In Search of the Fountain of Youth

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In Greek mythology, Klotho spins the thread of life. In human biology, Klotho was named when researchers discovered that mutations that interrupt its normal function result in a shorter thread of life that is attributable to accelerated aging.1 From its initial discovery, the kidney and its role in phosphate and calcium handling have been at the epicenter of the Klotho story.

α, β, and γ Klotho are single-pass transmembrane proteins that serve as coreceptors for the fibroblast growth factor 19 (FGF-19) subgroup of endocrine FGFs.2 Heterodimeric complexes of Klotho and FGF receptors at the cell surface increase the binding affinity of ubiquitously expressed FGF receptors for specific FGF ligands and thereby confer the end-organ specificity of the main actions of the endocrine FGFs.3 βKlotho serves as the coreceptor for FGF-19, which regulates biliary tract function, and for FGF-21, which regulates hepatic and adipocyte energy metabolism.2 The functions of γKlotho are poorly understood. αKlotho (shortened to Klotho below) is the coreceptor for FGF-23 and is the Klotho protein that is most relevant to normal renal function and to diseases of the kidney.

Klotho is most highly expressed in the kidney, parathyroid glands, and choroid plexus.4 Activation of FGF receptor-Klotho complexes by FGF-23 regulates phosphate homeostasis through effects on proximal tubular expression of the sodium-phosphate cotransporters, NaPi2a and NaPi2c.4 FGF-23 also helps regulate calcium homeostasis by influencing parathyroid hormone (PTH) secretion and by regulating circulating levels of 1,25-dihydroxyvitamin D via Klotho-dependent inhibition of CYP27B1 and stimulation of CYP24A1.4 The actions of Klotho in the brain are unknown.

In addition to its 130-kD transmembrane form, a slightly shorter, 120- to 130-kD soluble form of Klotho that lacks the short transmembrane and intracellular domains circulates freely.2 Soluble Klotho can be derived from alternative splicing of the Klotho gene or from proteolytic cleavage of the extracellular domains of membrane-bound Klotho from the cell surface. Soluble Klotho appears to exert its own FGF-23–independent effects on mineral metabolism through enzymatically cleaving sugar residues from phosphate and calcium transporters that result in increased urinary phosphate excretion and decreased urinary calcium excretion.5 In addition to these effects, the literature abounds with reports of protein actions of soluble Klotho too vast to comprehensively detail in this space, but broadly including antibacterial, anticalcification, anti-inflammatory, antimetastasis, and antioxidant effects, among others.5

Despite our growing understanding of Klotho biology, numerous fundamental questions remain unanswered. High on the list is resolving the relative contributions of the kidney versus other organs to the overall systemic effects of Klotho and to the pool of circulating soluble Klotho. In this issue of JASN, Lindberg et al report on the role of the kidney using Cre-LoxP recombination technology to create a kidney-specific deletion of Klotho (Six2-KL−/−) that they contrasted with a global Klotho knockout.6 In the kidney-specific Six2-KL−/− mice, Klotho mRNA expression was reduced by approximately 80%, but Klotho protein was completely undetectable by immunofluorescence staining of kidney sections and by Western blotting of kidney extracts. In addition, unlike explanted wild-type kidneys, explanted Six2-KL−/− kidneys shed no soluble Klotho into culture medium. Consistent with the absence of soluble Klotho shedding from Six2-KL−/− kidneys ex vivo, Six2-KL−/− mice demonstrated 80% reductions in circulating soluble Klotho levels in vivo. The authors confirmed that they successfully deleted Klotho only in the kidney by demonstrating unperturbed Klotho expression in the parathyroid glands and choroid plexus.

Metabolically, the kidney-specific deletion of Klotho caused hyperphosphatemia due to defective FGF-23–mediated renal phosphate excretion, which caused FGF-23 levels to rise dramatically while NaPi2a expression remained abundant in the proximal tubular brush border. Renal Klotho deletion also caused hypercalcemia due to increased levels of 1,25-dihydroxyvitamin D, which could not be adequately suppressed by FGF-23 in the absence of Klotho. Hypercalcemia suppressed PTH levels despite severe hyperphosphatemia, consistent with the known effect of PTH to primarily respond to calcium rather than phosphate when they exert opposing demands on PTH secretion. In summary, kidney-specific deletion of Klotho reproduced the biochemical phenotype of mutations that globally interrupt Klotho function.1

Systemically, kidney-specific deletion of Klotho caused severe growth retardation, inanition, kyphosis, bone de-mineralization, soft tissue and arterial calcifications, skin atrophy, infertility, emphysema, and premature death. In summary, kidney-specific deletion of Klotho also reproduced the systemic phenotype of global Klotho mutations.1 The
authors justifiably conclude that the kidney is unquestionably the most important source of Klotho.

The authors note that their study could not determine the extent to which the severe phenotype of Klotho deficiency is due to lack of renal transmembrane Klotho versus lack of circulating soluble Klotho. Stated another way, they could not determine whether the severe extrarenal manifestations observed in Six2-KL/−/− and global Klotho-deficient mice are mediated by direct adverse effects of soluble Klotho deficiency versus downstream consequences of the biochemical alterations inflicted by lack of transmembrane Klotho: notably, hyperphosphatemia, hypercalcemia, FGF-23 excess, and vitamin D intoxication. Prior studies that demonstrated rescue of the Klotho-deficient phenotype by compound deletion of NaPi2a or CYP27B1 suggest that the systemic effects are downstream consequences of the biochemical alterations,7,8 but administering exogenous soluble Klotho to Six2-KL/−/− mice would address this important point more directly. The authors dismiss this possibility on the grounds that the phenotype appears so early in the animals’ lifespan. Perhaps inducible deletion of renal Klotho expression later in the animals’ life coincident with initiation of exogenous soluble Klotho administration could be a useful approach.

The results of this important study should also be considered in the context of another similarly substantial contribution from this group in which Klotho was specifically deleted from only the parathyroid glands.9 Surprisingly, given what were thought to be “known” effects of Klotho in the parathyroid glands, deletion of parathyroid Klotho resulted in no change in calcium homeostasis, and Klotho was not required for FGF-23 to suppress PTH secretion, which was mediated by activation of calcineurin-nuclear factor of activated T cells signaling similar to Klotho-independent FGF-23 signaling in the heart.10 If Klotho is indeed less important to parathyroid function than previously thought, what is its role in the glands? Is it possible that the parathyroid glands are the secondary source of soluble Klotho after the kidney that accounted for the 20% residual soluble Klotho levels observed in the Six2-KL/−/− mice in this study? If so, perhaps parathyroid Klotho serves as a second reservoir of protein that can be cleaved and released into the circulation in response to stimuli that are sensed by the parathyroid glands as part of their critical contribution to regulating calcium and phosphate homeostasis.

The kidney is the major source of Klotho, as Lindberg et al.6 convincingly show, and Klotho deficiency is a consequence of CKD that could explain several of its complications, including hyperphosphatemia, elevated FGF-23 levels, arterial calcification, and early mortality. However, because Six2-KL/−/− and global Klotho-deficient mice differ from humans with CKD in several important ways, it would be premature to label CKD an all-inclusive Klotho deficiency state. Unlike in Klotho-deficient animals, human CKD does not typically feature hypercalcemia, elevated levels of 1,25-dihydroxyvitamin D, or suppressed PTH levels. Furthermore, FGF-23 reversibly inhibits 1,25-dihydroxyvitamin D production in CKD.11 This suggests that renal Klotho expression is adequate to support this classic FGF-23 action when FGF-23 levels are already elevated in early and moderate CKD. Thus, it remains a matter of debate whether decreased Klotho expression in CKD stimulates secondary increases in FGF-23 or whether primary increases in bone production of FGF-23 in CKD downregulate renal Klotho expression. In an alternative hypothesis that could unify several seemingly disparate aspects of the current literature, perhaps kidney injury stimulates Klotho cleavage and release of the soluble form. This could reconcile the paradoxically increased levels of soluble Klotho that have been observed in early human CKD.12,13 Because high soluble Klotho levels can stimulate osteocyte production of FGF-23,14 perhaps increased cleavage of transmembrane Klotho would raise soluble Klotho and thus FGF-23 levels while simultaneously decreasing abundance of transmembrane Klotho on kidney tubular cells, consistent with studies that demonstrated its reduction in the CKD kidney.15 Further research is needed to define the pathophysiologic cascade that culminates in Klotho deficiency and FGF-23 excess so that novel therapeutic strategies can be defined, including perhaps Klotho replacement therapy as the authors propose.

At medical conferences and cocktail parties, it is not uncommon to overhear many specialists, including nephrologists, jokingly touting their organ as the most important and least dispensable. While these light-hearted quibbles are inherently absurd, the data presented by Lindberg et al. finally provide the nephrology community with objective fodder for the faux fight. At least we can now claim, backed by data reported in this issue of JASN, that in their long apocryphal search for the fountain of youth, explorers need not have looked further than their own retroperitoneum. The fountain is the kidney.

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t-RNA Fragmentation as an Early Biomarker of (Kidney) Injury

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Definitions, diagnosis, and monitoring of AKI still rely on serum creatinine levels and urinary output, both of which are markers of kidney function but not kidney injury.1 This makes it difficult to distinguish renal hyperperfusion without renal cell injury from renal cell necrosis for diagnosis, and it limits predictions on short-term and long-term renal outcomes of AKI. Although several candidate markers of kidney injury have been proposed,2 none has yet been broadly implemented in AKI management.3

In this issue of JASN, Mishima et al. describe not only a novel biomarker of cell stress but also its pathophysiology and putative diagnostic and prognostic use at multiple evidence levels.4 The authors’ concept is based on a recent finding that one of the first events during cell stress is a proteolytic cleavage of transfer (t)-RNAs by an enzyme called angiogenin.5 t-RNAs shuttle single amino acids to ribosomes, where the coding sequence of mRNA determines the sequence of different amino acids to form a protein (i.e., protein translation). Under physiologic conditions, angiogenin-receptor signaling promotes protein translation via protein kinase B/AKT-mediated transcription of ribosomal RNA.6 However, under conditions of cell stress, angiogenin cleaves t-RNA, which stops protein translation as a central energy-consuming process of cell metabolism, function, and growth.6

The novelty in the authors’ work relates to the generation of a monoclonal antibody specific for the mononucleoside 1-methyladenosine (mA) epitope.4 In a series of complex, well controlled experiments (including Northern blot, immunostaining, and immunogold electron staining), the authors demonstrate that the mA antibody detects the unfolded mammalian t-RNA in vitro as well as in vivo (e.g., in postischemic or toxic kidney injury as well as brain and liver injury). Immunopositivity for mA, implying t-RNA degradation, was evident as early as 30 minutes after reperfusion of liver ischemia, while nuclear chromatin injury have been proposed,2 none has yet been broadly implemented in AKI management.3

Subsequently, the authors demonstrated that t-RNA derivates were detectable as early as 15 minutes after renal ischemia-reperfusion in pigs. Even more exciting, plasma t-RNA derivative levels increased within 10 minutes after vascular reperfusion upon human aortic surgery, while urinary kidney injury molecule-1 (KIM-1) levels were increased only at 60 minutes (urinary KIM-1 may also have been increased earlier, but this was not tested). Unfortunately, the authors did not further evaluate the prognostic significance of these data for subsequent AKI or CKD, which would be important to establish t-RNA degradation as an early biomarker of AKI episodes. However, the authors report that plasma-free mA levels predicted mortality in the general population at a relative hazard risk of death of 2.99 upon multivariate analysis that corrected for numerous cardiovascular risk factors, comorbidity, and medications. This, however, was not related or referred to any form of kidney disease.