Octa Journal of Biosciences
Octa J. Biosci. Vol. 7(2):113-118

International peer-reviewed journal
July-Dec 2019

Octa Journal of Biosciences
ISSN 2321 – 3663
journal homepage: www.sciencebeingjournal.com

Review Article

Received:12/07/2019 / Revised:23/10/2019 / Accepted:18/11/2019 / Published on-line:30/12/2019

The role of the Klotho protein in the function of aging and neurodegenerative disorders

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ABSTRACT

Klotho, an anti-aging protein (soluble or membrane-bound) plays a prominent role in understanding the aging process. It exerts various protective activities for neurons and multiple organs. Klotho-deficient mice show accelerated aging and Klotho overexpression prolongs lifespan. The altered expression of Klotho produces premature aging syndrome in humans and various neurological dysfunctions such as cognitive deficits. It reduces oxidative stress and cell death by suppressing the insulin-like growth factor–1 signaling pathway, and consist of a neuroprotective effect on hippocampal neurons by preventing amyloid and glutamate toxicity. It usually does this by activating the antioxidant enzyme system. Klotho is a multifunctional protein that controls the metabolism of phosphate, calcium, and vitamin D. For the various functions of Klotho, it became a potential target for the therapeutic approach to eliminate the risky effects of aging. In this review, we have focused on the structure and molecular mechanism of Klotho function with its role in human aging and age-related neurodegenerative disorders.

Keywords:*Klotho; anti-aging protein; structure and expression of Klotho; neurological dysfunctions; neuroprotective activity*

1. INTRODUCTION

Product of an aging suppressor gene: Klotho was identified by Kuro-o et al in 1997 (Kuro et al., 1997). Klotho, the name was adopted from Greek mythology. According to mythology, Clotho, the daughter of Zeus and Themis, weaves the thread of life (Paluszczak et al., 2018). The name of this anti-aging protein comes from the name of the Greek goddess Clotho. As this protein has anti-aging properties, it is the best option to be analyzed in the field of neurobiology. Originally, the Klotho gene was discovered in transgenic mice. A genetic mutation in Klotho increases the rate of aging by decreasing the life expectancy of mice. It affects molecular pathways that are directly related to chronic age-related ailments e.g. kidney disease, diabetic retinopathies, tissue dysfunction, neurodegeneration, deficiencies in mitochondrial function, and deficiencies in muscle regeneration (Smith et al., 2019).

An insertion mutation in the α-Klotho gene of homozygous hypomorphic allelic mice has shown multiple symptoms of premature aging, such as the initial signs of aging, atherosclerosis, osteopenia, skin atrophy, organ atrophy, thymic atrophy, vascular calcification, pulmonary emphysema, hearing impairment, motor neuron degeneration, sarcopenia, hypoglycemia, hyperphosphatemia, gonadal dysplasia, infertility, and an overall short life expectancy. These symptoms are more related to the disorders of human aging (Kim et al., 2015). In contrast, overexpression of Klotho prolongs the lifespan of female mice ~ 19% and male mice ~ 31% longer than in wild-type mice by reducing oxidative stress (Kurosu et al., 2009). In this sense, the Klotho protein acts as a hormone by binding to membrane receptor and inhibiting intracellular signal of insulin-like growth factor–1 (Kurosu et al., 2005). In this way, it exerts an age-suppressing activity in mice. The α-Klotho gene is highly conserved in humans, mice, rats, and Caenorhabditis elegans. The α-Klotho protein exhibits a high level of homology between species. The α-Klotho protein shares 98% of the sequence similarity between humans and mice.

To recognize the aging progression and the ailments related to aging, the study and research of the α-Klotho gene are gaining significant importance. In humans, the serum concentration of α-Klotho decreases with age after 40 years (Yamazak et al., 2010; Pedersen et al., 2013). This reduced concentration of α-Klotho causes aging-related diseases including cancer, hypertension, and kidney disease (Siahanidou et al., 2012; Wang et al., 2009). Clinical analysis revealed that a single nucleotide polymorphism (SNP) of the promoter of the α-Klotho gene was directly related to

essential hypertension, specifically in aged subjects (Yamazak et al., 2010). Recently, the potential of Klotho protein as an anti-aging agent has greatly increased for the therapeutic application of degenerative disorders related to aging. Aging is a global phenomenon in the population; age-related neurodegenerative disorders outnumber cancer. The late onset Alzheimer's disease (LOAD) is a vital neurodegenerative disease related to aging. A study identified that the Klotho level in the CSF of Alzheimer's disease (AD) patients is higher in older patients than in younger ones. In contrast, women show a lower Klotho concentration than men (Dote-Montero et al., 2019; Semba et al., 2014).

2. STRUCTURE AND EXPRESSION OF KLOTHO

The Klotho gene (in both mice and humans) occurs on chromosome 13q12. It is composed of 5 exons and 4 introns of 50 kb long. Both humans and mice Klotho produce two transcripts, one for the Klotho membrane and the other for the Klotho secretory protein, which is derived from the same gene by alternative RNA splicing. Transcription generates a single-pass membrane protein (KL) consisting of an N-terminal signal sequence, an extracellular domain with two internal repeats (KL1 and KL2), a single-pass transmembrane protein, and a small intracellular carboxy-terminal domains located in the cell membrane and Golgi apparatus (Shiraki-Iida et al., 1998; Imura et al., 2007). The tiny non-functional intracellular domain containing ~ 10 amino acids.

The two internal repeats of the extracellular domains (i.e. KL1 and KL2) share a common amino acid sequence with the family 1 enzyme glycosidase (Tohyama et al, 2004). The extracellular Klotho domain can be digested by membrane proteases (ADAM 10 and 17) and the released portion can be observed in blood, urine, or cerebrospinal fluid (CSF) (Chen et al., 2007; Bloch et al., 2009). Secretary Klotho acts as endocrine, paracrine, or autocrine hormones in target cells (Kurosu et al., 2005; Imura et al., 2007). The Klotho protein is of three types, i.e., a cell membrane-associated, an intracellular, and a secretory form. Membrane-associated Klotho (transmembrane Klotho) present in the kidneys, pituitary gland, inner ear, brain, parathyroid glands, pancreas, large intestine, skeletal muscles, bladder, ovaries, testes, and epithelial cells of the breast (Kameron et al., 2002). There are elevated levels in the distal convoluted tubules, kidney, and choroid plexus of the brain. Klotho is directly associated in the renal metabolism of Ca^{2+}, PO_3^-, and Vit. D. The membrane Klotho has the affinity of binding to fibroblast growth factor (FGF) and fibroblast growth factor receptor (FGFR) (Kim et al., 2015). Klotho bound to the FGF receptor protein represses PO_3^- reabsorption in the proximal tubule of the kidney and maintains Ca^{2+} uptake by stabilizing the Ca^{2+} transient receptor potential vanilloid 5 (TRPV5) channel in the distal tubule plasma membrane. Hence, hyperphosphatemia, hypercalcemia, elevated plasma calcitriol, vascular calcification, and premature aging can be distinguished in Klotho-deficient mice (Drüeke et al., 2007).

Furthermore, two other Klotho proteins, i.e. β-Klotho and γ-Klotho proteins are in the Klotho protein family. Both are a type 1 single-pass transmembrane glycoprotein and, to some extent, share a similar sequence with α-Klotho (Duce et al., 2008). β-Klotho, comprises of the KL1 and KL2 domains, has 41% amino acid similarity to α-Klotho and is present in the liver, gastrointestinal tract, spleen, kidney, and adipose tissue. γ-Klotho is consist of the KL1 domain and occurs in adipose tissue, eyes, and kidneys (Kim et al., 2015).

3. KLOTHO'S ANTIOXIDANT AND ANTI-AGING ACTIVITY

The expression and concentration of Klotho are down-regulated with age (King et al, 2012; Zhu et al, 2018). It has been proposed that the Klotho knockout mouse is a suitable subject organism for studying the aging of the central nervous system (CNS). Studies have developed CNS alterations such as hypomyelination, neurofilament overexpression/phosphorylation, synaptic loss, and behavioral alterations in Klotho-deficient mice, as well as age-related human diseases (Shiozaki et al., 2008; Nagai et al., 2003). There is a direct connection between aging and neuropathological changes, including cognitive deficits, oxidative stress. Oxidative stress is one of the most important factors causing dementia and Alzheimer's disease (Viswanathan et al., 2009). It exerts its neuroprotective activity, the regulation of oxidative stress, inflammation, and fibrosis by suppressing the signaling pathways of insulin/insulin-like growth factor-1 (IGF-1) and transforming growth factor-β1 (TGF-β1) (Doi et al., 2011; Bartke et al., 2006; Thurston et al., 2010). Klotho shows resistance to oxidative stress and anti-aging effect by suppressing the insulin/IGF-1/PI3K signaling pathway, which stimulates FoxOs, and increases the expression of manganese superoxide dismutase (MnSOD) (a superoxide neutralizer) (Dalton et al., 2017; Zhang et al., 2011).

In 2017, tacrolimus-induced oxidative stress in mice has been identified to be decreased by using Klotho. Klotho has been observed to downregulate the PI3K/Akt pathway and successively stimulated FoxO-mediated expression of MnSOD. Similarly, soluble recombinant Klotho can arrest apoptosis and attenuate lipid oxidation in HeLa cells treated with paraquat (Lim et al., 2017). There is a second way to influence the aging process in the brain by regulating the synthesis of vitamin D by Klotho. Vitamin D3 deficiency and hypervitaminosis D are a probability factor for age-related cognitive decline (Schlögl et al., 2014; Maji, 2012; Ellis et al., 2018). Here, Klotho controls this by inhibiting the enzyme 1-α-hydroxylase that catalyzes the formation of 1,25-dihydroxy vitamin D3, the bioactive form of Vit. D. Therefore,

Klotho has the prominent role of controlling the appropriate mechanism of the CNS (Yoshida et al., 2002; Pathare et al., 2019).

4. KLOTHO'S NEUROPROTECTIVE ACTIVITY

Klotho and IGF-1 deficiency in the hippocampus of the rat model of dementia reported that this protein has a significant impact on dementia. It has been revealed that a high level of Klotho strengthens synapses and increases synaptic GluN2B (a subunit of the N-methyl-D-aspartate receptor and vital functions of learning and memory). A comparative study between Klotho overexpressing mice and control mice in their study showed that increasing Klotho stimulates learning and memory in various tasks positively without any behavioral alteration. The researcher has suggested that Klotho is necessary for postnatal neurogenesis, including the propagation and evolution of neural stem cells during the transition to adult brain functionality. Furthermore, they showed that Klotho deficiency in the hippocampus led to fewer neural stem cells with reduced proliferation rate and impaired neuronal maturation, which collectively led to premature aging. Furthermore, recombinant Klotho can control this (Laszczyk et al, 2017). The schematic representation has been shown for the possible mechanism of neuroprotection in Figure 1.

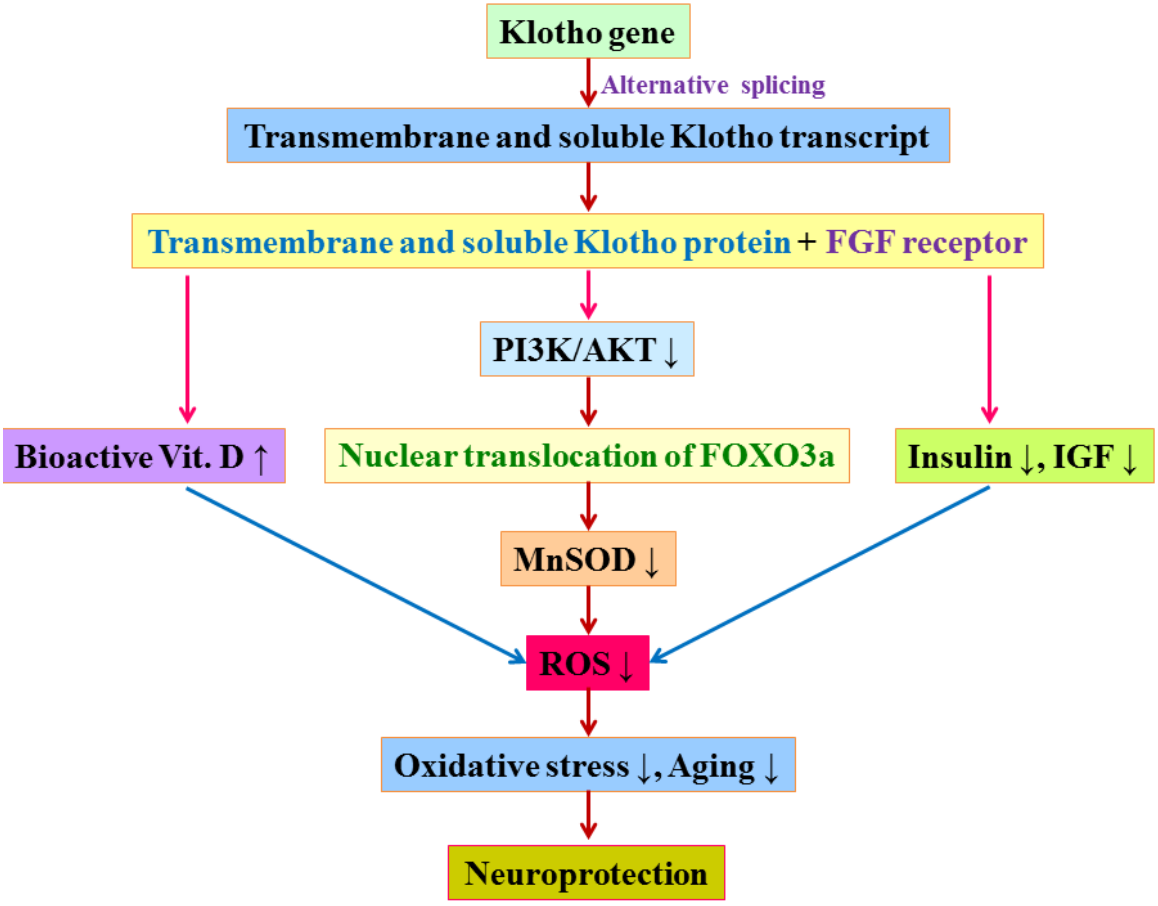

Figure 1. Schematic representation of neuroprotection mechanism

5. ROLE OF KLOTHO IN NEURODEGENERATIVE DISORDERS

Alzheimer disease

The formation of β-amyloid (Aβ) plaques is a vital feature of the pathogenesis of Alzheimer's disease due to the cause of mainly amyloid precursor protein (APP). This protein produces a secreted form of ectodomain fragments (APPsα and APPsβ), and an intracellular domain of APP by amyloidogenic processing (Li et al., 2010). Overexpression of APP causes a neurotrophic effect on neural cell proliferation (Oh et al., 2009). Along with this, reactive oxygen species (ROS) generations are directly related to neurodegenerative disorders such as Alzheimer's disease by generating oxidative damage and neuronal cell death. Klotho is a target of APP, which expression completely depends on APPsβ. The derivatives of the extracellular APP ectodomain induce Klotho expression and protect against Aβ neurotoxicity during aging (Li et al., 2010). Furthermore, L-glutamate (an important excitatory neurotransmitter) and Aβ are models of in vitro neurodegeneration of common oxidative stressors. The neurotoxic effect of Aβ is also associated with a cellular injury resulting from exposure to reactive oxygen species. It has been reported that Klotho has a protective effect on hippocampal neurons, which is vulnerable to oxidative stress, due to glutamate and oligomeric Aβ-related toxicity. This protection could be achieved by stimulating exogenous Klotho or by increasing levels of

endogenous Klotho as found in mice that overexpress Klotho (Zeldich et al., 2014). The significantly lower CSF Klotho concentrations have been observed in elderly patients with AD. They also found that CSF Klotho concentrations are lower in older people compared to younger people (Semba et al., 2014).

Parkinson's disease

The antioxidant activity of Klotho can be used to treat aging-related movement disorder, Parkinson's disease (PD). Clinically, PD is characterized by impaired motor functions such as resting tremor, impaired coordination, muscle stiffness, stooped posture, and bradykinesia (Sveinbjornsdottir, 2016). Increased oxidative stress, reduced antioxidant status, and inflammation-mediation via protein kinase-A dependent cascade plays a major part in the pathogenesis of PD (Mosley et al., 2006). All the alteration of dopamine neurons in the Substantianigra pars compacta (SNc) is one of the vital pathological features (Dauer et al., 2003). The nigrostriatal pathway is vital in establishing a connection between the SNc and the dorsal striatum. Klotho prevents the disruption of tyrosine hydroxylase-positive neurons in the SNc. Also, Klotho significantly ameliorates α-synuclein, glial fibrillary acidic protein, phosphorylated cAMP-response element-binding protein (CREB), DNA fragmentation, and oxidative stress through the PKA/CaMKII/CREB signaling cascade. Furthermore, the positive effect of Klotho was reported to be prevented by the administration of a PKA inhibitor and a Ca^{2+}/calmodulin-dependent protein kinase II (CamKII) inhibitor. Therefore, the neuroprotective activity of Klotho in the 6-OHDA rat model of PD appears to be dependent on the PKA/CaMKII/CREB signaling cascade (Baluchnejadmojarad et al, 2017).

Multiple sclerosis

Multiple sclerosis is a common neurodegenerative disease with demyelinated lesions in the brain, spinal cord, and optic nerve caused by myelin autoimmunity (Gallardo et al., 2018; Yi et al., 2019). A direct connection has been reported between the Klotho protein, the dissemination, and myelination of oligodendrocytes of the CNS (Chen et al., 2013). Furthermore, the Klotho knockout mice exhibited a significantly low concentration of major myelin protein and mature oligodendrocytes. Therefore, Klotho might be the best target to protect myelin to preserve age-dependent brain alterations.

6. CONCLUSIONS

The Klotho has been evidenced as an antioxidant, antiapoptotic, and neuroprotective activity. It plays an important role in counteracting brain aging and protecting neuronal damage as well as neurodegeneration from oxidative stress. It has some immunoregulatory role to protect against cognitive decline and age-related neurodegenerative diseases. Increasing the Klotho level could be the most appropriate and prospective therapeutic target to prevent aging and neurodegenerative disorders. New small Klotho enhancer molecules that can travel across the blood-brain barrier need to be synthesized to treat neurodegeneration.

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6. ACKNOWLEDGEMENTS

The authors are grateful to the Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry, India and Department of Chemistry, Uttaranchal University, Dehradun, Uttarakhand, India.