**Translational Nephrology**

Klotho spins the thread of life—what does Klotho do to the receptors of fibroblast growth factor-23 (FGF23)?*

Tilman B. Drüeke\(^1\) and Dominique Prié\(^2\)

\(^1\)Inserm Unité 845 and Service de Néphrologie and \(^2\)Service de Physiologie-Explorations Fonctionnelles and Inserm Unité 845, Hôpital Necker, Assistance Publique-Hôpitaux de Paris and Faculté de Médecine René Descartes Paris 5, Paris, France

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Klotho is one of the three goddesses of the Moirae, who in Greek mythology control the life and destiny of everyone. She is the goddess who helps life to unfold, in contrast to the (apoptotic) goddess Atropos, who cuts the thread of life.

Prolonging lifespan is probably the most important role of the ageing-suppressor gene *Klotho*, named after the Greek goddess. *Klotho* was identified 10 years ago, by the Japanese group of Kuro-o et al. [1]. They reported that a defect in *Klotho* gene expression in the mouse resulted in a syndrome that resembled human ageing, including a short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema. The gene encoded a membrane protein that shared sequence similarity with the beta-glucosidase enzymes. This led them to conclude that the protein might function as part of a signalling pathway involved in the regulation of ageing and related diseases. One year later, the same group isolated the human homologue of the *Klotho* gene and determined its structure [2].

Why is Klotho of particular interest for nephrology?

First of all, Klotho is involved in the renal control of calcium, phosphate and vitamin D metabolism. It suppresses phosphate re-absorption in renal proximal tubule, by directly binding to FGF receptors [3]. It regulates Ca\(^{2+}\) re-absorption in the distal convoluted tubule by stabilizing the TRPV5 Ca\(^{2+}\) channel in the plasma membrane [4]. It inhibits renal 1-alpha 25 hydroxylase activity and thereby decreases circulating calcitriol levels [5]. It therefore appears to synergize with the renal tubular effects of parathyroid hormone (PTH) on Ca\(^{2+}\) and phosphate transport, whereas it antagonizes the stimulatory effect of PTH on calcitriol synthesis by the kidney (Figure 1).

Second, *Klotho*-deficient mice and fibroblast-growth factor-23 (FGF23)-deficient mice have an identical phenotype including hyperphosphataemia, hypercalcaemia, elevated plasma calcitriol and vascular calcification, in addition to premature ageing [1,6,7]. In contrast, over-expression of the *Klotho* gene extends the lifespan and increases resistance to oxidative stress [8,9]. These observations were highly suggestive of a close cooperation between Klotho and FGF23 and/or its receptor(s). Others have shown that FGF23 binds to multiple FGF receptors (FGFRs) [10]. Table 1 provides a summary of the presently known role of Klotho in numerous systemic and organ functions and of the biochemical expression of, respectively, its absence and its overfunction in animal models and in man.

Of note, Klotho was the topic of a Translational Nephrology article in a recent issue of *NDT*, with particular focus on its role in the control of calcium homeostasis [11].

**Regulation of FGF23 signalling by Klotho**

The common phenotypes of *Klotho* and FGF23 over-expression and deletion, respectively, led to the postulate of a common signal transduction pathway. Kurosu et al. [3] showed that Klotho protein directly bound to multiple FGFRs. The Klotho–FGFR complex bound to FGF23 with higher affinity than FGFR or Klotho alone. In addition, Klotho significantly enhanced the ability of FGF23 to induce phosphorylation of FGF receptor substrate and ERK in various types of cells. Very recently, Urakawa et al. [12] made an important further contribution to our understanding of this interaction. They reasoned that the

renotropic activity of circulating FGF23 was compatible with the presence of a unique, FGF23-specific receptor in the kidney. In order to prove their hypothesis, they chose different experimental approaches \textit{in vitro} and \textit{in vivo}.

Conversion by Klotho of canonical FGF receptor into FGF23-specific receptor

Over-expression of FGF23 in animals and in humans does not reproduce the effects of other FGF molecules, suggesting the existence of a specific FGF receptor (FGFR) for FGF23. Although 22 different FGF molecules have been identified to date, only four genes encode FGFRs with a few additional isoforms by alternative RNA splicing. This suggests that co-receptors could modulate FGFR affinity for the various FGFs. Several lines of evidence support the role of Klotho as one of these co-receptors.

First, Urakawa \textit{et al.} [12] forced the expression of Klotho respectively in ovary cells and renal cells, which are normally devoid of Klotho. This enabled high affinity binding of FGF23 to the cell surface and made the cells responsive to FGF23 treatment, as demonstrated by the activation of FGF23-dependent post-receptor events, such as the phosphorylation of extracellular signal-regulated kinase (ERK) protein and enhanced mRNA and protein expression of the gene early growth-responsive 1 (Egr-1).

Second, the serum concentration of FGF23 in Klotho-deficient mice was 2000 times higher than in wild-type mice, and they did not respond to pharmacological doses of exogenous FGF23.

Third, an anti-Klotho monoclonal antibody specifically antagonized FGF23 effects \textit{in vitro}, in Klotho-expressing cells, and \textit{in vivo} in mice, despite an increase in circulating FGF23 concentration. Although Kurosu \textit{et al.} recently showed, by immunoprecipitation experiments, that Klotho can bind to various FGFRs [3], the results by Urokawa \textit{et al.} suggest that Klotho specifically interacts with the FGFR1(IIIc) subtype. Coexpression of Klotho with FGFR1(IIIc) in various cell lines specifically enabled FGF23 signalling. Furthermore the addition of heparin stabilized the FGF23-FGFR1(IIIc)-Klotho complex but did not substitute for Klotho. Figure 2 provides a schematic view of the interaction between FGF23, Klotho and its receptor.

Table 1. Multiple functions of Klotho in health and disease

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<tr>
<th>Klotho</th>
<th>Systemic effects</th>
<th>Biochemical effects</th>
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<tr>
<td></td>
<td>Ageing</td>
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<td>Deletion</td>
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<td>Over-expression</td>
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<td>Association of human polymorphisms</td>
<td>Life span</td>
<td>Coronary artery disease</td>
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Fig. 1. Effect of FGF23 + Klotho on renal tubular re-absorption of inorganic phosphate (iP) and synthesis of 1,25 dihydroxy vitamin D (calcitriol). FGF23 + Klotho act synergistically with parathyroid hormone (PTH) to reduce tubular iP re-absorption. However, FGF23 + Klotho inhibit tubular calcitriol synthesis, in contrast to PTH which stimulates it. In end-stage renal disease (ESRD), the physiological inhibition of tubular iP re-absorption by both PTH and FGF23 + Klotho becomes ineffective. The concomitant increase in PTH secretion leads to excessive iP release from bone into the extracellular space. The clinical consequence is hyperphosphataemia.

Fig. 2. Schematic view of the interaction between FGF23, Klotho and their receptor, FGFR1(IIIc).
Conclusion

The mystery of the synergy between Klotho, FGF23 and its receptor has been progressively unravelled. The recent demonstration, that Klotho transforms one of the canonical receptors for various FGFs, namely FGFR1(IIIc), into a specific receptor for FGF23, is a major breakthrough. It is still uncertain, however, whether this effect of Klotho is strictly limited to this FGFR subtype. The unique biological activity of FGF23 in the kidney appears to depend on the limited local availability of Klotho. However, in the kidney, FGF23 effects are mainly observed in the proximal tubule, while Klotho expression is limited to the distal tubule.

From a more general point of view, the discovery of the conversion by Klotho of a non-specific to a specific FGF receptor may apply to other FGF-FGF receptor systems. As pointed out by Urakawa et al. [12], this form of conversion may represent a new type of receptor modulation and illustrates the potential diversity of biological responses in the presence of similar, and apparently redundant ligand and receptor subtypes.

Conflict of interest statement. None declared.

References


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