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REVIEW

What's new in FGF23 research?

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FGF23 is a hormone that regulates phosphate and vitamin D metabolism by binding to Klotho-fibroblast growth factor (FGF) receptor complex. Excess actions of FGF23 cause several kinds of hypophosphatemic diseases. The mechanism of overproduction of FGF23 in some of these diseases is becoming clear, whereas it is not yet completely understood. Several specific methods to inhibit FGF23 actions have been reported as candidates for new therapies for these FGF23-related hypophosphatemic diseases. On the other hand, many epidemiological studies indicated the association between high FGF23 levels and several adverse events in cardiovascular system, kidney, bone and mortality. FGF23 was recently shown to induce ventricular hypertrophy in a Klotho-independent manner. However, it is not yet shown whether this Klotho-independent action of FGF23 can explain all the results of epidemiological studies.

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Introduction

In the previous perspective in *IBMS BoneKEy*, I summarized the physiological functions of FGF23, diseases caused by aberrant actions of FGF23, the role of FGF23 in chronic kidney disease (CKD) and several epidemiological studies concerning FGF23.[1] Since then, many important papers have been published. However, there still remain several questions to be answered. In this follow-up review, I want to focus on several recent findings concerning FGF23.

FGF23 and Disorders of Phosphate Metabolism

FGF23 is a hormone that reduces serum phosphate level by inhibiting renal proximal tubular phosphate reabsorption. At the same time, FGF23 reduces serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) by inhibiting its synthesis and also by stimulating its metabolism into more hydrophilic metabolites. It has been shown that excess actions of FGF23 result in several hypophosphatemic diseases. These include genetic hypophosphatemic rickets, such as X-linked hypophosphatemic rickets (XLHR), autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets 1 and 2 (ARHR 1, 2), and several acquired diseases, such as tumor-induced rickets/osteomalacia and hypophosphatemic diseases caused by intravenous administration of iron polymaltose or saccharated ferric oxide.[1,2] The responsible genes for XLH, ADHR, and ARHR1 and 2 are the *phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), FGF23, dentine matrix protein 1 (DMP1)* and *ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)*, respectively. Although high circulating FGF23 levels were reported in patients with XLHR, ARHR1 and 2, and FGF23 was shown to be overexpressed in model mice for XLHR and ARHR1,[3,4] it has been unknown how inactivating mutations in *PHEX*, *DMP1* or *ENPP1* results in overproduction of FGF23. Using *Hyp* mice, which have a deletion of *Phex* and thus are model mice for XLHR, and *Dmp1*-null mice, Martin *et al.*[5] showed that compound-mutant *Hyp*/*Dmp1*$^{-/-}$ mice had similar phenotypes to those of single mutant, suggesting that Phex and Dmp1 work in a common signaling pathway. They also showed that activation of FGF receptor pathway was involved in the overexpression of FGF23 in bone in these mice.[5] The involvement of fibroblast growth factor (FGF) receptor signaling in the transcription of *Fgf23* was also reported by another group.[6] Therefore, it is likely that the activation of FGF receptor somehow enhances FGF23 production in bone. However, it is not directly shown that PHEX and/or DMP1 interact with FGF receptor. In addition, it is not known how this regulation of FGF23 production by FGF receptor signaling fits to the role of FGF23 as a phosphotropic hormone.

ADHR is caused by missense mutations in *FGF23* that prevent the processing of FGF23 protein into inactive fragments.[7] In patients with ADHR, it was reported that low serum iron was associated with elevated FGF23.[8] In addition, Farrow *et al.*[9] created a model mouse of ADHR by knocking in a mutant *Fgf23*. They found that iron-deficient diet increased Fgf23 and lowered serum phosphate in this model mice, again indicating that iron

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deficiency induced hypophosphatemic disease by enhancing Fgf23 production.[9] In contrast, intravenous administration of polymaltose or saccharated ferric oxide was shown to cause hypophosphatemic disease with high FGF23 levels.[2,10] It is not clear why iron deficiency rather than iron administration causes overexpression of FGF23 only in patients with ADHR and model mice for ADHR.

Treatment of FGF23-Related Hypophosphatemic Diseases

Tumor-induced osteomalacia can be cured by removal of responsible tumors. In addition, hypophosphatemic disease by intravenous administration of iron polymaltose or saccharated ferric oxide rapidly improves after the cessation of these drugs. On the other hand, neutral phosphate and active vitamin D_3 are the standard medical treatment for other FGF23-related hypophosphatemic diseases. Although these medications certainly improve clinical symptoms, several adverse events, such as hypercalcemia, nephrocalcinosis, diarrhea and secondary–tertiary hyperparathyroidism, can be induced by these drugs. Therefore, periodic monitoring of biochemical parameters are necessary during the treatment.[11] In addition, it was reported that these medications increased FGF23 levels in patients with XLHR.[12,13]

Several options have been reported to specifically inhibit the actions of FGF23 (**Figure 1**). First, C-terminal fragment of FGF23 was shown to inhibit FGF23 signaling by competing with full-length FGF23 for binding to the FGF receptor–Klotho complex.[14] In addition, injection of C-terminal fragment of FGF23 into rats was shown to reduce renal phosphate excretion and increase serum phosphate level.[14] Second, the inhibitor of FGF receptor was shown to increase serum phosphate and $1,25(OH)_2D$ in wild-type mice, indicating that the actions of endogenous FGF23 was inhibited.[6] Third, FGF23 was shown to activate mitogen-activated protein kinase pathway after binding to the FGF receptor–Klotho complex. The inhibition of this pathway in *Hyp* mice improved deranged phosphate and vitamin D metabolism, and mineralization of bone.[15,16] Finally, some anti-FGF23 antibodies were shown to inhibit the activity of endogenous FGF23.[17] These antibodies were shown to increase serum phosphate and $1,25(OH)_2D$ levels also in *Hyp* mice. In addition, repeated injections of anti-FGF23 antibodies improved mineralization of bone, shortened growth plate thickness and enhanced longitudinal growth of long bones.[18] Furthermore, these antibodies were shown to improve grip strength and enhanced spontaneous movement of *Hyp* mice.[19] Therefore, it is possible that biochemical, histological and clinical improvements are obtained by these methods. In addition, another potential drug target would be Klotho. However, long-term effects of these methods need to be examined. Furthermore, it is necessary to specifically affect FGF23 signaling in the case of inhibiting FGF receptor and mitogen-activated protein kinase pathway. Further studies are clearly necessary to develop specific drugs for FGF23-related hypophosphatemic diseases.

FGF23 in Epidemiological Studies

Many cross-sectional and cohort studies have been published regarding the relationship between FGF23 levels and adverse events in cardiovascular system, kidney, bone and mortality. Some of these results are mentioned in the previous perspective[1] and updated more recently in a review.[20] Even since then, several papers appeared in the literature. In patients with CKD, high FGF23 was shown to be associated with aortic calcification, lower ejection fraction, higher troponin T, left ventricular mass, cardiovascular events, higher mortality, progression of CKD, increase of albuminuria and dialysis initiation.[21–29] It was also reported that high FGF23 was associated with cardiovascular diseases in women over 70 years, calcification of abdominal aorta in men over 60 years, and higher mortality in patients with heart failure, although some of these subjects have CKD.[30–32] However, the association between high FGF23 and various adverse events have not been reported in all studies.[20] For example, both negative and positive correlations between FGF23 and bone mineral density have been reported. In addition, it was reported that FGF23 was not associated with the index of vascular stiffness and the progression of CKD.[33,34] As the participants and methods of these studies are quite variable, it is not surprising that several somewhat contradictory results have been reported. Nonetheless, there are more than 40 epidemiological studies, indicating the association between high FGF23 and adverse events, such as cardiovascular events, higher mortality, progression of CKD, fractures and so on. Therefore, it is reasonable to assume that FGF23 levels are associated with at least some of these adverse events, especially in patients with CKD. However, the association does not indicate the direct cause–effect relationship. What we do not precisely know is the reason of this association.

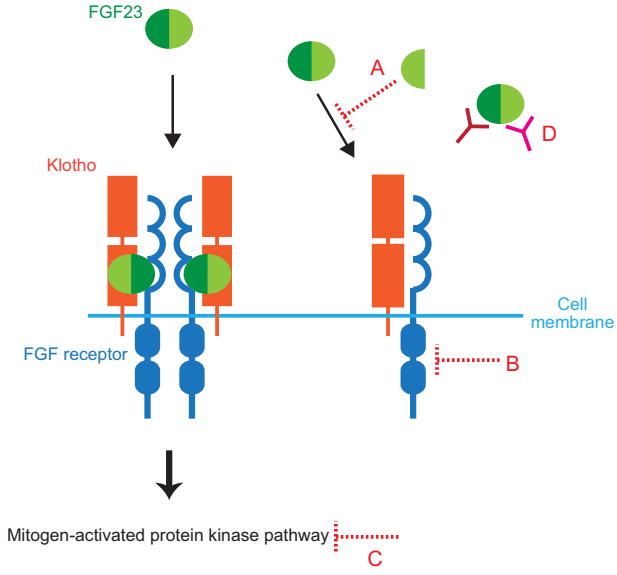

Figure 1 Reported methods to inhibit FGF23 signaling. FGF23 binds to Klotho–FGF receptor complex and activates mitogen-activated protein kinase pathway in kidney and parathyroid glands (left). Several methods have been reported as potential new therapy for FGF23-related hypophosphatemic diseases. C-terminal fragment of FGF23 competes with full-length FGF23 for binding to the Klotho–FGF receptor complex (A). The inhibition of FGF receptor (B) and mitogen-activated protein kinase (C) can inhibit FGF23 signaling. Anti-FGF23 antibodies also antagonize actions of FGF23 (D).

FGF23 and Cardiovascular System

One of the possibilities to explain the association between FGF23 levels and cardiovascular adverse events is that FGF23 acts on these tissues. Using data obtained in Chronic Renal

Insufficiency Cohort study, Faul *et al.*[35] showed that elevated FGF23 levels are associated with left ventricular hypertrophy assessed by echocardiography in 3070 participants. In addition, they also showed that FGF23 induced hypertrophic genes in neonatal rat ventricular cardiomyocytes in *in vitro* experiments. As Klotho was not detected in cardiomyocytes, this effect was considered to be Klotho-independent. Examining signal transduction pathways indicated that FGF23 induced hypertrophic genes through calcineurin. Furthermore, they also showed that intramyocardial and intravenous injections of FGF23 in mice resulted in left ventricular hypertrophy, and left ventricular hypertrophy in animal model of CKD was inhibited by an inhibitor of FGF receptor, PD173074. Collectively, they postulate that FGF23 induces cardiac hypertrophy through FGF receptor–phospholipase Cγ-calcineurin pathway in a Klotho-independent manner.[35] This report is quite interesting considering above-mentioned epidemiological studies linking higher FGF23 levels with cardiovascular adverse events, especially in patients with CKD.

However, this study also raises several questions. If FGF23 can activate intracellular signaling pathways independent of Klotho, it is likely that FGF23 affect many organs in patients with end-stage renal disease, whose FGF23 levels are sometimes extremely high. It is currently unknown whether this is actually happening. In addition, clinical data indicate that the association between higher FGF23 and left ventricular hypertrophy begins in subjects with FGF23 within or near the upper limit of the reference range.[35] This suggests that FGF23 can physiologically affect cardiomyocytes. It is not known whether FGF23 can activate intracellular signaling pathways in a Klotho-dependent and Klotho-independent manner in the similar dose–response relationship. Furthermore, it is not clear whether high FGF23 alone can explain left ventricular hypertrophy in patients with CKD, because it is not known that left ventricular hypertrophy is common in patients with FGF23-related hypophosphatemic rickets/osteomalacia, such as XLHR and tumor-induced rickets/osteomalacia.[35] Despite these questions, the paper by Faul *et al.*[35] certainly proposed attractive hypothesis to explain the effects of FGF23 on other organs than kidney and parathyroid glands, and will stimulate further research on FGF23.

Conclusion

Identification of FGF23 and its signaling pathway opened the way to develop new drugs for several hypophosphatemic diseases, and promising results have been already reported. Still, there remain several important questions about FGF23. For example, we do not precisely know why FGF23 level is associated with various adverse events. In addition, it is not evident how signaling through FGF receptor in bone is connected to phosphate metabolism, either. Future studies will clarify these unanswered questions and will make new drugs targeting FGF23 and its signaling pathway available.

Conflict of Interest

Dr Fukumoto reports that he receives a consulting fee from Kyowa Hakko Kirin Co., Ltd.

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