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Klotho: An antiaging protein involved in mineral and vitamin D metabolism

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Klotho gene mutation leads to a syndrome strangely resembling chronic kidney disease patients undergoing dialysis with multiple accelerated age-related disorders, including hypoactivity, sterility, skin thinning, muscle atrophy, osteoporosis, vascular calcifications, soft-tissue calcifications, defective hearing, thymus atrophy, pulmonary emphysema, ataxia, and abnormalities of the pituitary gland, as well as hypoglycemia, hyperphosphatemia, and paradoxically high-plasma calcitriol levels. Conversely, mice overexpressing klotho show an extended existence and a slow aging process through a mechanism that may involve the induction of a state of insulin and oxidant stress resistance. Two molecules are produced by the klotho gene, a membrane bound form and a circulating form. However, their precise biological roles and molecular functions have been only partly deciphered. Klotho can act as a circulating factor or hormone, which binds to a not yet identified high-affinity receptor and inhibits the intracellular insulin/insulin-like growth factor-1 (IGF-1) signaling cascade; klotho can function as a novel β-glucuronidase, which deglycosylates steroid β-glucuronides and the calcium channel transient receptor potential vallinoid-5 (TRPV5); as a cofactor essential for the stimulation of fibroblast growth factor (FGF) receptor by FGF23. The two last functions have propelled klotho to the group of key factors regulating mineral and vitamin D metabolism, and have also stimulated the interest of the nephrology community. The purpose of this review is to provide a nephrology-oriented overview of klotho and its potential implications in normal and altered renal function states.

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In Greek mythology, the duration of life is controlled by the three daughters of Zeus and Themis: Klotho (Clotho) who combs and spins the thread of life, Lachesis who determines the length of life by measuring the threads length, and Athropos who cuts the string causing a life to end. In science, a Japanese group, which was exploring aging mechanisms, conferred the name of Klotho to a gene that they fortuitously discovered in 1997.[1]

Indeed, the klotho gene was disrupted or mutated in its 5′-flanking promoter region by the random insertion of an exogenously introduced nonfunctional gene, the rabbit Na/H exchanger under the control of the human elongation factor promoter.[1] The coding region of this mutated klotho gene was still preserved, but its expression was markedly reduced generating a mouse strain with a strong hypomorphic allele. Homozygous mice, for this hypomorphic allele, showed shortened lifespan and a syndrome strangely resembling chronic kidney disease patients undergoing dialysis with multiple accelerated age-related disorders, including hypoactivity, sterility, skin thinning, muscle atrophy, osteoporosis, vascular calcifications, soft tissue calcifications, defective hearing, thymus atrophy, pulmonary emphysema, ataxia, and abnormalities of the pituitary gland, as well as hypoglycemia, hyperphosphatemia, and paradoxically high-plasma calcitriol levels.

Conversely, mice overexpressing klotho showed an extended existence and a slow aging process through a mechanism that may involve the induction of a state of insulin and oxidant stress resistance.[2] Moreover, several single-nucleotide polymorphisms in the human klotho gene have been found to be associated with lifespan, osteoporosis, stroke, and coronary artery diseases. All these observations support the suggestion that klotho plays an important role in aging and senescence-related disorders.

Nephrologists have recently been extremely interested by the possible physiological functions of klotho because of its predominant renal expression, its colocalization together with the epithelial calcium channel transient receptor potential vallinoid-5 (TRPV5) in kidney distal tubular cells,[3] and its interaction with fibroblast growth factor-23 (FGF23).[4] The purpose of this review is to provide a nephrology-oriented overview of klotho and its potential implications in normal and altered renal function states.

MOLECULAR CHARACTERISTICS OF KLOTHO: GENE, mRNA, AND PROTEIN

The human klotho gene is a 5-exon gene located on chromosome 13q12 within a region longer than 50 kb. Its promoter region lacks a TATA-box consensus sequence and contains four potential binding sites for SP1.[5] Two transcripts arise from this single gene; one full-length transcript of 5.2 kb encoding a 1012-aminoacid (130 kDa), single-pass, membrane protein. This membrane form can be released into the circulation after losing its short transmembrane domain and slightly lowering its molecular weight. Moreover, it is possible that the secreted form is ultimately metabolized to a smaller size protein of 65–70 kDa. The other transcript derived from an alternative mRNA splicing, encodes the N-terminal half of klotho, a protein of 549 amino acids with a molecular weight of approximately 65–70 kDa.[5–7]

On the basis of their predicted structures, both proteins belong to the β-glycosidase family. The expression of the secreted form predominates over that of the membrane form. The human protein shows 86% of amino-acid identity with the mouse klotho protein. The extracellular domain of klotho is composed of two internal repeats (KL1, KL2), each one of approximately 450 amino acids long with a similarity of 21% to each other. These two domains form a butterfly-shaped molecule on the surface of the cellular membrane. They share 20–40% sequence identity with the β-glucosidase of both bacteria and plants and with mammalian lactase glycosylceramidase.[6,7] Another speciality of klotho proteins is that the secreted form and the membrane form develop oligomeric complexes, suggesting a post-translation klotho processing and possible regulatory mechanisms for klotho secretion *in vivo*.

The tissue distribution of klotho mRNAs expression reveals that it is expressed, in descending order, in kidney, brain, reproductive organs, pituitary gland, parathyroid glands, urinary bladder, skeletal muscle, placenta, thyroid gland, and colon.[1] In the kidney, klotho mRNAs and proteins are localized in the distal tubular cells. In these cells, klotho is diffusely expressed in the cytoplasm and not at the apical side.[8] It is colocalized with other proteins involved in tubular calcium reabsorption such as the epithelial calcium channel TRPV5 and calbindin 28 K,[3,8] suggesting that klotho is implicated in renal calcium homeostasis (Figures 1 and 2).

In brain, klotho is expressed at the apical plasma membrane of ependymal cells in the choroids plexus of both the lateral ventricles and the third ventricle. Klotho protein is also expressed in the stria vascularis and spiral ligament of the inner ear probably serving as a modulator of ion transport as in the renal distal tubular cells.[9] In the heart, klotho expression is recognized exclusively in the sinoatrial node region, where it plays an essential role in sinoatrial node function as a dependable pacemaker under conditions of stress.[10] In reproductive organs, in the testis, klotho is expressed in the inner layers of seminiferous tubules containing elongating spermatids or mature germ cells. It is absent in spermatogonia, primary spermatocytes, rounds spermatids, and Sertoli cells. In the ovary, klotho is expressed exclusively in the most mature follicles; it is absent or weakly expressed in primary and secondary follicles.[8]

MODE OF ACTION OF KLOTHO

Nine years after its identification, the exact biological role and molecular function of klotho have been only partly deciphered. For instance, first, klotho can act as a glycosidase because of its high similarity with other members of the glycosidase family. However, this has been questioned because klotho lacks glutamic acid residues that are responsible for the catalytic activity of this enzyme family. Nonetheless, recent results obtained in *in vitro* experiments support the glycosidase activity of klotho; when a purified chimeric klotho-human IgG1 Fc protein is incubated in the presence of a series of 4-methylumbelliferyl β-glycosides serving as putative substrates, an enzymatic activity of klotho is demonstrated only with the 4-methylumbelliferyl β-D-glucuronide.[6] This enzymatic activity of klotho-human IgG1 Fc protein is reduced by the addition of specific inhibitors of β-glucuronidase. Furthermore, naturally occurring β-glucuronides such as β-estradiol 3-β-D-glucuronide, strone 3-β-D-glucuronide, and estriol 3-β-D-glucuronide are also hydrolyzed by klotho-human IgG1 Fc protein.[6] In addition, klotho hydrolyses sugar residues on TRPV5, avoiding its retrieval from the cell surface. Interestingly, this stimulatory effect of klotho can be entirely mimicked by a purified bovine β-glucuronidase and blocked by the D-saccharic acid 1,4-lactone, a klotho inhibitor.[3] Collectively, these data strongly suggest that klotho functions as a novel β-glucuronidase, and steroid β-glucuronides and calcium channels TRPV5 are potential candidates for klotho actions.

Second, klotho can act as a circulating factor and this is supported by the fact that klotho protein, probably resulting from the secretion of the membrane form, is detectable in urine, serum, and cerebrospinal fluid.[3] This protein binds to a high-affinity but yet not identified cell-surface klotho receptor and activates the protein kinase C (PKC) pathway in kidney and testicular cells; klotho also stimulates cAMP pathway in several cell types.[11] The activation of this receptor by klotho leads to the suppression of tyrosine phosphorylation of insulin/insulin-like growth factor (IGF-1) receptors and insulin receptor substrates, association of insulin receptor substrates with phosphatidylinositol 3-kinase, and serine phosphorylation of Akt/PKB.[2] Therefore, klotho protein is a circulating factor that inhibits the intracellular insulin/IGF-1 signaling cascade. This activity probably contributes to the antiaging effects of klotho, because inhibition of insulin-like signaling is an evolutionarily conserved mechanism for extending lifespan.[2,12]

Third, klotho can act as a coreceptor or a cofactor for other proteins such as FGF23. It has been recently demonstrated that klotho directly binds to multiple FGF receptors (FGFRs) and that the klotho/FGFR complex binds to FGF23 with higher affinity than FGFR or klotho alone. Furthermore, klotho enhanced significantly the ability of FGF23 to induce