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Klotho: a possible role in the pathophysiology of nephrotic syndrome

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ABSTRACT

Klotho, encoded by the *klotho* gene, is associated with phosphate homeostasis. Klotho acts as a co-receptor for FGF23 for binding to its receptors. With FGF23, klotho regulates the systemic mineral homeostasis by regulation of vitamin D and parathyroid hormone. The anti-inflammatory, antifibrotic and antioxidant properties of klotho give it a cardinal role in the development of various renal diseases. The protective effect of klotho has been evident in different types of nephropathy, including diabetic nephropathy, cyclosporine A-induced nephropathy, Calcineurin inhibitors-induced nephropathy, and renal ischemic-reperfusion injury. Nephrotic syndrome is distinguished by hypoproteinemia, proteinuria, and hypercholesterolemia as a result of the aberration of the glomerular filtration barrier. The various factors and pathways associated with the pathophysiology of the nephrotic syndrome have similarities with other types of nephropathy. Despite these similarities,

the role of klotho in the pathology of nephrotic syndrome remains still unexplored. This mini-review builds the case for the possible role of klotho in nephrotic syndrome. The review explores the possible pathways where klotho can play a major role by identifying the similarities in the pathophysiology of nephrotic syndrome and other types of nephropathy.

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INTRODUCTION

Klotho, a 135 kDa transmembrane protein, is associated with the aging process and is involved in phosphate metabolism, and regulates the activity of fibroblast growth factors (FGF) [1]. The word klotho has been derived from the name of the Greek goddess Clotho who is believed to spin the "thread" of human fate. The derivation of the word signifies a metaphor for the life span of an individual as the *klotho* gene (KL) is associated with aging. The role of klotho in aging was first reported in mouse studies where KL deficient mice had a decreased life span of fewer than eight weeks [2]. Similarly, an increased expression of the KL gene had positive effects on the life span of the organism [3]. Animal studies demonstrated an association between klotho protein deficiency and an increase in phosphate levels in the blood that is attributed to be one of the mechanisms involved in the decreasing life span in the animals.

The changes observed in klotho deficient animals are comparable to the age-related senile changes observed in humans [4, 5]. These major changes included plaque formation in the arteries, weakening of bones, fat and muscle loss, hasty shrinkage of the thymus resulting in changes in the architecture of the thymus, and decrease in tissue mass leading to gradual deterioration of the immune system; which are similar to senile changes observed in the elderly.

The klotho gene family comprises α-klotho, β-klotho, and γ-klotho [6]. α-KL gene, located on Chromosome 13, comprises three exons and two introns [7] and encodes a transmembrane protein with a smaller cytoplasmic domain and a larger extracellular domain. There are three distinct types of α-klotho protein: transmembrane klotho, secretory klotho, and soluble klotho [7]. Metalloproteinases enzymes ADAM 10 and 17 act on the extracellular domain of klotho to release it from its membrane sites [8-10]. The enzymatic cleavage by the sheddase enzyme at the extracellular domain leads to the generation of soluble klotho form. Secretory klotho, having a molecular weight of 70 kDa, is another form of klotho protein formed by alternate splicing of KL exons. Fibroblast Growth Factor-23 (FGF23) plays a crucial role in phosphate and vitamin D metabolism requires transmembrane α-klotho protein as its co-receptor [11]. β-klotho and γ-Klotho are also transmembrane proteins [6]. β-klotho acts as a co-receptor for FGF19 and FGF21 regulating bile acid synthesis and energy metabolism [12]. Both the transmembrane form of α-klotho protein (acting as a co-receptor for FGF23) and the soluble form of klotho has been demonstrated to be involved in pathways whose aberration can lead to nephrogenic effects [13,14].

Klotho has been directly implicated in the development of chronic kidney disease (CKD) [15]. A decrease in klotho levels succeeded by a rise in serum FGF23 indicated deterioration of kidney function in chronic kidney disease. Further, FGF23, produced from bones, regulates mineral metabolism [11].

Increased excretion of protein in urine and the resultant edema and hypoalbuminemia characterize the nephrotic syndrome. Of all types of nephrotic syndrome, some are steroid-resistant and some are steroid responsive. Minimal change disease, the commonest cause, is steroid responsive.

Steroid resistance includes focal segmental glomerulosclerosis that has a significant risk of kidney failure [16]. Systemic diseases such as Lupus can also cause nephrotic syndrome.

The primary and secondary causes of nephrotic syndrome should be distinguished and management strategies appropriately tailored. Immunosuppressive medications are the mainstay of treatment. The various causes of nephrotic syndrome have been categorized and listed in Table 1.

Klotho has significant beneficial effects on the kidney by alleviating oxidative stress and by its antiapoptotic properties. This protective effect of klotho in different types of nephropathy, including diabetic nephropathy, has been demonstrated in multiple studies.

The pathologic alterations observed in different types of nephropathy such as increased oxidative stress, and inflammation, have also been demonstrated in nephrotic syndrome. However few studies have explored the role of klotho in nephrotic syndrome.

KLOTHO IN DIABETIC NEPHROPATHY

The role of klotho in the development of diabetic nephropathy has been explored in various animal models. A decreased klotho level in animal models increased the purinergic receptor $P2X_7$ culminating in cell death by apoptosis or necrosis in diabetic nephropathy [18]. Klotho was also demonstrated to suppress the hyperglycemia-mediated glomerular endothelial injury and activation of the Wnt/β-catenin pathway in mice models of diabetic nephropathy [19]. The aetiological role of klotho in DN was further expounded by the attenuation of apoptosis of renal tubular cells by the drug atrasentan that acts by decreasing the expression of miR-199b-5p and thus increasing its target, klotho [20]. A reduction in the odds of early nephropathy in T2DM patients was observed at a higher concentration of FGF-23, which correlated positively with sKL in diabetic patients [21]. Interestingly, nephrotic syndrome, when compared with controls is associated with decreased levels of FGF23. The reduction in Vitamin D levels and the loss of FGF23 in urine are thought to contribute to lowered levels observed [22].

Table 1 List of various causes of nephrotic syndrome categorized based on etiology

Genetic	Infectious causes	Idiopathic	Others
• Diffuse mesangial sclerosis (DMS) • Epidermolysis bullosa associated • Steroid-resistant nephrotic syndrome • Familial focal segmental glomerulosclerosis (FSGS)	• Congenital infections including syphilis, toxoplasmosis, and HIV • Cytomegalovirus • HIV-associated nephropathy	• Minimal change nephropathy • Focal segmental glomerulosclerosis • Diffuse mesangial hypercellularity • Membranous glomerulonephritis • Membranoproliferative GN	• Lupus nephropathy • IgA nephropathy • Drugs • Malignancies • Hemolytic uremic syndrome (HUS)

INFLAMMATION AND OXIDATIVE STRESS IN NEPHROPATHY

The protective effect of klotho has also been explored in other types of nephropathy. Increased klotho levels, in cyclosporine A-induced nephropathy, regulate cytokine expression and modulate the inflammation via PDLIM2/NF-kB p65 pathway resulting in beneficial effects [23]. It also has a crucial role in protection against Calcineurin inhibitors-induced nephropathy [24]. The decreased klotho levels make the kidney vulnerable to oxidative stress-induced organ injury [25]. Klotho mitigates oxidative stress by increasing the manganese superoxide dismutase expression via suppression of the PI3K-AKT signaling pathway [26]. Further, klotho also prevents Calcineurin inhibitors-induced nephropathy by improving autophagy clearance and preventing autophagy cell death [27]. In renal ischemic-reperfusion injury, klotho was demonstrated to have an inhibitory effect on oxidative stress in tubular epithelial cells thus preventing necroptosis [28]. Besides, the inflammatory environment also adversely affects the klotho expressions via NFκB-dependent mechanism as shown by the downregulation of klotho expression by inflammatory cytokines, such as TWEAK and TNFα [29].

Similar to the aforementioned types of nephropathy, the nephrotic syndrome also has been correlated with increased oxidative stress and inflammatory state. Patients with steroid-sensitive nephrotic syndrome had higher plasma levels of advanced oxidation protein products and malondialdehyde indicating oxidative stress in them [30]. The increased pro-oxidant status in nephrotic children leads to considerable change in antioxidant concentrations [31]. The presence of oxidative stress and abnormality in the antioxidative system has been verified in adult nephrotic syndrome patients also [32, 33]. The increased activity of GSH-Px and selenium content in polymorphonuclear leukocytes (PMNLs) in nephrotic syndrome also indicates the presence of oxidative stress in these patients [34]. The decrease in NF-kappaB p65 in addition to the up-regulation of IL-2 are mechanisms hypothesized to initiate glucocorticoid resistance in steroid-resistant nephrotic syndrome [35]. Similarly, elevated serum TNFα levels in nephrotic syndrome are associated with a lack of response to steroids [36]. Further, In vitro study has demonstrated the increased expression by TWEAK of PLA2R as well as NFKB1 and IRF4 which are linked to membranous nephropathy [37].

FACTORS AFFECTING KLOTHO EXPRESSION

Albuminuria, in cultured tubular cells, decreased the expression of klotho [38]. Concurrently, in CKD animal models with frank albuminuria, the klotho expressions were found to be suppressed indicating a possible role that klotho may have in the pathogenesis of CKD.

Proteinuric kidney disease is associated with endoplasmic reticulum (ER) stress. Animal models of albuminuria demonstrated features of ER stress in renal tubular cells which are instigated by the albumin and mediated via ATF3/ATF4 activation. The induction of ATF3 and ATF4 leads to enhanced binding to the promoter region effectuating altered transcription of the klotho gene culminating in the suppression of klotho expression [39]. ATF3 is also induced in renal ischemia-reperfusion injury, where klotho is observed to be downregulated [40]. Further, ER stress accentuates klotho degradations via proteasome and lysosome. Hence, proteinuria (especially albuminuria) leads to decreased klotho protein half-life as well as the genetic expression [41].

Further, the downregulation of klotho in tubular epithelial cells is also associated with the aggravation of renal fibrosis [42]. Although solu-

ble α-klotho positively correlated with eGFR in patients with CKD, the efficacy of klotho in improving renal function in CKD patients is still under investigation [43]. Further, the expression of klotho in kidneys is also affected by excessive renin–angiotensin–aldosterone system (RAAS) activation. In vitro experiments demonstrated Angiotensin II to have a suppressive effect on klotho through PPAR-γ downregulation [44] (Figure 1).

The aforementioned factors affecting klotho expression (albuminuria and Angiotensin II) have been implicated in the pathophysiology of nephrotic syndrome in various studies. The glomerular-derived angiotensinogen has been thought to have a significant effect on glomerular dysfunction in nephrotic syndrome. As a result, the ARB treatment is beneficial in slit diaphragm injury by inhibiting the positive feedback loop of the activated local Ang II action [45]. During podocyte injury, a vicious loop that stimulates the intrarenal generation of Angiotensin II is activated aggravating the development of glomerular dysfunction [46]. Further, PPARγ agonists decrease the proteinuria in acute nephrotic syndrome by regulating the expression of multiple genes like actinin-4 and nephrin and leading to the restoration of podocyte structure [47]. Hence, activation of PPARγ and inhibition of ANGPTL4 is associated with a better prognosis in patients with nephrotic syndrome [48].

CONCLUSION

Figure 2 summarizes the possible mechanisms by which klotho may play a cardinal role in the pathophysiology of nephrotic syndrome.

The various factors associated with the pathophysiology of nephrotic syndrome, including oxidative stress and inflammation, are intertwined with klotho expression and its downstream effects. The multitude of studies demonstrating the association of klotho and these factors in other types of nephropathy warrants its investigation into the nephrotic syndrome. The insights thus obtained would help in designing therapeutic strategies involving klotho and its downstream effectors for nephrotic syndrome.

Figure 1 **Depiction of factors and their modes of influencing the expression of klotho. A: Albuminuria; B: Angiotensin II**

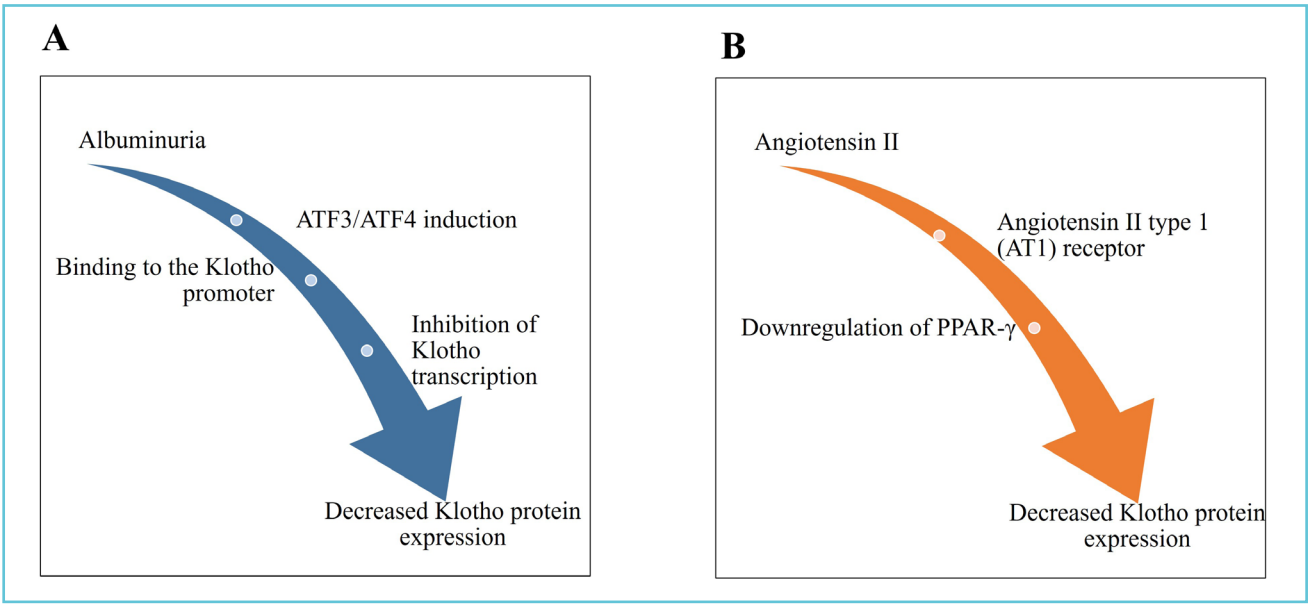
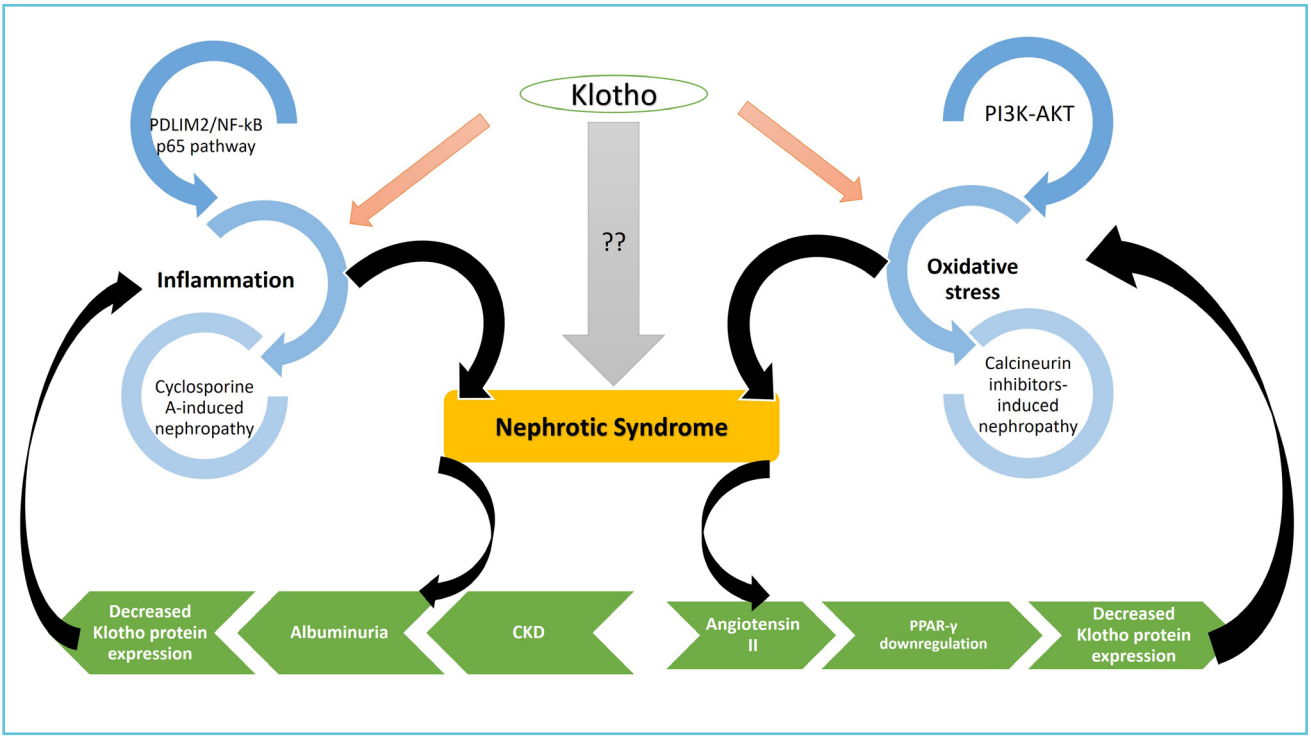


Figure 2 Illustration of the possible roles of klotho in nephrotic syndrome



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