REVIEW

FGF23 signalling and physiology

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Abstract

Fibroblast growth factor 23 (FGF23) is a phosphotropic hormone that belongs to a subfamily of endocrine FGFs with evolutionarily conserved functions in worms and fruit flies. FAM20C phosphorylates FGF23 post-translationally, targeting it to proteolysis through subtilisin-like proprotein convertase *FURIN*, resulting in secretion of FGF23 fragments. O-glycosylation of FGF23 through *GALNT3* appears to prevent proteolysis, resulting in secretion of biologically active intact FGF23. In the circulation, FGF23 may undergo further processing by plasminogen activators. Crystal structures show that the ectodomain of the cognate FGF23 receptor *FGFR1c* binds with the ectodomain of the co-receptor *alpha-KLOTHO*. The *KLOTHO-FGFR1c* double heterodimer creates a high-affinity binding site for the FGF23 C-terminus. The topology of FGF23 deviates from that of paracrine FGFs, resulting in poor affinity for heparan sulphate, which may explain why FGF23 diffuses freely in the bone matrix to enter the bloodstream following its secretion by cells of osteoblastic lineage. Intact FGF23 signalling by this canonical pathway activates *FRS2/RAS/RAF/MEK/ERK1/2*. It reduces serum phosphate by inhibiting 1,25-dihydroxyvitamin D synthesis, suppressing intestinal phosphate absorption, and by downregulating the transporters *NPT2a* and *NPT2c*, suppressing phosphate reabsorption in the proximal tubules. The physiological role of FGF23 fragments, which may be inhibitory, remains unclear. Pharmacological and genetic activation of canonical FGF23 signalling causes hypophosphatemic disorders, while its inhibition results in hyperphosphatemic disorders. Non-canonical FGF23 signalling through binding and activation of *FGFR3/FGFR4/calcineurin/NFAT* in an *alpha-KLOTHO*-independent fashion mainly occurs at extremely elevated circulating FGF23 levels and may contribute to mortality due to cardiovascular disease and left ventricular hypertrophy in chronic kidney disease.

Key Words

- ▶ FGF23
- ▶ FGF receptor
- ▶ *KLOTHO*
- ▶ *PTH*
- ▶ phosphate homeostasis
- ▶ phosphate signalling
- ▶ mineralisation
- ▶ erythropoietin
- ▶ iron
- ▶ hematopoiesis
- ▶ CKD

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Introduction

Fibroblast growth factor 23 (FGF23) is a phosphotropic hormone that belongs, along with FGF19 and 21, to a subfamily of endocrine FGFs (Château *et al.* 2010). FGF23 is secreted by osteoblasts and osteocytes into the systemic circulation and acts in the kidney, parathyroid, heart, bone and possibly other organs (Shimada *et al.* 2004, Krajisnik *et al.* 2007, Faul *et al.* 2011, Murali *et al.* 2016b).

This property sets FGF23 apart from paracrine FGFs, which mediate cell and organ differentiation locally. FGF23 also stands apart from the intracrine FGFs, which act intracellularly independently of an FGF receptor (FGFR) by interacting with voltage-gated sodium channels and neuronal scaffolding proteins to potentiate neuronal excitability (Goldfarb *et al.* 2007, Itoh *et al.* 2016).

FGF23 was first discovered in the murine brain (Yamashita *et al.* 2000) and soon thereafter loss-of-function (LOF) mutations were identified by linkage analysis to be the cause of autosomal dominant hereditary rickets (ADHR) (ADHR-Consortium 2000) and tumour-induced osteomalacia (TIO) (Shimada *et al.* 2001). ADHR and TIO are rare renal phosphate wasting disorders characterised by, through different mechanisms, hypophosphatemic rickets and osteomalacia, which leads to bowing of the long bones, short stature, bone pain, muscle weakness and fractures (Carpenter *et al.* 2020).

FGF23 has since been shown to be a critical phosphaturic hormone which, along with parathyroid hormone (PTH), regulates phosphate recycling and synthesis of calcitriol (1,25-Dihydroxyvitamin D or 1,25(OH)₂D) in the kidneys (Shimada *et al.* 2004). Canonical FGF23 signalling requires the obligatory co-receptor alpha KLOTHO (KL), a transmembrane protein with extracellular glucuronidase activity (Tohyama *et al.* 2004), for binding to the FGF receptor 1c (FGFR1c) (Urakawa *et al.* 2006). Still, some FGF23 signalling occurs independently of KL and is often referred to as non-canonical FGF23 signalling.

Here we review the current understanding of the actions of FGF23 and the signalling pathways involved. FGF23, in turn, is regulated by phosphate, inflammation and energy metabolism, which we will not discuss here

because it has been addressed in several recent reviews (Faul 2012, Clinkenbeard & White 2017, Chande & Bergwitz 2018, Michigami *et al.* 2018, Takashi & Fukumoto 2020).

Evolutionary conservation of KL/FGF23 function

Orthologs of the FGF signalling pathway are found in *Caenorhabditis elegans* and *Drosophila melanogaster* (Château *et al.* 2010). Even KL and FGF23 functions are evolutionary conserved. *C. elegans* has two FGF orthologs, egg-laying-defective 17 (Egl-17) (Burdine *et al.* 1997) and lethal-756 (Let-756) (Roubin *et al.* 1999); one FGFR ortholog, Egl-15 (DeVore *et al.* 1995); and two KL orthologs, Klotho-1 and Klotho-2 (Table 1) (Château *et al.* 2010). Egl-15 activity is negatively regulated by N-glycosylation, which inhibits ligand and heparan sulphate binding to this receptor (Polanska *et al.* 2009), in a similar manner to human FGFR activity (Duchesne *et al.* 2006). Egl-15 has also been shown to associate with Klotho-1 by co-immunoprecipitation using monoclonal Klotho or Egl-15 antibodies (Polanska *et al.* 2011), though further work is necessary to determine, if Egl-15 binds to Klotho-1 in a fashion similar to KL and FGFR, and if Klotho-1 and Klotho-2 are necessary for Egl-15 activity. Activation of

Table 1 FGF, FGFR, and KL Orthologs in *Caenorhabditis elegans* and *Drosophila melanogaster*.

Species	Ortholog	General function	Gain/loss of function consequences
<i>C. elegans</i>	FGF: Egl-17 Let-756 (Szewczyk and Jacobson 2003)	Egl-17: Longevity, oxidative stress resistance Let-756: Fluid homeostasis	LOF Egl-17: defective migration and differentiation of sex myoblasts LOF Let-756: larval developmental arrest LOF both Egl-17 and Let-756: suppression of muscle lysis
	FGFR: Egl-15 (Szewczyk and Jacobson 2003)	Receptor for Egl-17 and Let-756	GOF: Muscle lysis, abnormal fluid filling in pseudocoelom
	KL: Klotho-1	Improve longevity and resistance to oxidative stress	Unknown
	Klotho-2 (Château <i>et al.</i> 2010)		
<i>D. melanogaster</i>	FGF: bnl, pyr, ths (Rose <i>et al.</i> 2019)	bnl: promote Pi excretion	LOF during development: absent trachea resulting in death Injection of human FGF23 in adults: increased MFS2 expression, decreases blood Pi
	FGFR: btl, htl (Rose <i>et al.</i> 2019)	btl: bnl receptor htl: pyr and ths receptor	LOF during development: absent trachea resulting in death LOF in adults: blocks hypophosphatemic response to injection of human FGF23
	KL: CG7901 (Rose <i>et al.</i> 2019)	Unknown	Unknown

Orthologs to human FGF, FGFR, and KL in *C. elegans* and *D. melanogaster*, including their general function and consequences of alterations of activity, are listed as mentioned in the text.

bnl, branchless; btl, breathless; Egl-15/17, egg-laying-defective 15/17; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GOF, gain-of-function mutation; htl, heartless; KL, Klotho; Klotho-1, Klotho-ortholog; Let-756, lethal-756; LOF, loss-of-function mutation; MFS2, major facilitator superfamily 2; pyr, pyramus; ths, thisbe.

Egl-15 by Egl-17 or Let-756 results in muscle lysis (Szewczyk & Jacobson 2003). Loss-of-function mutation in Egl-17 leads to defective migration and differentiation of sex myoblasts (Burdine *et al.* 1997), and loss-of-function mutation in Let-756 leads to larval developmental arrest (Roubin *et al.* 1999). These effects resemble the roles of FGFs in morphogenesis in higher species and suggest these orthologs may share properties similar to those of multiple FGFs in higher species. Interestingly, like ablation of FGF23 and KL in higher species, LOF mutations in Klotho-1 and Klotho-2 also reduced stress tolerance and longevity in *C. elegans* (Polanska *et al.* 2009, Chateau *et al.* 2010). Conversely, the gain of function in Egl-15 leads to fluid accumulation in the animal's pseudocoelom, hinting at the roles of FGF23 and KL in mineral excretion in higher species (Huang & Stern 2004).

Likewise, *D. melanogaster* has three FGF orthologs, branchless (bnl), pyramus (pyr), and thisbe (ths); two FGFR orthologs, breathless (btl) and heartless (htl); and one KL ortholog (CG9701) which were identified through the DRSC integrative ortholog prediction tool (DIOPT) (Table 1) (Rose *et al.* 2019). As in humans, these FGFR orthologs signal via the MAPK pathway (Sen *et al.* 2011). We recently showed that flies microinjected with human FGF23 become hypophosphatemic. This hypophosphatemia appears to be due to upregulation of the type I sodium-dependent Pi co-transporter major facilitator superfamily 2 (*MFS2*) in their Malpighian tubules (Rose *et al.* 2019). Ablation of *MFS2* prevents this FGF23 effect and also blocks the formation of fly calcium Pi kidney stones.

Based on expression in the tubule and stimulation of *MFS2* gene expression by dietary Pi and FGF23, *MFS2* may function as an excretory Pi-transporter in the Malpighian tubules. This function is different from the situation in mammalian kidneys, where the type II sodium-dependent Pi co-transporters NPT2a and NPT2c help reclaim Pi from the urine and may reflect the unique anatomy of the fly renal system, which lacks a connection to a glomerulus as seen in higher species (Rose *et al.* 2019), and absence of skeletal mineral stores for Pi. The roles of the FGF, FGFR, and KL orthologs in *D. melanogaster* in Pi homeostasis require further study, although in particular bnl may promote Pi excretion as transgenic overexpression of bnl increases *MFS2* expression (Rose *et al.* 2019).

Structure and biochemistry

FGF23 is a 251-amino acid, 32 kDa protein, with high N-terminal homology to other FGFs (Yamashita *et al.* 2000). Phosphorylation of FGF23 through the extracellular kinase family member 20C (FAM20C) at amino acid S180 prevents post-translational O-glycosylation by polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3) (Fig. 1). Lack of O-glycosylation makes FGF23 susceptible to proteolysis by the subtilisin-like proprotein convertase *FURIN*. Proteolysis of FGF23 results in the secretion of inactive FGF23 fragments (Tagliabracci *et al.* 2014). FGF23 phosphorylation can be detected in bone sections and FAM20C-transfected cells (Lindberg *et al.* 2015) underlining its biological importance. Conversely,

Figure 1

Post-translational modification of FGF23. FGF23 is a 251-amino acid 32 kDa protein. Phosphorylation by FAM20C at amino acid S180 is cleaved by *FURIN* into biologically inactive FGF23 fragments. Unprocessed transcripts can also be O-glycosylated by GALNT3 into biologically active FGF23. Cleavage by *FURIN* is suppressed by O-glycosylation of FGF23 and may be regulated by the elusive endocrine Pi sensor. The C-terminal fragment may serve as the endogenous inhibitor of KL-FGFR complex formation. FGF23, fibroblast growth factor 23; FAM20C, extracellular kinase family member 20C; *FURIN*, subtilisin-like proprotein convertase; GALNT3, N-acetylgalactosaminyltransferase 3; KL-FGFR, Klotho-fibroblast growth factor receptor complex.

phosphorylation and proteolysis by *FURIN* are prevented by O-glycosylation at position T178 through *GALNT3*, which results in the secretion of the biologically active intact FGF23 (Tagliabracci *et al.* 2014). The regulation of transcription, production, and post-translational modification of FGF23 has been recently reviewed in greater detail (Edmonston & Wolf 2020).

Regulation of FGF23 bioactivity by *GALNT3* and *FAM20C* is still poorly understood but may be controlled by the elusive endocrine Pi-sensor (Takashi & Fukumoto 2020). Impaired processing and inactivation of FGF23 play an essential pathophysiological role in the development of FGF23 excess in chronic kidney disease (CKD). Excess circulating FGF23 causes left ventricular hypertrophy (LVH) in CKD patients (Faul *et al.* 2011) and the autosomal dominant version of hypophosphatemic rickets (ADHR) as recently reviewed (Wolf & White 2014).

Furthermore, C-terminal fragments of FGF23 may serve a physiological role as an endogenous inhibitor to the formation of the KL-FGFR complex (Goetz *et al.* 2010) and to full-length FGF23 signalling, which may subsequently

induce hypophosphaturia (Goetz *et al.* 2010) and raise serum iron levels (Agoro *et al.* 2020), but this and the signalling pathways involved remain poorly understood.

Likewise, whether biologically meaningful cleavage and inactivation of intact FGF23 in the circulation occurs is poorly understood. FGF23 cleavage and inactivation may involve the actions of both tissue-type and urokinase plasminogen activators. Genetic ablation of *Pai-1*, an inhibitor of plasminogen activator, for example, can rescue the phenotype of the *Kl*-null mouse (Eren *et al.* 2017). As a result, these authors speculated that inhibition of *PAI-1* could be used pharmacologically to prevent FGF23 excess and the development of LVH in patients with CKD.

FGF23 signalling

FGFs signal through the four FGF tyrosine kinase receptors (FGFR1–4) and thereby activate the RAS-MAPK and the PI3K-AKT pathways (Ornitz & Itoh 2015). While FGF23 can activate FGFR3 and FGFR4 in a KL-independent fashion (Grabner *et al.* 2015, Murali *et al.* 2016b),

Figure 2

Canonical and non-Canonical FGF23 Signaling. FGF23 is a peptide hormone secreted by bone that affects multiple organ systems. (A) In the proximal tubules, FGF23 caused internalisation of sodium type 2 co-transporter NPT2a in a FGFR1c/KL-mediated manner by activation of ERK1/2 and SGK1 (red, canonical). FGF23 also decreased activation of vitamin D in a KL-independent fashion (blue, non-canonical). (B) In the distal tubules, FGF23 causes KL-dependent stimulation of calcium and sodium reabsorption. (C) In the parathyroid glands, FGF23 causes suppression of PTH secretion, likely through KL-dependent and -independent pathways. (D) In the heart, FGF23 causes LVH in a KL-independent manner in a FGFR4 mediated fashion mediated by PLCγ/NFAT/calcineurin. (E) In osteoblasts, FGF23 inhibits bone mineralisation by decreased expression of the phosphatase TNAP in a KL-independent fashion. FGF23, fibroblast growth factor 23; FGFR, fibroblast growth factor receptor; NPT2a, sodium-phosphate cotransporter 2a; ERK1/2, extracellular signal-regulated kinases 1/2; SGK1, serum/glucocorticoid regulated kinase 1; WNK4, serine/threonine-protein kinase WNK4; NFAT, nuclear factor of activated T-cells; PLCγ, phosphoinositide phospholipase C γ; TNAP, tissue nonspecific alkaline phosphatase; TRPV5, transient receptor potential vanilloid 5 (calcium channel protein); NCC, sodium chloride cotransporter.

Table 2 Serum and urine biochemistries of mouse models of disorders in phosphate homeostasis.

Model	Serum					Urine	
	Calcium	Phosphate	FGF23	PTH	1,25(OH) ₂ D	Calcium	Phosphate
<i>Kl</i> ^{-/-} (Yoshida <i>et al.</i> 2002, Segawa <i>et al.</i> 2007)	↑	↑	↑↑	↓	↑	↑	—
<i>Kap</i> - <i>Kl</i> ^{-/-} (Ide <i>et al.</i> 2016)	—	—	—	—	—	—	—
<i>Pepck</i> - <i>Kl</i> ^{-/-} (Ide <i>et al.</i> 2016)	—	—	↑↑	—	—	—	↓
<i>Slc34a1</i> - <i>Kl</i> ^{-/-} (Ide <i>et al.</i> 2016)	↑	↑	—	—	—	—	↓↓
<i>Ksp</i> - <i>Kl</i> ^{-/-} (Olauson <i>et al.</i> 2012)	—	↑	↑	↓	—	↑	—
<i>Fgf23</i> ^{-/-} (Shimada <i>et al.</i> 2004, Hesse <i>et al.</i> 2007)	↑	↑	↓↓	↓	↑	—	—
<i>Hyp</i> (Eicher <i>et al.</i> 1976, Murali <i>et al.</i> 2016a)	↓	↓	↑	—	—	N.d.	↑
<i>Fgfr1</i> ^{-/-} (Gattineni <i>et al.</i> 2009)	N.d.	—	↑	—	—	N.d.	N.d.
<i>Fgfr3</i> ^{-/-} (Gattineni <i>et al.</i> 2009)	N.d.	—	—	—	↑	N.d.	N.d.
<i>Fgfr4</i> ^{-/-} (Gattineni <i>et al.</i> 2009)	N.d.	—	↑	—	—/↑	N.d.	N.d.
<i>Ksp</i> - <i>Fgfr1</i> ^{-/-} (Han <i>et al.</i> 2016)	—	↓	—	↑	—	↑	↑
<i>gGt</i> - <i>Fgfr1</i> ^{-/-} (Han <i>et al.</i> 2016)	—	↑	—	—	—	—	—
<i>Ndr1</i> -CreERT2; <i>Fgfr1</i> -4 ^{-/-} (Takeshita <i>et al.</i> 2018)	—	↑	↑	↓	↑	N.d.	N.d.

Overview of the mouse models that have contributed to our understanding of phosphate homeostasis. Each strain is compared to its appropriate WT control. 1,25-(OH)₂D, calcitriol, 1,25-dihydroxyvitamin D; FGF23, fibroblast growth factor 23; *Fgfr*^{-/-}, fibroblast growth factor receptor-null mouse model; *gGt*-*Fgfr1*^{-/-}, *Fgfr1*-ablation in the proximal tubule by gamma-glutamyltransferase (gGt)-Cre-recombinase; *Hyp*, Phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX)-deficient mouse model of X-linked hypophosphatemia (XLH); *Kap*-*Kl*^{-/-}, *Kl*-ablation in the proximal tubules mediated by Kidney androgen-regulated protein (*Kap*)-Cre-recombinase; *Kl*^{-/-}, *Kl*-null mouse model; *Ksp*-*Fgfr1*^{-/-}, *Fgfr1*-ablation in the distal tubule by Kidney-specific cadherin (*Ksp*)-Cre-recombinase; *Ksp*-*Kl*^{-/-}, *Kl*-ablation in the distal tubules mediated by kidney-specific cadherin (*Ksp*)-Cre-recombinase; N.d., no data; *Ndr1*-CreERT2; *Fgfr1*-4^{-/-}, Tamoxifen-induced *Fgfr1*-4-ablation in the proximal tubule by N-myc downstream-regulated gene-1 (*Ndr1*)-Cre-recombinase; *Pepck*-*Kl*^{-/-}, *Kl*-ablation in the proximal tubules mediated by Phosphoenolpyruvate carboxykinase (*Pepck*)-Cre-recombinase; PTH, parathyroid hormone; *Slc34a1*-*Kl*^{-/-}, *Kl*-ablation in the proximal tubules mediated by Solute carrier family 34 member 1 (*Slc34a1*)-Cre-recombinase.

FGFR1c appears to be the primary endocrine receptor for FGF23 which requires the presence of KL (Kurosaki *et al.* 2006). Recent crystal structure data suggest that this receptor isoform, together with KL, but independent of its beta-glucuronidase enzymatic activity, forms a binding pocket for the C-terminal region of c-FGF23 (Chen *et al.* 2018). Heparan sulphate, which unlike other paracrine FGFs has a weak affinity to FGF23 itself, facilitates the dimerisation of two 1:1:1 FGF23-FGFR1c-KL complexes into a symmetric 2:2:2:2 FGF23-FGFR1c-KL-HS signal transduction unit (Chen *et al.* 2018). The osteocyte extracellular matrix is a heparan sulphate-rich environment, which limits systemic diffusion of paracrine FGFs, and these authors hypothesise that reduced affinity to heparan sulphate therefore promotes systemic circulation of FGF23 (Beenken & Mohammadi 2012).

Commonly, FGF23 signalling through KL-dependent binding to FGFR1c is referred to as 'canonical'. Conversely, FGF23 signalling through KL-independent binding to FGFR3 and FGFR4 is referred to as 'non-canonical'. Figure 2 for details of canonical and non-canonical signalling and Table 2 for the various animal models that helped shape the current understanding of FGF23 signalling and physiology.

Canonical FGF23 signalling

The phosphaturic effects of FGF23 in the kidney tubules are KL-dependent. In the proximal renal tubules,

membrane levels of the sodium-phosphate co-transporters, NPT2a and NPT2c, are reduced by FGF23. The internalisation and degradation of these phosphate transporters increases phosphate excretion and was shown to depend on the activation of the extracellular signal-regulated kinases 1/2 (ERK1/2) and serum/glucocorticoid-regulated kinase-1 (SGK1) pathways (Andruxhova *et al.* 2012), resulting in phosphorylation of the Na⁺/H⁺ exchange regulatory factor (NHERF1) (Fig. 2A) (Deliot *et al.* 2005). An initial question was the site of action of FGF23 in the kidneys since KL is expressed highly in the distal renal tubules (Kuro-O *et al.* 1997). KL was furthermore shown to co-localise with phosphorylated-ERK1/2 in the distal tubules following FGF23 injection of mice (Farrow *et al.* 2009). This was corroborated by co-localisation of high KL expression with the calcium channel protein transient receptor potential vanilloid 5 (TRPV5) in the distal tubules (Chang *et al.* 2005). Furthermore, targeted deletion of KL in the distal tubule with *Ksp*-Cre elevated serum Pi, which is consistent with resistance to FGF23 in the distal tubules, while interestingly 1,25(OH)₂D remained normal (Table 2) (Olauson *et al.* 2012).

However, low levels of KL expression in the proximal tubule was ultimately demonstrated along with FGFR1, 3, and 4 expression (Andruxhova *et al.* 2012). Multiple groups have now shown that FGF23 stimulates both fibroblast growth factor receptor substrate 2 (FRS2) and ERK1/2 in the proximal tubule (Urakawa *et al.* 2006, Andruxhova

et al. 2012). Targeted ablation of KL using three proximal tubule-specific promoters driving Cre, *Kap*, *Pepck*, and *SLC34a1* (Table 2), increased serum Pi and reduced urine Pi, suggesting that FGF23 regulates re-absorption of Pi in a KL-dependent manner in the proximal tubules (Ide *et al.* 2016). Likewise, conditional ablation of *Fgfr1* in the distal tubule (*Ksp-Cre*) caused hypophosphatemia and hypercalciuria whereas ablation in the proximal tubule (*gGt-Cre*) resulted in hyperphosphatemia (Fig. 2B). At the same time, 1,25(OH)₂D levels were unchanged (Han *et al.* 2016). Tamoxifen-induced conditional ablation of *Fgfr1–4* in the proximal tubule (*Ndr1-CreERT2*) likewise caused hyperphosphatemia, although along with an increase in 1,25(OH)₂D levels, which will be discussed further (Takeshita *et al.* 2018).

Conversely, global ablation of *Fgfr3* and 4 decreased 1,25(OH)₂D levels in mice (Table 2) (Gattineni *et al.* 2009). These results suggest that 1,25(OH)₂D and 1- α -hydroxylase regulation may be mediated through FGFR3 and FGFR4 in a KL-independent manner, which would formally be considered non-canonical (see below). However, KL-deficient mice have highly increased serum 1,25(OH)₂D and 1- α -hydroxylase levels, which resemble findings in FGF23-deficient mice. This notion argues that FGF23 suppresses 1- α -hydroxylase at least in part in a KL-dependent fashion (Shimada *et al.* 2004).

FGF23 affects calcium homeostasis in the distal tubules, which express the calcium channel protein transient receptor potential vanilloid 5 (TRPV5) in the apical membrane, a transporter that reabsorbs calcium from the urine (Hoenderop *et al.* 1999). KL deglycosylates and thereby retains TRPV5 in the apical distal tubule membrane (Chang *et al.* 2005). This process appears to be stimulated by FGF23: recombinant FGF23 injections increased membrane TRPV5 expression and decreased urinary calcium excretion in WT but not *Klotho*-deficient mice, which appears to be mediated by FGFR, ERK1/2 and serum/glucocorticoid-regulated kinase-1 (SGK1) (Andrukhova *et al.* 2012). Furthermore, KL-deficient and FGF23-deficient mice showed profound hypercalciuria. FGF23 stimulates formation of TRPV5-serine/threonine-protein kinase 4 (WNK4) complexes (Fig. 2B).

Sodium is reabsorbed in the distal tubules by a thiazide-sensitive sodium-chloride cotransporter (NCC) (Gamba *et al.* 1993). This transporter appears to be regulated by FGF23 in a KL-dependent fashion. Expression of NCC and thus sodium reabsorption is decreased in an FGF23-deficient mouse model, and conversely injection of FGF23 into WT mice results in increased sodium reabsorption and volume expansion, an effect not observed with

inhibition of ERK1/2 or SGK1 or following the injection of FGF23 into KL-null mice (Andrukhova *et al.* 2014). FGF23 has also been shown to reduce expression of angiotensin-converting enzyme 2 (ACE2), a component of the renin-angiotensin system, and thus has been proposed to participate systemically in blood pressure regulation and to prevent angiotensin-related cardiac remodeling as recently reviewed (Kovesdy & Quarles 2016). The contribution of FGF23 excess to volume expansion seen in CKD may warrant further study.

Parathyroids in *Kl*-null mice have a reduced set-point for calcium (Mace *et al.* 2018) which may be due to decreased expression of Na/K-ATPase and is independent of FGF23 (Imura *et al.* 2007). In addition, FGF23 reduces PTH secretion in the parathyroid gland, which may have KL-dependent and -independent mechanisms (Fig. 2C). A murine chronic kidney disease (CKD) model exhibited decreased *Kl* and *Fgfr1* expression (Ben-Dov *et al.* 2007). Presence of secondary hyperparathyroidism in these mice was interpreted by the authors as indirect evidence for *Klotho*-dependence of PTH suppression by FGF23. However, FGF23 was able to suppress PTH in a parathyroid-specific *Kl*-null mouse model (Olauson *et al.* 2013). Suppression of PTH secretion by FGF23 was abolished by addition of the calcineurin-NFAT pathway inhibitor cyclosporine A, arguing that this action of FGF23 is, at least partially, KL-independent (Olauson *et al.* 2013). Furthermore, broad FGFR inhibition by PD173074 in Wistar rats (Mace *et al.* 2018) and inhibition of ERK1/2 by U0126 (Ben-Dov *et al.* 2007) or by recombinant dual-specific phosphatases (Dusp) (Román-García *et al.* 2012) in *ex vivo* parathyroid cultures prevented suppression of PTH secretion in response to recombinant FGF23.

There is some evidence that canonical FGF23 signalling may finally play a role in the pathophysiology of tumour-induced osteomalacia (TIO). TIO is caused by mesenchymal tumours often described as hemangioperitomas, which secrete FGF23 and cause a paraneoplastic renal phosphate wasting syndrome with osteomalacia and muscle weakness (Carpenter *et al.* 2020). RNA-seq analysis of FGF23-producing tumours revealed fibronectin (*FN*)-*FGF1* or *FN*-*FGFR1* gene rearrangements, which may activate FGFR1 in an autocrine fashion that stimulates FGF23 production by these tumours (Lee *et al.* 2016). Increased KL expression may constitute a second positive feedback loop to increase FGF23 production in TIO tumours, suggesting that the autocrine stimulation of FGF23 and the manifestations of TIO may be KL-dependent, however this requires further study (Kinoshita *et al.* 2019).

Non-canonical FGF23 signalling

KL-independent activation of the MAPK, calcineurin/NFAT, and PLC pathways by FGF23 is essential for the actions of FGF23 other than regulation of renal phosphate excretion and is referred to as 'non-canonical' FGF23 signalling. The first evidence for KL-independent effects of FGF23 was the discovery that this hormone can cause LVH in *Kl*-null mice, which have extremely elevated circulating FGF23 levels (Faul *et al.* 2011, Grabner *et al.* 2015). Changes consistent with LVH in isolated rat cardiomyocytes can be prevented by treatment with inhibitors of PLC γ and calcineurin/NFAT (Faul *et al.* 2011). It was later shown that this effect of FGF23 is mediated by FGFR4, and in turn, genetic ablation or pharmacological inhibition of FGFR4 reduced the development of LVH in isolated murine cardiomyocytes and rats with CKD, respectively (Fig. 2D) (Grabner *et al.* 2015). Myocardocyte-specific conditional ablation of FGFR4 in a mouse model also prevents FGF23-induced LVH (Han *et al.* 2020). Interestingly, LVH is not observed in the Hyp mouse model of X-linked hypophosphatemia (XLH) (Liu *et al.* 2018), which may be because FGF23 in Hyp and XLH is only mildly elevated and because hyperphosphatemia, a possible co-factor for the development of LVH in CKD, is missing in this disorder.

Skeletal muscle like cardiac muscle expresses FGFR1–4, KL, and even FGF23; however, no effect of FGF23 on fibre diameter and *ex-vivo* excitation-contraction testing was shown (Avin *et al.* 2018).

KL-independent signalling was also described in the skeleton. FGF23 inhibits expression of tissue nonspecific alkaline phosphatases (TNAP) (Murali *et al.* 2016b) in femurs obtained from newborn WT and *Kl*-null mice (Fig. 2E). Further work using specific FGFR inhibitors showed that TNAP suppression depends on FGFR3 and activation of ERK1/2 (Murali *et al.* 2016b). Suppression of TNAP is also thought to be a possible mechanism for the mineralisation defects seen in XLH and inhibiting FGF23 or FGF23-FGFR3 binding was able to partially restore TNAP expression and the mineralisation defect in Hyp mice (Murali *et al.* 2016a). Furthermore the osteoclast markers matrix metalloproteinase 13 (MMP13) and cathepsin K (CTSK) are elevated in Hyp mice and thought to be one mechanism why treatment with anti-FGF23 antibodies decreased bone resorption (Tokarz *et al.* 2018). Further work is needed to show which signalling pathway is involved and if this is a Klotho-independent effect.

FGF23 may also play a role as a negative regulator of erythropoiesis. This role was initially discovered by the

observation of increased red blood counts in FGF23-null mice, which may be mediated by increased circulating erythropoietin (EPO) levels (Coe *et al.* 2014). Conversely, injection of FGF23 into WT mice decreased both EPO and functional erythroid cell counts (Coe *et al.* 2014). Elevated levels of FGF23 in patients with CKD who suffer from anaemia may contribute to failure of the kidneys to produce erythropoietin (EPO) (Babitt & Lin 2012). Thus EPO closes a feedback loop whereby iron deficiency stimulates EPO and FGF23, which in turn inhibits EPO and erythropoiesis. KL and FGFR1, 3, and 4 are highly expressed in erythroid cells (Coe *et al.* 2014). *Kl*-null mice have increased EPO and erythrocyte counts, although hematopoiesis is also impaired in KL deficiency (Vadakke Madathil *et al.* 2014). However, whether regulation of EPO by FGF23 is KL-dependent and mediated by one of the FGFRs remains to be seen.

FGF23 finally impairs neutrophil activity in partially nephrectomised mice, a model for CKD, that was restored by neutralising FGF23 or by inhibition of FGFR2. Binding of FGF23 to FGFR2 was shown to be KL-independent in neutrophils, and leads to activation of protein kinase A and repression of the small GTPase Rap1, resulting in suppression of chemokine- and selectin-mediated integrin activation in neutrophils (Rossaint *et al.* 2016). Impaired neutrophil activity may be part of the impaired inflammatory responses commonly seen in CKD (Recio-Mayoral *et al.* 2011).

Summary and future questions

FGF23 is an endocrine FGF secreted by bone, which stimulates phosphate excretion in the kidneys like PTH, while different from PTH, FGF23 also inhibits synthesis of 1,25(OH) $_2$ D. Both FGF23 actions lower Pi levels in the circulation and require canonical signalling through FGFR1c and the co-receptor KL. Conversely, FGF23 actions in the heart, parathyroids, bones, and erythrocytes are mediated mainly through FGFR3 and 4 in a KL-independent fashion, which is also referred to as non-canonical FGF23 signalling. FGF23 plays a significant role in the pathophysiology of many phosphate concentration disorders, including CKD, XLH, and TIO. We still need to understand better the functions of FGF23 fragments and the signal transduction pathways involved particularly for non-canonical FGF23 signalling to treat these disease processes better. It is also unclear why iron and phosphate homeostasis are co-regulated through FGF23 and EPO and what role inflammation plays for the regulation of FGF23 bioactivity.

Declaration of interest

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