

Klotho, an Anti-Aging Protein with Significant Potential in Regenerative Medicine

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ABSTRACT

Aging is a continuous physiological process that results in senescence or a decline in biological functions. This process is triggered by changes in the body's ability to respond to stress and to adapt to metabolic shifts. As a natural course of events, it leads to the emergence of various health issues and limitations in the capacity to repair tissues and organs. Contemporary medicine aims to delay or modify this process and minimize the consequences of alterations in physical activity, nutritional parameters, and risks. The success of this protective approach is largely dependent on the restoration of the production of anti-aging agents. The klotho protein is one such agent and has fundamental importance in this regard. A decline in klotho protein levels has been clearly linked to aging events, and an increase in its levels has a significant impact on the health of older individuals. Klotho deficiency has been observed in several experimental and clinical disease models. Restoring pre-aging conditions can enhance the regenerative capacity of organisms, which is why klotho protein can be considered for use in regenerative medicine processes across different conditions and not just in older patients. This paper provides an overview of the characteristics and functions of the klotho protein and suggests its potential application in regenerative medicine.

Keywords: Aging • Senescence • Repair • Regeneration • Klotho

INTRODUCTION

In 1997, *Kuro-O M, et al.* and colleagues discovered the klotho gene, which encodes an aging-suppressor protein [1]. Animals with a mutation of this gene within chromosome 13 displayed signs of premature aging and a shortened lifespan. A year later, the human klotho gene was identified, showing a homology with mouse genes of over 80%. Thus, findings in mice provide valuable information for human translation [2].

There are three klotho subfamilies: α -klotho, β -klotho, and γ -klotho. α -klotho is a co-receptor of Fibroblast Growth Factor (FGF) 23 which is subject to ectodomain shedding to release soluble klotho [3,4]. β -klotho serves as a co-receptor for FGF19 and FGF21. [5] γ -klotho is expressed in the kidneys and skin with its functions yet to be defined [6,7]. α -klotho is a membrane-bound protein that is linked to β -glucuronidase and is present in the human Cerebrospinal Fluid (CSF), blood plasma, and urine. It is known to promote longevity and delay the onset of multiple systemic aging in both mice and humans.

Unless otherwise specified, the term “klotho” generally refers to the α -klotho subfamily,

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which functions as a soluble endocrine or a paracrine factor.

Overexpression of klotho results in suppression of aging-associated conditions and a significant delay in animal death [8]. Klotho secretion decreases with age [9].

KLOTHO ACTION MECHANISMS

The klotho transmembrane protein cleavage by α -secretases is subsequently released into the systemic circulation, cerebrospinal fluid, and urine [10-13]. The secreted protein is known as soluble klotho [14]. This is the predominant klotho gene product and acts as a paracrine or endocrine factor that mediates several key klotho effects. FGF23 is a potent inhibitor of klotho transcription [15]. In a similar manner, inflammatory processes lead to a decrease in klotho expression, and the protein has anti-inflammatory activity that blocks nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling [16-18]. Klotho functions as an endogenous antioxidant under various conditions [19]. One of its main effects is the regulation of extracellular calcium levels, and it also regulates phosphate reabsorption and excretion in the kidney [10,20,21].

Klotho expression is universally suppressed in various types of cancer, such as breast, pancreatic, ovarian, lung, colorectal, and melanoma [22]. As a modulator of different growth factor pathways, Klotho acts as a tumor suppressor in pancreatic, breast, and liver cancers and inhibits different growth factors such as Insulin Growth Factor I (IGF-1) and Transforming Growth Factor Beta (TGF β) 1 [4,23-26].

Klotho has a key mechanism of action that involves the restoration, preservation, and/or stimulation of cellular autophagy, which is one of its major functions.

Its actions extend to nearly all organs, although its synthesis varies across different systems. In a comprehensive list, *H. Olauson et al.* specified the tissues that exhibited klotho expression and described the relevance of production in each case. In rodents, the kidneys, parathyroid gland, choroid plexus, and sinoatrial node are the primary sources of klotho. Meanwhile, intermediate levels of klotho expression are observed in the central nervous, endocrine, and respiratory systems; gastrointestinal and genitourinary tracts; and skeletal muscle. Lastly, the authors mentioned tissues with low or no klotho expression, including connective tissue and skin, cartilage, adipose tissue, cardiovascular and immune system, blood, salivary glands, and liver. However, reports on the gallbladder, genitourinary tract, and uterus are inconclusive [27].

KLOTHO EFFECTS IN HEALTH AND DISEASE

The absence of klotho in animal models results in premature aging, which is associated with organ regression and health changes that are indicative of human aging. These alterations are also linked to different pathologies that appear at different stages of life, offering insight into the potential therapeutic uses of klotho.

Kidney

The kidney is the principal organ responsible for klotho production and mediates many of its effects [28-30]. In aging rodents, serum creatinine levels are elevated, and renal klotho expression is decreased [31]. In individuals with chronic kidney disease, there is a deficiency of klotho, both locally and systemically. The decrease in klotho levels can serve as a sign of disease progression and is linked to the development of chronic kidney disease (CKD), including renal fibrosis, declining kidney function, and cardiovascular dysfunction [29,32-35]. Klotho's systemic availability and endocrine signaling are affected by its reduction in the kidneys [29]. Klotho has been shown to reduce renal fibrosis by suppressing TGF- β signaling and mitigating organ senescence by reducing p21-cip1 mRNA levels [36]. Systemic klotho treatment has been found to improve uremic complications in animal models of CKD and may be a promising strategy to prevent, retard, and decrease comorbidities in CKD [32,37,38]. Klotho is also an important regulator of vitamin D metabolism and blood phosphate levels, directly associated with aging process [28,39]. In addition, klotho regulates calcium homeostasis with a direct effect on kidney calcium reabsorption [40, 41]. Although the exact mechanism of action of klotho is not fully understood, klotho treatment has been shown to significantly reduce both renal and aortic calcium deposits [36]. Klotho deficiency has also been linked to cardiac hypertrophy, vascular calcification, endothelial dysfunction, salt-sensitive hypertension, and renal damage due to inflammation and hyperparathyroidism secondary to CKD [30,42-46].

In studies involving mice with rhabdomyolysis-induced acute kidney injury, the use of bone marrow mesenchymal stem cells transfected with recombinant adenoviruses expressing the klotho gene enhanced recovery [47]. It has been proposed that klotho modulates compensatory renal hypertrophy after nephrectomy by suppressing the IGF-1 signaling pathway [48].

Klotho has been shown to reduce the severity of diabetic nephropathy in mice. Researchers have proposed that a decrease in the excretion of klotho in the urine may serve as an early indicator of diabetic nephropathy and administering klotho may have several positive effects on renal function

in this condition [49,50].

Heart

Research indicates that insufficient levels of klotho are connected to both essential and renovascular hypertension. In a prior study, it was determined that supplementing with klotho prevented the increase in blood pressure caused by high salt intake. Moreover, when administered via adeno-associated virus in mice, klotho supplementation proved effective in reducing blood pressure, suggesting a promising long-term treatment for the condition [51,52]. Klotho may also serve as a potential biomarker for the early detection and progression evaluation of hypertension. In a mouse model, klotho supplementation decreased blood pressure and improved renin-angiotensin system activity [53,54]. Klotho gene delivery, another strategy, has demonstrated positive effects on endothelial function and has been proposed as a possible therapeutic alternative for modulating vascular remodeling in arterial hypertension [55].

Low levels of klotho have been linked to hypertension during pregnancy, and its concentration in the placenta may help to predict preeclampsia risk [56-58].

Klotho is expressed in human arteries and Vascular Smooth Muscle Cells (VSMCs), and its knockdown results in VSMCs calcification in vitro [59]. This further supports the endothelial protective effects of klotho associated with its antioxidant activity [60]. Klotho regulates intracellular calcium levels by affecting the ejection rate, speed of contraction and relaxation, and alleviation of fibrosis and remodeling of the myocardium in a rat model of heart failure [61].

Klotho and TGF- β interaction results in decreased proliferation and hypertrophy of cardiomyocytes, as well as fibrosis of the cardiac connective tissue, with a clear impact on cardiac remodeling.

Klotho deficiency has been linked to several age-related vascular defects, including arterial dilatation, vascular calcification, an increased collagen-to-elastin ratio, endothelial dysfunction, inflammation, and hypertrophy of vascular smooth muscle cells [62,63]. Recent studies indicate a considerable reduction of up to 45% in the serum levels of klotho in individuals with hypertension and heightened vascular stiffness [61,64]. Overexpression of klotho ameliorates arterial medial calcification and endothelial dysfunction [65]. This protective action, in CKD and age-related associated decline, could be mediated by the regulation of phosphate and vitamin D, as well as a direct effect on vascular smooth muscle cells [63]. The increase in autophagy is another mechanism induced by

klotho to ameliorate calcification through multiple possible mechanisms, including IGF-1 and the Mammalian Target of Rapamycin (mTOR) phosphorylation [66]. The relationship between serum klotho levels and pulse pressure has been found to be inverse and independent, implying that klotho may be associated with arterial stiffness [67]. Klotho deficiency has also been linked to decreased arterial stiffening and subsequent hypertension through minor autophagic activity [68].

The anti-inflammatory properties of klotho play an essential role in preventing the production of pro-inflammatory cytokines in the peri-infarct regions. This was demonstrated by the administration of recombinant klotho, which significantly reduced apoptosis and intracellular reactive oxygen species in myocardial ischemia and reperfusion injury [69]. The release of klotho into the intercellular space by ischemic cardiac tissue suggests a protective mechanism. Furthermore, the elevated levels of serum Klotho in patients with heart failure may make it a promising biomarker for assessing heart injury [70,71].

Klotho has been proposed to have an anti-apoptotic effect as well as a positive effect on angiogenesis associated with its anti-senescence action [72-74].

Metabolic syndrome has been found to be a significant risk factor for cardiovascular disease and mortality, and studies have suggested that individuals with high klotho levels may have a reduced risk of developing this condition [75].

Research has also revealed an inverse association between klotho gene expression and hyperlipidemia [76].

Brain

In the brain, klotho is expressed by choroid plexus epithelial cells, and in the cerebellum, by Purkinje neurons. Klotho exerts a protective effect on hippocampal neurons against oxidative stress similar to that observed in the kidney [77]. Klotho promotes neurogenesis in the adult hippocampal area as well as hippocampal-dependent cognition, whereas decreased klotho expression leads to decreased hippocampal-dependent memory [78].

Klotho protein decreased with aging in the prefrontal cortex, cerebral cortex, and hippocampus [79].

It has been suggested that the level of circulating klotho, which does not pass through the Blood-Brain Barrier (BBB), serves as a clinical indicator of vascular cognitive impairment. This is because reduced circulating klotho levels have been found to correspond with the degree of deep white matter lesions in the brain, which are associated with cognitive decline [80]. The use of klotho fragments

in peripheral administration has been found to improve synaptic plasticity and cognitive function in aged mice. Additionally, increased serum klotho levels have been shown to positively impact intrinsic connectivity in key functional brain networks that are vulnerable to aging and Alzheimer's Disease (AD) [81,82]. A single subcutaneous administration of a low dose of klotho has been shown to enhance memory in nonhuman primates [83]. A possible explanation for these effects is that peripheral klotho may indirectly modulate brain function through signals that can cross the BBB [81]. The exact circuits by which systemic klotho influences brain function, health, and aging remain to be determined.

It has shown that rats exposed to chronic unpredictable stress exhibit reduced expression of klotho in the choroid plexus, along with other proteins [84].

The pretreatment of neurons with klotho has been shown to prevent the harmful effects of amyloid- β and glutamate, which are associated with AD pathogenesis [85]. Klotho levels in the cerebrospinal fluid are lower in patients with AD and older adults than in younger adults. [86] In aged mice, overexpression of klotho in the brain and serum improved amyloid- β clearance and cognitive deficits by reducing neuronal and synaptic loss [87]. Klotho also reduced oxidative stress in the brain, which is associated with sporadic AD [88]. Klotho serum levels are known to be correlated with cerebrospinal fluid levels and are predictive of cognitive function. Furthermore, serum klotho levels are highly reflective of cerebrospinal fluid levels, making klotho an important biomarker for cognitive health and neurodegeneration [89].

Klotho stimulates oligodendrocyte maturation and plays a crucial role in myelin biology, making it a promising therapeutic target for protecting brain myelin against age-dependent changes and for promoting repair in multiple sclerosis. Additionally, it enhances myelination in the central nervous system, which protects against age-associated demyelination and other neurological diseases. [90] Myelination is highly dependent on oligodendrocyte mitochondrial function and is strongly enhanced by klotho activity.

Klotho has also been proposed as a potential protective factor against retinopathy in patients with type 2 diabetes [91]. A reduction in intraocular klotho level is associated with oxidative stress, inflammation, and the development of age-related macular degeneration [92]. Klotho may also play a role in hearing function and auditory disorders by modulating endolymph composition [93]. Klotho protein in the inner ear may potentially delay the onset of age-related hearing loss and support auditory capacity [94].

The significant influence of klotho on neuropsychiatric disorders further highlights its significance in nervous system function. Prolonged mental stress is linked to hastened aging, untimely sickness, and mortality. The levels of klotho in chronic stress are lower than those in low-stress conditions. Similar results have been reported for patients with depressive symptoms. It has been suggested that klotho reduction plays a role in the pathogenesis of depression, as its overexpression produces an antidepressant effect in normal mice and ameliorates behavioral responses in susceptible mice [95]. Klotho can be considered as a potential marker of psychological health that can monitor or predict the evolution of stress and depression [96]. Klotho is elevated in the cerebrospinal fluid of elderly patients with depression undergoing Electroconvulsive Therapy (ECT). This effect, which is similar to that observed in vitro when cells are electro-stimulated, has been linked to the modulation of neuroinflammatory processes that contribute to the antidepressant effects of ECT [97].

Plasma levels of klotho may serve as a promising candidate for a novel biomarker for sexual desire and function [98]. Noting its potential influence on male sexual activity, it is essential to recognize that the klotho gene has previously been associated with the occurrence of priapism [99].

Mothers of children with autism spectrum disorder under high chronic stress exhibit lower levels of klotho than low-stress mothers of typically developed children. Within the stressed group, those reporting more depressive symptoms had even lower klotho levels than the low-stress participants. These findings imply that klotho is involved in the relationship between stress and depression and may have a therapeutic role [96]. In a similar scope, *Gao et al.* proposed that klotho could serve as a connection between depression and dementia by regulating oxidative stress and inflammation [100].

It has been suggested that klotho may be implicated in schizophrenia pathogenesis, with increased klotho potentially acting as a compensatory factor to preserve cognitive function in individuals with this condition [101].

Lung

The anti-inflammatory properties of klotho have a substantial positive impact on the respiratory system.

Klotho has been characterized as a protective factor against oxidative stress in lung disease [1,102]. Studies suggest that individuals suffering from Chronic Obstructive Pulmonary Disease (COPD) and those who are exposed to ozone or cigarette smoke tend to have decreased levels of klotho in their airways [102, 103]. In a large sample population, it was

detected that serum klotho was lower in current smokers than in nonsmokers and quitters [104]. The protective effect of klotho on paraquat-induced lung injury has been observed as further evidence of its local anti-inflammatory activity and modulation of mitochondria-dependent apoptosis [105]. Klotho secretion inhibits Interleukin-8 (IL-8) secretion in cystic fibrosis airway epithelium, acting as an endocrine and local anti-inflammatory agent [106].

In a mouse model of chronic asthma induced by ovalbumin and utilizing BEAs-2B human bronchial epithelial cells, the klotho protein was discovered to be vital for suppressing fibrosis related to persistent airway diseases [107].

Klotho levels have also been found to be reduced in individuals with obstructive sleep apnea, and this reduction may contribute to the systemic inflammation associated with the condition [108].

Bone

Klotho plays a role in mouse limb development, chondrocyte differentiation, and cartilage formation, as well as in transient expression that occurs during in vitro chondrogenic differentiation of mesenchymal stem cells. The presence of senescent chondrocytes is a hallmark of age-related damage to the articular cartilage. In a mouse model of osteoarthritis, intra-articular gene transfer of klotho was shown to delay cartilage senescence and degradation [109].

Preliminary results showed that plasma klotho concentration was an independent predictor of changes in knee strength over time in older adults [110].

Altered spatial distribution of osteocytes and bone matrix proteins, as well as accelerated aging in bone cells, was observed in klotho-deficient mice [111]. In the opposite manner, higher serum klotho levels result in a lower incidence of osteoporosis in postmenopausal women [112].

Skin

Deficient klotho mice exhibited a noticeably slower rate of wound healing, accompanied by a decrease in collagen deposition and signs of skin deterioration that resembled age-related deficits in collagen 1 and 3 [113].

It has been hypothesized that klotho may function as a regulator of human hair growth and the hair cycle. Klotho is expressed in human hair follicles and its expression decreases with age. Klotho was found to extend human hair growth, whereas its inhibition had the opposite effect, promoting the onset of catagen [114].

Muscle

Klotho plays a crucial role in regulating muscle

physiology and is associated with the natural muscle dysfunction associated with aging. Exercise-induced klotho has been reported in several studies to reverse or delay muscle regression, leading to its classification as a novel ergonomic agent. Antioxidant protection and antifibrotic and anti-inflammatory mechanisms are believed to be key factors in the beneficial effects of klotho on muscle tissue [115].

Klotho has been found to be positively correlated with muscle strength and negatively correlated with osteoporosis, frailty, disability, and mortality, as indicated in a systematic review [116]. Furthermore, physical activity typically results in an increase in klotho levels and its deficiency has a significant impact on muscle strength and endurance in mouse models; this information is relevant for understanding the causes and consequences of age-related muscle decline and conditions, including sarcopenia [117]. Muscle stem cells are significantly decreased in klotho hypomorphic mice, and their function is altered due to the loss of klotho expression in vitro and in vivo. Klotho seems to exert its influence by suppressing Wnt signaling in aged stem cells, thereby facilitating the transformation of satellite cells into a myogenic lineage and diminishing fibrosis [118]. Klotho secretion increases after muscle damage as an apparent reparative mechanism that is reduced by aging. Consistent with this finding, klotho appears to be a fundamental mediator of skeletal muscle regeneration.

THERAPEUTICAL KLOTHO INDUCTION

Klotho administration or the use of genetic therapies have been effective in modulating klotho expression in various animal models. However, translating this into a human application is a complex and time-consuming process. Alternatively, natural inducers, such as exercise, lifestyle changes, energy stimulation, or plant and pharmaceutical agents, offer promising prospects for klotho application in human medicine.

It is reported that even short periods of moderate-intensity training can lead to an increase in klotho concentrations [119]. In rats, moderate aerobic training has been shown to attenuate aging-induced pathological cardiac hypertrophy, in part by restoring klotho levels, reducing oxidative stress, and decreasing the phosphorylation of ERK1/2, P38, and fibrosis [120]. The levels of klotho vary depending on exercise type and duration, but its inductive effect is undeniable [115]. The minor risk of chronic diseases seemingly associated with elite-level athletes' activities appears to be evidence of the antiaging effect of aerobic activity, and that klotho expression is associated with muscular contraction [121]. According to recent research,

klotho has been implicated as a key factor in the body's recovery process following strenuous exercise [122].

It is crucial to explore alternative methods for promoting klotho production in patients who are confined to bed for extended periods or have movement restrictions due to pathology. Passive mobilization or physical stimulation, such as electrical muscle stimulation, can potentially mitigate the negative effects of immobilization on klotho production. Previous research has shown that in vitro electrical stimulation of different cell types can increase klotho transcription (unpublished data). Electricity can stimulate the production of myogenic proteins, as previously observed for the in vitro induction of Follistatin (FST) [123].

Klotho levels have been strongly associated with lifestyle and stress. For instance, sleep quality has an impact on klotho plasma levels, making it a potential means of preventing age-related decline [124].

Natural geroprotectors, such as curcumin, ginseng, and resveratrol, have been shown to induce klotho expression in animal models [18,125]. Similar activity has been suggested for astaxanthin, which slows brain aging [126]. Ligustilide, a substance found in nature with neuro-anti-inflammatory properties that can cross the blood-brain barrier, has an effect similar to that of IGF-1 and cytokines. It increases the production of klotho by inducing the expression of α -secretases, and has shown a protective effect in a mouse model of AD [18,127].

Studies have shown that Vitamin D upregulates klotho transcription [128]. A strong positive correlation has been reported between dietary vitamin C consumption and serum klotho concentrations in the general adult population [129]. An antioxidant diet can also achieve similar results. A similar effect involves the inhibition of the mTOR, which activation suppresses klotho gene expression [130]. Peroxisome Proliferator-Activated Receptor- γ (PPAR γ) induces klotho expression [131]. Aldosterone and angiotensin II decrease klotho production. Statins stimulate klotho gene transcription diminishing angiotensin II response. Valsartan use in hypertensive patients is associated with an increase in soluble klotho levels [132,133].

KLOTHO AND REGENERATIVE MEDICINE

The use of klotho in tissue engineering and regenerative medicine remains limited. Given its ability to modulate the anti-aging process, klotho is a promising candidate for promoting tissue repair and regeneration. Its diverse actions on various cell types can aid tissue restoration, especially in individuals with chronic diseases, limited mobility, or

advanced age [134]. Klotho exerts various effects with significant implications in regenerative medicine, such as inhibition of apoptosis, modulation of inflammation, and stimulation of stem cell populations (Figure 1). Its therapeutic action can be achieved using biomaterials, cell therapies, or a combination of both strategies.

Klotho, when administered through a nanomaterial

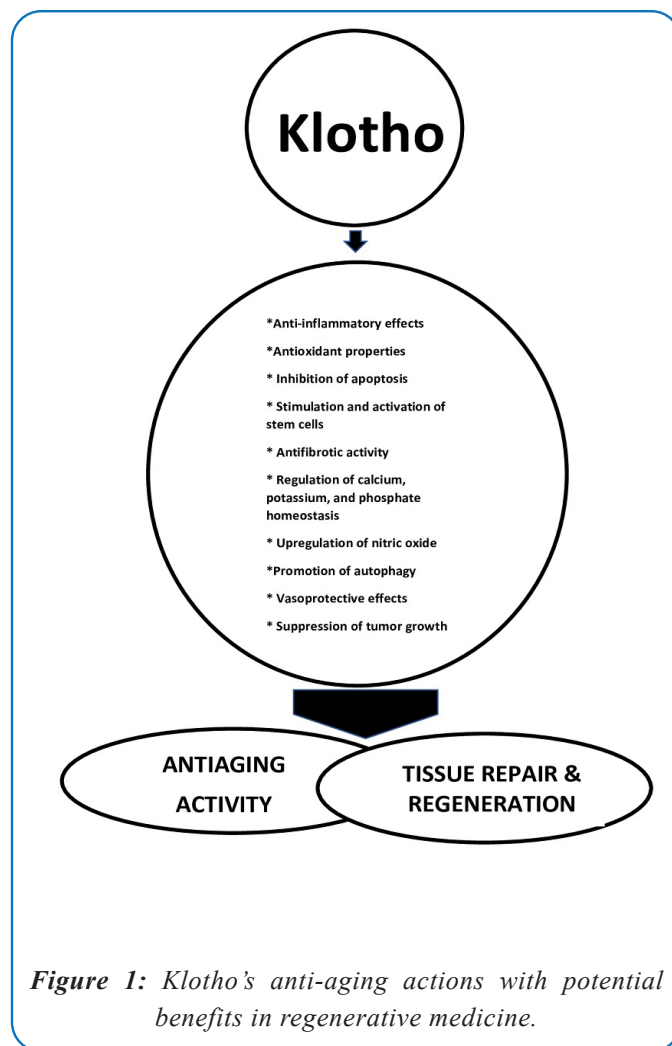


Figure 1: Klotho's anti-aging actions with potential benefits in regenerative medicine.

compound, has shown promising therapeutic effects on diabetic foot and atherosclerotic ulcers [135]. The incorporation of klotho within a bio-active reinforced hydrogel that contains Mesenchymal Stem Cells (MSC) resulted in a decrease in oxidative stress and local inflammation, thereby enhancing the regenerative effect on myotendinous junction injuries [136]. A portion of the protective effect exerted by klotho on stem cells is attributed to the restoration of mitochondrial function, which is coincident with the observed cell mitochondrial dysfunction in impaired muscle regeneration associated with klotho's age-related decline [137]. Klotho seems to play a significant role in the neural differentiation of bone marrow-derived MSCs, as cell changes are linked to an

increase in the protein [138].

Moreover, non-medicinal klotho inducers, such as electrical stimulation, appear to be potentially beneficial modulators of the regenerative process in the above-mentioned cases.

CONCLUSION

Klotho's potential to improve the quantity and functionality of stem cell populations and to manage processes such as inflammation, autophagy, and fibrosis undoubtedly displays therapeutic benefits. When utilized in regenerative procedures, this advantage can provide benefits that are yet to be fully understood. Many therapeutic targets in regenerative medicine and tissue engineering

are associated with conditions present in senescence. However, the use of klotho in regenerative medicine is still limited. The favorable effects of klotho and other anti-aging agents in regenerative medicine across various organs and systems, regardless of patient age, concurrent pathologies, physical conditions, or exercise capacity, present promising alternatives in a rapidly developing and broadly applicable field.

DECLARATION

The authors have issued patents on klotho's bioelectric expression in the USA and in Europe for klotho's expression in mesenchymal stem cells

References

1. Kuro-o M, Matsumura Y, Aizawa H, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*. 1997;390(6655):45-51.
2. Matsumura Y, Aizawa H, Shiraki-Iida T, Nagai R, Kuro-o M, Nabeshima Y. Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. *Biochem Biophys Res Commun*. 1998;242(3):626-630.
3. Kurosu H, Ogawa Y, Miyoshi M, et al. Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem*. 2006;281(10):6120-6123.
4. Kuro-O M. The Klotho proteins in health and disease. *Nat Rev Nephrol*. 2019;15(1):27-44.
5. Kilkenny DM, Rocheleau JV. The FGF21 Receptor Signaling Complex: Klotho beta, FGFR1c, and Other Regulatory Interactions. *Vitam Horm*. 2016;101:17-58.
6. Hu MC, Shiizaki K, Kuro-o M, Moe OW. Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol*. 2013;75:503-533.
7. Ito S, Kinoshita S, Shiraiishi N, et al. Molecular cloning and expression analyses of mouse betaklotho, which encodes a novel Klotho family protein. *Mech Dev*. 2000;98(1-2):115-119.
8. Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone Klotho. *Science*. 2005;309(5742):1829-1833.
9. Espuch-Oliver A, Vázquez-Lorente H, Jurado-Fasoli L, et al. Reference Values of Soluble alpha-Klotho Serum Levels Using an Enzyme-Linked Immunosorbent Assay in Healthy Adults Aged 18-85 Years. *J Clin Med*. 2022;11(9):2415.
10. Pavlatou MG, Remaley AT, Gold PW. Klotho: a humeral mediator in CSF and plasma that influences longevity and susceptibility to multiple complex disorders, including depression. *Transl Psychiatry*. 2016;6(8):e876.
11. Imura A, Iwano A, Tohyama O, et al. Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. *FEBS Lett*. 2004;565(1-3):143-147.
12. Dalton GD, Xie J, An SW, Huang CL. New Insights into the Mechanism of Action of Soluble Klotho. *Front Endocrinol* (Lausanne). 2017;8:323.
13. Xu Y, Sun Z. Molecular basis of Klotho: from gene to function in aging. *Endocr Rev*. 2015;36(2):174-193.
14. Hu MC, Shi M, Zhang J, et al. Renal Production, Uptake, and Handling of Circulating alpha Klotho. *J Am Soc Nephrol*. 2016;27(1):79-90.
15. Marsell R, Krajisnik T, Göransson H, et al. Gene expression analysis of kidneys from transgenic mice expressing fibroblast growth factor-23. *Nephrol Dial Transplant*. 2008;23(3):827-833.
16. Chen P, Tang Y, Luo L, Chen H, He X. Lower serum Klotho level and higher systemic immune-inflammation index: an inverse correlation. *BMC Geriatr*. 2023;23(1):650.
17. Zhao J, Jia Y, Zeng L, Huang H, Liang G, Hong K, et al. Interplay of Systemic Immune-Inflammation Index and Serum Klotho Levels: Unveiling a New Dimension in Rheumatoid Arthritis Pathology. *Int J Med Sci*. 2024; 21(2):396-403.
18. Prud'homme GJ, Kurt M, Wang Q. Pathobiology of the Klotho Antiaging Protein and Therapeutic Considerations. *Front Aging*. 2022;3:931331.
19. Daneshgar N, Dai DF. ROS, Klotho and mTOR in cardiorenal aging. *Aging (Albany NY)*. 2020;12(20):19830-19831.
20. Neyra JA, Hu MC, Moe OW. Klotho in Clinical Nephrology: Diagnostic and Therapeutic Implications. *Clin J Am Soc Nephrol*. 2020;16(1):162-176.
21. Nabeshima Y, Imura H. alpha-Klotho: a regulator that integrates calcium homeostasis. *Am J Nephrol*. 2008;28(3):455-464.
22. Rubinek T, Wolf I. The Role of Alpha-Klotho as a Universal Tumor Suppressor. *Vitam Horm*. 2016;101:197-214.
23. Abramovitz L, Rubinek T, Ligumsky H, et al. KL1 internal repeat mediates klotho tumor suppressor activities and inhibits bFGF and IGF-I signaling in pancreatic cancer. *Clin Cancer Res*. 2011;17(13):4254-4266.
24. Ligumsky H, Rubinek T, Merenbakh-Lamin K, et al. Tumor Suppressor Activity of Klotho in Breast Cancer Is Revealed by Structure-Function Analysis. *Mol Cancer Res*. 2015;13(10):1398-1407.
25. Tang X, Wang Y, Fan Z, et al. Klotho: a tumor suppressor and modulator of the Wnt/beta-catenin pathway in human hepatocellular carcinoma.

- Lab Invest.* 2016;96(2):197-205.
26. Wolf I, Levanon-Cohen S, Bose S, et al. Klotho: a tumor suppressor and a modulator of the IGF-1 and FGF pathways in human breast cancer. *Oncogene.* 2008;27(56):7094-7105.
 27. Olauson H, Mencke R, Hillebrands JL, Larsson TE. Tissue expression and source of circulating alpha Klotho. *Bone.* 2017;100:19-35.
 28. Kalaitzidis RG, Duni A, Siamopoulos KC. Klotho, the Holy Grail of the kidney: from salt sensitivity to chronic kidney disease. *Int Urol Nephrol.* 2016;48(10):1657-1666.
 29. Lindberg K, Amin R, Moe OW, et al. The kidney is the principal organ mediating klotho effects. *J Am Soc Nephrol.* 2014;25(10):2169-2175.
 30. Hu MC, Kuro-o M, Moe OW. Klotho and chronic kidney disease. *Contrib Nephrol.* 2013;180:47-63.
 31. Zuo Z, Lei H, Wang X, Wang Y, Sonntag W, Sun Z. Aging-related kidney damage is associated with a decrease in klotho expression and an increase in superoxide production. *Age (Dordr).* 2011;33(3):261-274.
 32. Hu MC, Shi M, Zhang J, Quiñones 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):1240-1251.
 33. Satoh M, Nagasu H, Morita Y, Yamaguchi TP, Kanwar YS, Kashiwara N. Klotho protects against mouse renal fibrosis by inhibiting Wnt signaling. *Am J Physiol Renal Physiol.* 2012;303(12):1641-1651.
 34. Guan X, Nie L, He T, et al. Klotho suppresses renal tubulo-interstitial fibrosis by controlling basic fibroblast growth factor-2 signalling. *J Pathol.* 2014;234(4):560-572.
 35. Sugiura H, Yoshida T, Shiohira S, et al. Reduced Klotho expression level in kidney aggravates renal interstitial fibrosis. *Am J Physiol Renal Physiol.* 2012;302(10):1252-1264.
 36. Chen TH, Kuro-O M, Chen Chet al. The secreted Klotho protein restores phosphate retention and suppresses accelerated aging in Klotho mutant mice. *Eur J Pharmacol.* 2013;698(1-3):67-73.
 37. Doi S, Zou Y, Togao O, et al. Klotho inhibits transforming growth factor-beta1 (TGF-beta1) signaling and suppresses renal fibrosis and cancer metastasis in mice. *J Biol Chem.* 2011;286(10):8655-8665.
 38. Neyra JA, Hu MC. Potential application of klotho in human chronic kidney disease. *Bone.* 2017;100:41-49.
 39. Kuro-o M. Klotho and aging. *Biochim Biophys Acta.* 2009;1790(10):1049-1058.
 40. Cha SK, Ortega B, Kurosu H, Rosenblatt KP, Kuro-O M, Huang CL. Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. *Proc Natl Acad Sci U S A.* 2008;105(28):9805-9810.
 41. Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG. The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science.* 2005;310(5747):490-493.
 42. Faul C, Amaral AP, Oskoueï B, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest.* 2011;121(11):4393-4408.
 43. Hu MC, Shi M, Zhang J, et al. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol.* 2011;22(1):124-136.
 44. Hu MC, Shi M, Cho HJ, et al. Klotho and phosphate are modulators of pathologic uremic cardiac remodeling. *J Am Soc Nephrol.* 2015;26(6):1290-1302.
 45. Xie J, Yoon J, An SW, Kuro-o M, Huang CL. Soluble Klotho Protects against Uremic Cardiomyopathy Independently of Fibroblast Growth Factor 23 and Phosphate. *J Am Soc Nephrol.* 2015;26(5):1150-1160.
 46. Zhou X, Chen K, Lei H, Sun Z. Klotho gene deficiency causes salt-sensitive hypertension via monocyte chemotactic protein-1/CC chemokine receptor 2-mediated inflammation. *J Am Soc Nephrol.* 2015;26(1):121-132.
 47. Ni W, Zhang Y, Yin Z. The protective mechanism of Klotho gene-modified bone marrow mesenchymal stem cells on acute kidney injury induced by rhabdomyolysis. *Regen Ther.* 2021;18:255-267.
 48. Nagasu H, Satoh M, Kuwabara A, et al. Overexpression of klotho protein modulates uninephrectomy-induced compensatory renal hypertrophy by suppressing IGF-I signals. *Biochem Biophys Res Commun.* 2011;407(1):39-43.
 49. Typiak M, Kulesza T, Rachubik P, et al. Role of Klotho in Hyperglycemia: Its Levels and Effects on Fibroblast Growth Factor Receptors, Glycolysis, and Glomerular Filtration. *Int J Mol Sci.* 2021;22(15):7867.
 50. Wang Q, Ren D, Li Y, Xu G. Klotho attenuates diabetic nephropathy in db/db mice and ameliorates high glucose-induced injury of human renal glomerular endothelial cells. *Cell Cycle.* 2019;18(6-7):696-707.
 51. Kawarazaki W, Mizuno R, Nishimoto M, et al. Salt causes aging-associated hypertension via vascular Wnt5a under Klotho deficiency. *J Clin Invest.* 2020;130(8):4152-4166.
 52. Wang Y, Sun Z. Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage. *Hypertension.* 2009;54(4):810-817.
 53. Takenaka T, Kobori H, Miyazaki T, et al. Klotho protein supplementation reduces blood pressure and renal hypertrophy in db/db mice, a model of type 2 diabetes. *Acta Physiol (Oxf).* 2019;225(2):e13190.
 54. Yang T, Xu C. Physiology and Pathophysiology of the Intrarenal Renin-Angiotensin System: An Update. *J Am Soc Nephrol.* 2017;28(4):1040-1049.
 55. Saito Y, Nakamura T, Ohyama Y, et al. In vivo klotho gene delivery protects against endothelial dysfunction in multiple risk factor syndrome. *Biochem Biophys Res Commun.* 2000;276(2):767-772.
 56. Uzun Cilindir I, Varol F, Gurkan H, et al. Placental and serum levels of human Klotho in severe preeclampsia: A potential sensitive biomarker. *Placenta.* 2019;85:49-55.
 57. Loichinger MH, Towner D, Thompson KS, Ahn HJ, Bryant-Greenwood GD. Systemic and placental alpha-klotho: Effects of preeclampsia in the last trimester of gestation. *Placenta.* 2016;41:53-61.
 58. Kanbay M, Demiray A, Afsar B, et al. Role of Klotho in the Development of Essential Hypertension. *Hypertension.* 2021;77(3):740-750.
 59. Lim K, Lu TS, Molostvov G, et al. Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation.* 2012;125(18):2243-2255.
 60. Yao Y, Wang Y, Zhang Y, Liu C. Klotho ameliorates oxidized low density lipoprotein (ox-LDL)-induced oxidative stress via regulating LOX-1 and PI3K/Akt/eNOS pathways. *Lipids Health Dis.* 2017;16(1):77.
 61. Tyurenkov IN, Perfilova VN, Nesterova AA, Glinka Y. Klotho Protein and Cardio-Vascular System. *Biochemistry (Mosc).* 2021;86(2):132-145.
 62. Saito Y, Yamagishi T, Nakamura T, et al. Klotho protein protects against endothelial dysfunction. *Biochem Biophys Res Commun.* 1998;248(2):324-329.

63. Shroff R, Shanahan CM. Klotho: an elixir of youth for the vasculature? *J Am Soc Nephrol*. 2011;22(1):5-7.
64. Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. *J Am Coll Cardiol*. 2011;57(14):1511-1522.
65. Mencke R, Hillebrands JL; NIGRAM consortium. The role of the anti-ageing protein Klotho in vascular physiology and pathophysiology. *Ageing Res Rev*. 2017;35:124-146.
66. Li L, Liu W, Mao Q, et al. Klotho Ameliorates Vascular Calcification via Promoting Autophagy. *Oxid Med Cell Longev*. 2022;2022:7192507.
67. Alkalbani M, Prabhu G, Lagbo J, Qayyum R. Serum Klotho and pulse pressure; insight from NHANES. *Int J Cardiol*. 2022;355:54-58.
68. Chen K, Sun Z. Autophagy plays a critical role in Klotho gene deficiency-induced arterial stiffening and hypertension. *J Mol Med (Berl)*. 2019;97(11):1615-1625.
69. Myung J, Beom JH, Kim JH, et al. Recombinant Klotho Protein Ameliorates Myocardial Ischemia/Reperfusion Injury by Attenuating Sterile Inflammation. *Biomedicines*. 2022;10(4):894.
70. Olejnik A, Krzywonos-Zawadzka A, Banaszkiwicz M, Bil-Lula I. Klotho protein contributes to cardioprotection during ischaemia/reperfusion injury. *J Cell Mol Med*. 2020;24(11):6448-6458.
71. Paula RS, Souza VC, Machado-Silva W, et al. Serum Klotho (but not haplotypes) associate with the post-myocardial infarction status of older adults. *Clinics (Sao Paulo)*. 2016;71(12):725-732.
72. Ikushima M, Rakugi H, Ishikawa K, et al. Anti-apoptotic and anti-senescence effects of Klotho on vascular endothelial cells. *Biochem Biophys Res Commun*. 2006;339(3):827-832.
73. Markiewicz M, Panneerselvam K, Marks N. Role of Klotho in migration and proliferation of human dermal microvascular endothelial cells. *Microvasc Res*. 2016;107:76-82.
74. Maekawa Y, Ohishi M, Ikushima M, et al. Klotho protein diminishes endothelial apoptosis and senescence via a mitogen-activated kinase pathway. *Geriatr Gerontol Int*. 2011 Oct;11(4):510-516.
75. Yuguang L, Chang Y, Chen N, et al. Serum klotho as a novel biomarker for metabolic syndrome: findings from a large national cohort. *Front Endocrinol (Lausanne)*. 2024;15:1295927.
76. Yan S, Luo W, Lei L, Zhang Q, Xiu J. Association between serum Klotho concentration and hyperlipidemia in adults: a cross-sectional study from NHANES 2007–2016. *Front Endocrinol*. 2023;14:1280873.
77. Cheng MF, Chen LJ, Niu HS, Yang TT, Lin KC, Cheng JT. Signals mediating Klotho-induced neuroprotection in hippocampal neuronal cells. *Acta Neurobiol Exp (Wars)*. 2015;75(1):60-71.
78. Salech F, Varela-Nallar L, Arredondo SB, et al. Local Klotho Enhances Neuronal Progenitor Proliferation in the Adult Hippocampus. *J Gerontol A Biol Sci Med Sci*. 2019;74(7):1043-1051.
79. Torbus-Paluszczak M, Bartman W, Adamczyk-Sowa M. Klotho protein in neurodegenerative disorders. *Neurol Sci*. 2018;39(10):1677-1682.
80. Kuriyama N, Ozaki E, Mizuno T, et al. Association between alpha-Klotho and Deep White Matter Lesions in the Brain: A Pilot Case Control Study Using Brain MRI. *J Alzheimers Dis*. 2018;61(1):145-155.
81. Leon J, Moreno AJ, Garay BI, et al. Peripheral Elevation of a Klotho Fragment Enhances Brain Function and Resilience in Young, Aging, and alpha-Synuclein Transgenic Mice. *Cell Rep*. 2017;20(6):1360-1371.
82. Yokoyama JS, Marx G, Brown JA, et al. Systemic klotho is associated with KLOTTHO variation and predicts intrinsic cortical connectivity in healthy human aging. *Brain Imaging Behav*. 2017;11(2):391-400.
83. Castner SA, Gupta S, Wang D, et al. Longevity factor klotho enhances cognition in aged nonhuman primates. *Nat Aging*. 2023;3(8):931-937.
84. Sathyanesan M, Girgenti MJ, Banasr M, et al. A molecular characterization of the choroid plexus and stress-induced gene regulation. *Transl Psychiatry*. 2012;2(7):e139.
85. Paroni G, Panza F, De Cosmo S, Greco A, Seripa D, Mazzoccoli G. Klotho at the Edge of Alzheimer's Disease and Senile Depression. *Mol Neurobiol*. 2019;56(3):1908-1920.
86. Semba RD, Moghekar AR, Hu J, et al. Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. *Neurosci Lett*. 2014;558:37-40.
87. Zhao Y, Zeng CY, Li XH, Yang TT, Kuang X, Du JR. Klotho overexpression improves amyloid-beta clearance and cognition in the APP/PS1 mouse model of Alzheimer's disease. *Ageing Cell*. 2020;19(10):e13239.
88. Adeli S, Zahmatkesh M, Tavosoidana G, Karimian M, Hassanzadeh G. Simvastatin enhances the hippocampal klotho in a rat model of streptozotocin-induced cognitive decline. *Prog Neuropsychopharmacol Biol Psychiatry*. 2017;72:87-94.
89. Kundu P, Zimmerman B, Quinn JF, et al. Serum Levels of alpha-Klotho Are Correlated with Cerebrospinal Fluid Levels and Predict Measures of Cognitive Function. *J Alzheimers Dis*. 2022;86(3):1471-1481.
90. Chen CD, Sloane JA, Li H, et al. The antiaging protein Klotho enhances oligodendrocyte maturation and myelination of the CNS. *J Neurosci*. 2013;33(5):1927-1939.
91. Ji B, Wei H, Ding Y, et al. Protective potential of klotho protein on diabetic retinopathy: Evidence from clinical and in vitro studies. *J Diabetes Investig*. 2020;11(1):162-169.
92. Ma Z, Liu J, Li J, Jiang H, Kong J. Klotho Levels are Decreased and Associated with Enhanced Oxidative Stress and Inflammation in the Aqueous Humor in Patients with Exudative Age-related Macular Degeneration. *Ocul Immunol Inflamm*. 2022;30(3):630-637.
93. Kamemori M, Ohyama Y, Kurabayashi M, Takahashi K, Nagai R, Furuya N. Expression of Klotho protein in the inner ear. *Hear Res*. 2002;171(1-2):103-110.
94. Yuan N, Qiu S, Wang Q, et al. Hearing analysis in heterozygous and homozygous klotho gene deficient mice. *J Otol*. 2018;13(4):131-134.
95. Wu HJ, Wu WN, Fan H, et al. Life extension factor klotho regulates behavioral responses to stress via modulation of GluN2B function in the nucleus accumbens. *Neuropsychopharmacology*. 2022;47(9):1710-1720.
96. Prather AA, Epel ES, Arenander J, et al. Longevity factor klotho and chronic psychological stress. *Transl Psychiatry*. 2015;5(6):e585.
97. Hoyer C, Sartorius A, Aksay SS, et al. Electroconvulsive therapy enhances the anti-ageing hormone Klotho in the cerebrospinal fluid of geriatric patients with major depression. *Eur Neuropsychopharmacol*. 2018;28(3):428-435.
98. Dote-Montero M, De-la-O A, Castillo MJ, Amaro-Gahete FJ. Predictors of Sexual Desire and Sexual Function in Sedentary Middle-Aged Adults: The Role of Lean Mass Index and S-Klotho Plasma Levels. The FIT-AGEING Study. *J Sex Med*. 2020;17(4):665-677.
99. Nolan VG, Baldwin C, Ma Q, et al. Association of single nucleotide polymorphisms in klotho with priapism in sickle cell anaemia. *Br J*

- Haematol.* 2005;128(2):266-272.
100. Gao X, Li Y, Sun Z, et al. Could alpha-Klotho Unlock the Key Between Depression and Dementia in the Elderly: from Animal to Human Studies. *Mol Neurobiol.* 2021;58(6):2874-2885.
 101. Xiong JW, Zhan JQ, Luo T, et al. Increased Plasma Level of Longevity Protein Klotho as a Potential Indicator of Cognitive Function Preservation in Patients With Schizophrenia. *Front Neurosci.* 2020;14:610.
 102. Richter B, Haller J, Haffner D, Leifheit-Nestler M. Klotho modulates FGF23-mediated NO synthesis and oxidative stress in human coronary artery endothelial cells. *Pflugers Arch.* 2016;468(9):1621-35.
 103. Li L, Wang Y, Gao W, et al. Klotho Reduction in Alveolar Macrophages Contributes to Cigarette Smoke Extract-induced Inflammation in Chronic Obstructive Pulmonary Disease. *J Biol Chem.* 2015;290(46):27890-900.
 104. Liu T, Song M, Li J, Zhao Y, Zhong W. Association between smoking, smoking cessation and serum alpha-klotho levels among American adults: National Health and Nutrition Examination Survey. *PLoS One.* 2024;19(3):e0300562.
 105. Zhang Z, Nian Q, Chen G, Cui S, Han Y, Zhang J. Klotho Alleviates Lung Injury Caused by Paraquat via Suppressing ROS/P38 MAPK-Regulated Inflammatory Responses and Apoptosis. *Oxid Med Cell Longev.* 2020;2020:1854206.
 106. Krick S, Baumlin N, Aller SP, et al. Klotho Inhibits Interleukin-8 Secretion from Cystic Fibrosis Airway Epithelia. *Sci Rep.* 2017;7(1):14388.
 107. Shin IS, Shin HK, Kim JC, Lee MY. Role of Klotho, an antiaging protein, in pulmonary fibrosis. *Arch Toxicol.* 2015;89(5):785-795.
 108. Pákó J, Kunos L, Mészáros M, et al. Decreased Levels of Anti-Aging Klotho in Obstructive Sleep Apnea. *Rejuvenation Res.* 2020;23(3):256-261.
 109. Chuchana P, Mausset-Bonnefont AL, Mathieu M, et al. Secreted alpha-Klotho maintains cartilage tissue homeostasis by repressing NOS2 and ZIP8-MMP13 catabolic axis. *Aging (Albany NY).* 2018;10(6):1442-1453.
 110. Semba RD, Ferrucci L, Sun K, et al. Low Plasma Klotho Concentrations and Decline of Knee Strength in Older Adults. *J Gerontol A Biol Sci Med Sci.* 2016;71(1):103-108.
 111. Suzuki H, Amizuka N, Oda K, et al. Histological evidence of the altered distribution of osteocytes and bone matrix synthesis in klotho-deficient mice. *Arch Histol Cytol.* 2005;68(5):371-381.
 112. Jiang J, Liu Q, Mao Y, et al. Klotho reduces the risk of osteoporosis in postmenopausal women: a cross-sectional study of the National Health and Nutrition Examination Survey (NHANES). *BMC Endocr Disord.* 2023;23(1):151.
 113. Yamashita K, Yotsuyanagi T, Yamauchi M, Young DM. Klotho mice: a novel wound model of aged skin. *Plast Reconstr Surg Glob Open.* 2014;2(1):e101.
 114. Jin XH, Pi LQ, Lee WS. Expression Pattern and Role of Klotho in Human Hair Follicles. *Ann Dermatol.* 2019;31(5):511-517.
 115. Arroyo E, Troutman AD, Moorthi RN, Avin KG, Coggan AR, Lim K. Klotho: An Emerging Factor With Ergogenic Potential. *Front Rehabil Sci.* 2022;2:807123.
 116. Veronesi F, Borsari V, Cherubini A, Fini M. Association of Klotho with physical performance and frailty in middle-aged and older adults: A systematic review. *Exp Gerontol.* 2021;154:111518.
 117. Phelps M, Pettan-Brewer C, Ladiges W, Yablonka-Reuveni Z. Decline in muscle strength and running endurance in klotho deficient C57BL/6 mice. *Biogerontology.* 2013;14(6):729-739.
 118. Ahrens HE, Huettemeister J, Schmidt M, Kaether C, von Maltzahn J. Klotho expression is a prerequisite for proper muscle stem cell function and regeneration of skeletal muscle. *Skelet Muscle.* 2018;8(1):20.
 119. Middelbeek RJW, Motiani P, Brandt N, et al. Exercise intensity regulates cytokine and klotho responses in men. *Nutr Diabetes.* 2021;11(1):5.
 120. Baghaiee B, Karimi P, Siahkouchian M, Pescatello LS. Moderate aerobic exercise training decreases middle-aged induced pathologic cardiac hypertrophy by improving Klotho expression, MAPK signaling pathway, and oxidative stress status in Wistar rats. *Iran J Basic Med Sci.* 2018;21(9):911-919.
 121. Paffenbarger, RS. A history of physical activity, cardiovascular health and longevity: the scientific contributions of Jeremy N Morris, DSc, DPH, FRCP. *Int J Epidemiol.* 2001. 30(5):1184-1192.
 122. Rao Z, Zheng L, Huang H, Feng Y, Shi R. alpha-Klotho Expression in Mouse Tissues Following Acute Exhaustive Exercise. *Front Physiol.* 2019;10:1498.
 123. Genovese JA, Spadaccio C, Ravello HG, Toyoda Y, Patel AN. Electrostimulated bone marrow human mesenchymal stem cells produce follistatin. *Cytotherapy.* 2009;11(4):448-456.
 124. Mochón-Benguigui S, Carneiro-Barrera A, Castillo MJ, Amaro-Gahete FJ. Is Sleep Associated with the S-Klotho Anti-Aging Protein in Sedentary Middle-Aged Adults? The FIT-AGEING Study. *Antioxidants (Basel).* 2020;9(8):738.
 125. Lim SW, Shin YJ, Luo K, et al. Ginseng increases Klotho expression by FoxO3-mediated manganese superoxide dismutase in a mouse model of tacrolimus-induced renal injury. *Aging (Albany NY).* 2019;11(15):5548-5569.
 126. Sorrenti V, Davinelli S, Scapagnini G, Willcox BJ, Allsopp RC, Willcox DC. Astaxanthin as a Putative Geroprotector: Molecular Basis and Focus on Brain Aging. *Mar Drugs.* 2020;18(7):351.
 127. Kuang X, Chen YS, Wang LF, et al. Klotho upregulation contributes to the neuroprotection of ligustilide in an Alzheimer's disease mouse model. *Neurobiol Aging.* 2014;35(1):169-178.
 128. Forster RE, Jurutka PW, Hsieh JC, et al. Vitamin D receptor controls expression of the anti-aging klotho gene in mouse and human renal cells. *Biochem Biophys Res Commun.* 2011;414(3):557-562.
 129. Wang Y, Wu M, Xiang L, et al. Association of Dietary Vitamin C Consumption with Serum Klotho Concentrations. *Foods.* 2023; 12(23):4230.
 130. Zhao Y, Zhao MM, Cai Y, et al. Mammalian target of rapamycin signaling inhibition ameliorates vascular calcification via Klotho upregulation. *Kidney Int.* 2015 Oct;88(4):711-721.
 131. Zhang H, Li Y, Fan Y, et al. Klotho is a target gene of PPAR-gamma. *Kidney Int.* 2008;74(6):732-739.
 132. Narumiya H, Sasaki S, Kuwahara N, et al. HMG-CoA reductase inhibitors up-regulate anti-aging klotho mRNA via RhoA inactivation in IMCD3 cells. *Cardiovasc Res.* 2004;64(2):331-136.
 133. Karalliedde J, Maltese G, Hill B, Viberti G, Gnudi L. Effect of renin-angiotensin system blockade on soluble Klotho in patients with type 2 diabetes, systolic hypertension, and albuminuria. *Clin J Am Soc Nephrol.* 2013;8(11):1899-1905.
 134. Ullah M, Sun Z. Stem cells and anti-aging genes: double-edged sword-do the same job of life extension. *Stem Cell Res Ther.* 2018;9(1):3.

135. Tang R, Zhao G, Wang Y, Zhang R. The effect of Klotho protein complexed with nanomaterials on bone mesenchymal stem cell performance in the treatment of diabetic ischaemic ulcer. *IET Nanobiotechnol.* 2022;16(9):316-324.
136. Sun Y, Sheng R, Cao Z, et al. Bioactive fiber-reinforced hydrogel to tailor cell microenvironment for structural and functional regeneration of myotendinous junction. *Sci Adv.* 2024;10(17):eadm7164.
137. Sahu A, Mamiya H, Shinde SN, et al. ge-related declines in alpha-Klotho drive progenitor cell mitochondrial dysfunction and impaired muscle regeneration. *Nat Commun.* 2018;9:4859.
138. Naeeni B, Taha MF, Emami Aleagha MS, Allameh A. The expression of anti-aging protein Klotho is increased during neural differentiation of bone marrow-derived mesenchymal stem cells. *Cell Biochem Funct.* 2023;41(2):243-253.