

Review

Klotho-Dependent Role of $1,25(\text{OH})_2\text{D}_3$ in the Brain

Miribane Dërmaku-Sopjani^a Fatbardhë Kurti^b Nguyen Thi Xuan^c
Mentor Sopjani^b

^aDepartment of Chemistry, University of Prishtina, Prishtinë, Kosova, ^bFaculty of Medicine, University of Prishtina, Prishtinë, Kosova, ^cInstitute of Genome Research, Vietnam Academy of Science and Technology, Cau Giay, Hanoi, Vietnam

Key Words

Brain • $1,25(\text{OH})_2\text{D}_3$ • Klotho • Fibroblast growth factor

Abstract

The antiaging protein Klotho is encoded by the *Klotho* gene first identified as an ‘aging suppressor’, in mice. Klotho deficiency is involved in premature aging and early death, while its overexpression is related to longevity. Klotho is mostly expressed in the kidney, but also in the brain, and in other organs. Two forms of Klotho, the cell membrane and secreted form, have pleiotropic activities that include regulation of general metabolism, oxidative stress, and mineral metabolism that correlates with its effect on accelerating aging. Membrane Klotho serves as an obligate co-receptor for the fibroblast growth factor (FGF), while secreted Klotho plays its role as a humoral factor. Klotho protein participates in the regulation of several biological activities, including regulation of calcium-phosphate homeostasis and PTH as well as vitamin D metabolism. The active form of vitamin D, $1,25(\text{OH})_2\text{D}_3$ (1,25-dihydroxy-vitamin D3 = calcitriol), acts as a neurosteroid that participates in the regulation of multiple brain functions. It provides neuroprotection and suppresses oxidative stress, inhibits inflammation and inflammatory mediators, and stimulates various neurotrophins. Calcitriol is involved in many brain-related diseases, including multiple sclerosis, Alzheimer’s disease, Parkinson’s disease, and schizophrenia. This review covers the most recent advances in Klotho research and discusses Klotho-dependent roles of calcitriol in neuro-psycho-pathophysiology.

© 2021 The Author(s). Published by
Cell Physiol Biochem Press GmbH&Co. KG

Introduction

Aging is a complex and multifactorial process characterized by age-related changes. This biological phenomenon is driven by the delicate interaction between multiple genetic and environmental factors [1, 2]. The intrinsic complexity of aging still remains a challenge to be fully elucidated. However, it is well known that age-related processes are influenced by the alteration of particular gene expression and involve numerous intracellular signaling

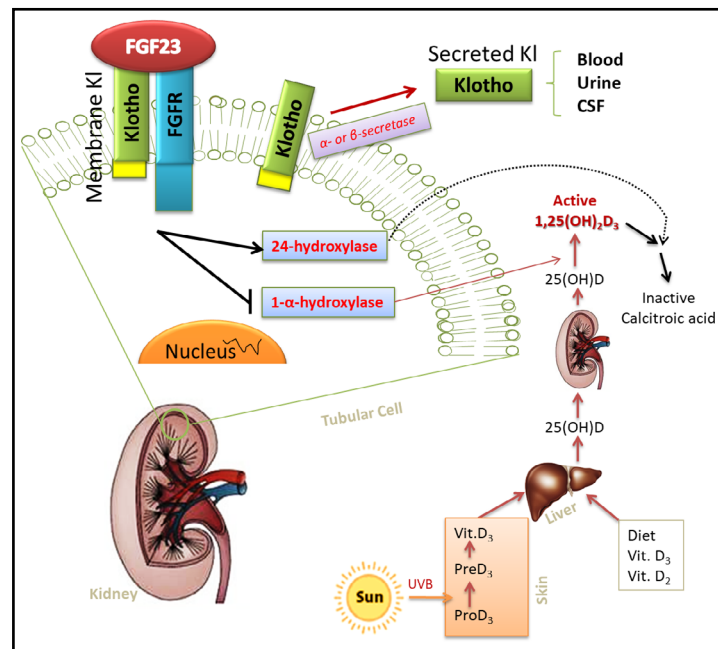
pathways. A complex interplay between age-related genes and various signaling pathways needs to be better understood. It is well known that aging is characterized by an increased incidence of various human diseases, including diabetes [3], hypertension [4], neurological disorders [5], chronic kidney disease (CKD) [6], and increased risk of cardiovascular diseases [7]. One of the genes involved in aging is the Klotho gene, an aging suppressor gene. Therefore, potential medications targeting the Klotho expression may slow down the process of aging and postpone the onset of age-dependent diseases. The therapeutic intervention of Klotho may have significant clinical relevance.

Klotho gene encodes a single-pass transmembrane protein with multiple anti-aging effects [8]. The Klotho family of proteins has three members with pleiotropic functions: α Klotho (known as Klotho) encoded by the main α Klotho gene, and two other Klotho proteins β Klotho and γ Klotho, encoded by two other Klotho-related genes, β -Klotho and γ -Klotho [9]. The α Klotho gene is primarily expressed in the kidney, more in the distal convoluted tubule (DCT) cells, and less in the renal proximal convoluted tubule (PCT) cells [8-11]. Klotho is synthesized in large amounts in the brain by the brain choroid plexus epithelial cells [12, 13], hippocampal neurons, and Purkinje EC cells. To a lesser extent, Klotho is also present in the cerebral white matter [14]. Low expression of α Klotho has been reported in other body parts, including the pituitary gland, thyroid gland, urinary bladder, placenta, skeletal muscle, aorta, ovary, colon, pancreas, and testis [8, 15, 16].

Similar to other signaling molecules such as AMP-activated protein kinase [17-22] and Janus kinase 2 [23-27], Klotho is involved in various processes, including regulation of cellular transport systems and cell volume regulation [9, 11, 28-31]. Noteworthy, the Klotho gene was first identified in mice, as an 'aging suppressor'. A Klotho gene-deficient mouse has phenotypes resembling human premature aging [9], while gene overexpression in mice is characterized by an increased lifespan [32]. The mouse *klotho* (*mKL*) gene and the human α Klotho gene (*hKL*) are very similar [33].

Two protein molecules are produced by the Klotho gene, the membrane-bound, and the secreted form. Secreted Klotho protein, known as soluble or circulating Klotho, arises either by proteolytic cleavage of the extracellular domain of the full length α Klotho just above the cell surface [8, 34] or by alternative splicing of mRNA Klotho gene that generates a secreted Klotho isoform 70 kDa [35] (Fig. 1). Although both forms of Klotho protein have their distinct biological activities, most of the Klotho functions are attributed to secreted Klotho [9, 36] that may function in different roles, including its role as a humoral factor [30, 37] through an unknown plasma membrane receptor [38], and as an enzyme regulating the various plasma membrane glycoproteins [39, 40], and through it influencing several signaling pathways [41, 42]. The main function of membrane Klotho protein is to serve as an obligatory co-receptor for fibroblast growth factor 23 (FGF23) through forming a complex with FGF receptors on the cell membrane, which participates in various biological processes, including the regulation of P_i and 1,25(OH) $_2$ D $_3$ metabolism, which is a critical hallmark in the development of chronic diseases. As many recent studies revealed, the biological effects of Klotho proteins appear to be much broader. The underlying molecular mechanisms of Klotho effects are not fully understood. However, Klotho is involved in different intracellular signaling pathways, as reviewed elsewhere [9, 33], such as regulating FGF23-mediated signaling, cAMP, p53/p21, PKC, insulin/insulin-like growth factor-1 (insulin/IGF-1), and Wnt signaling pathways. Klotho is involved in various different physiological and pathological processes. There are still poor data about the direct role of Klotho in the human central nervous system, brain diseases respectively. In older human community-dwelling adults, lower plasma Klotho has been reported to be associated with a significant increase in all-cause mortality, including cognitive impairment, but the biological mechanisms remain unclear [43]. However, Klotho is critical for the maturation of oligodendrocytes, myelin integrity, and prevent myelin degeneration [44]. The neural Klotho protein can protect hippocampal neurons against amyloid formation, glutamate toxicity [44], and against the development of neurodegenerative diseases related to aging as confirmed in mice [45]. Calcitriol is an important regulator of mineral metabolism [46]. Due to its ability to induce

Fig. 1. Schematic diagram indicating the main functions of FGF23/Klotho/FGFR complex in vitamin D production [9, 15]. The complex of membrane Klotho-FGFR forms a high-affinity receptor for FGF23 that uses this binary complex to mediate intracellular signaling, including vitamin D metabolism. By serving as an obligate co-receptor for FGF23, Klotho participates in vitamin D metabolism. The biologically active form ($1,25(\text{OH})_2\text{D}_3$) of vitamin D plays many important roles in the body, including controlling the mineral metabolism and influencing brain health. The ectodomain shedding, between KL1 and KL2 domains, of membrane Klotho by α - and β -secretases causes the release of the secreted Klotho that can be predominantly detected in blood, urine, and cerebrospinal fluid. FGF23/Klotho regulates the level of the active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$) in circulation. This effect can be reached either by downregulating the expression levels of enzyme 1- α -hydroxylase that catalyzes the synthesis of $1,25(\text{OH})_2\text{D}_3$, or by upregulating the enzyme 24-hydroxylase that catalyzes the breakdown of $1,25(\text{OH})_2\text{D}_3$ into inactive calcitric acid. See the figure and the text for further details.



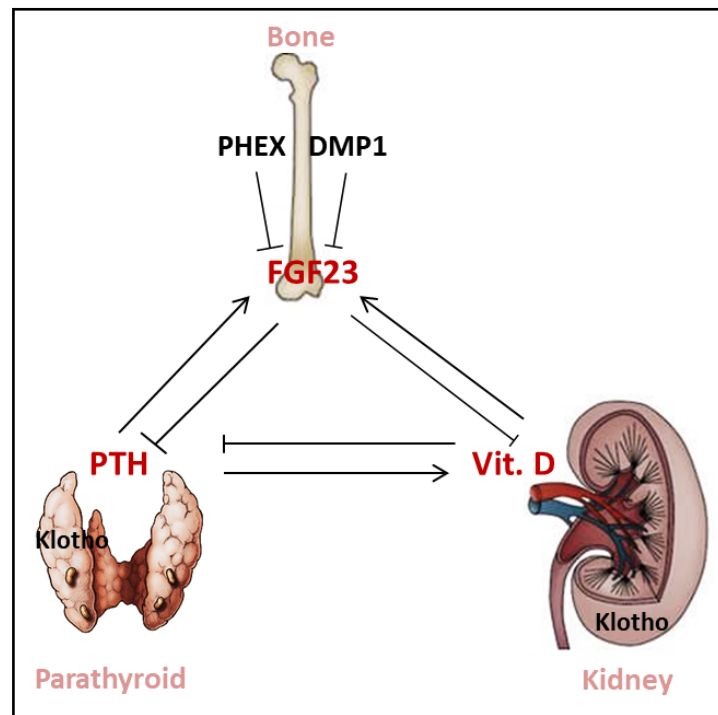
Klotho, calcitriol has anti-aging/wellness functions. Therefore, normal levels of calcitriol are associated with anti-aging effects [47-49]. The plasma levels of calcitriol and Klotho are decreased with age.

The role of Klotho in calcitriol synthesis

FGF23, a protein, is a member of the FGF19 subfamily, composed of 251-amino acids in length (approx. 32 kDa), synthesized and secreted by bone cells, predominantly osteoblast [50, 51]. FGF23 has an N-terminal fragment binding domain for the FGF receptor (FGFR) and a C-terminal binding domain for Klotho [52, 53]. Klotho is involved in the FGF23-signaling. Specifically, FGF23 requires a binary complex of membrane α Klotho protein as an obligate co-receptor and FGFR for activation of FGFR/ α Klotho binary receptor complexes located on the cell membrane [54]. These FGF23/ α -Klotho co-mediated activities have been implicated in several functions. Therefore, the cell membrane expression of both FGFR and Klotho proteins defines the target tissues for FGF23. Synthesis and secretion of FGF23 from the bone are stimulated by the parathyroid hormone (PTH), vitamin D ($1,25(\text{OH})_2\text{D}_3$), and by dietary and serum phosphate levels [55]. In the bone FGF23 synthesis is inhibited the phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX) and by dentin matrix protein 1 (DMP1) [56] (Fig. 2).

The Klotho/FGFR complex is obligatory for eliciting the FGF23-induced intracellular signaling events [57]. This pathway participates in several diseases [58-60] and controlling numerous biological functions, including $1,25(\text{OH})_2\text{D}_3$ synthesis and its breakdown. FGF23 overexpression inhibits biosynthesis of $1,25(\text{OH})_2\text{D}_3$ and the renal phosphate reabsorption [60, 61], whereas FGF23 deficiency results in much higher levels of serum $1,25(\text{OH})_2\text{D}_3$, thus

Fig. 2. Schematic representation of interplay between the FGF23/Klotho, PTH, and Vitamin D. Klotho protein participates in the regulation of numerous signaling pathways, including aging through phosphate and calcium regulation. See the text for further details.



causing hyperphosphatemia, accompanied by soft-tissue calcification [1, 54, 55]. FGF23 overexpression mediated hypophosphatemia is caused by down-regulating the expression of renal sodium-coupled phosphate cotransporter, NaPi2a, and NaPi2c [62], resulting in elevated phosphate excretion through the urine. The antiaging protein Klotho has a key role in calcium homeostasis, an effect that is realized either through $1,25(\text{OH})_2\text{D}_3$ inhibition or by other pathways [63, 64].

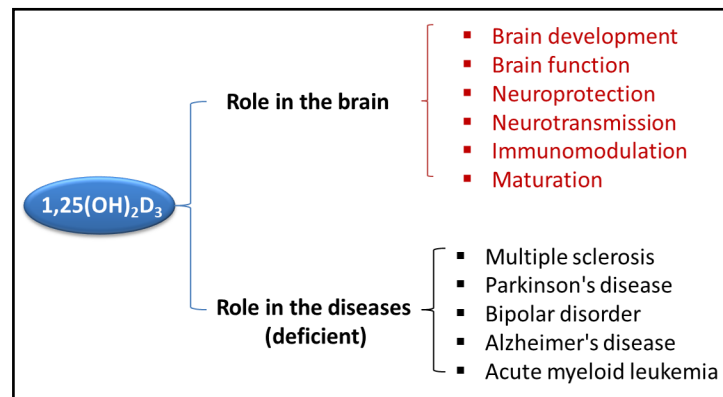
The FGF23/Klotho pathway regulates $1,25(\text{OH})_2\text{D}_3$ levels by modulating the expression levels of enzymes involved in the synthesis and degradation of $1,25(\text{OH})_2\text{D}_3$ in the kidney (Fig. 1). Specifically, FGF23/Klotho downregulates the 25-hydroxyvitamin D 1- α -hydroxylase (Cyp27b1) that catalyze the synthesis of the active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$) and upregulates the 1,25-dihydroxyvitamin D 24-hydroxylase (Cyp24a1) that catalyzes the breakdown of the $1,25(\text{OH})_2\text{D}_3$ into inactive calcitric acid [65]. FGF23/Klotho pathway functions as a counter-regulatory phosphaturic hormone for vitamin D.

Multiple roles of $1,25(\text{OH})_2\text{D}_3$ in the brain

The active form of vitamin D and vitamin D receptors (VDR: vitamin D receptor; PDIA3: Protein-Disulphide-Isomerase, family A member 3) are reported to be expressed throughout the brain tissues [66], particularly in regions that are central to learning and memory. This has led to the paradigm that preventing calcitriol deficiency or insufficiency may have a key role in preserving cognitive function, indicating that $1,25(\text{OH})_2\text{D}_3$ may prevent, or even treat, age-related cognitive diseases [49, 67].

The $1,25(\text{OH})_2\text{D}_3$ is considered a neurosteroid that participates in multiple brain functions. However, the cerebral expression of $1,25(\text{OH})_2\text{D}_3$ -associated enzymes and receptors remains unclear [68]. Like other nuclear steroids, within the brain, $1,25(\text{OH})_2\text{D}_3$ may trigger both genomic and major auto-/paracrine non-genomic actions. An overview of the roles of the $1,25(\text{OH})_2\text{D}_3$ in the brain is presented in Fig. 3.

Fig. 3. The schematic view of the role of $1,25(\text{OH})_2\text{D}_3$ in the brain functions and some diseases fostered by $1,25(\text{OH})_2\text{D}_3$ deficiency. See the text for further details.



The role of $1,25(\text{OH})_2\text{D}_3$ in brain development and function

Over the last decade, numerous studies reported an important role of $1,25(\text{OH})_2\text{D}_3$ in brain development and function [49, 69]. Studies suggest $1,25(\text{OH})_2\text{D}_3$ plays a neuroprotective role through withstanding greater oxidative stress, as reported in experiments using primary cortical neuronal cultures and of different biomarkers of oxidative stress [69]. Additionally, $1,25(\text{OH})_2\text{D}_3$ is reported to be a vital factor for the growth, survival, and proliferation of the neurons [69], neurotransmission, brain development, and immunomodulation [68, 70], with a potential to treat various neurodegenerative diseases.

$1,25(\text{OH})_2\text{D}_3$ in the neuropsychiatric and other diseases

Optimal levels of $1,25(\text{OH})_2\text{D}_3$ are important for normal functions of the brain, but either deficient and excessive levels of vitamin D may lead to brain dysfunctioning [49]. The antiaging properties of $1,25(\text{OH})_2\text{D}_3$ are primarily attributed to its ability to induce Klotho. The decreased levels of $1,25(\text{OH})_2\text{D}_3$ are followed by a decrease in phosphate and calcium plasma concentrations [39, 57], while deficient levels of phosphate and calcium induce $1,25(\text{OH})_2\text{D}_3$ formation. The hypocalcemia-induced parathyroid hormone leads to $1,25(\text{OH})_2\text{D}_3$ formation as well. Conversely, phosphate excess stimulates FGF23, which in turn inhibits $1,25(\text{OH})_2\text{D}_3$ formation. FGF23 requires Klotho to become effective [9, 33, 71]. This vitamin has powerful effects in the brain unnecessary related to mineral metabolism. $1,25(\text{OH})_2\text{D}_3$ is involved in many processes in the brain function [49] as well as in neuropsychiatric diseases [49, 67, 72, 73] (Fig. 3) such as Multiple sclerosis (MS) [74], a progressive disease of the central nervous system (CNS), which is characterized by damage to the myelin sheath surrounding axons of nerve cells. The immunomodulatory effects of $1,25(\text{OH})_2\text{D}_3$ have been widely reported during the last years. The results of the last study using the animal model of CNS inflammation reported that calcitriol downregulates both, blood-brain barrier disruption and local macrophage/microglia Activation, and prevents neuroinflammation [74]. $1,25(\text{OH})_2\text{D}_3$ may potentially serve as a therapy for treating MS patients. $1,25(\text{OH})_2\text{D}_3$ deficiency is closely associated with depressive symptoms, especially in older adults. Another neurodegenerative disease where $1,25(\text{OH})_2\text{D}_3$ is involved is Parkinson's disease (PD). The progressive neurodegeneration in PD is characterized by neuroinflammation and endothelial vascular impairment. The vitamin D receptor (VDR) is expressed in the brain including the dopamine neurons and brain endothelial cells; however, its role in the regulation of endothelial biology has not been clearly characterized in terms of PD. A recent study reported that brain endothelial P-glycoprotein (P-gp, encoded by *MDR1a* gene) level is down-regulated in PD through the VDR-mediated pathway [75]. This result indicates that a dysfunctional VDR-P-gp pathway may be used as a possible target for the maintenance of vascular homeostasis during pathological conditions in PD. $1,25(\text{OH})_2\text{D}_3$ has also been used for neuroprotective treatment for COVID-19 [72], a disease caused by infection with a novel coronavirus SARS-CoV-2 [71]. Recent clinical trials suggest the importance of

using $1,25(\text{OH})_2\text{D}_3$ supplementation to reduce the incidence of acute respiratory infection and the severity of the respiratory tract in COVID-19 adults and children.

The most common cause of dementia is Alzheimer's disease (AD), a progressive disorder that degenerates brain cells. One of the major pathological features of AD is the accumulation of Amyloid-beta ($\text{A}\beta$). The $1,25(\text{OH})_2\text{D}_3$ through its nuclear hormone receptor, VDR, may be exploited for the treatment of $\text{A}\beta$ pathology [73]. The mechanisms of action of $1,25(\text{OH})_2\text{D}_3$ are at least partly understood. Specifically, $1,25(\text{OH})_2\text{D}_3$ exerts its role *via* an interplay with glial cell line-derived neurotrophic factor (GDNF)-signaling as well as through restoring the downregulation of GDNF and inhibiting the phosphorylation of the phosphatidylinositol 3 kinase (PI3K)/protein kinase B/Akt/glycogen synthase kinase-3 β (GSK-3 β) signaling pathway. There is an association between $1,25(\text{OH})_2\text{D}_3$ deficiency and psychiatric disorders. A similar correlation has been suggested regarding $1,25(\text{OH})_2\text{D}_3$ and bipolar disorder (BD). However, in most of the studies, no significant differences in the levels of $1,25(\text{OH})_2\text{D}_3$ between BD patients and other psychiatric disorders were confirmed [67]. This does not rule out that an appropriate $1,25(\text{OH})_2\text{D}_3$ status may have a positive role in mood balance due to its immunomodulatory, antidepressant, and other functions.

$1,25(\text{OH})_2\text{D}_3$ plays a role in brain metastatic cancer and acute myeloid leukemia (AML) [76]. A combination of $1,25(\text{OH})_2\text{D}_3$ and the hypomethylating agent (HMA) 5-Azacytidine (AZA) Increases cytotoxicity and differentiation, as well as decreases the proliferation of primary AML patient samples and several AML cell lines used in that study.

An ischemic stroke leads to blood-brain barrier (BBB) dysfunction, which is a physical and biochemical barrier that precisely maintains cerebral homeostasis. $1,25(\text{OH})_2\text{D}_3$ has been reported to protect against cerebral ischemia by maintaining BBB permeability, increasing the level of brain-derived neurotrophic factor (BDNF) in their brains, and decreasing PPAR γ -mediated neuroinflammation [77]. $1,25(\text{OH})_2\text{D}_3$ -induced regulation of GDNF/Ret signaling is also involved in dopaminergic neurons [78]. Hence, indicating its important role in dopamine physiology. Ret, a receptor for GDNF-family ligands, is directly regulated by $1,25(\text{OH})_2\text{D}_3$ through VDR in dopaminergic neurons. $1,25(\text{OH})_2\text{D}_3$ -mediated effect *via* VDR is implied in the expression of various dopaminergic-associated genes [70], and, thus, in dopamine neuronal development and maturation.

Conclusion

The antiaging protein Klotho is implied in numerous processes, including the development of multiple-age-related diseases when deficient. Klotho has been reported to be involved in various biological functions and in the regulation of many intracellular signaling pathways [9, 33], including cAMP, p53/p21, PKC, and Wnt. Klotho is an obligatory cofactor for the activation of FGF23-dependent intracellular signaling. Klotho, in an FGF23-dependent and FGF23-independent manner, participates in the regulation of $1,25(\text{OH})_2\text{D}_3$ formation and phosphate and calcium metabolism [9, 11, 47, 79]. Klotho increases the resistance to oxidative stress and protects cells and tissues from oxidative damage [80, 81]. Klotho participates in the regulation of $1,25(\text{OH})_2\text{D}_3$. This vitamin is implied in numerous brain functions, under physiological and pathophysiological conditions [49, 69, 70, 72, 73, 76, 78]. Due to its reported role in cognitive impairments and neurodegenerative diseases, the therapeutic use of $1,25(\text{OH})_2\text{D}_3$ or related agonists in the treatment of several neuropsychiatric disorders and other conditions may represent great potential. $1,25(\text{OH})_2\text{D}_3$ mediated brain actions could be genomic and non-genomic [49, 69]. However, the interaction of genomic and non-genomic processes of $1,25(\text{OH})_2\text{D}_3$ is largely unexplored. The detailed molecular mechanisms by which $1,25(\text{OH})_2\text{D}_3$ exerts its actions in the brain remains incompletely understood, especially regarding cell-specific variations and the stage of development in the brain. This needs further study efforts to better understand the precise mechanisms associated with specific functional outcomes.

Acknowledgments

Author Contributions

MS drafted the initial manuscript and coordinated finalizing. MDS helped in drafting and contributed to finalizing the manuscript. FK and XNT contributed to finalizing the manuscript.

Funding

This work was supported by the Italmed, Kosova.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

- 1 Razzaque MS, Sitara D, Taguchi T, St Arnaud R, Lanske B: Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. *FASEB J* 2006;20:720-722.
- 2 Tsujikawa H, Kurotaki Y, Fujimori T, Fukuda K, Nabeshima Y: Klotho, a gene related to a syndrome resembling human premature aging, functions in a negative regulatory circuit of vitamin D endocrine system. *Mol Endocrinol* 2003;17:2393-2403.
- 3 Mochly-Rosen D, Das K, Grimes KV: Protein kinase C, an elusive therapeutic target? *Nat Rev Drug Discov* 2012;11:937-957.
- 4 Satoh M, Nagasu H, Morita Y, Yamaguchi TP, Kanwar YS, Kashihara N: Klotho protects against mouse renal fibrosis by inhibiting Wnt signaling. *Am J Physiol Renal Physiol* 2012;303:F1641-F1651.
- 5 Dehvari N, Cedazo-Minguez A, Isacson O, Nilsson T, Winblad B, Karlström H, Benedikz E, Cowburn RF: Presenilin dependence of phospholipase C and protein kinase C signaling. *J Neurochem* 2007;102:848-857.
- 6 Hu MC, Shi M, Zhang J, Quiñones H, Griffith C, Kuro-o M, Moe OW: Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol* 2011;22:124-136.
- 7 Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, Guralnik JM, Ferrucci L: Plasma klotho and cardiovascular disease in adults. *J Am Geriatr Soc* 2011;59:1596-1601.
- 8 Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997;390:45-51.
- 9 Dërmaku-Sopjani M, Kolgeci S, Abazi S, Sopjani M: Significance of the anti-aging protein Klotho. *Mol Membr Biol* 2013;30:369-385.
- 10 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;78:1240-1251.
- 11 Dërmaku-Sopjani M, Sopjani M, Saxena A, Shojafard M, Bogatkov E, Alesutan I, Eichenmüller M, Lang F: Downregulation of NaPi-IIa and NaPi-IIb Na-coupled phosphate transporters by coexpression of Klotho. *Cell Physiol Biochem* 2011;28:251-258.
- 12 John GB, Cheng CY, Kuro-o M: Role of Klotho in aging, phosphate metabolism, and CKD. *Am J Kidney Dis* 2011;58:127-134.
- 13 Zacchia M, Capasso G: Dehydration: a new modulator of klotho expression. *Am J Physiol Renal Physiol* 2011;301:F743-F744.
- 14 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;56:106-117.
- 15 Kuro-o M: Klotho in health and disease. *Curr Opin Nephrol Hypertens* 2012;21:362-368.
- 16 Manya H, Akasaka-Manya K, Endo T: Klotho protein deficiency and aging. *Geriatr Gerontol Int* 2010;10:S80-S87.

- 17 Dërmaku-Sopjani M, Abazi S, Faggio C, Kolgeci J, Sopjani M: AMPK-sensitive cellular transport. *J Biochem* 2014;155:147-158.
- 18 Munoz C, Sopjani M, Dërmaku-Sopjani M, Almilaji A, Föller M, Lang F: Downregulation of the osmolyte transporters SMIT and BGT1 by AMP-activated protein kinase. *Biochem Biophys Res Commun* 2012;422:358-362.
- 19 Föller M, Jaumann M, Dettling J, Saxena A, Pakladok T, Munoz C, Ruth P, Sopjani M, Seeböhm G, Rüttiger L, Knipper M, Lang F: AMP-activated protein kinase in BK-channel regulation and protection against hearing loss following acoustic overstimulation. *FASEB J* 2012;26:4243-4253.
- 20 Sopjani M, Alesutan I, Dërmaku-Sopjani M, Fraser S, Kemp BE, Föller M, Lang F: Down-regulation of Na⁺-coupled glutamate transporter EAAT3 and EAAT4 by AMP-activated protein kinase. *J Neurochem* 2010;113:1426-1435.
- 21 Dërmaku-Sopjani M, Almilaji A, Pakladok T, Munoz C, Hosseinzadeh Z, Blecu M, Sopjani M, Lang F: Down-regulation of the Na-coupled phosphate transporter NaPi-IIa by AMP-activated protein kinase. *Kidney Blood Press Res* 2013;37:547-556.
- 22 Dërmaku-Sopjani M, Sopjani M: Intracellular signaling of the AMP-activated protein kinase. *Adv Protein Chem Struct Biol* 2019;116:171-207.
- 23 Bhavsar SK, Hosseinzadeh Z, Brenner D, Honisch S, Jilani K, Liu G, Sztejn K, Sopjani M, Mak TW, Shumilina E, Lang F: Energy-sensitive regulation of Na⁺/K⁺-ATPase by Janus kinase 2. *Am J Physiol Cell Physiol* 2014;306:C374-C384.
- 24 Hosseinzadeh Z, Sopjani M, Pakladok T, Bhavsar SK, Lang F: Downregulation of KCNQ4 by Janus kinase 2. *J Membr Biol* 2013;246:335-341.
- 25 Sopjani M, Morina R, Uka V, Xuan NT, Dërmaku-Sopjani M: JAK2-mediated intracellular signaling. *Curr Mol Med* 2020;21:417-425.
- 26 Sopjani M, Konjufca V, Rinnerthaler M, Rexhepaj R, Dërmaku-Sopjani M: The Relevance of JAK2 in the Regulation of Cellular Transport. *Curr Med Chem* 2016;23:578-588.
- 27 Sopjani M, Thaci S, Krasniqi B, Selmonaj M, Rinnerthaler M, Dërmaku-Sopjani M: Regulation of Ion Channels, Cellular Carriers and Na⁽⁺⁾/K⁽⁺⁾/ATPase by Janus Kinase 3. *Curr Med Chem* 2017;24:2251-2260.
- 28 Sopjani M, Alesutan I, Dërmaku-Sopjani M, Gu S, Zelenak C, Munoz C, Velic A, Föller M, Rosenblatt KP, Kuro-o M, Lang F: Regulation of the Na⁺/K⁺ ATPase by Klotho. *FEBS Lett* 2011;585:1759-1764.
- 29 Sopjani M, Alesutan I, Wilmes J, Dërmaku-Sopjani M, Lam RS, Koutsouki E, Jakupi M, Föller M, Lang F: Stimulation of Na⁺/K⁺ ATPase activity and Na⁺ coupled glucose transport by beta-catenin. *Biochem Biophys Res Commun* 2010;402:467-470.
- 30 Almilaji A, Pakladok T, Muñoz C, Elvira B, Sopjani M, Lang F: Upregulation of KCNQ1/KCNE1 K channels by Klotho. *Channels (Austin)* 2014;8:222-229.
- 31 Sopjani M, Millaku L, Nebija D, Emini M, Rifati-Nixha A, Dërmaku-Sopjani M: The Glycogen Synthase Kinase-3 in the Regulation of Ion Channels and Cellular Carriers. *Curr Med Chem* 2019;26:6817-6829.
- 32 Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP, Kuro-o M: Suppression of aging in mice by the hormone Klotho. *Science* 2005;309:1829-1833.
- 33 Sopjani M, Rinnerthaler M, Kruja J, Dërmaku-Sopjani M: Intracellular signaling of the aging suppressor protein Klotho. *Curr Mol Med* 2015;15:27-37.
- 34 Chen CD, Podvin S, Gillespie E, Leeman SE, Abraham CR: Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. *Proc Natl Acad Sci U S A* 2007;104:19796-19801.
- 35 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;242:626-630.
- 36 Kuro-o M: Disease model: human aging. *Trends Mol Med* 2001;7:179-181.
- 37 Li BS, Ma HX, Wang YJ, Wu P: [Klotho gene attenuates the progression of hypertension and heart damage in spontaneous hypertensive rats]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2012;29:662-668.
- 38 Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, Lanske B, Razzaque MS, Rosenblatt KP, Baum MG, Kuro-o M, Moe OW: Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J* 2010;24:3438-3450.
- 39 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;105:9805-9810.

- 40 Wang Y, Sun Z: Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage. *Hypertension* 2009;54:810-817.
- 41 Doi S, Zou Y, Togao O, Pastor JV, John GB, Wang L, Shiizaki K, Gotschall R, Schiavi S, Yorioka N, Takahashi M, Boothman DA, Kuro-O M: Klotho inhibits transforming growth factor-beta1 (TGF-beta1) signaling and suppresses renal fibrosis and cancer metastasis in mice. *J Biol Chem* 2011;286:8655-8665.
- 42 Wolf I, Levanon-Cohen S, Bose S, Ligumsky H, Sredni B, Kanety H, Kuro-o M, Karlan B, Kaufman B, Koeffler HP, Rubinek T: Klotho: a tumor suppressor and a modulator of the IGF-1 and FGF pathways in human breast cancer. *Oncogene* 2008;27:7094-7105.
- 43 Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, Guralnik JM, Ferrucci L: Plasma klotho and mortality risk in older community-dwelling adults. *J Gerontol A Biol Sci Med Sci* 2011;66:794-800.
- 44 Abraham CR, Mullen PC, Tucker-Zhou T, Chen CD, Zeldich E: Klotho Is a Neuroprotective and Cognition-Enhancing Protein. *Vitam Horm* 2016;101:215-238.
- 45 Massó A, Sánchez A, Gimenez-Llort L, Lizcano JM, Cañete M, Garcia B, Torres-Lista V, Puig M, Bosch A, Chillón M: Secreted and Transmembrane α Klotho Isoforms Have Different Spatio-Temporal Profiles in the Brain during Aging and Alzheimer's Disease Progression. *PLoS One* 2015;10:e0143623.
- 46 Razzaque MS: Osteo-renal regulation of systemic phosphate metabolism. *IUBMB Life* 2011;63:240-247.
- 47 Anour R, Andrukhova O, Ritter E, Zeitz U, Erben RG: Klotho lacks a vitamin D independent physiological role in glucose homeostasis, bone turnover, and steady-state PTH secretion *in vivo*. *PLoS One* 2012;7:e31376.
- 48 Haussler MR, Whitfield GK, Kaneko I, Forster R, Saini R, Hsieh JC, Haussler CA, Jurutka PW: The role of vitamin D in the FGF23, klotho, and phosphate bone-kidney endocrine axis. *Rev Endocr Metab Disord* 2012;13:57-69.
- 49 Lang F, Ma K, Leibrock CB: 1,25(OH)2D3 in Brain Function and Neuropsychiatric Disease. *Neurosignals* 2019;27:40-49.
- 50 Takeuchi Y, Suzuki H, Ogura S, Imai R, Yamazaki Y, Yamashita T, Miyamoto Y, Okazaki H, Nakamura K, Nakahara K, Fukumoto S, Fujita T: Venous sampling for fibroblast growth factor-23 confirms preoperative diagnosis of tumor-induced osteomalacia. *J Clin Endocrinol Metab* 2004;89:3979-3982.
- 51 Khosravi A, Cutler CM, Kelly MH, Chang R, Royal RE, Sherry RM, Wodajo FM, Fedarko NS, Collins MT: Determination of the elimination half-life of fibroblast growth factor-23. *J Clin Endocrinol Metab* 2007;92:2374-2377.
- 52 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;444:770-774.
- 53 Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, et al.: FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011;121:4393-4408.
- 54 Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M: Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem* 2006;281:6120-6123.
- 55 Liu S, Zhou J, Tang W, Jiang X, Rowe DW, Quarles LD: Pathogenic role of Fgf23 in Hyp mice. *Am J Physiol Endocrinol Metab* 2006;291:E38-E49.
- 56 Liu S, Zhou J, Tang W, Menard R, Feng JQ, Quarles LD: Pathogenic role of Fgf23 in Dmp1-null mice. *Am J Physiol Endocrinol Metab* 2008;295:E254-E261.
- 57 Bergwitz C, Jüppner H: Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annu Rev Med* 2010;61:91-104.
- 58 Fukumoto S, Yamashita T: Fibroblast growth factor-23 is the phosphaturic factor in tumor-induced osteomalacia and may be phosphatonin. *Curr Opin Nephrol Hypertens* 2002;11:385-389.
- 59 Sitara D, Razzaque MS, Hesse M, Yoganathan S, Taguchi T, Erben RG, Jüppner H, Lanske B: Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in Phex-deficient mice. *Matrix Biol* 2004;23:421-432.
- 60 Shimada T, Urakawa I, Yamazaki Y, Hasegawa H, Hino R, Yoneya T, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T: FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. *Biochem Biophys Res Commun* 2004;314:409-414.
- 61 Bai X, Miao D, Li J, Goltzman D, Karaplis AC: Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders. *Endocrinology* 2004;145:5269-5279.
- 62 Gattineni J, Bates C, Twombly K, Dwarakanath V, Robinson ML, Goetz R, Mohammadi M, Baum M: FGF23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia *in vivo* predominantly via FGF receptor 1. *Am J Physiol Renal Physiol* 2009;297:F282-F291.

- 63 Borst O, Münzer P, Schmid E, Schmidt EM, Russo A, Walker B, Yang W, Leibrock C, Szteyn K, Schmidt S, Elvers M, Faggio C, Shumilina E, Kuro-o M, Gawaz M, Lang F: 1, 25(OH)₂ vitamin D₃-dependent inhibition of platelet Ca²⁺ signaling and thrombus formation in klotho-deficient mice. *FASEB J* 2014;28:2108-2119.
- 64 Imai M, Ishikawa K, Matsukawa N, Kida I, Ohta J, Ikushima M, Chihara Y, Rui X, Rakugi H, Ogihara T: Klotho protein activates the PKC pathway in the kidney and testis and suppresses 25-hydroxyvitamin D₃ 1α-hydroxylase gene expression. *Endocrine* 2004;25:229-234.
- 65 Perwad F, Zhang MY, Tenenhouse HS, Portale AA: Fibroblast growth factor 23 impairs phosphorus and vitamin D metabolism *in vivo* and suppresses 25-hydroxyvitamin D-1α-hydroxylase expression *in vitro*. *Am J Physiol Renal Physiol* 2007;293:F1577-F1583.
- 66 Stephenson A, Mamo JCL, Takechi R, Hackett MJ, Lam V: Genetic, environmental and biomarker considerations delineating the regulatory effects of vitamin D on central nervous system function. *Br J Nutr* 2019;123:41-58.
- 67 Cereda G, Enrico P, Ciappolino V, Delvecchio G, Brambilla P: The role of vitamin D in bipolar disorder: Epidemiology and influence on disease activity. *J Affect Disord* 2021;278:209-217.
- 68 Landel V, Stephan D, Cui X, Eyles D, Feron F: Differential expression of vitamin D-associated enzymes and receptors in brain cell subtypes. *J Steroid Biochem Mol Biol* 2018;177:129-134.
- 69 AlJohri R, AlOkail M, Haq SH: Neuroprotective role of vitamin D in primary neuronal cortical culture. *eNeurologicalSci* 2019;14:43-48.
- 70 Pertile RA, Cui X, Eyles DW: Vitamin D signaling and the differentiation of developing dopamine systems. *Neuroscience* 2016;333:193-203.
- 71 Dërmaku-Sopjani M, Sopjani M: Molecular characterization of SARS-CoV-2. *Curr Mol Med* 2020;21:589-595.
- 72 Xu Y, Baylink DJ, Chen CS, Reeves ME, Xiao J, Lacy C, Lau E, Cao H: The importance of vitamin d metabolism as a potential prophylactic, immunoregulatory and neuroprotective treatment for COVID-19. *J Transl Med* 2020;18:322.
- 73 Lin CI, Chang YC, Kao NJ, Lee WJ, Cross TW, Lin SH: 1, 25(OH)₂D₃ Alleviates Aβ₂₅₋₃₅-Induced Tau Hyperphosphorylation, Excessive Reactive Oxygen Species, and Apoptosis Through Interplay with Glial Cell Line-Derived Neurotrophic Factor Signaling in SH-SY5Y Cells. *Int J Mol Sci* 2020;21:4215.
- 74 de Oliveira LRC, Mimura LAN, Fraga-Silva TFC, Ishikawa LLW, Fernandes AAH, Zorzella-Pezavento SFG, Sartori A: Calcitriol Prevents Neuroinflammation and Reduces Blood-Brain Barrier Disruption and Local Macrophage/Microglia Activation. *Front Pharmacol* 2020;11:161.
- 75 Kim H, Shin JY, Lee YS, Yun SP, Maeng HJ, Lee Y: Brain Endothelial P-Glycoprotein Level Is Reduced in Parkinson's Disease via a Vitamin D Receptor-Dependent Pathway. *Int J Mol Sci* 2020;21:8538.
- 76 Xu Y, Payne K, Pham LHG, Eunwoo P, Xiao J, Chi D, Lyu J, Campion R, Wasnik S, Jeong IS, Tang X, Baylink DJ, Chen CS, Reeves M, Akhtari M, Mirshahidi S, Marcucci G, Cao H: A novel vitamin D gene therapy for acute myeloid leukemia. *Transl Oncol* 2020;13:100869.
- 77 Guo T, Wang Y, Guo Y, Wu S, Chen W, Liu N, Wang Y, Geng D: 1, 25-D₃ Protects From Cerebral Ischemia by Maintaining BBB Permeability via PPAR-γ Activation. *Front Cell Neurosci* 2018;12:480.
- 78 Pertile RAN, Cui X, Hammond L, Eyles DW: Vitamin D regulation of GDNF/Ret signaling in dopaminergic neurons. *FASEB J* 2018;32:819-828.
- 79 Woudenberg-Vrenken TE, van der Eerden BC, van der Kemp AW, van Leeuwen JP, Bindels RJ, Hoenderop JG: Characterization of vitamin D-deficient klotho^{-/-} mice: do increased levels of serum 1, 25(OH)₂D₃ cause disturbed calcium and phosphate homeostasis in klotho^{-/-} mice?. *Nephrol Dial Transplant* 2012;27:4061-4068.
- 80 Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffey PJ, Huang TT, Bos JL, Medema RH, Burgering BM: Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 2002;419:316-321.
- 81 Yamamoto M, Clark JD, Pastor JV, Gurnani P, Nandi A, Kurosu H, Miyoshi M, Ogawa Y, Castrillon DH, Rosenblatt KP, Kuro-o M: Regulation of oxidative stress by the anti-aging hormone klotho. *J Biol Chem* 2005;280:38029-38034.