Klotho to Treat Kidney Fibrosis

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Sixteen years ago, defective expression of the murine *klotho* gene was found to result in a syndrome resembling human aging.[1] Tubular kidney cells were the main sites of Klotho expression. Rescue of the phenotype by expression of a Klotho transgene outside the kidney suggested a humoral regulation of aging.[1] These findings supported the concept of Klotho as a kidney-secreted hormone like erythropoietin. Klotho is now known to be both a membrane-bound and a secreted protein. A key function of Klotho is to regulate phosphate metabolism by both being a necessary coreceptor for the phosphaturic hormone fibroblast growth factor-23 and directly inhibiting tubular phosphate reabsorption by the sodium-phosphate cotransporter NaPi2a.[2,3] In addition, a growing list of actions of soluble Klotho depend on its glycosidase activity or binding to cell membrane receptors and transporters and as reported by Zhou *et al.*,[2,4] soluble ligands. Thus, Klotho regulates insulin/IGF-1, Wnt, and TGF-β1 signaling as well as renal outer medullary potassium channel (ROMK), transient receptor potential channel 5 (TRPC5), and TRPC6 availability.[2]

Loss of Klotho may contribute to the aging-like features of human CKD and progression of CKD.[5–7] The initial description of the mouse Klotho gene reported normal serum creatinine in mutant mice.[1] However, Klotho$^{-/-}$ mice develop renal failure characterized by kidney calcification and increased renal cell apoptosis, suggesting that Klotho deficiency is deleterious for the kidney.[6] The loss of Klotho during kidney disease coupled with a negative impact of Klotho deficiency on kidney disease may potentially generate a vicious circle where kidney injury results in low kidney Klotho and Klotho downregulation favors progression of kidney injury. In the current issue of *JASN*, Zhou *et al.*[4] now provide evidence supporting the existence of such a vicious circle leading to kidney fibrosis, characterized by the interplay of Klotho, TGF-β1, and Wnt/β-catenin signaling.[4] Either preventing Klotho downregulation or supplementing the missing Klotho may interrupt the vicious circle.[4]

The observation that urinary Klotho is reduced already in stage I human CKD suggests that there are factors that reduce Klotho synthesis by tubular epithelium beyond the loss of Klotho-producing cells.[5] TNF superfamily inflammatory cytokines, TGF-β1 or angiotensin II, decrease Klotho expression in cultured tubular cells.[2,3,7,8] Zhou *et al.*[4] confirm a TNF-independent Klotho-lowering effect of TGF-β1 in cultured renal cells. Zhou *et al.*[4] propose that TGF-β1 could be the culprit behind Klotho depletion in diseased kidneys. However, key experiments were missing to fully support this notion. Thus, the effect of systemic TGF-β1 administration or neutralization on kidney Klotho expression was not studied. In this regard, systemic delivery of TWEAK (TNF-like weak inducer of apoptosis) or angiotensin II decreases levels of kidney Klotho, and targeting of TWEAK, TNF, or the renin-angiotensin system prevents kidney Klotho downregulation in animal models of kidney injury or systemic inflammation.[3,8,9] Similar functional studies should define the relative *in vivo* contribution of TGF-β1 to the regulation of kidney Klotho levels. Currently available data support a model where multiple tubular cell stressors decrease Klotho synthesis. Thus, constitutive transcription of *Klotho* in tubular epithelial cells is downregulated by transcription factors (NF-κB or Smad-3) or epigenetic modulation and rapidly results in decreased protein levels.[4,8]

Animal models of AKI and CKD have uniformly shown decreased kidney Klotho as well as a nephroprotective role of Klotho.[2–5,7,8,10,11] Klotho sits at the AKI to CKD interface. Kidney Klotho is decreased early in the course of experimental AKI and persists decreased well beyond the recovery of normal renal function,[8] which was reproduced by Zhou *et al.*[4] In addition, kidney Klotho is decreased in experimental CKD of ischemic, unilateral ureteral obstruction (UUO), or glomerular origin.[4,11] Zhou *et al.*[4] now add to the literature on the role of Klotho in CKD and kidney fibrosis resulting from these causes.[4,5,7,10,11]

Klotho deficiency aggravates fibrosis resulting from UUO.[7] Klotho overexpression in ICR-derived GN mice leads to improved renal function and decreased apoptosis and fibrosis both in the tubular and glomerular compartments.[11] In a model of angiotensin II administration, an adenovirus harboring the mouse *klotho* gene improves creatinine clearance and a tubulointerstitial injury score that includes thickening of the tubular basement membrane.[12] Administration of secreted Klotho to mice immediately after the procedure suppressed renal fibrosis induced by UUO.[10] Genetic low Klotho levels impair renal function and proteinuria, and high Klotho expression protects from both in CKD induced by ischemia reperfusion in a solitary kidney.[5]

Where is the novelty of the current report? Zhou *et al.*,[4] for the first time, show that overexpression of exogenous Klotho at late time points, when kidney lesions are already established, is still therapeutically effective to prevent fibrosis.[4] This finding is a significant advance, because clinic diagnosis is frequently delayed, and therapy is applied when some degree of kidney injury has already occurred and not prophylactically. Thus, this clear step is in the direction of clinical studies on Klotho therapy for CKD.

Kidney protection by Klotho overexpression extends beyond the tubulointerstitium and into the glomerulus in adriamycin nephropathy, tending to reduce proteinuria and preserving

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nephrin expression.[4] Whether Klotho might even restore nephrin expression should be further studied. Glomerular protection is in accordance to observations of Klotho reducing glomerulosclerosis in ICR-derived GN mice and reducing proteinuria in ischemia reperfusion-induced CKD.[5,11] This finding suggests podocyte actions of Klotho. Indeed, in addition to protecting from angiotensin II or TGF-β1–induced cell stress, Klotho downregulates the TRPC6 channel. TRPC6 hyperactivity of genetic origin promotes podocyte injury in humans.

Klotho decreases oxidative stress, apoptosis, and proinflammatory and profibrotic responses in kidney cells.[3] Zhou *et al.*[4] further address the potential mechanisms of nephroprotection by Klotho, focusing on TGF-β1 and Wnt/β-catenin signaling and kidney fibrosis. In Klotho-deficient mice, persistent Wnt activation leads to stem cell depletion in some tissues, but the kidney and fibrosis were not studied.[13] A reduction of Wnt signaling and kidney fibrosis was recently reported in UUO mice treated with a Klotho-encoding plasmid.[14] Wnt3a induced prolonged tubular cell cycle arrest at the G(2)/M phase, a condition known to promote the release of TGF-β1.[14] Zhou *et al.*[4] thus provide a link between two known antifibrotic actions of Klotho: inhibition of TGF-β1 and Wnt signaling.[4] Klotho bound to Wnt ligands and repressed profibrotic Wnt-induced transcription of β-catenin targets in response to TGF-β1 in tubular epithelial cells.[4] This mechanism should be added to the already known capacity of Klotho to directly bind to and inhibit the TGF-β type II receptor.[10] In murine CKD, Klotho was downregulated in the same tubules where β-catenin was active. Furthermore, *in vivo* expression of secreted Klotho inhibits the activation of renal β-catenin and myofibroblasts, decreasing extracellular matrix deposition.[4]

Zhou *et al.*[4] emphasize a key difference between Klotho and other Wnt antagonists.[4] Secreted antagonists of Wnt signaling are generally upregulated by Wnt/β-catenin signaling, which leads to increased expression of these molecules in injured kidney and may contribute to limited Wnt signaling. By contrast, Klotho is downregulated during kidney injury, further favoring Wnt/β-catenin signaling and additional tissue injury. In this regard, exogenous soluble Klotho induced the expression of endogenous, full-length Klotho expression *in vivo* and even restored the expression of endogenous Klotho in injured kidneys.[4]

As with any novel research, new questions arise. Is there a role for Klotho downregulation in TGF-β1 or Wnt/β-catenin–induced fibrogenesis in other organs? Could Klotho be protective in these other organs? A bidirectional relationship has been described between Klotho in both inflammation and fibrosis. Inflammatory and profibrotic factors downregulate Klotho expression. Klotho may downregulate fibrosis and inflammation. Is fibrosis or inflammation the main target of Klotho? What is the relative contribution of these two effects of Klotho to tissue protection? Is there any relationship between the present observation and phosphate metabolism? Other than preventing Klotho downregulation or supplementing the missing Klotho, as illustrated by Zhou *et al.*,[4] there is a third potential way to intervene on the Klotho–kidney axis. This way consists of preventing the consequences of Klotho deficiency. Renal failure in Klotho$^{-/-}$ mice is dependent on abnormal phosphate disposal based on the results of dietary or genetic manipulation.[6] Early mortality and kidney injury in Klotho$^{-/-}$ mice improves when the *Npt2a* gene encoding a key proximal tubular phosphate transporter is targeted, leading to hypophosphatemia. In this regard, higher serum phosphate levels are associated with a decreased nephroprotective response to renin-angiotensin system targeting in clinical trials.[15] Additional studies should unravel the relationship between this clinical observation, our current understanding of the role of Klotho in kidney injury and phosphate regulation, and the antifibrotic actions observed in cultured renal cells in the absence of modulation of phosphate levels.

In conclusion, an expanding number of factors leading to kidney injury suppresses the transcription of *Klotho* in tubular cells. In turn, Klotho downregulation allows the development of a full-blown profibrotic response.[4,10] The finding that delayed therapy with soluble Klotho prevents tubulointerstitial and glomerular injury and fibrosis may help design clinical interventions. Eventual interventional studies in humans may either target the factors that decrease Klotho expression or use of recombinant Klotho or Klotho-derived peptides to treat kidney injury.

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DISCLOSURES

None.

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See related article, "Loss of Klotho Contributes to Kidney Injury by Derepression of Wnt/β-Catenin Signaling," on pages 771–785.

Physical Performance and All-Cause Mortality in CKD

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It is well documented that measures of exercise capacity and physical performance are impaired in people with ESRD who are undergoing maintenance dialysis therapy.[1–3] Several studies have also described impaired exercise capacity in CKD patients who have lesser degrees of reduced GFR, and who are not receiving chronic dialysis treatment.[4,5] The causes for impaired exercise capacity and physical performance are not entirely clear. A number of adverse conditions have been associated epidemiologically with these impairments, including physical deconditioning, muscle atrophy, anemia, a propensity toward increased serum inflammatory markers, and lower quality of life.[4,6,7] The relative contributions of these putative causes to impaired exercise capacity and reduced physical performance are not well defined.

Another poorly explored area is the clinical consequences to CKD patients who manifest these disorders. No one, to my knowledge, has previously reported whether reduced exercise capacity or physical performance associates with increased morbidity or mortality in nondialyzed CKD patients. The article published by Roshanravan *et al.* in this issue of *JASN* is unique in that it is the first to address the question of whether physical performance is associated with mortality rates. [8] It examined this question in 385 patients who were not receiving chronic dialysis therapy, but had stage 2–4 CKD. Patients were recruited from two prospective cohorts: the Seattle Kidney Study and the University of Maryland Study of Chronic Kidney Disease. There were some differences in the characteristics of the patients in these two separate cohorts, but these differences would not be expected to invalidate the results of the study.[8] Physical performance was measured by usual gait speed (walking 4 m at the patient's usual pace), timed up and go test (TUAG) (time to stand from a seated position and walk around a cone placed 4 m distant), 6-minute walking distance, and handgrip strength (HGS). For some study participants, the reduction in GFR was rather modest. The inclusion criteria required the estimated GFR (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration study equation[9] to be $<$90 ml/min per 1.73 m^2. Other inclusion criteria were that the patient was not receiving chronic renal replacement treatment at the time of the physical performance assessment, did not have a stroke, was not using a wheel chair, and had completed at least one physical performance measurement. Mortality rate was monitored over a median duration of 3 years.

The mean age of the participants was 61$\pm$13 years, and the mean eGFR was 41$\pm$19 ml/min per 1.73 m^2. The results of this innovative study indicate that measures of physical performance of the lower extremities were at least 30% below predicted values and were strongly associated with mortality rates. Each 0.1-m/s decrement in gait speed was associated with a 26% higher risk for all-cause death (hazard ratio, 1.26; 95% confidence interval, 1.09 to 1.47). Each 1-second longer TUAG was associated with an 8% higher risk for all-cause death (hazard ratio, 1.08; 95% confidence interval, 1.01 to 1.14). In contrast, HGS was relatively well preserved and not