Regulation of ion channels by secreted Klotho: mechanisms and implications

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Klotho is an anti-aging protein predominantly expressed in the kidney, parathyroid glands, and choroid plexus of the brain. It is a single-pass transmembrane protein with a large extracellular domain. The extracellular domain of Klotho is cleaved and released into extracellular fluid, including blood, urine, and cerebrospinal fluid. The membrane-bound full-length Klotho and secreted extracellular domain of Klotho have distinct functions. Membrane Klotho interacts with fibroblast growth factor (FGF) receptors to form high-affinity receptors for FGF23. Secreted Klotho functions as a humoral factor that regulates several ion channels and transporters, and other processes, including insulin and insulin-like growth factor signaling. This mini-review focuses on the mechanisms of regulation of cell-surface abundance of ion channels by secreted Klotho and the importance of these effects of Klotho in physiology and pathological conditions.

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Klotho is type-1 transmembrane protein predominantly expressed in the kidney, parathyroid glands, and the epithelial cells of choroid plexus of the brain.[1] Mice with disruption in the *Klotho* gene display multiple phenotypes closely resembling human aging, including shortened lifespan, muscle and skin atrophy, pulmonary emphysema, osteopenia, infertility, hyperphosphatemia, and vascular and soft tissue calcification.[1] In support of its function in aging suppression, overexpression of Klotho in mice extends their lifespan.[2]

The *Klotho* gene, in mice and humans as well, contains 5 exons and encodes a single-pass transmembrane polypeptide of 1012 amino acids (for human Klotho; 1014 amino acids for mouse Klotho).[1] The majority of amino acids in the Klotho peptide ($\sim$980 residues) reside in the amino-terminal extracellular domain, which is followed by 21 amino acids membrane-spanning domain, and a 11 amino acid short intracellular carboxyl terminus (Figure 1a).[1] The extracellular domain consists of two internal repeat sequences of 440 amino acids, named KL1 and KL2, respectively.[1,3] The linker region between the two internal repeats contains a stretch of four basic amino acids (Lys-Lys-Arg-Lys) that forms a potential site for proteolytic cleavage. A secreted form of Klotho consisting of the full-length extracellular domain is detected in the blood, urine, and cerebrospinal fluid (Figure 1b).[2,4] Recently, Chen *et al.*[5] reported that the extracellular domain is cleaved by the metalloproteases ADAM10 and ADAM17 and that insulin stimulates the cleavage of Klotho.

Both human and mouse Klotho gene are alternatively spliced.[6] Besides the transcript that encodes the full-length Klotho of 1012-1014 amino acids, an alternatively spliced form of Klotho mRNA encoding the KL1 repeat of Klotho is detected in humans and mice.[6] Imura *et al.*[4] reported the existence of the entire extracellular domain of Klotho, but not KL1 or KL2 fragment, in serum and cerebrospinal fluid. KL1 and KL2 fragments, however, can be detected in the mouse and human urine (Kuro-o M, personal communication). The existence of KL1 and KL2 fragments in the urine is likely a result of cleavage of the full-length extracellular domain at the linker region by proteases. However, the possibility that the alternatively spliced KL1 transcript produces a peptide, which is secreted into urine, cannot be excluded.

The KL1 and KL2 repeats each share amino-acid sequence homology to family 1 glycosidases.[1,3] Members of family 1 glycosidases contain two highly conserved glutamate residues

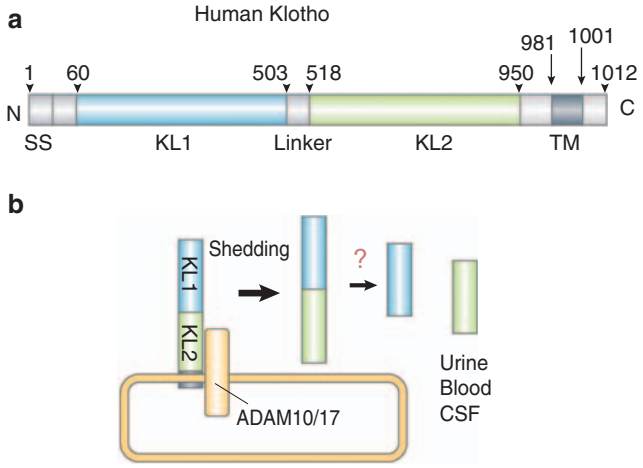

Figure 1 | Domain structure and topology of Klotho. (**a**) Domain structure of human Klotho. Not drawn to scale. SS, KL1, KL2, TM indicate signal sequence, first and second Klotho repeat, and transmembrane domain, respectively. (**b**) Membrane topology of Klotho. The entire extracellular domain consisting of KL1 and KL2 repeats is cleaved by ADAM10 or ADAM17 and released into urine, blood, or cerebrospinal fluid (CSF). In the urine, KL1 and KL2 are also detected, likely due to cleavage at the linker region by yet unspecified protease(s). See text for details.

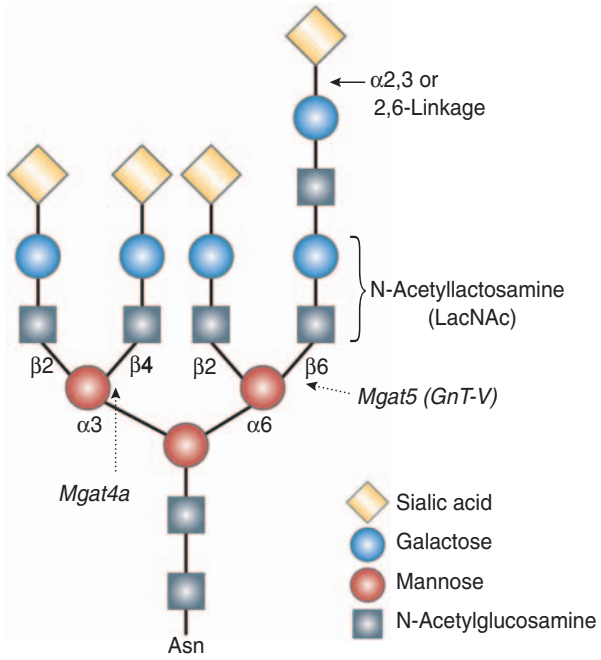

Figure 2 | Structure of the typical complex type tetra-antennary N-glycans. See text for details. *N*-acetyllactosamine (LacNAc) is the ligand for galectins. Multiple LacNAc repeats may be present in the β6 branch (formed from underlying α6-mannose), thereby increasing the affinity of N-glycans for galectins. *Mgat5* (also known as *GnT-V*), β1,6 *N*-acetylglucosaminyltransferase V, is responsible for synthesis of the β6 *N*-acetylglucosamine linkage on the α6-mannose; *Mgat4a*, β3,4 *N*-acetylglucosaminyltransferase IVa, is responsible for synthesis of the β4 *N*-acetylglucosamine linkage on the α3-mannose. Altered expression of these enzymes changes the number of branches of N-glycans and the number of LacNAc they carry, thus affecting binding of glycoprotein to galectin lattice on the cell surface. See text for specific examples of conditions/diseases that alter the expression of these enzymes.

critical for the enzymatic activity.[3,7] One of these acts as a nucleophile, the other as an acid-base catalyst. In Klotho, the two glutamate residues, however, are replaced by an asparagine at the acid-base catalyst position in the KL1 repeat and an alanine or a serine at the nucleophile position in the KL2 repeat, respectively.

Since the recognition of homology to family 1 glycosidases, much effort has been devoted to investigate whether Klotho has enzymatic glycosidase activity. Tohyama *et al.*[8] first showed that secreted Klotho exhibits weak enzymatic activity toward β-glucuronic acids (hydrolyzing glycosidic bond at 73 pmol/h/μg for Klotho vs 1940 pmol/h/μg for purified liver β-glucuronidase). They suggested that secreted Klotho is a novel β-glucuronidase that catalyzes glucuronylation of circulating steroids or related substances to increase their water solubility, thus enhancing their clearance by the kidney. Recent reports suggest that secreted Klotho also exhibits a sialidase activity and by which regulates cell-surface expression of TRPV5 and ROMK channels (see below).[9,10]

REGULATION OF TRPV5 CHANNEL BY SECRETED KLOTHO

Secreted Klotho increases cell-surface abundance of TRPV5 by removing terminal sialic acids from its N-glycan

The regulation of the epithelial Ca^{2+} channel TRPV5 by secreted Klotho was first reported by Chang *et al.*[11] They showed that treatment with secreted Klotho increases cell-surface abundance of TRPV5 expressed in human embryonic kidney cells and that mutation of a single asparagine for N-glycosylation prevents the effect of Klotho. This study provides the first evidence that secreted Klotho regulates ion channels by modifying N-glycans. On the basis of the finding that purified bovine liver β-glucuronidase also regulates TRPV5, Chang *et al.*[11] suggested that the action of Klotho is through hydrolysis of glucuronic acids from N-glycans of the channel. Similarly, TRPV6, a channel closely related to TRPV5 and may co-assemble with it in the distal renal tubules, is regulated by secreted Klotho.[12] Although glucuronic acids may be sugar moieties of cell-membrane protein in the form of heparin sulfate proteoglycans,[13] they are extremely uncommon moieties of N-glycan chains of membrane glycoproteins such as TRPV5 channels.[14,15]

Recently, Cha *et al.*[9] investigated the sugar substrates of the N-glycans of TRPV5 for Klotho and provided compelling evidence that secreted Klotho regulates TRPV5 by a sialidase, rather than glucuronidase activity. As shown in Figure 2, the complex type N-glycan chains of glycoproteins such as TRPV5 are comprised of up to four branches.[14,15] Each branch is initiated by the attachment of *N*-acetylglucosamine to underlying α3 or α6 mannose by β2, β4, or β6-linkage, followed by the addition of galactose to form the disaccharide galactose-*N*-acetylglucosamine or *N*-acetyllactosame (LacNAc). Galactoses may be the terminal residues or they can be