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Potential Application of Klotho in Human Chronic Kidney Disease

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Abstract

The extracellular domain of transmembrane alpha-Klotho (αKlotho, hereinafter simply called Klotho) is cleaved by secretases and released into the circulation as soluble Klotho. Soluble Klotho in the circulation starts to decline early in chronic kidney disease (CKD) stage 2 and urinary Klotho possibly even earlier in CKD stage 1. Therefore soluble Klotho could serve as an early and sensitive marker of kidney function decline. Moreover, preclinical animal data support Klotho deficiency is not just merely a biomarker, but a pathogenic factor for CKD progression and extrarenal CKD complications including cardiovascular disease and disturbed mineral metabolism. Prevention of Klotho decline, re-activation of endogenous Klotho production or supplementation of exogenous Klotho are all associated with attenuation of renal fibrosis, retardation of CKD progression, improvement of mineral metabolism, amelioration of cardiomyopathy, and alleviation of vascular calcification in CKD. Therefore Klotho is not only a diagnostic and/or prognostic marker for CKD, but the treatment of Klotho deficiency may be a promising strategy to prevent, retard, and decrease the burden of comorbidity in CKD.

1. INTRODUCTION

The *Klotho* gene was discovered in 1997 when mice with serendipitous silencing of this gene developed multiple organ dysfunction and failure with shortened life span [1]. Subsequently, two other paralogs βKlotho [2] and γKlotho [3] were identified, then Klotho was designated αKlotho [4]. For the sake of simplicity, αKlotho is referred hereinafter as Klotho throughout this manuscript.

Klotho is highly expressed in the kidney, brain and to a lesser extent in other organs including parathryoid [1, 5]. The extracellular domain of membrane Klotho consisting of two repeat sequences (Kl1 and Kl2) can be shed by secretases and released into the

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circulation as cleaved Klotho [6–9] (Figure 1). Another form of Klotho protein in the circulation is Kl1 fragment which is generated by alternative transcript splicing called secreted Klotho (Figure 1) [1, 10–12]. In concert with cleaved Klotho, these are collectively termed soluble Klotho. But in this review, soluble Klotho is only strictedly used for cleaved full lengh of extracelluar domain of membrane Klotho. Soluble Klotho is a main functional form in the circulation [6, 13–16] and is also present in cerebrospinal fluid [17][16, 18–21] and urine of mammals [15, 22–24]. At physiologic condition, the kidney is a major contributor to maintaining soluble Klotho levels [6, 25], but other organs may participate in maintaining soluble Klotho in chronic kidney disease (CKD) and end-stage renal disease (ESRD) [23]. Soluble Klotho functions as a circulating substance exerting multiple biological actions on distant organs [26–31].

CKD is characterized by progressive deterioration of renal function with high risk of ESRD. CKD risk increases with age, and about half of the CKD stage ≥3 cases occurs in subjects >70 years old. CKD can be viewed as a state of accelerated aging [32, 33]. The relative risk for cardiovascular mortality of a 25 to 34-year-old dialysis patient is similar to a non-CKD patient of >75 years of age [34]. Cardiovascular disease is the principal killer in CKD and ESRD patients. The fact that Klotho-deficient mice and CKD subjects have similar phenotypes also suggests a potential pathogenic role of Klotho deficiency in CKD development and progression [4, 24, 35–37].

Since the kidney is the main origin for circulating Klotho [6, 25] [38, 39], it is not surprising that CKD and ESRD patients have low renal Klotho expression and low levels of circulating Klotho. Renal Klotho deficiency in early stages of CKD may be attributed mainly to suppression of Klotho expression rather than loss of viable renal tubules. Several intermediates are shown to be involved in the reduction of Klotho expression: high serum phosphate [40], hypermethylation [41–45] and hyper-deacetylation [46] in Klotho gene promoter induced by inflammatory cytokines and the uremic toxin, indoxyl sulfate (Figure 2). Furthermore, dialysis patients still have detectable circulating Klotho suggesting that renal Klotho expression is not completely suppressed, and Klotho may come from extra-renal source(s), although its origin is not clear to date (Figure 1) [23]. Establishing extra-renal sources of Klotho and characterizing how this can be up-regulated when renal production fails is of paramount importance.

Klotho deficiency is not only an early biomarker of CKD (to be discussed in detail below), but also a pathogenic intermediate for CKD development and progression (Figure 2), and extrarenal complications [24, 47, 48]. It has been shown that Klotho deficiency is associated with stem cell dysfunction and depletion which is part of normal aging [49]. Furthermore, Klotho deficiency in CKD could enhance renal tubular and vascular cell senescence induced by oxidative stress, uremic toxins such as indoxyl sulfate, and high phosphate (Figure 2) [50–57]. In addition, Klotho deficiency promotes renal fibrosis in several kidney disease models [58–60]. Klotho deficiency also results in defective endothelial function and impaired vasculogenesis [61], and Klotho protein protects vascular endothelium by inhibition of endothelial inflammation [62]. Klotho deficiency directly and indirectly contributes to uremic cardiomyopathy which can be prevented or attenuated by supplementation of soluble Klotho [47, 48, 63, 64]. Therefore, soluble Klotho protein may

be a novel therapeutic agent for CKD patients. We will first discuss the recent literature about Klotho deficiency as a biomarker for CKD and its role in CKD-mineral and bone disorder (MBD) development, then summarize preclinical results about Klotho supplementation as a therapeutic agent for prevention of CKD progression and amelioration of cardiovascular disease (Figure 2).

2. KLOTHO DEFICIENCY AS A BIOMARKER FOR CKD

There is an urgent need to identify diagnostic and prognostic biomarkers for CKD which are both more sensitive (diagnostic value) and/or more specific (treatment effect value) than those currently used. The early identification of CKD onset and the risk-stratification of CKD progression and/or CKD-related complications are essential to ameliorate the comorbidity burden of CKD patients, particularly cardiovascular disease, and prevent the development of ESRD [65–67]. Certain characteristics are crucial for CKD biomarkers to be incorporated into clinical practice. These include the ability to improve current predictive clinical models of CKD onset or progression; the characterization of the severity of CKD stage; the reliability across species; and the accessibility to be measured in body fluids or tissues.

In this context, Klotho has emerged as both a promising biomarker and potential therapeutic agent for CKD [35]. Importantly, innovative translational efforts to confirm and validate animal data in different human CKD models are evolving. In the following sections, clinical data of Klotho measurements in CKD patients will be reviewed.

2.1. Klotho deficiency is associated with eGFR decline in CKD patients

CKD is a known state of Klotho deficiency [35, 68]. Klotho mRNA expression in kidney was found to be significantly lower in CKD patients than healthy controls and positively correlated with estimated glomerular filtration rate (eGFR) in a small number of CKD patients [69, 70]. Similarly, Klotho mRNA levels in parathyroid gland were shown to decline in parallel with decreasing eGFR [71]. In a larger sample of CKD patients (stages 1 to 5D) with available kidney biopsies, Klotho mRNA levels were also positively correlated with eGFR in multiple regression analysis that adjusted for age and CKD-MBD parameters, such as intact parathyroid hormone (iPTH), fibroblast growth factor 23 (FGF-23), 1,25-dihydroxy vitamin D3 (1,25 $VitD_3$), corrected serum calcium, and serum phosphorus [72]. Importantly, Klotho mRNA in the kidney was the only independent predictor of serum Klotho across all CKD stages. Renal Klotho was significantly correlated with serum calcium, serum phosphorus, 1,25 $VitD_3$, FGF23, and iPTH [72].

In another study, serum Klotho levels were significantly lower in CKD patients than in healthy controls and were shown to be progressively lower with more advanced CKD stage [73]. The adjusted mean serum Klotho decrease was 3.2 pg/mL for each 1 mL/min/1.73 m^2 eGFR decrease. Age and eGFR were independently associated with serum Klotho levels [73]. A similar finding of progressive Klotho decline with worsening CKD was observed by Kim and coworkers in a *post-hoc* analysis of CKD patients [74]. Serum Klotho was independently associated with eGFR in multivariable linear regression analysis. In addition, serum Klotho was negatively correlated with FGF-23 and serum phosphate [74]. Several

other studies have confirmed the positive correlation between Klotho levels (serum and urine) and eGFR in adult CKD patients [22, 24, 74–76]. Consistently, both serum and urine Klotho levels have been independently associated with eGFR in CKD patients [22, 74]. A similar positive correlation between plasma Klotho levels and eGFR was shown in children with CKD [77]. The decline in Klotho levels was associated with increased FGF23 and iPTH levels in this study [77]. Furthermore, serum Klotho levels in children on chronic peritoneal dialysis were significantly lower than in healthy controls [78].

The very early decline of serum Klotho levels in adults with incipient CKD i.e. CKD stages ≤2, suggests that this change antecedes the increase in serumFGF23, iPTH, and hyperphosphatemia. It may therefore constitute an early marker of progressive CKD and CKD-MBD disturbances [68, 74, 79, 80]. The early occurrence of Klotho deficiency in human CKD needs to be further confirmed with other assay(s) (the limitation of current ELISA kit will be discussed below) and its validity to predict adverse CKD-related outcome further evaluated.

2.2. Klotho deficiency and possible association with cardiovascular disease in CKD patients

A cross-sectional study of CKD patients with modest decline in renal function revealed that serum Klotho is associated with arterial stiffness measured by ankle-brachial pulse waive velocity [75]. This association was independent of age, gender, mean blood pressure, use of antihypertensive drugs, tobacco smoking, ethanol use, non-HDL cholesterol, statin use, diabetes status, eGFR, albuminuria, and hemoglobin [75]. In contrast, a larger cohort study of CKD stages 2 – 4 showed that plasma Klotho levels (highest vs lowest tertile) did not predict atherosclerotic or acute heart failure events or death at 2.6 years follow-up [81].

Serum Klotho levels were found to be significantly reduced in hypertensive (essential and renovascular) patients with mild CKD when compared to healthy controls, even after adjustment by eGFR [82]. The proposed cross-talk between the renin-angiotensin-aldosterone system and the vitamin D-FGF23-Klotho pathways supports the hypothesis that modulation of one system can have positive effects on the other [83, 84], [85]. In this context, a *post hoc* analysis of the ESCAPE trial in children with CKD (all received fixed dose of ramipril 6 mg/m^2 per day) showed that 25 (OH)-D ≥50 nmol/L was associated with greater preservation of renal function. Interestingly, ramipril therapy significantly increased serum Klotho levels without any associated changes in serum calcium or phosphate [86].

Despite evolving experimental data showing that Klotho deficiency may be an intermediate mediator of the pathologic vascular calcification, endothelial dysfunction, cardiac remodeling, and cardiac hypertrophy observed in CKD [24, 47, 48, 64] an association between Klotho levels and cardiovascular disease has been only observed in small studies utilizing surrogate markers of cardiovascular disease rather than hard outcomes such as major cardiovascular events or death. Larger studies with well-defined cardiovascular disease outcome are needed to fully elucidate the role of Klotho as a prognostic marker of cardiovascular disease in human CKD.

2.3. Klotho deficiency may be a prognostic biomarker of progressive CKD

The role of Klotho as a predictor of adverse outcomes in CKD was examined in a *post hoc* cohort study of adult patients with CKD (stages 1 to 5). Patients with acute coronary syndrome, ischemic stroke or progressive CKD within 3 months prior to the study were excluded. Importantly, serum Klotho levels independently predicted the composite outcome of doubling serum creatinine (S_{Cr}), ESRD, or death in Cox regression time-to-event analysis that adjusted for age, diabetes, mean arterial pressure, eGFR, proteinuria, and iPTH [74]. If serum Klotho levels were ≤396.3 pg/mL, 35.2% of patients reached the composite outcome (doubling S_{Cr}, ESRD, or death), whereas only 15.7% of patients reached this adverse composite outcome if serum Klotho levels were >396.3 pg/mL. The areas under the curve (a measure of discrimination, that is, the ability of Klotho to correctly classify those with and without the outcome) for 1/serum Klotho to predict the composite outcome (doubling SCr, ESRD, or death) were 0.81, 0.78, and 0.72 at 12, 24, and 36 months [74]. Currently, these data constitute the most conclusive evidence of Klotho candidacy as a prognostic biomarker of progressive CKD or death. Although, these results are encouraging given the hard composite outcome utilized, this study was performed in a small sample of CKD patients and further validation is urgently needed.

3. CURRENT LIMITATIONS TO THE USE OF KLOTHO AS A BIOMARKER IN CKD

It is imperative to recognize that current Klotho clinical data are derived from small observational studies, despite being promising and somehow consistent, and still require subsequent extrinsic validation. Moreover, the utilization of hard cardiovascular and CKD outcomes will further test Klotho candidacy as a potential biomarker of CKD progression and/or CKD-related cardiovascular complications. There is a dire need for the development of collaborative research that expedite translational research in Klotho biology and kidney disease. Prospective studies with novel enrichment tools may be required to achieve adequate outcome events and the necessary power to test performance or reclassification metrics incorporating clinical and other biomarker data.

An additional difficulty that impaired progress in this field was the lack of a standardized assay to measure circulating Klotho, often yielding contradictory results [68, 87]. Immuno-Biological Laboratories Co generated ELISA kit that gave higher readings in fresh serum samples because Klotho ELISA kit may measure Klotho and other cross-reacting proteins in fresch samples, but gave lower readings in the stored samples when compared to immunoprecipitation-immunoblot assay [67] The development of a reliable assay is mandatory to accelerate human studies in CKD. Furthermore, the distinction and characterization of circulating free Klotho, complexed Klotho, cleaved Klotho, secreted Klotho, and the Kl1 and Kl2 Klotho sequences may be biologically necessary to fully unveil the role of Klotho in kidney disease. Finally, other clinical parameters known to influence Klotho, such as high phosphate diet, FGF-23, vitamin D supplementation and certain medications, cannot be addressed in observational studies and may exert influence in our interpretations. Prospective studies need to be carefully designed to address some of these difficulties.

Despite the stated caveats, Klotho has gained attention and raised a significant amount of enthusiasm in the nephrology community because of its candidacy as a potential biomarker and/or therapeutic agent in CKD. This is particularly important in an era of recurrent disillusionment when validating CKD biomarkers that are readily applicable in clinical practice.

4. KLOTHO DEFICIENCY AS A PATHOGENIC INTERMEDIATE FOR BONE DISORDERS IN CKD

When Klotho was discoved as an aging suppressor, the association of Klotho deficiency with osteoporesis was readily documented [1]. Shortly Klotho deficiency was confirmed to lead to low-turnover bone metabolism and consequently cause oseoporesis and osteopenia [88–93]. Clinical observational studies showed that the severity of bone loss was associated with Klotho gene G395A and C1818T polymorphisms in Caucasian and Japanese postmenopausal women [94]. Reversal of renal Klotho by demethylation of Klotho gene promoter led to significant improvement in disturbed serum biochemistry and bone damage in CKD mice [95].

CKD-MBD is one of the striking features associated with high morbidity and mortality of cardiovascular events In CKD and ESRD [96–107]. Abnormal mineral metabolism including high serum phosphate, FGF23, and PTH as well as low serum 25- $VitD_3$, and 1,25-$VitD_3D_3$, is not only a hallmark of CKD-MBD, but also an intiator or accelerator to bone disease development in CKD. Moreover, Klotho deficiency is proposed to be associated with or even induce high serum phosphate, FGF23, and PTH [35–37, 108–112]. On the other hand, high serum phosphate will further downregulate the renal Klotho expression [47]. These factors can exacerbate and also interact with each other. Together, they constitute a self-amplifying downhill vortex that drives disturbed mineral metabolism worse and worse. Given that CKD-MBD contributes to the high morbidity and mortality of cardiovascular disease in CKD/ESRD patients [97, 113–116], the increase in soluble Klotho in the circulation should aid in the disruption of this downhill vortex and improvement of CKD-MBD, and hence potentially reduces the risk for cardiovascular morbidfity and mortality [23, 117]. Therefore, FGF23-FGF receptor/Klotho pathway can be a new drug target for disorders of phosphate and bone metabolism [118].

5. POTENTIAL TREATMENT STRATEGY FOR CHRONIC KIDNEY DISEASE

The kidney is confirmed as the major organ that contributes to circulating Klotho [6, 25] and decreased Klotho production renders the kidney more susceptible to a variety of kidney insults [15, 30], retards kidney recovery from acute kidney injury (AKI) [85], promotes fibrosis [58] and AKI-to-CKD transition [119], and exacerbates uremic cardiomyopathy [47] and vascular calcification [24]. On the other hand, Klotho repletion through genetic manipulation, viral delivery, or administration of Klotho protein has been shown to improve multiple renal and extrarenal complications in both acute and chronic loss of kidney function [15, 24, 47, 58, 72, 74, 80, 120]. Therefore, Klotho deficiency may not only be a pathogenic intermediate for accelerating CKD progression, but also a major contributor to chronic complications such as secondary hyperparathyroidism and cardiovascular disease in CKD.

Conceivably, any therapy that restores klotho levels through re-activation of endogenous Klotho or administration of exogenous Klotho might be a novel treatment strategy for CKD (Figure 2).

5.1. Re-Activation of Endogenous Klotho Expression dependent of epigenetic mechanism

5.1.1. Demethylation—Epigenetic modulation of Klotho was proposed to contribute to renal Klotho deficiency in certain instances including Klotho hypomorphic mice which was originally thought to result from interruption of the promoter of *Klotho* gene by the exogenous transgene [1], because there was aberrant Klotho promoter methylation due to exogenous transgene in *kl/kl* mice. The methylation of the *Klotho* gene promoter reduced its activity by 30–40%, whereas DNA demethylation increased *Klotho* expression 1.5- to 3.0-fold [44]. Moreover, uremic toxins – indoxyl sulfate or p-cresyl sulfate – induced hypermethylation of the *Klotho* gene, and decreased Klotho expression in renal tubules and kidney cell lines, which could be reversed by demethylation of the *Klotho* gene. Animal experiments clearly showed that demethylation of Klotho gene promoter remarkably reversed renal Klotho deficiency and reduced renal fibrosis [95]. Therefore, hypermethylation may be one of the mechanisms of *Klotho* gene expression inhibition in CKD [41, 42, 121].

5.1.2. Deacetylation—The hyperacetylation of histone in Klotho promoter was also proposed as a possible mechanism for Klotho silencing in many types of cancer cell lines [122, 123]. Furthermore, cytokines-induced elevation of histone acetylation is shown to be associated with downregulation of Klotho expression, because TNF and TNF-like weak inducer of apoptosis (TWEAK)-induced downregulation of Klotho expression in the kidney and kidney cell lines could be blunted by inhibition of histone deacetylase [46]. The effect of histone acetylation of Klotho promoter on the regulation of Klotho expression was shown to be implicated in several types of cancer development [45, 122, 123], but its role in renal Klotho deficiency and the therapeutic effect of inhibition of histone acetylation on upregulation of renal klotho is worthy to confirm in kidney disease subjects.

5.2. Re-Activation of Endogenous Klotho Expression Independently of Epigenetics

To date, several categories of drugs in the market including peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonists [124–127], Angiotensin II-type I receptor antagonists [128–130], 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors (statin) [131], vitamin D active derivatives [23, 85, 132–134], and intermedin [135, 136] have been shown to up-regulate Klotho expression *in vivo* and/or *in vitro*. Peritoneal injection of vitamin D receptor agonists robustly increased circulating Klotho levels, reduced serum creatinine, and attenuated vascular calcification in mice with CKD [23]. Hence, the up-regulation of Klotho doubtlessly has positive beneficial effect on target organs of experimental CKD models although the detailed molecular mechanisms whereby Klotho is upregulated remain to be explored [23].

Taken together, re-activation of Klotho production through demethylation and deacetylation of Klotho gene promoter, or other yet-to-be identified mechanism(s) may be an easy and useful strategy to restore renal Klotho production in patients with early CKD and

consequently increase circulating Klotho levels. Higher Klotho in the kidney and the circulation is doubtlessly of help in maintenance of mineral metabolism homeostasis, prevention of CKD progression, and protection of vasculature and heart. Their clinical efficacy is worthy of being confirmed.

5.3. Delivery of Klotho cDNA

Klotho gene delivery via viral carrier was shown to effectively rescue many phenotypes observed in Klotho-deficient mice [137], attenuating the progression of hypertension and kidney damage in spontaneously hypertensive rats [138, 139], improving kidney function in AKI [120], ameliorating Angiotensin II-induced kidney injury [140], improving endothelial function [141], and protecting from uremic cardiomyopathy [142]. More recently, an injection of cDNA coding for full-length extracellular domain of rat Klotho under the control of the cytomegalovirus promoter was shown to ameliorate pathologic cardiac hypertrophy in *Klotho*-deficient CKD mice [61]. Although gene therapy is effective in animal studies, its safety is still questionable and human clinical application is not in the proximity.

5.4. Administration of soluble Klotho Protein

The supplementation of Klotho via gene delivery systems is not ready for clinical use, but it proves the concept that restoration of circulating Klotho is effective [117, 143]. The alternative option to increase circulating Klotho is the administration of soluble Klotho protein (cleaved full length of extracellular domain of membrane Klotho) which is more direct, safer, and an easier modality to restore endocrine Klotho deficiency than viral carrier delivery. Animal studies have already provided encouraging proof-of-concept data showing that bolus administration of soluble Klotho protein is safe and effective in protection against kidney injury [15] and induction of phosphaturia [144]. Furthermore, repeated administration of soluble Klotho protein attenuates AKI-to-CKD progression [119].

It has been shown that ischemia-reperfusion induced AKI is a state of acute Klotho deficiency. The single administration of soluble Klotho protein immediately after kidney injury effectively amelioratedkidney damage and preserves kidney function [15]. Furthermore, repeated administration of soluble Klotho protein post surgery inhibited renal fibrosis in a unilateral ureteral obstruction kidney injury model [58]. More recently, repeated administration of soluble Klotho protein starting one day after IRI-induced AKI (S_{cr} at peak levels) promoted kidney recovery, suppressed renal fibrosis, accelerated the removal of deposited collagen through upregulation of autophagy, and consequently retarded AKI-to-CKD progression [119]. The effective therapeutic results in two different kidney disease models highly support the concept that Klotho protein administration is a promising strategy which can be used to prevent acute kidney injury when given early and also mitigate AKI-to-CKD progression when given post AKI. Thus, although Klotho protein administration has not been approved to treat AKI or CKD patients, the pre-clinical data clearly support the therapeutic potential of soluble Klotho protein to attenuate adverse renal outcome regardless of etiology.

The identification of AKI phenotypes (combining clinical and biomarker data) of patients at high risk of recurrent AKI or incomplete AKI recovery with rapid transition to CKD may help the identification of high-risk patients eligible for therapeutic Klotho trials in which renal and non-renal hard outcomes could be feasibly tested. The parallel development of Klotho therapeutic strategies (e.g., soluble Klotho protein administration) and more specific Klotho assays makes the design of Klotho trials foreseeable in the near future. Ongoing collaborative Klotho research in human AKI and CKD will aid confirmatory testing of circulating Klotho assays and the recognition of therapeutic windows for potential Klotho administration. In this context, recurrent soluble Klotho administration guided by serum or urine Klotho levels could be of paramount strategic importance. A highly relevant human model of disease is AKI-to-CKD progression in which Klotho administration could be initiated in the early post-AKI period in individuals at high risk of adverse renal outcome. It is expected that the development of Klotho therapies accelerates in the near future and therefore pragmatic clinical trials of Klotho administration in human disease are no longer utopic in the scientific community.

6. SUMMARY

Several beneficial roles of Klotho have been proposed in clinical nephrology based on pre-clinical studies (Table 1): (1) Klotho may be a sensitive marker for early diagnosis of CKD and a reliable prognostic marker for the prediction of CKD progression, the development of ESRD, and CKD-related cardiovascular disease. However, the biomarker candidacy of serum or urine Klotho needs to be further evaluated; (2) Klotho could serve as a prophylactic agent to prevent AKI in patients at high risk for this complication such as those with underlying CKD; (3) Klotho holds a promising therapeutic effect to mitigate CKD progression, AKI-to-CKD progression, and alleviate cardiovascular disease, being the latter the principal culprit of elevated mortality in CKD/ESRD patients. However, the efficacy and safety of Klotho therapy in different stages of human CKD still needs to be demonstrated.

The molecular mechanisms of Klotho’s renoprotection and cardioprotection are still being unraveled. Whether the effect of Klotho on the kidney and heart is organ-specific or shares similar signaling pathways is not known. Obviously, better understanding of the molecular mechanism(s) of Klotho curing kidney disease and preventing cardiovascular disease will help in developing and implementing novel therapeutic strategies.

Klotho therapy to arrest or attenuate the development or progression of CKD or CKD-related complications is definitely in the pipeline of development. Collaborative and translational research are crucial to enhance bench-to-bedside transition. Novel circulating Klotho assays should be tested for generalizability across the spectrum of kidney disease and heterogeneity of sample characteristics. Exogenous soluble Klotho administration may not be the sole agent to prevent or ameliorate the burden of CKD but definitely could be a key player in conjunction with FGF-23 antagonism, vitamin D supplementation and phosphate control to restore mineral metabolism homeostasis and halt CKD-related complications, because high phoshage, low serum vitamin D and and high FGF23 are negative regulators for Klotho expression in the kidney and circulating Klotho [16,33~35,85]. A key human disease model that can potentially unveil the pathobiology of Klotho in renal disease is AKI-

to-CKD progression. Longitudinal follow-up of AKI patients at high risk of CKD should be carefully instituted for state-of-the-art patient care and research utilizing biological samples and clinical data. The time has come for translational and clinical research to support the vast animal data in Klotho biology and renal disease.

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Highlights

- CKD is a state of pan-Klotho deficiency
- Klotho deficiency is a biomarker for CKD and a predictor for CKD progression
- Klotho deficiency is an intermediate for CKD progression and CKD-MBD
- Soluble Klotho protein is novel and promising therapeutic agent for CKD/ESRD

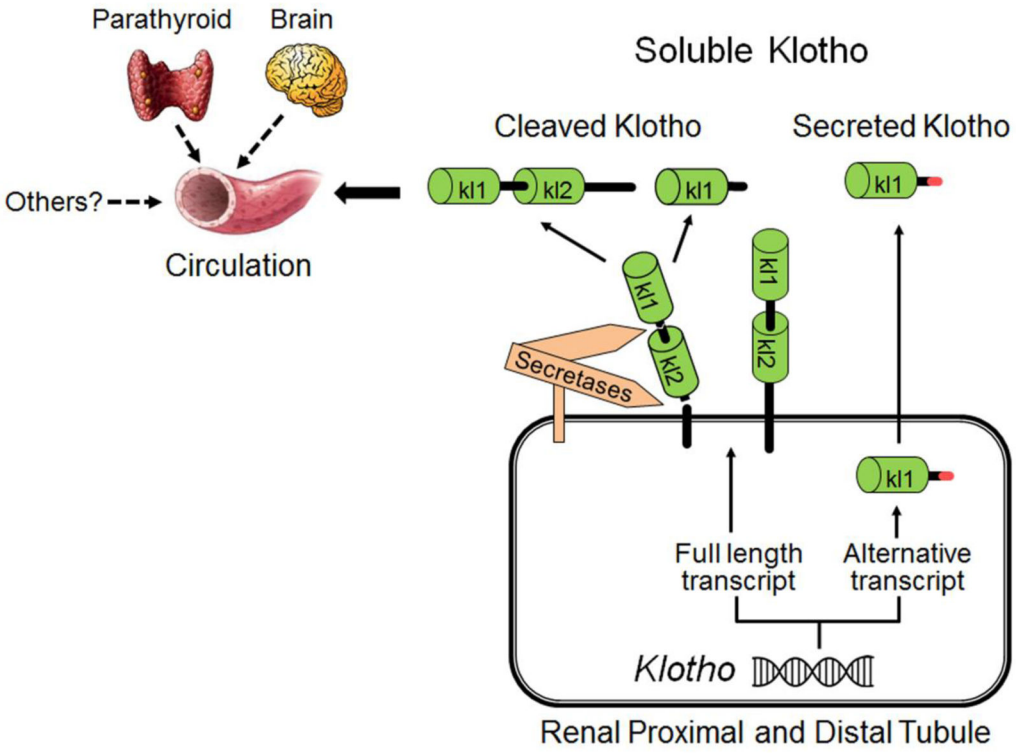

Figure 1. Source of soluble Klotho

The kidney is the main source of circulating Klotho under physiological conditions. Both renal proximal and distal tubules express membrane Klotho protein and may also produce a secreted Klotho protein through alternative splicing. The secreted Klotho only contains Kl1 domain and is directly secreted into the blood circulation. But its biologic function is not clear yet. Extracellular domain of membrane Klotho containing Kl1 and Kl2 repeats is shed and cleaved by secretases into either full extracellular domain or Kl1 repeat. Both cleaved Klotho fragments are present in the circulation. A few extra-renal organs including parathyroid gland and brain express Klotho protein as well, but their contribution to circulating Klotho in CKD/ESRD (dash line) remains to be confirmed.

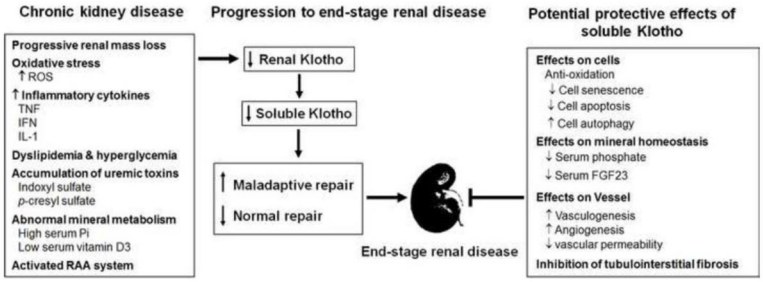

Figure 2. Potential mechanisms of Klotho downregulation in CKD, and beneficial effects of soluble Klotho on CKD

Left panel: Loss of renal mass, over production of reactive oxygen species (ROS) as well as pro-inflammatory cytokines including tumor necrosis factor (TNF), interferon (IFN) and interleukin 1 (IL-1), dyslipidemia and hyperglycemia, and elevation of uremic toxins including indoxyl sulfate and *p*-cresyl sulfate may contribute to or participate in downregulation of renal Klotho. Furthermore, high serum phosphate and FGF23 as well as low serum 1,25-Vit.D_3 inhibit renal Klotho expression. Low serum 1,25-Vit.D_3 not only reduces Klotho expression, but also stimulates renin-aldosterone-angiotensin (RAA) system which further suppresses Klotho production. **Middle panel:** Reduced Klotho expression in the kidney would lead to endocrine Klotho deficiency in CKD. Low soluble Klotho promotes CKD progression to ESRD through impaired normal renal repair process and induction of maladaptive repair process. **Right panel:** Supplementation of soluble Klotho protein retards CKD progression through multiple biologic actions: (1) cytoprotection via anti-oxidation, reduction of cell senescence and apoptosis, and upregulation of autophagy, hence accelerating renal tubule regeneration; (2) correction of high serum phosphate and FGF23; (3) maintenance of peritubular capillary formation and function; and (4) inhibition of tubuloinsterstitial fibrosis.

Table 1

Potential applications of circulating Klotho in clinical nephrology

Biomarker			Therapeutic agent		
Diagnostic	•	Prediction of early onset CKD	Prophylactic	•	Prevention of post-AKI CKD development in high risk groups
				•	Prevention of CVD development in CKD patients
Prognostic	•	Prediction of CKD progression	Therapeutic	•	Ameliorates hyperphosphatemia toxicity
	•	Prediction of ESRD development		•	Ameliorates CKD-MBD
	•	Prediction of CVD		•	Attenuates CVD

AKI: acute kidney injury; CKD: chronic kidney disease; CKD-MBD: chronic kidney disease-mineral and bone disorder; CVD: cardiovascular disease; ESRD: end-stage renal disease

Table 2

Potential strategies to upregulate Klotho for treatment of human CKD

Epigenetic increase of endogenous Klotho	•	Demethylation of Klotho gene promoter
	•	Deacetylation of Klotho gene promoter
Non-epigenetic increase of endogenous Klotho	•	PPAR-γ agonist
	•	Angiotensin II-type I receptor antagonist
	•	Statins
	•	Active vitamin D derivatives
	•	Intermedin
Delivery of Kotho gene *in vivo*	•	Viral carrier of Klotho cDNA
	•	Klotho cDNA
Administration of exogenous Klotho protein	•	Recombinant Klotho protein

CKD: chronic kidney disease;