

Mini Review

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Recent Advances in Fibro-blast Growth Factor-23 Functions

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ABSTRACT

During the past decade a lot of work has been done to better understand the roles of fibroblast growth factor-23 (FGF-23); a relatively newly discovered endocrine hormone, in multiple organ systems in the body. This review focuses on expressions of FGF-23, co-expressions of α -Klotho and FGF receptors, and FGF-23 mediated end-organ effects in the physiological and pathological conditions. We also discuss the controversial reports regarding α -Klotho-dependent and α -Klotho-independent functions of FGF-23.

EXPRESSION OF FGF-23, α -KLOTHO, AND FGF RECEPTORS

FGF-23

FGF-23 is a family member of 22 fibroblast growth factors (FGFs) including FGF-1-FGF-23, all of which are not only structurally but also evolutionarily related proteins. These FGFs have been classified as being paracrine (15 FGFs), endocrine (3 FGFs), or intracrine (4 FGFs)¹ and using a mammalian (murine) model the endocrine FGFs, especially FGF-23 an approximately 32 kD protein,² have been thoroughly investigated in recent years.

FGF-23 is expressed in multiple cells in the body. It has been conclusively shown to be secreted in bone by cells of the osteoblast lineage and osteocytes, as a part of the lacuna-canalicular system, under the influence of phosphate.³ Local factors derived from bone itself also regulate its expression as seen in cases of inactivating mutations of *PHEX* which results in increased transcription and circulating levels of FGF-23.⁴ It is also expressed in the pericyte-like endothelial cells surrounding the venous sinusoids in bone marrow⁵ and in the thymus.² FGF-23 also plays a part in the body's immune response since its expression is induced in activated dendritic cells and macrophages in response to inoculation with *E. coli* and *S. aureus* via nuclear factor-kappa B (NF- κ B) signaling.⁶ FGF-23 is found to be expressed in the ventero-lateral (VL) thalamic nucleus as well. In addition, FGF-23 is also expressed in heart by cardiac interstitial fibroblasts.^{7,8} Apart from these tissues, there is also FGF-23 expression seen in the muscle, spleen, skin, lung, testes, kidney, and liver to a much lesser extent.⁷

α -Klotho

α -Klotho is a unique molecule that establishes a regulatory system of calcium homeostasis by affecting transepithelial transport of calcium, parathyroid hormone secretion, and FGF-23 signal transduction.⁹ Evolutionarily the FGF-23- α -Klotho system is part of a major milestone in vertebrate evolution that started in the ocean when the early piscine ancestors acquired the bony endoskeleton.¹⁰ α -Klotho has been demonstrated to enhance FGF-23 activity over 10-fold¹¹ and has also been identified as a necessary co-receptor for FGF-23 binding due to the phenotypic similarity observed in α -Klotho and FGF-23 knockout mice, i.e.; hyperphosphatemia and hypercalcemia.¹¹

α -Klotho is expressed in high levels in kidney, parathyroid gland (PTG), testis, ovary, brain, pituitary, and apical plasma membrane of ependymal cells in the choroid plexus but

not in bone, lung, liver, skin, spleen, small intestines, or adrenal glands.¹² The identification of these sites of expression is important because these must be compared to the sites of expression of FGFRs, since FGF-23 has the majority of its effects through the FGFRs α -Klotho complexes pathway.¹³

Expression of FGF Receptors

There are four single pass transmembrane proteins called the fibroblast growth factor receptors (FGFRs 1-4),¹⁴ which are involved with important biological processes ranging from cell division and maturation to formation of blood vessels, wound healing, and embryonic development.¹⁵ Local and systemic secreted FGFs bind FGFRs to dimerize them, followed by activation of intracellular FGF signaling pathways including RAS-RAF-MAPK, PI3K-AKT, STAT, and PLC γ pathways.¹

FGFR has been shown to be highly expressed in the skin and heart with moderate expression in the ovary.¹⁶ Some degree of expression has also been shown in the kidney and urinary bladder.¹⁷ {Luqmani, 1992 #67} Other than these sites, FGFR-1 expression was noticed in low levels in the breast, lung spinal cord, adrenal, thyroid, ileum, colon, and stomach.¹⁸ FGFR-2 expression has also been detected in these tissues except for the heart. A particularly high level of expression of FGFR-2 was noted in stomach and in the thyroid.^{16,18} FGFR-3 shows very little expression, if any, throughout the human body. However, high expression levels have been noted in the skin.¹⁹ FGFR-4 shows moderate levels of expression in the lung and low levels in the ovary, kidney, intestines, and the liver. Nonetheless, other than these tissues there is limited expression seen in the body.²⁰ Tissue specific FGFR expression and activation has been shown to be modulated by heparin, heparan sulfate, or other glycosaminoglycan chains.²¹ FGFRs 1-3 show an alternative splicing pattern which leads to formation of 'b' and 'c' isoforms. The 'b' isoform is expressed preferentially in epithelial tissues while 'c' is found in mesenchymal tissues.²² This, along with the different sites of expression of different FGFs, renders specificity to the FGF signaling system.¹⁴

Co-expression of FGFRs and α -klotho Utilized by FGF-23

As noted above, FGFR-1, FGFR-2, and FGFR-4 have some sites of expression in common with α -Klotho, particularly the kidneys and the ovary. FGF-23 was found to act preferentially via FGFR-1c, FGFR-3c, and FGFR-4 since α -Klotho forms complexes with them.^{11,14} However, a single or double depletion of FGFR-3 and FGFR-4 does not lead to defects in phosphate homeostasis.²³ A deletion of FGFR-1 however, is embryonically lethal in mice²⁴ and FGF-23 expression is notably increased when FGFR-1 is activated in rats with normal kidney function and *in vitro* in osteoblast-like cells derived from bone.²⁵ This increased expression also occurs in cases of activating mutations of FGFR-1 as evident in osteoglophonic dysplasia.³ At the same time a conditional deletion of FGFR-1 in the osteocytes of Hyp mice showed decreased FGF-23 expression.⁵ This leads to the

conclusion that FGFR-1c is the most significant binding site for FGF-23.

END-ORGAN EFFECTS OF FGF-23 IN THE PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

Aside from the issues discussed above, there are also some gaping holes in the research regarding FGF-23 that have not been investigated yet and could possibly lead to answers that will not only link multiple effects and disorders caused by FGF-23 in the body but also provide more paradigm-shifting knowledge regarding the complex metabolic balance in our bodies and the hitherto unknown roles of multiple organs in this. These issues are briefly discussed here according to the organs that they concern most directly.

Kidney

FGF-23 acts *via* the α -Klotho:FGFR-1 complex in the renal tubules. The highest expression of these complexes has been seen in the distal tubule indicating that this is where the initial effect of FGF-23 takes place. However, FGF-23 was found to inhibit sodium-dependent phosphate reabsorption *via* decreased expression of NPT-2a and NPT-2c in the proximal tubule.^{26,27} FGF-23 also promotes calcium reabsorption in the distal tubule *via* the TRPV-5 channel.²⁸ It is also linked to vitamin D3 levels since 1, 25(OH)2D administration stimulates FGF-23 expression.²⁹ Furthermore, FGF-23 inhibits 1 α -hydroxylase expression in the proximal tubule while simultaneously increasing 24-hydroxylase expression leading to formation of a less active form of vitamin D,^{5,30} setting up an effective feedback loop. FGF-23 also decreases the expression of α -Klotho by the kidney, establishing a complete feedback loop regarding its effects in the kidney.³¹ Meanwhile the serum levels of FGF-23 have also been established as biomarkers for renal failure.³²

Gastrointestinal Tract

FGF-23 has been shown to inhibit expression of the intestinal phosphate transporter NPT-2b,³³ thus leading to a decrease in serum phosphate levels. As an indirect effect of FGF-23 we also see that due to decreased activation of 1, 25(OH)2D from the kidney, there is decreased absorption of calcium and phosphate from the intestine.³⁴

Parathyroid Gland

α -Klotho and FGFR-1 are both expressed in high levels in the PTG.³⁵ However, many different studies have reached different conclusions regarding FGF-23 and its effects on the PTG. This has raised a lot of questions and will be discussed in the following section. The one undisputed fact is that PTH acts on bone to cause an increased expression of FGF-23³⁶ and FGF-23 decreases PTH expression,³⁷ setting up a feedback inhibition loop.

Bone

The bone is the major origin of FGF-23. However, α -Klotho is not expressed in bone,³⁸ but all of FGF-23's receptors (FGFR-1c, FGFR-2c, and FGFR-3c) are expressed in osteoblasts.³⁹ FGF-23's importance as a vital hormone came to light when the FGF-23 gene was identified as the one linked to Autosomal Dominant Hypophosphatemic Rickets (ADHR).⁴⁰ This discovery prompted further research and FGF-23 was also found to be the causative agent behind human tumor-induced osteomalacia which is a disease linked to hypophosphatemia due to renal phosphate wasting,⁴¹ thus leading to the discovery of its role as a part of a bone-kidney-parathyroid axis. Given FGF-23's obviously important role in bone-related pathologies, and the work already done on the indirect effects it has on the bone *via* its effects on the other endocrine organs, much more work needs to be done on the direct effects FGF-23 has on bones.

Thalamus, Choroid Plexus, and Pituitary Gland

The venterolateral (VL) thalamic nucleus is related to the motor system and is an important relay for deep cerebellar nuclei to the motor cortex which is in fact where, at the time of FGF-23's discovery, it was thought it might have its effects.² Despite that, not much research has been carried out to assess FGF-23's function there. The choroid plexus and pituitary gland both exhibit high levels of α -Klotho expression and thus are potential targets for FGF-23.¹² Following FGF-23 injections in mice in both of these organs, there is induction of early growth response-1 (EGR-1) expression as well as phosphorylation of the extracellular signal-regulated kinase (ERK).⁴² The choroid plexus and the pituitary gland are both sites of α -Klotho expression¹² but FGF-23's function in these organs is still unknown. The choroid plexus is involved in the composition of the cerebrospinal fluid (CSF) and the pituitary gland is a major endocrine gland. As such, the knowledge of how FGF-23 is involved in the functioning of both these organs is of vital importance if we are to understand completely the diverse effects it can cause physiologically as well as in certain pathologies.

Heart

Cardiac tissue is one of the sites of FGF-23 expression,⁸ but there is no α -Klotho expression in the heart tissues.⁴³ Single nucleotide polymorphisms (SNPs) in FGF-23 have been identified as potential risk factors for cardiac abnormalities in Kawasaki disease,⁴⁴ and the serum levels of FGF-23 have been recognized as biomarkers for cardiovascular failure.⁴⁵ FGF-23 had also been linked to greater risks of left ventricular hypertrophy in patients with CKD.⁴⁶ However, no conclusion has been reached whether this effect is *via* α -klotho independent pathways or indirectly due to the renal effects of FGF-23. This is also discussed in the next section.

Ear

FGF-23 is expressed throughout the cochlea and while

α -Klotho also has receptors in the ear, FGF-23's specific effects were determined by the fact that functional assessments of hearing in FGF-23 null mice did not match the auditory phenotype of α -Klotho null mice. There is also an overlap of initiation of FGF-23 activity and the development of the eustachian tube during embryogenesis which could be the cause of middle ear malformations in FGF-23 deficiency. FGF-23 heterozygous knockout mice have been found to be deaf with close to normal morphology, while homozygous knockout mice have dysplastic bulla and ossicles.⁴⁷ There is a difference in auditory phenotype between the FGF-23 null mice and α -Klotho null mice.⁴⁷ Investigating this further can not only help us find out how FGF-23 might be carrying out α -Klotho independent functions in the ear, but also set a template for investigation into α -Klotho independent functions of FGF-23 in other organs.

Prostate

FGF-23 is heavily linked with prostate cancer. It has been shown that it is not only expressed as an autocrine factor in prostate cancer cells but also enhances proliferation, invasion and anchorage when given exogenously. FGF-23 knock down was shown to decrease *in vivo* tumor growth.⁴⁸ Single nucleotide polymorphisms in FGF-23 have also been linked to a risk of prostate cancer.⁴⁹

Vasculature

The role of FGF-23 in causing vascular calcification has also been studied recently. Nevertheless, the scientific community has reached a consensus neither regarding the exact effect of FGF-23 on vasculature nor regarding the mechanism of such an effect. This issue is also outlined in the following sections. Meanwhile the serum levels of FGF-23 have been definitely established as biomarkers for stroke.⁵⁰ While previous studies have shown that ablation of FGF-23 leads to increased serum phosphate levels and thus vascular calcification and death,⁵¹ a new study states that FGF-23 enhances phosphate induced calcification in the aortic rings of rats by promotion of osteoblastic differentiation involving the ERK1/2 pathway.⁵² However, there are other studies which do not support α -Klotho mediated effects of FGF-23 in the vasculature.⁵³ Studies looking into this could not only help understand the link between FGF-23 and cardiovascular abnormalities in the body but also FGF-23's link with strokes.

Immune System (Macrophages)

FGFR-1c is expressed in macrophages and FGF-23 has been shown to not only increase the number of macrophages but also to induce TNF* expression in the macrophages.⁶ FGF-23 might play a key role in inflammation *via* its effects on macrophages as discussed above.⁶ However much more work needs to be done in this respect, since inflammation could also account for, or at least play a major role in, many different complications associated with disease states with elevated FGF-23 levels.

Indirect Effects via The RAAS Pathway

FGF-23 has been shown to stimulate the rennin-angiotensin-aldosterone-system (RAAS) by suppression of ACE-2 expression in renal tissue, independent of other bone-mineral disorder abnormalities.⁵⁴ Renin expression in the kidney also increases indirectly due to the inhibition of 1, 25(OH)2D by FGF-23.³⁴ Through this stimulation of RAAS, FGF-23 leads to numerous adverse effects like hypertension, diabetic nephropathy, baroreceptor dysfunction, activation of sympathetic system, endothelial dysfunction, atherosclerotic progression, and fibrinolytic system inhibition.⁵⁵ This also helps link FGF-23 to many of its observed adverse effects.

KLOTHO-DEPENDENT AND KLOTHO-INDEPENDENT FUNCTIONS OF FGF-23

As discussed above, FGF-23 is linked deeply with many of the organ systems in the physiological conditions to contribute to the body's overall endocrine homeostasis and is also responsible for diseases linked to disturbances in the pathophysiological conditions. So far the new focus of research regarding FGF-23 is concerned with the identification of α -Klotho dependent and α -Klotho independent effects of FGF-23. It has been reported that besides acting as a co-receptor of FGF-23 binding to FGFRs, α -Klotho also acts as a molecular switch. Its presence or absence determines which intracellular signaling pathways will be recruited downstream of the FGFRs. Thus disease states with α -Klotho deficiency may not involve global FGF-23 resistance, but rather they may in fact promote a switch of the FGF-23-induced signaling towards different cellular responses and outcomes in cells which express FGFRs, but not α -Klotho.⁵⁶

Previously FGF-23 functions on major organs, directly or indirectly, were considered only when α -Klotho was co-expressed along with FGFRs in the target tissue,⁴² but with new studies that have been conducted exploring different mechanisms of action of FGF-23, researchers need to reassess the role FGF-23 plays in the organs and how that is achieved. There is clearly a delicate balance between FGF-23's α -Klotho dependent actions on the FGFRs and its α -Klotho independent actions on FGFR's throughout the body that need to be considered simultaneously and further studied. We discuss these issues here according to the target organ they impact.

Kidney

What needs to be clarified regarding FGF-23's role in the kidney by future studies is how exactly FGF-23 stimulation of the distal renal tubule leads to regulation of proximal tubule function, and which signaling pathways are involved in signal transduction from the distal to the proximal tubule. It has been shown that murine proximal tubular epithelium also expressed α -Klotho and that FGF-23 acts directly on these proximal tubular cells to down regulate membrane expression of NPT-2a

via ERK-1/2 and SGK-1.⁵⁷ This suggests that FGF-23's actions in the kidney are all reliant on α -Klotho-dependent stimulation of FGFR's, in the proximal as well as the distal tubule, with no observable α -Klotho-independent actions. However the above observation can possibly be confounded by the use of FGF-23 amounts high enough to activate α -Klotho-independent receptors of FGF-23, and by the authenticity of the proximal tubular phenotype in the cell culture model which could have been either contaminated with distal tubular cells or have undergone dedifferentiation. Thus where we already know of the effects down-stream of α -Klotho-dependent stimulation by FGF-23 at the distal tubular cells, further research should focus on possible α -Klotho-independent receptor stimulation of FGF-23 at proximal tubular cells.

Bone

Despite being the site of greatest expression and production of the hormone, little is known about the direct effects of FGF-23 on the bone itself. Though the absence of α -Klotho indicates a greatly reduced affinity of FGF-23 for its receptors, studies have shown that FGF-23 might directly inhibit bone formation *via* weak activation of FGFR signaling.^{39,58} This could be due to a α -Klotho-independent action of FGF-23 on bone cells. There have been defects in bone mineralization noted in FGF-23 null mice but these might be secondary to the elevated levels of 1, 25(OH)2D in these mice since the deletion of the vitamin D receptor rescues the phenotype of FGF-23 null mice.⁵⁹ Further research in this aspect would serve to further the understanding of the role that bone plays in the delicate endocrine axis and the role of α -Klotho, if any, in this regard.

Parathyroid Gland

In human disease conditions and in murine models, FGF-23 has been linked to increased levels of PTH,⁶⁰ while in more recent studies FGF-23 has been shown to decrease PTH expression and secretion from the PTG.³⁷ However due to lack of research into FGF-23's Klotho-independent signaling *via* FGFRs in the PTG, it is as of yet unknown whether FGF-23 directly causes an increase in PTH, or if the increase in PTH might be a misleading finding due to FGF-23 induced down regulation of α -Klotho in the PTG rendering FGF-23 unable to exert its suppressive effects on PTH *via* a α -Klotho-dependent mechanism. It is also possible, as discussed above, that the down regulation of α -Klotho in the PTG enables FGF-23 to activate different downstream signaling pathways that lead to different effects than those seen in the presence of α -Klotho. Another theory is that only the extremely elevated levels of FGF-23 in disease states, like chronic kidney disease (CKD), lead to a 1, 25(OH)2D level low enough to cause not only the release of PTG from the FGF-23 induced inhibition, but also to cause a subtle hypocalcemia which chronically stimulates the PTG to cause a secondary hyperparathyroidism.⁶¹ This is supported by the fact that among the factors known as chronic kidney disease related mineral and bone disorders (CKD-MBD), FGF-23 has also been noted to be

the first to increase in concentration before changes in levels of PTH, 1, 25, (OH)2D, or serum phosphate,^{62,63} pointing to it having a causal role in this disease. It is also possible that the hypophosphatemia that results from elevated FGF-23 levels leads to a decreased PTH secretion, since phosphorus levels *in vitro* in rat PTGs have been linked directly to PTH secretion.⁶⁴ Further studies need to work specifically on this aspect of FGF-23's actions since knowledge of how exactly the FGF-23-PTG axis is set up and the role of α -Klotho-dependent and α -Klotho-independent receptors will help clarify the development and mechanism of many pathologies associated with variance in FGF-23 levels.

Heart

Left ventricular hypertrophy (LVH) is a serious mortality causing condition linked to chronic kidney disease (CKD) and the elevated FGF-23 levels that it is associated with. Since there is a scarcity of research into the α -Klotho-dependent and α -Klotho-independent actions of FGF-23 in the heart muscle, and since α -Klotho is absent in cardiomyocytes, the current thought is that LVH in CKD must be a complication due to the indirect effects of FGF-23 on the kidneys.

However, studies have demonstrated the role of FGF-23 in causing pathological hypertrophy in isolated rat cardiomyocytes *via* an α -Klotho independent, FGFR-4 dependent activation of calcineurin-NFAT pathway.^{56,65} Activation of this FGFR-4/calcineurin/NFAT pathway has been shown to be enough to cause cardiac hypertrophy in mice while FGFR-4 blockade, even with high serum FGF-23 levels, attenuates cardiac hypertrophy in rats with CKD.⁵⁶ In another study FGF-23 has been shown to induce cardiomyocyte hypertrophy *via* PLC- γ signaling, upstream of calcineurin/NFAT, independent of Klotho.⁴³ These studies bring to light questions regarding α -Klotho independent functions of FGF-23 in the heart that must be further investigated. Another thing that needs to be clarified by future research is whether the FGF-23 of cardiac origin plays a role in causing LVH in a paracrine manner.

CONFLICTS OF INTEREST: None.

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