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COMMENTARY

What can FGF23 do without Klotho?

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Fibroblast growth factor (FGF) family members are humoral factors that have diverse functions and are divided into several subfamilies.[1] FGF23 belongs to the FGF19 subfamily, together with FGF19 and FGF21. The prototypical FGFs like FGF1 (acidic FGF) and FGF2 (basic FGF) work as local factors.[2] These FGF family members show high affinity for heparan sulfate. Because of this binding to heparan sulfate, the prototypical FGFs are believed to be trapped in the extracellular matrix surrounding cells producing FGFs. In contrast to these FGFs working as local factors, FGF19 family members have been shown to work as systemic factors.[3] In addition, FGF19 family members were shown to have low affinity for heparan sulfate.[4] FGF23 is produced by bone and the main targets of FGF23 have been considered to be kidney and parathyroid glands. FGF23 inhibits proximal tubular phosphate reabsorption by suppressing the expression of type 2a and 2c sodium-phosphate cotransporters.[5] FGF23 also reduces 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] by modifying the expression of enzymes involved in vitamin D metabolism.[5] Owing to these functions in the kidney, FGF23 reduces serum phosphate level by suppressing proximal tubular phosphate reabsorption and intestinal phosphate absorption by reducing 1,25$(OH)_2$D. Furthermore, FGF23 was shown to inhibit the production and secretion of parathyroid hormone (PTH).[6]

FGF family members were shown to function by binding to FGF receptors. There are four FGF receptor genes and alternative splicing from these genes produces several kinds of FGF receptors.[1] However, the affinity of FGF23 to these FGF receptors was quite low.[7] In addition, the expression of these FGF receptors is not tissue-specific. However, the functions of FGF23 had been reported only in a few limited organs. These results suggested that there must be a high affinity-specific receptor for FGF23 in kidney and parathyroid glands because FGF23 is a systemic factor.

Klotho, also called α-*Klotho*, was discovered as a gene whose expression was markedly suppressed in genetically engineered *Klotho* mice.[8] Because *Klotho* mice showed several phenotypes such as short life span, infertility, emphysema and organ atrophy, Klotho protein was proposed to be related to senescence. In addition, *Klotho* mice were shown to have hyperphosphatemia and high 1,25$(OH)_2$D levels.[9] The expression of *Klotho* was observed in a limited number of tissues, including kidney and parathyroid glands.[8] After the cloning of *FGF23*, *FGF23* knockout mice were found to have hyperphosphatemia and high 1,25$(OH)_2$D, exactly the same phenotypes as those in *Klotho* mice.[10] These results indicated that FGF23 is a physiological humoral factor regulating serum phosphate and 1,25$(OH)_2$D levels. Furthermore, Klotho protein was found to bind to FGF23 in the kidney during an effort to identify the receptor for FGF23.[7] Subsequent studies established that FGF23 works by binding to the Klotho–FGF receptor complex.[7,11] Therefore, the expression of *Klotho* had been considered to determine the tissue specificity of FGF23's functions. Klotho in parathyroid glands was also shown to induce PTH secretion in response to hypocalcemia by enhancing Na^+,K^+-adenosine trisphosphatase expression at the plasma membrane.[12] On the other hand, β-Klotho has been proposed to work as a co-receptor for FGF19 and FGF21.[3]

However, this 'classic' view of FGF23's functions through Klotho has been challenged by several papers. FGF23 was shown to induce hypertrophy of cardiomyocytes mainly through the phospholipase Cγ–calcineurin pathway in a Klotho-independent way.[13] In addition, many epidemiological studies reported associations between high FGF23 levels and various adverse events, including higher mortality, cardiovascular events, left ventricular hypertrophy, progression of chronic kidney disease and fractures.[14] These associations might be explained by FGF23's functions in various tissues without *Klotho* expression. However, there are also papers that could not find any associations between FGF23 levels and adverse events.[14] Furthermore, inhibition of FGF23's function did not modify the expression of hypertrophic marker genes in a rat model of chronic kidney disease.[15] Therefore, further studies are necessary to confirm whether FGF23 can work in organs without *Klotho* expression.

A recent paper by Olauson *et al.* indicated another possibility.[16] They created mice with parathyroid-specific deletion of *Klotho* (*PTH-KL*$^{-/-}$) to examine the significance of Klotho in parathyroid glands. *PTH-KL*$^{-/-}$ showed normal gross phenotype and survival. Serum calcium, PTH and FGF23 in *PTH-KL*$^{-/-}$ were not different from those of wild-type mice. Parathyroid size and histology were not affected by *Klotho*

deletion. Changes in PTH in response to hypocalcemia and hypercalcemia were also similar in wild-type and *PTH-KL*$^{-/-}$ mice. Furthermore, the induction of renal failure by adenine similarly caused secondary hyperparathyroidism in these mice. These results suggested that Klotho does not play major roles in the regulation of PTH secretion. However, FGF23 was shown to decrease PTH levels in both wild-type and *PTH-KL*$^{-/-}$ mice, suggesting Klotho-independent function of FGF23 in parathyroid glands. While phosphorylation of ERK was markedly blunted, the calcineurin pathway was shown to be enhanced in *PTH-KL*$^{-/-}$ mice treated with FGF23. In fact, inhibition of the calcineurin pathway by cyclosporine A abrogated the suppressive effect of FGF23 on PTH secretion both *in vivo* and in thyro-parathyroid explants in *PTH-KL*$^{-/-}$ mice. From these results, they propose that FGF23 can suppress PTH secretion through both the Klotho-dependent ERK pathway and Klotho-independent calcineurin pathways.

These results are intriguing and may be important to understand the detailed functions of FGF23. However, these results also evoke several questions. As mentioned above, the affinity of FGF23 for FGF receptors is low and FGF23 has not been considered to work on FGF receptors alone.[7] In the case of cardiomyocytes, unidentified heparan sulfate proteoglycan was proposed to increase the affinity of FGF23 for FGF receptor.[13] Is a similar mechanism working in parathyroid glands? Still, the affinity of FGF23 for heparan sulfate was shown to be low by surface plasmon resonance analysis.[4] In this sense, Klotho-independent functioning of FGF23 in parathyroid glands may be limited to patients with quite high FGF23 levels, such as those with end-stage renal disease.[17] In addition, serum PTH and FGF23 were not different in wild-type and *PTH-KL*$^{-/-}$ mice treated by adenine, and FGF23 caused a similar decrease of PTH in these mice. These results suggest that there are no additive effects of Klotho-dependent and Klotho-independent functions of FGF23 in parathyroid glands on PTH levels, and argue against the physiological role of the Klotho-independent pathway. However, PTH levels are determined by several factors, including serum calcium, phosphate and FGF23. While the effects of cyclosporine were examined only in short-term experiments in this paper, it would be interesting to examine the *in vivo* effects of longer exposure to this drug in both wild-type and *PTH-KL*$^{-/-}$ mice. Finally, no clear different phenotypes between *Klotho* and *FGF23* knockout mice have been reported except for cardiac hypertrophy in *Klotho* mice in one paper, while FGF23 was shown to be extremely high in *Klotho* mice.[13] It would be useful to examine the phenotypes of these mice in more detail to analyze the Klotho-independent functions of FGF23.

Conflict of Interest

The author declares no conflict of interest.

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