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FGF23 in Skeletal Modeling and Remodeling

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Abstract

Fibroblast growth factor 23 (FGF23), a hormone primarily produced in bone cells, targets the kidney to accelerate phosphate excretion into the urine and suppresses vitamin D synthesis, thereby inducing a negative phosphate balance. Excessive serum FGF23 due to hereditary disorders such as hypophosphatemic rickets leads to phosphate wasting and impaired bone mineralization. In contrast, deficiencies in FGF23 are associated with hyperphosphatemia, elevated 1,25(OH)₂D₃, ectopic ossification in soft tissues, and defects in skeletal mineralization. Recent studies of human genetic disorders and genetically engineered mice, as well as the in vitro approaches, have clarified some mysteries in FGF23 regulation and its potential roles in bone modeling and remodeling, which are summarized in this review article.

Keywords

FGF23; Hyp; DMP1; Hypophosphatemic rickets; Phosphate homeostasis; Modeling and remodeling

Introduction

FGF23 is a novel member of the fibroblast growth factor (FGF) family. It was originally cloned from mouse skin complementary DNA by its homology with *Fgf15* [1]. The *Fgf23* gene encodes a 251-amino acid protein with a signal peptide comprised of 24 amino acids [1]. It has been identified as the causative gene mutated in autosomal-dominant hypophosphatemic rickets (ADHR) [2] and overproduced in tumor-induced osteomalacia (TIO) [3]. In addition, FGF23 has also been found to be elevated in X-linked hypophosphatemic rickets (XLH), which is caused by mutations in a phosphate-regulating gene with homologies to endopeptidases on the X chromosome (*Phex*) gene [4]. More recently, an increase in FGF23 has been observed in autosomal-recessive hypophosphatemic rickets (ARHR) induced by mutations in dentin matrix protein 1 (*Dmp1*) gene [5–9]. In contrast, *Fgf23*-deficient mice manifest a reciprocal phenotype to ADHR and TIO patients, including hyperphosphatemia, due to increased renal phosphate reabsorption, elevated circulating 1,25(OH)₂D₃ levels, as well as skeletal abnormalities [10, 11].

FGF23 is primarily produced by osteoblasts/osteocytes in bone [5, 11–13] with low levels in other tissues such as the ventrolateral thalamic nucleus of the brain [1, 3]. Both systemic and local factors have been shown to regulate FGF23 homeostasis directly or indirectly. As a potent phosphaturic factor, FGF23 targets the kidney to regulate phosphate homeostasis and vitamin D metabolism [3, 10]. Therefore, the skeletal abnormalities associated with aberrant

FGF23 action are often secondary to the abnormal phosphate homeostasis and vitamin D metabolism. However, some new studies suggest that FGF23 also plays a direct role in skeletal modeling and remodeling. This brief review summarizes recent insights into these two aspects: FGF23 regulation and the indirect and direct roles of FGF23 in the skeletal modeling and remodeling process.

FGF23 Regulation

FGF23 is regulated at multiple levels, including its expression, post-translational modification, secretion, and extracellular processing. Several pieces of evidence support the stimulatory effects of 1,25(OH)₂D₃ on *Fgf23* expression in bone. First, in vivo administration of 1,25(OH)₂D₃ increases FGF23 serum levels and *Fgf23* mRNA levels in bone in both wild-type and *Hyp* mice [14]. Second, the FGF23 serum level in the vitamin D receptor (VDR) null mice is extremely low, and these null mice fail to respond to the stimulatory effect of 1,25(OH)₂D₃ on FGF23 and phosphate [15]. Third, in vitro studies show that 1,25(OH)₂D₃ increases endogenous *Fgf23* mRNA levels in osteoblast cell culture [14], and upregulates the *Fgf23* promoter activity in both osteoblast and non-osteoblast cell cultures [14, 16]. These observations suggest that 1,25(OH)₂D₃ directly upregulates *Fgf23* expression.

Dietary phosphate is another key regulator of FGF23 secretion. In animal studies, a decrease in the dietary intake of phosphate from 1.65% to 0.02% for 5 days leads to a reduction of serum FGF23 over a sevenfold range in a linear, dose-dependent fashion [17]. In human studies, Burnett et al. [18] showed that dietary phosphate loading increases circulating FGF23 but has little effect on serum phosphate levels. Conversely, dietary phosphate deprivation reduces the levels of both serum phosphate and circulating FGF23 within 48 h. Because the change in FGF23 in response to dietary phosphate depletion is much smaller than that caused by dietary phosphate loading, these authors propose that FGF23 mainly promotes phosphate wasting. The regulation of FGF23 by phosphate seems independent from 1,25(OH)₂D₃, because a diet with high calcium and phosphate normalizes the serum phosphate level, and increases the circulating *Fgf23* levels in VDR null mice [19]. Note that the mechanism by which phosphate regulates FGF23 remains unknown, despite the fact that high phosphate levels could significantly enhance the effects of 1,25(OH)₂D₃ on *Fgf23* promoter activities in vitro [16].

Recent studies also suggest that parathyroid hormone (PTH) plays a direct role in the regulation of FGF23, although these data are still controversial. The evidence for this regulation includes 1) chronic kidney disease patients with hyperphosphatemia display high levels of FGF23 and PTH [20]; 2) kidney failure rat models, induced either by administering an adenine high-phosphorus diet or by removing more than four fifths of the kidney tissue plus administering a high phosphate diet, exhibit a significant increase in FGF23 serum levels. Performing a parathyroidectomy fully prevents or corrects the elevated FGF23 levels within these rat models [21•, 22]; 3) A Jansen's meta-physeal chondrodysplasia patient with a constitutively active PTHR1 receptor mutant exhibits high FGF23 serum level, despite low phosphate and normal 1,25(OH)₂D₃ levels; and 4) PTH infusion for 3 days to wild-type mice increases both serum FGF23 and calvaria FGF23 mRNA levels; and adding PTH to rat osteoblast-like UMR106 cells increases FGF23 mRNA up to fourfold [21•]. The data against PTH direct regulation of FGF23 are 1) exogenous 1,25(OH)₂D₃ could increase the circulating FGF23 level in *Gcm2* null mice that lack parathyroid glands [14] or in thyroparathyroidectomized rats [15] (note that the thymus also produces PTH [23]), and 2) addition of PTH to the in vitro cultured calvaria has no effect on the medium FGF23 concentration or the *Fgf23* mRNA levels in bone, although addition of 1,25(OH)₂D₃ increases FGF23 in the medium and in the bone [24].

FGF23 could also be regulated by PHEX or DMP1 due to the following evidence: 1) *Phex* null mice or *Hyp* mice [25] or *Dmp1* null mice [5] exhibit high *Fgf23* expression in bone and elevated circulating FGF23 level; 2) a single injection of FGF23 antibodies corrects the hypophosphatemia, and inappropriately normal serum 1,25-dihydroxyvitamin D, which are correlated by an increase of *NAPI2A* (type IIa sodium-phosphate cotransporter) and 1 α -hydroxylase (25-hydroxyvitamin-D-1 α -hydroxylase) in the kidney [26•]; 3) repeated injections of FGF23 antibodies in young *Hyp* mice ameliorate impaired bone growth, defective mineralization, and abnormal cartilage development [26•]; and 4) treatment of *Dmp1* null mice with the same FGF23 antibodies restores phosphate levels, and greatly improves the rickets phenotype, including osteoblast extracellular matrix mineralization, growth plate maturation, secondary ossification, and osteoblast differentiation [27••]. However, there is no evidence to support the hypothesis that PHEX or DMP1 directly regulates FGF23. For example, overexpression of the full-length DMP1 or the 57 kDa C-terminal of DMP1 in osteoblast/osteocytes has no effect on *Fgf23* expression in the wild-type background, although the DMP1 transgene fully rescues the *Dmp1* null phenotype [28•]. Similarly, despite repeated efforts by multiple laboratories, no evidence yet exists to prove that PHEX, an endopeptidase on the osteoblast/osteocyte membrane, cleaves FGF23 [29, 30].

It is well documented that there are dramatic decreases in osteoblast-specific gene expression levels and metabolic activity during the maturation of osteoblasts into osteocytes. This process is rather complex and is still largely undefined. Recent studies suggest that DMP1 plays an important role in controlling this process, as there are abnormal increases in many osteoblast-specific genes in the *Dmp1* null osteocyte, including a sharp increase in FGF23 [5], which are fully restored when the *Dmp1* transgene is re-expressed in these null cells [28•]. These findings may explain in part why DMP1 has no direct role in the regulation of FGF23 but deletion of *Dmp1* leads to a sharp increase in FGF23 (see a working model in Fig. 1).

Skeletal Abnormalities Associated with Aberrant FGF23 Action

Although FGF23 is maintained at a low level in normal conditions, elevated FGF23 serum levels have been observed in patients with ADHR, TIO, XLH, and ARHR. ADHR patients carry missense mutations in the *FGF23* gene, which results in the resistance of the mutant FGF23 to proteolytic degradation [2, 31]. Patients afflicted with TIO bear tumors overproducing FGF23 [3]. XLH and ARHR are caused by mutations in the *Phex* gene and *Dmp1* gene, respectively [4–6]. All patients with elevated FGF23 share a similar serum biochemical profile and clinical phenotype, characterized by a low serum phosphate concentration, inappropriately low or normal 1,25(OH) $_2$ D $_3$ levels, rickets, osteomalacia, lower extremity deformities, and short stature. Transgenic mice overexpressing *Fgf23* are phenocopies of the clinical characteristics of ADHR, TIO, and XLH [5, 32–34]. In addition, administration of either recombinant wild-type FGF23 or the ADHR mutant form of FGF23 to wild-type mice induces hypophosphatemia with increased renal phosphate clearance [3, 32]. Conversely, *Fgf23* null mice display hyperphosphatemia, ectopic mineralization in soft tissues, and possess a poorly formed skeleton with an extremely low PTH level and an elevated 1,25(OH) $_2$ D $_3$ level [10–12]. These human genetic studies and various mouse models provide firm evidence that possessing either an excess or a deficiency in *Fgf23* results in skeletal abnormalities.

Studies on *Hyp* and *Dmp1* null mice shed more light on the pathologic actions of excessive FGF23 in vivo. Aono et al. [26•] showed that applications of neutralizing anti-FGF23 monoclonal antibodies into *Hyp* mice, which blocks endogenous FGF23 action, increases expression of *NaPi2a* and 1 α -hydroxylase, and suppresses expression of 24-hydroxylase in

the kidney. As a result, hypophosphatemia and inappropriately normal serum 1,25-dihydroxyvitamin D are corrected, and the rachitic bone phenotypes typically observed in *Hyp* mice (eg, impaired longitudinal elongation, defective mineralization, and abnormal cartilage development) are ameliorated [26•, 35•]. Similarly, the treatment of *Dmp1* null mice with the same anti-FGF23 antibodies fully corrects hypophosphatemia, and greatly improves osteoblast extracellular matrix mineralization, growth plate maturation, secondary ossification center formation, and osteoblast differentiation [27••]. However, osteomalacia is only partially ameliorated in both animal models where there is a sharp increase in FGF23. These data point out the pathogenic role of elevated FGF23 in mediating the degree of hypophosphatemia and skeletal abnormalities within *Hyp* and *Dmp1* null mice.

Excessive FGF23 combined with hypophosphatemia (a consequence of a high FGF23 level) also impairs bone modeling (ie, reshaping of bone by independent action of osteoblasts and osteoclasts during birth to adulthood) and bone remodeling (ie, replacement of old bone by new bone, a process occurring primarily in the adult skeleton with the coupling of bone formation and bone resorption), where osteoclast numbers are sharply reduced [27••, 36]. Our data show that both *Hyp* (left) and *Dmp1* null mice (right) display a severe defect in bone modeling by radiographs (Fig. 2a). This modeling defect is likely caused by a defect in osteoclastogenesis based on the following evidence. Hayashibara et al. [36] documented a significant reduction of osteoclast number in *Hyp* mice. Similarly, the number of cells staining positive for tartrate-resistant acid phosphatase (a marker enzyme of osteoclasts) is sharply reduced within the *Dmp1*-knockout (KO) long bone in both the epiphyses and metaphyses at 3 weeks (Fig. 2b) [27••]. Consistent with this observation, the number of osteoclasts formed in bone marrow cultures from *Dmp1*-KO mice is less than 50% of that in the wild-type control. However, *Dmp1*-KO osteoclast precursors can differentiate normally when provided with the necessary factors such as receptor activator of nuclear factor- κ B ligand (RANKL) and macrophage colony-stimulating factor. Our laboratory further showed that the *Dmp1*-KO long bone expresses less RANKL and three times more osteoprotegerin, with a one-fifth ratio of RANKL to osteoprotegerin, than in the control, which explains why the osteoclast number is reduced in *Dmp1*-KO mice [27••]. Note that the remodeling process is severely impaired in 1-year-old *Dmp1*-KO mice (Fig. 2c, right panel). Taken together, these data suggest that the abnormalities in modeling/remodeling in *Dmp1*-KO mice are related to both the alteration in osteoblast function and the reduction in osteoclast number [27••]. It appears that a low level of phosphate caused by elevated FGF23 levels plays a key role in the defect of bone modeling/remodeling in animal models of hypophosphatemic rickets. For example, Murshed et al. [37] demonstrated that a high phosphate diet significantly improves the bone phenotype in *Hyp* mice. Similar findings have also been observed in *Dmp1*-null mice [5, 27••].

Fgf23-null mice show skeletal abnormalities (accompanied by hyperphosphatemia), an extremely low PTH level, and an elevated 1,25(OH) $_2$ D $_3$ level [10–12]. Ablation of 1 α -hydroxylase gene, which catalyzes metabolic activation of 25-hydroxyvitamin D $_3$ into 1,25(OH) $_2$ D $_3$ (an active form of vitamin D), in *Fgf23* null mice completely reverses the severe hyperphosphatemia, and ameliorates the ectopic calcification of soft tissues. Conversely, the abnormal bone mineralization persists [38]. In addition, the bone phenotype in mice lacking both the *Fgf23* and the 1 α -hydroxylase genes also resembles that of the 1 α -hydroxylase mutant mice, possessing a reduced total bone mineral content and bone mineral density [38]. Studies by Hesse et al. [39] further validate the pathologic role of VDR signaling by generating compound mutants deficient in both the *Fgf23* and *VDR* genes. The compound mutants present normal serum calcium and phosphate levels, normal body weight, no signs of ectopic calcifications, and significantly improved bone mineralization when fed with the rescue diet high in calcium, phosphate, and lactose [39]. Taken together, these in vivo findings, using compound mutant mice, suggest that excessive vitamin D

signaling plays a key role in the abnormalities of mineral homeostasis and the bone phenotype within *Fgf23* null mice.

Direct Effects of FGF23 on Bone

Does FGF23 directly target bone? Accumulating evidence suggests that imbalanced phosphate homeostasis and disturbed vitamin D metabolism play essential roles in skeletal abnormalities associated with aberrant FGF23 action. The direct effects of FGF23 on bone are not excluded. It is known that FGF23 is primarily expressed in osteoblasts/osteocytes in bone [5, 11, 12], and all receptors of FGF23 (*Fgfr1c*, *Fgfr2c*, and *Fgfr3c*) are expressed in osteoblasts [40•, 41], although Klotho, the essential co-receptor of FGF23, is not expressed in bone [42] and FGF23 has low affinity to FGFRs in the absence of Klotho [43]. Therefore, FGF23 might exert its effects on osteoblasts weakly through direct activation of FGF signaling.

This possibility has been supported by a number of in vitro studies. Wang et al. [40•] investigated the effects of FGF23 on isolated fetal rat calvarial cells in vitro. Overexpression of human *Fgf23* using adenoviral-mediated approach has no effect on cell proliferation, but markedly inhibits the differentiation and mineralization of these cells [40•]. A similar result is also obtained in wild-type mouse calvaria treated with recombinant FGF23 protein [44•]. In addition, Wang et al. [40•] demonstrated that FGF23 enhances the phosphorylation of FGFRs, and that SU5402, an inhibitor of the tyrosine kinase activity of FGFR1, reverses the inhibitory effects of excessive FGF23 on matrix mineralization. These in vitro studies provide evidence that FGF23 might directly inhibit bone formation via its activation of FGF signaling.

Studies performed on *Fgf23* null mice further show that FGF23 might play a direct role on bone modeling/remodeling. Administering a phosphate-deficient diet to *Fgf23* null mice does not correct their persistent bone mineralization defects, but does completely reverse hyperphosphatemia, prevent ectopic calcifications from forming, and greatly improve the survival of these mice. [45]. Sitara et al. [44•] recently generated a mouse model deficient in both *Fgf23* and *NaPi2a* (the key phosphate cotransporter in the kidney) genes. These double mutant mice are viable and exhibit normal physical activities. Unexpectedly, the skeletal phenotype in these double null mice resembles that of *Fgf23* null mice, despite the fact that the phosphate level is reversed compared with that in *NaPi2a* null mice. Of note, the *NaPi2a* null mouse exhibits hypophosphatemia but has no apparent bone phenotype. These studies suggest that the bone defects observed in *Fgf23* null mice are due to a FGF23 deficiency and are independent of systemic phosphate homeostasis [44•].

Conclusions and Perspective

As a potent phosphaturic hormone, FGF23 has attracted extensive research on its regulation and function since its discovery. Studies performed have demonstrated that FGF23 is tightly regulated at the transcriptional and posttranscriptional levels by systemic as well as local factors, and the kidney has been identified as its main target organ. An excess or deficiency in FGF23, caused by its disturbed regulation, would result in imbalanced phosphate homeostasis and inappropriate vitamin D metabolism, which contributes significantly to the appearance of skeletal abnormalities. However, direct roles of aberrant FGF23 action on bone have also been suggested. Therefore, FGF23 may control bone formation and modeling/remodeling via both direct and indirect mechanisms.

Although there is an increase in the knowledge of FGF23 regulation and function, many challenging questions remain to be resolved. For example, the detailed mechanisms negatively regulating FGF23 by dietary intake and serum phosphate levels are unknown.

Also, it remains to be completely understood as to how loss-of-function *DMP1* and *PHEX* results in an increase in *FGF23* gene transcription. Although there is in vitro evidence that FGF23 could target bone, the mystery is that Klotho, the essential co-receptor of FGF23, is not expressed in bone. Another perplexing puzzle is that an increase in FGF23 is the primary cause of hypophosphatemic rickets in all animal models, but an increase of FGF23 is not linked with all cases of hypophosphatemic rickets in humans. By continuing to elucidate these issues, we will gain more novel insights into the biological function and regulation of FGF23.

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Fig. 1.

Effects of dentin matrix protein 1 (DMP1) on osteocyte maturation. The proposed model is that gene expression levels are high in matrix producing osteoblasts, whereas gene expression levels are reduced during osteocyte maturation. DMP1 is a key player during this downregulation process. A loss of DMP1 disrupts normal osteoblast to osteocyte maturation and mineralization, and expression of many genes, such as *Fgf23* and *Col1*, remain abnormally elevated in the knockout (KO) osteocytes. WT—wild-type. (Part of the figure is adapted from Feng et al. [5] and Zhang et al. [27••])

Fig. 2. Abnormal bone modeling/remodeling in the *Hyp* and *Dmp1* null mice. **a**, Radiographs of 6-week-old femurs obtained from wild-type (WT) controls (Cont), hyp mice, and *Dmp1*-knockout (KO) showing a severe defect in modeling and length. **b**, Tartrate-resistant acid phosphatase (TRAP) stained 3-week-old femurs showing a sharp reduction in osteoclast number (*right panel*). **c**, Photographs of the 1-year-old *Dmp1*-KO (*right panel*) versus WT Cont (*left panel*) show a severe defect in remodeling. (*Figures B and C* are adapted from Zhang et al. [27••])