The Roles of Specific Genes Implicated as Circulating Factors Involved in Normal and Disordered Phosphate Homeostasis: Frizzled Related Protein-4, Matrix Extracellular Phosphoglycoprotein, and Fibroblast Growth Factor 23

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Normal serum phosphate (Pi) concentrations are relatively tightly controlled by endocrine mediators of Pi balance. Recent data involving several disorders of Pi homeostasis have shed new light on the regulation of serum Pi balance. It has been hypothesized that circulating phosphaturic factors, or phosphatonin, exist that, when present at high serum concentrations, directly act on the kidney to induce renal Pi wasting. This review will focus upon recently discovered factors that are overexpressed in tumors associated with tumor-induced osteomalacia and have reported activity consistent with effecting Pi balance in vivo. Currently, the best-characterized group of phosphatonin-like polypeptides includes secreted frizzled related protein-4, matrix extracellular phosphoglycoprotein, and fibroblast growth factor-23. Our understanding of these factors will, in the short term, aid us in understanding normal Pi balance and, in the future, help to design novel therapeutic strategies for disorders of Pi handling.

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Abbreviations: ADHR, Autosomal-dominant hypophosphatemic rickets; ASARM motif, acidic serine-aspartate-rich MEPE-associated motif; BMD, bone mineral density; BMP-2, bone morphogenetic protein 2; CHO, Chinese hamster ovary; CKD, chronic kidney disease; FD, fibrous dysplasia; FGF, fibroblast growth factor; FGFR, FGF receptor; FRP4, frizzled related protein-4; GFR, glomerular filtration rate; LNSS, linear nevus sebaceous syndrome; LRP, low-density lipoprotein receptor-related protein; MAS, McCune-Albright syndrome; MEPE, matrix extracellular phosphoglycoprotein; OGD, osteoglophonic dysplasia; 1α(OH)ase, 25-hydroxyvitamin D 1-α hydroxylase; 1,25-(OH)2D, 1,25-dihydroxyvitamin D; PHEX, phosphate-regulating gene with homologies to endopeptidases on the X chromosome; pHPT, primary hyperparathyroidism; Pi, serum phosphate; SPC, subtilisin-like proprotein convertase; TC, familial tumoral calcinosis; TIO, tumor-induced osteomalacia; %TRP, percent tubular reabsorption of Pi; UTR, untranslated region; VDR, vitamin D receptor; XLH, X-linked hypophosphatemic rickets.

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MAINTENANCE OF PROPER serum phosphate (Pi) concentrations is required for normal skeletal development and for preservation of bone integrity. In addition, Pi is required for cellular processes such as energy provision in the form of ATP, is an integral molecule in DNA and RNA, and serves as the substrate for kinase and phos-
phatase regulation of intracellular signaling. Recent advances in our understanding of disorders involving Pi metabolism have shed light on the underlying mechanisms that control Pi homeostasis in normal and in disordered states. Tumor-induced osteomalacia (TIO) is an acquired disorder of isolated renal Pi wasting that is associated with tumors, often arising from a mesenchymal origin. TIO patients present with hypophosphatemia with inappropriately suppressed 1,25-dihydroxyvitamin D [1,25-(OH)2D] concentrations and elevated alkaline phosphatase levels (1). Osteomalacia is seen upon bone biopsy. Clinical symptoms include gradual onset of muscle weakness, fatique, and bone pain especially from ankles, legs, hips, and back (1–3). Insufficiency fractures are common, and proximal muscle weakness can become severe enough for patients to require a wheelchair or become bed bound (1).

The study of TIO introduced the idea for the existence of possible circulating factors, referred to as “phosphatonin,” produced by the tumor, that act upon the renal proximal tubule to decrease Pi reabsorption (4, 5). Support for these factors comes primarily from the knowledge that if the responsible neoplasm is surgically removed, the abnormalities in Pi wasting and in vitamin D metabolism are rapidly corrected, as well as the fact that PTH, which decreases renal Pi reabsorption, is usually within normal reference ranges in TIO patients. Other studies have supported this hypothesis by indicating that tumor extracts and conditioned media from TIO tumor cell cultures inhibit Pi transport in cells that are a model for the proximal tubule (4), and implantation of tumor tissue into nude mice resulted in increased urinary Pi excretion (6).

Another disorder of renal Pi wasting that has provided insight into the regulation of serum Pi is X-linked hypophosphatemic rickets (XLH, OMIM no. 307800). XLH is an X-linked dominant disorder and the most common form of heritable rickets (7). XLH patients present with laboratory findings that include hypophosphatemia with normocalcemia, and inappropriately normal or low 1,25-(OH)2D concentrations. Skeletal defects include lower extremity deformities from rickets, bone pain, osteomalacia, fracture, and enthesopathy (calcification of the tendons and ligaments) (7). It was determined by the Hyp Consortium that XLH is caused by inactivating mutations in PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) (8). Based upon sequence homology, PHEX encodes a protein that is a member of the M13 family of membrane-bound metalloproteases. Other members of this enzyme class include neutral endopeptidase and endothelin converting enzymes 1 and 2 (8, 9). This protease family is known to cleave small peptide hormones; therefore it is likely that PHEX has similar activity. More than 160 inactivating PHEX mutations have been described in XLH patients, including genomic variations that cause missense, nonsense, frame shift, and splicing changes (see PHEX Locus database: www.phexdb.mcgill.ca). Of note, although XLH is a renal Pi wasting disorder, PHEX shows the highest expression in bone cells such as osteoblasts, osteocytes, and odontoblasts in teeth, as well as lower expression in the parathyroid glands, lung, brain, and skeletal muscle, but no expression in kidney (10, 11).

Taken together with the biochemical phenotype of XLH, PHEX protein homology and tissue expression are consistent with the hypothesis that PHEX interacts with small, circulating factors outside of the kidney to directly or indirectly control renal Pi homeostasis.

A valuable tool for the study of the pathophysiology of XLH has been the Hyp mouse, which has 3’-deletion in the Phex gene from intron 15 through the 3’-untranslated region (UTR) (12), and does not make a stable Phex transcript (10). This rodent model parallels the XLH phenotype, characterized by hypophosphatemia with inappropriately normal 1,25-(OH)2D levels and normal serum calcium, as well as growth retardation and bone mineralization defects (14). Parabiosis studies between the Hyp mouse and a normal mouse pointed to the presence of a humoral factor, a phosphatonin, being transferred through the circulation of the Hyp mouse to the normal mouse to cause isolated renal Pi wasting (15). After the identification of PHEX/Phex, it was logically postulated that the enzyme may directly degrade a phosphaturic substance; however, recent studies point to a more complex pathophysiology.

This review will focus upon the identification of secreted frizzled related protein-4 (FRP4), matrix extracellular phosphoglycoprotein (MEPE), and fibroblast growth factor-23 (FGF23) as potential phosphatonin and their relationships to disorders associated with impaired Pi handling.

II. Pi Homeostasis

Control of serum Pi concentrations involves hormonal regulation at the level of the intestine, skeleton, and kidneys. The skeleton represents the largest stores of Pi, primarily complexed with calcium in hydroxyapatite crystals, which constitute the main inorganic component of the mineralized bone matrix. Normal serum concentrations of Pi are relatively tightly regulated and range in adults from 2.5–4.5 mg/dl. Pi values in children are substantially higher and vary with age (the younger the child, the higher the Pi). Pi absorption in the intestine is directly proportional to the dietary intake but is also influenced by 1,25-(OH)2D. In normal individuals, the active form of vitamin D, 1,25-(OH)2D, converted from 25-hydroxy vitamin D by the 25-hydroxyvitamin D 1-alpha hydroxylase enzyme [1α(OH)ase] in the kidney proximal tubule, regulates serum Pi concentrations by increasing intestinal calcium and Pi absorption and, at high concentrations, by increasing Pi mobilization from bone (16). This rise in serum calcium concentrations decreases PTH secretion, which in turn increases the expression of the primary transport protein in proximal tubule, the type II sodium-dependent Pi cotransporter, NPT2a (17–19). Acute changes in serum Pi concentrations are largely controlled by the proximal tubule of the kidney. The abundance of apical expression of NPT2a in the brush-border membrane of proximal tubular cells is the main determinant of percent tubular reabsorption of Pi (%TRP) (20, 21). Increased abundance of the NPT2a protein confers increased %TRP and consequently raises serum Pi levels and vice versa. Accordingly, in normal individuals, hypophosphatemia increases the expression of NPT2a, thereby raising serum Pi levels. Conversely, hyper-
phosphatemia results in deceased expression of NPT2a, thus leading to reduced serum Pi levels.

Expression studies in TIO tumors revealed constitutively high expression of several genes that potentially are involved in the systemic regulation of Pi and calcitriol levels, including FRP4, MEPE, and FGF23. Although much is known regarding the effects of vitamin D and PTH on Pi handling, the study of the phosphatonin proteins that play roles in the rare disorders described herein points to novel mechanisms involved in Pi homeostasis.

III. FRP4

A. FRP and Wnts

Secreted FRP4 is a secreted member of the Frizzled protein family and contains a cysteine-rich ligand-binding domain as well as a hydrophilic C-terminal region (22, 23). The secreted FRP proteins share homology with the extracellular domain of the membrane-bound frizzled receptors. These membrane-bound frizzled receptors bind Wnt proteins in tandem with the cell surface LRP (low-density lipoprotein receptor-related proteins) family of coreceptors. Nineteen human Wnt ligands have been identified and play important roles in a variety of metabolic processes including cellular differentiation, proliferation, and apoptosis (24–27). The biological actions of the secreted FRPs are believed to arise mainly from inhibition of Wnt signaling through direct association with Wnt proteins. The secreted FRPs are thought to compete for Wnt binding to the cell surface frizzled receptors, as some, but not all, of the FRPs act as soluble decoy receptors (23, 28). The binding of Wnt proteins to frizzled receptors and the LRP-5/6 coreceptors in heterotrimeric complexes on the cell surface leads to stabilization of intracellular β-catenin and a complex network of signaling cascades. The significance of Wnt proteins in bone biology was revealed by the human syndrome, osteoporosis pseudoglioma. This disorder is characterized by congenital blindness and severe childhood-onset osteoporosis and is caused by a loss of function mutation in the lipoprotein receptor-related protein-5 (LRP-5) gene (29). Conversely, gain-of-function mutations in the same gene are associated with high bone mineral density in patients with autosomal dominant high bone mass (30, 31).

B. FRP4 expression

FRP4 was first identified as having a role in TIO by serial analysis of gene expression. This gene was reported as a highly expressed transcript in each tumor from a group of hemangiopericytomas that resulted in TIO and was found to be absent from control hemangiopericytomas (32, 33). A differential expression cloning approach also identified FRP4 as highly expressed in TIO tumors (34). The gene encoding FRP4 is located on chromosome 7p14.1 and is comprised of six coding exons, spanning approximately 10.8 kb of genomic sequence (GenBank accession nos. NT_007819, NM_003014). The translated protein product consists of 346 amino acids, of which the first 21 residues constitute the predicted signal peptide. The molecular mass of FRP4 is approximately 40 kDa, but it circulates as a 48-kDa protein due to posttranslational glycosylation of the mature polypeptide. FRP4 is ubiquitously expressed, and importantly has been found to be present in bone cells, thus indicating that this protein may have autocrine or paracrine effects in the skeleton. FRP4 is also detectable in the circulation of healthy individuals at a mean level in five subjects of 34 ± 13 ng/ml but was not elevated when measured in one TIO patient (32).

C. FRP4 and Pi metabolism

The identification of the role of FRP4 in Pi homeostasis comes largely from a publication by Berndt et al. (32). In this regard, these investigators initially revealed the capacity of FRP4 to inhibit sodium-dependent Pi uptake in opossum kidney cells in vitro. Furthermore, iv infusion of FRP4 (0.3 μg/kg/h) in normal or parathyroidectomized mice caused an increase in the fractional excretion of Pi and caused subsequent hypophosphatemia, indicating that FRP4 inhibits renal Pi reabsorption, at least in part, by PTH-independent mechanisms (32). Western analysis of kidney β-catenin indicated that FRP4 infusion resulted in the increased production of phosphorylated β-catenin, which was consistent with FRP4 actions on renal Pi transport by inhibition of Wnt signaling. However, infusion of FRP4 failed to decrease serum levels of 1,25-(OH)2D, although an expected reciprocal increase in the face of hypophosphatemia was blunted. This observation suggests that FRP4 may act predominantly on the Pi-related portion of the TIO phenotype, and other factors expressed by the tumors may act on the vitamin D pathways. Finally, serum concentrations of FRP4 were reported to be normal in at least one TIO patient receiving vitamin D and Pi treatment (11 pg/ml) (32).

One important question is why FRP4 mRNA and protein are markedly overexpressed within TIO tumors. Speculatively, FRP4 may act as a local autocrine or paracrine factor to sustain tumor development and cell growth, because dysregulation of Wnt signaling is known to promote tumor progression in various types of cancers (35–37). In the case of TIO, FRP4 overexpression could induce diminished Wnt signaling in the tumors, possibly leading to decreased apoptosis of the tumor cells. Alternatively, the abundant expression of FRP4 in TIO tumors may be a compensatory response to increased activation of frizzled receptors, as FRP4 normally attenuates Wnt signaling, thus counteracting tumor growth. Indeed, aberrant methylation resulting in silencing of FRP4 transcription and increased activation of Wnt pathways are associated with several types of cancers (38). As yet, to our knowledge, no studies have been conducted to dissect a role of any of the proposed phosphatonin proteins in development and growth of TIO tumors. Finally, FRP4 may have other direct actions on the kidneys, including during renal tubular epithelial injury. Indeed, it was reported that FRP4 may have a role in suppressing the fibrosis associated with unilateral ureteral obstruction (39).

In summary, evidence to date indicates that FRP4 is highly expressed in TIO tumors and influences sodium-dependent Pi transport in vitro and in vivo. Future studies, including animal models describing transgenic expression or ablation of the FRP4 gene, will be needed to further establish the
regulatory functions of FRP4 in Pi and in vitamin D homeostasis.

IV. MEPE

A. MEPE identification and expression

MEPE was identified in tumor-conditioned medium and osteosarcoma cell lines using polyclonal antibodies raised against the preoperative serum from a TIO patient (40). Screening an expression library derived from a TIO tumor followed by 5′-PCR subsequently allowed isolation of the full-length human MEPE cDNA (GenBank accession no. AJ276396). Independently, MEPE was also confirmed as one of the most frequently expressed mRNAs in another set of tumors causing TIO (33). The mouse homolog of MEPE was cloned by subtractive hybridization based upon its high expression in bone marrow-derived osteoblasts and was originally designated OF45 (osteoblasts/osteocyte factor of 45 kDa) (41). It is now established that OF45 and Mepe possess identical amino acid sequences (GenBank accession no. NM_053172) (33). The MEPE gene is located on chromosome 4q22.1, encodes four exons, and spans 13.8 kb in genomic sequence. Isolated MEPE transcripts are approximately 2.0 kb in length and contain an open reading frame of 525 amino acids with a predicted molecular mass of 58 kDa. The encoded polypeptide possesses an N-terminal 16-amino acid signal peptide as well as attachment sites for glycosaminoglycans, allowing for posttranslational modification of the mature protein.

As in the case of FRP4, MEPE is strongly expressed at the mRNA level in TIO tumors (33, 34). Additionally, in humans, MEPE is predominately expressed in odontoblasts and osteocytes embedded in mineralized matrix (42, 43), whereas in mice high levels are detected both in odontoblasts, osteoblasts, and osteocytes (42, 44–46). In mouse skeleton, MEPE is detected as early as 2 d postpartum (42) and increases expression levels throughout life; it peaks in bone and teeth in a maturation-dependent manner and reaches its maximum during bone matrix maturation (44, 47). This is in contrast to in vitro data from human osteoblast cell cultures, where MEPE expression was the highest during the mineralization phase (48). High levels of Mepe have also been reported in callus tissue of fractured bone and are presumably involved in both endochondral and intramembranous ossification (42). There are several known regulators of Mepe mRNA expression. In vitro experiments have demonstrated that FGF2 down-regulates Mepe mRNA levels in a dose-dependent manner in rat bone marrow-derived osteoblastic cells, at least in part by decreasing mRNA stability through a MAPK pathway-dependent mechanism (49). Moreover, short-term stimulation with bone morphogenetic protein 2 (BMP-2) decreases MEPE mRNA in primary human osteoblasts in vitro (48). Finally, injection of 1,25-(OH)2D into rodents suppresses Mepe expression, a finding supported by a reciprocal up-regulation of Mepe mRNA transcripts in the vitamin D receptor (VDR) null mice (50).

B. MEPE homology

Portions of the MEPE polypeptide share homology with specific domains of other proteins known to be involved in bone mineralization. MEPE displays similarities with genes encoding bone-tooth mineral matrix phosphoglycoproteins and belongs to the short integrin-binding ligand-interacting glycoprotein (SIBLING) family, which also includes dentin sialophosphoprotein, osteopontin, and bone sialoprotein (40). Notably, this family of genes maps to a defined region of chromosome 4q and plays a role in bone matrix mineralization and/or modulating osteoblasts and osteoclast function. An important structural feature of the mature MEPE polypeptide is the acidic serine-aspartate-rich MEPE-associated motif (ASARM motif) consisting of the last 18 C-terminal amino acids of the MEPE protein, which is also a feature present in the above-mentioned proteins. The ASARM motif may be critical to MEPE function because this region is characterized by resistance to proteolytic degradation. The ASARM motif is suggested to play an important role in preventing calcification of the urinary tract (52, 53) as well as inhibiting precipitation of calcium/Pi in supersaturated saliva solution (54, 55).

C. MEPE and bone

Multiple experimental observations support a role for MEPE as a mineralization inhibitor (designated “minhibin”). First, the ASARM fragment is normally released through cathepsin C-mediated proteolytic cleavage of MEPE (56), and loss of function mutations in the cathepsin C gene, which lead to a relative lack of the ASARM peptide, have been postulated to partially account for the ectopic and intracranial calcification in Papillon-Lefevre syndrome (57). Second, MEPE demonstrates a dose-dependent inhibition of BMP-2-mediated mineralization of a murine osteoblast cell line in vitro, an effect attributed to the ASARM peptide, and direct sc administration of the ASARM peptide into mice also resulted in impaired skeletal mineralization (58). Third, primary osteoblasts derived from Mepe−/− mice cultured ex vivo produced more mineralized nodules than did osteoblasts from wild-type littermates when cultured in medium supplemented with 5 mmol/liter β-glycerophosphate and 25 µg/ml of ascorbic acid (50).

MEPE is also likely to affect the bone formation and resorption process. Importantly, Mepe null mice display increased trabecular and cortical bone mass (59), due to increases in both osteoblast number and activity, and Mepe-deficient mice are also resistant to age-dependent trabecular bone loss (59). This is, however, in contrast to another recent report that did not reveal any difference in bone mineral density (BMD) between Mepe null mice and wild-type littermates (50). This apparent discrepancy could possibly be explained by the use of different background strains or variations in the age at which the animals were analyzed. Interestingly, injection of a synthetic peptide, containing an integrin-binding RGD (arginine-glycine-aspartate) motif and the glycosaminoglycan-attachment motif, corresponding to residues 242–264 of MEPE (designated “AC-100”), stimulated new bone formation and increased the number of os-
Evidence supporting either a direct or indirect role for MEPE in the systemic regulation of Pi and vitamin D levels has been reported. Human MEPE inhibited sodium-dependent Pi uptake in primary human proximal tubule cells and a human renal cell line (Hu-CL8) in a dose-dependent manner (58). Additionally, a study reported that ip injection of full-length MEPE into mice (40 μg/kg/30 h) resulted in hypophosphatemia secondary to an increase in the fractional excretion of Pi, which may be due to an accumulation of the ASARM peptide localizing to the proximal tubule (58, 62). At higher MEPE doses (400 μg/kg/30 h), mice also demonstrated increased renal phosphorus clearance. Of significance, healthy human subjects that were given a high-Pi diet exhibited a concomitant reduction of circulating MEPE, instead of an expected compensatory increase (63), and Chinese hamster ovary (CHO) cells overexpressing MEPE did not lead to renal phosphate wasting when injected into nude mice (34). These data point to the possibility that MEPE may not regulate serum Pi levels but, rather, its expression could be directly or indirectly influenced by serum Pi levels.

Although the phosphatonin group is classically thought to be associated with hypophosphatemia and reduced serum 1,25-(OH)2D in TIO patients, the hypophosphatemia after MEPE injections resulted in a concomitant increase in serum 1,25-(OH)2D (58), indicating that MEPE, like FRP4, could be responsible for a portion of the renal Pi wasting in TIO patients, but not the inappropriately suppressed circulating 1,25-(OH)2D concentrations.

D. MEPE and Pi handling

In summary, evidence to date demonstrates that MEPE is highly expressed in tumors that cause TIO and supports the role of MEPE as an important gene for the negative regulation of mineralization in the skeleton. MEPE may have direct roles in the decreased skeletal mineralization associated with disorders of Pi wasting; however, powerful genetic experiments to date suggest that MEPE may have only a limited impact on the systemic levels of serum Pi or vitamin D.

F. Genetic studies

In contrast, Phex was found to inhibit Mepe cleavage by blocking cathepsin-like enzymatic activity, an effect independent of endogenous Phex enzymatic activity (56). Further studies with surface plasmon resonance have demonstrated that MEPE binds directly to PHEX via the ASARM motif, thus preventing MEPE cleavage and subsequent release of the ASARM peptide (64). Based upon these findings, a theoretical model explaining the intrinsic mineralization defect in XLH and Hyp mice has been proposed, i.e., loss of functional PHEX protein leads to increased ASARM levels, which in turn may inhibit bone mineralization.

V. Fibroblast Growth Factor-23 (FGF23)

Autosomal-dominant hypophosphatemic rickets (ADHR, OMIM no. 193100) is a rare disorder characterized by laboratory findings of low serum Pi concentrations due to decreased maximum tubular reabsorption of phosphate/glomerular filtration rate (GFR) and inappropriately low or normal circulating vitamin D concentrations (65). ADHR was
first described in a small family (66). We subsequently evaluated a large ADHR kindred with many affected individuals. This kindred provided us with an opportunity to explore the phenotypic variability of this disease in a large number of individuals with the same mutation. Similar to XLH, we found no evidence of genetic anticipation or imprinting. In contrast to XLH, ADHR displays incomplete penetrance and variable age of onset. Important to the diagnosis and clinical management of ADHR, we observed that this expanded ADHR family contains two subgroups of affected individuals. One subgroup consists of patients who presented during childhood with Pi wasting, rickets, and lower extremity deformity in a pattern similar to the classic presentation of XLH. The second group consists of individuals who presented as adolescents or adults. These individuals complained of bone pain, weakness, and insufficiency fractures but did not have deformities of the lower extremity (67). The patients with adult-onset ADHR had clinical presentations that were essentially identical to those of patients who present with TIO, although none of the ADHR patients were ever found to develop tumors. The molecular mechanisms for early-onset ADHR resembling XLH, and the late-onset ADHR resembling TIO are currently unknown. To date, all of the patients that have been described with delayed onset of clinically evident disease are female. In addition to these two groups, we found unaffected individuals who are carriers for the ADHR mutation and two patients (65) who were treated for hypophosphatemia and rickets, but later lost the Pi-wasting defect. Thus, the clinical manifestations of ADHR are even more variable than those observed in XLH.

To identify the gene for ADHR, the ADHR Consortium undertook a positional cloning strategy. A genome-wide linkage scan in the large ADHR kindred mentioned above demonstrated significant linkage to a locus on chromosome 12p13.3 (68). Direct sequencing of exons from four ADHR families revealed three missense changes affecting two arginines, which are separated by two amino acids. Two kindreds shared the same change from R176Q (527G→A), whereas, other families had R179W (535C→T) and R179Q (536G→A) substitutions (69). These changes segregated with the disease, and all mutations were ruled out as polymorphisms by analysis of control individuals. FGF10, which is the gene mutated in autosomal-dominant aplasia of lacrimal and salivary glands (70), and FGF14, which has been identified as the gene for autosomal-dominant cerebellar ataxia (71), are the only FGF family members other than FGF23 associated with disease-causing mutations.

To determine whether FGF23 could be involved in TIO as phosphatonin, we tested RNA from five TIO tumors as well as several control tissues by Northern blot for the presence of FGF23 mRNA in tumors that cause TIO. FGF23 was detected in all tumors tested after only 30-min exposure and was not detected in control RNAs, providing the first evidence for overexpression of FGF23 in TIO tumors. In additional studies, serial analysis of gene expression indicated that there was increased expression of FGF23 mRNA in a majority of TIO tumors assessed (33), which supports immunohistochemical data demonstrating that FGF23 protein was detectable in most TIO tumor samples (73, 74).

A. FGF23 gene and mRNA structure

FGF23 most likely arose from gene duplication (75), as the gene lies 54 kb telomeric of FGF6 and is similarly comprised of three coding exons spanning approximately 11 kb of genomic sequence (69). The longest human FGF23 mRNA obtained is 3018 bp (GenBank accession no. NM_020638), and contains a predicted open reading frame of 251 amino acids. The 5′-UTR consists of 146 bp with no in-frame stop codon present upstream of the predicted start site. The 3′-UTR consists of 2116 bp with at least one potential polyad-
FGF23-CHO mice. Bone mineral content was reduced in femurs (roentgenogram of mice with control cells (left) or FGF23-CHO cells (right)). There is markedly reduced bone mineral content in the FGF23-CHO mice. Bone mineral content was reduced in femurs from FGF23-CHO mice (right two femurs) compared with controls. D, Widening of the growth plate and increased osteoid was present in FGF23-CHO implanted mice (lower panel). [Reproduced with permission from: T. Shimada et al. Proc Natl Acad Sci USA 98:6500–6505, 2001 (34).]

FIG. 2. FGF23 overproduction in vivo resembles TIO patient phenotypes. Nude mice were injected with CHO cells overexpressing FGF23 or control cells. A, Appearance of mice bearing the FGF23-CHO cells on d 45 after implant. Note marked kyphosis and leanness. B, Soft roentgenogram of mice with control cells (left) or FGF23-CHO cells (right). C, Bone mineral content was reduced in femurs (two femurs) compared with controls. D, Appearance of mice bearing the FGF23-CHO cells (red). Bone mineral content was reduced in femurs from FGF23-CHO mice (right two femurs) compared with controls. D, Widening of the growth plate and increased osteoid was present in FGF23-CHO implanted mice (lower panel). [Reproduced with permission from: T. Shimada et al. Proc Natl Acad Sci USA 98:6500–6505, 2001 (34).]

C. ADHR mutation effects on FGF23

The mutations in FGF23 that result in the ADHR phenotype replace arginine (R) residues in FGF23 within a subtilisin-like protease convertase (SPC) cleavage site, 174RXXR179/S180 (34, 78–80). The SPCs are a family of serine proteases, involved in the processing of a wide variety of polypeptides including neuropeptides, peptide hormones, growth factors, membrane-bound receptors, blood coagulation factors, and plasma proteins (81). SPC substrates are cleaved C-terminal to the basic motif K/R-X-X-K/R, where X = two, four, or six residues of any amino acid, although rarely Cys (82, 83). The SPCs, such as the furin protease, are largely expressed in the trans-Golgi network, and possess similar, but not exact, substrate specificities.

To begin to understand the molecular mechanisms of ADHR, investigators have used site-directed mutagenesis to insert the ADHR mutations into the wild-type FGF23 cDNA, followed by transient or stable expression in mammalian cells. As assessed by Western analysis, wild-type FGF23 is secreted from cells as two major polypeptides, a 32-kDa species and a 12-kDa species (34, 74, 78–80). Peptide sequencing confirmed that the 32-kDa isoform corresponded to full-length FGF23 after cleavage of the signal peptide (residues 25–251) and that the 12-kDa isoform was the C-terminal portion of FGF23 immediately downstream from the furin cleavage site after R179 (residues 180–251) (76). After mutational replacement of either R176 or R179, or both residues in tandem, the FGF23 secreted from the cells was primarily full length (74, 76, 80, 85). In additional studies, high-affinity monoclonal antibodies recognizing a FLAG epitope incorporated into the N-terminal portion of FGF23 revealed that, although the majority of ADHR mutant FGF23 is indeed secreted in vitro as full-length polypeptide, a smaller proportion of proteolytic fragments are still detectable, indicating that mutant FGF23 is not completely resistant to intracellular cleavage (80). As further evidence that FGF23 is processed intracellularly, investigators have shown that the cleavage of wild-type FGF23 between R179/S180 can be inhibited by a nonspecific furin competitive inhibitor, Dec-RVKR-CMK, at concentrations between 25–50 μM (78, 86). These studies provide further support that the RXXR motif in FGF23 is central to its intracellular processing.

The SPC family is usually associated with the production of the mature, active form of their substrate polypeptides. Many SPC substrates are involved in bone and mineral homeostasis, such as the BMPs (87, 88) and PTH (89). However, there is evidence that cleavage of FGF23 at the RXXR motif appears to be inactivating. When recombinant full-length FGF23, or N-terminal (residues 25–179) and C-terminal (residues 180–251) fragments were injected into rodents, only the

treatment signal 831 bp downstream of the stop codon. Prediction analyses also indicated that FGF23 contained a signal peptide, with cleavage most likely occurring between the alanine residue at position 24 and the tyrosine at position 25, a finding confirmed by peptide mapping of recombinant FGF23 (76). FGF23 has also been identified from bovine (GenBank accession no. XP_608704), rhesus monkey (BV210513), mouse (NM_022657), chicken (XP_425663), zebrafish (Pan troglodyte chimpanzee (AY412417), rat (NM_130754), human (NM_000022657), monkey (NM_022657), and pufferfish (AAV97593) sequences.

B. FGF23 tissue expression profile

FGF23 is transcribed at low levels in specific tissues. Hybridization of multiple-tissue Northern blots containing control human and mouse RNAs were negative for FGF23 transcripts (69). In contrast, a Northern blot of cancer cell RNAs (34, 69).

By RT-PCR, FGF23 could be amplified from human heart, myelogenous leukemia line K562, whereas several other tumor cell lines displayed positive mRNAs of 3 and 1.3 kb in the chronic myelogenous leukemia line K562, whereas several other tumor cell lines expressed only the 3.0- or 1.3-kb transcript. By RT-PCR, FGF23 could be amplified from human heart, liver, thyroid/parathyroid, intestine, and skeletal muscle RNAs (34, 69).

In subsequent studies, it has become clear that the tissue with the highest FGF23 expression is bone. Using in situ hybridization of adult trabecular bone, Riminucci et al. (77) demonstrated that FGF23 mRNA was observed in osteocytes and flattened bone-lining cells. In regions of active bone formation, a strong hybridization signal can be seen in os-
full-length protein was capable of lowering circulating phosphate concentrations (76). In addition, it has been reported that ADHR mutant FGF23 may be more potent than the less stable wild-type FGF23 (85); however, given the labile nature of wild-type FGF23, this paper may not have ensured delivery of equal amounts of full-length wild-type and mutant protein. Because the full-length form of FGF23 induces hypophosphatemia, it is likely that the ADHR mutations increase the biological activity of FGF23 by stabilizing the full-length form and increasing its concentrations in the serum. Indeed, severely affected ADHR patients have increased circulating levels of FGF23 (our unpublished data).

D. XLH and FGF23

As described above, patients with XLH have overlapping phenotypes with ADHR patients. Because XLH results from a mutation in PHEX, which shares homology to a family of extracellular proteases, and ADHR arises from mutations in a protease-cleavage site, it was logically hypothesized that circulating FGF23 would be cleaved and inactivated by PHEX. Thus, by mutational inactivation of PHEX in XLH, serum FGF23 concentrations would then elevate and cause renal Pi wasting. As described earlier, this hypothesis was further supported by parabiosis studies between Hyp and normal mice, which pointed to the presence of a humoral phosphaturic factor in the Hyp mouse that was transferred to the normal mouse. However, evidence to date has not supported a direct enzyme-substrate relationship between FGF23 and PHEX. In this regard, Liu et al. (78) demonstrated that recombinant PHEX did not cleave FGF23, but did cleave a positive control substrate. Furthermore, another report provided evidence that recombinant FGF23 was not cleaved by PHEX in cultured human embryonic kidney 293 cells expressing the proteins (86). To ensure that the additional residues did not cause conformational changes within FGF23 and interfere with potential PHEX activity, this latter study (86) expressed native FGF23 that was not epitope tagged.

Several reports have established that FGF23 is elevated in many XLH patients (90–92). To understand the possible relationship between PHEX and FGF23, Liu et al. (78) used quantitative real-time RT-PCR to test Hyp bone for the level of FGF23 mRNA vs. levels in wild-type bone. Interestingly, FGF23 mRNA in bone tissue from Hyp mice was elevated compared with levels present in control mice, and serum concentrations of FGF23 have been reported to be 10-fold higher in Hyp mice when compared with normal mice (our unpublished results and Ref. 93). This finding provides support for the idea that there is a cellular connection between inactive PHEX mutants (or lack of Phex expression in Hyp mice) and the up-regulation of FGF23 mRNA in bone cells. The elevated FGF23 mRNA levels may indicate that the increase in serum FGF23 in XLH patients is due to overproduction and secretion of FGF23 by skeletal cells, as opposed to the alternative hypothesis of a decreased rate of FGF23 degradation by cell surface proteases after secretion into the circulation. Although the interactions between FGF23 and PHEX are most likely indirect, the encoded proteins are coexpressed in osteoblasts and osteocytes (10, 77, 78). At present, the PHEX substrate and the mechanisms for phosphate sensing are unknown.

The current therapy for XLH, ADHR, and TIO includes oral replacement of phosphorus in combination with high-dose 1,25-(OH)2-D. This regimen “treats” XLH by increasing serum Pi concentrations and ameliorates much of the metabolic bone disease, but does not directly “cure” the disorder by reversing the underlying molecular defects in kidney and in bone. In this regard, several studies have attempted to reverse the XLH phenotype. Transgenic expression of wild-type PHEX under the control of the bone-specific mouse pro-α(I) collagen gene (94) and the osteocalcin (OG2) (95) promoters on the Hyp background was undertaken. Interestingly, the defective mineralization of bone and teeth in the Hyp mice was partially resolved with PHEX under the regulation of the collagen promoter, and dry ash weight increased with the OG2 PHEX, indicating improved mineralization. However, the hypophosphatemia was not normalized in either study, indicating that expression of PHEX under the temporal regulation of an osteoblast-specific promoter is not sufficient to rescue the Hyp phenotype. Furthermore, expression of PHEX to levels observed in wild-type animals was not obtained in all studies. Importantly, a recent report of a transgenic model overexpressing PHEX in the Hyp mouse using the human β-actin promoter for directing expression in a wider tissue distribution (bone, skin, lung, muscle, heart) produced similar results as the bone-specific promoter studies (96), further demonstrating that proper spatial-temporal expression of Phex is critical for normal mineral metabolism.

E. FGF23 and Pi metabolism

The primary transport protein responsible for phosphate reabsorption in the kidney is the type II sodium-phosphate cotransporter, or NPT2a, which is localized to the apical membrane of the proximal tubule. In support of this, Npt2a null mice had approximately 80% less renal sodium-dependent phosphate reabsorption capacity compared with wild-type mice (20). High circulating FGF23 concentrations led to renal phosphate wasting through the down-regulation of Npt2a. This was clearly demonstrated by the finding that mice transgenic for human FGF23 had markedly increased Pi excretion secondary to decreased proximal tubule Npt2a expression (97, 98). Certainly, NPT2a is a primary hormone-regulated renal Pi transporter; however, it is also plausible that FGF23 controls Pi metabolism through additional mechanisms. In this regard, dietary Pi loading causes a significant down-regulation of Npt2a (99). However, in mice fed a high-Pi diet, a single injection of FGF23 was able to decrease serum Pi concentrations (100); thus other renal phosphate transporters may be responsive to FGF23. It was subsequently shown that FGF23 also down-regulated Npt2c, a distinct apical membrane sodium-phosphate cotransporter in the kidney that shares homology to Npt2a (97). Taken together, these results demonstrate that FGF23 controls Pi homeostasis by controlling renal reabsorption through regulation of the expression of the renal sodium-Pi cotransporter family members. Importantly, it was previously demonstrated that Hyp mice have a 50% reduction in proximal tubule Npt2a expression (101), which is responsible for the
low serum phosphate observed in these animals. The fact that Hyp mice have approximately 10-fold greater serum Fgf23 concentrations compared to wild-type mice, as well as the fact that early parabiosis experiments implicated a circulating factor as causing the renal phosphate leak in Hyp, is consistent with the idea that elevated Fgf23 concentrations in Hyp mice are most likely responsible for the decreased abundance of Npt2a levels, and further supports a central role for FGF23 in XLH.

**F. FGF23 and vitamin D metabolism**

In normal individuals, hypophosphatemia is a strong positive stimulator for increased serum 1,25-(OH)_{2}D (102). However, in TIO, ADHR, and XLH patients, there are parallel observations of hypophosphatemia with low or inappropriately normal 1,25-(OH)_{2}D concentrations. 1,25-(OH)_{2}D controls its own negative feedback mechanism by down-regulating the 1α(OH)ase through the VDR (103, 104). The observation that the TIO patient profiles paralleled those of the FGF23-CHO cell-injected mice led Shimada et al. (34) to test the expression levels of the 1α(OH)ase and the catabolic 24(OH)ase enzyme. These animals had markedly suppressed 1α(OH)ase expression when compared with animals carrying native CHO cells. Additional animal studies demonstrated that despite severe hypophosphatemia, FGF23 transgenic mice also demonstrated decreased renal levels of the 1α(OH)ase (97, 98, 105). FGF23 appears to regulate vitamin D metabolism more rapidly than Pi homeostasis because injections of purified FGF23 resulted in a maximal effect on vitamin D 3 h after injection, whereas serum Pi reached maximal suppression at 9 h after injection (100). Thus, the effects of FGF23 on the renal vitamin D metabolic enzymes is most likely responsible for the reductions in serum 1,25-(OH)_{2}D concentrations observed in TIO, ADHR, and XLH patients.

**G. Fgf23 knockout mice**

To further understand the role of FGF23 in mineral homeostasis, Fgf23 null mice were generated by targeted ablation of the Fgf23 gene as reported by two independent groups. These mice are remarkable for several findings including hyperphosphatemia, elevated 1,25-(OH)_{2}D levels, immature reproductive organs, atrophy of the thymus, low serum triglycerides, elevated serum cholesterol, and hypoglycemia (106, 107). These Pi and vitamin D phenotypes observed are the biochemical reciprocals of the Hyp mouse. The additional phenotypes beyond Pi homeostasis indicate that FGF23 may have tissue-specific roles in development as a local factor, similar to many of the other FGFs, or that renal failure in the mice caused by the excess serum Pi and 1,25-(OH)_{2}D produced secondary phenotypes.

Sitara et al. (107) used the Fgf23 null mouse in a genetic strategy to address the question of whether Fgf23 was the primary phosphatonin responsible for XLH. These investigators mated the Fgf23 null mouse with the Hyp mouse (which is null for Phex), to remove the Fgf23 alleles from the Hyp genetic background and create an animal that could not express Fgf23, but would express the other phosphatins. The authors reasoned that if Fgf23 is the central phosphatonin in Hyp/XLH, then ablation of Fgf23 from the Hyp mouse should reverse the hypophosphatemic phenotype. This cross produced a Hyp/Fgf23 null offspring that had a similar phenotype to the Fgf23 null mouse, including hyperphosphatemia with increased circulating 1,25-(OH)_{2}D. Because the only genetic change in this offspring mouse was the biallelic deletion of Fgf23 (and not deletion of FRP4 or MEPE), this strongly implicates the elevation of Fgf23 as responsible for the Hyp phenotype. If elevated FRP4 or MEPE were responsible for the low serum phosphate in the Hyp mouse, then these Hyp/Fgf23 null mice would have retained the hypophosphatemic phenotype because FRP4 and MEPE were still carried normally in the Hyp genome. Thus, this study indicates that Fgf23 is necessary to reduce serum Pi and 1,25-(OH)_{2}D concentrations in Hyp mice and demonstrates that Fgf23 has a central role in the pathogenesis of XLH.

**H. Regulation of FGF23 in vivo**

Because Fgf23 affects both Pi and vitamin D homeostasis in the renal proximal tubule, investigators have reasoned that these metabolites may influence the serum concentrations of Fgf23. Understanding the regulation of FGF23/Fgf23 in vivo has been undertaken in both human and animal studies. In a small study, Larsson et al. (108) did not find a change in FGF23 in human subjects due to low or high Pi diets; however, Ferrari et al. (109) were able to show an inverse correlation between FGF23 serum concentrations and serum phosphorus levels. In order to blunt the possible confounding regulation of serum Pi by PTH, these investigators used dietary supplementation to maintain constant serum calcium levels (109). In animal studies, the Fgf23 response to serum Pi has been more consistent than in the human studies. Of note, mice given high and low Pi diets produced the expected correlations between Fgf23 and dietary Pi intake (110, 111). The effects of vitamin D on Fgf23 expression in mice have been examined by Shimada et al. (100). Interestingly, injections of 20–200 ng 1,25-(OH)_{2}D led to dose-dependent increases in serum Fgf23 concentrations, as measured by ELISA. Of significance, these changes in serum Fgf23 at the low doses of vitamin D occurred before detectable changes in serum Pi concentrations, indicating that Fgf23 may be directly regulated by vitamin D concentrations. Physiologically, this would be consistent with results examining the role of Fgf23 in vitamin D metabolism. Fgf23 has been shown to down-regulate the 1α(OH)ase mRNA (97, 100); thus as 1,25-(OH)_{2}D concentrations rise in the blood as a product of 1α(OH)ase production, vitamin D would then stimulate Fgf23 production, which would complete the feedback loop and down-regulate 1α(OH)ase expression to lower circulating vitamin D concentrations. Consistent with these observations, Ito et al. (110) demonstrated a dose-dependent increase in FGF23 promoter activity in the chronic myelogenous leukemia cell line K562 with 1,25-(OH)_{2}D, despite the absence of a classical vitamin D response element within the FGF23 promoter. In confirmation of these findings, cotransfection of the K562 cells with the human VDR further increased FGF23 promoter activity. Of note, there was also an
increase in activity under high Pi cell culture conditions, which was synergistic in the presence of 1,25-(OH)₂D₃. Interestingly, administration of calcitriol to thyroparathyroidectomized rats results in a dose-dependent increase in FGF23 levels (112).

Other investigators have used a genetic approach to attempt to dissociate the effects of Pi and of vitamin D on serum Fgf23 concentrations. Using the VDR null mouse, a model for vitamin D-dependent rickets type II (113), Yu et al. (111) determined that on a standard diet, VDR null mice have markedly reduced serum Fgf23 concentrations when compared with wild-type mice, most likely due to severe hypophosphatemia. When the VDR null mice are returned to normophosphatemia by a high-calcium, high-phosphorus “rescue” diet, Fgf23 is significantly elevated. These results indicate that Pi-mediated regulation of Fgf23 can be regulated independently from the genomic effects of vitamin D.

I. FGF23 transgenic animals

Further sources of evidence in support of a fundamental role for FGF23 in TIO are several recently reported animal models. At least three FGF23 transgenic mice have been developed, and in addition to subtle differences between mice possibly due to differing gene targeting approaches, these mice are phenocopies of TIO patients and provide opportunities to study the effects of elevated FGF23 in vivo. In the first reported mouse, human FGF23 was expressed via a non-tissue-selective chicken β-actin promoter (98). FGF23 was detected in the heart, brain, and thymus, but not in some tissues such as small intestine and liver. In the second FGF23 transgenic mouse, human FGF23 was under the control of the type I collagen promoter to target expression to cells of the osteoblastic lineage (97). Both mice had growth retardation, hypophosphatemia secondary to renal Pi wasting, low levels of circulating 1,25-(OH)₂D₃, and rickets and osteomalacia. The two animal models had virtually identical phenotypes, although one mouse had a more widespread expression pattern of FGF23. These results supported the earlier in vivo findings in nude mice bearing CHO cells expressing FGF23 (34, 85) as well as experiments infusing recombinant FGF23 (34, 76, 85).

J. Serum assays

In a study with a large number of controls (n = 147 adults, 26 children) and TIO patients (n = 17), a C-terminal FGF23 ELISA (with both the capture and detection antibodies targeting C terminal of the R179 cleavage site) was used to assess the role of FGF23 in TIO and XLH (90). This study indicated that FGF23 could be detected in the circulation of normal individuals and demonstrated that the mean FGF23 was greater than 10-fold elevated in the TIO patients tested compared with controls and rapidly fell after tumor resection. Of significance, many XLH patients (13 of 21) had elevated serum concentrations of FGF23 compared with control individuals (90) when assessed with this assay. Although some of the patients did not have concentrations above control levels, in the face of hypophosphatemia, the concentrations of FGF23 may be considered inappropriately elevated because FGF23 is a phosphaturic substance; the assay also points to a possible defect in serum Pi-sensing in XLH patients or indicates that the defect in PHEX overwhelms the Pi regulatory system. Because the C-terminal assay recognizes full-length FGF23 as well as C-terminal fragments, it is unknown whether the high FGF23 concentrations observed in some XLH patients is a true reflection of circulating levels of active protein or are the result of an increased breakdown of FGF23 in the serum. In a smaller study using the same ELISA, a significant difference between serum FGF23 concentrations in normal individuals and in XLH patients was not found; however, FGF23 concentrations were inversely correlated with serum Pi levels (91). It is difficult to determine with these assays whether the patients with normal FGF23 values have increased clearance of FGF23, or whether the proportion of full-length FGF23 to C-terminal fragments is altered in XLH and TIO. Furthermore, the FGF23 serum assays may need refinement and may not be adequate to detect elevated FGF23 in all cases. The fall of FGF23 post-tumor resection and rapid rise in serum Pi concentrations was also confirmed by findings in subsequent TIO cases (91, 115, 116).

An “intact” FGF23 ELISA assay was also developed that used conformation-specific monoclonal antibodies to N-and C-terminal portions of FGF23 (117). This study also showed that FGF23 could be detected in normal individuals with a mean circulating concentration of approximately 30 pg/ml, and that there was no correlation between FGF23 concentrations and age. The single TIO patient tested in this study had a serum FGF23 concentration approximately 9-fold above control concentrations before tumor resection. After tumor removal, circulating FGF23 concentrations decreased within 30 min, and serum Pi improved within 6 h (92). In the same study, six XLH patients (from four unrelated families) showed significant elevations in FGF23 concentrations when compared with 104 control individuals (92). Although more study is required, the results of the two assays generally agree with regard to the relative range of FGF23 concentrations in XLH and in TIO patients, and that FGF23 is elevated in most XLH patients (90, 92). Taken together, these studies provide support for a key role of FGF23 in TIO and XLH. In this regard, FGF23 mRNA and protein are up-regulated in tumors that cause TIO, FGF23 blood concentrations are markedly elevated in TIO patients’ circulation preoperatively and fall to normal concentrations within hours of tumor removal, and FGF23 concentrations are also elevated in most XLH patients as well as in the Hyp mouse.

K. Effects of FGF23 on the skeleton

FGF23 is largely produced in bone; however, whether FGF23 has a direct role in skeletal biology is unclear. The skeletal phenotypes of the FGF23 transgenic mice included growth retardation, a disorganized growth plate, and osteomalacia (97, 98). In these models, it is unknown whether the high FGF23 concentrations observed in TIO patients or indicates that the defect in PHEX overwhelms the Pi regulatory system. Because the C-terminal assay recognizes full-length FGF23 as well as C-terminal fragments, it is unknown whether the high FGF23 concentrations observed in some XLH patients is a true reflection of circulating levels of active protein or are the result of an increased breakdown of FGF23 in the serum. In a smaller study using the same ELISA, a significant difference between serum FGF23 concentrations in normal individuals and in XLH patients was not found; however, FGF23 concentrations were inversely correlated with serum Pi levels (91). It is difficult to determine with these assays whether the patients with normal FGF23 values have increased clearance of FGF23, or whether the proportion of full-length FGF23 to C-terminal fragments is altered in XLH and TIO. Furthermore, the FGF23 serum assays may need refinement and may not be adequate to detect elevated FGF23 in all cases. The fall of FGF23 post-tumor resection and rapid rise in serum Pi concentrations was also confirmed by findings in subsequent TIO cases (91, 115, 116).

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levels in the long bones and in cultured osteoblasts when compared with wild-type littermates (78). Osteoblasts from the H\textsubscript{yp} mouse have been reported to have an intrinsic mineralization defect when transplanted into normal mice (118, 119); thus FGF23 could have direct effects on bone to reduce mineralization \textit{in vivo} although further data are required to validate this hypothesis.

Regarding bone biology, it is interesting that the Fgf23 null mice exhibited a disorganized growth plate and accumulation of unmineralized osteoid, in a manner similar to the transgenic FGF23 mice. Histomorphometry showed that osteoblast and osteoclast surface areas were reduced in the null mice, which indicates that bone turnover was suppressed by loss of function of Fgf23. These data support the idea that FGF23 may have a direct action on bone metabolism, although the possibility exists that FGF23 may be acting in a developmental manner or that the Fgf23 null mice suffer from secondary consequences due to renal failure.

VI. Tumoral Calcinosi.s: Is Glycosylation the Link?

Familial tumoral calcinosis (TC, OMIM 211900) is a heritable autosomal recessive metabolic disorder characterized by ectopic calcified tumoral masses, dental abnormalities, as well as soft tissue periarticular and vascular calcification (120–122). Biochemical abnormalities include hyperphosphatemia, increased %TRP, and inappropriately normal or elevated 1,25-(OH)\textsubscript{2}D concentrations. Calcium and PTH are usually within the normal reference range, although suppressed PTH levels also occur (121). It was initially demonstrated that TC is caused by recessive mutations in GALNT3, the UDP-N-acetyl-\alpha-D-galactosamine/polypeptide N-acetylgalactosaminyl transferase-3 enzyme responsible for initiating mucin-like O-linked glycosylation in the Golgi apparatus (123). Moreover, in another family with a reported autosomal dominant trait, TC was, in fact, caused by two different biallelic GALNT3 mutations, further supporting an autosomal recessive inheritance of this disorder (124). Topaz et al. (123) also reported that the TC patients had serum FGF23 levels approximately 30-fold above normal when assessed with the C-terminal FGF23 ELISA. This finding indicates that FGF23 may be increased in response to the hyperphosphatemia associated with GALNT3-TC, or that FGF23 may be inactive in the absence of GALNT3.

The TC phenotype closely resembles that of the Fgf23 knockout mice (106, 107) and is basically a mirror image metabolic profile of the Pi-wasting disorder ADHR (65, 66), caused by activating mutations in FGF23 (69). Importantly, it was also discovered that the TC syndrome is caused by recessive homozygous mutations in the FGF23 gene, giving rise to an amino acid change from a serine to a glycine at residue 71 (S71G) (126, 127), or from a serine to a phenylalanine at residue 129 (S129F) (128). Serum from patients with S71G and S129F mutations revealed low-normal intact FGF23 levels (127, 128), whereas C-terminal FGF23 concentrations were markedly elevated (126–128). These observations suggested a different processing or secretion of TC mutant FGF23 to that of wild-type protein. Indeed, it has been demonstrated that these mutations destabilize the protein structure of FGF23, thus leading to increased intracellular proteolytic degradation of FGF23 (126–128). This is, at least in part, mediated by furin-like proteases, which is consistent with the serum biochemistry and calcification profiles observed in TC patients (129) (Fig. 3).

One question that remains to be answered is whether a direct enzyme-substrate relationship between GALNT3 and FGF23 exists. A direct interaction between these proteins is an intriguing thought, as loss of function of either results in a TC phenotype, but also because all known FGF23 mutations causing TC occur at serine residues, thus potentially affecting O-linked glycosylation (126–128) (Table 1). Earlier studies have indeed demonstrated that FGF23 is a target of O-linked glycosylation and that ADHR mutant FGF23 is differentially glycosylated compared with its native form (76). The serine at residues 71 and 129 do not, however, constitute predicted glycosylation motifs (127, 128), and molecular modeling suggests a decrease in protein stability rather than a defect in glycosylation (129).

Therefore, it is possible that any potential substrate that works upstream of FGF23 may be a GALNT3 substrate, thus leading to defective regulation in the production/secretion of FGF23. Indeed, a marked elevation of C-terminal FGF23 levels in TC...
patients harboring GALNT3 mutations (>30 times normal) was reported by Topaz et al. (123). Intact FGF23 levels have not yet been reported in these patients; thus a direct link between FGF23 and GALNT3-TC has not been established. Although further study is required, the hallmark of the syndromes associated with FGF23 (ADHR, XLH, TIO, fibrous dysplasia) is the improper regulation of Pi in parallel with improper regulation of 1,25-(OH)2D. Thus, it is possible that disordered FGF23 glycosylation in TC could also have functional consequences, such as an inhibitory effect on the ability of FGF23 to bind to its cognate receptor or the inability to secrete full-length FGF23.

The discovery that mutations in GALNT3 may have effects on FGF23 production and processing has substantial impact on the understanding of the molecular pathophysiology of other disorders of Pi homeostasis. In particular, the ADHR mutants created from site-directed mutagenesis of FGF23 are destabilized and may exist for FGF23 within the kidney. Indeed, seven major isoforms potentially mediate the activity of FGF23 in vivo (133). These investigators reported that opossum kidney cells, which possess a proximal tubule cell-like phenotype, express mRNA encoding Fgfr3b and Fgfr3c, with Fgfr3c appearing to be the dominant isoform. Using surface plasmon resonance, murine FGF23 was shown to bind to Fgfr2c and 3c, but not to 1c. The apparent affinity for the Fgfr3-Fgfr3c interaction was approximately 18 nM (133). Because FGF23 bound to Fgfr2c and 3c, it is possible that FGF23 may activate several FGFR isoforms in vitro to produce physiological responses regulating Pi homeostasis as well as other metabolic processes. Consistent with the observations that FGF23 may interact with multiple FGFRs, Fgfr3 knockout mice have interesting phenotypes in addition to those involved in mineral metabolism, such as underdeveloped organs, thymus atrophy, low serum triglycerides with elevated cholesterol, and hypoglycemia (106, 107). These widespread effects of Fgf23 gene ablation are suggestive of FGF23 acting through multiple developmental processes, or could be due in part to the secondary effects of renal failure. Determining the FGFRs that specifically interact with FGF23 in kidney and skeleton could potentially be important for designing FGF23 inhibitors for disorders associated with increased FGF23 activity, such as ADHR, XLH, and TIO, as well as selective agonists for disorders of hyperphosphatemia, such as TC and renal failure.

Activating mutations in FGFR1, 2, and 3 give rise to skeletal disorders involving craniosynostosis and dwarfism (for reviews see Refs. 75 and 134); however, the association of these skeletal disorders with Pi wasting has not been reported. In addition, mouse models involving the FGFRs have failed to shed light on the Fgfr3 receptor. Of note, Fgfr1 and Fgfr2 knockout mice are embryonic lethal (135, 136), and mice null for Fgfr3 and Fgfr4 do not have Pi phenotypes (12, 137). The fact that FGF23 may interact with multiple FGFRs, taken together with immunohistochemical analyses indicating that several FGFRs are expressed within kidney tubule segments (138–140), highlights the possibility that FGF redundancy may exist for FGF23 within the kidney. Indeed, seven major FGFR isoforms can be detected by RT-PCR in RNA isolated from kidney cortex (139); thus it is possible that FGF23 could

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VII. FGF23 Receptors

The identification of the FGF receptors (FGFRs) activated by FGF23 within the proximal tubule will be critical for understanding the molecular mechanisms of FGF23 actions on Pi and vitamin D metabolism. FGFR1-4 are receptor tyrosine kinase family members and comprise an extracellular ligand binding domain, a single transmembrane domain, and an intracellular split tyrosine kinase region (130). Alternative RNA splicing within this receptor family gives rise to many FGFR isoforms, each of which exhibits distinct tissue expression patterns and also possesses different ligand binding and activation specificities (131). The extracellular region of the FGFRs contains three Ig-like domains (D1–D3). One of the most significant splicing events in FGFR1–3 utilizes one of two mutually exclusive exons, exon 8 or exon 9, within D3. Inclusion of exon 8 results in the "b" FGFR isoform, which is largely present in epithelial cell lineages, and inclusion of exon 9 gives rise to the "c" isoform, which is generally thought to be restricted to mesenchymal cell lineages (131, 132). These isoforms, originating from the same FGFR gene, have dramatically different activation properties for their cognate ligands (131).

The current evidence indicating that known FGFR isoforms potentially mediate the activity of FGF23 is largely based upon the work of Yamashita et al. (133). These investigators reported that opossum kidney cells, which possess a proximal tubule cell-like phenotype, express mRNA encoding Fgfr3b and Fgfr3c, with Fgfr3c appearing to be the dominant isoform. Using surface plasmon resonance, murine FGF23 was shown to bind to Fgfr2c and 3c, but not to 1c. The apparent affinity for the Fgfr3-Fgfr3c interaction was approximately 18 nM (133). Because FGF23 bound to Fgfr2c and 3c, it is possible that FGF23 may activate several FGFR isoforms in vitro to produce physiological responses regulating Pi homeostasis as well as other metabolic processes. Consistent with the observations that FGF23 may interact with multiple FGFRs, Fgfr3 knockout mice have interesting phenotypes in addition to those involved in mineral metabolism, such as underdeveloped organs, thymus atrophy, low serum triglycerides with elevated cholesterol, and hypoglycemia (106, 107). These widespread effects of Fgf23 gene ablation are suggestive of FGF23 acting through multiple developmental processes, or could be due in part to the secondary effects of renal failure. Determining the FGFRs that specifically interact with FGF23 in kidney and skeleton could potentially be important for designing FGF23 inhibitors for disorders associated with increased FGF23 activity, such as ADHR, XLH, and TIO, as well as selective agonists for disorders of hyperphosphatemia, such as TC and renal failure.

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signal through multiple FGFRs to regulate Npt2a and Pi homeostasis, and the lack of expression of one Fgfr may be compensated by activation of a different Fgfr isoform within the proximal tubule. Whether a single FGFR isoform controls Pi and vitamin D metabolism, or whether multiple FGFRs independently regulate Pi and vitamin D metabolism, is currently unknown. Either wild-type or ADHR mutant FGFR23 may circulate at elevated levels in patients with disorders of Pi handling such as ADHR, XLH, and TIO. Indeed, native and ADHR-mutant FGFR23 were capable of inducing hypophosphatemia when injected into mice (76, 85). Thus, it is a reasonable hypothesis that the ADHR mutants bind to the same FGFRs, and activate similar intracellular mechanisms, as the wild-type protein to influence serum Pi concentrations.

**VIII. Chronic Kidney Disease (CKD)**

As described, a vast amount of evidence supports the idea that FGF23 is a Pi-regulating hormone; it was hypothesized that serum FGF23 levels are altered as a physiological response in pathological states in which disturbances in serum Pi levels occur. In human disease, the most common form of pathological change in serum Pi is hyperphosphatemia from renal insufficiency. The positive Pi balance in these patients results from several different mechanisms (141). The most important factor is a diminished renal elimination rate of Pi, which cannot compensate for dietary Pi intake, resulting in hyperphosphatemia. A further aggravating factor may be secondary hyperparathyroidism (142), which is seen in many patients with CKD, and may result in PTH-mediated bone resorption and increased efflux of Pi from the skeleton to the blood.

It was initially demonstrated that serum levels of FGF23 were markedly elevated when measured in a few patients with end-stage renal disease receiving hemodialysis (90, 91). These findings were supported by additional studies, where high serum FGF23 levels were found in CKD patients with various degrees of renal function (108, 143). Because all FGF23 measurements in these studies were performed using an assay that recognizes both intact FGF23 as well as biologically inactive C-terminal FGF23 fragments, however, it was not possible to establish whether endogenous FGF23 production was increased as a response to chronic hyperphosphatemia or whether the elevation of FGF23 levels was due to retention of C-terminal breakdown products. The notion that C-terminal FGF23 fragments were retained due to reduced GFR in CKD patients is favored by the strong correlation between serum FGF23 and creatinine (91, 108, 143). Immunoprecipitation studies also revealed increased FGF23 immunoreactivity in the secondary urine output of dialysis patients compared with that of healthy controls (108), further suggesting a renal clearance of FGF23. This question was partially resolved when increased amounts of intact FGF23 were detectable in the circulation of dialysis patients, as demonstrated by immunoprecipitation of serum from CKD subjects and healthy controls (91, 108). Later, it was indeed confirmed that there is a significant elevation of intact FGF23 in CKD subjects when measured with an intact FGF23 ELISA, but also that intact serum FGF23 increases in parallel with the decline of GFR (144). Additionally, one preliminary report described gradually increasing intact FGF23 levels in rats made uremic by antiglomerular basement membrane antibodies, with subsequent progressive hyperphosphatemia, reduced 1,25-(OH)₂D₃ levels, and increased PTH production (145). In summary, the hyperphosphatemia associated with CKD most likely triggers FGF23 production to promote renal Pi excretion, reflected by the severely elevated FGF23 levels in CKD subjects. However, given the history of protein hormone assays in patients with renal failure, these findings should be interpreted with caution, as even “intact” hormone levels may not accurately reflect true physiology in renal disease.

The calcium × Pi product may be the best predictor of serum FGF23 levels in end-stage renal disease (91, 144), which is consistent with a potential interaction between FGF23 and calcium metabolism. This is further supported by the significant correlations between FGF23, Pi, calcium, PTH, and 1,25-(OH)₂D₃ found in CKD subjects (108, 143, 144), but also the development of secondary hyperparathyroidism, a common complication in patients with renal failure. Secondary hyperparathyroidism is, in part, a physiological response to the prevailing hyperphosphatemia, which stimulates PTH production through several different mechanisms. First, precipitation of calcium and Pi is more likely to occur in soft tissues, which leads to a decrease in serum calcium and thereby increased PTH secretion (146). Second, Pi per se can increase PTH synthesis by posttranslational mechanisms (142, 147) but can also stimulate parathyroid cell proliferation directly (148). Finally, high serum Pi inhibits renal 1α(OH)ase activity and serum 1,25-(OH)₂D₃ levels, which normally has a direct suppressive effect on PTH production and secretion (149–151).

The role of FGF23 in the development of secondary hyperparathyroidism is still not understood. One plausible scenario is that FGF23 production gradually increases as a physiological response to the hyperphosphatemia when renal function declines in CKD subjects. When this model is applied to earlier stages of CKD, there are enough viable nephrons that respond to increased FGF23 levels and are thus capable of promoting Pi excretion and maintaining normal serum Pi levels. However, in later stages of CKD, the number of nephrons is insufficient to excrete adequate quantities of Pi, even when maximally stimulated by FGF23, leading to hyperphosphatemia and low 1,25-(OH)₂D₃ levels, a setting that strongly promotes the development of secondary hyperparathyroidism.

One important question to be addressed is whether FGF23 has a direct stimulatory effect on the parathyroid glands, or whether PTH modulates the production and/or the biological activity of FGF23. Excess FGF23 in vivo could potentially directly signal through the parathyroid cells to increase PTH production, as a part of a counterregulatory feedback loop to protect vitamin D and calcium levels, as FGF23 has a profound effect on down-regulating renal expression of the 1α(OH)ase (85, 97, 98, 100, 105–107). Several in vivo animal models overexpressing FGF23 exist, all of which cause parathyroid hyperplasia and increased PTH levels (85, 97, 105). A more likely scenario is that the increase in PTH levels and
parathyroid hyperplasia observed in these animals could also be mediated by the calcium-sensing receptor, again protecting systemic calcium levels, as the suppressed 1,25-(OH)2D3 levels would otherwise lead to persistent hypocalcemia. Indeed, mice expressing high systemic levels of FGF23(R176Q) demonstrate hypocalcemia and subsequent development of secondary hyperparathyroidism, even though elevated PTH levels likely further deteriorate the prevailing hypophosphatemia (105).

In support of PTH-mediated effects on FGF23 production is that increased FGF23 levels induced by a high dietary Pi intake were abolished by parathyroidectomy, both in normal and uremic rats (145). Moreover, injection of PTH into sham-operated mice increased serum Fgf23 levels, whereas a corresponding increase was blunted when injected into parathyroidectomized rats (145). Finally, patients with advanced secondary hyperparathyroidism due to CKD, who underwent total parathyroidectomy, reduced their elevated circulating FGF23 levels, although these levels were not completely normalized postoperatively (152). This may suggest either that intact parathyroid glands are a prerequisite for normal FGF23 production or that low calcium levels function as an inhibitor of FGF23 production. The latter hypothesis again implies that systemic calcium levels affect the expression of FGF23 and support the notion that the calcium × Pi product rather than Pi alone exerts an internal “set point” for FGF23 production. Recently, two genetic studies were performed to dissect Pi and vitamin D-dependent effects on Fgf23 production. Importantly, both studies revealed that serum Fgf23 levels were undetectable in VDR null mice on a standard diet but increased markedly when these mice were given a rescue diet containing 2% calcium and 1.25% phosphorous (111) or a high calcium diet only (153), an effect that was not observed when mice were fed on a high Pi diet only (153). This further supports the concept that hypocalcemia is a negative regulator of Fgf23 production and that calcium homeostasis always is prioritized before Pi homeostasis. For example, in chronic hypoparathyroidism or in CKD subjects, FGF23 levels are increased to reduce the prevailing hyperphosphatemia, but not at the expense of lowering serum calcium levels, because high FGF23 levels would further decrease serum calcitriol and calcium levels. Therefore, the development of secondary hyperparathyroidism in FGF23 transgenic models may be an inevitable consequence to protect systemic calcium levels, despite the fact that this scenario likely causes a further aggravation of the FGF23-induced hypophosphatemia. A key genetic study that demonstrated the importance of protecting calcium metabolism was undertaken by Bai et al. (154), who back-crossed Pth null mice into a Hyp background. The resulting Pth/Hyp double-null mutant mice resulted in an early lethal phenotype, most likely due to severe hypocalcemia.

The parathyroid glands are not the source of increased FGF23 production, as enlarged parathyroid glands from patients with severe secondary hyperparathyroidism do not express FGF23 mRNA (155). Additional evidence from two recent reports supports the involvement of FGF23 in secondary hyperparathyroidism: one suggested that serum FGF23 levels are the most valuable factor in predicting future refractory secondary hyperparathyroidism in long-term dialysis patients (155), and the other showed that pretreatment serum FGF23 levels also predicted the efficacy of calcitriol treatment in patients with advanced secondary hyperparathyroidism (156). These studies suggest that measurement of serum FGF23 may be a useful tool in future clinical practice in patients with CKD.

IX. Other Disorders Involving FGF23

A. Fibrous dysplasia (FD)

FD is a disorder caused by activating, somatic mutations in the GNAS1 gene, encoding the α-subunit of the stimulatory G protein, Gαs (157, 158). FD is characterized by fibrous lesions and colonized mineralization defects of the skeleton, which contribute to significant morbidity among these patients (159). A significant proportion of FD patients suffer from various degrees of renal Pi wasting and subsequent hypophosphatemia (160), and some of these patients also develop hypophosphatemic rickets and osteomalacia. When extraskeletal clinical manifestations of FD occur, such as abnormal skin pigmentation, premature sexual development, and hyperthyroidism, the disease is then referred to as McCune-Albright syndrome (MAS). In one study of FD patients, increased FGF23 serum levels correlated negatively to serum Pi and 1,25-(OH)2D3, but positively to skeletal disease burden (77). FGF23 mRNA and protein were localized to fibrous cells in the fibrous bone lesions of FD, as well as osteogenic and endothelial cells associated with microvascular walls in bone (77). Therefore, it is likely that FGF23 plays an important role in the pathogenesis of the phosphate wasting that is often seen in FD/MAS.

FD patients are often treated with bisphosphonates, which generally leads to some improvement of symptoms. Recently, it was reported that three MAS patients significantly reduced their elevated FGF23 levels after pamidronate therapy, further supporting a central role of FGF23 in FD/MAS (161). The mechanism of action for this reduction of serum FGF23 levels is unclear, but one plausible explanation is that osteogenic cells overproducing FGF23 in FD/MAS may undergo apoptosis, thus leading to decreased production of FGF23.

The reason why bone lesions of FD occur and why FGF23 is overexpressed in cells associated with these lesions is not understood. Normally, FGF23 is expressed in osteogenic cells (77, 78), but it is possible that improperly differentiated cells of FD lesions may have lost the proper regulatory mechanisms required for normal FGF23 production. Speculatively, this is analogous to FGF23 overexpression in TIO tumors, which are not subject to normal regulatory mechanisms (73).

B. Osteoglophonic dysplasia (OGD)

Activating mutations in fibroblast growth factor receptors 1–3 (FGFR1–3) are responsible for a diverse group of skeletal disorders. In general, mutations in FGFR1–2 cause the majority of syndromes involving craniosynostosis, whereas the dwarfing syndromes are largely associated with FGFR3 mutations. OGD (OMIM no. 166250) is a “crossover” disorder
that possesses skeletal phenotypes associated with FGFR1–2 mutations as well as with FGFR3 mutations. Indeed, OGD patients present with craniosynostosis, prominent supraborital ridge, and depressed nasal bridge, as well as with rhizomelic dwarfism and nonossifying bone lesions characteristic for the disorder (162). We demonstrated that OGD is caused by missense mutations in highly conserved residues that make up the ligand-binding and transmembrane domain of FGFR1, thus defining novel roles for this receptor as a negative regulator of long bone growth. Of significance, three of the four OGD patients studied had isolated renal Pi wasting with inappropriately low 1,25-(OH)2D3 concentrations (163). In one of these patients, a sample was available for analysis of plasma FGF23 concentrations, which were significantly elevated above control levels (163). It was hypothesized that the associated metaphealseans, which may be similar to FD lesions, produce FGF23, which leads to renal Pi wasting. Although only a few patients were analyzed, OGD may have parallels with FD whereby the lesonal burden of a patient is proportional to the FGF23 production and the extent of Pi wasting.

C. Linear nevus sebaceous syndrome (LNSS)

LNSS is a rare congenital disorder, involving cutaneous lesions characterized by papillomatous epidermal hyperplasia and excess sebaceous glands (164, 165). However, additional defects are often present in LNSS patients, including neural developmental defects of the brain, which are associated with seizures, as well as eye complications (166). An additional rare association with LNSS is hypophosphatemic rickets, which usually manifests within the first years of life, often as skeletal pain and insufficiency fractures (167). The primary cause of LNSS in currently unknown, but a recent report described elevated serum FGF23 levels in a LNSS child with simultaneous therapy-resistant hypophosphatemic rickets (168). Treatment with octreotide and excision of the nevus were followed by normalization of serum FGF23 and regression of clinical symptoms, implying a putative role of FGF23 in the development of hypophosphatemia and rickets in this disorder (168).

D. Primary hyperparathyroidism (pHPT)

A potential role of FGF23 in pHPT has been proposed, because FGF23 may be implicated in the development of secondary hyperparathyroidism and other disorders of calcium and Pi disturbances. However, serum FGF23 levels are normal in most patients with pHPT (125, 169), and the few pHPT subjects with reported elevated FGF23 concentrations suffered from impaired renal function and reduced GFR (125), which likely caused the observed increase in FGF23 levels. Total parathyroidectomy in pHPT subjects, which reduced levels of PTH, calcium, and 1,25-(OH)2D3 and increased serum Pi, did not have any obvious effect on serum FGF23 concentrations (125). Hence, PTH may be the major regulator of serum Pi in patients with pHPT, and FGF23 does not appear to play a significant role in the pathogenesis of this disorder. As the calcium × Pi product strongly correlates to serum FGF23 levels in subjects with secondary hyperparathyroidism (91, 155, 156), it may offer an explanation for the observed normal serum FGF23 in pHPT. The calcium × Pi product is commonly not altered in mild pHPT, because PTH exerts opposite effects on serum calcium and Pi. Thus, elevated FGF23 levels in pHPT would further deteriorate the hypophosphatemia (possibly in a synergistic manner), but also reduce the inhibition of 1,25-(OH)2D3 on PTH synthesis and secretion.

E. Hypoparathyroidism

Subjects with hypoparathyroidism offer a unique opportunity to study FGF23 levels in the setting of hyperphosphatemia and low serum PTH and calcium, thus allowing separation of the phosphaturic effects of FGF23 and PTH. FGF23 levels are significantly elevated in patients with chronic hypoparathyroidism (114), suggesting that FGF23 production is increased as part of a physiological response to the hyperphosphatemia and that FGF23 regulates serum Pi in this disorder. However, the elevation of FGF23 is insufficient to restore euvphosphatemia, indicating that FGF23, in the absence of normal or elevated PTH levels, cannot by itself correct the hyperphosphatemic state (114). This is in agreement with an additional study demonstrating that injection of FGF23 into aparathyroid rats lowered serum Pi, although it did not return it to normal (100), and the same FGF23 dose was sufficient to induce hypophosphatemia in normal rats (100). These findings are intriguing because they suggest that FGF23 acts partially through a PTH-dependent mechanism. Alternatively, hypocalcemia may serve as a “brake” on FGF23 because a further increase in FGF23 levels would be detrimental to serum calcium concentrations, i.e., FGF23 may further suppress 1,25-(OH)2D3 levels, an undesirable consequence in the setting of hypocalcemia.

X. Potential Therapeutic Avenues

As evidence accumulates supporting the role of FGF23 in rare, as well as more common, disorders of phosphate homeostasis, this molecule is becoming an attractive therapeutic target. Using a novel approach to understanding the mechanisms underlying XLH, presented in preliminary form, neutralizing antibodies targeting FGF23 were administered to Hyp mice (93). After 4 wk of treatment, injection of the monoclonal antibodies resulted in complete normalization of the serum Pi and 1,25-(OH)2D3 concentrations. Additionally, the Hyp rachitic lesions were ameliorated, and bone and tail length increased (93). When the mechanisms for these physiological changes were explored, it was found that the inactivation of FGF23 in the mice led to an increase in Npt2a protein and in 1α(OH)ase mRNA in the renal proximal tubule (93). These studies reinforce the concept that FGF23 acts partially through a PTH-dependent mechanism. Using a novel approach to understanding the mechanisms underlying XLH, presented in preliminary form, neutralizing antibodies targeting FGF23 were administered to Hyp mice (93). After 4 wk of treatment, injection of the monoclonal antibodies resulted in complete normalization of the serum Pi and 1,25-(OH)2D3 concentrations. Additionally, the Hyp rachitic lesions were ameliorated, and bone and tail length increased (93). When the mechanisms for these physiological changes were explored, it was found that the inactivation of FGF23 in the mice led to an increase in Npt2a protein and in 1α(OH)ase mRNA in the renal proximal tubule (93). These studies reinforce the concept that FGF23 acts partially through a PTH-dependent mechanism.
Ameliorating the symptoms of TIO by using the FGF23 antibodies until the tumor is found, or treating XLH early in life, could alleviate potentially harmful effects of long-term Pi and vitamin D therapy, such as hyperparathyroidism and nephrocalcinosis with resulting renal insufficiency.

Renal failure is a common disorder that often results in elevated serum Pi concentrations due to the loss of the ability of the kidney to normally control Pi handling. Understanding the mechanisms responsible for Pi homeostasis in this patient group is essential because hyperphosphatemia is associated with an increase in mortality risk due to arterial calcification and uremic bone disease (84). A number of studies have demonstrated that FGF23 is elevated in renal failure patients, often 100- to 1000-fold over normal concentrations (90, 91, 108, 143). Because these patients are hyperphosphatemic, FGF23 may be overproduced in response to the elevated serum phosphorus concentrations. The progressive loss of renal function is positively correlated with the rise of serum FGF23 concentrations (108, 143); therefore, it is difficult to determine whether the reported elevation in serum FGF23 is due to a compensatory mechanism to counter hyperphosphatemia, or whether the loss of renal function results in the retention of FGF23 proteolytic fragments normally processed by renal clearance. These fragments would then increase the apparent FGF23 concentration as determined by ELISA. Regardless, it is an attractive idea that recombinant FGF23 could potentially be used to increase Pi excretion, and balance serum Pi concentrations in early renal failure patients, who still have significant residual renal function. Lowering serum Pi could reduce arterial calcification by lowering the calcium × Pi product and may help to resolve secondary hyperparathyroidism. However, because FGF23 down-regulates the renal 1(α)OHase, FGF23 therapy may exacerbate the hyperparathyroidism associated with CKD.

Certainly, the most direct potential application for recombinant FGF23 could be in TC. Several groups have demonstrated that inactivating mutations in FGF23 lead to TC (126–128), thus delivering recombinant FGF23 may completely resolve the disorder by directly treating the molecular defect through replacement of missing or mutant FGF23. Additional data are required to determine whether FGF23 would be a potential treatment for GALNT3-mediated TC. Whether ADHR-mutant FGF23 (mutant at positions 176 and/or 179) would provide a “longer acting” form of therapy due to the stabilization of the full-length polypeptide compared with the labile wild-type form remains to be determined.

XI. Summary

The evidence to date supports the existence of several circulating factors, or phosphatonin, that play roles in Pi and vitamin D homeostasis under normal circumstances as well as in disease. FRP4 and MEPE were identified from tumors causing TIO, and their roles in normal Pi handling and mineral metabolism are active areas of investigation. Multiple lines of evidence point to the importance of FGF23 in normal and pathophysiologically Pi metabolism: mutations in FGF23 identified this factor as the causative gene in at least two hereditary disorders of Pi balance; its overexpression in TIO tumors; its association with renal Pi homeostasis and with vitamin D metabolism after FGF23 delivery in vivo; and finally, the FGF23 transgenic and Fgfr3 knockout mouse phenotypes. Also, FGF23 may be a common denominator in other rare renal Pi wasting disorders. In sum, the discovery of the phosphotonins will provide likely candidates as therapeutic targets for many disorders of Pi handling.

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