Libyan Journal of Medical Research

www.ljmr.ly/

eISSN:2413-6096

Review Article

Klotho gene transfection into mesenchymal stem cells as a potential therapy for kidney disease

Zakya Shoub Elshari

Department of Immunology of Experimental Medicine, Istanbul
Istanbul University / Turkey
College of Medical Technology, Derna, Libya

Corresponding Author: Zakya Shoub Elshari, e-mail. Zakyashoub@gmail.com

Received:25/04/2025Accepted:28/06/2025Published:03/07/2025,DOI:https://doi.org/10.54361/LJMR.19.2.04

ABSTRACT:

Purpose: Chronic kidney disease (CKD) is a progressive condition associated with high morbidity and mortality. Traditional therapeutic strategies primarily aim to manage symptoms and slow disease progression, but often fail to reverse kidney damage. The mesenchymal stem cells (MSCs), known for their regenerative and immunomodulatory capacities, have emerged as promising candidates for renal repair. Meanwhile, the Klotho gene, recognized as an anti-aging and nephroprotective gene, is markedly downregulated in CKD. This review conducted the synergistic potential of combining MSC therapy with Klotho gene transfection. By enhancing MSCs with Klotho, their reparative effects, such as anti-fibrotic, anti-inflammatory, and antioxidant properties, can be boosted. **Materials and Methods**: the search process covered over 50 peer-reviewed articles and research papers, about the Mesenchymal stem cell therapy in kidney repair disease with different transfection protocols, and the same as the klotho gene, with an understanding of the therapeutic role. However, all databases in this review were estimated by PubMed and Google Scholar with keywords. **Conclude**: the biology of the Klotho gene, MSC-based therapeutic mechanisms, current transfection techniques, preclinical studies, and translational challenges. This approach may offer a novel and more effective therapeutic avenue for kidney disease management.

Keywords: Klotho gene, mesenchymal stem cells, kidney therapy, chronic kidney disease, gene transfection, renal regeneration, anti-aging gene

How to cite this article: ELSHARI Z.S, Klotho gene transfection into mesenchymal stem cells as potential therapy for kidney disease

Libyan 19.2

INTRODUCTION:

For more than half a century, mesenchymal stem cells (MSCs) have been a widely used cell-based therapy approach. Moreover, MSCs are heterogeneous cells with adherent properties and fibroblast-like morphology. [1] MSCs are mature multipotent stromal cells that originate from the mesenchyme lineage. However, these cells have the ability to differentiate into different tissue types, such as osteocytes, chondrocytes, adipocytes, tenocytes, and myocytes, MSCs can also be isolated from various tissues, such as bone marrow mesenchymal stem cells (BMMSCs), umbilical cord (UCMSCs), and Adipose tissue (ADMSCs). According to the International Society for Cellular Therapy ISTC, MSCs must fulfill minimum criteria: (i) plasticity and adherence ability, (ii) obtain several positive markers for differentiation they must express the surface markers CD105, CD73 and CD90 but not CD45, CD34, CD14, CD19 and HLA-DR, (iii) transform in vitro into adipocytes, chondrocytes and osteocytes. [2]

The mesenchymal stem cell co-transfection gene has potential for kidney disease therapy. Nevertheless, it is still challenging because of the multifactorial effect, microenvironment factors, survival, and proliferation rate, and also phenotypic and genetic stability. However, the promising novel approach for klotho-mesenchymal transfection has been assessed. Likewise, it has been demonstrated in many kidney disorders and may enhance both mesenchymal cell and kidney function. Due to the interaction between mesenchymal stem cells (MSCs) and Klotho. [3] However, the administration of klotho by MSCs is interesting for high transfection efficiency via the klotho gene. whereas Klotho blocks Wnt activation, which negatively impacts stem cell survival and inhibits cell senescence. [4] It provides support for the immunomodulatory activity. Meanwhile, Klotho Inhibits NF-κB in vitro and in vivo (Guo et al., 2018), which is an inflammation inducer and reduction of inflammatory cytokines in kidney diseases. [5] On the other hand, MSCs reduce inflammation, oxidative stress, and fibrosis and upregulate the renin-angiotensin-aldosterone system. The novel therapeutic target for gene therapy, expression of the Klotho gene transfection into mesenchymal stem cells, is a promising therapeutic strategy for treating kidney diseases. Subsequently, the low level of soluble klotho in the circulation is a biomarker of early kidney dysfunction. However, the potential administration of klotho, either from exogenous or endogenous sources, may help to prevent or mitigate the chronic kidney disease (CKD) and Acute kidney injury (AKI). [6] In this review, we will discuss the therapeutic role of klotho and the obstacles to reliable methods for preclinical and clinical therapies approached for mesenchymal transfection of the klotho gene in kidney disease.

1. The role of Klotho protein and biological functions in the kidney.

Klotho protein was identified by Kuro-o et al as an ageing-suppressor gene. However, the Klotho gene was observed first in mice. [4] The deficiency of this protein or overexpression is associated with chronic renal diseases and fibrosis with vascular calcification and hyperphosphatemia, as well as diabetic nephropathy [7]. The klotho protein is produced at a high level and expressed by the kidneys via renal tubules. [9] There are two distinct functions of klotho, which in the proximal tubules, decreases the impairment of phosphate reabsorption. While in the distal tubule, it promotes Ca^{+2} (calcium) reabsorption. Furthermore, Klotho can exist as a membrane-bound coreceptor for fibroblast growth factor 23 (FGF23) [10]. or a soluble endocrine mediator with many functions. [11] A low level of soluble klotho is associated with high oxidative stress and inflammation, cardiovascular disease in CKD patients. [12] Meanwhile, there are several studies to improve kidney function by targeting different promoters of the klotho gene via DNA methylation to upregulate their expressions. But unfortunately, the therapeutic strategy remains unclear. [13]

The klotho protein is a hypo-morphic gene allele which was first observed in mice ($Kl^{kl/kl}$). [7] This protein is involved in human aging, and the expression of the klotho gene declines with age, reducing lifespan. [14] The insufficiency of klotho protein is associated with age-related diseases such as renal dysfunction, diabetes mellitus, and neurodegenerative disease. [15] Additionally, klotho has several biological functions including anti-inflammatory and antioxidant effects, preventing apoptosis and cell senescence activities, and preservation of stem cells.

Moreover, the klotho protein is linked with parathyroid hormone regulation by controlling the phosphate hemostasis and preventing hyperphosphatemia and tissue calcification, and also regulates 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] activity[16].Regarding studies in Mice, the NF-κB, a nuclear factor, is inflammatory promotor that is inhibited by Klotho via administrate the nicotinamide in mice, the active form of vitamin B3. [17]

Researchers in transgenic Mice have shown that high levels of klotho are associated with the induction of autophagy and maintain collagen in renal kidney via a decrease in collagen type I (Col I). [18]
In a mouse model with AKI that suffers from klotho deficiency, there is also evidence of an increase in Wnt pathway, which promotes cell senescence. However, the klotho gene suppresses Wnt pathway [4]. Likewise, the Klotho molecule upregulates the FOXO3 (Forkhead Box O3) in induced-AKI. [19].
In diabetic kidney disease (DKD), the role of klotho is to decrease oxidative stress, regulate the function of the glomerulus and autophagy, however, High glucose levels cause the triggering of inflammation and apoptosis with kidney injury. Reduced klotho levels are associated with the destruction of podocyte pyroptosis in DKD. [20]
On the other hand, the upregulate of klotho protein suppresses the activation of the Wnt/β-catenin and renin–angiotensin system (RAS), which is a that exacerbates kidney fibrosis and a decline of renal function. and also regulate the mineral metabolism by binding to the Fibroblast Growth Factor 23 (FGF23) receptor. [21]

1.1The original source of klotho distribution

The klotho is distributed in multiple organs and tissues, since it is highly expressed in nephron tubules of the kidney, the choroid plexus of the brain, neurons and CSF, and also in the skin, blood vessels, and pancreas β cells. However, it has been confirmed that the klotho expression was observed in both the proximal and distal tubular epithelial cells of the kidney cortex. A highly amount of klotho is expressed in the medulla of distal tubules[22]. And it is also expressed in the ovary, placenta, and testis.

1.2The klotho types and feature's structure.

The klotho protein is 130 kDa protein with β-glucosidases activity, found in two isoform, two extracellular domain form membrane protein (*KL1/KL2*) act as coreceptor for fibroblast growth factor 23 (FGF23 and soluble α-Klotho form (s-Klotho) which is produced by enzymatic cleavage so as result, the soluble α-Klotho form is released into circulation and acts as an endocrine hormone and it has pleiotropic effects. [23], and also secreted α-Klotho (~65 kDa) is a short form from splicing the α-klotho protein. However, its function is less understood from the transmembrane α-kl0tho protein. Note that the soluble α-kl0tho protein is not filtered by the glomerulus, but it has been secreted into the urine through kidney tubules into the luminal [22].

In humans, the klotho protein has a very short intracytoplasmic domain with a weight (10 aa). In addition, it has been reported that the klotho protein has a crystal structure and binds to the two FGF receptors (especially FGFR1c) and FGF23. The portion of extracellular domain bound to cytoplasmic tail, a transmembrane segment (TM), and a short non-signaling cytoplasmic tail (CYT), since the soluble form it is cleaved by A Desintegrin and Metalloproteinase such as ADAM10 and ADAM17 (α-secretases). The cleaving process can be inhibited by a phosphoinositide 3-kinase (P mnI3K) inhibitor. [24]
It would be interesting to determine whether the biological properties of the KL1 and KL2 fragments differ from those of the major 130-kDa KL fragment or 130-kDa KL fragment. [25] Furth more, it has been reported that two other klotho protein, β klotho and γ klotho, that produced the 68-kDa fragment β-cut. In addition, β-Klotho is mostly expressed in the liver and white adipose tissues (pancreas, brain), where it can form complexes with FGFR1c and FGFR4 (FGF21-FGFR1c; FGF15/19-FGFR4) and trigger downstream signaling pathways. And also reduced the expression of pro-inflammatory factors (IL-1b, IL-6, IL-8, and TNF-a). Further, γ-Klotho is expressed in the skin and the kidney, and has only one extracellular structural domain with only one extracellular structural domain, Hence, it has not yet fully defined functions. [26]

1.2 Klotho genomic and molecular basis

KL gene is localized on chromosome 13q12 region in humans, which is homologous gene. There are five exons and four introns in the coding region of KL in humans, which transcribe 3036 nucleotide mRNAs. Understanding the molecular genetics of the klotho gene may help to promote endogenous transcription and promote exogenous transgene expression. [7].
Whereas, increases in the hypermethylation because of uremia toxins decrease the activity of klotho expression in renal tubules and also in the transfected kidney cell line. [27] Moreover, hyperacetylation of klotho histone contributes to klotho gene deactivation in the cancer cell line must be considered [28].
In a performed study, using physiological cells like the HK-2 cell for klotho production. However, the alternative klotho gene has 5exons, with 2 mRNA transcription by splicing. Furthermore, it cannot yield *klotho* mRNA functional protein in vitro because it is dysregulated and degraded. [29].

In contrast, the klotho has a short half-life, so its administration in preclinical studies are mostly direct by recombinant or peptide forms with no long-term effect and little toxicity. In particular, the klotho gene delivery may applicably. Henceforth, siRNA, shRNA, RNAi, and CRISPR-Cas9 have been used for klotho gene delivery by lentivirus and adenovirus as vectors. Hence, these delivery pathways are still of concern because of mutagenic and immunogenicity, and lower stability.

In addition, using natural vesicles for klotho transport to the target organ, the extravascular (EV) with MSC and Exosomes holds promise in improving therapy especially in fibrosis disease in the kidney.[30]

2. Potential therapy of Mesenchymal stem cells by klotho in kidney disease.

kidney diseases, are a life-threating and global health problem with high mortality and morbidity, current therapeutics approaches remain limited. It has been attempting to find innovative treatments to stimulate kidney tissue regeneration, Furthermore, the mesenchymal stem cell-based therapy, has been proposed for reno -protective, Moreover, most clinical trial studies proposed the safety and efficiency of stem cell treatment in kidney failure.[31]

2.1The Application of mesenchymal stem cell-klotho targeting therapy in kidney disease

2.1.1 Bone marrow mesenchymal stem cells (BMSCs)

The bone marrow-derived mesenchymal stem cell is the most frequently used stem cell-based therapy. They have generally been used to treat kidney injury in 5/6 nephrectomized Nx rats. as mediators of paracrine modulatory effects via exosome delivery, which carry proteins or complementary nucleic acid. The Sprague–Dawley rats were used to isolate the rat BMSC-derived exosome (Autologus BMSCs). Hence, Klotho (cDNA) was synthesized from RNA the rats' kidney tissue and used for transfection. The BMSCs derived exosomes have ameliorated kidney tissue and upregulate klotho expression. [32]. However, in Acute kidney injury studies (AKI)Klotho produced by Klotho-GFP-BMSCs, transfected them with Klotho-GFP-green fluorescent protein adenovirus which inhibited the Wnt/β-catenin pathway in renal tubular epithelial cells (TECs). Klotho-GFP-BMSCs showed increased proliferative capacity and stronger immunoregulatory capacity than GFP-BMSCs. [33] whereas, Klotho forward ACTACGTTCAAGTGGACACTACT and reverse 5′-GATGGCAGAGAAATCAACACAGT-3′was transfected to BMSCs, the protein expression of Klotho, measured using Western blot, and the mRNA expression of Klotho, quantified by qPCR, the klotho expression level in AKI+BMSCs-Klotho group was higher than AKI+BMSCs-EV and AKI+BMSCs. The results observed improvement of renal function and less kidney tissue damage. [34]

In a study, by allogenic rat model with Chronic allograft dysfunction (CAD, and induced the Renal ischemia-reperfusion injury (RIRI), the Klotho protects BMSCs against RIRI induced-toxicities in vitro .and also Klotho improves efficacy of BMSCs transplantation in a rat model of RIRI by intravenous. Bone marrow mesenchymal stem cells (BMSCs) were transfected with recombinant adenoviruses expressing the Klotho gene (BMSCs-Klotho) and those expressing empty vector (BMSCs-EV). And upregulating the fork head box protein O1 (FoxO1) and phosphorylated FoxO1 (p-FoxO1)[35].

1.2 Urine-derived stem cells (UDSCs)2

Klotho-enhanced UDSCs was confirmed by their trilineage differentiation (adipo-, osteo-, and chondrogenesis), however, by the expression level of both 130 kDa and 65 kDa forms of Klotho in UDSCs.

Although, in induced ischemia–reperfusion injury (IRI) vivo study, Urine-derived stem cells (UDSCs) had been used as a source for cell therapeutics in injured renal tissue. Nevertheless, klotho expression during fibrosis progression in renal tissue was reduced. especially the high secretion of collagen IV. Moreover, the results indicate that UDSCs,is perfectly homing to injured organs showing no retention, normal biodistribution, and safety. Additionally, the endogenous Klotho enhancement after intravenous administration using a GMP-regulation.[36]

Obviously, in another *vitro* model study, Urine-derived stem cell is isolated from urine and screened for surface markers such as CD73 and CD90, CD105, renal progenitor cell marker CD133, pericyte marker CD146, and pluripotent stem cell marker SSEA4. It was found that the klotho gene expression level was about 35 times higher in UDSCs compared with adipose-derived stem cells (ADSCs) and bone marrow-derived stem cells (BM-MSCs), and umbilical cord-derived stem cells (UC-MSCs), also it has been expressed The klotho protein by UDSCs using the HK-cell which is a renal proximal tubular cell line. However, UDSCs transfected with Klotho demonstrated anti-fibrotic activity which inhibit transforming growth factor (TGF)-β in HK-2. [37]

2.1. 3 Adipose-derived stem cells

The adipose-derived stem cells have regenerative potential to tissue renewal, but their ability to differentiate is limited due to the cell senescence. However, significantly the secreted Klotho (SKL) that had been transfected into (ADSCs), Klotho regulates adipogenic differentiation linages such as Oct-4, Nanog, and Sox-2. Hence, the TGFβ1 Signaling is inhibited by overexpression of SKL leading to upregulation of Peroxisome proliferator-activated receptor gamma PPARγ. In addition, the transfection of ADSCs achieved using the pAAVmSKL plasmid DNA (driven by the cytomegalovirus promoter) with (65 kDa) secreted Klotho protein expression. This study provides the first evidence that ADSCs may offer promising therapeutics potential for renal disease when enhanced with klotho expression. [38]

CONCLUSION:

Several limitations are related to the klotho protein only, as potential therapy strategy for kidney diseases. Such as the molecular basis and the effector mechanism, viral delivery limitation by their immunogenicity and cancer promoter, and also the route of administration. Conceivably, its safety in clinical trials on human need more investigation[6]. However, in vitro and vivo studies the transfection of into mesenchymal stem cell the integration of Klotho gene transfection into mesenchymal stem cells (MSC) therapy presents a compelling, next-generation approach for treating chronic kidney disease (CKD) and acute kidney injury (AKI). As demonstrated, MSCs alone provide immunomodulatory, anti-fibrotic, and regenerative effects; however, their therapeutic potential can be significantly enhanced by overexpressing the Klotho gene. This synergistic strategy addresses the multifactorial nature of renal pathophysiology—mitigating inflammation, oxidative stress, and cellular senescence while promoting tissue repair through modulation of Wnt/β-catenin, FOXO3, and NF-κB signaling pathways. [20]

Recent advances in gene delivery techniques, including viral vectors and exosome-based systems, have shown promise in achieving stable Klotho expression in various MSC subtypes such as bone marrow, adipose-derived, and urine-derived stem cells. Preclinical studies have consistently reported improved renal function and histological outcomes following MSC-Klotho therapy, laying a strong foundation for translational research.

Despite these promising results, clinical application remains challenged by gene delivery efficiency, immunogenicity of vectors, and long-term transgene expression. Addressing these hurdles with non-viral platforms and optimized exosome-mediated delivery could pave the way for safe and effective clinical implementation.

Ultimately, MSC-based Klotho gene therapy is a promising frontier in regenerative nephrology, with the potential to shift current treatment paradigms from disease management to true renal regeneration.

Acknowledgement

I am deeply grateful to [Faculty of Medical Technology- Lab Medicine Department] for their unwavering support and provision of resources. Their commitment to promoting research and academic excellence has been instrumental in the successful completion of this study.

I would like to extend my heartfelt appreciation to the anonymous peer reviewers who generously dedicated their time and expertise to review and provide constructive feedback on this research paper. Their insightful comments and suggestions have significantly enhanced the quality and rigor of this study.

I would like to express my deepest appreciation to my family, friends, and loved ones for their unwavering support throughout this research journey. Their love, understanding, and encouragement have been the pillars that have kept me motivated and focused during challenging times.

REFERENCES:

1. Rastegar F, Shenaq D, Huang J, Zhang W, Zhang BQ, He BC, Chen L, Zuo GW, Luo Q, Shi Q, Wagner ER. Mesenchymal stem cells: Molecular characteristics and clinical applications. World journal of stem cells. 2010 Aug 26;2(4):67.
2. Christoffers S, Seiler L, Wiebe E, Blume C. Possibilities and efficiency of MSC co-transfection for gene therapy.

Stem Cell Research & Therapy. 2024 May 23;15(1):150.
3. Franco ML, Beyerstedt S, Rangel ÉB. Klotho and mesenchymal stem cells: a review on cell and gene therapy for chronic kidney disease and acute kidney disease. Pharmaceutics. 2021 Dec 21;14(1):11.
4. Bian A, Neyra JA, Zhan M, Hu MC. Klotho, stem cells, and aging. Clinical Interventions in Aging. 2015 Aug 4:1233-43.
5. Guo Y, Zhuang X, Huang Z, Zou J, Yang D, Hu X, Du Z, Wang L, Liao X. Klotho protects the heart from hyperglycemia-induced injury by inactivating ROS and NF-κB-mediated inflammation both in vitro and in vivo. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2018 Jan 1;1864(1):238-51.
6. Neyra JA, Hu MC. Potential application of klotho in human chronic kidney disease. Bone. 2017 Jul 1;100:41-9.
7. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. nature. 1997 Nov 6;390(6655):45-51.
8. Gerald LitwackVitamins and Hormones,Chapter Twelve - Aging and FGF23-klotho system,Academic Press.2020.Volume 115,2021,Pages 317-332,ISSN
9. Lim K, Groen A, Molostvov G, Lu T, Lilley KS, Snead D, James S, Wilkinson IB, Ting S, Hsiao LL, Hiemstra TF. α-Klotho expression in human tissues. The Journal of Clinical Endocrinology & Metabolism. 2015 Oct 1;100(10):E1308-18.
10. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature. 2006 Dec 7;444(7120):770-4.
11. Dalton G, An SW, Al-Juboori SI, Nischan N, Yoon J, Dobrinskikh E, Hilgemann DW, Xie J, Luby-Phelps K, Kohler JJ, Birnbaumer L. Soluble klotho binds monosialoganglioside to regulate membrane microdomains and growth factor signaling. Proceedings of the National Academy of Sciences. 2017 Jan 24;114(4):752-7.
12. Wu SE, Chen WL. Soluble klotho as an effective biomarker to characterize inflammatory states. Annals of medicine. 2022 Dec 31;54(1):1520-9.
13. Li SS, Sheng MJ, Sun ZY, Liang Y, Yu LX, Liu QF. Upstream and downstream regulators of Klotho expression in chronic kidney disease. Metabolism. 2023 May 1;142:155530.
14. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I. Suppression of aging in mice by the hormone Klotho. Science. 2005 Sep 16;309(5742):1829-33.
15. Espuch-Oliver A, Vázquez-Lorente H, Jurado-Fasoli L, de Haro-Muñoz T, Díaz-Alberola I, López-Velez MD, de Haro-Romero T, Castillo MJ, Amaro-Gahete FJ. References values of soluble α-klotho serum levels using an enzyme-linked immunosorbent assay in healthy adults aged 18–85 years. Journal of clinical medicine. 2022 Apr 25;11(9):2415.
16. Martin A, David V, Quarles LD. Regulation and function of the FGF23/klotho endocrine pathways. Physiological reviews. 2012 Jan;92(1):131-55.
17. Lin W, Wu X, Wen J, Fei Y, Wu J, Li X, Zhang Q, Dong Y, Xu T, Fan Y, Wang N. Nicotinamide retains Klotho expression and ameliorates rhabdomyolysis-induced acute kidney injury. Nutrition. 2021 Nov 1;91:111376.
18. Shi M, Flores B, Gillings N, Bian A, Cho HJ, Yan S, Liu Y, Levine B, Moe OW, Hu MC. αKlotho mitigates progression of AKI to CKD through activation of

autophagy. Journal of the American Society of Nephrology. 2016 Aug 1;27(8):2331-45.
19. Zhu X, Lin Q, Yang Y, Li S, Shao X, Zhang W, Cai H, Li J, Wu J, Zhang K, Qi C. αKlotho modulates BNIP3-mediated mitophagy by regulating FoxO3 to decrease mitochondrial ROS and apoptosis in contrast-induced acute kidney injury. Cellular and Molecular Life Sciences. 2024 Dec;81(1):454.
20. Hajare AD, Dagar N, Gaikwad AB. Klotho antiaging protein: molecular mechanisms and therapeutic potential in diseases. Molecular biomedicine. 2025 Mar 22;6(1):19.
21. Muñoz-Castañeda JR, Rodelo-Haad C, Pendon-Ruiz de Mier MV, Martin-Malo A, Santamaria R, Rodriguez M. Klotho/FGF23 and Wnt signaling as important players in the comorbidities associated with chronic kidney disease. Toxins. 2020 Mar 16;12(3):185.
22. Lim K, Groen A, Molostvov G, Lu T, Lilley KS, Snead D, James S, Wilkinson IB, Ting S, Hsiao LL, Hiemstra TF. α-Klotho expression in human tissues. The Journal of Clinical Endocrinology & Metabolism. 2015 Oct 1;100(10):E1308-18.
23. Dalton GD, Xie J, An SW, Huang CL. New insights into the mechanism of action of soluble klotho. Frontiers in endocrinology. 2017 Nov 17;8:323.
24. Prud'homme GJ, Kurt M, Wang Q. Pathobiology of the klotho antiaging protein and therapeutic considerations. Frontiers in Aging. 2022 Jul 12;3:931331.
25. Kim JH, Hwang KH, Park KS, Kong ID, Cha SK. Biological role of anti-aging protein Klotho. Journal of lifestyle medicine. 2015 Mar 30;5(1):1.
26. Zhao X, Han D, Zhao C, Yang F, Wang Z, Gao Y, Jin M, Tao R. New insights into the role of Klotho in inflammation and fibrosis: molecular and cellular mechanisms. Frontiers in Immunology. 2024 Sep 6;15:1454142.
27. Young GH, Wu VC. KLOTHO methylation is linked to uremic toxins and chronic kidney disease. Kidney international. 2012 Apr 1;81(7):611-2.
28. Rubinek T, Shulman M, Israeli S, Bose S, Avraham A, Zundelevich A, Evron E, Nili Gal-Yam E, Kaufman B, Wolf I. Epigenetic silencing of the tumor suppressor klotho in human breast cancer. Breast cancer research and treatment. 2012 Jun;133:649-57.
29. Mencke R, Harms G, Moser J, van Meurs M, Diepstra A, Leuvenink HG, Hillebrands JL. Human alternative Klotho mRNA is a nonsense-mediated mRNA decay target inefficiently spliced in renal disease. JCI insight. 2017 Oct 19;2(20):e94375.
30. Zhao X, Han D, Zhao C, Yang F, Wang Z, Gao Y, Jin M, Tao R. New insights into the role of Klotho in inflammation and fibrosis: molecular and cellular mechanisms. Frontiers in Immunology. 2024 Sep 6;15:1454142.
31. Chen F, Chen N, Xia C, Wang H, Shao L, Zhou C, Wang J. Mesenchymal stem cell therapy in kidney diseases: Potential and challenges. Cell transplantation. 2023 Apr;32:09636897231164251.
32. Wan F, Yang RC, Tang YW, Tang XL, Ye T, Zheng J, Zhang HQ, Lin Y. BMSC-derived exosomes protect against kidney injury through regulating klotho in 5/6 nephrectomy rats. European Journal of Medical Research. 2022 Jul 11;27(1):118.
33. Zhang F, Wan X, Cao YZ, Sun D, Cao CC. Klotho gene-modified BMSCs showed elevated antifibrotic effects by inhibiting the Wnt/β-catenin pathway in kidneys after acute injury. Cell biology international. 2018 Dec;42(12):1670-9.
34. 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. Regenerative Therapy. 2021 Dec 1;18:255-67.

37. Fan J, Sun Z. The antiaging gene klotho regulates proliferation and differentiation of adipose-derived stem cells. Stem Cells. 2016 Jun 1;34(6):1615-25.

35. Xie LB, Chen X, Chen B, Wang XD, Jiang R, Lu YP. Protective effect of bone marrow mesenchymal stem cells modified with klotho on renal ischemia-reperfusion injury. Renal Failure. 2019 Jan 1;41(1):175-82.
36. Kim SH, Lee SH, Jin JA, So HJ, Lee JU, Ji MJ, Kwon EJ, Han PS, Lee HK, Kang TW. In vivo safety and biodistribution profile of Klotho-enhanced human urine-derived stem cells for clinical application. Stem Cell Research & Therapy. 2023 Dec 10;14(1):355.
38. Kim SH, Jin JA, So HJ, Lee SH, Kang TW, Lee JU, Choi DE, Jeong JY, Chang YK, Choi H, Lee Y. Urine-derived stem
39. cell-secreted Klotho plays a crucial role in the HK-2 fibrosis model by inhibiting the TGF-β signaling pathway. International Journal of Molecular Sciences. 2022 Apr 30;23(9):5012.