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FGF-23 and α -Klotho Co-Dependent and Independent Functions

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Abstract

Purpose of review: This review examines what is known about the FGF-23/ α -Klotho co-dependent and independent pathophysiological effects, and whether FGF-23 and/or α -Klotho are potential therapeutic targets.

Recent findings: FGF-23 is a hormone derived mainly from bone, and α -Klotho is a transmembrane protein. Together they form a trimeric signaling complex with FGFRs in target tissues to mediate the physiological functions of FGF-23. Local and systemic factors control FGF-23 release from osteoblast/osteocytes in bone, and circulating FGF-23 activates FGFR/ α -Klotho complexes in kidney proximal and distal renal tubules to regulate renal phosphate excretion, 1,25(OH)₂D metabolism, sodium and calcium reabsorption, and ACE2 and α -Klotho expression. The resulting bone-renal-cardiac-immune networks provide a new understanding of bone and mineral homeostasis, as well as identify other biological effects FGF-23. Direct FGF-23 activation of FGFRs in the absence of α -Klotho is proposed to mediate cardiotoxic and adverse innate immune effects of excess FGF-23, particularly in chronic kidney disease, but this FGF-23, α -Klotho independent signaling is controversial. In addition, circulating soluble Klotho (sKl) released from the distal tubule by ectodomain shedding is proposed to have beneficial health effects independent of FGF-23.

Summary: Separation of FGF-23 and α -Klotho independent functions has been difficult in mammalian systems and understanding FGF-23/ α -Klotho co-dependent and independent effects are incomplete. Antagonism of FGF-23 is important in treatment of hypophosphatemic disorders caused by excess FGF-23, but its role in chronic kidney disease is uncertain. Administration of recombinant sKl is an unproven therapeutic strategy that theoretically could improve the healthspan and lifespan of patients with α -Klotho deficiency.

Keywords

Fibroblast growth factor 23 (FGF-23); α -Klotho; fibroblast growth factor receptors; hypophosphatemia; innate immunity; cardiovascular; kidney; bone

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Conflicts of interest

Dr. Quarles has received honoraria from Amgen for serving on a scientific advisory board.

Introduction

FGF-23 is a member of the hormonal FGF subfamily that also includes FGF-19 and FGF-21 that emerged during the evolution of vertebrates to activate FGF receptors complexed with Klotho co-receptors [1]. FGF-23 activates FGFR 1c, -3c and -4, but not FGFR-2, in the presence of cell surface membrane α -Klotho [2].

α -Klotho is a single-pass transmembrane cell surface protein with extracellular KL1 (Glu-34 to Phe-506) and KL2 (Leu-515 to Ser-950) domains [3]. The N-terminus of FGF-23 and the KL2 domain of α -Klotho interact with FGFRs and the C-terminus of FGF-23 binds to a pocket created by the KL1 and KL2 domains to form the active ternary receptor complex [3]. This canonical FGF-23/FGFR/ α -Klotho co-dependent signaling limits FGF-23 effects to tissues expressing α -Klotho (Figure 1, middle circle) [2].

α -Klotho orthologues with a single KL1-like domain emerged during evolution before FGF-23, indicating the presence of FGF-23 independent functions [4]. In mammals, α -Klotho undergoes ectodomain shedding and subsequent proteolytic cleavage to generate soluble KL1 and KL2 (sKl) circulating protein fragments. Circulating sKl imparts a multitude of purported FGF-23 independent functions [5] (Figure 1, left circle). Recent studies suggest that FGF-23 can directly activate FGFs in the absence of membrane α -Klotho [6] (Figure 1, right circle).

Canonical FGF-23- α -Klotho dependent functions.

FGF-23 is expressed predominately in osteoblasts/osteocytes (Obs/Ocys) in bone, at low levels in venous sinusoids of bone marrow, thymus, and lymph nodes, and in ventral lateral thalamic nuclei of the brain; in disease states, FGF-23 may be ectopically expressed in tissues that do not normally express FGF-23 [7]. Established physiological functions of FGF-23 are mediated by activation of cell surface FGFR/ α -Klotho binary receptor complexes located in proximal and distal tubules of the kidney to regulate renal phosphate, sodium, and calcium reabsorption, 1,25(OH)₂D metabolism, angiotensin converting enzyme 2 and α -Klotho expression [7]. The co-dependent functions of FGF-23 and α -Klotho are supported by the fact that FGF-23^{-/-} and α -Kl^{-/-} mice are exact phenocopies, α -Kl^{-/-} mice are refractory to FGF-23 effects, and compound mutant FGF-23 and α -Klotho have non-additive effects [8–12].

Normal mineral homeostasis requires FGF-23 levels in a narrow range of 50 to 200 pg/ml. Elevations of FGF-23 beyond this range result in hypophosphatemia, low 1,25(OH)₂D levels and rickets/osteomalacia, while FGF-23 deficiency causes hyperphosphatemia, excess 1,25(OH)₂D, and soft tissue calcifications (i.e., tumoral calcinosis).

Transcriptional regulation plays a key role in determining circulating levels of FGF-23. The FGF-23 gene locus has a proximal promoter, four enhancers and two silencer regions that are conserved between mice and humans [13]. Local and systemic factors coordinately regulate FGF-23 gene transcription in Obs/Ocys to increase circulating FGF-23 that create afferent limbs of several endocrine networks [7]. Posttranslational processing of FGF-23, including phosphorylation, glycosylation, and proteolysis, also contributes to its circulating

bioactivity [14]. FGF-23 contains a R(176)XXR(179)/S(180)AE subtilisin-like proprotein convertase motif between its N- and C-terminus. Proprotein convertases cleave FGF-23 into inactive fragments in a pre-release degradative pathway. Polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3) mediates O-glycosylation of FGF-23 prevents cleavage, while the family with sequence similarity 20, member C (Fam20C), phosphorylates FGF-23 to prevent O-glycosylation and promote FGF-23 cleavage by unknown subtilisin-like proprotein convertases [15]. Recently, plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor (SERPIN), inhibits cleavage of FGF-23, presumably through inhibition of tPA and uPA [16].

This FGF23/ α -Klotho co-dependent functions have been implicated in: 1) mineral metabolism (i.e., local factors linked to mineralization of extracellular matrix, and systemic effects of PTH, 1,25(OH)₂D, and calcium) [17], 2) inflammation (i.e., LPS and TNF α)[18], 3) cardiovascular (CV) homeostasis (i.e., Renin-Angiotensin-Aldosterone and Sympathetic Nervous System)[19,20], 4) energy metabolism (i.e., insulin) [21,22], and 5) hematopoiesis (i.e., Hif1 α , iron deficiency and erythropoietin) [23,24].

Bone and mineral metabolism.

One established function of the FGF-23/FGFR/ α -Klotho ternary complex in all vertebrates from fish to humans is to coordinate bone mineral metabolism with renal handling of phosphate. FGF-23 production in bone is regulated by factors involved in extracellular matrix mineralization [17], including mutations in *Phex*, dentin matrix protein 1 (*Dmp1*), *Enpp1*, and *FAM20C*.

Phex and *Dmp1* work through a common pathway involving FGFR1, which also contributes to increased FGF-23 in chronic kidney disease (CKD) models [25–27]. In addition, HMW-FGF2, which is derived from alternative translation, functions as an intracellular ligand for FGFR1 in *Hyp* mice [28]. Transgenic HMW-FGF2 reproduce the phenotype of *Hyp* mice [26,29].

Other pathways linking bone mineralization and FGF-23 involve pyrophosphate (PPi) metabolism [30,31]. ENPP1 generates extracellular PPi, a physiochemical inhibitor of mineralization through the hydrolysis of extracellular ATP, and PPi is metabolized to phosphate (P) by tissue non-specific alkaline phosphatase (TNAP) to provide phosphate form mineralization. Inactivation of ENPP1 would be predicted to decrease local concentrations of PPi and P and increase local ATP levels [32], which might regulate FGF-23 through purinergic receptors [33] or unknown metabolic (intracellular) or endocrine (extracellular) phosphate sensor in osteoblasts [34–37].

Multiple systemic factors also converge to regulate FGF-23 gene transcription. 1,25(OH)₂D, extracellular calcium, and PTH/cAMP dependent signaling pathways, but surprisingly not phosphorus, stimulate FGF-23 production in Obs/Ocys [38]. The store operated Ca²⁺ entry (SOCE) also regulates FGF-23 expression in osteocytes [20]. The PTH receptor and G α S signaling regulates FGF-23 transcription in osteocytes [39]. Although serum phosphate levels positively correlate with elevations in FGF23 levels in ESRD [40], phosphate

restriction has variable, limited, and delayed effects to lower elevated FGF23 levels animal models and patients with CKD [41,42].

FGF-23 targets FGFR/ α -Klotho complexes in both the proximal and distal tubule. Recent studies show that conditional deletion of α -Klotho in the proximal tubules results in loss of FGF-23 effects on inhibit Npt2a and Npt2c sodium-dependent phosphate co-transporters and suppression of Cyp27b1 and upregulation of Cyp24a1 [43]. FGFR3 and FGFR4 preferentially regulate vitamin D metabolism in the proximal tubule [44], whereas FGFR1 is most important in mediating the effects of FGF-23 on phosphate and calcium transport in the proximal and distal tubules [45]. The mechanisms underlying these differential renal tubule effects of FGFR1, 3 and 4 are unknown.

Physiologically, local factors linked to mineralization of extracellular matrix in conjunction with PTH, 1,25(OH)₂D and circulating calcium, regulate FGF-23, which targets the kidney to match the phosphate buffering capacity/mineralization of bone [46]. There is also a FGF-23 vitamin D counter-regulatory loop, whereby 1,25(OH)₂D stimulates FGF-23 and FGF-23 suppresses 1,25(OH)₂D levels by inhibiting Cyp27B1 and stimulating Cyp24A1 in the renal proximal tubule [47]. In addition, there is a calcium-FGF-23 endocrine loop, whereby calcium stimulates FGF-23 in bone and FGF-23 stimulates calcium reabsorption in the distal tubule [45,48].

Originally it was proposed that PTH stimulates FGF-23 in bone and FGF-23 suppresses PTH production/secretion in the PTG to create a feed-back loop [49]. Recent studies that conditionally deleted FGFRs and α -Klotho in the PTG found that FGF-23 stimulates PTH secretion and cell proliferation [50]. Clinical disorders of excess FGF-23 are typically characterized by elevated PTH.

Canonical FGF-23/FGFR/ α -Klotho signaling is important in several diseases. Excess FGF-23 causes rare hereditary hypophosphatemic disorders in humans and mouse disease homologues, including X-linked hypophosphatemic rickets (XLH)/Hyp mice, caused by inactivating mutations of *Phex* [51], autosomal recessive hypophosphatemic rickets 1 (ARHR1), caused by inactivating mutations of *Dmp1* [52], ARHR2, caused by inactivating mutations in *Enpp1* [30], and Raine Syndrome (RNS), caused by inactivation mutations in FAM20C [53]. Tumor-induced osteomalacia (TIO) is an acquired paraneoplastic disorder caused by excess FGF-23 produced by tumors [40].

Increased FGF-23 also plays an adaptive role in maintaining phosphate homeostasis [40], and also suppresses 1,25(OH)₂D as an additional adaptive response to alter mineral metabolism in in early chronic kidney disease (CKD) [46,54]. It appears that increased FGF-23 gene transcription in bone is the initial event leading to reductions in 1,25(OH)₂D and secondary elevations in PTH in CKD.

There are several clinical disorders caused by defects in FGF-23 processing [16]. GalNAc-T3 inactivating mutations cause autosomal recessive familial tumoral calcinosis by reducing O-glycosylation leading to increased FGF-23 degradation [55]. In contrast, Raine's syndrome, a disorder of FGF-23 excess, is caused by inactivation mutations in FAM20C, leading to decreased phosphorylation of FGF-23, enhanced O-glycosylation and decreased

degradation of FGF-23 [14]. In some studies [56], but not others [40], FGF-23 degradation is reported to be inhibited in CKD.

Immunity and inflammation.

Elevated FGF-23 is linked to inflammation and adverse infection outcomes in CKD [57]. FGF-23 is proposed to impair host responses to infection and impair innate immune responses [58,59]. FGF-23 is expressed in endothelial cells in the venous sinusoid of bone marrow, as well as in the thymus and spleen [9]. The function of FGF-23 in these sites is not clear, but this location implicates a role of FGF-23 in regulating macrophage, neutrophils, T-cell and/or B-cell functions. FGF-23 effects to activate immune cells in the inflammatory milieu [7,18] may lead to adverse outcomes associated with bacterial infections.

A paracrine role for FGF-23 in regulation of innate immune responses has been described that consists of the ectopic production of FGF-23 and α -Kl in activated macrophages to reconstitute canonical FGFR/ α -Klotho signaling in the inflammatory milieu [7,18] (Figure 3). Resting macrophages do not normally express either FGF-23 or α -Klotho. Inflammation stimulates the production of FGF-23 locally in M1 activated macrophages [18]. As noted above, circulating FGF-23 is stimulated in both animal models and humans in response to infection, inflammation, and oxidative stress. TNF α also stimulates FGF-23 gene transcription in osteoblasts *in vitro* [18].

Systemic elevations of FGF-23 may indirectly impact innate immune responses through suppression of 1,25(OH) $_2$ D production by the kidney. Low vitamin D and high FGF-23 serum levels are associated with infectious and cardiac deaths in a large cohort of patients with end stage renal disease (ESRD) [60,61]. Microbiota induced inflammation in the germ free mice inhibits FGF-23 and suppresses TNF- α to modulate vitamin D homeostasis [62].

Cardiovascular homeostasis.

Chronic elevations of FGF-23 are linked to increased mortality and cardiovascular disease in CKD and in the normal population [63]. The cardiac effects of FGF-23 are potentially explained by a bone-renal-cardiac axis created by FGF-23 “on-target” activation of FGFR/ α -Klotho binary complexes in the kidney. The afferent limb of this bone-renal-cardiac axis consists of aldosterone, Ang II, and β -adrenergic pathways stimulate FGF-23 gene expression in bone [19,20]. The efferent limb consists of FGF-23 activation of FGFRs/ α -Kl in renal tubules through at least four mechanisms (Figure 4). First, FGF-23 could activate RAAS through either suppression of 1,25 (OH) $_2$ D, which would increase renin expression [64], and/or reduction of ACE2 expression. Both RAAS activation and ACE2 insufficiency are linked to cardiac hypertrophy and myocardial fibrosis, and deletion of ACE2 in the renal proximal tubule (PT) in mice results in HTN. Ang II and excess FGF-23 have additive effects on induction of LVH in mouse models [19,65]. Second, FGF-23 administration to mice induces HTN and LVH through stimulation of sodium-chloride cotransporter (NCC) in the renal distal tubule (DT) leading to sodium retention [66]. Third, FGF-23 and α -Klotho, which are not expressed in the normal heart, are ectopically expressed in the stressed myocardium [67], which may potentially reconstitute canonical signaling in a paracrine

manner. Fourth, FGF-23 suppresses kidney expression of α -Kl [65], which may lead to LVH through loss of soluble Klotho (sKl) cardioprotective effect (i.e., α -Klotho independent effects, *vide infra*) [68].

Other canonical effects.

Insulin suppresses FGF-23 production [21], which should lead to renal phosphate conservation to match insulin effects on cellular glucose and phosphate uptake homeostasis. FGF-23 may also induce insulin-resistance and glucose intolerance via effects on bone, liver, and kidney [69]. The role of FGF-23 in regulating energy metabolism, however, is not supported by clinical studies that show normal glucose levels in XLH patients [70].

FGF23/FGFR/ α -Klotho signaling has also been implicated in prostate cancer progression [71]. FGF-23 is ectopically expressed in prostate cancer cell lines, and FGF-23 stimulates prostate cancer cell proliferation *in vitro* and *in vivo* [71]. Release of FGF-23 from prostate cancer cells is a cause of paraneoplastic hypophosphatemia [72].

Factors that regulate erythropoiesis, including erythropoietin, iron deficiency, and HIF1 α , also stimulate FGF-23 expression in bone and bone marrow [23,24]. In mice, rhEPO is variably reported to induced FGF23 mRNA in bone and bone marrow in erythroid precursors, a site that does not normally express FGF-23 [73]. Parenteral iron administration transiently stimulates FGF-23 production and inhibits degradation, whereas iron deficiency also stimulates FGF23 production and bone FGF-23 mRNA expression, and correction of iron deficiency can suppress FGF-23 in ADHR [23].

FGF-23 is expressed in the ventrolateral thalamic nucleus in the brain and α -Klotho is expressed in the choroid plexus and hippocampal synaptosomes [74]. The function of FGF-23/FGFR/ α -Klotho in the central nervous system is not clear [75]. Phosphate concentrations are very low in the CSF, and it is tempting to speculate that a possible function is for FGF-23 to promote phosphate removal from the CSF via activation FGFR/ α -Klotho mediated phosphate transport. FGF-23 may also play a role in the maturation of hippocampal neurons and in cognition [76].

FGF-23 effect independent of α -Klotho (non-canonical effects of FGF-23).

Several groups have proposed that FGF-23 can activate FGFRs in the absence of α -Klotho. High concentrations of FGF-23 do activate FGFR signaling in the absence of α -Kl *in vitro*, under some conditions, but the effect is small compared to FGF-23 activation of FGFRs in the presence of α -Klotho [77]. Non-canonical effects of FGF-23 have been reported in the heart, PMNs and liver that do not normally express α -Klotho. Several studies suggest that FGF-23 directly activates FGFR4 in the myocardium to stimulate LVH [6,77] and in hepatocytes to stimulate cytokine production [78], and FGFR2 in PMNs to impair host response to infection [58].

Direct activation of FGFRs by FGF-23 in the absence of α -Klotho is inconsistent with the functional and structural data regarding the obligate requirement of α -Klotho for FGF-23 tissue restricted activation of FGFRs [3]. It is difficult to conceive how non-specific

activation of FGFRs by FGF-23 would be limited to only a few tissues. Low expression of α -Klotho, below the detection limit of immunohistochemistry, has been shown to impart canonical signaling in the proximal tubule and osteoblasts, and confounds attributing FGF-23 to direct activation of FGFRs [43,79]. It is also difficult to exclude α -Klotho dependent effects due to the ectopic expression of α -Klotho in the heart and immune cells and because sKl¹³⁰ may be able to substitute for membrane α -Klotho in FGF-23 dependent FGFR activation [3,18].

The evidence that FGFR4 is directly activated by FGF-23 in the myocardium and liver lacks rigor [77,78]. There are weaknesses in study designs, including failure to conditionally ablate FGFR4 in the heart or hepatocytes; and reliance on global FGFR4^{-/-} mice, which could have effects due to loss of FGFR4 in the kidney or other tissues. With regard to the cardiac effects, existing studies did not administer rFGF-23 to FGFR4^{-/-} mice to test if loss of FGFR4 prevents FGF-23 induced LVH; rather studies used dietary phosphate loading to secondarily elevate FGF-23 levels without controlling for confounding effects of hyperphosphatemia [77]. Circulating concentrations of FGF-21 are also increased in CKD, yet activation of FGFR4 signaling through FGF-21 is purported to have cardioprotective effects [80]. It is not clear why FGF-23, but not FGF-21, activation of FGFR4 would have different direct effects on the myocardium. If FGFR2 and FGFR4 activation by FGF-23 can occur in the absence of membrane α -Kl and/or presence of circulating s-Kl, new concepts are needed to explain the observed tissue restrictive functions of FGF-23 [2].

α -Klotho-independent effects.

α -Klotho orthologues emerged earlier in evolution than FGF-23, indicating the presence of FGF-23 independent functions. C50F7.10 and E02H9.5, the Klotho orthologues present in *Caenorhabditis elegans*, encode only the K1 domain and lacks the structural features necessary to form binary complexes with FGFRs. Klotho orthologues in these invertebrates inhibit DAF-2, which is homologous to IGF1 signaling in mammals. There is compelling evidence the α -Klotho has effects independent of FGF-23. For example, transgenic overexpression of α -Kl is associated with increased longevity [5], a phenotype distinct from FGF-23 excess [4]. Moreover, injection of sKl¹³⁰ elicits biological responses distinct from FGF-23. Enhancing Klotho levels in the brain are proposed to enhance cognition and prevent age-associated neurodegenerative, demyelinating [76].

α -Klotho is highly expressed in the distal tubule of the kidney; where its level of expression is more than what is needed to impart FGF-23 signaling, indicating that the distal tubule of the kidney is an endocrine organ that releases sKl into the circulation [12]. α -Klotho undergoes ADAM 10 and 17 dependent ectodomain shedding to release soluble K11+K12 proteins (sKl¹³⁰). In addition, a 176Arg-His-Thr-Arg179 proteolytic cleavage site allows genesis of distinct K11 (Glu-34 to Phe-506) and K12 (Leu-515 to Ser-950) fragments. There is also an alternatively spliced α -Klotho mRNA that may not be translated into a protein in mammalian systems [81,82]. The relative concentrations of the sKl¹³⁰ (sK11+ K12) and sKl⁶⁵ (sK11) proteins and their respective biological contributions remain unclear [83]. Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17 [84]. α -Klotho gene transcription in the kidney is also stimulated

by 1,25(OH)₂D [85] and suppressed by FGF-23 [65]. Reductions in sKl are observed in CKD [86].

There are several possible mechanisms mediating the FGF-23 independent effects of α -Klotho. The sKl soluble isoforms may act as a decoy receptor to disrupt IGF1, Wnt and TGF- β signaling [87]. sKl is reported to exert cardioprotective effects by downregulating TRPC6 channels in cardiomyocytes [88], and to inhibit RAAS and normalize blood pressure in CKD mouse models [89]. Other FGF-23 independent functions are likely due to α -Klotho sialidase activity that stabilizes membrane expression of transient receptor potential cation channel, subfamily V, member 5 (TRPV5) [90,91] and renal outer medullary potassium channel 1 (ROMK1) [92]. Finally, sKl is reported to bind to membrane lipid rafts to disrupt lipid organization and down-regulate raft-dependent PI3K/Akt signaling [93]. The biological effects of sKl differ from FGF-23. Moreover, FGF-23 mediated suppression of sKl, rather than direct effects of FGF-23 on FGFR4, may account for the cardiotoxic effects of FGF-23 on the heart [94].

Treatment considerations.

The ultimate goal of understanding the co-dependent and independent effects of FGF-23 and α -Klotho is to develop new treatments to modulate the activity of these signaling networks to correct the adverse effects observed in various disease states. These putative FGF-23 independent effects of α -Klotho, however, are difficult to quantify and separate from the co-dependent actions of FGF-23 using mouse genetic approaches [5]. The best evidence for separate functions of FGF-23 and sKl are derived from pharmacological studies that show that the administration of FGF-23 or sKl produce opposite cardiotoxic and cardioprotective effects [94], suggesting that potential therapies might be designed to inhibit FGF-23 and to administer recombinant sKl.

Treatment of the rare hereditary hypophosphatemic disorders traditionally consisted of 1,25(OH)₂D and phosphate supplements, which does not cure the disease, and is associated with toxicities related to excess phosphate and 1,25(OH)₂D, including nephrocalcinosis. Recently, KRN23 (Crysvita, burosumab) has been approved for treatment of XLH [95]. KRN23 is an FGF-23 blocking antibody. KRN23 subcutaneously administration improves rickets and increases serum phosphate levels in XLH [95]. The disadvantages of KRN23 are systemic administration, long-half life, and cost. Small molecule inhibitors of FGF-23 have also been identified that may offer advantage over a blocking antibody [96].

An open question is whether inhibition of FGF-23 has a role to prevent the cardiotoxic and impairment of immune response attributed to excess FGF-23. One problem is that a causative role of FGF-23 in cardiovascular complications remains controversial. Indeed, recent studies failed to identify LVH in either animal homologues of XLH [97] or patients with XLH [70]. Even if inhibition of elevated FGF-23 proves to be cardioprotective in diseases, such as CKD, the therapeutic window is narrow, and preclinical studies in CKD models show that inhibiting FGF-23 with a potent, long acting blocking antibody increases mortality due to oversuppression of FGF-23 [98].

sKl is suppressed in CKD, and administration of sKl prevents cardiovascular complications in mouse models. To date, sKl¹³⁰ has not been pursued as a therapy, possibly because of the potential of off-target effects from stimulating canonical FGF-23 signaling. Preclinical studies show that Ang II cardiotoxicity is exacerbated in transgenic α -Klotho mice [99], whereas other studies show that sKl¹³⁰ administration attenuates cardiovascular toxicity [100]. Studies are lacking that examine the therapeutic effects of the “ancestral” Kl⁶⁵ isoform that is most likely to have FGF-23 independent functions.

Conclusion.

A new understanding of mineral metabolism, cardiovascular homeostasis and innate immunity has been discovered by investigating the FGF-23 and α -Klotho co-dependent and independent physiological effects. Abnormalities of these signaling pathways underlie bone and mineral disorders as well as cardiovascular and infectious diseases. While the therapeutic role of FGF-23 inhibition has been established for hypophosphatemic disorders caused by excess FGF-23, the role of FGF-23 antagonists and recombinant sKl administration to prevent cardiovascular and innate immune disorders that occur CKD remain to be determined.

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Key points:

- Canonical FGF-23 and α -Klotho codependent signaling defines unexpected bone-renal endocrine networks regulating phosphate, calcium, and vitamin D metabolism.
- FGF-23 canonical signaling in the kidney creates a bone-renal-cardiac axis that regulates blood pressure and leads to left ventricular hypertrophy through effects to stimulate sodium reabsorption, suppress ACE2 expression and reduce α -Klotho transcription by the kidney.
- Ectodomain shedding of α -Klotho from the kidney generates sKl that acts as a circulating hormone with actions distinct from those of FGF-23.
- Antagonism of FGF-23 is a proven treatment for hypophosphatemic disorders caused by excess FGF-23.
- Chronic kidney disease is characterized by FGF-23 excess and sKl deficiency, but whether FGF-23 antagonists or sKl administration improve outcomes in CKD remains to be established.

Figure 1. Venn diagram of FGF-23 and α -KI signaling mechanisms and pathophysiological functions.

FGF-23/ α -KI co-dependent canonical signaling (middle circle), α -KI dependent/FGF-23 independent sKL signaling (right circle), and FGF-23 dependent/ α -KI independent non-canonical signaling (left circle).

Figure 2. Transcriptional control of FGF-23.

A) Cell signaling pathways and B) transcription factors and cis-elements in the proximal promoter regulating FGF-23 gene transcription. C) Mouse FGF-23 gene showing proximal promoter, silencer region, transcriptional repressor CTCF and four enhancers. D) Location of the potential enhancer regions by histone modifications and CTCF binding at -38, -16, -10, and +7kb from the TSS across the FGF-23 genetic locus.

Figure 3. FGF-23 regulates innate immune responses.

Inflammation stimulates FGF-23 expression in bone, and ectopic expression of FGF-23 in M1 macrophages to create both systemic and paracrine signaling. **a.** FGF-23 blocks macrophage transition to M2 and resolution of inflammation. **b.** FGF-23 activates FGFR/ α -Kl complexes in macrophages to stimulate TNF- α and promote inflammation. **c.** FGF-23 may activate FGFR2 to impair PMN recruitment. **d.** FGF-23 through FGFR/ α -Kl in the kidney suppresses 1,25(OH) $_2$ D, creating networks that further modulate host responses to infection.

Figure 4. Indirect and Direct Mechanisms of FGF-23 cardiac effects.

A) Canonical mechanisms whereby FGF-23 regulates cardiovascular functions through a bone-kidney-cardiac endocrine network. LVH could be due to effects of FGF-23 to suppress ACE2 expression or stimulate distal tubular sodium reabsorption. Alternatively, FGF-23 induced LVH could be mediated by suppression of sKL release by the kidney and the effect of sKL to increase TRP6 in the heart. B) Proposed non-canonical direct effects of FGF-23 on FGFR4 in the heart.