminal fragments (14).

There is also an association between increments in FGF-23 and hypophosphatemic disorders. ADHR is caused by missense mutations in FGF-23 at the 176-RXXR-179 motif that prevent the hydrolysis and inactivation of the full-length bioactive FGF-23 (13). The X-linked dominant hypophosphatemic disorder XLH, which is caused by inactivating mutations of the cell surface metalloprotease phosphatase-regulating endopeptidase (PHEX) (6, 18), also is characterized by increased circulating levels of FGF-23 that correlate with the degree of hypophosphatemia (19). Although initial studies suggested that FGF-23 might be a substrate for PHEX (20), additional studies have been unable to confirm this (17). Rather, quantitative real-time RT-PCR analysis of bone from the Hyp mouse homologue of XLH identifies significant increases in the expression of FGF-23 in bone as well as in Hyp-derived osteoblast cultures (17). Thus, increased FGF-23 production by bone may be the cause of increased circulating levels of FGF-23 in XLH, rather than decreased degradation. Regardless, additional intermediate steps involving PHEX substrates and/or interacting proteins are necessary to link inactivating PHEX mutations to increased FGF-23 production. FGF-23 has also been identified in the tumors of patients with TIO (9), and circulating levels of FGF-23 are increased in subjects with TIO (19, 21, 22).

In this issue of the JCI, Riminucci et al. have provided evidence that FGF-23 is implicated in the pathogenesis of phosphaturia in MAS (23). MAS, which is caused by activating mutations of Gα1 that lead to fibrous dysplastic lesions of bone, is often associated with impaired skeletal mineralization and humoral-induced phosphaturia (7, 8). The finding that circulating levels of FGF-23 are also increased in MAS is consistent with the notion that all of these phosphaturic disorders have a common pathogenesis mediated by elevated FGF-23. More importantly, the finding that FGF-23 is produced by normal and fibrous dysplasia osteo-progenitors and bone-forming cells in vivo and in vitro implicates bone and osteoblasts as the tissue/cell type that produces FGF-23, similar to findings of increased fgf23 expression in Hyp bone associated with inactivating Phex mutations. At present, FGF-23 is the leading candidate for phosphatonin.

**MEPE**

Differential gene-expression profiling of TIO tumors by a variety of techniques has also identified the matrix extracellular phosphoglycoprotein MEPE (9, 13), also named OF45 (24). MEPE was isolated and cloned from a TIO tumor cDNA library (11) and independently isolated and cloned from the rat and mouse based on its ability to regulate mineralization (25). MEPE belongs to the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of proteins. The SIBLING family of ECM proteins are involved in regulating the mineralization of ECM and include bone sialoprotein, osteopontin, dentin sialophosphoprotein, and dentin matrix protein-1 (26). MEPE-null mice exhibit increased osteoblast-mediated mineralization, indicating that MEPE plays an inhibitory role in bone formation in mice (24). It has been postulated that MEPE may have phosphaturic actions (10); however, preliminary studies indicate that transfer of MEPE deficiency onto the Hyp mouse background fails to rescue the hypophosphatemia in Hyp mice, suggesting that MEPE is not the cause of phosphaturia in the setting of inactivating Phex mutations (27). Other studies suggest that Phex may modify the hydrolysis of MEPE (28). The biological significance of the later finding is uncertain, but it might implicate MEPE in the local regulation of mineralization through Phex-dependent mechanisms.

**Figure 1**

A bone-kidney axis regulating renal phosphate handling and skeletal mineralization. A phosphaturic hormone, phosphatonin, stimulates renal phosphate excretion. Osteoblasts in bone may be the source of phosphatonin. Increased release of phosphatonin leads to autocrine effects to regulate bone mineralization of ECM and systemic effects to cause phosphaturia.
Secreted frizzled-related protein-4 (sFRP-4) was also identified in serial analysis of gene expression of TIO (9). In this issue of the *JCI*, Berndt et al. have extended these observations by showing that sFRP-4 is present in normal human serum and is increased in the serum in TIO (29).

sFRP-4 is an extracellular antagonist of Wnt signaling. Wnts interact with a receptor complex containing a member of the frizzled family of heptahelical receptors and LDL receptor–related proteins (LRPs), which includes LRP-5 and LRP-6. The binding and sequestration of Wnts by sFRPs prevents Wnt-dependent activation of the frizzled/LRP receptor complex. Given the primary importance of Wnt pathways in the regulation of proliferation, differentiation, and apoptosis and the finding that sFRPs are expressed by nonphosphaturic tumors, it is difficult to conceive how sFRP-4 could selectively target the renal proximal tubule to inhibit sodium-dependent phosphate uptake. Indeed, there is no known precedent for Wnt-dependent regulation of phosphate homeostasis or for hormonal actions of sFRPs. Rather, sFRPs are more typically found at the plasma membrane and/or in the ECM. Thus, the initial bias is that sFRP-4 is an unlikely candidate for phosphatonin.

However, the study by Berndt et al. (29) requires that we reevaluate this preconception and consider the possible role of sFRP-4 in the pathogenesis of phosphaturia in TIO and other hypophosphatemic disorders. In this regard, the authors provide compelling evidence that sFRP-4 has characteristics of phosphatonin. Recombinant sFRP-4 inhibits sodium-dependent phosphate transport in cultured opossum renal epithelial cells, indicating a possible direct action of sFRP-4 on proximal tubular phosphate transport. In addition, the systemic administration of recombinant sFRP-4 caused phosphaturia in normal rats without stimulating 1α-hydroxylase activity in the kidney. The inhibition of renal phosphate reabsorption was associated with evidence for sFRP-4 antagonizing Wnt-dependent β-catenin pathways in the kidney. These observations support the existence of more than one phosphatonin (30).

**A bone-kidney axis regulating phosphate homeostasis and mineralization**

How do we reconcile the observations that FGF-23 and sFRP-4 are both phosphatomins with the hypothesis that hereditary and acquired hypophosphatemic disorders share a common pathogenesis? One possibility is that these factors are integrated at the level
of the osteoblast into a novel phosphate-regulating hormonal axis that controls the renal tubular reabsorption of phosphate and mineralization (Figures 1 and 2).

Several compelling observations support the central role of bone in coordinating renal phosphate handling to meet the needs for mineralization. First, the association between increased FGF-23 transcripts in bone, increased circulating FGF-23, and hypophosphatemia provides a strong case for a possible endocrine function of the skeleton to inhibit sodium-dependent phosphate reabsorption by the renal proximal tubule through the production and secretion of FGF-23 (Figure 1). Indeed, osteoblasts derived from Hyp mice produce a factor capable of inhibiting sodium-dependent phosphate transport in renal tubular cells (31), implicating bone as a possible source of phosphatonin. The current findings by Rimanucci et al. (23) and recent studies in Hyp mice (17) indicate that osteoblasts are a source of FGF-23 in bone. Second, there is evidence for an intrinsic defect in osteoblast-mediated mineralization. In this regard, the severity of rickets and osteomalacia in XLH, ADHR, and TIO appears to be greater than can be explained by hypophosphatemia. Hypophosphatemia in mice lacking the renal proximal tubule sodium-dependent phosphate transporter Npt2 does not cause rickets and osteomalacia as severe as in the hereditary hypophosphatemic disorders (32). Hyp-derived osteoblasts lacking a functional Phex also secrete a putative factor, referred to as minihbin (33), which inhibits mineralization independent of hypophosphatemia.

The severe bone phenotype and apparent intrinsic abnormalities of osteoblast-mediated mineralization in Hyp mice suggest local regulation of the mineralization process. This might occur via autocrine effects of FGF-23 to regulate skeletal mineralization and/or actions of Phex on the expression and/or metabolism of ECM proteins involved in mineralization (Figure 2). There is indirect evidence for both mechanisms in that FGF receptors are present in osteoblasts and chondrocytes that might be targets of FGF-23 actions. Increased mepe transcripts in bone of Hyp mice are consistent with involvement of this ECM protein in mineralization. The further observation that increments in mepe are highly correlated with the level of fgf23 expression in Hyp bone (17) suggests that FGF-23 may have an autocrine effect to increase MEPE production by osteoblasts. The current investigation by Rimanucci et al. (23), showing an association between activation of Gα and FGF-23 production, also opens the possibility of PTH stimulation of FGF-23 by osteoblasts, thereby linking FGF-23 to the PTH-vitamin D axis.

Could sFRP-4 also indirectly affect renal phosphate transport and mineralization through a primary skeletal effect? This possibility is suggested by the importance of osteoblastic Wnt signaling in the control of bone mass. In this regard, a Gly171Val mutation in LRP5 results in autosomal dominant high bone density due to augmented osteoblast-mediated bone formation (34, 35), whereas disruption of LRP5 leads to a decrease in osteoblast proliferation and low bone mass in mice (36). Based on these observations, sFRP-4 inhibition of Wnt-dependent bone formation might secondarily reduce the need for renal phosphate conservation. It would be of interest to determine whether sFRP-4 is regulated in response to stimuli that lead to alterations in bone mass. It also would be of interest to determine whether Wnt-dependent signaling affects PHEX activity, particularly since the C-terminal half of sFRPs contains a motif found in tissue inhibitors of metalloproteases. In addition, the effects of sFRP-4 and Wnt on osteoblast-derived phosphaturic and mineralization-inhibiting factors need to be investigated.

Regardless, a bone-kidney hormonal axis would provide a mechanism for the skeleton to communicate with the kidney to coordinate the mineralization of ECM with the renal handling of phosphate. Osteoblasts are well suited for coordinating systemic phosphate homeostasis and mineralization, since they express all of the implicated components of a possible bone-kidney axis, including PHEX, FGF-23, MEPE, and LRP-5/Wnt, as well as frizzled, FGF, and PTH receptors (Figure 2). In addition, autocrine effects of phosphatonin on osteoblasts could regulate the production of ECM proteins that regulate mineralization. Proving the existence of this bone-kidney axis and defining its physiological role will require additional investigations. The available data, however, are not inconsistent with the possibility that FGF-23 produced by osteoblasts has phosphaturic actions on the kidney and autocrine effects on osteoblasts to modulate the mineralization of bone (Figure 1).

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