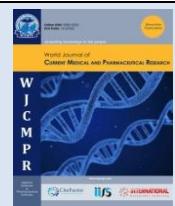




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THE VERSATILITY OF KLOTHO PROTEIN: INSIGHTS INTO ITS MULTIFACETED FUNCTIONS IN HEALTH AND DISEASE

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Article History	Abstract
Received on: 05-05-2024	The Klotho protein, initially discovered for its role in aging and calcium metabolism, has emerged as a multifunctional regulator with diverse impacts on health and disease. Beyond its functions in maintaining phosphate homeostasis and suppressing aging pathways, Klotho exhibits pleiotropic effects in various physiological processes, including cognition, cardiovascular health, and cancer suppression. This review elucidates the mechanisms underlying Klotho's versatile functions, shedding light on its involvement in modulating signaling pathways such as insulin/IGF-1, Wnt, and fibroblast growth factor (FGF) pathways. Furthermore, we explore the therapeutic potential of Klotho modulation in combating age-related diseases, neurodegeneration, and metabolic disorders. Understanding the complexities of Klotho biology unveils novel strategies for therapeutic interventions and highlights its significance in promoting healthy aging and disease prevention.
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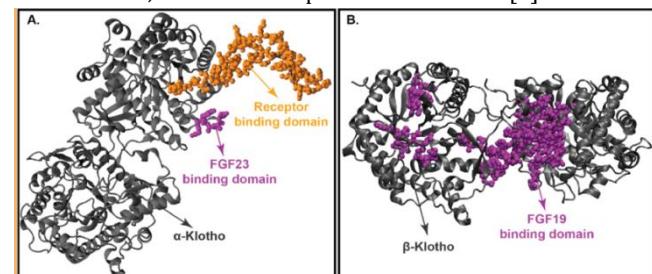
Introduction

The Klotho gene on chromosome 13, flanked by PDS5B and STARD13 sequences, was discovered for the first time from mouse strains that displayed phenotypes similar to human aging, such as growth retardation, vascular calcification, and osteoporosis. Two members of the Klotho family were identified and named Alpha and Beta Klotho. Alpha Klotho is highly conserved in humans, mice, and rats. Klotho is usually referred to as Alpha Klotho because it is the more common and abundant type [1]. The other Klotho members namely, Beta and Gamma Klotho are also Type I, single-pass transmembrane proteins. Beta Klotho contains a β -glycosidase-like domain (KL1 and 2 domains) and shares 42% amino acid sequence homology with Alpha Klotho. Gamma Klotho is a comparatively shorter single-pass transmembrane protein, composed of only the KL1 domain and a short intracellular domain [2].

Structure

Structurally, Klotho is a Type I, single-pass transmembrane protein located in the cell membrane and plasma membrane. Its intracellular domain is very short and without any functional domain. The Klotho protein when cleaved by

proteases (ADAM10 and ADAM17) and secreted, functions as an endocrine, autocrine and paracrine hormone [1].



Crystal structure of Klotho proteins. (A): Crystal structure of KLA. (B): Crystal structure of KLB. The backbone is represented in grey - backbone, magenta - protein binding domain (FGF23 for KLA and FGF19 for KLB), and yellow - the receptor binding domain

The Klotho gene is expressed in human tissues by alternate splicing and is detectable in blood, urine, and Cerebrospinal Fluid (CSF). Notably, Klotho expression is seen at higher levels in the distal convoluted tubule in the kidneys and in the choroid plexus of the brain. It is also expressed in the renal proximal tubule, parathyroid gland and several sex organs including the ovary, testis, placenta, and adventitial area of the aorta [2].

Physiological functions of Klotho

Klotho, a pivotal regulator of aging and various physiological processes, plays a fundamental role in maintaining

homeostasis within the body. Its significance lies in its multifaceted functions across different organ systems, [3]. Klotho exerts pleiotropic effects, encompassing regulation of mineral metabolism, antioxidant activity, anti-inflammatory properties, and modulation of insulin sensitivity [4, 5]. In the kidneys, Klotho acts as a co-receptor for fibroblast growth factor 23 (FGF23), facilitating phosphate excretion and maintaining phosphate homeostasis [5]. Moreover, Klotho exerts protective effects on the cardiovascular system by promoting endothelial function, suppressing vascular calcification, and attenuating oxidative stress. In the brain, Klotho modulates synaptic plasticity, neuroprotection, and cognitive function, thereby influencing central nervous system homeostasis [6]. This review aims to provide a comprehensive overview of the role of Klotho protein in various diseases, encompassing its involvement in CKD, cardiovascular diseases, neurodegenerative disorders, cancer, and emerging therapeutic avenues.

Role of Klotho in Disease Conditions

1. Cardiovascular Diseases

Cardiovascular diseases (CVD) include hypertension, coronary artery disease, stroke, heart failure, and peripheral artery disease. Klotho holds multifaceted effects on the cardiovascular system by enhancing endothelial function and promoting the production of nitric oxide and vasodilation while it inhibits the vascular calcification via antagonism of the Wnt/β-catenin signaling pathway [7]. Moreover, Klotho exerts antioxidant and anti-inflammatory properties, controlling oxidative stress and inflammation in the vasculature [8, 9]. Klotho changes the key signal pathways like TGF-β and IGF-1, thus suppressing cardiac fibrosis, hypertrophy, and remodeling while promoting cardiomyocyte survival [10]. Furthermore, Klotho is also involved in interaction with TRP channels, influencing the contractility and endothelial function of vascular smooth muscle [11]. Augmentation of Klotho or its activity, including recombinant Klotho protein administration and gene therapy, holds immense potential as a potential therapeutic intervention. Yet, there persists challenges like mode of delivery and off-target effects that requires further investigation [12, 13].

2. Occult Coronary Artery Disease (CAD)

Occult Coronary Artery Disease (CAD) refers to a condition where there is evidence of significant coronary artery disease, such as plaque buildup or narrowing of the arteries, without corresponding symptoms or signs of the disease. Although the targets of Klotho in a cell have not been identified yet, it has been proved to alter the function of genes such as angiotensin-converting enzyme (MIM 106180) and plasminogen activator inhibitor type 1 (PAI-1 [MIM 173360]). Klotho is known for its protective effects against atherosclerosis, the underlying pathology of CAD. It inhibits vascular smooth muscle cell proliferation and migration, reduces oxidative stress, and promotes endothelial function, thereby attenuating the progression of atherosclerotic lesions and inhibits vascular calcification, a common feature of advanced atherosclerosis and CAD.

Studies suggest that Klotho is involved in the modulation of the renin-angiotensin system, which plays a significant role in cardiovascular homeostasis and the pathogenesis of CAD. It

acts by enhancing the activity of angiotensin-converting enzyme 2 (ACE2), leading to an enhanced conversion of angiotensin II to angiotensin-(1-7), which has vasodilatory and anti-inflammatory properties. Such multifaceted functions of Klotho highlight its potential therapeutic relevance.

2. Chronic Kidney Disease (CKD)

(b) Vascular calcification (VC)

Because of the similarity between human CKD and murine experimental Klotho deficiency, it has been postulated that Klotho may be responsible for VC in CKD, which is the cause of death in CKD patients [14]. To confirm, CKD models were developed using disease models such as uninephrectomy along with ischemia-reperfusion injury in the contralateral kidney. The disease was developed in two transgenic animals, one with overexpression of Klotho, EFmKL46 and combined genetic background from C57BL/6J and 129 and the other heterozygous for Klotho-deficient mice (KI), with genetic background from C57BL/6J and C3H/J.

(b) Disruption of Phosphate Homeostasis

FGF23 is a member of the FGF family of receptors, which down-regulates the production of 1α,25-dihydroxyvitamin D3 (1,25(OH)2D) thereby increasing the excretion of phosphate in the kidney and suppressing intestinal phosphate absorption. Klotho is an essential co-receptor for FGF23 because the FGF23 lacks heparan sulphate-binding domain. Research findings indicate that deletion of only Klotho in proximal renal tubules does not elevate the renal phosphate excretion *in vivo* [15]. This suggests that the impact of FGF23 on phosphate excretion is restricted by the deficiency of Klotho in proximal tubules. Moreover, soluble Klotho plays a direct role in regulating phosphorus excretion within the kidney and contributes to systemic mineral balance by controlling the activity of 1α-hydroxylase as well as the secretion of parathyroid hormone (PTH) and FGF23 [16, 17].

(c) Inflammation

As renal function declines in CKD, expression of numerous proinflammatory factors such as interleukin (IL)-6, serum fetuin-A, and tumor necrosis factor (TNF) progressively increase [18]. The nuclear factor κB (NF-κB) regulates various cellular functions, including antiapoptotic responses, oxidative stress management, and inflammatory reactions. Research has demonstrated a dual association between Klotho and NF-κB. Klotho expression is suppressed via an NF-κB-dependent mechanism. Decreased levels of Klotho have been observed in both blood and urine samples from individuals with CKD [19, 20]. However, blocking TNF-related weak inducer of apoptosis (TWEAK), a member of the TNF superfamily was found to restore kidney Klotho levels and preserve renal function. Moreover, Klotho can inhibit the expression of NADPH oxidase 2 (Nox2) protein, thereby mitigating oxidative stress in rat aortic smooth muscle cells. Additionally, it can suppress inflammation mediated by retinoic acid-inducible gene-I (RIG-I) [21, 22].

4. Cancer

Interestingly, Klotho exhibits a complex and dual role in cancer progression wherein it acts as both a tumor suppressor and an oncogenic factor. Its tumor-suppressing functions are well-documented in lung, colon and breast cancer. With studies, evolving evidence suggests that Klotho can also exhibit

oncogenic effects including promoting tumor growth and progression in some specific cases [23,24]. The properties of Klotho to inhibit cellular proliferation, promotion of apoptosis, and inhibition of epithelial-mesenchymal transition (EMT) promote its role as a tumor-suppressing protein [25,26]. Mechanistically, the suppression of tumor and thus metastasis is primarily due to the modulation of various signaling pathways by Klotho including the transforming growth factor-beta (TGF- β) pathway, insulin-like growth factor 1 (IGF-1) pathway, and Wnt/ β -catenin pathway,[24](25). Klotho promotes the proliferation of cancer cells, their survival, and resistance to drugs. Moreover, Klotho may promote cancer stemness and metastasis by activating the signaling pathways related to stem cells, like Notch and Hedgehog pathways [27]. The dual role of Klotho protein in cancer highlights the need of further study to understand the molecular mechanisms involved so as to overcome the clinical hurdles of cancer therapeutics.

Renal cell carcinoma (RCC) is the third most frequent form of cancer in urological oncology. Klotho has been reported to suppress the invasive action of renal cancer cells in RCC by inhibition of the Epithelial-Mesenchymal transition (EMT) by altering the PI3K / Akt /GSK3b signal activation by suppressing EMT phenotypic switch of the cellular RCC via signal downregulation. Klotho is pivotal for the endogenous FGF23 (FGF family) [28]. Although studies suggest that the role of FGF in renal cancer remains limited but an increase in levels of basic FGF serum seem to be associated with poor survival rate in RCC. [29, 30].

5. Aging

Following the groundbreaking discoveries by Kuro-o et al. in 1997 and Kurosu et al. in 2005, significant advancements have been achieved in understanding the mechanisms and functions of Klotho. Notably, Duce et al. (2008) [31] Age-related alterations were observed in the expression of the Klotho (KL) gene, telomeric repeat binding factor 1, and mitochondrial polymerase gamma, all of which are linked with brain degeneration and decreased lifespan. Notably, Klotho expression decreased with age in brain white matter by approximately 1.5-fold. Serum levels of KL decline with age in humans [32], bonobos and chimpanzees [33]. Additionally, researchers have noted lower KL levels in older individuals with Alzheimer's disease (AD) compared to those without AD ($p = 0.02$), and lower levels in older adults compared to younger adults ($p = 0.005$) [34]. In the following sections, we delve into the variations in Klotho levels across a range of diseases, all of which are linked to the aging process.

6. Neurodegeneration

Klotho protein has emerged as a significant factor in neurodegenerative diseases by inhibition of oxidative stress, attenuation of neuroinflammation, and promotion of the synaptic plasticity along with neuronal survival [35]. Furthermore, Klotho increases the clearance of amyloid-beta (A β) peptides, which marks a hallmark of AD pathology [35]. Preclinical studies that utilize mice overexpressing. Beyond AD, potential implications of Klotho in other neurodegenerative diseases such as Parkinson's disease and multiple sclerosis have also been observed. These findings encourage the therapeutic potential of targeting Klotho.

7. Wound Healing

Klotho has recently emerged as a significant player in wound healing processes. Research suggests that it exerts multifaceted effects on various stages of wound repair, such as inflammation, tissue regeneration, and remodeling.

1. **Anti-inflammatory Properties:** Inflammation is the primary phenomenon of wound formation and healing progression. Klotho suppresses inflammation by inhibiting pro-inflammatory cytokines such as (IL-6) and (TNF- α) [36, 37].
2. **Promotion of Cell Proliferation and Migration:** Klotho facilitates the proliferation and migration of various immune cells to initiate wound repair and tissue regeneration, including fibroblasts, keratinocytes, lymphocytes, and endothelial cells [38].
3. **Enhancement of Extracellular Matrix Formation:** Klotho contributes to the synthesis and deposition of extracellular matrix (ECM) proteins such as collagen and fibronectin, crucial for structural support and guiding tissue regeneration at the wound site [10, 39].
4. **Angiogenesis:** Klotho promotes angiogenesis, the process of new blood vessel formation, which is essential for delivering oxygen and nutrients to the wound area, and facilitating tissue repair and regeneration. Klotho upregulates pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS).
5. **Modulation of Oxidative Stress:** Klotho exerts antioxidant effects by scavenging reactive oxygen species (ROS) and enhancing the activity of antioxidant enzymes [40, 41].
6. **Regulation of Immune Responses:** Klotho modulates immune responses during wound healing. It promotes the transition from a pro-inflammatory to a pro-resolution phase, facilitating the clearance of apoptotic cells while promoting tissue remodeling [39].

Future of Klotho

Emerging research on Klotho has unveiled novel insights into its diverse physiological functions and therapeutic potential, paving the way for future investigations. Recent studies have highlighted the multifaceted roles of Klotho, including its involvement in stem cell biology, immune regulation, and tissue regeneration [42]; [43]. For instance, Klotho has been implicated in the modulation of immune responses, with studies demonstrating its regulatory effects on T-cell activation, macrophage polarization, and cytokine production [44, 45]. These findings can hypothesize Klotho as a potential therapeutic candidate for disease such as T cell malignancies, autoimmune diseases and systemic inflammatory disorders. Moreover, emerging evidence suggests that Klotho may play a critical role in tissue repair and regeneration [46, 47]. Future directions in Klotho research encompass several key areas, including its molecular mechanisms, identifying novel therapeutic targets and exploring the utility of Klotho as a diagnostic biomarker and therapeutic agent [48]. Overall, the research on Klotho underscores its significance as a master regulator of health and disease.

Conclusion

In conclusion, the multifaceted role of Klotho in various diseases underscores its significance as a pivotal regulator of health and disease pathogenesis. Emerging research on Klotho has provided novel insights into its mechanisms of action and therapeutic potential, offering promising avenues for the development of innovative diagnostic and therapeutic strategies. However, several key challenges and opportunities lie ahead in Klotho's research. Further elucidation of the molecular mechanisms underlying Klotho's diverse functions, as well as its intricate interplay with other signaling pathways and regulatory molecules, is essential for a comprehensive understanding of its physiological roles and therapeutic implications. Continued research efforts aimed at exploring the clinical utility of Klotho as a diagnostic biomarker and therapeutic agent, as well as the development of Klotho-based therapeutics, are warranted to harness its full potential in combating age-related diseases and promoting healthy aging.

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Ethical approval

This work adheres to the highest ethical standards in research and writing. All content presented within has been produced with integrity and honesty. Any external sources or references used have been appropriately credited. Furthermore, this work respects the principles of academic integrity, ensuring that all contributions, ideas, and findings are accurately attributed. It is the author's commitment to transparency, accuracy, and accountability that underpins the ethical foundation of this work. Any concerns regarding the ethical integrity of this work are welcomed and will be addressed with utmost seriousness.

Informed consent

I, Sonali Raj, hereby acknowledge that I am the sole contributor to the work titled. I understand that this project is self-funded and independent of any external financial support. I am aware that my work may contain opinions, interpretations, or findings solely my own and may not reflect the views of any other individual, institution, or organization.

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Author Contribution

Both Sonali Raj, Mehak Ahuja are contributed equally.

References

1. Kim JH, Hwang KH, Park KS, Kong ID, Cha SK. Biological Role of Anti-aging Protein Klotho. *J Lifestyle Med.* 2015 Mar 31;5(1):1-6.
2. Arking DE, Becker DM, Yanek LR, Fallin D, Judge DP, Moy TF, et al. KLOTHO Allele Status and the Risk of Early-Onset Occult Coronary Artery Disease. *Am. J. Hum. Genet.* 2003.
3. Kuro-o M. Klotho and aging. *Biochimica et Biophysica Acta - General Subjects.* 2009. p. 1049-58.
4. Wang Y, Sun Z. Antiaging gene Klotho regulates endothelin-1 levels and endothelin receptor subtype B expression in kidneys of spontaneously hypertensive rats. *J Hypertens.* 2014;32(8):1629-36.
5. Hu MC, Shi M, Zhang J, Addo T, Cho HJ, Barker SL, et al. Renal Production, Uptake, and Handling of Circulating oklotho. *Journal of the American Society of Nephrology.* 2016 Jan 1;27(1):79-90.
6. Dubal DB, Yokoyama JS, Zhu L, Broestl L, Worden K, Wang D, et al. Life Extension Factor Klotho Enhances Cognition. *Cell Rep.* 2014 May 22;7(4):1065-76.
7. Kuro-O* M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *NATURE.* 1997.
8. Haruna Y, Kashihara N, Satoh M, Tomita N, Namikoshi T, Sasaki T, et al. Amelioration of progressive renal injury by genetic manipulation of Klotho gene [Internet]. 2007. Available from: www.pnas.org/cgi/doi/10.1073/pnas.0611079104
9. Saito Y, Yamagishi T, Nakamura T, Ohyama Y, Aizawa H, Suga T, et al. Klotho Protein Protects against Endothelial Dysfunction. *BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS.* 1998.
10. Xie J, Cha SK, An SW, Kuro-O M, Birnbaumer L, Huang CL. Cardioprotection by Klotho through downregulation of TRPC6 channels in the mouse heart. *Nat Commun.* 2012;3.
11. Avin KG, Coen PM, Huang W, Stoltz DB, Sowa GA, Dubé JJ, et al. Skeletal muscle as a regulator of the longevity protein, Klotho. *Front Physiol.* 2014;5 JUN.
12. Novo-Rodríguez C, García-Fontana B, De Dios Luna-Del Castillo J, Andújar-Vera F, Avila-Rubio V, García-Fontana C, et al. Circulating levels of sclerostin are associated with cardiovascular mortality. *PLoS One.* 2018 Jun 1;13(6).
13. Hu MC, Shi M, Zhang J, Addo T, Cho HJ, Barker SL, et al. Renal Production, Uptake, and Handling of Circulating oklotho. *Journal of the American Society of Nephrology.* 2016 Jan 1;27(1):79-90.
14. Parikh NI, Hwang SJ, Larson MG, Levy D, Fox CS. Chronic Kidney Disease as a Predictor of

Cardiovascular Disease (From the Framingham Heart Study).

15. Ide N, Olauson H, Sato T, Densmore MJ, Wang H, Hanai J, et al. In vivo evidence for a limited role of proximal tubular Klotho in renal phosphate handling. *Kidney Int.* 2016 Aug 1;90(2):348-62.

16. Smith RC, O'Bryan LM, Farrow EG, Summers LJ, Clinkenbeard EL, Roberts JL, et al. Circulating α Klotho influences phosphate handling by controlling FGF23 production. *Journal of Clinical Investigation.* 2012 Dec 3;122(12):4710-5.

17. Sakan H, Nakatani K, Asai O, Imura A, Tanaka T, Yoshimoto S, et al. Reduced renal α -Klotho expression in CKD patients and its effect on renal phosphate handling and vitamin D metabolism. *PLoS One.* 2014 Jan 23;9(1).

18. Zoccali C, Vanholder R, Massy ZA, Ortiz A, Sarafidis P, Dekker FW, et al. The systemic nature of CKD. Vol. 13, *Nature Reviews Nephrology.* Nature Publishing Group; 2017. p. 344-58.

19. Izquierdo MC, Perez-Gomez M V, Sanchez-Niño MD, Sanz AB, Ruiz-Andres O, Poveda J, et al. Klotho, phosphate and inflammation/ageing in chronic kidney disease. Vol. 27, *Nephrology Dialysis Transplantation.* 2012.

20. Hu MC, Shi M, Zhang J, Quñones H, Kuro-O M, Moe OW. Klotho deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective. *Kidney Int.* 2010;78(12):1240-51.

21. Wang Y, Kuro-O M, Sun Z. Klotho gene delivery suppresses Nox2 expression and attenuates oxidative stress in rat aortic smooth muscle cells via the cAMP-PKA pathway. *Aging Cell.* 2012 Jun;11(3):410-7.

22. Liu F, Wu S, Ren H, Gu J. Klotho suppresses RIG-I-mediated senescence-associated inflammation. *Nat Cell Biol.* 2011 Mar;13(3):254-62.

23. Chen B, Wang X, Zhao W, Wu J. Klotho inhibits growth and promotes apoptosis in human lung cancer cell line A549. *Journal of Experimental and Clinical Cancer Research.* 2010;29(1).

24. Wolf I, Levanon-Cohen S, Bose S, Ligumsky H, Sredni B, Kanety H, et al. Klotho: A tumor suppressor and a modulator of the IGF-1 and FGF pathways in human breast cancer. *Oncogene.* 2008 Nov 27;27(56):7094-105.

25. Mota J, Lima AMM, Gomes JIS, Souza de Andrade M, Brito HO, Silva MMAL, et al. Klotho in Cancer: Potential Diagnostic and Prognostic Applications. Vol. 13, *Diagnostics.* Multidisciplinary Digital Publishing Institute (MDPI); 2023.

26. Xie B, Chen J, Liu B, Zhan J. Klotho acts as a tumor suppressor in cancers. Vol. 19, *Pathology and Oncology Research.* 2013. p. 611-7.

27. Li XX, Huang LY, Peng JJ, Liang L, Shi DB, Zheng HT, et al. Klotho suppresses growth and invasion of colon cancer cells through inhibition of IGF1R-mediated PI3K/AKT pathway. *Int J Oncol.* 2014;45(2):611-8.

28. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature.* 2006 Dec 7;444(7120):770-4.

29. B B Cell Definition.

30. Torres PU, Prié D, Molina-Blétry V, Beck L, Silve C, Friedlander G. Klotho: An antiaging protein involved in mineral and vitamin D metabolism. Vol. 71, *Kidney International.* 2007. p. 730-7.

31. Duce JA, Podvin S, Hollander W, Kipling D, Rosene DL, Abraham CR. Gene profile analysis implicates Klotho as an important contributor to aging changes in brain white matter of the rhesus monkey. *Glia.* 2008 Jan 1;56(1):106-17.

32. Shardell M, Semba RD, Kalyani RR, Bandinelli S, Prather AA, Chia CW, et al. Plasma Klotho and Frailty in Older Adults: Findings from the InCHIANTI Study. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2019 Jun 18;74(7):1052-8.

33. Behringer V, Stevens JMG, Deschner T, Sonnweber R, Hohmann G. Aging and sex affect soluble alpha klotho levels in bonobos and chimpanzees. *Front Zool.* 2018 Sep 19;15(1).

34. Semba RD, Moghekar AR, Hu J, Sun K, Turner R, Ferrucci L, et al. Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. *Neurosci Lett.* 2014 Jan 13;558:37-40.

35. Zeldich E, Chen C Di, Colvin TA, Bove-Fenderson EA, Liang J, Tucker Zhou TB, et al. The neuroprotective effect of Klotho is mediated via regulation of members of the redox system. *Journal of Biological Chemistry.* 2014;289(35):24700-15.

36. Kuro-O* M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Vol. 390, *NATURE.* 1997.

37. Mitani H, Ishizaka N, Aizawa T, Ohno M, Usui SI, Suzuki T, et al. In vivo klotho gene transfer ameliorates angiotensin II-induced renal damage. *Hypertension.* 2002;39(4):838-43.

38. Sugiura H, Yoshida T, Tsuchiya K, Mitobe M, Nishimura S, Shirota S, et al. Klotho reduces apoptosis in experimental ischaemic acute renal failure. *Nephrology Dialysis Transplantation.* 2005 Dec;20(12):2636-45.

39. Doi S, Zou Y, Togao O, Pastor J V, John GB, Wang L, et al. Klotho inhibits transforming growth factor- β 1 (TGF- β 1) signaling and suppresses renal fibrosis and cancer metastasis in mice. *Journal of Biological Chemistry.* 2011 Mar 11;286(10):8655-65.

40. Kuro-o M. Klotho as a regulator of oxidative stress and senescence. Vol. 389, *Biological Chemistry.* 2008. p. 233-41.

41. Yamamoto M, Clark JD, Pastor J V, Gurnani P, Nandi A, Kurosu H, et al. Regulation of Oxidative Stress by the Anti-aging Hormone.

42. Yu LX, Li SS, Sha MY, Kong JW, Ye JM, Liu QF. The controversy of klotho as a potential biomarker in chronic kidney disease. Vol. 13, *Frontiers in Pharmacology.* Frontiers Media S.A.; 2022.

43. Paquette JS, Rhéaume C, Cordeau P, Moulin JA, Audet-Walsh E, Blanchette V, et al. The Longevity Protein Klotho: A Promising Tool to Monitor Lifestyle Improvements. Vol. 13, Metabolites. Multidisciplinary Digital Publishing Institute (MDPI); 2023.
44. Nakao VW, Mazucanti CHY, de Sá Lima L, de Mello PS, de Souza Port's NM, Kinoshita PF, et al. Neuroprotective action of α -Klotho against LPS-activated glia conditioned medium in primary neuronal culture. *Sci Rep.* 2022 Dec 1;12(1).
45. Zhou L, Mo H, Miao J, Zhou D, Tan RJ, Hou FF, et al. Klotho ameliorates kidney injury and fibrosis and normalizes blood pressure by targeting the renin-angiotensin system. *American Journal of Pathology.* 2015 Dec 1;185(12):3211-23.
46. Prud'homme GJ, Kurt M, Wang Q. Pathobiology of the Klotho Antiaging Protein and Therapeutic Considerations. Vol. 3, *Frontiers in Aging.* Frontiers Media S.A.; 2022.
47. Komaba H, Kaludjerovic J, Hu DZ, Nagano K, Amano K, Ide N, et al. Klotho expression in osteocytes regulates bone metabolism and controls bone formation. *Kidney Int.* 2017 Sep 1;92(3):599-611.
48. Brobey RK, Dheghani M, Foster PP, Kuro-O M, Rosenblatt KP. Klotho regulates 14-3-3 ζ monomerization and binding to the ASK1 signaling complex in response to oxidative stress. *PLoS One.* 2015 Oct 30;10(10).