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KLOTHO AN ANTI- AGING GENE

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ABSTRACT

The **klotho gene**, identified by insertional mutagenesis in mice, is a suppressor of the expression of multiple aging phenotypes. The klotho gene plays a critical role in regulating aging and the development of age-related diseases in mammals: Loss of klotho can result in multiple aging-like phenotypes, while overexpression of klotho gene extends lifespan by 20-30%. Mice lacking KL exhibit many changes that occur during aging, including atherosclerosis, osteoporosis, infertility, and cognitive decline. The gene for the mammalian KL has two transcripts that encode a long type I transmembrane protein and a short secreted protein. The long isoform of KL, originating from the transmembrane isoform, is found in serum and cerebrospinal fluid (CSF), suggesting that the extracellular domain of KL is cleaved and released from the cell membrane.

KEY WORDS

klotho, aging, insulin signaling cascade, wnt signaling.

INTRODUCTION

Aging is defined as the age-related decline in physiological functions necessary for survival and fertility [1]. Nobody can predict when and where senescence starts to be realized in the body. Thus, the aging process is stochastic, and it contrasts with the programmed processes undertaken during early development. On the other hand, the fact that no one in the history of the human being has ever lived beyond 130 years old suggests an absolute (or maximum) life span. It also tells us that there are extrinsic and intrinsic factors acting on senescence. A family lineage exhibiting *progeria* (premature aging) indicates that senescence is, at least to some extent, controlled genetically [2, 3].

The **klotho gene**, identified by insertional mutagenesis in mice, is a suppressor of the expression of multiple aging phenotypes. The klotho gene plays a critical role in regulating aging and the development of age-related diseases in mammals: Loss of klotho can result in multiple aging-like phenotypes, while overexpression of klotho gene extends lifespan by 20-30%. The klotho gene is composed of 5 exons and encodes a type-I single pass transmembrane protein (1014-amino acid-long). The intracellular domain is short (10-amino acid-long) and no known functional domains exist. The extracellular domain is composed of two domains, termed **KL1** and **KL2**, with weak homology. Each domain has homology to family 1 glycosidases, including lactose-phlorizin hydrolase of mammals and β-glucosidases of bacteria and plants. These enzymes have exoglycosidase activity that hydrolyzes β-glucosidic linkage in saccharides, glyco-proteins, and glycolipids [4].

Klotho (KL), an antiaging protein, was named after the goddess who spins the threads of life. Mice lacking KL exhibit many changes that occur during aging, including atherosclerosis, osteoporosis, infertility, and cognitive decline. They also have a short life span [5]. In contrast, mice overexpressing KL live 30% longer than wild-type mice and are more resistant to oxidative stress [6] (fig-1). The gene for the mammalian KL has two transcripts that encode a long type I transmembrane protein and a short secreted protein. The long isoform of KL, originating from the transmembrane isoform, is found in serum and cerebrospinal fluid (CSF), suggesting that the extracellular domain of KL is cleaved and released from the cell membrane [7]. Identifying the proteases responsible for KL shedding may lead to research designed to regulate the aging process. The extracellular domains of numerous integral membrane proteins such as KL are released from the cell surface by enzymes called **sheddases** [8]. The sheddases include members of various proteinase families, such as metalloproteinases, A Disintegrin and Metalloproteinases (ADAMs) [9,10], and serine proteases [11]. ADAMs are particularly important for ectodomain shedding of proteins such as Notch, the amyloid-β precursor protein (APP), and TGF-α, which, as a result of shedding, transactivate surface receptors in autocrine or paracrine mechanisms [12-14].

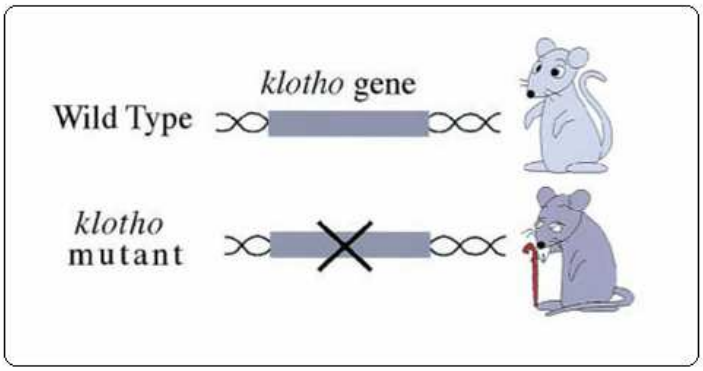

Fig.1

A mutant model mouse is useful for studies of aging. The klotho phenotype (premature aging) is caused by a disruption of the single gene, klotho.

Possible biological roles of Klotho protein

Study reveals that serum levels of 1,25-$(OH)_2D$ in *kl*−/− mice are greatly elevated. This deterioration in the Vitamin D3 endocrine system may participate in many of the phenotypes in *kl*−/− mice via toxicity due to increased levels of calcium, phosphorus and 1,25-$(OH)_2D$ (Fig. 2).

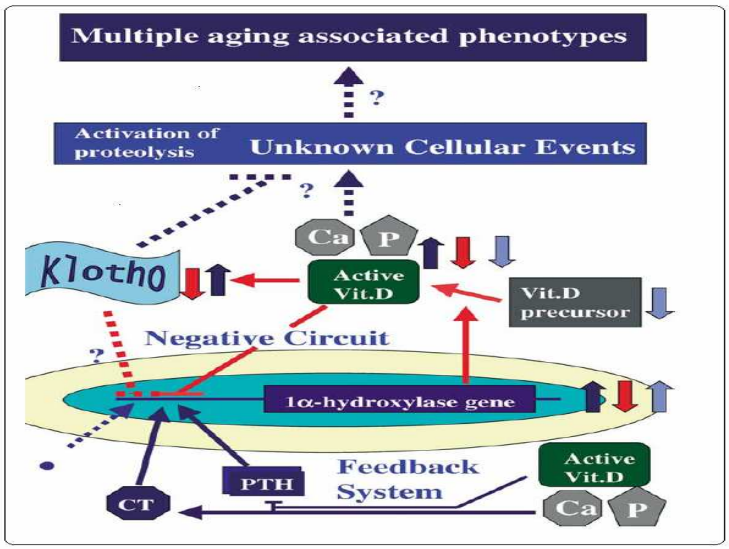

Fig. 2

The mutual and coordinated actions of PTH, CT, and 1, 25-$(OH)_2D3$ are illustrated. Klotho may be a member of the negative regulatory circuit of Vitamin D metabolism and protect cells from morphological and functional deterioration. The deficiency of Klotho protein may trigger deterioration in cells and cause subsequent severe tissue damage in conjunction with the toxicity from the increased calcium, phosphorus and 1,25-$(OH)_2D$ in serum [15].

It should be noted that when serum concentrations of calcium, phosphorus and 1,25-$(OH)_2$D are restored to normal levels, many of phenotypes are improved despite Klotho protein deficiency. Therefore, the increased activation of Vitamin D3, due to the impaired regulation of 1α-hydroxylase and 24-hydroxylase, could be a major indispensable cause of multiple abnormalities in *kl*−/− mice.The next question to be elucidated is whether the abnormalities observed in *kl*−/− mice are solely dependent on the increased levels of calcium, phosphorus and 1,25-$(OH)_2$Dor due to both, the combination of the increased serum levels and the deficiency of Klotho protein. Two classes of phenotypes may exist; those that are solely due to the toxic action of increased calcium, phosphorus, and 1,25-$(OH)_2$D and those that require the additional lack of Klotho. If the latter is true, Klotho may play another role in addition to that as a regulator of calcium homeostasis. The deficiency of Klotho protein may trigger a morphological and functional deterioration of cells and tissues which causes subsequent severe tissue damage together with the toxic action of increased calcium, phosphorus and 1,25-$(OH)_2$D in serum (Fig. 2) [15].

Regulation of Oxidative Stress by Klotho:-

The *klotho* gene encodes a single-pass transmembrane protein and is expressed only in limited tissues, notably the distal convoluted tubules in the kidney and the choroid plexus in the brain. The shedded portion of klotho binds to a high affinity but as yet unidentified cell-surface Klotho receptor and signals suppression of tyrosine phosphorylation of insulin/ IGF-1 receptors and insulin receptor substrates (IRS), association of IRS with phosphatidylinositol 3-kinase (PI3K), and serine phosphorylation of Akt/PKB. Thus, Klotho protein is a hormone that inhibits the **intracellular insulin/IGF-1 signaling cascade**. To dissect the mechanism by which Klotho protein increases resistance to oxidative stress, the scientists investigated a potential link between the intracellular insulin/IGF-1/PI3K/Akt signaling pathway and the metabolism of reactive oxygen species. Recent studies have identified FoxO forkhead transcription factors (FOXOs) as downstream targets of insulin- like signaling that regulate organismal aging. FOXOs are negatively regulated by phosphorylation at three conserved serine/threonine residues by Akt. Once activated by suppression of insulin/ IGF-1 signaling, FOXOs up-regulate expression of the manganese superoxide dismutase (MnSOD or SOD2) gene encoding a mitochondrial enzyme that detoxifies superoxide. These observations have led to the hypothesis that the anti-aging hormone Klotho, possessing a potent activity that inhibits insulin/IGF-1 signaling, may activate FOXOs and increase SOD2 expression, thereby facilitating removal of reactive oxygen species and increasing resistance to oxidative stress (fig-3) [16].

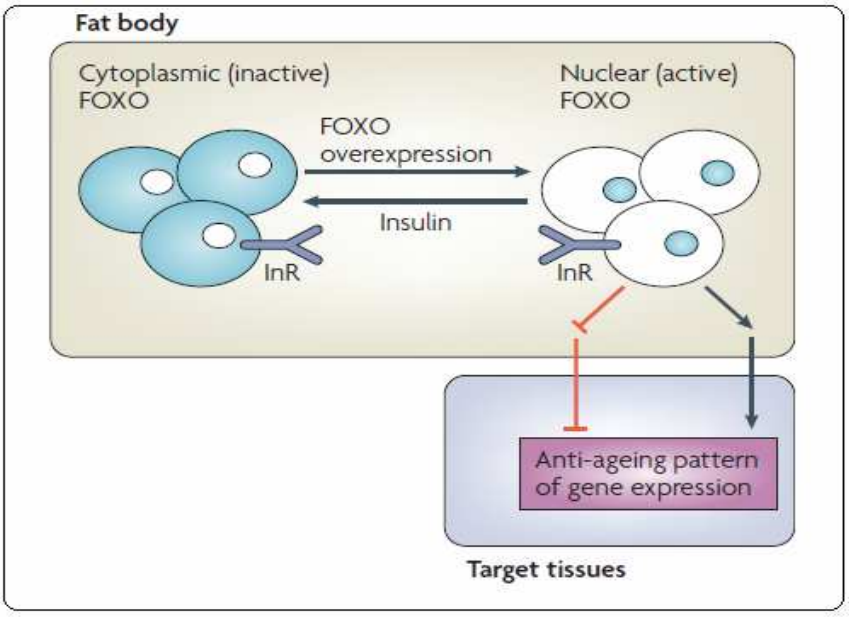

Fig. 3

Overexpression or activation of the class O forkhead box transcription factor FOXO localized to the fat body results in longevity. Diminished insulin signalling, overexpression of FOXO or expression of a constitutively active FOXO mutant all result in nuclear localization of FOXO in fat-body cells. Once in the nucleus and transcriptionally active, FOXO might stimulate the production of humoral factors in the fat body that promote an anti-ageing pattern of gene expression in other tissues. Alternatively, FOXO might inhibit the production of humoral factors that repress an anti-ageing pattern of gene expression in target tissues. InR, insulin receptor [17].

Regulation of angiogenesis by the klotho

Investigations by scientists showed that disruption of the klotho gene is associated with decreased vasodilatation in response to acetylcholine which reflects decreased production of nitric oxide (NO) in endothelial cells. Attenuated levels of NO were also seen in klotho disrupted mice. Interestingly, this endothelial dysfunction was rescued by parabiosis between wild type mice and mice heterozygously deficient for the klotho gene suggesting that the klotho gene product may be a humoral factor. Adenovirus-mediated transfer of klotho into rats with endothelial dysfunction (e.g. Ohtsuka– Long Evans–Tokushima Fatty rats) ameliorated this endothelial function as well as the reduced nitric oxide levels. These findings collectively showed that klotho, an aging-suppressor gene, is involved in the regulation of endothelial function likely through a pathway mediated by NO.

Involvement of age-related endothelial dysfunction, including reduced NO production , impaired proliferation and migration of endothelial cells has been implicated in impaired angiogenesis in aged animals, and importantly as these pathogenic characteristics closely parallel the actions of klotho, it suggests that klotho is likely to play a role in angiogenesis, and if so, although further

investigation is required, would be a promising potential therapeutic factor for use in therapeutic angiogenesis, especially in an elderly population [18].

KLOTHO as the secreted Wnt antagonist

The Wnt family of secreted cysteine-rich glycosylated proteins has emerged as versatile targets for a variety of conditions that involve cardiovascular disease, aging, cancer, diabetes, neurodegeneration, and inflammation. In particular, modulation of Wnt signaling may fill a critical void for the treatment of disorders that impact upon both cellular survival and cellular longevity. Yet, in some scenarios, Wnt signaling can become the catalyst for disease development or promote cell senescence that can compromise clinical utility [19].

Typically, in human cells, Wnt signaling maintains proliferation of stem cells by inhibiting cell differentiation and apoptosis and stimulating cell division. Scientist identified an additional mechanism by which Wnt signaling can promote cell proliferation, by inhibiting senescence of primary fibroblasts and epithelial cells. Researchers present three lines of evidence to show that Wnt signaling regulates senescence in fibroblasts and epithelial cells. First, Wnt2-dependent signaling is down regulated in senescent cells. Second, premature inhibition of Wnt signaling induces a premature senescence. Third, forced activation of the canonical Wnt pathway delays senescence [20].

Klotho Protein Promotes Adipocyte Differentiation

The differentiation of adipose precursor cells can be divided into early and late events, a phase of proliferation and a phase of differentiation. A number of genes have been shown to be differentially expressed during adipocyte differentiation. Some of them are involved in lipid synthesis and storage, and others, such as novel transcription factors, are induced during differentiation.

Researchers investigated klotho expression during the differentiation induced by several stimulations. Furthermore, to elucidate the role of klotho in the differentiation of adipocytes, they investigated the effects of suppression and overexpression of klotho gene on adipocyte differentiation.

Further they suggest that klotho promotes adipocyte differentiation, especially in the period of transient proliferation, early period of differentiation. The salient findings in this study are that: 1) klotho mRNA expression increased during adipocyte differentiation; 2) the PPAR-γ agonist pioglitazone increased klotho mRNA ex-pression; 3) regarding the function of up-regulated klotho, suppression by siRNA for klotho gene decreased markers of adipocyte differentiation, such as C/EBPα, C/EBPβ, C/EBPδ, PPAR-γ, and aP2; and 4) both overexpression of klotho gene and recombinant Klotho protein increased differentiation markers. Then, the role of klotho in adipocyte differentiation by using overexpression of the klotho gene was investigated by researchers.They found that klotho overexpression resulted in the increased expression of adipocyte differentiation markers in the early phase, indicating that klotho may promote adipocyte differentiation. Adipocytes are known to contribute to the development of vascular diseases closely related to aging disorders via production of adipocytokines [21].

Klotho Protein Deficiency leads to Overactivation of μ-Calpain

Calpain, a calcium-dependent cytosolic cysteine protease, is involved in many physiological and pathological processes [22-24]. Calpain mediates proteolysis of various cellular proteins, including cytoskeletal proteins, and causes irreversible cell damage

[25-30]. Thus, calpain overactivation may contribute to the pathology of cerebral and cardiac ischemia, Alzheimer's disease, arthritis, and cataract formation [31].. Calpain has been shown to be regulated by both calcium ion and calpastatin [32]. Two types of isozymic calpain, *μ-calpain* and *m-calpain*, are ubiquitously distributed in mammalian cells. The former is activated by micromolar concentrations of calcium and the latter is activated by millimolar concentrations of calcium. Calpastatin is an endogenous inhibitor specific for calpain, but is slowly degraded by calpain [33].

The overactivation of calpains, in particular, has figured prominently in hypotheses of cellular aging beginning with the observation that levels of soluble calpain activity in brain extracts strongly correlate inversely with lifespan across several orders of mammals. Although in vitro measurements do not necessarily predict in vivo calpain activity, these initial observations presaged a growing number of studies that link increased calpain activity to aging-related phenomena in various tissues and to the pathogenesis of degenerative diseases of late-age onset. Consistent with observations of increased intracellular or calcium influx in aging tissues, calpain activities also rise during aging. The basis for the elevated calpain activity with aging is most commonly increased activation without a change in overall expression. Increased calpain activation, even in the presence of lowered calpain levels, is associated with reduced levels of calpastatin in the kidney of normal aged rodents and in erythrocytes from aged individuals. The calpain-dependent cleavage of certain substrates may also be altered by changes in the post-translational modification of the substrate that arise as cells age. For example, phosphorylation of p35 suppresses calpain-mediated generation of the cdk5 activator p25. The decreased phosphorylation of p35 in the adult brain promotes calpain-dependent activation of cdk5 during brain maturation [34].

An over-active calpain system contributes to the degenerative phenomena of aging. A well-established example is the process of cataract formation. This process seems to result from the specific proteolytic cleavage of α-crystallin leading to its denaturation and of α-crystallin, which normally acts as a chaperone to reduce α-crystallin denaturation [34].

Band 3 protein degradation by calpain is enhanced in erythrocytes of old people:

Band 3 protein may be involved in the generation of the senescence signals that lead to the recognition and removal of old erythrocytes from the circulation. It has been proposed that degradation of band 3 protein plays a role in the generation of a senescence signal. Some degradation of band 3 protein seems to occur in vivo during erythrocyte aging in the circulation, but the relationships of the degradation to erythrocyte removal and the proteinase(s) responsible are not known. A shortened erythrocyte survival has been found in old individuals [35].

Klotho in chronic kidney disease

The bone–kidney endocrine axis mediated by FGF23 and Klotho has emerged as an essential component in the regulation of phosphate homeostasis. Serum phosphate levels are determined by a counterbalance between absorption of dietary phosphate from the intestine, mobilization from bone (the reservoir of phosphate and calcium) and excretion from the kidney into urine. When phosphate is in excess, FGF23 is secreted from bone and acts on the kidney where Klotho is expressed. As a phosphaturic hormone, FGF23 reduces the amount of sodium phosphate co-transporter type-2a (NaPi-2a) on the brush border membrane of proximal

tubules, thereby promoting renal phosphate excretion. As a counter-regulatory hormone for vitamin D, FGF23 suppresses synthesis and promotes inactivation of 1, 25- dihydroxyvitamin D3 in proximal tubules. FGF23 suppresses expression of the *Cyp27b1* gene that encodes 1α- hydroxylase, the enzyme that converts vitamin D from an inactive form (25-hydroxyvitamin D3) to the active form (1,25-dihydroxyvitamin D3). In addition, FGF23 increases expression of the *Cyp24* gene that encodes 24-hydroxylase, the enzyme that inactivates 1,25-dihydroxyvitamin D3. The ability of FGF23 to reduce serum 1, 25-dihydroxyvitamin D3 levels also contributes to induce a negative phosphate balance through limiting phosphate absorption from the intestine. Importantly, 1,25-dihydroxyvitamin D3 induces expression of the FGF23 gene and closes a negative feedback loop (Fig 4) [36].

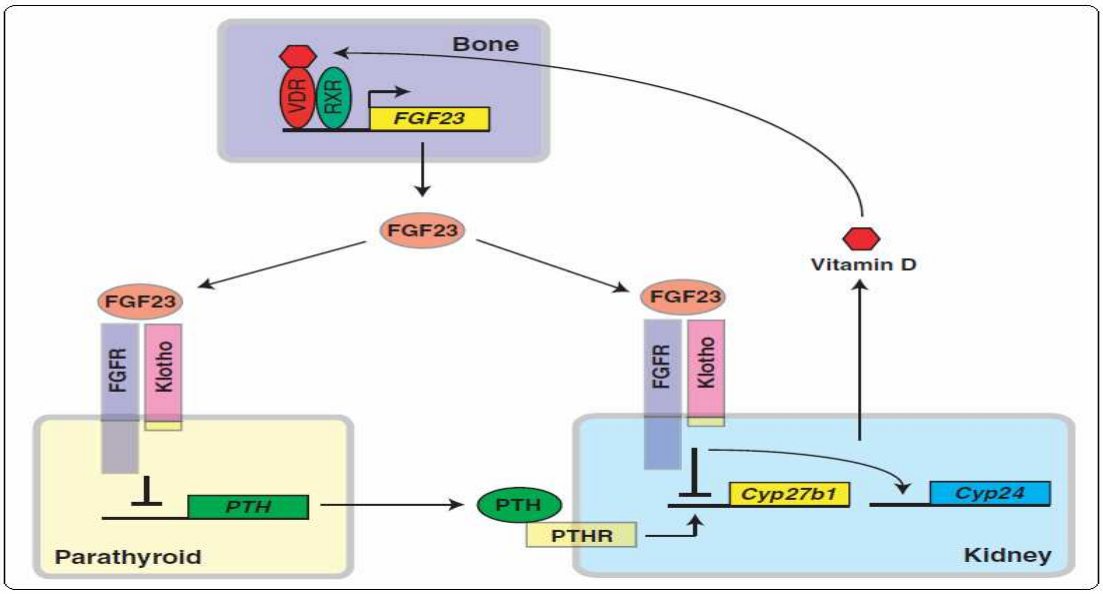

Fig. 4

The bone–kidney–parathyroid endocrine axes mediated by FGF23 and Klotho. Active vitamin D (1, 25-dihydroxyvitaminD3) binds to the vitamin D receptor (VDR) in osteocytes. The ligand-bound VDR forms a heterodimer with a nuclear receptor RXR and transactivates the FGF23 gene expression. FGF23 secreted from bone acts on the Klotho–FGFR complex in kidney (the bone–kidney axis) and parathyroid gland (the bone–parathyroid axis). In kidney, FGF23 suppresses synthesis of active vitamin D by down-regulating expression of the Cyp27b1 gene and promotes its inactivation by up-regulating expression of the Cyp24 gene, thereby closing a negative feedback loop for vitamin D homeostasis. In the parathyroid gland, FGF23 suppresses production and secretion of PTH. Since PTH is a potent inducer of Cyp27b1 gene expression, suppression of PTH by FGF23 reduces expression of the Cyp27b1 gene as well as serum levels of 1, 25-dihydroxyvitamin D3, which closes another long negative feedback loop for vitamin D homeostasis. Klotho and FGF23 are indispensable for the regulation of vitamin D metabolism because defects in either Klotho or FGF23 cause hypervitaminosis D [36].

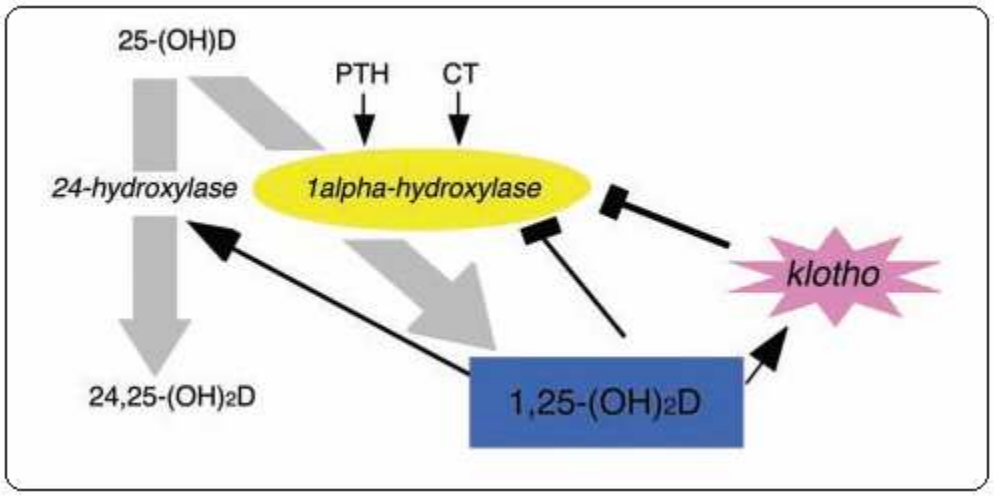

Fig. 5

Model for the Role of Klotho in the Vitamin D Feedback Loop- Loss of klotho in mice results in the abnormal elevation of 1α-hydroxylase gene expression, but normal responses to known regulators, such as 1,25-$(OH)_2$D, PTH, and CT are intact, suggesting that signaling by these regulators are independent of klotho. Klotho expression is up-regulated by 1, 25-$(OH)_2$D, suggesting that klotho participates in a vitamin D feedback loop. A novel negative regulatory circuit for the regulation of vitamin D activity that involves klotho function was proposed by researchers [37].

Phosphate toxicity

Disruption of the bone–kidney endocrine axis mediated by Klotho and FGF23 results in hyperphosphataemia, hypercalcaemia and hypervitaminosis D associated with multiple ageing-like phenotypes. These observations raised the possibility that toxicity of phosphate, calcium and/or vitamin D may be responsible for the premature ageing syndrome observed in Klotho- and FGF23-deficient mice [36].

CONCLUSION

It was clearly demonstrated that klotho have roles in many endogenous regulatory mechanisms which are mainly responsible for aging in mmamals so we suggest klotho as a potential target for preventimg the aging as well as it helps in treating atherosclerosis, osteoporosis, infertility, cognitive decline, diabetes, cancer, obesity and alzheimer. So we suggest that klotho is a new prospective target for fighting against the various metabolic disorders that generally occurs due to aging in mammals.

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