Circulating Micro–RNAs in Acute Kidney Injury: Early Observations

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Acute kidney Injury (AKI) remains a diagnostic and therapeutic dilemma. Translation of excellent basic science information into therapeutic advances in AKI now depends on early diagnosis and drug delivery before elimination of the potential for this target to influence cell injury. For instance, treatment of ischemic AKI with small interfering RNA to P53 depends on glomerular filtration followed by proximal tubule uptake and release into the cytosol before ischemia-mediated upregulation of P53 (1). Therefore, it is of paramount clinical importance to make the diagnosis of AKI early during the course of injury for development of successful therapies. To this end, the search for clinical, structural, biochemical, functional, and inflammatory biomarkers of AKI for risk assessment, diagnosis, prognosis, and severity of injury has been a major thrust in AKI research (2). It is exciting that the article by Lorenzen et al. (3) in this issue of CJASN adds yet another class of compounds, that being microRNAs (miRNAs), to the growing list of potentially useful biomarkers.

miRNAs are endogenously expressed 18– to 24-nucleotide single-strand RNA molecules that principally function by binding to the 3′ untranslated region of mRNAs and repress the expression of their gene products. Initially discovered in the context of Caenorhabditis elegans development, miRNAs are highly conserved across species. Unlike small interfering RNAs in plants, miRNAs do not require complete complementarity to bind their target. Evidence suggests that the seed sequence (nucleotides 2 through 8 of the miRNA) is the most important region for binding to and regulation of target genes. Once bound, miRNAs induce repression by blocking the initiation or elongation of translation or through deadenylation of the mRNA transcript. Because miRNAs do not require complete complementarity to repress gene expression, one miRNA can regulate multiple mRNA transcripts and one mRNA transcript can be repressed by multiple miRNAs (4).

miRNAs are encoded in the genome, transcribed by RNA polymerase II and regulated tightly at the transcriptional and posttranscriptional levels. DNA encoding miRNAs can have their own promoter and open reading frame or can be situated within other genes, in intronic regions. Expression of miRNAs occurs after transcription and cleavage by two RNase III enzymes, Drosha and Dicer. Drosha cleaves the primary transcript of a miRNA, the pri-miRNA, into an approximately 70-nt stem-loop intermediate, the pre-miRNA. The pre-miRNA is exported from the nucleus and cleaved by Dicer into a single-stranded mature miRNA. Pri-miRNAs can encode either an individual miRNA or a polycistronic cluster of two or more miRNAs (5).

Since the discovery of miRNAs a little more than 10 years ago (6), researchers have discovered that miRNAs are essential for normal development and physiology. Expression of miRNAs is altered in many pathophysiologic conditions, and regulation of miRNAs by drugs is essential to drug activity (7). To date, the majority of miRNA-based research has focused on their mechanism of action, expression patterns, and the gene products that they regulate. In addition to uncovering the underpinnings of miRNA function, miRNAs are being investigated as biomarkers. In 2008, several groups demonstrated that miRNAs circulate in the bloodstream and that alterations in the expression of individual miRNAs can serve as biomarkers of specific cancers (8–10). After this work, other groups used similar techniques to demonstrate elevation of specific miRNAs in the circulation after myocardial injury and liver injury (11,12). Recent evidence also suggests that miRNA expression levels can be used as prognostic markers. In B cell chronic lymphocytic leukemia, expression alterations in 13 miRNAs distinguished good and poor prognoses for patients (13).

miRNAs travel along with mRNAs and cytosolic constituents in microvesicles (MVs) that are released by all cells. Circulating MVs derived from platelets, white blood cells, and endothelial cells are always present and increase during and after infection and tissue injury (14,15). Cell surface receptors and similar surface membrane lipid components of the MV allow for mediation of cell–cell communication, fusion, and epigenetic reprogramming of cells (14,16). They may also serve to transfer membrane receptors and infections (17). MVs are believed to mediate protection and repair after release from mesenchymal stem cells in AKI (18,19). The observed protective effect of the MVs on AKI was lost when the MVs were pretreated with RNase, suggesting that the mRNAs and/or miRNAs...
present in the MVs were essential for the observed protection (18).

In the study by Lorenzen et al. (3), a global miRNA expression analysis of serum revealed 13 miRNAs with differential regulation between AKI and healthy control patients. Five miRNAs showed induction and eight showed repression in patients with AKI. The authors then quantified three miRNAs in the plasma of 77 patients with AKI requiring renal replacement therapy (RRT), just before the start of RRT, and compared these results with healthy control subjects and patients with acute myocardial infarctions. miR-210, an miRNA known to be upregulated in endothelial cells in association with tissue hypoxia (20), was found upregulated in patients with AKI, whereas miR-320 and miR-16 both were severely reduced. They also compared the three miRNAs in surviving and nonsurviving patients with AKI using an end point of survival at 4 weeks after initiation of RRT. Nonsurvivors had increased levels of all three miRNAs compared with survivors, and multivariate analysis for survival revealed that only miR-210 showed increased levels with a significant Kaplan-Meier curve.

Although Lorenzen et al. (3) do not establish the tissue of origin for miR-210, miR-320, and miR-16 or whether they play an active role in the progression of AKI, these are interesting future directions, in light of the mounting evidence for the importance of circulating miRNAs. One potential explanation is that these miRNAs are released from the kidney after injury. This would correlate with the findings by Lorenzen et al. that all three are elevated in nonsurviving patients, compared with survivors. Alternatively, however, these miRNAs could be contained within MVs and worsen AKI. If this were the case, then it could prove to be a useful therapeutic target in the treatment of AKI, in addition to the description by Lorenzen et al. of miR-210 as a prognostic biomarker. Therefore, many questions remain about miRNAs in AKI. Perhaps a significant role for urinary miRNAs, especially considering transfer of genetic information between tubular epithelial cells, will evolve. The potential for such cell–cell communication by this route seems worthy of further investigation.

Interestingly, the criteria used to initiate RRT included a decrease in estimated GFR of 30% resulting in a wide variation in the level of kidney injury in the AKI group requiring RRT. Five patients had an R classification in the RIFLE system, 21 had injury (I), and 50 had failure (F). It is widely known the RIFLE classification system correlates with survival, yet the authors did not include this in the multivariate analysis. Could it be that the miRs reflect severity of injury and add little to this well-established parameter? Could less damage to the kidney result in less release of miRs? Could remaining kidney function clear miRs and therefore limit serum accumulation in patients with AKI?

Whether the findings are “AKI specific” remains to be determined. Perhaps a better comparator group would have been patients who had similar disease processes but not AKI, not a group of patients with acute myocardial infarction. The presence of systemic multiorgan involvement could be a critical component for an unbiased comparison. Finally, receiver operating characteristic area under the curve values were not >0.7 and were similar to SOFA score predictions. Both SOFA and APACHE II scores were significant with univariate analysis, as was sepsis. Therefore, the overlap of miRNAs within the surviving and nonsurviving groups is large, thereby potentially limiting the utility of such observations in the individual patient. However, these exciting early data should stimulate further studies into this rapidly evolving area.

Disclosures
None.

References
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See related article, “Circulating miR-210 Predicts Survival in Critically Ill Patients with Acute Kidney Injury” on pages 1540–1546.