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Role of α Klotho and FGF23 in Regulation of Type II Na-dependent Phosphate Co-transporters

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Abstract

Alpha-Klotho is a member of the Klotho family consisting of two other single-pass transmembrane proteins- β Klotho and γ Klotho; only α Klotho has been shown to circulate in the blood. Fibroblast growth factor (FGF)23 is a member of the FGF superfamily of 22 genes/proteins. α Klotho serves as a co-receptor with FGF receptors (FGFRs) to provide a receptacle for physiological FGF23 signaling including regulation of phosphate metabolism. The extracellular domain of transmembrane α Klotho is shed by secretases and released into blood circulation (soluble α Klotho). Soluble α Klotho has both FGF23-independent and dependent roles in phosphate homeostasis by modulating intestinal phosphate absorption, urinary phosphate excretion, and phosphate distribution into bone in concerted interaction with other calciophosphotropic hormones such as PTH, and 1,25-(OH)₂D. The direct role of α Klotho and FGF23 in maintenance of phosphate homeostasis is partly mediated by modulation of type II Na⁺-dependent phosphate cotransporters in target organs. α Klotho and FGF23 are principal phosphotropic hormones and the manipulation of the α Klotho-FGF23 axis is a novel therapeutic strategy for genetic and acquired phosphate disorders and for conditions with FGF23 excess and α Klotho deficiency such as chronic kidney disease.

Keywords

α Klotho; FGF23; FGF receptor; Phosphate homeostasis; Na-dependent phosphate co-transporter

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Conflict of interest

There are no conflicts of interest.

I. Introduction

Alpha-Klotho (α Klotho) and fibroblast growth factor23 (FGF23) were independently discovered in 1997 [52] and 2000 [19], and was labelled as an “anti-aging” protein, and a novel phosphaturic hormone respectively. Interestingly, the FGF23-null mouse phenocopies almost all features observed in α Klotho-null mouse including short lifespan, hyperphosphatemia, and ectopic calcification, suggesting that α Klotho and FGF23 might share common downstream pathways, at least in the maintenance of phosphate (Pi) metabolism [75]. Shortly after, two research laboratories independently confirmed that membrane α Klotho functions as a mandatory co-receptor for FGF23 and along with the FGF receptor (FGFR) to transduce FGF23 signaling to modulate Pi metabolism as a phosphotropic hormone [53, 95].

The extracellular domain of membrane α Klotho can be shed by secretases and released into the circulation and urine [8, 15, 42, 44, 97]. This cleaved α Klotho protein is hereinafter referred as soluble α Klotho. Recently, a different model was presented where soluble α Klotho can also sustain FGF23 signaling by acting as a “deliverable” coreceptor [16]. The current model of this complex based on the crystal structure is a 2:2:2 ternary complex of α Klotho, the FGFR1c, and FGF23. In this complex, α Klotho simultaneously tethers the D3 domain of FGFR1c, and FGF23 via the FGF23 Cterminal tail, thus implementing FGF23-FGFR1c proximity and conferring stability. This model does not formally exclude other possibilities that other higher ratio of this complex or soluble α Klotho alone can exert as functional unit for Pi metabolism.

The SLC34 family of type II Na^+ -driven Pi cotransporters (NaPi) includes NaPi-2a, NaPi2b, and NaPi-2c. Both NaPi-2a and NaPi-2c are mainly expressed in renal tubules [26, 27, 61], and NaPi-2b in more widely distributed tissues including intestinal epithelial apical membrane [73, 74, 88]. Type II NaPi cotransporters are primary regulators of maintenance of Pi balance [100]. This review updates recent progress in the knowledge of the physiological role of α Klotho and FGF23 in modulation of epithelial NaPi-2a, 2b, and 2c, although there is evidence showing both α Klotho and FGF23 regulate type III Na-Pi cotransporters (PIT-1 and PIT-2) [9, 39, 93, 99]; this will not be addressed in this review.

II. FGF23, FGFRs, α Klotho expression and regulation

FGF23 expression:

The FGF's are a highly conserved superfamily of genes divided into seven subfamilies. FGFs exert pleiotropic effects on extremely broad biological processes, including development, organogenesis, and metabolism, through binding and activation of FGF receptor (FGFR) tyrosine kinases [40]. The three members FGF23, FGF21, and FGF19/15, constitutes the endocrine FGF19 family.

FGF23 is a genuine osteogenic endocrine hormone, primarily secreted from the bone (osteocytes and osteoblasts) and has a myriad of effects, and it acts on kidney to promote phosphaturia and suppression of active vitamin D (1,25-dihydroxyvitamin D₃, 1,25-(OH)₂D) synthesis and intestinal Pi absorption, thereby promoting negative external Pi

balance [40]. FGF23 is a 32-kDa 251 amino acids glycoprotein with a signal sequence (24 amino acids), an N-terminal FGF core homology domain (155 amino acids), and a C-terminal domain (72 amino acids) unique to FGF23 [5, 40, 68, 80]. The C-terminal domain is essential for interaction with and activating the FGFR-Klotho complex [16]. Between the N- and C-terminal domains is a proteolytic site (176^{RXXR}179), and FGF23 is inactivated by subtilisin-like proprotein convertase and plasminogen activators, resulting in two inactive N- and active C-terminal fragments [10, 21, 23, 22, 43, 72, 90, 102]. The C-terminal fragment can competitively interfere the formation of intact FGF23/ α Klotho/FGFR complex, thus acting as a natural FGF23 antagonist [34].

FGFR expression:

FGFRs are single-pass receptor tyrosine kinases transmembrane proteins. There are four cell surface FGFR isoforms (FGFR1–4 with further subdivision from splice variants), which are ubiquitously expressed but differentially activated by different FGF ligands in conjunction and complexed with heparan sulfate proteoglycan and with α Klotho and β Klotho [16, 40, 56]. Because of universal expression of FGFRs and restricted expression of membrane α Klotho, tissue specificity of FGF23 action is proposed to be conferred by the presence of transmembrane α Klotho protein through formation of the FGFR/FGF23/ α Klotho tertiary complex [16, 40, 56].

Although soluble α Klotho and FGF23 are present in the circulation and soluble α Klotho is also able to form similar complex as membrane α Klotho [16], distinct expression pattern of FGFR isoforms and their different affinity of binding to α Klotho may provide certain degree of specificity to FGF23 action.

α Klotho expression:

α Klotho is predominantly expressed in renal distal convoluted tubules with lower abundance in proximal convoluted tubules, and in parathyroid chief cells, making the kidney and parathyroid gland the primary FGF23 target organs for mineral metabolism [4, 38]. In addition to the kidney and parathyroids, α Klotho is also present in osteocytes and osteoblasts, which regulates FGF23 synthesis [46, 50]. To date, there is no evidence showing α Klotho presence in intestinal epithelium, another crucial organ regulating Pi absorption. Animal experiments showed that administration of FGF23 protein increases membrane α Klotho in parathyroid gland [4] and in the kidney with an increase in soluble α Klotho [91].

The transmembrane α Klotho protein does not only constitute the cellular basis for initiation of FGF23 signal in specific tissues and also provides circulating soluble α Klotho which can reduce serum Pi and suppress the secretion of PTH and 1,25(OH)₂D [94], thus indirectly influencing the production of FGF23. FGF23 and α Klotho can reciprocally modulate each other to both directly and indirectly participate in Pi homeostasis (Figure 1).

III. α Klotho and FGF23 action on phosphate transport in the kidney, intestine, and bone

Pi is vital for many biological functions including energy metabolism, intracellular signaling, structural composition of many cellular components, and bone mineralization. Pi homeostasis is regulated by the coordinated interplay of different organs including intestine, kidney and bone, and endocrine networks consisting of PTH, 1,25-(OH)₂D, FGF23, and α Klotho (Figure 1 and 2).

Type II Na⁺-dependent Pi co-transporters (NaPi-2) are responsible for active uptake of extracellular Pi into polarized epithelial cells [6, 61, 63, 79]. NaPi-2a and NaPi-2c are expressed in apical brush border membranes (BBM) of the renal proximal tubules and play a major role in active Pi reabsorption in the kidney [12, 60, 98] and NaPi-2b present in the luminal membrane of the ileum which mediates active phosphate absorption in the intestine [37, 78] (Table 1). Type III cotransporters including PiT-1 and PiT-2 are expressed broadly. PiT-1 exists in bone and kidney and PiT-2 in intestine and bone. They participate Pi absorption in the intestine, Pi reabsorption in the kidney, cellular uptake of Pi, and Pi release and storage in the bone [27, 63, 79, 101]. The role and regulation of PiT-1 and PiT-2 are not discussed in this manuscript.

Pi balance is achieved through modulation of intestinal uptake of Pi from diet, renal excretion of Pi from urine, and bone Pi uptake/release via regulation of expression and activity or several Na⁺-coupled Pi transporters. One interesting difference between the kidney and the intestine is the mirror image of the distribution of transcellular and paracellular transport of calcium and phosphate. Calcium absorption is exclusively transcellular in the gut and mostly paracellular in the kidney. In contrast, phosphate absorption is exclusively transcellular in the kidney and largely paracellular in the gut. Both active Na⁺-dependent transcellular Pi and passive paracellular Pi transport occur across the apical membrane in the intestine [79]. Both FGF23 and α Klotho regulate Na⁺-dependent Pi transport across the apical membrane [3, 30, 41] (Figure 2) but the mechanism of Pi efflux across the basolateral membrane remains to be identified.

FGF23/ α Klotho effect on renal phosphate reabsorption:

In the kidney, NaPi-2a and 2c expression and function in proximal tubules have been well characterized (Table 1 and Figure 2). High Pi diet causes gradual down-regulation of Pi reabsorption mediated by decrease in NaPi-2a (< 1 hour) followed by delayed and eventual down-regulation of PiT-2 (> 8 hours) and NaPi-2c (> 24 hours) in normal rats [65].

FGF23, as a phosphaturic hormone, leads to negative Pi balance by reducing the expression of NaPi-2a and NaPi-2c in renal proximal tubule and by reducing serum 1,25-(OH)₂D levels [3, 30, 31]. An important study by Baum et al. used isolated tubules from *Hyp* mice with high FGF23 levels, and showed that the decreased Pi transport can recover *in vitro* with time accompanied by increased apical membrane NaPi-2a after removal of the tubule from the high FGF23 *Hyp* milieu; and the recovery can transpire in the absence of translation [3]. This finding suggests that FGF23 maintains a tonic shift of NaPi-2a away from the cell

surface. Between FGFR3, and R4 global null mice, and in conditional renal tubular FGFR1 null mice, there were no differences in serum Pi levels, NaPi-2a, and NaPi-2c expression in the renal BBMVs compared to their *WT* littermates. Administration of FGF23 to FGFR3 null mice or FGFR4 null mice induced hypophosphatemia and a decrease NaPi-2a and NaPi-2c protein expression in renal BBMVs. In contrast, injection of FGF23 into FGFR1 null mice had no effect on serum phosphorus levels or NaPi-2a and NaPi-2c expression in BBM indicating FGFR1 is the principal receptor for FGF23 to induce hypophosphatemia, while FGFR4 is a minor player [30].

α Klotho deficiency up-regulates NaPi-2a expression in the kidney and NaPi transport activity [66, 83]. Increasing α Klotho in the kidney and urine chronically by transgenic overexpression of α Klotho or acutely by intravenous infusion of α Klotho caused hypophosphatemia, phosphaturia from decreased proximal phosphate reabsorption, and decreased activity and protein of the principal renal phosphate transporter NaPi-2a. In addition, α Klotho deficiency is associated with up-regulation of NaPi-2c in the kidney [83], which should exacerbate hyperphosphatemia in α Klotho-deficient mice. However, those intact animal experiments cannot discern direct vs. indirect effect of α Klotho on modulation of renal NaPi transport.

Direct inhibition of NaPi-2a by α Klotho was shown in cultured cells and in cell-free membrane vesicles characterized by acute inhibition of Na-dependent Pi transport activity followed by decreased cell surface protein [38]. The Pi transport inhibition can be mimicked by recombinant β -glucuronidase and is associated with proteolytic degradation and reduced surface NaPi-2a. The inhibitory effect of α Klotho on NaPi-2a was blocked by β -glucuronidase inhibitor but not by protease inhibitor, suggesting that one mechanism of α Klotho-induced phosphaturia is enzymatic action on the proximal tubule urinary lumen through modifying glycans (not on NaPi-2a), consequently decreasing transporter activity, promoting NaPi-2a proteolytic degradation possibly followed by internalization of NaPi-2a from the apical membrane [38].

FGF23/ α Klotho effect on intestinal phosphate absorption:

Functional NaPi-2b is present in ileum in mice [62] and is thought to be most active in modulating intestinal transcellular Pi absorption. Under physiological conditions, dietary Pi is absorbed in the small intestine by two distinct mechanisms: paracellular transport, which is dependent on electrochemically driven passive diffusion and transcellular transport, which takes place through NaPi-2b. Genetic deletion of NaPi-2b gives rise to low absorption of dietary Pi suggesting that NaPi-2b contributes to the maintenance of systemic Pi homeostasis [78].

In normal mice fed with normal chow, injection of FGF23 plasmids [64] or recombinant protein [34] decreased intestinal Na⁺-dependent Pi transport activity and the amount of NaPi-2b protein in BBM vesicles [64], and consequently reduced serum Pi. To test the effect of FGF23 on plasma Pi reduction through modulation of intestinal NaPi-2b, Tomoe and colleagues treated *WT* mice and NaPi-2a^{-/-};NaPi-2c^{-/-} double knockout mice by administering a plasmid encoding mutant FGF23^{R179Q}. In *WT* mice, FGF23 induced hypophosphatemia and reduced renal NaPi transport activity. NaPi-2a^{-/-};NaPi-2c^{-/-} double

knockout mice have severe hypophosphatemia at baseline but no additional reduction of plasma Pi and of intestinal NaPi-2b protein. Interestingly, double knockout mice had decreased plasma 1,25(OH)₂D [93], suggesting that FGF23 is not able to further inhibit intestinal Pi NaPi-2b expression and intestinal Pi absorption when there is renal Pi wasting and low 1,25(OH)₂D, which are known stimulators of NaPi-2b expression [28] (Figure 2). However, it has not been established whether FGF23-reduced NaPi-2b is associated with decreased 1,25(OH)₂D synthesis. In the presence of significant paracellular Pi transport [79], it may be difficult to evaluate the effect of modulation of NaPi-2b activity on intestinal Pi transport.

αKlotho-deficient mice displayed an increased activity of intestinal NaPi transport, and increased levels of NaPi-2b protein compared with *WT* mice [83], indicating that upregulation of NaPi-2b protein and activity may be one of the molecular mechanisms of hyperphosphatemia in αKlotho-deficient mice. *In vivo* studies could not provide convincing evidence supporting direct effect of αKlotho on NaPi-2a. Dermaku-Sopjani and coworkers used electrophysiologic approaches to determine Pi-induced current and found αKlotho decreased maximal Pi-induced current in both NaPi-2a- and NaPi-2b-expressing *Xenopus* oocytes. Treatment of NaPi-2a- or NaPi-2b-expressing oocytes with αKlotho protein similarly decreased Pi-induced current [20]. Therefore, αKlotho can directly downregulate intestinal NaPi-2b-mediated Pi absorption as well as renal NaPi2a-mediated Pi reabsorption (Figure 2).

FGF23/αKlotho effect on the phosphate transport in the bone:

Both NaPi-2a and NaPi-2b were recently found in osteoblast-like cell lines and may play a role in Pi flux to modulate mineralization but *in vivo* confirmation is not available [59]. Their responses to Pi challenge differ, as Pi supplementation only up-regulated NaPi-2a, and not NaPi-2b, whereas Pi deprivation did not change either one. Therefore, these isoforms may play distinct roles in Pi trafficking across the bone individually, or in concert at different scenarios. Whether FGF23 and αKlotho modulate NaPi-2a and 2b in the bone, remains to be explored (Figure 2).

In addition to mechanical support, bone also contributes to the maintenance of circulating Pi and calcium as the largest exchangeable depot, a target organ of several calciophosphotropic hormones such as 1,25-(OH)₂D, PTH, FGF23, and αKlotho, and the source of FGF23 (Figure 1). It has been well established that bone is not only a source of FGF23 but also a target of FGF23 to participate Pi homeostasis [58]. High FGF23 is associated with bone demineralization, osteoporosis, and fractures [17, 18, 49, 105]; suggesting FGF23 can act as a mineralization inhibitor by controlling Ca and Pi entrance and deposit in the bone. In general speaking, FGF23 action on bone has systemic and local modes [69, 82]. Obviously FGF23 can modulate PTH, 1,25(OH)₂D, and αKlotho to regulate Pi entrance to bone and mineralization [81, 89, 96, 104, 105] (Figure 1). However, direct action of FGF23 on bone mineralization independent of its effect on systemic Pi homeostasis has been documented in *in vitro* with cultured rat calvarial cells and osteoblastic MC3T3-E1 cell line [36, 82, 84], but the mechanism has not been elucidated.

The bone phenotype in α Klotho-deficient mice has been recognized for nearly two decades [52]. Mice with α Klotho deficiency have osteopenia characterized by low turnover [48, 47, 107, 106, 108]. However, bone phenotypes can be attributable to either low α Klotho, or high FGF23 or high Pi or high 1,25(OH) $_2$ D or their combinational effect. *In vitro* studies with human bone marrow mesenchymal stem cells provide convincing evidence support direct effect of α Klotho on osteogenesis [110]. Zhang et al. found soluble α Klotho protein promoted human bone marrow mesenchymal stem cells proliferation, decreased osteoblast-specific genes (Runx, ALP, and Col1a1) expression and calcium deposition as well as FGFR1 expression [110]. Unfortunately, this study did not measure Pi flux in human bone marrow mesenchymal stem cells. Therefore, whether α Klotho modulates Pi uptake by osteoblasts/osteocytes is still not entirely elucidated.

IV. Molecular mechanisms of α Klotho and FGF23 effect on type II NaPi cotransporters

The three members of SLC34 family of Na $^+$ -driven phosphate cotransporters, NaPi-2a, NaPi-2b, and NaPi-2c, mediate the translocation of divalent Pi (HPO $_4^{2-}$) together with two (NaPi-2c) or three Na $^+$ (NaPi-2a and NaPi-2b), respectively. No matter where they are predominantly expressed, the abundance and activity of these transporters are mostly regulated by changes in their expression at the cell surface and are determined by interactions with proteins involved in scaffolding, trafficking, or intracellular signaling. All three transporters are regulated by factors including dietary Pi status and hormones like PTH, 1,25-(OH) $_2$ D, FGF23, or α Klotho [100].

Indirect effects of FGF23 and α Klotho on NaPi-2 via PTH and 1,25-(OH) $_2$ D

The phosphoregulatory factors interact with each other so it is often difficult to discern the direct vs. indirect effects of a certain regulator of Pi metabolism. It is well documented that FGF23 production is regulated by dietary Pi intake, serum Pi, 1,25(OH) $_2$ D, PTH, and α Klotho [33, 31, 71, 85, 86] (Figure 1). The concept that FGF23 targets FGFRs through formation of a tertiary complex with membrane α Klotho protein to inhibit renal Na-dependent Pi transport activity and 1,25-(OH) $_2$ D production in the kidney is challenged by *in vitro* experiments showing that supraphysiologic concentrations of soluble α Klotho protein is able to form similar complex as membrane α Klotho probably to transduce FGF23 signal [16]. However, there is no *in vivo* experiment to confirm whether soluble α Klotho/FGF23/FGFR1 complex formation is mandatory or unique way for soluble α Klotho protein to modulate mineral metabolism.

FGF23 inhibits PTH production: FGF23 can act via secondary effects of modulation of PTH. PTH is a phosphaturic hormone and its synthesis by parathyroid chief cells is regulated by interplay between FGF23 and normal presence of membrane α Klotho and FGFR1 [4, 72] (Figure 1). PTH reduces tubular Pi reabsorption through promoting endocytosis of NaPi-2a and 2c in proximal tubule, thus increasing urinary Pi excretion [7, 25, 32]. PTH also stimulates bone turnover and release of calcium and phosphate, enhances intestinal absorption of calcium and phosphate, and increases renal calcium reabsorption while decreasing urinary phosphate reabsorption [55]. High PTH can stimulate the secretion

of FGF23 and 1,25-(OH)₂D and [11, 72], which increases intestinal Pi absorption (Figure 2). While FGF23 inhibits PTH production, PTH stimulates FGF23 production in the bone, completing a negative and positive feedback loops (Figure 1).

FGF23 inhibits 1,25-(OH)₂D synthesis: FGF23 null mice have high 1,25-(OH)₂D indicating that FGF23 is a 1- α -hydroxylase suppressor [75] (Figure 1). In *WT* mice, FGF23 reduces serum Pi and 1,25-(OH)₂D and NaPi-2b protein in intestinal BBMVs [64]. In VDR-null mice prior to FGF23 administration, there was low serum Pi, Ca, Na⁺-dependent Pi cotransport activity and NaPi-2b protein in the gut, and higher serum PTH and 1,25-(OH)₂D levels compared to *WT* mice. After FGF23 plasmids injection, VDRnull mice had no change in NaPi cotransport activity and NaPi-2b protein. So FGF23-induced modulation of NaPi-2b activity and protein is mediated through reduction of 1,25-(OH)₂D [64]. Therefore, FGF23 inhibits 1,25-(OH)₂D synthesis, consequently reducing intestinal NaPi transport activity and NaPi-2b protein levels [64] (Figure 1, and 2). Whether FGF23 can directly suppress NaPi-2b is to be explored.

α Klotho inhibits 1,25-(OH)₂D synthesis: Serum 1,25-(OH)₂D levels are dramatically increased in α Klotho-deficient mice suggesting that 1,25-(OH)₂D synthesis in the kidney can be suppressed by α Klotho [52, 109]. Active vitamin D stimulates α Klotho production in the kidney and soluble α Klotho [54, 70, 76], which consequently suppresses Pi reabsorption (Figure 2). Independent of changes in intestinal calcium absorption and serum calcium, 1,25-(OH)₂D represses transcription of *PTH* and decreases renal Pi excretion [51]. High vitamin D also increase FGF23 levels to counteract PTH effect on limiting phosphate excretion [70].

Taken together, almost all players implicated in phosphate homeostasis including PTH, 1,25(OH)₂D, FGF23, and α Klotho that regulate phosphate metabolism independently but are also highly interrelated through modulation of other hormones' metabolism (Figure 1 and 2).

Direct effects of α Klotho and FGF23 on type II Na-dependent Pi cotransporters.

The FGF23- α Klotho coreceptor model: The strikingly similar physical, morphological, and biochemical phenotypes of FGF23 and α Klotho-deficient mice suggested that they may share common signaling pathways [67]. *In vivo* study further showed that FGF23 injection induce low serum Pi in both *WT* and *FGF23*-null mice, but not in *FGF23*/ α *Klotho* double-knockout mice or α *Klotho* knockout alone, suggesting that FGF23 regulation of Pi metabolism depends on the presence of α Klotho [67]. *In vitro* studies from two laboratories independently confirmed the formation of FGF23/ α Klotho/FGFR1 [53, 95]. *Hyp* mice bearing a mutation of PHEX have higher FGF23, severe hypophosphatemia due to urinary Pi wasting. Deletion of α Klotho causes severe hyperphosphatemia and increased renal NaPi-2a protein expression in *Hyp* mice despite high serum FGF23. Interestingly injection of bioactive PTH downregulated renal NaPi-2a and consequently reduced serum Pi in *Hyp*/ α *Klotho*^{-/-} mice. These results suggest that in the absence of α Klotho, FGF23 is unable to regulate systemic Pi homeostasis [66].

FGF23-independent effects of α Klotho: In general, type II NaPi co-transporter activity is regulated through down or upregulation of abundance of their protein and trafficking to and from the cell membrane, but not through change in their mRNA, suggesting posttranscriptional and protein distribution control. Soluble α Klotho suppresses NaPi transport activity, when directly added to cultured proximal tubule-like cells, and even in cell-free BBM vesicles without possibility of protein trafficking suggesting a direct gating effect on NaPi's which is unique for regulators of NaPi-2a and 2c. A second important finding is that the cell culture and the BBM vesicle experiments did not contain FGF23 indicating that α Klotho exerts an FGF23-independent effect. In support of that notion is the fact that the *FGF23*-null mice preserve the ability to increase urine Pi excretion in response to exogenous soluble α Klotho [38].

In addition to the acute direct gating of NaPi's, the more chronic α Klotho-induced suppression of NaPi transport is associated with reduced surface expression NaPi-2a internalization and degradation through modification of moieties of sugar chain in cell surface protein [38]. The glycan-modifying activity of α Klotho is based on one study with direct measurement [92] and numerous papers using sialidase and glucuronidase inhibitors [1, 14, 13, 38, 57, 103]. However, recently one *in vitro* study proposed that soluble α Klotho is unlikely to be a glycan-modifying enzyme [16]. Whether the enzymatic model can be employed to α Klotho effect on NaPi-2a and 2b in renal proximal tubules remains to be clarified.

α Klotho-independent effects of FGF23: There is suggestion that FGF23 may also directly decreases type II NaPi transport activity. To explore its direct effect, Andrukhova and colleagues conducted *in vitro* experiment with culture proximal tubular cells and dissected proximal tubular segments [2]. They found that FGF23 decreased NaPi-2a protein expression in dissected proximal tubular segments isolated from *WT* mice. FGF23 at low concentration (<10 ng/ml) could not, but only at high concentration (>10 ng/ml) could reduce NaPi-2a protein in proximal tubules isolated from α Klotho-deficient mice suggesting that perhaps high FGF23 is able to exert α Klotho-independent reduction of NaPi-2a. It is possible that FGF23 at low concentration directly acts on proximal tubular epithelial NaPi-2a through its canonical, α Klotho/FGFR-dependent signaling pathway. FGF23 at high concentration also directly inhibits NaPi-2a protein in renal tubules through a non-canonical α Klotho independent signaling pathway [2], which still needs to be defined. In the heart, FGF23 has been proposed to trans-activate the FGFR4 [35, 77].

5. Conclusion and Perspectives

Abnormal Pi metabolism is associated with cardiovascular disease and lifespan [24, 29, 45, 87]. The study of the regulation and function of the phosphoregulatory factors FGF23 and α Klotho has led to the great advances in the cellular and molecular mechanisms of Pi homeostasis maintained by an interacting network of hormones including FGF23, α Klotho, PTH and 1,25-(OH)₂-VD involving the gut, kidney, bone and parathyroid glands. The balance of Pi metabolism by modulation of NaPi-2 in gut, kidney, and bone is important for normal cell metabolism, skeleton architecture, and for general cell health. The addition of FGF23 and soluble α Klotho to this network establishes a new conceptual framework for

understanding the pathogenesis of genetic and acquired phosphate disorders. The manipulation of NaPi-2 activity and/or expression by change in FGF23 and/or α Klotho axis signal activity as well as modification of PTH and 1,25-(OH)₂D will constitute a novel strategy for those genetic and acquired hyper- or hypophosphatemia and even more importantly to prolong human life.

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Figure 1: A network consisting of α Klotho, FGF23, PTH and 1,25-(OH)₂D to maintain Pi homeostasis.

PTH stimulates 1 α -hydroxylase activity, and increase serum 1,25-(OH)₂D, which feeds back to suppress PTH. FGF23 produced by bone targets the kidney, leading to reduction in 1,25-(OH)₂D levels by inhibiting 1 α -hydroxylase activity. 1,25-(OH)₂D stimulates FGF23 synthesis in the bone and membrane α Klotho in the kidney. FGF23 may also directly decrease renal expression of membrane α Klotho (dash line), but this effect still awaits definitive confirmation. FGF23 inhibits PTH secretion, consequently turning off the stimulatory effect of PTH on FGF23 synthesis. In addition to FGF23, α Klotho also suppresses 1,25-(OH)₂D production in the kidney to prevent over absorption of Pi from gut. Interestingly, soluble α Klotho protein shed from membrane Klotho in the kidney is able to suppress 1,25-(OH)₂D synthesis and may also inhibit FGF23 production in the bone (dash line). Therefore, FGF23, α Klotho, PTH and 1,25-(OH)₂D form several interconnecting negative and positive feedback loops to tightly control Pi metabolism. Dash line: putative effect yet to be experimentally confirmed. Solid line: experimentally confirmed results.

Figure 2: The role of α Klotho, PTH, FGF23, and 1,25-(OH) $_2$ D in modulation of NaPi-2a, 2b, and 2c.

Phosphate absorption from small intestine is mainly controlled by NaPi-2b, which is negatively regulated by soluble α Klotho and probably FGF23 (dash line), but positively by 1,25-(OH) $_2$ D. Urinary Pi excretion through the kidney is mainly controlled by NaPi-2a and NaPi-2c. Their expression and activity are negatively regulated by several calciophosphotropic hormones (PTH, FGF23, and soluble α Klotho). Pi flux in the bone contributing to Pi homeostasis, is presumably suppressed by FGF23 in an autocrine manner as well as soluble α Klotho (dash line), but await to be proved. Dash line: putative effect yet to be experimentally confirmed. Solid line: experimentally confirmed results.

Table 1:

α Klotho and FGF23 inhibit NaPi-2a, 2b and 2c expression and activity

		NaPi-II in the kidney		NaPi-II in gut
		NaPi2a	NaPi2c	NaPi2b
	Location in mouse	S1~3	S2~3	ileum
α Klotho	Expression	↓	↓	↓
	Activity	↓	↓	↓
FGF23	Expression	↓	↓	↓
	Activity	↓	↓	↓

S: Segment of the renal proximal tubule, which is subdivided into three segments. S1: first portion of proximal convoluted tubule. S2: latter portion of proximal convoluted tubule and first portion of proximal straight tubule. S3: latter portion of proximal straight tubule.