Overview of Soluble Klotho in Diabetic Nephropathy Patients

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Abstract

Diabetic nephropathy (DN) is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. it is characterized by nephrotic syndrome and diffuse glomerulosclerosis. It is due to longstanding diabetes mellitus. DN is the leading cause of chronic kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality. The goals of treatment are to slow the progression of kidney damage and control related complications. Kidney cells are the main source of soluble Klotho, but it can also be shed from ependymal cells of the choroid plexus.The reason why soluble Klotho can target tissues and affect various signaling pathways is unknown because no specific receptor for soluble Klotho has yet been identified. The aim of the article to review the role of the soluble klotho in the management of DN patients.

Keywords: Diabetic Nephropathy; Soluble Klotho; Management

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Introduction

Diabetic nephropathy (DN) is defined in both type 1 and type 2 diabetes as the presence of persisting severely elevated albuminuria of more than 300 mg/24 h (or >200 μg/min) or an albumin creatinine ratio > 300 mg/g creatinine, confirmed in at least two out of three samples, with concurrent presence of diabetic retinopathy and absence of signs of other forms of renal disease. As such, it is a clinical diagnosis, requiring little more than basic clinical and laboratory evaluations. Normal value for albuminuria has been defined as <30 mg/g (or 30 mg/24 h), and abnormal values above 30, but albuminuria is a continuous measurement and increasing values within the normal and abnormal range are associated with elevated risk for renal and cardiovascular disease **(1).**

The number of people with type II diabetes is increasing in every country. The prevalence of diagnosed diabetes is higher in racial and ethnic minorities among people aged > 20 years, affecting approximately 15.9% of Native Americans, 13.2% of African Americans, and 12.8% of Hispanics **(2)**. DN can be characterized by several morphological changes that involve all sections of the kidney, which affect the function of the organ. The series of alterations include the thickening of the basement membrane and the formation of nodular glomerulosclerosis within

the glomeruli. The transformation in nature of the glomerular basement membrane (GBM) from its normal collagen chains, a1 (IV) and a2 (IV) to more restricted collagen chains, a3 (IV) and a4 (IV) results in the accumulation of type IV collagen in the lamina of the GBM **(3).**

Risk factors

The two main risk factors for DN are hyperglycemia and arterial hypertension. However, DN develops in only about 40% of patients, even in the presence of hyperglycemia and elevated BP for long periods of time. This observation raised the concept that DN will develop only in a susceptible subset of patients. Furthermore, family studies have confirmed a genetic contribution for the development of DN in both type I and type II DM **(4).**

1-Increased albuminuria: increase excretion of albumin in the urine is a major risk factor for the development and progression of kidney disease in people living with diabetes. It is characterized by increased excretion of albumin/g creatinine in the urine referred to as microalbuminuria (30–300 mg/g) or macroalbuminuria (> 300 mg/g) **(4).**

2-Hyperglycemia: is considered as one of the most prominent and independent risk factors of DN. It increases the worsening of renal function by altering the antioxidant system which leads to the increased ormation of advanced glycation end products. Polyol pathway activation is also postulated in the pathogenesis of DKD. Variability in glycated hemoglobin (HbA1c) is associated with the development and progression of nephropathy in both type 1 DM patients and T2DMM patients. A similar finding was reported by the Renal Insufficiency and Cardiovascular Events (RIACE). Evidence from trials found beneficial effects of intensive glucose control in the delayed onset as well as in preventing the progression of albuminuria in T2DMM patients **(5).**

3-Arterial hypertension: is a pivotal risk factor for diabetic nephropathy. Hypertension is significantly associated with the development of diabetic nephropathy as confirmed by a recent meta-analysis. Hypertensive patients are at higher risk of developing diabetic nephropathy as compared to non-hypertensive patients with an odds ratio of 1.67 (95% CI: 13.1–2.14) **(5).** This was further confirmed by a population-based prospective study from china which states that hypertension control can reduce the incidence of end stage kidney failure by 23% **(4).**

4-Dyslipidaemia: plays an important role in the development and progression of DKD. The impact of dyslipidemia on renal function impairment was described by the lipid nephrotoxicity hypothesi.In people with diabetes, dyslipidemia is characterized by a decrease in high density lipoprotein, and an increase in triglycerides, low-density lipoprotein, and very low density lipoprotein **(1).** Dyslipidemia has a role in the development of DN by causing apoptosis of podocytes, macrophage infiltration, and production of extracellular matrix. Hyperglycemia and insulin resistance could aggravate dyslipidemia in DN patients **(4).**

5-Obesity: evidence suggests a strong association between obesity and DN. The mechanism by which obesity leads to DN is not clear but it is presumed that obesity leads to glomerular injury, glomerular hypertrophy, and proteinuria **(4).**

6-Smoking: is considered as an independent risk factor in the development and progression of diabetic nephropathy. The pathogenic role of smoking in the development of diabetic

nephropathy is multifactorial including oxidative stress, hyperlipidemia, deposition of advanced end glycation products, and glomerulosclerosis.Evidence form a finish diabetic nephropathy study on 3613 type 1 DM patients found a higher risk of albuminuria and end stage renal disease in smokers as compared to non-smokers **(6).** The risk of diabetic nephropathy was found to be increased with the dose of smoking. This was also confirmed by a recent meta-analysis based on the pooling of nine cohort studies which concludes that smoker T2DM patients are at an increased risk of developing diabetic nephropathy **(7).**

7-Dietary factors: increased dietary protein intake seems to be associated with the presence of higher UAE values, at least in patients with type I DM. In patients with type II DM, this association has not been documented. The source of proteins in the diet also seems to be related to the presence of DN. A higher intake of fish protein is related to a lower risk of microalbuminuria in type I DM patients. The mechanisms involved in these findings are unknown but probably related to hemodynamic factors **(8).**

Regarding the dietary lipid content, an association has been observed between the higher intake of saturated fat and the presence of microalbuminuria in patients with type I DM. In patients with type II DM, it was observed that the presence of microalbuminuria was associated with the lower content of polyunsaturated fatty acids, especially those of vegetal origin **(8).**

8-Genetic risk factors: the exact genetic model underlying DN susceptibility is uncertain, but theoretically few genes with a major contribution and some with minor interaction with the environment could cause DN. Unfortunately, no gene with a major effect had been identified so far. The knowledge of which gene(s) predisposes to DN will allow the identification of patients at high risk for this complication, and adoption of preventive measures **(5).** Advanced technology makes easier to look for regions in whole genome linked to different DN phenotypes (**Rukov et al., 2016**). Moreover, new targets for drug development may come into sight, since some of the genes found are novel and have not been previously implicated in the pathogenesis of DN **(5).**

9-Oxidative stress: has been associated with the pathogenesis and progression of DN, and it is suggested that antioxidants might inhibit the progression of kidney dysfunction. In animal models, antioxidants have been shown to be effective in treating DN. Bilirubin was an important factor of the endogenous antioxidant system in the human body. As the bilirubin level decreased, antioxidant capacity fell down. Several studies had shown that there was a negative relationship between the total bilirubin level (TBL) and diabetic microvascular complications **(10).**

Treatment:

There are two overarching aims in the management of DN: preserving renal function to reduce the risk of ESKD; and reducing the risks of cardiovascular events and mortality. In addition, people with DKD are also more likely to experience retinopathy, neuropathy and foot ulcers so increased vigilance for these complications is important **(11).**

The main treatment, once proteinuria is established, is Angiotensin converting enzyme (ACE) inhibitor drugs, which usually reduces proteinuria levels and slows the progression of diabetic nephropathy. Several effects of the Angiotensin converting enzyme inhibitors (ACEIs) that may contribute to renal protection have been related to the association of rise in Kinins which is also responsible for some of the side effects associated with ACEIs therapy such as dry cough. The

renal protection effect is related to the antihypertensive effects in normal and hypertensive patients, renal vasodilatation resulting in increased renal blood flow and dilatation of the efferent arterioles **(12).**

Metformin is the standard therapy for patients with type II DM and will only be briefly discussed here. Metformin is contraindicated when serum creatinine is above 1.5 mg/dl in men and 1.4 mg/dl in women due to the increased risk of lactic acidosis. However, these values are being questioned. In these creatinine ranges, some subjects will be using metformin on chronic renal disease stages II and III **(1).** Certain hypoglycemic agents have been shown to confer independent renoprotective effects beyond their hypoglycemic action. For instance, peroxisome proliferator activator receptor-gamma (PPAR-γ) agonists, known as thiazolidinediones (TZD), have direct renoprotective effects in experimental models. However, reports from clinical studies have been varied, with some achieving encouraging results by lowering proteinuria **(13).**

Sodium-glucose cotransporter 2 inhibition therapeutic class has been approved for the treatment of patients with T2DM with an eGFR of ≥ 45 mL/min/1.73 m2. To date, however, just canagliflozin has been evaluated, showing safety and efficacy in a subset of patients with stage 3 CKD **(14).**

Role of Klotho in DN patients

α-Klotho was discovered in 1997 and simply named Klotho because no other Klotho proteins were identified at that time. It was shown to be an antiaging molecule. Mice that exhibited Klotho deficiency presented a premature aging phenotype, whereas Klotho overexpression extended their lifespan by up to 30% and protected them against many pathological phenotypes, especially renal disease **(15).**

Klotho is expressed mostly in the kidneys and choroid plexus in the brain. In the kidneys, Klotho is present in podocytes, the (mostly) apical and basolateral membrane, and intracellularly in cells that comprise the proximal tubule of the nephron. Klotho is also shed to the proximal tubule lumen **(16).**

The pleiotropic effects of soluble Klotho are hypothesized to be based on its capacity to bind sialic acid and targetmonogangliosides (e.g., mono sialo tetra hexosyl ganglioside GM1and monosialodihexosylganglioside-GM3) that are enriched in lipid rafts of cell membranes. Soluble Klotho can also interact with numerous intracellular proteins **(17).**

Two or three forms of α-Klotho protein exist. Membrane-bound Klotho is a 130 kDa single-pass transmembrane protein that is encoded by a *KL* gene. The extracellular domain of the Klotho protein can be cleaved and separated from the cell membrane by the metalloproteinases ADAM-10 and ADAM-17 (ADAM-a disintegrin and metalloproteinase domain-containing protein) and β-secretase 1 (BACE1). This proteolytic cleavage by ADAM-10 and ADAM-17 can be stimulated inter alia by insulin. The extracellular domain of Klotho contains two homologous repeat sequences (KL1 and KL2), which are separated from each other by unknown proteases. Both the whole released extracellular domain and separated KL1 and KL2 are referred to as soluble Klotho particles **(18).** Both transmembrane Klotho and soluble Klotho serve as coreceptor proteins for fibroblast growth factor (FGF) 23 (binding to FGF receptors 1–4), which

promotes phosphaturia to control phosphate metabolism , also Klotho functions independently to regulate many signaling pathways because it is evolutionarily older than FGF23 **(19).**

Klotho regulates not only phosphate metabolism but also calcium metabolism. Furthermore, it inhibits the insulin/insulin-like growth factor 1 (IGF-1) signaling pathway and activates forkhead box (FoxO) transcription factors, resulting in the production of antioxidant enzymes (e.g., catalase and manganese-dependent superoxide dismutase and reduction of oxidative stress through the removal of ROS, thereby downregulating apoptosis. It also suppresses tumor necrosis factor α (TNF-α)-induced oxidative damage and prevents the translocation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), a transcription factor of many proinflammatory genes, including TNF-α, to the nucleus. Klotho also suppresses the profibrotic transforming growth factor β1 (TGF-β1) and Wnt/β-catenin signaling **(20).**

The disruption of phosphate and calcium metabolism, oxidative stress, inflammation, the fibrotic process, and an increase in the ratio of β-cell and podocyte loss through apoptosis can result in pathologies that resemble premature aging. Klotho protects cells against accelerated aging and damage during the course of DM and diabetic nephropathy Moreover, a reduction of plasma and urine levels of circulating soluble Klotho was observed during the aging process in cells with short telomeres or stress-induced premature senescence, widespread tissue injury, inflammation, oxidative stress and vascular calcification, which occur during the course of chronic kidney disease (CKD). Additionally, in patients with CKD, low levels of calcitriol (i.e., the bioactive form of vitamin D3) were found to intensify renal Klotho deficiency **(21).**

Low levels of Klotho mRNA and protein were also expected to be observed in diabetic nephropathy. This assumption was confirmed in diabetic mice. In two models of T1DM, Klotho deficiency promoted the apoptosis of insulin-producing β-cells, which were protected against this process after Klotho overexpression. Furthermore, Klotho improved the β-cell function and prevented the development of type 2 DM. Low plasma, but not urine, levels of Klotho predicted the progression of nephropathy in T2DM patients and were negatively correlated with a decrease in the glomerular filtration rate (GFR) **(16).** Moreover, diabetes-induced proteinuria, oxidative stress (reflected by intracellular ROS levels), podocyte injury, and apoptosis that was caused by protein kinase Cα (PKCα) activation were aggravated by Klotho deficiency and partially ameliorated by Klotho overexpression **(22).**

Both plasma and urine levels of soluble Klotho were lower in intrinsic AKI patients compared with prerenal AKI patients, together with an increase in levels of proinflammatory ligands (i.e., S100A8/A9 calgranulins) of Toll-like receptor 4 (TLR4). Therefore, plasma or urine levels of the Klotho protein were proposed to serve as a biomarker of early kidney injury in diabetic patients **(23).**

Surprisingly, it has been suggested that patients with poor glycemic control may have higher s-klotho levels, possibly because glycosuria increases the metabolic demands of the renal tubules, leading to increased expression and/or cleavage of klotho. It should be noted that low levels of circulating klotho have a strong association with deteriorating renal function in patients with T2DM, with this trend being more pronounced in those receiving hemodialysis. Furthermore,

studies have indicated that the expression of klotho in both plasma is reduced in the early stages of DN and that a further decrease may indicate the development of DN **(24).**

It has been observed that the levels of klotho decrease with increasing levels of inflammation and oxidative stress in DN patients **(25).**

Studies have shown that inflammatory factors, including tumor necrosis factor-α (TNF-α), and the uremic toxin indolyl sulfate, can increase ROS production and downregulate klotho expression through a nuclear factor-kappaB (NF-κB)-dependent mechanism **(26).** In addition, epigenetic involvement in klotho gene expression, such as m6A modification of the methyltransferase complex, has been found to reduce klotho expression and exacerbate renal injury and inflammation in DN **(27).**

Increased levels of klotho help to control levels of inflammation and oxidative stress. Klotho attenuates the inflammatory response induced by hyperglycemia by directly or indirectly regulating NF-κB, thereby reducing renal damage **(28).** It can be argued that klotho is an anti-inflammatory modulator that negatively regulates NF-κB, and thus reduces the transactivation of pro-inflammatory genes, primarily through a mechanism involving phosphorylation of Ser (536) in the transactivating structural domain of RelA . Klotho also inhibits the translocation of NF-κB to the nucleus and attenuates the activity of the NF-κB pathway, thereby reducing the production of downstream inflammatory factors **(15).**

Activation of TLR4 is also known to contribute to the transcription of pro-inflammatory genes, production of inflammatory factors, and ROS, promoting the development of systemic inflammation through paracrine and systemic effects. Subsequent investigations have demonstrated that klotho and TLR4 counteract each other via a protein hydrolysis process related to deglycosylation, whereby klotho mitigates the downstream inflammatory response associated with TLR4 and reverses acute kidney inflammation **(29).**

In early DN, IGF-1 activity is increased and interacts with the RAS to co-regulate renal hemodynamics **(30).** In renal tubular epithelial cells, IGF-1 induces elevated levels of ROS, NADPH oxidase activity, and fibronectin expression, which trigger renal inflammation. Klotho inhibits intracellular insulin/IGF-1 signaling and enhances tissue resistance to oxidative stress by suppressing tyrosine phosphorylation of insulin and IGF1 receptors **(31).**

Furthermore, overexpression of klotho has been shown to significantly induce the signaling of nuclear factor erythroid 2-related factor 2 (Nrf2), a core transcription factor that regulates antioxidant responses. By regulating Nrf2 and cytoprotective antioxidant enzymes, klotho inhibits high glucose-induced oxidative stress and podocyte apoptosis, thereby ameliorating DN **(32).**

A decrease in klotho levels can have a significant impact on endocrine signaling in the body, and a reduced abundance of klotho in the kidneys may directly lead to renal fibrosis and impaired renal function. In other words, low levels of klotho can activate the TGF-β1 signaling pathway, which contributes to diabetes-related renal fibrosis **(33).**

Notably, when klotho is deficient, a large increase in circulating levels of FGF23 can also target cell types that lack klotho, and this becomes the pathological basis for promoting fibrosis.

However, it has been demonstrated that recombinant klotho protein or exogenous klotho supplementation can inhibit high glucose-induced TGF-β signaling, downregulate the expression of pro-fibrotic genes in renal mesenchymal fibroblasts, and ultimately reverse fibrotic lesions. Egr-1 is expressed in renal tubular fibroblasts, glomerular mesangial cells, and several other cell types, making it a critical factor in the EMT of DN **(27).**

In particular, klotho has been found to directly target the Egr1/TLR4/mammalian target of rapamycin (mTOR) axis, which reduces inflammation and fibrosis in the glomerulus, suggesting that it has multi-targeted effects **(34).**

Importantly, klotho has significant upstream regulatory effects on TGF-β1. For example, klotho can downregulate TGF-β1 and connective tissue growth factor expression by inhibiting RhoA/ROCK activity, thereby reducing tubulointerstitial fibrosis and proteinuria in DN. The Wnt/β-catenin proteins frequently interconnect with other signaling pathways, including TGF-β1, that jointly contribute to a complex mechanism promoting interstitial fibrosis in the kidney. The endogenous antagonist klotho blocks Wnt-triggered β-catenin protein activation and nuclear translocation, inhibiting podocyte dedifferentiation and mesenchymal transformation and ultimately restoring podocyte integrity **(35).**

In another example, klotho-derived peptide 6 has been shown to possess similar properties to klotho by inhibiting the expression of downstream target genes of the Wnt/β-catenin pathway. This effect has been shown to improve DN and reduce lesions of glomerulosclerosis and interstitial fibrosis **(36).** It is worth noting that klotho can further suppress renin-angiotensin-aldosterone system (RAAS) activation by inhibiting the Wnt/β-catenin pathway, as the overexpression of β-catenin or Wnt ligands can activate all RAS genes **(5).**

Previous research has shown that klotho gene expression is downregulated in several conditions of endothelial dysfunction associated with vascular disease, including DN. In addition, studies have shown that klotho protein or its metabolites promote endothelial NO production in small arteries through a humoral pathway, thereby maintaining normal endothelial function. Further studies have shown that klotho plays an important role in the regulation of vascular function, vascular remodeling **(Figure 1)**, and prevention of perivascular fibrosis through the upregulation of oxidative scavengers and activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/nitric oxide synthase (eNOS) pathway, as well as stimulation of the MAPK/ERK kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway to attenuate endothelial apoptosis and senescence **(37).**

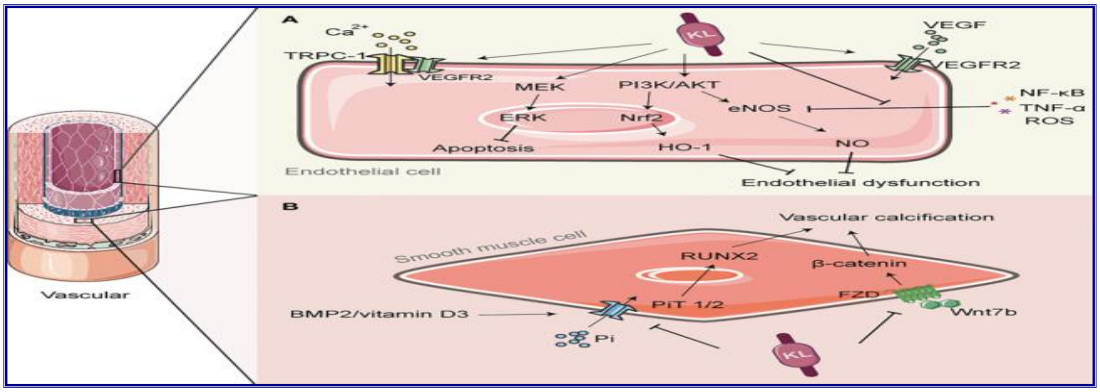

Fig. (1)The role of Klotho in vascular protection. (A) Klotho can activate PI3K/AKT/Nrf2/HO-1 to enhance endothelial antioxidant defense; It can also activate PI3K/Akt/eNOS pathway, and alleviate the inhibition of eNOS phosphorylation by inflammatory factors such as TNF-α, thereby promoting NO production and preventing endothelial cell dysfunction. Klotho is involved in the transmission of VEGF signaling and regulates Ca2+ influx, which helps to maintain endothelial integrity. (B) Klotho inhibits phosphate uptake by vascular smooth muscle cells (VSMCs), leading to improved vascular calcification. It also inhibits the PiT2/RUNX2 signaling pathway, which improves extracellular matrix calcification. Additionally, Klotho inhibits the Wnt7b/β-catenin signaling pathway, which prevents VSMC calcification. NO, nitric oxide; eNOS, endothelial nitric oxide synthase; PI3K, Phosphoinositide 3-kinase; AKT, protein kinase B; Nrf2, Nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; TRPC-1, transient-receptor potential canonical Ca(2+) channel 1; VEGF, vascular endothelial growth factor; PiT, Pi transporter; RUNX2, runt-associated transcription factor 2.

In addition, klotho inhibits the expression of vascular cell adhesion molecule-1 (VCAM-1) and suppresses the inflammatory response and vascular damage caused by the adhesion of leukocytes to endothelial cells; at the same time, klotho also deregulates the inhibition of eNOS phosphorylation by TNF-α, maintains NO production and regulates endothelial inflammation **(38).** Overall, klotho may indirectly reduce endothelial dysfunction induced by inflammatory factors and uremic toxins through its anti-inflammatory and antioxidant effects **(39).**

As an important pathway for calcium and phosphorus excretion, increased urinary phosphorus excretion by the kidneys seems to guarantee a lower risk of chronic kidney disease (CKD) progression in T2DM patients **(40).**

The mechanism by which klotho regulates calcium and phosphate homeostasis involves the binding of skeletal-produced FGF23 to the klotho/FGFR1c receptor in the renal tubules, which leads to increased phosphate excretion in the urine **(41).** However, one study has reported that klotho can transport renal phosphate independently of FGF23 **(42).**

Downregulation of the klotho gene in chronic renal failure is thought to be associated with the development of vascular calcification (VC) Specifically, defects in klotho lead to phosphate retention, and elevated blood phosphate concentrations trigger calcium phosphate precipitation, a product that is adsorbed by the serum protein fetuin-A and induces ectopic calcification **(43).**

In the early stages of renal disease, a reduction in the co-receptor klotho can lead to an increased risk of vascular calcification and vascular senescence. This risk is further amplified by a compensatory increase in F23. However, it is unclear whether this effect is due to circulating klotho or localized klotho in the vascular system. Rukov et al. demonstrated that neither normal nor calcified vascular systems in chronic renal failure express klotho **(9).** However, this finding contradicts the findings of Donate-Correa et al., who reported that klotho is expressed in calcified human arteries **(44).**

Klotho can improve vascular calcification by inhibiting phosphate uptake by vascular smooth muscle cells (VSMC) and increasing urinary phosphorus excretion. Further studies have revealed that this process may also involve the regulation of the Wnt7b/β-catenin signaling pathway by klotho, which indirectly inhibits hyper phosphate-induced VSMC calcification **(45).**

In diabetic rat kidneys, insulin-like growth factor 1 (IGF1) and insulin-like growth factor-1 receptor (IGF-1R) expression are significantly elevated, which can lead to lipid accumulation in rat renal mesangial cells and dysfunction. However, overexpression of klotho in mice can bind to cell surface receptors and inhibit the intracellular transmission of insulin and IGF1 **(46).**

Klotho antagonizes IGF-1R and subsequently inhibits downstream PI3K/AKT/mTOR signaling, which contributes to increased peroxisome proliferator-activated receptor-α(PPAR-α)transcriptional activity of genes that regulate metabolism, improve insulin sensitivity, and maintain glucolipid homeostasis .While klotho overexpression has been shown to inhibit insulin signaling, it does not necessarily result in insulin resistance and can still effectively harness its anti-aging properties. On the contrary, a deficiency in klotho has been demonstrated to worsen insulin resistance and raise blood glucose levels **(33).**

The discovery of klotho in the cerebral choroid plexus revealed that its functions are not limited to regulating glucose metabolism in the peripheral system. According to a study, central klotho does not have any impact on fasting insulin or glucose levels. Instead, its influence on insulin secretion may depend on glucose levels. Furthermore, the study suggests that this regulatory effect remains unaffected by any changes in body weight **(46).**

Autophagy is a lysosomal degradation pathway that aids cells in adapting to or counteracting stress responses, primarily by breaking down and recycling cytoplasmic components. Nutrient-sensing pathways, such as the IGF-1 signaling pathway, the mammalian target of rapamycin (mTOR) signaling pathway, and the adenylate-activated protein kinase (AMPK) pathway, regulate autophagy, with the target of mammalian rapamycin complex 1 (mTORC1) being the primary negative regulator of autophagy. A significant amount of evidence supports a regulatory role for autophagy in DN. For instance, cells exposed to high glucose levels for a prolonged period experience inhibited autophagy, which accelerates the development of DN **(47).**

However, klotho can upregulate autophagy and mitigate renal cell injury and subsequent renal fibrosis **(Fig. 2).** More specifically, klotho regulates the upregulation of autophagic flux by inhibiting the AKT/mTOR pathway or the IGF-1-mediated PI3K/Akt/mTOR pathway to improve diabetes and kidney injury **(48).**In parallel, the AMPK and MAPK pathways are also targets of klotho's intervention to inhibit hyperglycemic damage to renal tubules through improved autophagy **(49).**

In addition to the autophagic pathways mentioned above, klotho also regulates Beclin-1, a crucial autophagy regulator, and may modulate autophagy levels by affecting the relative levels of the Beclin-1/Bcl-2 protein complex **(50).**

Also, klotho increases the binding of Beclin-1 to Bcl-2 and decreases the interactions of Beclin-1 with other autophagy-related proteins, thereby inhibiting autophagic activity in DN. It is important to note that the autophagic process is not only regulated by klotho but also influenced by Pi, which promotes the binding of Beclin-1 to its negative regulator BCL-2, impairing autophagic flux **(51).**

In addition to its effects on the autophagic pathway, klotho is also known to rescue hyperglycemia-mediated apoptosis of podocytes, glomerular endothelial cells, and renal tubular epithelial cells. Klotho can even inhibit apoptosis or necrosis caused by ischemia-reperfusion injury and nephrotoxicity **(52).**

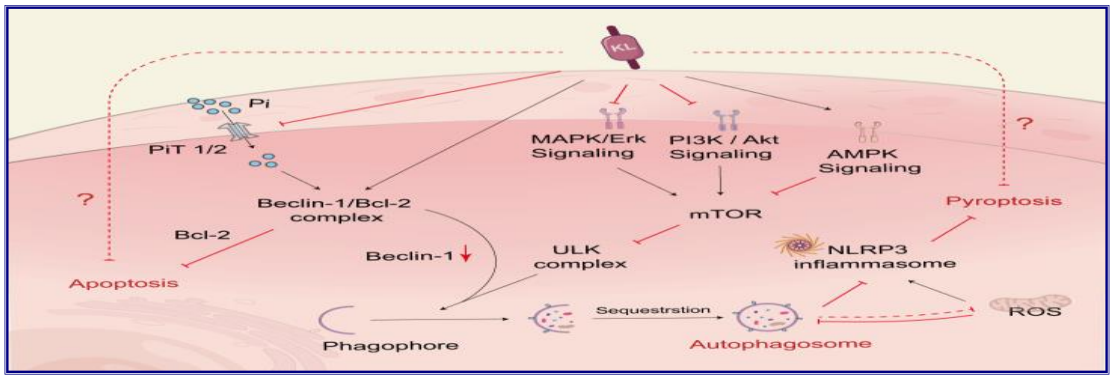

Fig. (2): Klotho regulates cell death. Klotho regulates autophagic flux through PI3K/AKT/mTOR, MAPK, and AMPK pathways. Klotho can increase the binding of Beclin-1 to Bcl-2, reduce Beclin-1's interaction with other autophagy-related proteins, and thereby regulate autophagy. Additionally, Klotho indirectly upregulates autophagy by inhibiting the activity of NLRP3 inflammatory vesicles, which helps prevent renal tubular cell death. Klotho also indirectly regulates autophagy by controlling the excretion of Pi. PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; AMPK, adenosine monophosphate-activated protein kinase; NLRP3, nucleotide-binding oligomerization domain-like pyrin domain-containing protein 3; PiT, phosphate transporter.

It is widely recognized that apoptosis is primarily mediated by the mitochondrial pathway, the death receptor pathway, and endoplasmic reticulum stress. However, studies investigating the mechanisms by which klotho intervenes in apoptosis have only begun to scratch the surface of this complex process. As a molecular chaperone of klotho, HSP expression increases in response to various stresses and thus functions as a cytoprotective agent. Studies have shown that klotho reduces apoptosis via HSP-70 in experimental ischemic acute kidney injury, and the mechanism may involve HSP70 relying on the immunoprecipitated anti-apoptotic proteins Bcl-2 and Bcl-xl to provide protecti to cells. Recent research has also shown that HSP70 promotes renal cell survival by inhibiting nucleophosmin (NPM) phosphorylation and reducing itaccumulation in the cytoplasm **(53).**

Pyroptosis is a type of programmed cell death that is characterized by cell lysis and is triggered by inflammatory vesicles. The canonical pathway for inducing pyroptosis is through activation of the nucleotide-binding oligomerization domain-like pyrin domain-containing protein 3(NLRP3) inflammasome-mediated caspase-1. It is important to note that both pyroptosis and necroptosis pathways essentially result in inflammatory lytic cell death **(54).**

In fact, autophagy, apoptosis, and pyroptosis are all part of programmed cell death. On the one hand, autophagy inhibits apoptosis as a pathway for cell survival; while on the other hand, autophagy itself or autophagy and apoptosis acting together can induce cell death. Apoptosis can be converted to pyroptosis via the cysteine-3-GSDME (caspase-3) signaling pathway and autophagy in turn can inhibit pyroptosis by reducing mitochondrial ROS to suppress NLRP3 inflammatory vesicle activation **(55).**

Therefore, these three mechanisms can act as both allies and adversaries, and the dominant mechanism is determined by the cellular environment in which the cell exists. Klotho is closely associated with cell fate and can exert its influence on the regression of DN by interfering with all three signaling pathways. However, the specific mechanisms through which it regulates cell death in the context of DN remain to be investigated in depth.

Klotho is found in every nuclear layer of the retina and is strongly linked to retinal function. For example, in klotho knockout mice, the amplitude of a- and b-waves is reduced, retinal signaling is weakened, and synaptic function is altered. Although there is no evidence of retinal degeneration, there is a definite tendency toward faster retinal aging **(56).**

However, only a small number of therapeutic approaches targeting klotho have been explored for the treatment of DR. Late-stage diabetes can result in microvascular complications such as DR and DN. It is important to recognise that these complications share common pathogenic mechanisms and are often predictive of each other. Consistent with our expectation that decreased levels of klotho are also found in DR, and low levels of klotho are strongly associated with a high risk of progression to DR **(57).**

Given the various advantageous effects of klotho on DN, it is speculated that klotho may also have a positive impact on DR . Several studies revealed that decreased levels of α-klotho in the lens of diabetic rats lead to a reduction in Nrf2-mediated antioxidant defense, thereby contributing to increased NF-κB-mediated inflammatory responses. On the contrary, when klotho was supplemented, it resulted in elevated levels of antioxidants such as superoxide dismutase, glutathione peroxidase, glutathione, and other oxidants, thus effectively preventing oxidative stress and inflammation in the lens of diabetic rats **(58).**

The retinal pigment epithelium (RPE) is a polarized layer of cells containing pigment that is essential for normal visual function in the retina. The protein klotho plays a crucial role in maintaining the viability of the RPE by promoting mitochondrial biogenesis and scavenging ROS **(48).**

It is worth mentioning that klotho can exert a protective effect against DR by inhibiting retinal endothelial cell apoptosis. It achieves this by affecting the Bcl-2/Bax ratio and activating the PI3k/Akt signaling pathway **(59).**

However, there is still a lack of studies on the effects of klotho on microvascular endothelial cells in DR. But the specific mechanisms that contribute to this remain unknown. Given that klotho has demonstrated the ability to intervene in DN through various mechanisms and that there are similarities in the pathological mechanisms between DR and DN, targeting klotho as a therapeutic option may have the potential to intervene in DR.

In summary, all these results suggest that klotho plays a key role in the development of DN. So, directing therapeutic efforts towards klotho presents a promising avenue for treating patients with DN and may also offer benefits for individuals with T2DM.

Conclusion:

The levels of serum soluble klotho have a positive correlation with the estimated glomerular filtration rate and a negative correlation with urinary albumin, particularly in patients with diabetes. Conversely, increasing klotho levels helped to delay the progression of diabetes and postpone the onset of DN complications.

All studies conducted thus far have confirmed the beneficial effects of klotho on vascular calcification. Klotho may have the immunotherapeutic potential for the treatment of diabetes and its associated nephropathy by controlling levels of inflammation and oxidative stress.

No Conflict of interest.

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