

Biological Role of Anti-aging Protein Klotho

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Klotho-deficient mice have accelerated aging phenotypes, whereas overexpression of Klotho in mice extends lifespan. Klotho is an anti-aging single-pass membrane protein predominantly produced in the kidney, with shedding of the amino-terminal extracellular domain into the systemic circulation. Circulating levels of soluble Klotho decrease with age, and the *klotho* gene is associated with increased risk of age-related diseases. The three forms of Klotho protein have distinct functions. Membrane Klotho forms a complex with fibroblast growth factor (FGF) receptors, functions as an obligatory co-receptor for FGF23, which is involved in aging and the development of chronic diseases via regulation of P_i and vitamin D metabolism. Secreted Klotho functions as a humoral factor with pleiotropic activities including regulation of oxidative stress, growth factor signaling, and ion homeostasis. Secreted Klotho is also involved in organ protection. The intracellular form of Klotho suppresses inflammation-mediated cellular senescence and mineral metabolism. Herein we provide a brief overview of the structure and function and recent research about Klotho.

Key Words: Klotho, Aging, Age-related diseases

INTRODUCTION

All living organisms age and die. In ancient Greek mythology the Moirai or Three Fates, Klotho (or Clotho), Lachesis and Atropos, were the daughters of Zeus and Themis and determined the duration of life. It was believed that Klotho spins the thread of life, Lachesis determines its length and Atropos cuts it. The *klotho* gene (symbol, *kl*) is named after the Moirai who spins the thread of life [1].

The *klotho* gene was originally identified as being mutated in a mouse strain in which inherited phenotypes closely resemble human aging [1]. Mice homozygous for a hypomorphic *klotho* allele (*kl/kl*) displayed multiple aging-like

phenotypes including growth retardation, vascular calcification and osteoporosis and died prematurely at around two to three months of age [1]. Conversely, overexpression of the *klotho* gene extends the life span in mice, which supports the notion that *klotho* is an aging-suppressor gene [2]. Since the discovery of Klotho, two related paralogs, β Klotho and γ Klotho (or Lct1 or KLPH), have been identified as Klotho family members [3,4]. Klotho is also called α Klotho in order to distinguish it from the other two members [5]. In this review, Klotho is simply used to refer to α Klotho. This review introduces the structure and function of Klotho and summarizes the current knowledge of Klotho as it relates to human aging and disease.

STRUCTURE AND EXPRESSION OF KLOTHO

The *klotho* gene is composed of five exons [6,7] and encodes a type 1 single-pass transmembrane glycoprotein (1014 and 1012 amino acids in mouse and human, respectively)

Received: December 17, 2014, Accepted: February 16, 2015

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that is located at the plasma membrane [1,7] and Golgi apparatus [8]. The intracellular domain is very short (~10 amino acids) without functional domains. The extracellular domain has two internal repeats, KL1 and KL2, which have amino-acid sequence homology to family 1 glycosidases that hydrolyze β -glycosidic linkage in saccharides, glycoproteins and glycolipids [1,9,10]. The linker region between two internal repeats contains four basic amino acids (Lys-Lys-Arg-Lys) that form a potential site for proteolytic cleavage [11,12]. Despite the sequence homology to glycosidase, glycosidase enzymatic activity is not detectable in recombinant Klotho protein [1,10] probably because critical amino acid residues in putative active centers of the Klotho protein diverge from those of β -glycosidase enzymes [1,9,10]. Indeed, Klotho exhibits weak β -glucuronidase activity *in vitro* [10] and elicits biological effects through its β -glucuronidase and/or sialidase activity [13-15].

The extracellular domain of Klotho can be cleaved by membrane proteases such as ADAM10 and ADAM17 (ADAM metalloproteinase domain 10 and 17) and released into blood, urine and cerebrospinal fluid [11,12]. Cleaved Klotho functions as an endocrine, autocrine and paracrine hormone on target cells [1,2,8]. In addition, secreted Klotho is generated through alternative transcriptional termination of the *klotho* gene lacking exons 4 and 5 in mice [7]. Secreted Klotho is detected in the blood, urine and cerebrospinal fluid [2,16].

Klotho is expressed in multiple tissues and cell types and at particularly high levels in the kidney. Klotho is abundantly expressed in the distal convoluted tubule in the kidney and choroid plexus in the brain [1]. It is also expressed in the renal proximal tubule [17], parathyroid gland [7,18,19] and several sex organs including the ovary, testis and placenta [1]. Recently, Klotho was found to be locally expressed in the adventitial area of the aorta, supporting the vascular protective effect of the Klotho protein [20]. The list of tissue-specific expression of Klotho is currently being updated.

Other Klotho family members, β Klotho and γ Klotho, are also type 1 single-pass transmembrane proteins [21]. β Klotho is composed of a β -glycosidase-like domain (KL1 and 2 domains) and shares 42% amino acid sequence homology with Klotho [4,22]. β Klotho is expressed mostly in the

liver, followed by the gastrointestinal tract, spleen and kidney [22]. γ Klotho, a shorter type 1 single-pass transmembrane protein, is made of a family 1 glycosidase-like extracellular domain (KL1 domain) and a short intracellular domain [3]. γ Klotho is highly expressed in the kidney and skin [3]. Recently, it has been reported that γ Klotho is undetectable in skin, but is abundantly expressed in the eye [23]. Klotho is present in secreted (or soluble) form; however, there has only been one report about the soluble form of β and γ Klotho until now [21].

FUNCTION OF KLOTHO

1. Membrane bound Klotho

Three Klothos form a constitutive obligatory receptor complex with fibroblast growth factor receptors (FGFRs), thereby providing the selective binding affinity of FGFRs to endocrine FGFs. The endocrine FGF family is also comprised of three members, FGF15 (the mouse ortholog of human FGF19), FGF21 and FGF23. Classic FGFs elicit their biological activity in an autocrine and/or paracrine manner [23]. Endocrine FGFs lack heparin-binding domain function as a humoral factor.

Klotho forms complexes with diverse FGFRs (FGFR1c, FGFR3c and FGFR4) and increases their affinity selectively to FGF23, a bone-derived phosphaturic hormone. FGF23 acting on the Klotho-FGFRs complex plays an important role in Ca^{2+} and phosphate homeostasis [18,24]. Klotho converts canonical FGFRs into specific receptors for FGF23 [18,24]. FGF23 not only inhibits inorganic phosphate (P_i) reuptake in the renal proximal tubules by inhibiting NaPi-IIa, but also downregulates 1α -hydroxylase (CYP27B1) expression. 1α -hydroxylase is a key enzyme for the synthesis of biologically active 1,25-dihydroxyvitamin D_3 (calcitriol), which stimulates P_i absorption in the gut. Membrane-bound Klotho is involved in FGF23 action, thereby promoting P_i excretion followed by low serum P_i . In addition, FGF23 acting on the Klotho-FGFRs complex at the basolateral side stimulates renal Ca^{2+} reabsorption via the TRPV5 channel, which is expressed in the apical membrane of the distal convoluted tubule. The Klotho-FGFRs complex activates signaling cascades involving Erk1/2, SGK-1 and WNK4 for TRPV5-mediated Ca^{2+} reabsorption [25]. Thus, membrane-bound Klotho func-

tions as an obligatory co-receptor for FGF23 and regulates P_i and Ca^{2+} homeostasis.

β Klotho contributes to the regulation of energy metabolism as an obligatory co-receptor for FGF15 (the mouse ortholog of human FGF19) and FGF21 [24,26]. Expression of FGF15/19 in the intestine is regulated by bile acid [27]. This intestine-liver endocrine axis mediated by FGF19 and β Klotho is indispensable for maintaining bile acid homeostasis, as evidence by the fact that mice lacking FGF15, β Klotho or FGFR4 exhibit increased *Cyp7a1* expression and bile acid synthesis in the liver [27-29]. By contrast, FGF21 is secreted from the liver upon fasting and acts on adipose tissue to promote lipolysis [30]. Thus, β Klotho is required to regulate energy metabolism in the fasting state.

γ Klotho forms complexes with FGFR1b, FGFR1c, FGFR2c and FGFR4 that increase FGF19 activity [23]. γ Klotho is highly and selectively expressed in brown adipose tissue and the eye and can function as an additional co-receptor for FGF19 in cultured cells [23]. However, the biological function of γ Klotho remains largely elusive.

2. Intracellular Klotho

Although Klotho is present on the cell surface, large amounts of Klotho immunoreactivity are detectable in the cytoplasm in mouse kidneys and human parathyroid glands [8]. In these tissues, Klotho binds Na^+K^+ -ATPase and stimulates its surface abundance and activity. Klotho interacts physically with Na^+K^+ -ATPase in intracellular organelles, not at the plasma membrane. The intracellular negativity and low $[Na^+]_i$ created by Na^+K^+ -ATPase activation provide driving force for transepithelial Ca^{2+} transport in the choroid plexus and the kidney [8].

It is well established that senescence is associated with increased expression of pro-inflammatory cytokines such as IL-6 and IL-8, which is mediated by retinoic-acid-inducible gene-I (RIG-I). Recently, intracellular Klotho, but not secreted Klotho, was shown to bind RIG-I and block its multimerization [31]. Klotho suppresses RIG-I-mediated senescence-associated inflammation, suggesting that Klotho functions as an intracellular anti-inflammatory and anti-aging factor. Although the majority of Klotho immunoreactivity is detectable in the cytoplasm in multiple tissues, the physiological roles of intracellular Klotho are largely

unknown.

3. Secreted Klotho

The secreted (or soluble) form of Klotho functions as a humoral factor that targets multiple tissues and organs independent of FGFRs. Although Klotho functions as a co-receptor for FGF23, secreted Klotho may not function as a soluble receptor for FGF23 [24]. The Klotho-FGFR complex has high affinity for FGF23, but not secreted Klotho or FGFR alone, indicating that secreted Klotho exerts its biological effect independent of FGF23 [24].

Secreted Klotho exerts anti-aging and organ protection effects role with pleiotropic actions. First, secreted Klotho downregulates the signaling of growth factors and cytokines such as insulin, IGF-1, TGF- β and IFN γ [2,32,33]. Overexpression of Klotho extends life by attenuating generation of reactive oxygen species evoked by insulin and IGF-1 signaling [2,34]. Wnt, TNF α and IFN γ signaling are augmented in Klotho-deficient mice, which contribute to accelerated aging [32,35]. TNF α and IFN γ signaling downregulates Klotho, which is an anti-inflammatory protein. Augmented Wnt signaling induces stem and progenitor cell dysfunction and depletion leading to cell senescence. Klotho binds to various Wnt proteins and suppresses the activity of endogenous and exogenous Wnt [35]. Klotho deficiency leads to premature aging and colitis with ion imbalance [32,36]. A recent study demonstrated that Klotho ameliorates renal fibrosis and cancer metastasis by inhibiting TGF- β -induced epithelial-to-mesenchymal transition (EMT) responses [33]. Multiple studies clearly demonstrate that secreted Klotho might function as an anti-aging and organ protection factor by inhibiting signaling of multiple growth factors.

Second, secreted Klotho maintains ion homeostasis by regulating ion channels and/or transporters. Secreted Klotho modifies the *N*-glycan of channels and transporters via its β -glucuronidase and/or sialidase activity [13-15,17,37]. Klotho-deficient mice develop severe defects in the homeostasis of ions such as P_i and Ca^{2+} [1,14]. Secreted as well as membrane-bound Klotho can directly inhibit both renal (NaPi-IIa) and intestinal (NaPi-IIb) phosphate transporters resulting in low plasma phosphate concentration [17,37]. Secreted Klotho reduces the cell surface abundance of

NaPi-IIa through its β -glucuronidase activity independent of FGF23 [17].

Several studies argue that Klotho exhibits β -glucuronidase activity because glucuronic acids are not common moieties of *N*-glycans of mammalian cell surface proteins [14]. The extracellular domain of Klotho is shed into the extracellular fluid where secreted Klotho cleaves terminal sialic acids from the *N*-glycan of TRPV5 and ROMK channels [14,15]. Removal of sialic acids exposes the underlying galactose, a ligand for galactose-binding lectin galectin-1. Binding to extracellular galectin-1 forms a lattice in the extracellular matrix leading to increased cell surface abundance of the channel by inhibition of its endocytosis [14,15]. These findings provide evidence that modification of *N*-glycan increases the residence time of cell surface proteins including growth factor and cytokine receptors. Klotho is involved in the modification of mature *N*-glycans at the cell surface. This action represents a novel mechanism for the regulation of cell surface proteins. New cell surface or soluble glycoproteins and glycolipids modified by Klotho should be examined in future studies.

KLOTHO IN HUMAN AGING AND DISEASES

Klotho is an anti-aging protein with pleiotropic actions that exerts organ protection [1,21]. Several lines of evidence support the notion that Klotho functions as a human aging-suppression molecule. Polymorphisms of *KLOTHO* are correlated with life span [38], coronary artery disease [39], atherosclerosis [39] and osteoporosis [40] in humans. Klotho is also associated with severe calcinosis and stroke [41,42]. Klotho deficiency is involved in acute and chronic kidney diseases [37], cancers [43] and salt-sensitive hypertension [44]. Actually, the serum level of Klotho decreases with aging in humans [45]. However, the biological function of Klotho and the way in which Klotho deficiency contributes to age-related diseases remain elusive.

CONCLUSION

Accumulating evidence indicates that the anti-aging function of Klotho plays an important role in human aging and age-related diseases. Klotho deficiency is strongly associated

with human diseases related to aging such as cancer, chronic kidney disease, ataxia, diabetes and skin atrophy. Klotho is an evolutionarily highly conserved protein related to aging suppression and organ protection. However, the physiological role and regulation mechanism of Klotho have been ill-defined. Studies examining the organ protective and anti-aging effects of the Klotho protein are still needed.

ACKNOWLEDGMENTS

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2010-0024789 to S.-K.C and NRF-2013R1A1A2060764 to I.D.K.).

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