No. 1138

Regulation of Sodium Dependent Phosphate Cotransporters by Klotho, an Antiaging Factor
Control of Renal Phosphate Reabsorption

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Summary

The *Klotho* gene was identified from a mouse strain in which *Klotho* mutant (kl/kl) mice present senescence manifestation such as growth retardation, short lifespan, osteoporosis, ectopic calcification because of autosomal recessive inheritance. In addition, the serum phosphate, calcium and 1,25$(OH)_2$ vitamin D_3 levels are significantly increased in kl/kl mice relative to that of wild type mice. Overexpression of Klotho extends lifespan in mice and, the serum phosphate level is significantly lower relative to that of wild-type mice. Thus, it is guessed that Klotho is an important factor of phosphate homeostasis and these mineral abnormalities cause senescence manifestation of kl/kl mice.

Fibroblast growth factor 23 (FGF23) acts on kidney to induce phosphaturia by suppressing NaPi-IIa and NaPi-IIc and to reduce serum 1,25$(OH)_2$ vitamin D_3, which in tern reduces phosphate absorption from intestine. FGF23 acting through FGF receptor (FGFR), and FGF23 require transmembrane form of Klotho for binding to FGFR because of any known FGFR had extremely low affinity to FGF23. Thus, transmembrane form of Klotho function as an obligatory co-receptor for FGF23. In addition, a cross-talk between the distal and proximal tubules was postulated for FGF23-induced phosphaturia based on the original notion that Klotho is exclusively expressed in the distal convoluted tubule and Pi reabsorption and regulation solely resides in the proximal tubule.

On the other hand, the extracellular domain of transmembrane form of Klotho (soluble Klotho) is cleavage from membrane and which is detected in blood, urine and cerebrospinal fluid and may function as an endocrine and/or paracrine hormone. The soluble Klotho protein cannot function as a coreceptor for FGF23 because Klotho protein alone cannot bind to FGF23 with high affinity, suggesting that soluble Klotho may have function independent of FGF23. Soluble Klotho has amino acid sequence homology to family 1 glycosidase.

NaPi-IIc is principal phosphate transporter in kidney and plays an important role in renal phosphate handling and may be a key determinant of plasma phosphate level in humans. NaPi-IIc may be an important potential target molecule of soluble Klotho, because NaPi-IIc is a membrane glycoprotein and plays an important role in phosphate homeostasis, but effect of soluble Klotho for NaPi-IIc has not clarified. To reveal the relationship between phosphate homeostasis and Klotho, it is necessary to clarify the effect of soluble Klotho for NaPi-IIc.

In the present study, we investigated the role of *N*-glycan chain of NaPi-IIc and the effect of soluble Klotho

on NaPi-IIc function in OK cells and *Xenopus laevis* oocyte. Based on a secondary structure model of the human and mouse NaPi-IIc, we identified the three consensus *N*-glycosylation sites (Asn-264, Asn-267, and Asn-299) in the putative second extracellular domain. Mutation of *N*-glycosylation sites of NaPi-IIc decreased the protein expression on the apical membrane, and reduced its membrane stability and phosphate transport activity. Next, we investigated the effect of soluble Klotho on the function of NaPi-IIc in Xenopus oocytes. The soluble Klotho elicited a dose-dependent decrease in the NaPi-IIc activity. However, soluble Klotho did not affect the double (2NQ) and triple *N*-glycosylation mutants (3NQ) of NaPi-IIc. In addition, immunohistochemical analysis showed the presence of Klotho protein in the basolateral membrane of proximal tubular epithelial cells induced by the feeding of a low Pi diet. After feeding of a high Pi diet, we found the elevation of soluble Klotho levels in the urine. These data suggest that the appearance of Klotho in the proximal lumen can be due to cleavage and /or secretion by the proximal tubule, or possibly by transcytosis of plasma- or distal tubule-derived Klotho. Finally, the present study indicates that soluble Klotho functions from the outside of cell as a humoral factor and by modifying *N*-glycan chain of NaPi-IIc at the cell surface.