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Update on FGF23 and Klotho signaling

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ABSTRACT

Fibroblast growth factor-23 (FGF23) is a bone-derived hormone known to suppress phosphate reabsorption and vitamin D hormone production in the kidney. Klotho was originally discovered as an anti-aging factor, but the functional role of Klotho is still a controversial issue. Three major functions have been proposed, a hormonal function of soluble Klotho, an enzymatic function as glycosidase, and the function as an obligatory co-receptor for FGF23 signaling. The purpose of this review is to highlight the recent advances in the area of FGF23 and Klotho signaling in the kidney, in the parathyroid gland, in the cardiovascular system, in bone, and in the central nervous system. During recent years, major new functions of FGF23 and Klotho have been discovered in these organ systems. Based on these novel findings, FGF23 has emerged as a pleiotropic endocrine and auto-/paracrine factor influencing not only mineral metabolism but also cardiovascular function.

1. Introduction

Gain-of-function mutations in fibroblast growth factor-23 (FGF23) were discovered as the genetic cause of autosomal dominant hypophosphatemic rickets in the year 2000 (The ADHR Consortium, 2000). Soon after this discovery it was shown that FGF23 is a phosphaturic hormone, reducing phosphate reabsorption from urine through a downregulation of sodium phosphate co-transporters in renal proximal tubular epithelial cells (Shimada et al., 2001, 2004a, 2005). There is solid evidence from a number of different diseases and disease models that excessive amounts of circulating intact FGF23 lead to renal phosphate wasting as long as kidney function is normal (Martin et al., 2012). FGF23 also down-regulates 1α-hydroxylase expression in renal proximal tubules, thereby suppressing the production of the biologically active vitamin D hormone, 1α,25-dihydroxyvitamin D_3 (Shimada et al., 2001, 2004a, 2005).

Osteoblasts and osteocytes probably are the major sources for circulating FGF23 *in vivo* (Martin et al., 2012). The secretion of FGF23 in bone is stimulated by the vitamin D hormone and by increased extracellular phosphate (Fig. 1), forming a feedback loop between bone and kidney (Juppner, 2011; Martin et al., 2012; Kaneko et al., 2015). In addition, increased extracellular calcium is able to augment FGF23 secretion (Quinn et al., 2013). Because activating mutations in FGF receptor 1 (*FGFR1*) in patients with osteoglophonic dysplasia can lead to increased FGF23 secretion (White et al., 2005) and ablation of *Fgfr1* in bone partially rescues the excessive Fgf23 secretion in *Hyp* mice (Xiao et al., 2014), FGFR1 signaling appears to be involved in the regulation of FGF23 secretion in osteoblasts and osteocytes. However, the intracellular pathways downstream of FGFR1 that regulate FGF23 transcription are currently unknown. There is also accumulating evidence that iron deficiency (Wolf and White, 2014) and pro-inflammatory stimuli enhance FGF23 secretion from bone (Ito et al., 2015; David et al., 2016; Pathak et al., 2016).

To protect FGF23 from intracellular cleavage by the subtilisin-like proprotein convertase furin during the secretory process, FGF23 needs to be O-glycosylated at threonine^{178} within the cleavage site by polypeptide N-acetylgalactosaminyltransferase 3 (GalNT3). Because only the intact FGF23 molecule is biologically active, failure of glycosylation in loss-of-function mutations of GalNT3 results in secretion of mostly cleaved FGF23, leading to an FGF23 deficiency-like phenotype in men and mice (Topaz et al., 2004; Kato et al., 2006; Ichikawa et al., 2009). More recently, it was discovered that O-glycosylation of FGF23 needs to be counterbalanced by phosphorylation of serine^{180} near the glycosylation site by family with sequence similarity 20, member C (FAM20C). Loss of function in Fam20C leads to increased circulating intact

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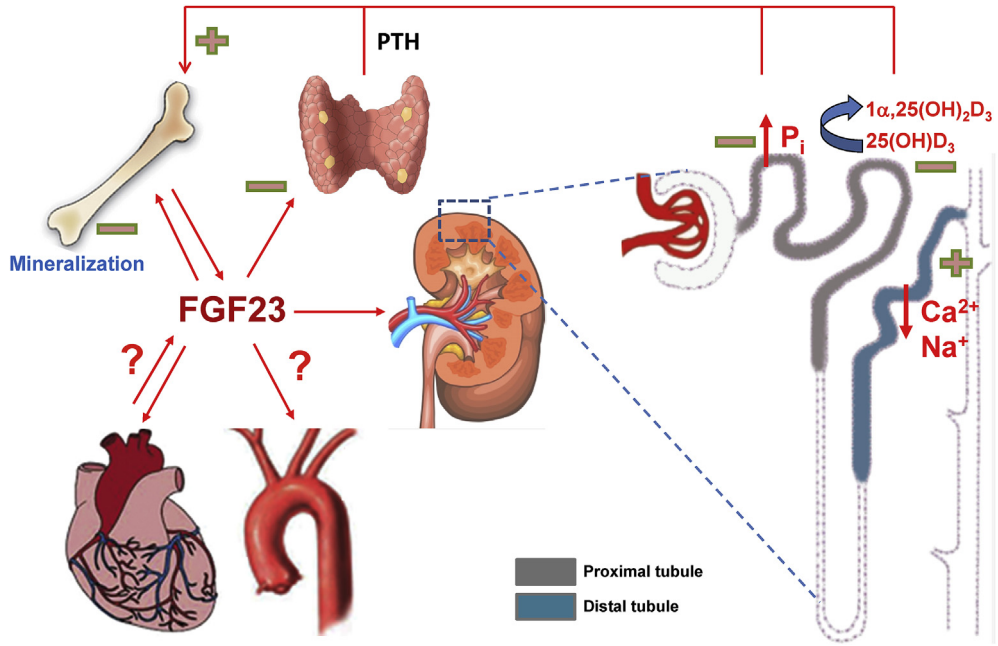

Fig. 1. Pleiotropic endocrine and auto-/paracrine functions of FGF23. FGF23 is mainly produced in bone by osteoblasts and osteocytes. Bony secretion of FGF23 is stimulated by phosphate, parathyroid hormone (PTH), and by the vitamin D hormone $1\alpha,25(OH)_2D_3$. FGF23 acts independently on renal proximal and distal tubules in a Klotho dependent fashion. In renal proximal tubules, FGF23 inhibits phosphate re-uptake and expression of 1α-hydroxylase, the rate-limiting enzyme for vitamin D hormone production. In distal tubules, FGF23 increases reabsorption of calcium and sodium which may indirectly contribute to vascular calcification and may put additional strain on the heart by salt and volume retention. Furthermore, FGF23 inhibits PTH secretion in parathyroid glands by a Klotho independent signaling mechanism. Recent evidence also suggests that FGF23 induces hypertrophy by a direct, Klotho independent action on cardiomyocytes. Because cardiac expression of FGF23 is increased after experimental myocardial infarction and in chronic kidney disease-induced left ventricular hypertrophy, the heart may also become a source of circulating FGF23 in these conditions. It has recently been uncovered that FGF23 is an auto-/paracrine inhibitor of bone mineralization by suppressing alkaline phosphatase in a Klotho independent fashion. It is still controversial whether FGF23 has direct effects on blood vessels, or whether the vascular effects of FGF23 are mediated indirectly through renal calcium retention and suppression of vitamin D hormone production which may in turn promote endothelial dysfunction.

Fgf23 and hypophosphatemic rickets (Wang et al., 2012). Therefore, both phosphorylation and glycosylation of FGF23 are physiologically essential processes, and it is currently thought that the balance between phosphorylation and glycosylation determines the relative amounts of intact and cleaved FGF23 secreted by osteoblasts and osteocytes (Tagliabracci et al., 2014). Collectively, the recent findings in this area underscore that better insight into the regulation of posttranslational processing of FGF23 is of crucial importance for a more complete understanding of FGF23 biology.

High affinity binding of FGF23 to target cells requires a receptor complex consisting of FGF receptors and the transmembrane protein αKlotho (Klotho) (Kurosu et al., 2006; Urakawa et al., 2006; Goetz et al., 2012). FGF receptors are tyrosine kinase receptors, leading to phosphorylation of downstream molecules after activation through ligand binding. There are 4 different FGFRs (FGFR1, 2, 3, and 4), and it is still controversial which FGFRs are responsible for the actions of FGF23 in different cell types. There is very good evidence that FGF23 signals through a FGF receptor-1c/Klotho complex (Urakawa et al., 2006; Goetz et al., 2012), but Klotho may also bind to FGFR3 and 4 (Kurosu et al., 2006).

Klotho is a single pass transmembrane protein that shares sequence homology with family I β-glycosidases (Kuro-o et al., 1997). There is only one mammalian *αKlotho* gene, but there are three isoforms of Klotho protein, namely the transmembrane form, a shed soluble form, and a truncated soluble form produced by alternative splicing of *Klotho* mRNA (Xu and Sun, 2015). The extracellular domain of Klotho, consisting of the two type I β-glycosidase domains KL1 and KL2, can be shed from the cell surface by membrane-anchored proteolytic enzymes, and released into the extracellular fluid and subsequently the blood stream (Imura et al., 2007). In addition, a soluble truncated Klotho protein isoform can be produced by alternative splicing of the *Klotho* mRNA, lacking exons 4 and 5 in mice and KL2 due to a premature stop codon in man, respectively (Matsumura et al., 1998; Shiraki-Iida et al., 1998). Therefore, both the human and the murine soluble truncated Klotho protein isoforms consist of KL1 only. Main sites of Klotho expression are renal proximal and distal tubules, the choroid plexus in the brain, and parathyroid glands (Kuro-o et al., 1997; Shiraki-Iida et al., 1998; Urakawa et al., 2006; Imura et al., 2007; Hu et al., 2010; Andrukhova et al., 2012).

Klotho was originally discovered as an anti-aging factor (Kuro-o et al., 1997). In agreement with this notion, *Klotho* and *Fgf23* deficient mice are characterized by a severe aging-like phenotype associated with runting, premature death, ectopic calcifications, organ atrophy, and osteomalacia (Kuro-o et al., 1997; Shimada et al., 2004b; Sitara et al., 2004). However, ablation of vitamin D signaling, using mice lacking a functioning vitamin D receptor ($VDR^{\Delta/\Delta}$), completely rescues the premature aging phenotype in $Klotho^{-/-}$ and $Fgf23^{-/-}$ mice (Hesse et al., 2007; Anour et al., 2012; Streicher et al., 2012). Notably, $Klotho^{-/-}$ and $Fgf23^{-/-}$ mice produce excessive amounts of $1,25(OH)_2D$ due to the lacking suppressive effect of Fgf23 on renal 1α-hydroxylase activity. The anti-aging function of Klotho was initially thought to be based on an inhibitory role of soluble Klotho for insulin signaling (Kurosu et al., 2005). Indeed, $Klotho^{-/-}$ and $Fgf23^{-/-}$ mice are characterized by increased peripheral insulin sensitivity (Kurosu et al., 2005; Hesse et al., 2007), and the phenotype of $Klotho^{-/-}$ mice can be partially rescued by insulin receptor substrate-1 (IRS1) haploinsufficiency (Kurosu et al., 2005). However, it was later shown that lack of *Klotho* does not alter glucose homeostasis in $Klotho^{-/-}/VDR^{\Delta/\Delta}$ compound mutant mice (Anour et al., 2012), indicating that the enhanced insulin sensitivity in $Klotho^{-/-}$ mice is secondary to disturbed mineral