Editorial: Tumoral Calcinosis: A Look into the Metabolic Mirror of Phosphate Homeostasis

Phosphorus is a critical element in skeletal development, bone mineralization, membrane composition, nucleotide structure, and cellular signaling. Similar to calcium, the serum phosphorus level is maintained within a narrow range through a complex interplay between intestinal absorption, exchange with intracellular and bone storage pools, and renal tubular reabsorption. The principal organ that regulates phosphate homeostasis is the kidney. Hypophosphatemia stimulates 1,25-dihydroxyvitamin D (calcitriol) synthesis via the 25(OH)D-1α-hydroxylase in the kidney, leading to increased calcium and phosphorus absorption in the intestine and enhanced mobilization of calcium and phosphorus from bone. In addition, hypophosphatemia is a potent stimulator of an increase in maximal tubular reabsorption of phosphate. The resulting increased serum calcium inhibits PTH secretion with a subsequent increase in urinary calcium excretion and an increased tubular reabsorption of phosphate. Thus, normal serum calcium levels are maintained and serum phosphorus levels are returned to normal. PTH regulates phosphate reabsorption, but its principal function is to maintain calcium homeostasis. PTH increases urinary phosphate excretion through reduced expression of the type IIa sodium-phosphate transporter. This effect is rapid and is achieved by internalization of the transporter from the brush border membrane and enhanced lysosomal degradation (1).

The classical PTH/vitamin D axis does not fully account for the complexities of phosphate homeostasis. For example, patients with inherited and acquired hypophosphatemic rickets display profound renal phosphate wasting and disruption of compensatory increases in calcitriol but maintain normal serum PTH and calcium levels. Recent advances in understanding the molecular basis of these hypophosphatemic syndromes have implicated novel regulators of phosphate homeostasis that may either act in concert or independently of the classical phosphate-homeostatic hormones. Through the study of rare syndromes of disordered phosphate homeostasis, fibroblast growth factor 23 (FGF23) excess or deficiency has emerged as a common theme. Tumor-induced osteomalacia (TIO) is a paraneoplastic syndrome of renal phosphate wasting. TIO is characterized by low serum phosphorus concentrations secondary to reduced renal reabsorption, inappropriately low calcitriol levels, but normal calcium and PTH. The biochemical derangements are accompanied by defective bone mineralization. Clinical and experimental studies have implicated the humoral factor(s) (termed “phosphatonin”) propagated by mesenchymal tumors in the profound biochemical and skeletal alterations observed in TIO (2). Efforts to identify phosphatonin were accelerated by using gene expression profiles of these tumors (3, 4). FGF23 was among the first phosphatonin candidates identified and is supported by the strongest experimental evidence. FGF23 is expressed at very low levels in normal tissue but is highly expressed in TIO tumors. Serum levels of FGF23 are markedly elevated in patients with TIO and plummet after surgical resection of the culprit tumor (5). Furthermore, the biochemical and skeletal abnormalities of transgenic mice that overexpress FGF23 mimic human TIO (6, 7).

FGF23 is also implicated in the pathogenesis of two inherited renal phosphate wasting syndromes: autosomal dominant hypophosphatemic rickets (ADHR) and X-linked hypophosphatemic rickets (XLH). Missense mutations at arginine 176 or 179 of FGF23 have been identified in affected members of ADHR families (8). These mutated arginine residues prevent the degradation of FGF23, resulting in prolonged and/or enhanced FGF23 action. Amassing evidence suggests that FGF23 is central in the pathogenesis of XLH. XLH is caused by mutations in the PHEX gene (phosphate-regulating gene with homologies to endopeptidases on the X chromosome), which encodes an M13 metalloprotease (9). Speculation about the function of PHEX, paired with data that implicate both an intrinsic osteoblast defect and a humoral factor in the pathogenesis of XLH, led to the hypothesis that FGF23 is either directly or indirectly regulated by PHEX. The hyp mouse, the murine homolog of XLH, has elevated serum FGF23 levels and increased FGF23 mRNA expression in the skeleton, providing support for the role of FGF23 in the pathophysiology of this disorder. Most XLH patients have elevated serum FGF23 levels, albeit to a more modest degree than TIO (5). In addition to XLH and TIO, patients with extensive fibrous dysplasia of bone that develop renal phosphate wasting exhibit elevated serum FGF23 levels that correlate with the extent of bony involvement (10).

Familial tumoral calcinosis (FTC) is the metabolic opposite of TIO, XLH, and ADHR. FTC is characterized by ectopic calcification (periarticular or dermal), hyperphosphatemia owing to enhanced renal phosphate retention, and inappropriately normal or elevated calcitriol. Patients with FTC may exhibit dental defects with short bulbous roots, pulp stones, and swirled radicular dentin deposits. Given that FGF23 excess is the common molecular basis for several forms of hypophosphatemic rickets, it was tempting to speculate that the molecular defect in FTC would be FGF23 deficiency. Surprisingly, the genetic defect in FTC was identified as biallelic loss-of-function mutations in the GALNT3 gene. GALNT3 encodes UDP-N-acetyl-o-D galactosamine: polypeptide N-acetylgalactosaminyltransferase (ppGaN-
Tase-T3), the glycosyltransferase responsible for initiating mucin-type O-glycosylation (11). In this issue, Ichikawa et al. (12) describe GALNT3 mutations in a multigenerational African-American family that was previously thought to have autosomal dominant FTC. Through careful molecular and clinical characterization, the investigators determined that the family was segregating two distinct GALNT3 mutations and that the proband was a compound heterozygote for a splice site mutation in exon 1 that resulted in a skipped exon and that the proband was a compound heterozygote for a splice site mutation in exon 1 that resulted in a skipped exon 2 and a nonsense mutation (484C>T; R162X). Subtle biochemical manifestations of FTC by family members that were later found to carry heterozygous GALNT3 mutations led to the initial assignment of autosomal dominant inheritance in this family. In retrospect, it can now be appreciated that individuals demonstrating any biochemical or physical manifestations of FTC were assigned as affected even though they did not display the full phenotype. Only the proband and his maternal aunt that harbored mutations on both GALNT3 alleles exhibited the full syndrome including the biochemical features and ectopic calcification. Thus, these elegant molecular studies confirm that this family, initially thought to have autosomal dominant FTC, actually segregates two GALNT3 mutations and the full features of FTC are manifested only in those individuals that have biallelic GALNT3 mutations inherited in an autosomal recessive fashion.

Although the GALNT3 gene defect in FTC had been identified, FGF23 remained a compelling candidate for FTC in patients without GALNT3 mutations. Furthermore, mouse models of FGF23 deficiency exhibit hyperphosphatemia with renal phosphate retention and elevated calcitriol similar to patients with FTC (13, 14). However, unlike patients with FTC, growth retardation, early lethality, and skeletal manifestations exhibited by these mice are distinct from those observed in humans. fgf23-null mice do display vascular, renal, and cardiac calcifications but not the typical periarticular or cutaneous calcification observed in humans.

Recently, the long-suspected relationship between FTC and loss-of-function mutations in FGF23 was confirmed. Benet-Pages et al. (15) reported a homozygous missense mutation (S71G) in FGF23 in a boy with hyperphosphatemia, renal phosphate retention, painful swelling of elbows and tibia, and dental pulp stones. Expression of the mutant FGF23-S71G protein in mammalian cells demonstrated that the full-length FGF23 was retained intracellularly within the Golgi complex and was not secreted. Similar results were found with a FGF23-S129F mutation identified in family with FTC (16). In this issue of JCEM, Larsson et al. (17) report the same biallelic FGF23-S71G mutation in two sisters with FTC. In addition to the typical biochemical manifestations of FTC and soft tissue calcification, the sisters exhibited mild hypercalcemia. Serum levels of intact FGF23 were low-normal, consistent with loss-of-function mutations in FGF23. However, serum FGF23 concentrations were markedly elevated when measured with an assay that detects the C-terminal portion of FGF23. A similar pattern of intact and C-terminal FGF23 levels was observed in the FTC family with the S129F mutation. Taken together with the studies of Benet-Pages et al., which demonstrate that FGF23-S71G is retained in the Golgi complex, this suggests that predominantly truncated C-terminal FGF23 fragments circulate and that full-length FGF23 is relatively deficient. It is speculated that C-terminal FGF23 levels are elevated as a physiological response to hyperphosphatemia in an effort to enhance urinary phosphate excretion.

Attempting to unify the two molecular defects identified in FTC is challenging. It is tempting to speculate that ppGaNTase-T3 O-glycosylates FGF23 and that appropriate glycosylation is critical for the stability, maturation, and/or function of FGF23. FGF23 does contain O-glycosylation sites; however, there is no evidence that ppGaNTase-T3 glycosylates FGF23. Furthermore, the serine residues (S71 and S129) that are mutated in FTC are not predicted glycosylation sites, are solvent inaccessible, and sterically impede O-glycosylation. Alternatively, ppGaNTase-T3 may modify phosphonins other than FGF23 such as FRP-4 (18). Additional studies are needed to determine whether O-glycosylation is important to the stability and/or function of FGF23 and whether O-glycosylation of FGF23 is mediated by ppGaNTase-T3.

Clearly, FGF-23 is implicated in disorders of phosphorus deficiency and excess, but is it a physiological regulator of phosphate homeostasis? Several lines of evidence support the notion that it is. In rodents, modulation of phosphate intake via phosphate-rich and phosphate-deplete diets appear to result in substantial increases and decreases in serum FGF23, respectively. Serum FGF23 rises in response to a phosphate load and promotes phosphorus excretion, whereas serum FGF23 falls with phosphate restriction to reduce renal phosphorus excretion. Studies in humans, however, have been inconsistent, with some showing changes in serum FGF23 levels with phosphate loading and depletion (19) whereas others do not (20).

The study of hypo- and hyperphosphatemic disorders has led to a new understanding of the hormonal regulators of phosphate homeostasis. Mutations in FGF23 or dysregulation of its expression provide a unifying explanation for these syndromes at a molecular level. TIO, XLH, and ADHR have overlapping phenotypic features but all share the common feature of hypophosphatemia due to decreased renal phosphate reabsorption. FGF23 excess is central to at least four distinct disorders of renal phosphate wasting. In TIO, tumors produce excess FGF23 that exerts its activity at the proximal renal tubule to inhibit tubular reabsorption of phosphate and down-regulate the 25-hydroxyvitamin D-1-α-hydroxylase resulting in hypophosphatemia and osteomalacia. In ADHR, FGF23 accumulates due to mutations that enhance its biological activity and render it resistant to inactivation by proteolytic cleavage. In XLH, mutated PHEX directly or indirectly leads to the accumulation of FGF23. In fibrous dysplasia of bone, fibrodysplastic bone appears to produce excess FGF23 that, when reaching a critical level, results in renal phosphate wasting. Conversely, FTC is the metabolic mirror image of XLH and ADHR with features of hyperphosphatemia secondary to enhanced renal phosphate re-absorption, elevated calcitriol levels, and ectopic calcification. Through work published in this month’s issue of JCEM, we now recognize that FTC can arise from two distinct but possibly interrelated mechanisms: 1) biallelic loss-of-function mutations in FGF23 that result in altered maturation and reduced secretion and 2) biallelic loss-of-function mutations
in GALNT3, an enzyme important in O-glycosylation of proteins that may include FGF23. Although it is gratifying to identify the underlying cause of these disorders, the real power of these discoveries is in the insight that is gained about the physiological regulation of phosphate homeostasis.

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