Possible Roles of Fibroblast Growth Factor 23 in Developing X-Linked Hypophosphatemia

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Abstract. Fibroblast growth factor 23 (FGF23) was identified as a causative factor of autosomal dominant hypophosphatemic rickets (ADHR) and tumor-induced osteomalacia (TIO). Continuous administration of FGF23 by transplanting the FGF23-expressing CHO cells reproduced typical features observed in TIO patients, such as hypophosphatemia, low serum 1,25-dihydroxyvitamin D level, and osteomalacia in nude mice. A series of in vivo studies have shown that FGF23 can reduce sodium dependent phosphate co-transporter (NaPi2a) protein amount and 1α-hydroxylase mRNA level, and increase 24-hydroxylase mRNA levels in kidney. Fgf23 knockout mice demonstrated severe hyperphosphatemia, significant elevation in serum 1,25-dihydroxyvitamin D level and abnormal skeletal development. In addition, vitamin D treatment or dietary phosphate loading have been shown to stimulate FGF23 production. These evidences suggest that FGF23 plays an essential role in regulating phosphate and vitamin D metabolisms in normal physiology. On the other hand, the elevation of serum FGF23 levels in patients with X-linked hypophosphatemic rickets (XLH) has suggested important roles of FGF23 in developing XLH as well as ADHR and TIO. In our recent preliminary study, administration of anti-FGF23 neutralizing antibody ameliorated hypophosphatemia and rachitic bone characters in Hyp mice. These findings indicate a pathological contribution of FGF23 in development of XLH and may provide new insights to its therapy.

Key words: FGF23, phosphate, 1,25-dihydroxyvitamin D, NaPi2a, 1α-hydroxylase

FGF23, a Causative Factor of TIO

FGF23 (fibroblast growth factor 23), the latest member of the FGF family, was first isolated as a homolog of FGF15 by Yamashita et al. (1). FGF23 consists of 251 amino acids and its C-terminal portion is unique in the FGF family, while the N-terminal domain shows a high similarity to FGF21 and FGF15/19. Yamashita et al. reported that FGF23 was expressed weakly in the brain, thymus, small intestine, and heart. It was not long before FGF23 was identified as a causative factor of two independent hypophosphatemic diseases, autosomal dominant hypophosphatemic rickets (ADHR) and tumor-induced osteomalacia (TIO) (2, 3).

TIO (alternatively called oncogenic hypophosphatemic osteomalacia) is characterized by hypophosphatemia, significantly low serum levels of vitamin D, osteomalacia, and association of a benign tumor. Since surgical resection of the associated tumor corrects hypophosphatemic

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osteomalacia, a humoral factor secreted from the tumor, called phosphatonin, has been considered to elicit these disorders (4). To identify the phosphatonin in TIO, we picked up a group of genes that were highly expressed in the causative tumor, and identified FGF23 as the most possible candidate of phosphatonin in TIO (3). Significantly high amount of FGF23 transcripts were detected in the causative tumor but not in the peripheral normal tissue isolated from same TIO patient. To evaluate biological activities of FGF23, we prepared the Chinese hamster ovary cells (CHO) expressing full-length of FGF23, and subcutaneously transplanted these cells to nude mice, in which the transplanted CHO cells formed the tumor that secreted FGF23 protein into the circulation. The mice bearing the FGF23-expressing CHO cells demonstrated a gradual decrease in serum phosphate and 1,25-dihydroxyvitamin D levels (Fig. 1). In addition, histological analysis of the tibia isolated at 45 days after transplantation showed an abnormal increase in the thickness of the epiphyseal growth plate, a decrease in the mineralized bone, as well as an increased osteoid deposition (Fig. 1). These phenotypes are similar to the typical features observed in TIO patient. The patient with TIO showed several folds of increase in serum FGF23 level compared to the normal subjects (5). However, the resection of the TIO tumor immediately and dramatically lowered serum FGF23 level, and followed by the normalization of serum phosphate level within two weeks (5). Therefore, these evidences indicate that in patients with TIO, high levels of FGF23 in the circulation derived from the tumor had induced hypophosphatemia, a low serum vitamin D level, and osteomalacia.

**Molecular Mechanism of FGF23 Action**

To evaluate molecular mechanism of FGF23 action, we analyzed rapid changes in phosphate and vitamin D metabolisms within 24 hrs by intravenously administrating the purified recombinant full-length of FGF23 into normal mice as a rapid bolus injection (6). In the mice of the FGF23-treated group, the serum phosphate concentration significantly lowered from 9 hrs after the administration in comparison with the vehicle-treated group (Fig. 2). The renal fractional excretion of phosphate for 12 hrs in the FGF23-treated group mice was significantly larger than that of the mice in the vehicle-treated group (Fig. 2). These results indicate that the development of hypophosphatemia in the FGF23-treated mice was induced by the decreased renal phosphate reabsorption. Since renal phosphate reabsorption is carried by type IIa sodium-dependent phosphate co-transporter (NaPi2a), we monitored effect of FGF23 on its expression. At 8 hrs after the administration of FGF23, almost at the same time when the lowering in serum phosphate began, a significant down-regulation of the NaPi2a protein was observed (Fig. 3). The down-regulation of
NaPi2a occurred in proximal tubular epithelial cells, as demonstrated by immunohistochemistry of the kidneys of the FGF23 transgenic mouse (7) (Fig. 3).

Prior to the changes in phosphate metabolism, significant decrease in serum 1,25-dihydroxyvitamin D level was already observed at 3 hrs after the FGF23 administration and these low levels lasted for about 10 hrs (Fig. 4). Serum 1,25-dihydroxyvitamin D level is mainly determined by 25-OH-vitamin D-1α-hydroxylase (1αOHase) and 1α,25-dihydroxyvitamin D-24-hydroxylase (24OHase) activities. In the mice treated with FGF23, marked decrease of 1αOHase mRNA and increase of 24OHase mRNA were observed at 1 hr after injection (Fig. 5). These findings indicate that FGF23 is a potent negative regulator of vitamin D metabolism by suppressing the synthesis and at the same time by enhancing the inactivation.

Thus, FGF23 negatively regulates both phosphate and vitamin D metabolisms. Although parathyroid hormone (PTH) also promotes renal phosphate excretion via down-regulating NaPi2a protein as well as FGF23, PTH stimulates 1αOHase expression to positively regulate serum 1,25-dihydroxyvitamin D. Therefore, FGF23 plays a unique role in regulating mineral metabolism.
By means of the development of ELISA that can measure serum FGF23 level, it revealed that the FGF23 levels in normal healthy persons (male 30, female 74) were constantly maintained in the range of $10^{5}$–$50$ pg/ml (5). This finding suggests a certain physiological role of FGF23 in mineral homeostasis. To address this issue, we established the $Fgf23$ knockout mouse (8). The growth of the homozygous founders was rather normal until 10 days of age, but it ceased thereafter. No homozygous mice were survived longer than three months. Thus, FGF23 is essential for normal growth and life span. The serum levels of phosphate and 1,25-dihydroxyvitamin D were both remarkably elevated after 10 days of age. The homozygous mice demonstrated increases in renal NaPi2a protein amount and of 1αOHase mRNA level. These results are mirror images of those seen in the FGF23-treated mice, confirming an important role of FGF23 in phosphate and vitamin D metabolisms. In addition, recent studies demonstrated that high phosphate diet feeding or administration of 1,25-dihydroxyvitamin D could stimulate Fgf23 production in rats (9). These findings suggest an existence of the feedback regulation of Fgf23 production. Thus, it is now evident that FGF23 plays a crucial role to maintain serum phosphate and vitamin D levels in normal physiology.

Fig. 5 FGF23 regulated 1αOHase and 24OHas expressions. Northern blot analysis of the kidneys prepared from 1, 5, 9 and 13 hrs after administration of rhFGF23 or vehicle.

Insights from the studies of anti FGF23-Neutralizing Antibody

The serum levels of FGF23 were higher in patients with XLH, suggesting the pathological contribution of FGF23 in developing XLH as well as ADHR and TIO (5). To further explore this hypothesis, we measured serum FGF23 levels in Hyp mice. Hyp is a murine homolog to XLH with a deletion in the Phex gene (10). As shown in Fig. 6, serum FGF23 levels in Hyp mice were significantly higher as seen in XLH patients.

We developed several mouse monoclonal anti-FGF23 antibodies that can neutralize endogenous FGF23 action in mouse and rat. Administration of anti-FGF23 neutralizing antibody into normal mice resulted both increases in serum phosphate and 1,25-dihydroxyvitamin D levels as seen in the $Fgf23$ knockout mice. In our preliminary study, anti-FGF23 neutralizing antibody could ameliorate hypophosphatemia, inappropriately low vitamin D level and defects in bone mineralization in Hyp mice. These results may indicate the pathological contribution of FGF23 in XLH/Hyp, and the FGF23 neutralizing antibody may possibly become one of the therapeutic tools in the treatment of XLH. At present, however, the direct action of FGF23 on bone, and the relationship between the PHEX/Phex gene defect
and the enhanced production of FGF23 remain unclear. In addition, contributions of other candidates of phosphatonin, such as MEPE and FRP-4 in XLH should be considered. Further studies should be necessary to clarify these issues.

References