FGF23-related hypophosphatemic rickets/osteomalacia: diagnosis and new treatment

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Abstract

FGF23 is a phosphaturic hormone produced by bone. FGF23 reduces serum phosphate by suppressing proximal tubular phosphate reabsorption and intestinal phosphate absorption. After the identification of FGF23, several kinds of hypophosphatemic rickets/osteomalacia such as X-linked hypophosphatemia (XLH) and tumor-induced osteomalacia (TIO) have been shown to be caused by excessive actions of FGF23. Circulatory FGF23 is high in patients with these hypophosphatemic diseases while FGF23 is rather low in those with chronic hypophosphatemia from other causes such as vitamin D deficiency. These results indicate that FGF23 measurement is useful for the differential diagnosis of hypophosphatemia. Chemiluminescent enzyme immunoassay for FGF23 has been approved for clinical use in Japan. The first choice treatment for patients with TIO is complete removal of responsible tumors. However, it is not always possible to find and completely remove responsible tumors. Phosphate and active vitamin D have been used for patients with hypophosphatemic diseases caused by excessive actions of FGF23 including TIO patients with unresectable tumors. However, these medications have limited effects and several adverse events. The inhibition of excessive FGF23 actions has been considered to be a novel therapy for these hypophosphatemic diseases. Human MAB for FGF23, burosumab, has been shown to improve biochemical abnormalities, roentgenological signs of rickets, growth, fracture healing and impaired mineralization in patients with XLH. Burosumab has been approved in several countries including Europe, North America and Japan. Long-term effects of burosumab need to be addressed in future studies.

Introduction

FGF23 was cloned as a responsible gene for autosomal dominant hypophosphatemic rickets (ADHR) (ADHR Consortium 2000). FGF23 was also identified as a responsible humoral factor for tumor-induced osteomalacia (TIO), a rare paraneoplastic syndrome characterized by hypophosphatemia (Shimada et al. 2001). Since then it has been shown that FGF23 works as a hormone regulating phosphate and vitamin D metabolism. In addition, several other kinds of hypophosphatemic rickets/osteomalacia have been shown to be caused by excessive actions of FGF23 (Fukumoto & Martin 2009). Recently, a new treatment for these hypophosphatemic diseases has become available in several countries. I will summarize recent findings concerning FGF23-related hypophosphatemic diseases.
Function of FGF23

After the cloning of FGF23, the function of FGF23 was examined using recombinant FGF23. Results indicated that FGF23 suppresses the expression of type 2a and 2c sodium–phosphate cotransporters in the brush border membrane of renal proximal tubules and inhibits proximal tubular phosphate reabsorption (Shimada et al. 2004). About 80–90% of phosphate filtered from glomeruli is reabsorbed in proximal tubules through these sodium–phosphate cotransporters. In addition, FGF23 suppresses the expression of CYP27B1 which encodes an enzyme that converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (1,25(OH)₂D) (Shimada et al. 2004). FGF23 also enhances CYP24A1 expression coding, an enzyme that works to reduce circulatory level of 1,25(OH)₂D (Shimada et al. 2004). From these changes of the expression of vitamin D-metabolizing enzymes, FGF23 reduces serum 1,25(OH)₂D level. 1,25(OH)₂D enhances the expression of type 2b sodium–phosphate cotransporter in the brush border membrane of intestinal cells and stimulates intestinal phosphate absorption (Xu et al. 2002). Therefore, FGF23 reduces serum phosphate level by inhibiting both proximal tubular phosphate reabsorption and intestinal phosphate absorption through reducing 1,25(OH)₂D level (Fig. 1).

Physiological sources of FGF23 are believed to be bone, osteoblasts and osteocytes (Liu et al. 2003). On the other hand, FGF23 works in kidney indicating that there is a specific receptor for FGF23 in kidney. FGF23 belongs to FGFR family. There are 22 FGFR family members in mouse and human. Human FGFR1 is an ortholog of murine Fgf15. FGF family members have been shown to bind to FGF receptors (FGFRs). There are four FGFR genes and alternative splicing form genes producing many FGFR subtypes (Itoh & Ornitz 2004). However, the expression of these FGFR subtypes is basically tissue non-specific. Investigation of binding proteins to FGF23 in kidney identified Klotho (Urakawa et al. 2006). Subsequent studies indicated that FGF23 binds to Klotho/FGF receptor 1 complex for its action (Kurosu et al. 2006, Urakawa et al. 2006) (Fig. 1).

FGF23 activates several signal transduction pathways in the downstream of FGFR1 including extracellular signal-regulated kinase (ERK), AKT and phospholipase Cγ (Goetz & Mohammadi 2013). It is likely that the renal actions of FGF23 are mainly mediated by ERK pathway (Zhang et al. 2012) (Fig. 1).

Posttranslational modification of FGF23

FGF23 encodes a protein with 251 amino acids (Shimada et al. 2001). The N-terminal 24 amino acids compose a signal peptide. A part of FGF23 protein is proteolytically cleaved between 179Arg and 180Ser before secretion (Shimada et al. 2001). This processing is mediated by enzymes that recognize R-X-X-R motif of FGF23 protein. While full length FGF23 has biological activities as shown above, the processed N-terminal and C-terminal fragments are inactive (Shimada et al. 2002) (Fig. 2). Posttranslational modification of FGF23 has been reported to affect this processing of FGF23. The attachment of mucin-type O-linked glycan to 178Thr prevents the processing between 179Arg and 180Ser (Kato et al. 2006, Frishberg et al. 2007). This glycation is initiated by an enzyme called polypeptide N-acetylgalactosaminyltransferase 3 encoded by GALNT3. On the other hand, phosphorylation of 180Ser by family with sequence similarity 20, member C (FAM20C) works to enhance the processing of FGF23 protein (Tagliabracci et al. 2014). Therefore, FGF23 concentration and activities can be regulated not only by FGF23 transcription and translation but also by the posttranslational modification of FGF23 protein.

Regulation of FGF23 production

The regulatory mechanisms of FGF23 production and serum FGF23 level have not been completely understood. Because FGF23 regulates serum phosphate level, it seemed likely that changes in serum phosphate affect FGF23 production and FGF23 level. While high phosphate diet
increased FGF23 levels in both mouse and human (Ferrari et al. 2005, Perwad et al. 2005, Antoniucci et al. 2006), i.v. infusion of phosphate and subsequent increase of serum phosphate for several hours did not change FGF23 concentration in healthy volunteers (Ito et al. 2007). In the regulation of serum calcium level, the increase of serum calcium inhibits PTH secretion within several minutes through calcium-sensing receptor on the surface of parathyroid cells (Brown et al. 1993). However, there seems to be no such rapid regulation of FGF23 level in the regulation of serum phosphate (Takashi et al. 2019). Recent study revealed the importance of posttranslational modification of FGF23 protein in response to phosphate. High phosphate diet for 2 weeks increased serum phosphate and FGF23 in mice as expected. However, the expression of Fgf23 in femur did not change by this diet. On the other hand, high phosphate diet increased the expression of Galnt3 in bone suggesting that high phosphate diet increased serum FGF23 by preventing the proteolytic processing of FGF23 protein. Further in vivo and in vitro analysis showed that phosphate enhances Galnt3 expression through FGFR1–FGF receptor substrate 2a–ERK pathway (Takashi et al. 2019). These results indicate that FGFR1 works at least as one component of phosphate-sensing mechanism in the regulation of FGF23 level in response to phosphate.

In addition to phosphate, 1,25(OH)2D and PTH have been reported to enhance FGF23 production (Saito et al. 2005, Rhee et al. 2011). FGF23 reduces 1,25(OH)2D level as mentioned previously and was reported to inhibit PTH synthesis and secretion (Ben-Dov et al. 2007). These results indicate that there are multiple negative feedback loops involving FGF23, phosphate, 1,25(OH)2D and PTH regulating phosphate metabolism.

On the other hand, FGF23 production has been shown to be affected by several other factors. Inflammatory cytokines such as tumor necrosis factor-a and interleukin-1b, iron deficiency and erythropoietin have been shown to enhance FGF23 production (Farrow et al. 2011, Ito et al. 2015, Daryadel et al. 2018). These factors are considered to be potential mediators for high FGF23 in patients with chronic kidney disease. In addition, a recent study identified lysophosphatidic acid as an inducer of FGF23 production in acute kidney injury (Simic et al. 2020). Furthermore, studies using genetically modified murine models indicated the involvement of membrane Klotho and some G protein-coupled receptor coupled with extra large as in the enhanced FGF23 production (He et al. 2019, Xiao et al. 2019). It has been also reported that insulin and energy deprivation reduce FGF23 production (Bar et al. 2018, Vidal et al. 2020). These factors activate quite different intracellular signaling pathways including JAK-STAT, hypoxia inducible factor 1a, ERK, phosphatidylinositol-3 kinase, and AMP-activated protein kinase. It is currently unknown how these different factors are involved in the physiological regulation of FGF23 production for the maintenance of phosphate and vitamin D metabolism.

**FGF23-related hypophosphatemic rickets/osteomalacia**

After the identification of FGF23, several kinds of hypophosphatemic rickets/osteomalacia other than ADHR and TIO have been shown to be caused by excessive actions of FGF23 (Table 1). Of these, X-linked hypophosphatemic rickets (XLHR) in children is by far the most prevalent cause of genetic hypophosphatemic rickets (Fukumoto & Martin 2009). The responsible gene for X-linked hypophosphatemia (XLH) in adults and children XLHR has been cloned by positional cloning and named PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) (The HYP Consortium 1995). PHEX is a type 2 membrane protein with a short N-terminal intracellular region. PHEX is mainly expressed in bone and teeth (Beck et al. 1997). Hyp mouse is a murine model of XLH and has a deletion in the 3’ region of Phex gene (Beck et al. 1997). FGF23 was shown to be overexpressed in bone of Hyp mice while the detailed mechanism of this FGF23 overexpression is not clear (Liu et al. 2003). Similarly, in other genetic
hypophosphatemic rickets such as autosomal recessive hypophosphatemic rickets 1 and 2 (ARHR 1, 2) (Feng et al. 2006, Lorenz-Depiereux et al. 2006, 2010, Levy-Litan et al. 2010). FGF23 is considered to be overexpressed in bone. However, it is largely unknown how the mutations in the responsible genes cause enhanced expression of FGF23.

Mutations in patients with ADHR change either 176Arg or 179Arg of FGF23 to other amino acids (ADHR Consortium 2000). These changes make the mutant protein resistant for the processing between 179Arg and 180Ser (White et al. 2001, Shimada et al. 2002). This resistance to the processing seems to contribute to increase serum FGF23 level. However, this resistance to the cleavage alone cannot explain high FGF23 and hypophosphatemia in patients with ADHR. FGF23 production is regulated by several factors as mentioned previously. Therefore, the physiological regulatory mechanism of FGF23 production should prevent the overproduction of FGF23 even when the patients have one mutant allele of FGF23. In patients with ADHR, other environmental factors seem to be necessary for the development of hypophosphatemia. Actually, it is known that the biochemical abnormalities and disease phenotype wax and wane in patients with ADHR (Imel et al. 2007). Especially, iron deficiency is considered to be one of these factors (Imel et al. 2011). Iron deficiency enhances both FGF23 transcription and the cleavage between 179Arg and 180Ser resulting in normal FGF23 and phosphate level in subjects without mutations in FGF23 (Wolf et al. 2013). However, in patients with ADHR, FGF23 increases because of the resistance of the mutant FGF23 protein for the processing. Theoretically, other non-physiological environmental factors that enhance FGF23 transcription can induce high FGF23 and hypophosphatemia in patients with ADHR.

TIO is a rare paraneoplastic syndrome. The most frequent causes are bone and soft tissue tumors whose pathological diagnosis is phosphaturic mesenchymal tumor, mixed connective tissue variant (PMTMCT) (Folpe et al. 2004). These tumors overproduce FGF23. However, the mechanism of this overproduction of FGF23 is not clear. Some tumors causing TIO have been shown to have fibronectin (FN)-FGFR1 or FN-FGFR1 fusion gene (Lee et al. 2015, 2016). In addition, Klotho was shown to be expressed in some tumors causing TIO (Kinoshita et al. 2019, Lee et al. 2020). It is possible that these fusion genes and signals from Klotho/FGFR1 complex are involved in the overproduction of FGF23. Some i.v. iron preparations have also been reported to cause FGF23-related hypophosphatemic diseases (Schouten et al. 2009a,b, Shimizu et al. 2009). It is proposed that i.v. iron administration prevents the processing of FGF23 protein in patients with iron deficiency anemia in whom iron deficiency has already enhanced FGF23 transcription and the processing (Wolf et al. 2013).

### Diagnosis of FGF23-related hypophosphatemic diseases

The characteristic biochemical features of patients with FGF23-related hypophosphatemic diseases are chronic hypophosphatemia, low tubular maxim transport of phosphate per glomerular filtration rate (TmP/GFR), low to low normal 1,25(OH)₂D and high or high normal FGF23 levels. After the cloning of FGF23, several kinds of ELISA for FGF23 have been developed (Yamazaki et al. 2001, Shimada et al. 2002). In patients with TIO, FGF23 is considered to be overexpressed. Some i.v. iron preparations have also been reported to cause FGF23-related hypophosphatemic diseases (Schouten et al. 2009a,b).
et al. 2002, Jonsson et al. 2003). The intact assay uses two monoclonal antibodies that detect N-terminal and C-terminal portion of the processing site of FGF23 (Fig. 3). This assay measures only biologically active FGF23 (Yamazaki et al. 2002). On the other hand, the C-terminal assay uses two kinds of antibodies against the C-terminal portion (Fig. 3). This assay detects both the intact and the processed C-terminal fragment of FGF23 (Jonsson et al. 2003). FGF23 level measured by C-terminal assay is considered to reflect the amount of FGF23 transcription or translation. FGF23 values by the intact and C-terminal assays usually correlate well (Ito et al. 2005). Both intact and C-terminal assay may be useful in clinical medicine. However, these values can be quite discrepant when the processing of FGF23 protein is accelerated (Fukumoto & Martin 2009). As shown below, intact assay has been approved for clinical use in Japan.

In contrast to patients with FGF23-related hypophosphatemic diseases, intact FGF23 levels were shown to be low in patients with chronic hypophosphatemia from other causes (Endo et al. 2008). In addition, FGF23 rapidly decreases after complete removal of responsible tumors in patients with TIO with a half-life of about 20–60 min and becomes undetectable in some patients (Takeuchi et al. 2004, Khosravi et al. 2007, Fukumoto 2014, Hana et al. 2017). These results indicate that chronic hypophosphatemia and/or other associated metabolic changes suppress FGF23 production. Therefore, FGF23 levels seem to be useful for the diagnosis of FGF23-related hypophosphatemic diseases. We are proposing that intact FGF23 levels of more than 30 pg/mL by Kainos assay in patients with chronic hypophosphatemia indicate the presence of FGF23-related hypophosphatemic diseases (Endo et al. 2008). An automated chemiluminescent enzyme immunoassay (CLEIA) for full-length FGF23 has been approved in 2019 and is now covered by public health insurance in Japan for the diagnosis and follow-up of patients with FGF23-related hypophosphatemic diseases (Shimizu et al. 2012). FGF23 measurement for hypophosphatemic patients is not clinically approved in other countries.

Vitamin D deficiency is an important cause for rickets/osteomalacia. Both patients with vitamin D deficient rickets and XLHR can present hypophosphatemia and high or slightly elevated PTH. While measurement of 25-hydroxyvitamin D [25(OH)D] has a pivotal role in the diagnosis of vitamin D deficient rickets, there was a considerable overlap of 25(OH)D levels in patients with vitamin D deficient rickets and XLHR (Kubota et al. 2014). In addition, there are also overlaps in serum calcium and PTH in these two diseases (Kubota et al. 2014). On the other hand, FGF23 levels completely discriminated these two diseases (Kubota et al. 2014).

### Treatment of FGF23-related hypophosphatemic diseases

The first choice for the treatment of patients with TIO is complete removal of responsible tumors. This can correct all the biochemical abnormalities and cure osteomalacia. However, the responsible tumors for TIO can be present anywhere in the body and are often difficult to detect. In addition, even if the tumors can be found, they may not be always resectable because of the localization of the tumors or health problems of the patients. For patients with FGF23-related hypophosphatemic diseases including TIO patients with unresectable tumors, phosphate and active vitamin D have been administered (Carpenter et al. 2011). These medications have shown to improve impaired mineralization of both children and adult patients with XLH and ameliorate symptoms of patients with TIO (Glorieux et al. 1980, Sullivan et al. 1992). However, the height of the treated XLHR patients with these medications is shorter than that of healthy control (Zivicnjak et al. 2011). In addition, these medications can induce several adverse events such as secondary–tertiary hyperparathyroidism, hypercalcuria, nephrocalcinosis, nephrolithiasis, diarrhea and so on (Carpenter et al. 2011).
Furthermore, phosphate needs to be administered several times per day frequently which causes a compliance problem. For these reasons, better treatment for these diseases has been wanted (Carpenter et al. 2011).

Because excessive actions of FGF23 are causing FGF23-related hypophosphatemic diseases such as XLH and TIO, the inhibition of FGF23 activities was considered to be a new candidate for the treatment of these diseases. FGF23 binds to Klotho/FGFR1 complex and activates intracellular signaling pathways including ERK. Therefore, the inhibition of FGF23 for the binding to Klotho/FGFR1 complex and inhibitors of FGFR or ERK pathway have been shown to suppress FGF23 actions and ameliorate hypophosphatemia in Hyp mice (Goetz et al. 2010, Zhang et al. 2012, Wöhrle et al. 2013). In addition, antibodies to FGF23 were shown to improve hypophosphatemia, rickets and impaired mineralization of bone and increase grip power in Hyp mice (Aono et al. 2009, 2011). Based on these preclinical results, human MAB to FGF23, burosumab, has been developed as a new therapeutic agent for FGF23-related hypophosphatemic diseases.

In a phase 1 clinical trial, single injection or infusion of burosumab increased serum phosphate and 1,25(OH)₂D in a dose-dependent manner in 38 adult patients with XLH (Carpenter et al. 2014). Subsequent phase 1/2 study indicated that s.c. injections of burosumab every 4 weeks induced prolonged increase in serum phosphate and 1,25(OH)₂D for more than 1 year in adult patients with XLH (Imel et al. 2015). Safety profiles were favorable and the most common drug-related adverse event was the injection site reaction. Phase 2 study in children with XLHR indicated that burosumab increased serum phosphate and 1,25(OH)₂D and induced radiographic improvement of rickets (Carpenter et al. 2018). This study showed that every 2 weeks injections were more effective than every 4 weeks dosings in child patients (Carpenter et al. 2018). Phase 3 study in child patients indicated that burosumab was more effective than conventional therapy with phosphate and active vitamin D in increasing serum phosphate, TmP/GFR and 1,25(OH)₂D, decreasing alkaline phosphatase, improving radiographic findings of rickets, and increasing recumbent length and standing height Z score (Imel et al. 2019). Phase 3 trial in adult patients with XLH indicated that burosumab improved bone mineralization, WOMAC physical function and stiffness scores, and induced fracture healing more effectively than placebo (Insogna et al. 2018, 2019). Burosumab was also shown to increase serum phosphate and 1,25(OH)₂D and improve osteomalacia in patients with TIO (https://ir.ultragenyx.com/news-releases/news-release-details/ultrageny x-and-kyowa-kirin-announce-us-fda-approval-crysvirtar). From these results, burosumab was approved for patients with XLH or XLHR since 2018 in several countries including Europe and USA. The approval of burosumab is now expanding to other countries in Asia, South America and Middle East (Table 2). The indication of burosumab is different depending on countries. In addition to XLH or XLHR, burosumab is approved for patients with TIO in several countries and for patients with FGF23-related hypophosphatemic diseases in Japan.

However, it is not currently known whether burosumab can normalize height of patients with XLHR. It is not clear either whether burosumab affects other associated conditions such as enthesopathy, dental problems and hearing disturbance in patients with XLH. Long-term safety of burosumab on several parameters such as renal function and ectopic calcification needs to be established in the future studies.

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<th>Region/country</th>
<th>Indications</th>
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<tr>
<td>European Union</td>
<td>X-linked hypophosphatemia with radiographic evidence of bone disease in children 1 year of age and older and adolescents with growing skeleton</td>
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<tr>
<td>United States of America</td>
<td>X-linked hypophosphatemia in adult and pediatric patients 6 months of age and older</td>
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<tr>
<td>Canada</td>
<td>X-linked hypophosphatemia in adult and pediatric patients 6 months of age and older</td>
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<td>United Arab Emirates</td>
<td>X-linked hypophosphatemia in adult and pediatric patients 1 year of age and older</td>
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<tr>
<td>Brazil</td>
<td>X-linked hypophosphatemia in adult and pediatric patients 1 year of age and older</td>
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<td>Japan</td>
<td>FGF23-related hypophosphatemic rickets/osteomalacia</td>
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<tr>
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<td>Bahrain</td>
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Conclusion

The cloning of FGF23 indicated that bone works as an endocrine organ. In addition, several kinds of hypophosphatemic rickets/osteomalacia previously classified as metabolic bone diseases are now considered to be caused by overproduction of FGF23. FGF32 measurement is useful for the diagnosis of FGF23-related hypophosphatemic diseases. While long-term effects of burosumab need to be addressed in future studies and the assay for FGF23 is not clinically available in most countries, it is desirable that such progress results in more efficient management of patients with hypophosphatemic diseases.

Declarations of interest

S F received consulting fee from Kyowa Kirin.

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