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Review Article

FGF23-Klotho signaling axis in the kidney

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ABSTRACT

Fibroblast growth factor-23 (FGF23) is a bone-derived hormone protecting against the potentially deleterious effects of hyperphosphatemia by suppression of phosphate reabsorption and of active vitamin D hormone synthesis in the kidney. The kidney is one of the main target organs of FGF23 signaling. The purpose of this review is to highlight the recent advances in the area of FGF23-Klotho signaling in the kidney. During recent years, it has become clear that FGF23 acts independently on proximal and distal tubular epithelium. In proximal renal tubules, FGF23 suppresses phosphate reabsorption by a Klotho dependent activation of extracellular signal-regulated kinase-1/2 (ERK1/2) and of serum/glucocorticoid-regulated kinase-1 (SGK1), leading to phosphorylation of the scaffolding protein Na^+/H^+ exchange regulatory cofactor (NHERF)-1 and subsequent internalization and degradation of sodium-phosphate cotransporters. In distal renal tubules, FGF23 augments calcium and sodium reabsorption by increasing the apical membrane expression of the epithelial calcium channel TRPV5 and of the sodium-chloride cotransporter NCC through a Klotho dependent activation of with-no-lysine kinase-4 (WNK4). In proximal and distal renal tubules, FGF receptor-1 is probably the dominant FGF receptor mediating the effects of FGF23 by forming a complex with membrane-bound Klotho in the basolateral membrane. The newly described sodium- and calcium-conserving functions of FGF23 may have major implications for the pathophysiology of diseases characterized by chronically increased circulating FGF23 concentrations such as chronic kidney disease.

Contents

1. Introduction

Genetic studies in patients with autosomal dominant hypophosphatemic rickets led to the discovery of fibroblast growth factor-23 (FGF23) in the year 2000 [1]. At the time of its discovery the function of FGF23 in health and disease was still unclear. However, follow-up studies soon uncovered that FGF23 is a phosphaturic hormone, downregulating the luminal membrane abundance of sodium phosphate co-transporters (NaPi) in renal proximal tubular epithelium [2–4]. Lower membrane abundance of phosphate-transporting molecules in the proximal nephron leads to reduced phosphate reabsorption from urine, and, thus, to increased urinary phosphate excretion. Excessive amounts of intact FGF23 in the blood stream such as those found in patients with autosomal dominant hypophosphatemic rickets, X-linked hypophosphatemic rickets (XLH) or tumor-induced osteomalacia lead to renal phosphate

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wasting and impaired bone mineralization [5]. In addition to its phosphaturic action, FGF23 also down-regulates renal proximal tubular expression of 1α-hydroxylase, the rate-limiting enzyme in the synthesis of the vitamin D hormone, 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2$D] [2–4]. Therefore, FGF23 not only increases urinary excretion of phosphate, it at the same time indirectly suppresses intestinal phosphate absorption by down-regulating the production of 1,25$(OH)_2$D. The net effect of both hormonal actions is to lower circulating phosphate concentrations, safeguarding against the deleterious effects of hyperphosphatemia.

Although other tissues may also contribute to circulating intact FGF23 concentrations [6], bone is probably the major source for circulating FGF23 under physiological circumstances *in vivo* [5]. There is good evidence that FGF23 is secreted by both osteocytes and osteoblasts in bone [7,8]. Bony FGF23 secretion is stimulated by 1,25$(OH)_2$D and by increased extracellular phosphate, thus forming a feedback loop between kidney and bone [5,9,10].

αKlotho (Klotho) is named after the Greek goddess spinning the thread of life, and was discovered as a gene regulating aging [11]. Klotho is a single pass transmembrane protein with a large extracellular domain, consisting of the two domains KL1 and KL2, which share sequence homologies with family I β-glycosidases [11]. Klotho exists in three isoforms, a transmembrane form, a shed soluble form consisting of KL1 and KL2 which is produced from transmembrane Klotho by protease-mediated cleavage of the extracellular domain of Klotho, and a truncated soluble form consisting of KL1 produced by alternative splicing of *Klotho* mRNA [12]. Klotho is expressed in several tissues. However, the main sites of Klotho expression are the kidney (distal and proximal renal tubules), the choroid plexus in the brain, and parathyroid glands [11,13–17]. Because the phenotype of mice with a kidney-specific ablation of *Klotho* is almost identical to the phenotype of global *Klotho* knockout mice, the kidney is probably the most important site of Klotho expression, and also the major source of circulating soluble Klotho [18]. The functional role of Klotho is still a matter of controversy. Klotho has been proposed to function as a hormone in its soluble forms, as a glycosidase, and as a co-receptor for FGF23 in its transmembrane form [12]. In the current review about renal FGF23-Klotho signaling, we naturally focus on the latter function.

FGFs signal through 4 different FGF receptors (FGFR), FGFR1-4, which are all tyrosine kinase receptors. Activation of FGFRs through ligand binding and dimerization leads to phosphorylation of downstream signaling molecules [19]. Endocrine FGFs such as FGF23 do not have a heparan sulfate binding domain, and require co-expression of membrane-bound Klotho for high affinity binding to ubiquitously expressed FGFRs in target tissues [14,20]. For example, the FGFR1c/Klotho receptor complex has a ~20-fold higher binding affinity for FGF23 compared with FGFR1c alone [21]. As described below, it is currently not entirely clear which FGFRs are responsible for mediating the actions of FGF23 in different tissues. Nevertheless, there is ample evidence that signaling through the FGFR1c/Klotho complex plays a pivotal role for many of the hormonal actions of FGF23 [14]. However, binding of Klotho to FGFR3 and 4 has also been reported [20].

The purpose of this review is to highlight the recent advances in the area of FGF23-Klotho signaling in the kidney. FGF23-independent effects of Klotho are not covered by this review. During recent years, significant progress has been made in the further characterization of FGF23-mediated signaling pathways in proximal and distal renal tubules, and new functions of FGF23 with potential major pathophysiological implications have been found.

1.1. Proximal tubular phosphate re-uptake

Unlike other minerals such as calcium, magnesium, potassium, sodium, or chloride, the endocrine regulation of phosphate reabsorption in the kidney takes place in the proximal renal tubule, and involves the regulation of the luminal membrane abundance of phosphate-transporting molecules in the epithelium [22]. Parathyroid hormone (PTH), the principal phosphaturic hormone, downregulates membrane expression of the key sodium-phosphate cotransporter, NaPi-2a, by a signaling cascade leading to phosphorylation of Na^+/H^+ exchange regulatory cofactor (NHERF)-1 as a final step. Because the scaffolding protein NHERF-1 is necessary to anchor NaPi-2a in the cell membrane, phosphorylation of NHERF-1 leads to internalization and degradation of NaPi-2a [23,24].

Based on the work of Shimada and coworkers [2] and on mouse models characterized by transgenic overexpression of FGF23 [25,26], it became clear soon after its discovery that FGF23 is a phosphaturic hormone, suppressing apical membrane expression of NaPi-2a and NaPi-2c in renal proximal tubules. However, the molecular mechanism underlying this effect remained elusive for many years. Studies using *in situ* hybridization [11] and immunohistochemistry employing a monoclonal antibody directed against the human KL1 domain [27] suggested that the main site of Klotho expression in the kidney is the distal renal tubule. In addition, the earliest increase in extracellular signal-regulated kinase (ERK) phosphorylation after injection of FGF23 into mice occurs in distal renal tubules [28]. These findings led to the hypothesis that binding of blood-borne FGF23 to distal tubular cells generates an unknown paracrine or endocrine signal which in turn acts on proximal tubular epithelium to suppress phosphate reabsorption [29,30]. However, this putative signaling molecule has never been found.

Using an antibody specifically directed against the membrane-bound Klotho isoform, we showed by immunohistochemistry and by Western blotting analysis of proximal and distal renal tubules harvested by laser capture microdissection that protein expression of membrane-bound Klotho is actually similar in proximal and distal tubules in the mouse [17]. It is likely that the discrepant findings regarding Klotho expression in the murine kidney [17,27] can be explained by differences in the anti-Klotho antibodies used which detect different isoforms of the protein. Extending the demonstration of the presence of the co-receptor Klotho in proximal renal tubules, we further showed that FGF23 can directly activate ERK1/2 and serum/glucocorticoid-regulated kinase-1 (SGK1) in isolated proximal tubular segments in a Klotho dependent fashion [17]. Activation of SKG1 in turn leads to phosphorylation of NHERF-1, and subsequent downregulation of the membrane expression of NaPi-2a (Fig. 1). Phosphorylation of NHERF-1 appears to be essential for the phosphaturic action of FGF23, because proximal tubular cells from NHERF-1 null mice are resistant to the FGF23-mediated inhibition of phosphate transport [31]. There is indirect evidence that Janus kinase 3 (JAK3) may somehow be involved in FGF23 signaling, because *Jak3*$^{-/-}$ mice show renal phosphate wasting and increased serum levels of Fgf23 and 1,25$(OH)_2$D, suggesting renal resistance to the phosphaturic effect of Fgf23 [32]. In addition, phosphate uptake in Xenopus oocytes was further stimulated when JAK3 was co-expressed together with NaPi-2a [32]. Collectively, these findings demonstrate that the phosphaturic hormones PTH and FGF23 share the common target NHERF-1 in the regulation of phosphate transport in proximal tubular epithelium [17,31]. The overlap between the PTH and FGF23 signaling pathways may also explain the clinical finding that the phosphaturic effect of FGF23 is decreased in patients with hypoparathyroidism [33–35], suggesting that basal levels of circulating PTH are required for efficient FGF23 signaling in humans. *Vice versa*, absence of Fgf23 signaling leads to renal PTH resistance in mice [36].

Our finding that the phosphaturic effect of FGF23 is based on a direct action on proximal tubules is strongly supported by two recent reports: i) Han and coworkers [37] showed that mice with a specific deletion of *Fgfr1* in proximal renal tubules are characterized by hyperphosphatemia and show resistance to the phosphaturic effect of FGF23. ii) Ide and coworkers [38] reported that mice with a specific deletion of Klotho in proximal tubules were unable to increase renal phosphate excretion in response to a high phosphate diet. The study by Han et al. [37] also sheds light on the question which FGF receptor is mainly mediating the phosphaturic actions of FGF23 signaling. Proximal tubular epithelial

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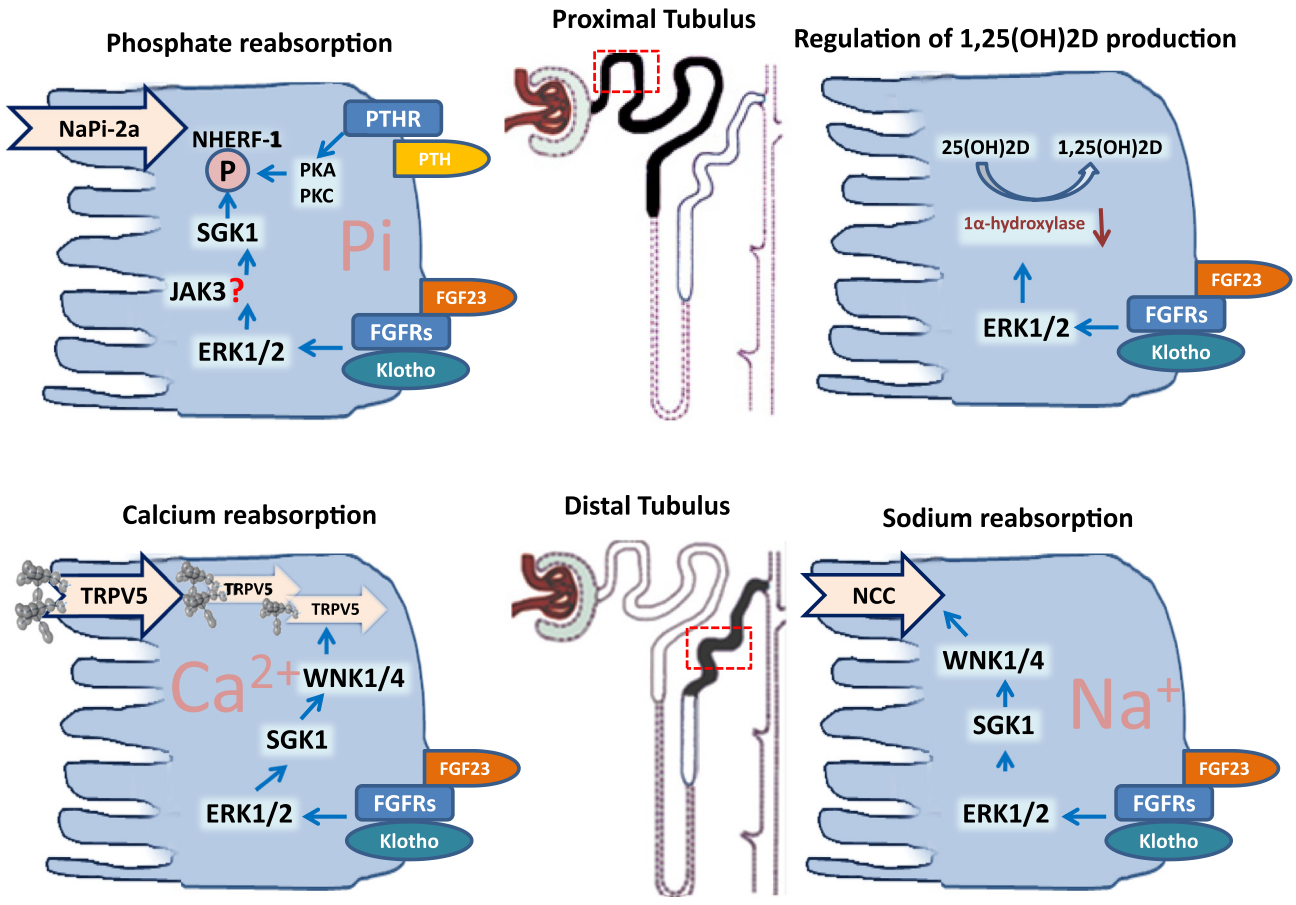

Fig. 1. FGF23-Klotho signaling in the kidney. In proximal renal tubules, blood-borne FGF23 binds to a receptor complex consisting of FGFRs and αKlotho (Klotho), and activates a signaling cascade involving ERK1/2 and SGK1. SGK1 in turn phosphorylates NHERF-1, leading to internalization and degradation of NaPi-2a. FGF23 signaling may also involve Janus kinase-3 (JAK3). PTH binds to the PTH receptor (PTHR), leading to activation of PKA and PKC, and subsequent phosphorylation of NHERF-1. FGF23- and PTH-induced phosphorylation of NHERF-1 decreases the membrane abundance of NaPi-2a, and leads to increased urinary phosphate excretion. The FGF23 signaling-induced mechanisms downstream of ERK1/2 which suppress the transcription of 1α-hydroxylase in proximal renal tubules are unknown. In distal renal tubules, FGF23 circulating in blood binds to the FGFR-Klotho receptor complex, and activates ERK1/2, SGK1, and the WNK1/4 complex. Activation of WNK signaling increases the luminal membrane abundance of glycosylated TRPV5 and of NCC, leading to increased distal tubular calcium and sodium reabsorption. PKA, protein kinase A; PKC, protein kinase C.

cells express FGFR1, 3, and 4, but not 2 [17,39]. Since conditional ablation of *Fgfr1* in proximal renal tubules largely blunts the hypophosphatemic action of FGF23 [37], FGFR1 is probably the predominant receptor responsible for the FGF23-mediated increase in urinary phosphate excretion *in vivo*, confirming earlier studies which compared the hypophosphatemic action of FGF23 in global *Fgfr4* or *Fgfr3* deficient mice and kidney specific *Fgfr1* knockout mice [39]. However, ablation of both *Fgfr1* and *Fgfr4* is necessary to completely abolish the phosphaturic action of FGF23 in mice [40], suggesting that FGFR4 also plays some, albeit minor role for FGF23-induced regulation of phosphate co-transporters in the kidney *in vivo*.

It is well known that FGF23 suppresses the apical membrane abundance of both NaPi-2a and NaPi-2c *in vivo* [3,25,26]. It has been shown in mice with a kidney-specific ablation of NaPi-2c that the phosphaturic action of FGF23 is mainly determined by downregulation of the apical membrane abundance of NaPi-2a, with NaPi-2c playing only a minor role [41]. In addition, the absence of renal NaPi-2c does not seem to be important for the control of phosphate homeostasis under physiological conditions in mice [41]. In contrast, humans with loss-of-function mutations in *NaPi-2c* are characterized by hypophosphatemia and renal phosphate wasting which obviously cannot be balanced by counter-regulatory changes in NaPi-2a [42]. Furthermore, in contrast to mice, loss-of-function mutations in *NaPi-2a* do not invariably lead to hypophosphatemia and renal phosphate wasting in humans [43]. Therefore, species specific differences in the importance of phosphate transporters in the kidney need to be considered.

In conclusion, FGF23 suppresses phosphate reabsorption in renal proximal tubular epithelium by a Klotho dependent, predominantly FGFR1-mediated signaling mechanism, involving activation of ERK1/2 and of SGK1 which in turn leads to phosphorylation of NHERF-1 (Fig. 1). It is currently unclear whether NHERF-1 is a direct target of SGK1, or whether SKG1 leads to activation of additional downstream kinases which subsequently phosphorylate NHERF-1.

1.2. Proximal tubular vitamin D hormone synthesis

The kidney is the major source of circulating $1,25(OH)_2D$ under physiological circumstances [44]. The key enzyme responsible for $1,25(OH)_2D$ production, 1α-hydroxylase (CYP27B1), is predominantly expressed in proximal tubular cells [45], and is strictly regulated by PTH, FGF23, and $1,25(OH)_2D$ itself [46]. Expression and activity of renal 1α-hydroxylase is suppressed by FGF23 and $1,25(OH)_2D$, and stimulated by PTH [46]. The essential function of FGF23-Klotho signaling for the regulation of renal 1α-hydroxylase is illustrated by the fact that despite hypercalcemia and suppressed PTH, 1α-hydroxylase expression remains inappropriately high in *Klotho* and *Fgf23* deficient mice [47–49]. Therefore, in the absence of the suppressive action of FGF23-Klotho signaling on renal 1α-hydroxylase expression, the complex system involved in the regulation of this enzyme fails. *Klotho*$^{-/-}$ and *Fgf23*$^{-/-}$ mice are characterized by unleashed production of the active vitamin D hormone, leading to hypercalcemia and hyperphosphatemia [47–50]. The sequelae of hypercalcemia and

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especially chronic hyperphosphatemia are growth retardation, premature death, ectopic calcifications, organ atrophy, and osteomalacia [11,48,50]. In analogy to the mouse models, loss-of-function mutations in *KLOTHO* [51] or *FGF23* [52] in humans cause tumoral calcinosis, a disease associated with increased $1,25(OH)_2D$ serum levels, hypercalcemia, hyperphosphatemia, and calcifications in soft tissues and blood vessels.

Whether FGF23 signaling also directly regulates 24-hydroxylase (CYP24A1), the most important enzyme initiating vitamin D degradation [53], in proximal renal tubules is a controversial issue. Our laboratory found unchanged CYP24A1 mRNA expression in the kidney of 4-week-old *Klotho*$^{-/-}$ and *Fgf23*$^{-/-}$ mice, relative to WT mice [49]. However, others reported increased CYP24A1 mRNA levels in kidneys of *Fgf23*$^{-/-}$ mice older than 3 weeks [48]. Moreover, injection of recombinant FGF23 into WT mice suppresses renal 1α-hydroxylase mRNA expression and at the same time upregulates 24-hydroxylase mRNA expression [3]. Therefore, FGF23 signaling may have direct, opposing effects on the regulation of renal 1α- and 24-hydroxylase. However, because $1,25(OH)_2D$ is a strong inducer of 24-hydroxylase in all tissues [53], it is difficult to separate direct effects of FGF23 signaling from indirect effects caused by altered $1,25(OH)_2D$ production *in vivo*. Notably, treatment with recombinant FGF23 suppressed renal 1α-hydroxylase mRNA expression in VDR-ablated mice characterized by deficient vitamin D signaling, but failed to upregulate 24-hydroxylase expression [4]. Another study in VDR-ablated mice also came to the conclusion that the FGF23-induced regulation of 24-hydroxylase is VDR dependent [54]. However, a potential caveat in the studies using VDR knockout mice is that 24-hydroxylase is profoundly suppressed in the absence of vitamin D signaling, and PTH, another suppressor of 24-hydroxylase, is usually elevated in these mice. Taken together, there is solid evidence that FGF23 signaling regulates 1α-hydroxylase in a $1,25(OH)_2D$ independent manner [48]. Whether FGF23 is able to regulate 24-hydroxylase in a vitamin D independent fashion still awaits further clarification. In this regard, more conclusive evidence will probably come from appropriate *in vitro* systems. So far, information from *in vitro* experiments is scarce, but in cultured murine proximal tubular cells, FGF23 had only weak and inconsistent effects on 24-hydroxylase expression [55].

The similarities between the phenotypes of *Klotho*$^{-/-}$ and *Fgf23*$^{-/-}$ mice strongly suggest that FGF23 regulates 1α-hydroxylase expression by a Klotho-dependent mechanism. This notion has recently been challenged by a report showing that mice characterized by a specific ablation of Klotho in proximal renal tubules had unchanged $1,25(OH)_2D$ levels under basal conditions [38]. The recombination efficiency in proximal tubules was between 50 and 95% for the different Cre mouse lines in the latter study [38]. Therefore, residual proximal tubular Klotho expression may account for the discrepancies between the phenotypes of mice with global *vs.* proximal tubular-specific Klotho ablation.

The intracellular signaling mechanisms involved in the FGF23-mediated suppression of renal 1α-hydroxylase are only partially known. In *Hyp* mice, a mouse model of the human disease XLH which is characterized by increased endogenous Fgf23 secretion, it was shown that the elevated serum Fgf23 levels are correlated with increased ERK1/2 signaling, and that blockade of ERK1/2 in the kidney of *Hyp* mice improves hypophosphatemia, $1,25(OH)_2D$ deficiency, and the skeletal mineralization defects [56,57]. The transcription factor egr-1 is a downstream target of FGF23-induced ERK1/2 activation [14]. However, a recent study using egr-1 null (*egr-1*$^{-/-}$) and *Hyp*/*egr-1*$^{-/-}$ mice suggested that egr-1 signaling is important for Fgf23-mediated changes in renal phosphate homeostasis, but not for $1,25(OH)_2D$ metabolism [58]. As mentioned above, *Jak3*$^{-/-}$ mice are characterized by increased serum $1,25(OH)_2D$ levels as well as increased 1α-hydroxylase expression in the kidney [32]. Therefore, JAK3 may also be involved in the FGF23-Klotho signaling axis regulating renal 1α-hydroxylase.

Regarding the FGF receptors responsible for the FGF23-mediated suppression of renal 1α-hydroxylase, it has recently been shown that treatment with recombinant FGF23 fails to induce a decrease in serum $1,25(OH)_2D$ levels in mice with a conditional deletion of *Fgfr1* in proximal tubules, indicating that, similar to the suppression of phosphate reabsorption by FGF23, FGFR1 may be the most important FGFR for the FGF23-mediated regulation of $1,25(OH)_2D$ production in proximal tubules [37]. In contrast, earlier studies using kidney-specific conditional *Fgfr1* knockout mice, as well as global *Fgfr3*$^{-/-}$ and *Fgfr4*$^{-/-}$ mutants treated with recombinant FGF23 showed a similar suppression of serum $1,25(OH)_2D$ levels by FGF23 in all three knockout lines [39], suggesting that more than one FGFR may mediate the effect of FGF23 on $1,25(OH)_2D$ metabolism. Later studies by the same group yielded conflicting results: one study showed that recombinant FGF23 had no effect on serum $1,25(OH)_2D$ levels in global *Fgfr3*$^{-/-}$/*Fgfr4*$^{-/-}$ compound mutants [59], whereas in a second study circulating $1,25(OH)_2D$ levels were elevated and the FGF23-induced suppression of $1,25(OH)_2D$ serum levels was blunted in compound mutant mice characterized by a kidney-specific conditional *Fgfr1* knockout and a global deletion of *Fgfr4* [40]. Experiments in which *Fgfr3* and *Fgfr4* were deleted in *Hyp* mice also suggested that the FGF23-induced suppression of renal hydroxylase expression is mediated through a combination of FGFR1, FGFR3, and FGFR4 signaling [60]. Collectively, the available data suggest that FGF23 suppresses renal 1α-hydroxylase expression by a Klotho dependent signaling mechanism involving FGFR1, 3 and 4. As mentioned above, it is not entirely clear whether Klotho associates with FGFR3 and 4 under physiological conditions. It is well established that FGF23 signaling activates ERK1/2. However, further details of the intracellular signaling pathways downstream of ERK1/2 are not known (Fig. 1).

What is currently known about FGF23 signaling in proximal tubular epithelium is schematically shown in Fig. 1. So far, the FGF23-induced intracellular signaling cascades regulating the membrane abundance of phosphate transporters as well as the expression of 1α-hydroxylase have only partially been characterized. It is an important goal to improve our understanding of the molecular mechanisms involved in FGF23 signaling in proximal tubular epithelium, because better insight in the signaling mechanisms involved may eventually lead to new possibilities in the treatment of phosphate-wasting disorders and of disorders involving alterations in renal $1,25(OH)_2D$ production.

1.3. Distal tubular calcium and sodium transport

Although it has long been known that Klotho is expressed in distal tubular epithelium [11], and that injection of FGF23 activates ERK1/2 within minutes in distal tubules *in vivo* [28], it was previously believed that FGF23 exclusively regulates phosphate reabsorption and $1,25(OH)_2D$ synthesis in proximal renal tubules. However, we recently reported that FGF23 also has physiologically relevant direct effects on distal tubular epithelium [61,62].

Calcium reabsorption from the urine is hormonally regulated in distal renal tubules by control of the apical membrane abundance and open probability of the epithelial calcium channel transient receptor potential vannilloid-5 (TRPV5) [63]. Calcium entry into the epithelial cells through the glycoprotein TRPV5 is the rate-limiting step in distal tubular transcellular calcium transport [63]. Based on our initial finding of renal calcium wasting in compound mutants characterized by a combined deficiency in *Klotho* or *Fgf23* and a functioning VDR, we identified FGF23 as a vitamin D independent regulator of TRPV5 in renal distal tubules [61]. FGF23 signaling leads to activation of ERK1/2, SGK1, and with-no-lysine kinase 4 (WNK4) in distal renal tubules, acting through the FGFR/Klotho receptor complex [61]. WNK kinases are key regulators of intracellular transport of membrane proteins such as TRPV5, and act as a complex of WNK1, 3, and 4 [64–67]. We found that FGF23 is a powerful regulator of TRPV5 expression and of calcium reabsorption in the kidney. Injection of mice with recombinant FGF23 led to increased membrane trafficking and distinctly upregulated TRPV5 membrane expression in distal renal tubules, together with profoundly reduced renal calcium excretion [61]. The FGF23-induced regulation of TRPV5 membrane abundance and calcium entry is a direct action on distal tubules,

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because it can also be demonstrated in isolated distal tubular segments [61]. Taken together, our recent findings demonstrate that FGF23, similar to the other phosphaturic hormone PTH, also acts as a calcium-conserving hormone in the distal nephron. This notion has recently been independently confirmed: similar to our *Klotho* and *Fgf23* loss-of-function models, conditional knockout mice with a specific deletion of *Fgfr1* in distal renal tubules are characterized by renal calcium wasting [37].

Because WNK4 is also a key regulator of distal tubular membrane abundance of the Na^+:Cl^- cotransporter NCC [68], the finding that FGF23 signaling activates WNK4 in distal tubular epithelium prompted us to examine FGF23-induced changes in renal sodium handling. NCC is a key molecule for distal tubular sodium and chloride reabsorption, and sodium reabsorption in the distal nephron is mainly regulated through NCC and the epithelial sodium channel ENaC. We found that *Fgf23* and *Klotho* deficient mice are characterized by lower distal tubular NCC expression, renal sodium wasting, lower blood volume, and hypotension despite a counter-regulatory increase in aldosterone secretion and ENaC expression [62]. Conversely, injection of recombinant FGF23 in wildtype but not Klotho deficient mice increased renal NCC expression, and caused renal sodium retention, plasma expansion, increased blood pressure, and heart hypertrophy [62]. Notably, co-treatment of FGF23-treated mice with the NCC inhibitor chlorothiazide completely prevented the FGF23-induced volume expansion, hypertension, and heart hypertrophy [62].

An interesting observation in this context was that a low sodium diet did not protect against, but actually aggravated the FGF23-induced hypertension [62]. A low sodium diet increases aldosterone secretion, which upregulates membrane abundance of NCC and ENaC in the distal nephron through the SGK1 - WNK4 - STE20/SPS-1-related proline/alanine-rich kinase (SPAK) signaling axis in an attempt to maximally conserve sodium [69–72]. Because aldosterone, similar to FGF23, also activates SGK1 in distal renal tubules, FGF23 and aldosterone signaling may have synergistic effects on NCC activation and volume homeostasis. The interaction between FGF23 and the renin-angiotensin-aldosterone-system (RAAS) has recently been indirectly supported by a study showing that FGF23 treatment interferes with the beneficial effects of angiotensin receptor blockade in mice subjected to unilateral ureteral obstruction as a model of renal fibrosis [73].

The novel link between FGF23 and calcium as well as sodium metabolism may have major pathophysiological implications for diseases in which FGF23 is chronically elevated such as in chronic kidney disease (CKD). The calcium-conserving function of FGF23 may not have negative health consequences in XLH patients and *Hyp* mice despite excessive FGF23 serum levels, because $1,25(OH)_2D$ production is suppressed and serum phosphate levels are low due to renal phosphate wasting. However, in CKD patients, the declining kidney function leads to hyperphosphatemia und secondary hyperparathyroidism. Hyperphosphatemia is an important risk factor for vascular calcification and cardiovascular disease [74,75]. In this situation, the FGF23-mediated increase in renal-tubular calcium reabsorption may contribute to calcium retention and vascular calcification. The recently reported positive association between aortic valve calcification and serum FGF23 as well as serum PTH in patients with CKD supports this line or argumentation [76].

Although we found increased renal sodium retention and slightly higher blood pressure in *Hyp* mice than in WT controls despite lower serum aldosterone [62], hypertension is not a common trait in XLH patients [77]. It is likely that the sodium-conserving effect of FGF23 can be largely counterbalanced by decreased aldosterone secretion and RAAS activity as long as kidney function is normal. Data about cardiovascular function in XLH patients are scarce, but some studies reported a high incidence of ventricular hypertrophy in XLH patients [77] which may reflect chronically increased volume load. Whether aldosterone serum levels are lower in XLH patients remains to be shown. In contrast, the presence of renal disease may interfere with the ability of the body to compensate chronic elevations in circulating FGF23. Driven by renal disease mechanisms, aldosterone levels are typically elevated in CKD patients due to activation of the RAAS [78]. Based on our data, increased serum aldosterone may further enhance the effects of FGF23 on sodium retention, volume homeostasis, and blood pressure in CKD patients in this situation. This mechanism may provide a tentative explanation why circulating FGF23 is positively and dose-dependently associated with mortality, CKD progression, left ventricular hypertrophy, and vascular calcifications in CKD patients [79–81]. It is also conceivable that high phosphate diets may predispose to the development of hypertension in normal subjects due to phosphate-induced stimulation of FGF23 secretion and subsequently increased renal sodium retention and volume load.

In conclusion, it has recently been established that FGF23 not only suppresses renal phosphate reabsorption in proximal renal tubules, but also regulates renal calcium and sodium handling in the distal nephron by activation of WNK signaling. Hence, FGF23 is not only a phosphaturic, but also a calcium- and sodium-conserving hormone. This novel paradigm is schematically shown in Fig. 1.

2. Conclusion

Recent advances in the field of FGF23-Klotho signaling in the kidney have shown that FGF23 acts independently on proximal and distal tubular epithelium. In proximal renal tubular epithelium, FGF23 suppresses phosphate reabsorption by a Klotho dependent activation of ERK1/2 and SGK1, leading to phosphorylation of NHERF-1 and subsequent internalization and degradation of NaPi-2a. In distal renal tubules, FGF23 augments sodium and calcium reabsorption by increasing the apical membrane expression of TRPV5 and NCC through a Klotho dependent activation of WNK4. In proximal and distal renal tubules, FGFR1 is probably the dominant FGF receptor mediating the renal effects of FGF23 by forming a complex with membrane-bound Klotho in the basolateral membrane. The newly described sodium- and calcium-conserving functions of FGF23 may have major implications for the pathophysiology of diseases characterized by chronically increased circulating FGF23 concentrations such as chronic kidney disease.

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Conflicts of interest

The authors declare no conflicts of interest.

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