Pathogenesis of FGF23-Related Hypophosphatemic Diseases

Subjects: Biology

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Since phosphate is indispensable for skeletal mineralization, chronic hypophosphatemia causes rickets and osteomalacia. Fibroblast growth factor 23 (FGF23), which is mainly produced by osteocytes in bone, functions as the central regulator of phosphate metabolism by increasing the renal excretion of phosphate and suppressing the production of 1,25-dihydroxyvitamin D. The excessive action of FGF23 results in hypophosphatemic diseases, which include a number of genetic disorders such as X-linked hypophosphatemic rickets (XLH) and tumor-induced osteomalacia (TIO). Phosphate-regulating gene homologous to endopeptidase on the X chromosome (PHEX), dentin matrix protein 1 (DMP1), ectonucleotide pyrophosphatase phosphodiesterase-1, and family with sequence similarity 20c, the inactivating variants of which are responsible for FGF23-related hereditary rickets/osteomalacia, are highly expressed in osteocytes, similar to FGF23, suggesting that they are local negative regulators of FGF23. Autosomal dominant hypophosphatemic rickets (ADHR) is caused by cleavage-resistant variants of FGF23, and iron deficiency increases serum levels of FGF23 and the manifestation of symptoms in ADHR. Enhanced FGF receptor (FGFR) signaling in osteocytes is suggested to be involved in the overproduction of FGF23 in XLH and autosomal recessive hypophosphatemic rickets type 1, which are caused by the inactivation of PHEX and DMP1, respectively. TIO is caused by the overproduction of FGF23 by phosphaturic tumors, which are often positive for FGFR. FGF23-related hypophosphatemia may also be associated with McCune-Albright syndrome, linear sebaceous nevus syndrome, and the intravenous administration of iron.

Keywords: fibroblast growth factor 23 ; osteocytes ; Rickets

1. Fibroblast Growth Factor 23 (FGF23)-Related Hypophosphatemic Diseases

Since the FGF23-αKlotho axis plays a central role in phosphate homeostasis, its disruption causes hyperphosphatemic conditions, as described in the previous section. On the other hand, the excessive action of FGF23 underlies various hypophosphatemic diseases, which are characterized by urinary phosphate wasting, hypophosphatemia, and inappropriately low levels of serum $1,25(OH)_2D$ [1][2]. Phosphate is indispensable for skeletal mineralization; therefore, chronic hypophosphatemia due to excessive FGF23 leads to rickets in children and osteomalacia in adults. The impaired production of $1,25(OH)_2D$ contributes to the resistance of FGF23-related hypophosphatemic rickets/osteomalacia to native vitamin D. FGF23-related hypophosphatemic rickets/osteomalacia include various conditions such as genetic diseases.

2. Autosomal Dominant Hypophosphatemic Rickets (ADHR)

ADHR is caused by missense variants in the FGF23 gene. Since the responsible variants occur at Arg^{176} or Arg^{179} within the RXXR/S motif recognized by subtilisin-like proprotein convertase, the resultant mutant FGF23 protein is resistant to cleavage-mediated inactivation [3]. However, this disease shows incomplete penetrance. Patients with ADHR variants do not always have high serum levels of intact FGF23, and the disease may occur with an early or delayed onset and variable expressivity [4]. Late-onset ADHR primarily manifests in post-pubertal women who are prone to iron deficiency [5]. Current findings suggest that iron deficiency increases the expression of *Fgf23* in bone and also that the FGF23 protein is cleaved in iron deficiency to maintain normal serum levels of FGF23 and normophosphatemia in control subjects, whereas the cleavage resistance of mutant FGF23 leads to the accumulation of intact FGF23 and hypophosphatemia in ADHR subjects [6].

3. X-Linked Hypophosphatemic Rickets (XLH)

XLH (OMIM #307800) is the most common form of hereditary hypophosphatemic rickets. Patients with XLH have elevated serum levels of FGF23, which result in urinary Pi wasting, hypophosphatemia, and inappropriately low levels of $1,25(OH)_2D$ [7]. XLH was initially called vitamin D-resistant rickets because of a poor response to treatment with native vitamin D at dosages that cure vitamin D-deficient rickets. XLH is caused by inactivating variants in the *phosphate-regulating gene homologous to endopeptidase on the X chromosome* (*PHEX*) located at Xp22.1, showing X-linked dominant inheritance [8]. Similar to FGF23, PHEX is expressed in osteoblast lineage cells and is more highly expressed in osteocytes [9][10]. Although its structure suggests that the product of *PHEX* functions as a cell surface-bound, zinc-dependent protease, its physiological substrates remain elusive. In hypophosphatemic *Hyp* mice, which harbor a large deletion in the *Phex* gene and are widely used as a model for XLH, the expression of *Fgf23* in osteocytes was found to be increased [9][11]; however, FGF23 did not serve as a substrate for PHEX [12]. Therefore, the regulation of FGF23 by PHEX may be indirect and involve other molecule(s).

As osteoblasts mature into osteocytes, the expression of *dentin matrix protein 1* (*Dmp1*) and *family with sequence similarity 20c* (*Fam20c*), which are responsible for autosomal recessive hypophosphatemic type 1 (ARHR1) and Raine syndrome (RNS), respectively, increased in both *Hyp* and wild-type cells, and these genes were up-regulated in *Hyp* cells, similar to *Fgf23* [9]. These findings indicated the critical roles of osteocytes in phosphate homeostasis and also suggested complex abnormalities in *Phex*-deficient osteocytes. The regulation of FGF23 production by FGFR signaling is also supported by osteoglophonic dysplasia, which is a rare skeletal dysplasia caused by activating mutations in *FGFR1* that is frequently associated with elevated serum FGF23 levels and hypophosphatemia [13].

Enhanced FGFR signaling in *Phex*-deficient osteocytes is of interest based on previous findings suggesting that FGFR plays a critical role in the transduction of signaling evoked by increased extracellular Pi [14][15][16]. In various cell types, treatment with high extracellular Pi activated FGFR for the regulation of gene expression. In an osteoblastic cell line, treatment with an FGFR inhibitor abolished the up-regulation of Dmp1 by increased extracellular Pi [15]. In HEK293 cells, the knockdown of FGFR1 diminished the Pi-induced phosphorylation of ERK1/2 [14]. More recently, the activation of FGFR1 by extracellular Pi was shown to increase the expression of *GalNt3* in bone, leading to an elevated serum level of FGF23 in mice [16]. Collectively, these findings suggest that FGFR1 is involved in the sensing of Pi availability. In consideration of this role of FGFR and enhanced FGFR signaling in the osteocytes of *Hyp* mice, abnormal Pi sensing may be involved in the pathogenesis of XLH.

Matrix extracellular phosphoglycoprotein (MEPE) is a member of the SIBLING (small integrin-binding ligand, N-linked glycoproteins) family, and was initially cloned from the tumor of a patient with TIO [17]. A genome-wide association research proposed MEPE as a factor influencing bone mineral density in humans [18]. MEPE contains an acidic serine-aspartate rich MEPE-associated motif (ASARM) consisting of 23 residues at the C terminus. ASARM peptides released from MEPE by cathepsin-mediated cleavage have been shown to inhibit mineralization. The ASARM motif is also present in other SIBLING proteins, such as DMP1 and osteopontin, and PHEX may bind to these proteins at these motifs [17]. A previous research implicated ASARM peptides released from these SIBLING proteins in defective mineralization in XLH [19].

Growth retardation is often observed in patients with XLH: however, the underlying mechanisms have not yet been elucidated in detail. A research published by Fuente et al. demonstrated marked alterations in the structure, dynamics, and maturation of growth plate cartilage in growth-retarded young *Hyp* mice [20]. In the growth plates of *Hyp* mice, both proliferation and apoptosis rates of chondrocytes were reduced, and the hypertrophy and maturation of chondrocytes were severely disturbed. The spatial organization of the chondro-osseous junction and the primary spongiosa trabeculae were markedly deformed. These alterations in the growth plates might be the mechanisms for the growth retardation in *Hyp* mice. The researchers also found an enhanced activation of the extracellular signal-regulated kinase (ERK)1/2 signaling pathway in the *Hyp* growth plates, implying an involvement of FGF23 in these abnormalities [20]. Reduction in caspase-mediated apoptosis of hypertrophic chondrocytes was also reported in rachitic mice with low-phosphate diet-induced hypophosphatemia as well as in *Hyp* mice, which suggests that hypophosphatemia impairs apoptosis of hypertrophic chondrocytes, leading to rickets [21].

Although chondrocytes do not express αKlotho, which is required for FGF23 to activate its downstream signaling pathways at physiological concentrations, soluble forms of αKlotho are present in serum and cerebrospinal fluid [22] and have been implicated in the regulation of FGF23 signaling in cells without the transmembrane form of αKlotho [23].

Since the placenta expresses FGFR1 and αKlotho, high levels of FGF23 in pregnant women with XLH may affect their fetuses. *Hyp* and wild-type female mice were mated with wild-type male mice, and the pregnant mothers and their male fetuses were subjected to analyses. FGF23 levels were higher in *Hyp* mothers than in wild-type mothers. *Hyp* fetuses and wild-type fetuses were obtained from mating between *Hyp* females and wild-type males. FGF23 levels in *Hyp* fetuses were approximately 20-fold higher than in their mothers, while wild-type fetuses from *Hyp* mothers had low levels of FGF23, as did fetuses from wild-type mothers, suggesting that FGF23 does not cross the placenta [24]. The expression of *Cyp24a1* was higher in the placentas of fetuses from *Hyp* mothers than in those of fetuses from wild-type mothers, which resulted in decreased levels of plasma 25-hydroxyvitamin D in fetuses from *Hyp* mothers. Therefore, increased levels of circulating FGF23 in *Hyp* mothers may exert direct effects on the placenta during pregnancy and alter fetal vitamin D metabolism via the regulation of *Cyp24a1* expression [24]. Further studies are needed to clarify whether similar phenomena occur with pregnancy in human patients with XLH.

The enthesis is a tissue that forms at the site of insertion of a tendon to bone and consists of a bony eminence, mineralized fibrocartilage, unmineralized fibrocartilage, and a tendon. It optimizes the transfer of mechanical force from muscle to bone, which is required for efficient movements [25]. Enthesopathy is a pathological change at the insertion of tendons and ligaments. Mineralizing enthesopathy is one of the complications of XLH and other types of FGF23-related hypophosphatemia and accounts for a high morbidity rate in adult patients [26]. Karaplis et al. previously reported that a transgenic mouse model overexpressing a secreted form of the human FGF23[p.R176Q] variant, which is resistant to cleavage, displayed mineralizing enthesopathy of the Achilles and planar facial insertions, suggesting the involvement of FGF23 in the development of mineralizing enthesopathy [27]. More recently, Liu et al. investigated the cellular and molecular mechanisms involved in the development of mineralizing enthesopathy in *Hyp* mice and reported that Achilles tendon entheses of *Hyp* mice showed the expansion of hypertrophic-appearing chondrogenic cells. In comparison with the entheses of wild-type mice, *Hyp* entheses exhibited the expansion of cells expressing the chondrogenic marker gene *Sox9* and enhanced bone morphogenetic protein and Indian hedgehog signaling pathways, both of which play critical roles in chondrocyte differentiation [28]. Although oral phosphate salts and active vitamin D metabolites are administered as conventional medical treatments for XLH to correct their deficiencies, it does not prevent or ameliorate enthesopathies [27]. Burosumab, a humanized monoclonal neutralizing antibody to FGF23, has recently become available as a new treatment for XLH [7]. In Japan, burosumab has been approved for the treatment of all types of FGF23-related hypophosphatemic rickets/osteomalacia. In pediatric patients with XLH, improvements in the severity of rickets and biochemical parameters were greater in patients treated with burosumab than in those who continued conventional therapy [29]. Further studies are needed to clarify the effects of burosumab on the prevention and treatment of enthesopathies.

4. Autosomal Recessive Hypophosphatemic Rickets Type 1 (ARHR1)

ARHR1 (OMIM #241520) is caused by inactivating variants of the *DMP1* gene [30][31]. DMP1 is an extracellular matrix protein belonging to the SIBLING family and is highly expressed in osteocytes as well as in dentin. Patients with ARHR1 manifest elevated FGF23 levels, hypophosphatemia, inappropriately low $1,25(OH)_2D$ levels, and skeletal hypomineralization, similar to patients with XLH. *Dmp1*-null mice reproduced the phenotype of ARHR1 and exhibited defective osteocyte maturation and the up-regulated expression of *Fgf23* in osteocytes [30][31]. Although the pathogenesis of ARHR1 remains largely unknown, the findings of studies using *Phex*-deficient *Hyp* mice and *Dmp1*-null mice suggest that the overproduction of FGF23 is attributable to enhanced FGFR signaling in bone in both mouse models [32].

5. Autosomal Recessive Hypophosphatemic Rickets Type 2 (ARHR2)

ARHR2 (OMIM #613312) also belongs to FGF23-related hypophosphatemic rickets and is caused by inactivating variants in the *ectonucleotide pyrophosphatase phosphodiesterase-1* (*ENPP1*) gene [33][34]. *ENPP1* encodes an enzyme that produces pyrophosphate (PPi), a potent inhibitor of mineralization, and inactivating variants in *ENPP1* are also responsible for hypermineralization disorders, such as generalized arterial calcification in infancy [35]. The ectoenzyme tissue non-specific alkaline phosphatase (TNSALP) facilitates skeletal mineralization by degrading PPi to produce Pi. Although PPi may regulate the production of FGF23, patients with hypophosphatasia, which is caused by inactivating variants in TNSALP, had normal levels of FGF23 despite elevated extracellular levels of PPi [36]. Therefore, the mechanisms by which ENPP1 deficiency results in the overproduction of FGF23 remain unclear. Since inactivating variants in ENPP1 cause conditions characterized by ectopic calcification and FGF23-related hypophosphatemia, a close relationship may exist between ectopic calcification and the overproduction of FGF23.

6. Raine Syndrome (RNS)

FAM20C, also known as *DMP4*, encodes a kinase that phosphorylates various secreted proteins. The proteins phosphorylated by FAM20C include FGF23 and members of the SIBLING family, such as DMP1, osteopontin, and MEPE [37][38]. Inactivating variants in the *FAM20C* gene are responsible for RNS. RNS is an autosomal recessive disease that is characterized by craniofacial malformation, osteosclerotic bone dysplasia, and a poor prognosis [39]. Surviving patients with mild RNS manifest hypophosphatemia due to elevated levels of FGF23 and dental anomalies [40][41]. *Fam20c*-null mice exhibited elevated levels of serum FGF23, hypophosphatemia, and dental anomalies [42]. These mice also showed low expression levels of *Dmp1* in osteocytes, which suggested that the down-regulated expression of DMP1 plays a causal role in the overproduction of FGF23 in RNS [42]. However, the overexpression of *Dmp1* failed to rescue the defects in *Fam20c*-null mice [43]. A previous research reported that FAM20C phosphorylated FGF23 on Ser^{180}, which inhibited the *O*-glycosylation of FGF23 on Thr^{178} by GalNAc-T3 and accelerated cleavage [37]. Therefore, inactivating variants in FAM20C may increase FGF23 levels by inhibiting its cleavage.

7. Tumor-Induced Osteomalacia (TIO)

TIO is a rare paraneoplastic syndrome characterized by urinary phosphate wasting, hypophosphatemia, and osteomalacia. Responsible tumors are generally benign, slow-growing phosphaturic mesenchymal tumors (PMT) [44]. The overproduction of FGF23 by tumors was previously shown to enhance the renal excretion of Pi and induce hypophosphatemia, low $1,25(OH)_2D$ levels, and osteomalacia, which were cured by the surgical removal of the responsible tumor [45][46]. Lee et al. identified the fusion genes *Fibronectin 1* (*FN1*)-*FGFR1* and *FN1-FGF1* in subgroups of PMT and showed that immunoreactivity for FGFR1 was positive in 82% of PMT [47][48]. These findings suggest the involvement of the FGF/FGFR signaling pathway in the development of PMT.

8. Other Causes of FGF23-Related Hypophosphatemia

McCune-Albright syndrome (MAS, OMIM #174800) is characterized by polyostotic fibrous dysplasia, café-au-lait skin pigmentation, and precocious puberty, and is caused by a somatic activating variant in *GNAS1* encoding the subunit of the stimulatory G protein. MAS is clinically heterogeneous and may manifest various endocrinological abnormalities. Some patients with MAS exhibit hypophosphatemia, which results from the overproduction of FGF23 by abnormal skeletal progenitor cells in the bone lesions of fibrous dysplasia [49]. Serum levels of FGF23 in MAS patients correlate with disease activity [49], and significant hypophosphatemia only occurs in patients with a severe disease burden. A previous research suggested that the ratio of the C-terminal fragment of FGF23 to intact FGF23 was elevated by accelerated cleavage in the bone lesions of fibrous dysplasia [50].

Linear sebaceous nevus syndrome, also called Schimmelpenning-Feuerstein-Mims (SFM) syndrome (OMIM #163200), is characterized by congenital linear nevus sebaceous and abnormalities in neuroectodermal organs and is caused by somatic variants in *RAS* genes, including *KRAS*, *HRAS*, and *NRAS*, which are detectable in skin lesions [51][52]. Hypophosphatemia due to elevated levels of FGF23 is rarely associated with SFM syndrome. Lim et al. suggested that the source of FGF23 in SFM syndrome was bone lesions carrying *RAS* variants rather than skin lesions [53].

Osteoglophonic dysplasia (OMIM #166250) is a rare autosomal dominant disease characterized by rhizomelic dwarfism, non-ossifying bone lesions, craniosynostosis, and face abnormalities, and is caused by activating variants in the *FGFR1* gene.

Jansen's metaphyseal chondrodysplasia is an autosomal dominant disease caused by an activating variant in the *PTH type 1 receptor* (*PTH1R*) gene [54]. Previous studies reported that FGF23-related hypophosphatemia may be associated with Jansen's metaphyseal chondrodysplasia [55]. This finding suggests that PTH signaling stimulates FGF23 production, which is also supported by the findings of several in vivo and in vitro studies [56][57][58].

FGF23-related hypophosphatemic rickets/osteomalacia may also be associated with the intravenous administration of saccharated ferric oxide or iron polymaltose [59][60]. The mechanisms by which these drugs cause the overproduction of FGF23 remain unclear; however, their discontinuance rapidly restores elevated FGF23 levels and hypophosphatemia.

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