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See related article, "The Kidney Is the Principal Organ Mediating Klotho Effects," on pages 2169–2175.

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 hypoperfusion 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 (m1A) epitope.[4] In a series of complex, well controlled experiments (including Northern blot, immunostaining, and immunogold electron staining), the authors demonstrate that the m1A 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 m1A, implying t-RNA degradation, was evident as early as 30 minutes after reperfusion of liver ischemia, while nuclear chromatin degradation as detected by terminal deoxynucleotidyl transferase–mediated digoxigenin-deoxyuridine nick-end labeling positivity, implying apoptosis or necrosis, occurred only after 8 hours. To establish t-RNA degradation as a potential biomarker to be used for diagnostic purposes, the authors developed an m1A antibody–based ELISA and validated the detection of t-RNA derivatives in plasma samples by ELISA using liquid chromatography–mass spectrometry as a specific reference method.

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 derivate 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 m1A 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.

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Can m1A levels be a useful biomarker to improve the management of AKI? The current answer is no. The recommendations of the 10th Acute Dialysis Quality Initiative consensus conference divide AKI biomarkers into the following categories: identifying patients at risk for AKI, diagnosing kidney injury versus functional GFR impairments, monitoring of injury/recovery, predicting prognosis, or identifying patients for targeted therapeutic interventions.[7] None of these are adequately addressed by m1A levels. Furthermore,

1. *Diagnostic use—specificity:* Because t-RNA degradation is not kidney-specific, its early detection is of no diagnostic use in terms of AKI versus other forms of tissue injury. Therefore, the authors' comparison of plasma m1A levels obtained after aorta clamping with urinary KIM-1 levels is problematic because m1A levels may not at all relate to kidney injury.

2. *Time factor:* Because AKI is asymptomatic, the relevance of the time factor applies only for predictable forms of AKI, such as renal transplantation, contrast media/toxin exposure, or cardiac surgery. Upon thoracic surgery, the time gain was 50 minutes compared with urinary KIM-1, but it could have been even less if KIM-1 had been tested also earlier. The aforementioned time points were those of sampling, but clinical decision making depends on when test results become available. M1A and KIM-1 ELISA results will be available only several hours later. It remains to be demonstrated that a time gain of 50 minutes will last for hours of sample processing and still be clinically relevant for decision making.

3. *Guiding therapy:* The clinical use of biomarkers for guiding therapy initially depends on available therapeutic options.[7] For example, in acute coronary syndrome the time factor is relevant because earlier angioplasty improves survival and long-term outcomes.[8] AKI management rarely involves such curative therapeutic interventions apart from stopping the underlying trigger of kidney injury and fluid therapy. It remains to be demonstrated that removing the trigger and fluid therapy (or any other intervention) 50 minutes earlier can improve short-term and long-term AKI outcomes.

4. *Prognosis:* The authors show that t-RNA degradation is associated with overall mortality in the general population. However, the same is true for many established predictors of mortality.[9] Neither the novelty of the identified mechanism nor the time factor provides any conceptual advance beyond those in terms of predicting overall mortality. A particular predictive quality in patients with kidney disease was not tested but is unlikely given the lack of specificity of the biomarker for kidney injury.

So what, then, is the importance of the work of Mishima *et al.*? The study very elegantly introduces t-RNA degradation as an early event occurring in postischemic and toxic (renal) cell stress *in vitro* and *in vivo*.[4] Using AKI models, their work validates m1A positivity as a biomarker of cell stress. Such m1A positivity, however, may reflect cell injury in extrarenal organs as well as in the kidney. These seminal findings published in *JASN* may thus be relevant, not just to kidney cell injury but also to cell injury in general. As such, they should be of broad biologic interest and appeal. The work of Mishima *et al.* will certainly trigger broad research activities and numerous follow-up studies on the greater susceptibility of RNA to indicate cell stress compared with DNA degradation. It will be necessary to determine whether t-RNA degradation precedes cell death or rather represents a transient or compensatory process of the cell that easily recovers. It may be possible that t-RNA degradation is conceptually similar to autophagy, a process that allows cells to save energy and avoid cell death in transient forms of stress.[10] It will be of interest to ascertain whether t-RNA derivatives are also detectable in the urine and whether this more specifically would indicate kidney injury. The authors developed tools to specifically identify conformational changes of t-RNA, namely the m1A antibody, and we can only hope that they will share the antibody with the research community to broadly explore its diagnostic use *in vitro* and *in vivo*. Finally, as the authors lay out in the discussion of their study, circulating t-RNA derivatives may serve as an unspecific surrogate marker of cell stress that could be useful in studies of specific therapeutic interventions to predict overall mortality. While they provided evidence from a clinical study using statins as a therapeutic intervention to improve cardiovascular mortality, one may speculate that further validation of this new biomarker could eventually be used as a surrogate marker for mortality in clinical AKI/CKD trials.[7,11,12]

Together, although it does not yet represent a clinically relevant step forward for patient management, the study of Mishima *et al.* offers exciting novel perspectives for further research at multiple levels—basic, preclinical, and clinical science—in nephrology and other disciplines.

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DISCLOSURES

None.

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See related article, "Conformational Change in Transfer RNA Is an Early Indicator of Acute Cellular Damage," on pages 2316–2326.

CKD and Risk of Renal Cell Carcinoma: A Causal Association?

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The increasing burden of CKD in the United States is an important public health concern. In addition to well established comorbidities, such as cardiovascular disease, patients with CKD may have an increased risk of renal cell carcinoma (RCC), whose incidence has also been rising in recent decades.[1] Although there is consistent evidence of an excess risk of RCC and other malignancies among patients with ESRD and kidney transplant recipients,[2–6] few studies have investigated cancer risk in relation to less severe forms of impaired renal function. The article by Lowrance *et al.*,[7] published in this issue of *JASN*, reports on the relationship between eGFR and risk of RCC and several other cancers among members of the Kaiser Permanente Northern California system, a large integrated health care network in the greater San Francisco Bay area.

The well maintained records of the Kaiser Permanente Northern California system are an ideal data source for evaluating cancer risk in relation to impaired renal function, and the authors have controlled for potential confounding factors to an extent not possible in previous studies of CKD or ESRD and cancer. Others strengths of this study include its large sample size and prospective, population-based design. The investigators observed increasing risk of RCC among those with lower eGFR; this association appeared to increase monotonically, with a >2-fold risk of RCC among individuals with eGFR<30 ml/min per 1.73 m^2 compared with those with eGFR of 60–89 ml/min per 1.73 m^2. Lower eGFR was also associated with an increased risk of urothelial (transitional cell) carcinomas, although the magnitude of this association was not as strong as that for RCC. The association with urothelial cancer is consistent with that seen in several studies reporting an increased risk of bladder cancer among patients with ESRD or those who received a transplanted kidney.[3–6]

Although the observed association between CKD and future RCC risk may be truly causal, several alternate explanations merit consideration. One possibility is that heightened medical surveillance of individuals with impaired renal function may lead to increased incidental detection of localized, indolent renal tumors discovered through abdominal imaging. However, the authors minimized the potential effect of detection bias on their results by adjusting for health care utilization, hematuria, and receipt of medical imaging in their statistical models. Furthermore, they noted that the association between CKD and RCC was still apparent in sensitivity analyses restricted to cases with nonlocalized renal tumors who did not have abdominal imaging; we might expect that such cases would be the least likely to have their renal tumors detected incidentally.

Another possibility is that impaired kidney function may be a prodromal effect of as-yet-undiagnosed renal tumors. Lowrance *et al.*[7] sought to account for bias resulting from reverse causation by restricting their analysis to individuals without a history of dialysis or renal transplantation before study entry, by excluding incident cancers diagnosed during the first 2 years of follow-up, and by excluding serum creatinine measurements obtained <3 months before an incident cancer diagnosis. Although these exclusion criteria should provide some reassurance that the observed association reflects an etiologic role of CKD in renal carcinogenesis, future studies characterizing kidney function earlier in time before RCC diagnosis would further strengthen the argument against reverse causation bias. It would also be informative for future studies to characterize RCC risk in relation to the change in kidney function over time using serial measurements from specimens collected at specific time intervals before RCC diagnosis.

As the authors note, the biologic mechanisms underlying the association between CKD and RCC risk (*e.g.*, pathologic

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