BoneKEy-Osteovision. 2006 August;3(8):14-17
http://www.bonekey-ibms.org/cgi/content/full/ibmske;3/8/14
DOI: 10.1138/20060225

COMMENTARIES

Disordered Secretion of FGF23 Links Three Disorders of Phosphate Transport

Gordon J. Strewler

Beth Israel Deaconess Medical Center and Harvard Medical School

Commentary on:

- Kato K, Jeanneau C, Tarp MA, Benet-Pages A, Lorenz-Depiereux B, Bennett EP, Mandel U, Strom TM, Clausen H. Polypeptide GalNAc-transferase T3 and familial tumoral calcinosis. Secretion of fibroblast growth factor 23 requires O-glycosylation. *J Biol Chem*. 2006 Jul 7;281(27):18370-7.

- 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.

Disordered processing of FGF23 leads to two mirror-image disorders. Mutations that impair *O*-glycosylation (1;2), as well as several missense mutations in the FGF23 sequence (3-5), lead to defective secretion of FGF-23, giving rise to familial tumoral calcinosis (OMIM 211900), a disorder characterized by hyperphosphatemia and massive deposition of calcium phosphate in soft tissues. The converse, excessive levels of FGF23 with phosphate wasting and rickets, occurs in autosomal dominant hypophosphatemic rickets because of defective cleavage of FGF23 at a prohormone convertase site (6). A recent paper (7) ties the two disorders directly together by showing that *O*-glycosylation of FGF23 occurs at the proprotein convertase cleavage site and prevents cleavage. The results indicate that this cleavage site is a control point for regulation of phosphate and vitamin D metabolism by FGF23. In another recent paper (8), the Hyp mouse model of X-linked hypophosphatemic rickets is also linked to disordered control of FGF23 secretion by the osteocyte.

To develop a model of glycosylation of FGF23, Kato *et al*. expressed FGF23 in the mutant CHO cell line IdlD, which is deficient in an epimerase which catalyzes the reaction from UDP-glucose to UDP-galactose. The defect can be complemented by addition of GalNac plus Gal. Secretion of FGF23 from CHO IdlD cells required *O*-glycosylation, as demonstrated by the requirement for addition of GalNAc and Gal to the mutant cells. Secretion of FGF23 was dramatically enhanced by coexpression of GalNAc-T3, the glycotransferase that was implicated in familial tumoral calcinosis (1;2). Thus, GHO IdlD cells do not produce an endogenous GalNAc-T3 with specificity for FGF23.

Next, the acceptor substrate site for *O*-glycosylation was identified by MALDI-TOF of model peptides from FGF23 that encompass the prohormone convertase site. Several recombinant GalNAc transferases targeted Thr^{171}, but only GalNAc-T3 incorporated GalNAc into Thr^{178}. The results agree with previous studies by Shimada *et al*. in which Thr^{178} and Ser^{180} were shown as the most likely sites of *O*-glycosylation of a similar peptide (9).

Thr^{178} is within the prohormone convertase site $R^{176}HTR^{179}$, raising the possibility that *O*-glycosylation of Thr^{178} affects the susceptibility of FGF23 to cleavage. In experiments to test this possibility, Kato *et*

al. showed that several glycopeptides modified at Thr^{178} could be efficiently cleaved by furin *in vitro*, but mono- and disialylated peptides were completely resistant to furin cleavage, suggesting that the major inhibitory effect of *O*-glycosylation is exerted by α2,6-sialylation of GalNAc (NeuAcα2-6GalNAcα1-*O*-Thr^{178}). Interestingly, however, α2,6-sialylation of *O*-glycans is rarely complete.

The results identify a novel mechanism for regulation of secretion of FGF23, in which competition between proteolytic cleavage and *O*-glycosylation determines the rate of secretion of FGF23. In the absence of glycosylation, secretion of FGF23 is virtually abolished because of efficient proteolysis of the full length molecule. Were glycosylation completely efficient, on the other hand, it is likely that mutations in R^{176} or R^{179} would not produce a phenotype. The fact that mutations in these arginines lead to FGF23 excess and a syndrome of phosphate wasting indicates that competition between proteolysis and glycosylation is indeed active in humans, and also suggests that the derangement in synthetic control that results from arginine mutations outflanks whatever homeostatic mechanisms regulate secretion of FGF23. That is, in general an increase in the efficiency of secretion of a hormone might be expected not to create a syndrome of hormone excess because negative feedback regulation should compensate, but negative feedback does not prevent phosphate wasting in autosomal dominant hypophosphatemic rickets.

The role of the C-terminal domain of FGF23 (aa180-251), which is unique among the FGFs, may be in part to stabilize FGF23 during biosynthesis and secretion. The C-terminal domain could have additional functions, e.g. to stabilize FGF23 in the circulation, or to bind klotho, a step that appears to be essential for the biological activity of FGF23 (10). It appears that other FGFs that lack this domain also interact with klotho, however. Another possibility is that the C-terminal domain, which accumulates in the blood of individuals with familial tumoral calcinosis, is itself a feedback signal to FGF23 synthesis.

A recent paper by Liu *et al*. (8) examines FGF23 secretion from bone cells. To understand better the regulation of *Fgf23* gene transcription at the cellular level, Liu *et al*. created a mouse reporter strain in which green fluorescent protein (eGFP) was knocked in to replace exon 1 of the FGF23 coding sequence. In these reporter mice, cells that express *Fgf23* gene transcripts are green, but the *Fgf23* gene is inactivated. The resulting *Fgf23*(+/-) and *Fgf23*(-/-) mice had phenotypes very similar to previous reports of removal of the *Fgf23* gene (11;12).

The *Fgf23*(-/+) reporter mice were crossed with *Hyp* mice. Hyp is a mouse model of X-linked hypophosphatemia (XLH) caused by loss-of-function mutations in a cell surface protease, Phex (13;14) and characterized by high levels of FGF23 (15). In keeping with earlier reports (12), the phenotype of the double knockout mice resembled that of *Fgf23*(-/-), with hyperphosphatemia and high levels of $1,25(OH)_2D$. Thus, FGF23 is required in order for the *Hyp* mutation to affect phosphate and vitamin D metabolism, placing *Fgf23* genetically downstream of *Hyp*. Rickets was also abolished in double knockout mice, consistent with a role of hypophosphatemia and/or low levels of $1,25(OH)_2D$ in the pathogenesis of rickets. The double knockout mice did not have excessive osteoid, as do *Hyp* mice, but mineralization was virtually absent. Therefore, FGF23 lack and the absence of Phex independently impair mineralization.

Reporter mice expressed *Ffg23* at two bone sites, osteocytes and CD31-positive endothelial cells in venous sinusoids of bone, but not in surface osteoblasts. A previous *in situ* hybridization study (16) localized FGF23 in osteocytes and venous sinusoids; active osteoblasts at fracture healing sites also expressed the *FGF23* gene. The reporter construct was also expressed in the thymus and ventrolateral hypothalamus. Introduction of the *Hyp* mutation into reporter mice led to a striking increase in *Fgf23* expression in osteocytes, but had no effect on expression in venous sinusoids. The increase in osteocyte FGF23 expression was greatest in *Fgf23*(-/-)/*Hyp*,

indicating that expression of FGF23 is not required in order for *Fgf23* gene transcription to be upregulated by the absence of Phex. This result puts to rest the attractive idea that FGF23 levels are high in Hyp and XLH because processing of FGF23 by Phex is impaired. Although intact FGF23 is not a substrate for Phex (15;17), internal Phex cleavage sites have been demonstrated in the FGF23 sequence, at least one of which would be exposed by prohormone convertase cleavage at $R^{176}HTR^{179}$ (18) , but the results of Liu *et al*. essentially rule out models of the pathogenesis of Hyp (or XLH) in which FGF23 peptides feed back to upregulate gene transcription.

Instead, the results of Liu *et al*. favor models in which Phex inhibits *Fgf23* gene transcription in osteocytes by cleaving a bone matrix substrate in their vicinity. Based on abstracts submitted to the 2006 Annual Meeting of the American Society for Bone and Mineral Research, dentine matrix protein 1 is a good candidate for the phex substrate, as its absence also leads to renal phosphate wasting.

The observation that *Fgf23*(-/-) mice have markedly impaired mineralization (8;11), despite dramatic hyperphosphatemia, raises the possibility that FGF23 has a local role in the control of mineralization, conceivably by regulating calcium or phosphate flux across mineralizing osteoblasts. Although Liu *et al*. prepared mineralizing bone nodules from *Fgf23*(-/-) mice (8), they did not comment on the ability of FGF23-null cells to mineralize their matrix *in vitro.* Alternatively, abnormalities of the extracellular milieu, such as the high levels of $1,25(OH)_2D$, could be responsible for the mineralization disorder in *Fgf23*(-/-) mice , as suggested by data in a recent review by Razzaque *et al*. (19).

Together, the work of Kato *et al*. and Liu *et al*. implicate disordered synthesis and secretion of FGF23 in three independent disorders of phosphate and vitamin D homeostasis. Osteocytes, now featured players in bone remodeling as well as mechanotransduction, are evidently featured players in phosphate and vitamin D homeostasis as well. Who would have thought?

Conflict of Interest: The author reports that no conflict of interest exists.

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