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COMMENTARIES

Bone Talk: Klotho and FGF23 Signaling

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Commentary on: Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature*. 2006 Dec 7;444(7120):770-4.

Fibroblast growth factor-23 (FGF23) was identified as a phosphate wasting factor, or phosphatonin, in 2000 (1), when the *FGF23* gene was shown to harbor gain-of-function mutations that cause autosomal dominant hypophosphatemic rickets (2) and FGF23 was determined to be a phosphate-wasting signal elaborated by tumors in oncogenous osteomalacia (3). FGFs and their receptors are ubiquitous, but the phosphaturic signal is specific: the clinical syndrome of FGF23 excess consists of phosphate wasting and impaired synthesis of the active vitamin D metabolite 1,25$(OH)_2$D, both functions of the proximal convoluted tubule of the kidney. Urakawa *et al*. (4) have now reported a startling explanation for the paradox of FGF23 specificity: the principal FGF23 receptor, FGFR1(IIIc), responds to FGF23 only when the Klotho protein is present as a coreceptor, and Klotho is present only in the kidney, parathyroid and pituitary. The *klotho* phenotype, though it was originally described as a form of premature aging, is the result of FGF23 resistance.

To address the renal specificity of FGF23 action, Urakawa et al. (4) used gene array analysis to pick out *Egr-1* as a kidney gene whose expression is rapidly upregulated by injection of FGF23. Although *Egr-1* is ubiquitous, FGF23 activated *Egr-1* only in kidney, pituitary and parathyroid, and not in other mouse tissues. To identify a renal molecule that might account for this tissue distribution, Urakawa and colleagues used FGF23 affinity chromatography to identify Klotho as the major FGF23-binding protein in renal homogenates. Cells exposed to FGF23 underwent ERK phosphorylation and increased their abundance of Egr-1 protein only when transfected with Klotho, similar to other recently reported results (5). Klotho is expressed in all three tissues that have an Egr-1 response to FGF23 – kidney, pituitary and parathyroid – but not in other mouse or rat tissues.

Klotho was characterized as an aging gene (6;7) because one of the mouse strains created in a program of random insertional mutagenesis had a reduced lifespan, atherosclerosis, osteopenia, skin atrophy, impaired sexual maturation and pulmonary emphysema (6). Since these traits were viewed as evidence of premature aging, the mutation was named *klotho,* after one of the three Fates who spins the thread of life. The *klotho* gene that was disrupted by insertional mutagenesis was shown to encode a cell surface protein with a short cytoplasmic tail, whose extracellular domain consists of tandem duplicated copies of a ß–glucosidase-like sequence, which can be released as a soluble form of Klotho.

FGF23 deficiency causes hyperphosphatemia and increased 1,25$(OH)_2$D synthesis, which leads to hypercalcemia and eventually to tissue damage and nephrocalcinosis (8;9). The biochemical profile of *klotho* mice is identical to this (4), but in contrast to *FGF23*(-/-) mice, *klotho* mice have markedly increased FGF23 levels, consistent with resistance to

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FGF23 action as their underlying disorder (4). These genetic data make a strong case that the Klotho protein is required for FGF23 action. Moreover, the short lifespan, infertility, osteoporosis, emphysema and skin changes of the *klotho* mouse are also present in *Fgf23*(-/-) mice and can be explained as the consequences of hyperphosphatemia and increased $1,25(OH)_2D$ levels, since the *Fgf23*(-/-) phenotype can be rescued by removal of either the *Cyp27B* gene, which encodes the vitamin D 1α–hydroxylase (10), or the vitamin D receptor (11).

In further studies, a neutralizing monoclonal antibody against the extracellular domain of Klotho was shown to inhibit FGF23 action in Klotho-expressing CHO cells. Injection of the antibody into mice caused a sharp increase in the serum concentration of $1,25(OH)_2D$, with subsequent increases in the serum concentration of phosphate and FGF23. This independently confirms the requirement of Klotho for successful FGF23 signaling.

To determine the role of canonical FGF receptors (FGFR) in FGF23 signaling, individual receptors and splice variants were used to complement Klotho in L6 cells, which are deficient in native FGF receptors. Only FGFR1(IIIc) in combination with Klotho was able to support significant FGF23 signaling in these experiments. In contrast to FGF23, basic FGF, a universal ligand for FGF receptors, was able to signal without coexpression of Klotho. Thus, Klotho selectively converts FGFR1(IIIc) into a specific FGF23 receptor.

Bone is the predominant source of FGF23, and recent studies point to the osteocyte as the site of FGF23 synthesis in another hereditary phosphate-wasting disorder, the *Hyp* mutation (12). In a *BoneKEy* Commentary in this issue (13), Caroline Silve discusses two recent papers implicating the bone matrix protein DMP1 in the FGF23 signaling pathway (14;15). These papers raise the possibility that osteocytes sample the state of bone matrix through DMP1 and then signal the kidney to dump phosphate and produce $1,25(OH)_2D$. FGF23 is thus a specific messenger from bone to kidney and perhaps other tissues and can be regarded as the first osteokine.

FGFs are heparin-binding molecules typically associated with the extracellular matrix (ECM). As a circulating cytokine, FGF23 is unusual among FGFs, but it is not unique. Other members of the same FGF subfamily – including FGF15, its human ortholog FGF19, and FGF21 – also seem to be able to deliver signals at a distance, circulating either in blood or lymph (see Table 1). What molecular adaptations are required for these FGFs to circulate? FGF19 has markedly reduced heparin binding, and structural studies of FGF19 show that ordered structure is not detected in a loop that includes strand 11, the principal heparin-binding domain of FGFs (16). Other members of the subfamily, including FGF23, are predicted to have similar structures; it may well be that all are adapted to circulate by reduced binding to the heparan sulfate proteoglycans of the ECM.

FGF	*Source*	*Target*	*Actions*	*Receptor*	*Refs*
FGF15/19*	Intestine	Liver Gallbladder	↑Bile acid synthesis ↓Gallbladder contractility	FGFR4 FGFR3	16-18
FGF21	Liver	Adipocytes Panc α-cells Panc β-cells	↑Glucose uptake ↓Glucagon release ↑Function/survival	FGFR1/2	20
FGF23	Bone	Kidney	↑Phosphate excretion ↓Vitamin D activation	FGFR1(IIIc)	4

Table 1. The FGF19 Family
*FGF15 is the mouse ortholog of human FGF19

The FGF19 subfamily may have another adaptation as well. Mice lacking β-Klotho, a second member of the Klotho family, have a marked increase in the synthesis and secretion of bile acids (17), much of which can be accounted for by impaired bile acid suppression of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid biosynthesis. This phenotype is quite similar to that resulting from removal of the gene encoding FGF15 (which is secreted by enterocytes and is the main feedback mechanism to regulate bile acid secretion by the liver (18)) or removal of the gene for FGFR4, the liver receptor for FGF15/19 (19). In addition, β-*klotho*(-/-) and *FGFR4*(-/-) mice both exhibit small gallbladders. It thus seems likely that Klotho and β-Klotho are both coreceptors for different FGFs, raising the possibility that the use of Klotho family members as coreceptors is another distinguishing feature of the FGF 15/19/21/23 subfamily (20). FGF21 does not bind to extracellular domains of FGFRs, though it activates FGFR1 and FGFR2 in adipocytes, suggesting that it also requires a binding partner (21). In addition to Klotho and β-Klotho, the family contains a third, more distantly related member, Klotho LPH-related protein (22), whose function and relationship to FGF signaling are unknown.

This beautiful work opens up many vistas. Is FGF23 signaling restricted to parathyroid, pituitary and kidney (where Klotho is predominantly expressed in distal rather than proximal tubule, leaving open a critical question about how the signal reaches the proximal tubule)? Or can FGF23 use other Klotho family members or soluble Klotho to signal in other tissues, such as bone itself? If FGFs 15, 19, 21, and 23 are all circulating signals, how is specificity maintained; are there specific partnerships between a given Klotho and FGFR or does the FGFR determine the tissue specificity of signaling? Finally, if the fatality of *klotho* mice is attributable to the consequences of FGF23 deficiency, such as hyperphosphatemia and hypercalcemia, then why is the mouse lifespan considerably increased by overexpression of FGF23 (23)?

References

1. White KE, Larsson TE, Econs MJ. The roles of specific genes implicated as circulating factors involved in normal and disordered phosphate homeostasis: frizzled related protein-4, matrix extracellular phosphoglycoprotein, and fibroblast growth factor 23. *Endocrine Rev*. 2006 May;27(3):221-41.

2. White KE, Evans WE, O'Riordan JL, Speer MC, Econs MJ, Lorenz-Depiereux B, Grabowski M, Meitinger T, Strom TM. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet*. 2000 Nov;26(3):345-8.

3. Shimada T, Mizutani S, Muto T, Yoneya T, Hino R, Takeda S, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Natl Acad Sci U S A*. 2001 May 22;98(11):6500-5.

4. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature*. 2006 Dec 7;444(7120):770-4.

5. Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M. Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem*. 2006 Mar 10;281(10):6120-3.

6. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*. 1997 Nov 6;390(6655):45-51.

7. Strewler GJ. The spinner meets the stone: Klotho and mineral metabolism.

BoneKEy-Osteovision. 2005 November;2(11):29-33. [Full Text]

8. Shimada T, Urakawa I, Yamazaki Y, Hasegawa H, Hino R, Yoneya T, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. *Biochem Biophys Res Commun*. 2004 Feb 6;314(2):409-14.

9. Sitara D, Razzaque MS, Hesse M, Yoganathan S, Taguchi T, Erben RG, Juppner H, Lanske B. Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in Phex-deficient mice. *Matrix Biol*. 2004 Nov;23(7):421-32.

10. Sitara D, Razzaque MS, St-Arnaud R, Huang W, Taguchi T, Erben RG, Lanske B. Genetic ablation of vitamin D activation pathway reverses biochemical and skeletal anomalies in Fgf-23-null animals. *Am J Pathol*. 2006 Dec;169(6):2161-70.

11. Hesse M, Frohlich LF, Zeitz U, Lanske B, Erben RG. Ablation of vitamin D signaling rescues bone, mineral, and glucose homeostasis in Fgf-23 deficient mice. *Matrix Biol*. 2006 Oct 20; [Epub ahead of print]

12. Liu S, Zhou J, Tang W, Jiang X, Rowe DW, Quarles LD. Pathogenic role of Fgf23 in Hyp mice. *Am J Physiol Endocrinol Metab*. 2006 Jul;291(1):E38-49.

13. Silve C. DMP1 and phosphate metabolism – matrix proteins go systemic. *BoneKEy-Osteovision*. 2006 December;3(12):30-35. [Full Text]

14. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet*. 2006 Nov;38(11):1310-5.

15. Lorenz-Depiereux B, Bastepe M, Benet-Pages A, Amyere M, Wagenstaller J, Muller-Barth U, Badenhoop K, Kaiser SM, Rittmaster RS, Shlossberg AH, Olivares JL, Loris C, Ramos FJ, Glorieux F, Vikkula M, Juppner H, Strom TM. DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis. *Nat Genet*. 2006 Nov;38(11):1248-50.

16. Harmer NJ, Pellegrini L, Chirgadze D, Fernandez-Recio J, Blundell TL. The crystal structure of fibroblast growth factor (FGF) 19 reveals novel features of the FGF family and offers a structural basis for its unusual receptor affinity. *Biochemistry*. 2004 Jan 27;43(3):629-40.

17. Ito S, Fujimori T, Furuya A, Satoh J, Nabeshima Y, Nabeshima Y. Impaired negative feedback suppression of bile acid synthesis in mice lacking betaKlotho. *J Clin Invest*. 2005 Aug;115(8):2202-8.

18. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson JA, Gerard RD, Repa JJ, Mangelsdorf DJ, Kliewer SA. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab*. 2005 Oct;2(4):217-25.

19. Yu C, Wang F, Kan M, Jin C, Jones RB, Weinstein M, Deng CX, McKeehan WL. Elevated cholesterol metabolism and bile acid synthesis in mice lacking membrane tyrosine kinase receptor FGFR4. *J Biol Chem*. 2000 May 19;275(20):15482-9.

20. Zhang X, Ibrahimi OA, Olsen SK, Umemori H, Mohammadi M, Ornitz DM. Receptor specificity of the fibroblast

growth factor family. The complete mammalian FGF family. *J Biol Chem*. 2006 Jun 9;281(23):15694-700.

21. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB. FGF-21 as a novel metabolic regulator. *J Clin Invest*. 2005 Jun;115(6):1627-35.

22. Ito S, Fujimori T, Hayashizaki Y, Nabeshima Y. Identification of a novel mouse membrane-bound family 1 glycosidase-like protein, which carries an atypical active site structure. *Biochim Biophys Acta*. 2002 Jul 19;1576(3):341-5.

23. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP, Kuro-o M. Suppression of aging in mice by the hormone Klotho. *Science*. 2005 Sep 16;309(5742):1829-33.