Role of microRNA in the detection, progression, and intervention of acute kidney injury

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Impact statement

Firstly, we have discussed the potential advantages and limitations of miRNA as biomarkers. Secondly, we have summarized the role of miRNA in the progress of AKI. Finally, we have made a vision of miRNA's potential and advantages as therapeutic target intervention AKI.

Abstract

Acute kidney injury, characterized by sharply decreased renal function, is a common and important complication in hospitalized patients. The pathological mechanism of acute kidney injury is mainly related to immune activation and inflammation. Given the high morbidity and mortality rates of hospitalized patients with acute kidney injury, the identification of biomarkers useful for assessing risk, making an early diagnosis, evaluating the prognosis, and classifying the injury severity is urgently needed. Furthermore, investigation into the development of acute kidney injury and potential therapeutic targets is required. While microRNA was first discovered in *Caenorhabditis elegans*, Gary Ruvkun's laboratory identified the first microRNA target gene. Together, these two important findings confirmed the existence of a novel post-transcriptional gene regulatory mechanism. Considering that serum creatinine tests often fail in the early detection of AKI, testing for microRNAs as early diagnostic biomarkers has shown great potential. Numerous studies have identified microRNAs that can serve as biomarkers for the detection of acute kidney injury. In addition, as microRNAs can control the expression of multiple proteins through hundreds or thousands of targets influencing multiple signaling pathways, the number of studies on the functions of microRNAs in AKI progression is increasing. Here, we mainly focus on research into microRNAs as biomarkers and explorations of their functions in acute kidney injury.

Keywords: Acute kidney injury, microRNA, biomarker

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Acute kidney injury

Acute kidney injury (AKI) is a clinical syndrome characterized by sharply decreased renal function, metabolite retention, and water, electrolyte and acid–base balance disorder.[1] AKI is a common and important complication with a high mortality rate in hospitalized patients.[2–5] Survivors of AKI are more likely to develop end-stage kidney disease.[6] There are no specific treatments for AKI other than routine supportive therapy and renal replacement therapy, and this is the main reason for AKI patients having a poor short-term prognosis.[7] Early diagnosis might lead to better prognosis. Recent studies of AKI have mainly focused on investigating diagnostic biomarkers, early interventions, and pathophysiological mechanisms.

Pathophysiology of AKI

The pathological mechanism of ischemic AKI is mainly related to immune activation and inflammation.[8–10] Accordingly, its progress can be divided into three stages: initiation, progress, and repair.[11–13] During initiation of the process, decreased renal perfusion leads to irreversible mitochondrial damage, resulting in endothelial damage, epithelial injury, and inflammatory infiltration. Inflammatory infiltration involves endothelial cell swelling, endothelial monolayer disruption, increased permeability, white blood cell emigration and micro-thrombus formation. Epithelial cell injury includes sub-lethal injury, apoptosis, and necrosis. In the sub-lethal injury process, loss of the apical brush border of proximal tubular cells, basement membrane exposure, and

cell polarity changes occur. Inflammatory cells are recruited to the injured area to release pro-inflammatory factors, chemokines, and costimulatory molecules, such as TNF-α, IL-1, IL-6, and MCP-1.[14–16] These molecules can not only worsen the damage to renal tubular epithelial cells and endothelial cells but can also further amplify the inflammatory response, promote inflammatory infiltration and facilitate the "inflammatory cascade effect." In the following repair process, renal tubular epithelial cell regeneration, cytoskeleton protein reconstruction and cell polarity reconstruction are initiated.[14] Septic AKI has a pathophysiological mechanism that clearly differs from that of ischemic AKI.[17,18] Alterations in renal blood flow, microcirculatory disturbances, and blood flow redistribution between the renal cortex and medulla are potentially important contributors to renal tubular injury and reduced glomerular filtration rate (GFR). Another theory posits that blood containing detrimental molecules, such as inflammatory and complement factors, are the main causes of the tubular injury and reduced GFR in septic AKI. Currently, due to the differences between animal models and septic patients, there is limited evidence indicating the pathophysiological mechanism of septic AKI. In addition, common causes of AKI include contrast nephropathy[19] and drug-induced kidney injury, the pathophysiological mechanisms of which differ.

As miRNAs were indicated could control the expression of multiple proteins through hundreds or thousands of targets influencing multiple signaling pathways, the number of studies on the functions of miRNAs in AKI progression is increasing. In addition, numerous studies have identified the potential that miRNAs as biomarkers for the detection and prognosis evaluation of AKI. Here, we mainly focus on research into miRNAs as biomarkers and explorations of their functions in AKI.

MicroRNA biogenesis

In 1993, Victor Ambrose's lab first discovered miRNA in *Caenorhabditis elegans*, while Gary Ruvkun's laboratory identified the first miRNA target gene.[20] Together, these two important findings confirmed the existence of a novel post-transcriptional gene regulatory mechanism. Over the past few years, miRNA research has been flourishing, and understanding of the role of non-coding RNA in biological processes has expanded. miRNA is encoded by a specific DNA region called a "mitron," and stem-loop pri-miRNAs with hundreds of base pairs are synthesized under the action of RNA polymerase II. Subsequently, the stem-loop pri-miRNAs are cleaved into single stem-loop pre-miRNAs under the action of the RNase III Drosha enzyme and cofactor DGCR8. Pre-miRNAs generally have approximately 60 to 70 nucleotides and are transported from the nucleus into the cytoplasm for processing by the Dicer enzyme into mature miRNAs, which can then perform their normal biological functions.[21–23]

Mature miRNAs bind to argonaute (AGO) proteins and form RNA-induced silencing complexes (RISCs), which act on mRNAs to negatively regulate gene expression.[24,25] The process is identified by miRNA-mRNA base pairing, and the degree of base complementarity of the pairing chain is important for target recognition: if the miRNA-mRNA pairing is perfect, AGO proteins in RISCs will exert their endonuclease effect and degrade the mRNA. This process is more common in plants. In mammals, as the miRNA-mRNA base complementarity is largely incomplete, the follow-up effect is mRNA translation inhibition. However, the manner in which RISCs and their targets inhibit protein translation is unclear.

miRNAs as biomarkers in AKI

Serum creatinine (Scr) is widely accepted as an overall index for kidney function but often fails in the early detection of AKI. Given the high morbidity and mortality rates of hospitalized patients with AKI, the identification of biomarkers useful for assessing risk, making an early diagnosis, evaluating the prognosis, classifying the injury severity is urgently needed.[26,27] miRNA is stable in body fluids, extremely sensitive to alterations within an organism, and exhibits increases that have been detected earlier than measurable Scr changes in many studies. Additionally, miRNA is detectable in urine, which can be sampled easily and noninvasively. Thus, miRNAs have great potential as biomarkers for disease detection.[28]

Because urine can be sampled easily and noninvasively, urine-based markers have been proposed as early indicators for identifying AKI. Moreover, miRNA is stable in urine.[29,30] Therefore, many researchers have become interested in determining whether miRNA expression levels in urine can be used as biomarkers for AKI. Our previous study[31] indicated that a group of miRNAs exhibited altered expression levels in urine in an ischemia animal model, and the earliest time points for miRNA alterations could be traced back to 2 h after the operation. Of course, these time points might be related to the times at which the samples were collected. Another study of miRNAs in the urine of ischemic animals found a significant difference in the miRNA profile at 1 h after the operation.[32] These findings indicate that miRNA is highly sensitive to injury-related factors. However, numerous studies have shown that the miRNA changes in these models are dynamic.[33] Thus, another problem in hospitalized patients who may have a higher sensitivity is that their urine miRNA levels are more likely to be affected by various factors, such as underlying disease, complications, of fluid input volume, and diuretics. In animal models, urine miRNA levels are also easily affected by urine volume, operation type, and fluid intake. For instance, one study[34] of miRNA profiles in a cisplatin-induced kidney injury animal model recommends the use of miR-15, miR-16, miR-20a, miR-192, miR-193, and miR-210 as biomarkers. Another similar investigation[35] indicated that mmu-let-7g-5p, rno-miR-93-5p, rno-miR-191a-5p, and rno-miR-192-5p might have the potential to serve as biomarkers. The inconsistency of these results can be explained by the differences between the sampling time points, species, and cisplatin dose. In other words, the discrepancy is due to a difference in sensitivity.

Of course, it is also important to consider miRNA levels in blood samples as biomarkers. miR-10a, miR-192, and

miR-194 were considered promising plasma biomarkers for ischemia-reperfusion (I/R)-induced kidney injury. However, the results were completely inconsistent among hospitalized patients. A screening of critically ill patients with AKI indicated that the plasma miR-16, miR-320, and miR-210 levels were altered. In contrast with previous studies, the study evaluated the 28-day patient prognosis and further explored the value of the miRNAs for assessing the prognosis. Because of the differences among studies, some researchers believe that multiple samples can be combined to account for these differences. In addition, studies have shown that the joint detection of miRNA in two or three sample types, such as urine and plasma or plasma and kidney tissue, leads to completely different results compared with detection in a single sample. One study using an animal model of I/R-induced kidney injury detected the miRNA levels in plasma and renal tissue. miRNAs exhibiting a change concordant with the extent of injury are considered potential biomarkers. miR-714, miR-1188, miR-1897-3p, miR-877*, and miR-1224 were found to be elevated consistently in plasma and renal tissue. Another study of patients who underwent cardiac surgery and developed AKI showed that the urine and plasma miR-21 levels were associated with severe AKI and other poor postoperative outcomes of cardiac surgery, indicating their potential for use as prognostic markers. In addition, one study of kidney transplant recipients with acute tubular necrosis indicated that miR-142-3p has a distinct expression pattern and could potentially be used as a non-invasive biomarker. We learned from the above studies that the reliability of miRNAs as biomarkers confirmed by two or three sample types is not superior to that of miRNAs detected in a single sample type. However, future research will likely shed light on these unknowns and differences. The discussed studies of miRNAs as biomarkers in AKI are summarized in Tables 1 and 2.

miRNA plays a role in the development of AKI

Disease development mechanisms are complicated and often involve changes in multiple proteins, coding genes, and signaling pathways. Thus, it is difficult to improve the prognosis of a disease by regulating a single molecule. miRNA is an important regulatory molecule in gene transcription and protein translation and acts as a hub in the processes of inflammation, apoptosis, proliferation, and angiogenesis, i.e. one miRNA can control the expression of multiple proteins through hundreds or thousands of targets, thus influencing multiple signaling pathways. For example, multiple studies have demonstrated miR-21 to be an important molecule involved in the progression of

Table 1. miRNA as a urine biomarker of AKI.

Species	Animal model/population	Sample type	miRNA involved	Method	Reference (PMID)
Rat/human	Bilateral renal ischemia for 45 min/cardiac operation-induced kidney injury	Urine	miR-30c-5p, miR-192-5p	Rattus norvegicus GeneChip miRNA 3.0 Array	28056546[31]
Rat	Gentamicin-induced kidney injury	Urine	mmu-miR-138-5p, mmu-miR-1971, mmu-miR-218-1-3p, rno-miR-489	TaqMan® Array Rodent miRNA Cards	27074385[36]
Rat	Gentamicin-induced kidney injury	Urine	let-7d, miR-203, miR-320	Next-generation sequencing (NGS)/ qPCR-based miRNA profiling	24942259[37]
Rat	Cisplatin-induced kidney injury	Urine	miR-15, miR-16, miR-21, miR-141, miR-146a, miR-184, miR-192, miR-193, miR-200b and other 18 miRNAs	TaqMan® Low-Density A Arrays (TaqMan® cards)	24880025[34]
Rat	Cisplatin-induced kidney injury	Urine	let-7g-5p, miR-93-5p, miR-191a-5p, miR-192-5p	TaqMan® Rodent miRNA PCR cards	24863737[35]
Human	Critically ill patients/cardiac surgery/biopsy-proven tubular injury to the transplanted kidney/ healthy volunteers	Urine	miR-21, miR-200c, miR-423, miR-4640	miScript miRNA PCR array human miRNome	24153252[38]
Human	Kidney transplant recipients who developed graft dysfunction	Peripheral blood and urinary cells	miR-142-3p	Real-time PCR	28380212[39]
Rat	Glomerulonephritis model/ cisplatin treatment	Urine/kidney tissue	miR-10 b, miR-100	TaqMan® Low-Density A Arrays (TaqMan® cards)	25758243[40]
Mouse/ human	Bilateral renal artery occlusion for 45 min/critical ill patients	Urine/kidney tissue	miR-494	Real-time PCR	23160513[32]

AKI: acute kidney injury.

Table 2. miRNA as a plasma/serum biomarker of AKI.

Species	Animal model/population	Sample type	miRNA involved	Method	Reference (PMID)
Human	Sepsis-induced AKI	Serum	miR-4321, miR-4270	miRCURY™ LNA Array	28296904[41]
Rat	Aristolochic acid-induced kidney injury	Plasma	miR-21-3p	Microarray assays	27422293[42]
Human	Acetaminophen-induced kidney injury	Plasma	A miRNA panel	miRNA profiling	26489516[43]
Human	Critical ill patients/cardiac surgery patients	Serum	miR-101-3p, miR-127-3p, miR-210-3p, miR-126-3p, miR-26b-5p, miR-29a-3p, miR-146a-5p, miR-27a-3p, miR-93-3p, miR-10a-5p	Taqman® Low-Density Arrays for miRNAs	26079930[44]
Rat	Bilateral ischemia of pedicle for 45 min	Plasma	miR-10a, miR-192, and miR-194	Microarray assay provided by LC Science	24553149[45]
Human	Critically ill patients	Plasma	miR-210, miR-16, miR-320	Genome-wide miRNA array analysis	21700819[46]
Human (Table 1)	Kidney transplant recipients who developed graft dysfunction	Peripheral blood and urinary cells	miR-142-3p	Real-time PCR	28380212[39]
Rat/human	Contrast-induced nephropathy	Plasma/kidney tissue	miR-30 family members	miRNA microarray assays	26337190[47]
Mouse	Bilateral renal ischemia for 27 min	Plasma/kidney tissue	miR-714, miR-1188, miR-1897-3p, miR-877*, miR-1224	Mouse miRNome miScript miRNA PCR Arrays	24695114[48]
Human	Cardiac surgery patients	Plasma/urine	miR-21	Taqman® miRNA assay kits	23717419[49]
Rat/human	Contrast-induced kidney injury	Plasma	miR-188, miR-30a, miR-30e	Agilent miRNAs microarray v.10.1	27528406[50]
Human	Cardiac surgery patients	Plasma	miR-21	Taqman® miRNA assay kits	26940124[51]

AKI: acute kidney injury.

AKI. One study found that miR-21 upregulation mediated a renal protective effect in an I/R animal model by delayed ischemic preconditioning.[52] The same team also found that xenon preconditioning could protect against sepsis-induced AKI through miR-21 activation in a mouse model.[53] In addition, miR-21 was found to play an important role in the renal tubular regeneration of fish after gentamicin-induced kidney injury.[54] These cases fully illustrate the multiformity of miRNA functions in AKI. This feature of miRNA can be further illustrated by the functions of miR-494. A study by Lan *et al.*[32] found that miR-494 could target the 3′ end non-transcribed region of active transcription factor 3 (ATF3) and induce the release of inflammatory mediators, such as IL-6, MCP-1, and P selectin, and miR-494 overexpression led to increased apoptosis and necrosis in a mouse model. Another study[55] on miR-494 found that this miRNA promoted the cyclosporine-induced epithelial-mesenchymal transition through targeting PTEN and might represent a novel druggable target for preventing renal injury. These studies show that various miRNA functions can be achieved through different target genes and that each miRNA typically performs remarkable functions in many biological processes.

As a negative regulator of gene expression that modulates the translational efficiency and/or stability of target mRNAs, miRNA is involved in the control of a wide range of biological functions and processes, such as development, differentiation, metabolism, growth, proliferation, and apoptosis. Sometimes the same miRNA plays opposite roles in different diseases, which can be demonstrated by the function of miR-30c. We found that miR-30c could decrease apoptosis and promote proliferation through its target in a cellular hypoxia-reoxygenation model (not published). Another study also found that miR-30c upregulation with a mimic significantly decreased the cisplatin-induced elevation in HK2 and NRK52E cell apoptosis.[56] As such, miR-30c exerts an anti-apoptotic effect in this kidney injury model. Additionally, there is evidence suggesting that miR-30-5p functions as a novel therapeutic tool for targeting the oncogenic Wnt/β-catenin/BCL9 pathway by promoting apoptosis.[56] Furthermore, miR-30c has been shown to directly target ADAM19 and inhibit cells that cause colon cancer; thus, it could serve as a promising strategy for treating colon cancer.[57] miR-30c exerts the opposite effects of inhibiting and promoting apoptosis in different diseases. The studies mentioned above regarding the roles miRNAs play in AKI are summarized in Table 3.

miRNAs in AKI interventions

Studies of whether miRNAs can be used as early diagnostic biomarkers or prognostic indicators and investigations of their specific mechanisms of action in AKI are performed with the goal of treating AKI early to improve patient survival and quality of life.

Table 3. miRNA in the development of AKI.

miRNA involved	Species	Model/population	Sample	Target	Reference (PMID)
miR-223	Mouse	miR-223 knockout mouse/sepsis model	Tissue		28515178[58]
miR-107	HK2	Septic kidney injury	Cells	DUSP7	28063928[59]
miR-204	Tubular epithelial cells	I/R-induced kidney injury	Cells	SP1	27959449[60]
miR-101	HuAECs	Co-culture with peripheral blood CD4+ T cells	Cells	c-Rel	27898347[61]
miR-46a	Mouse, cell, human	I/R-induced kidney injury	Tissue	/	27444565[62]
miR-146b	Rat	Cisplatin	Tissue	/	27400799[63]
miR-21	Rat, cell	I/R-induced kidney injury	Tissue	/	27152763[64]
miR-489	Mouse	I/R-induced kidney injury	Tissue/cells	PARP1	26975439[65]
miR-21	Fish	Gentamicin	Tissue	/	26577279[54]
miR-34a	Mouse	I/R-induced kidney injury	Tissue/cells	/	26406385[66]
miR-20a-5p	Mouse	I/R-induced kidney injury	Tissue	ATG16L1	26165754[67]
miR-21	Cell	Ischemia pretreatment	Tissue	/	26149640[68]
miR-150	Mouse	I/R-induced kidney injury	/	IGF-1R	26109086[69]
miR-21	Mouse	*Escherichia coli* lipopolysaccharide/ septic kidney injury	Tissue	/	25844699[53]
miR-494	Mouse	Cyclosporine-induced nephrotoxicity	Tissue	PTEN	25854542[55]
miR-26a	Rat	I/R-induced kidney injury	Renal vein	/	25728641[70]
miR-155	Mouse	Cisplatin-induced kidney injury	Tissue	c-Fos	25015656[71]
profile	Mouse	Cisplatin-induced kidney injury	Tissue	Foxo3	24759152[72]
miR-204/miR-211	Mouse	Candidemia-induced kidney injury	Tissue	Hmx1	24641951[73]
miR-126	Mouse	I/R-induced kidney injury	Tissue	/	24610930[74]
miR-17 family, miR-21	Mouse	I/R-induced kidney injury	Tissue	/	23988020[75]
miR-494	Mouse, human	I/R-induced kidney injury/ critical ill patients	Tissue, urine	ATF3	23160513[32]
miR-21	Mouse	I/R-induced kidney injury	Tissue	PDCD4	22785173[52]
miR-126/miR-296	Rat	I/R-induced kidney injury	Microvesicles	/	22495296[76]
miR-155	Rat	I/R-induced kidney injury	Tissue	FoxO3a	28006785[77]
miR-30c	HK2, NRK52E cells	Cisplatin	Cells	Bnip3L/Hspa5	28796263[56]

AKI: acute kidney injury.

Microvesicles (MVs), also called microparticles, are plasma membrane fragments ranging from 50 to 1000 nm in size that dissipate from various cell types to transduce signals between cells. MVs can transport mRNAs, miRNAs, and proteins and might contribute to the development and progression of inflammatory, vascular, malignant, and neurodegenerative diseases and infections.[78,79] Studies have shown that MVs can carry miRNAs into receptor cells that trigger downstream signaling events. Stefano Gatti *et al.*[80] performed a study on bone marrow mesenchymal stem cells (MSCs) in the treatment of I/R injury caused by AKI and chronic kidney disease and found that miRNA isolated from the MSC MVs could protect the cells from I/R injury; additionally, RNAase-treated MVs did not exhibit this protective effect, suggesting that miRNA may be involved. Cantaluppi *et al.*[76] found that injecting endothelial progenitor cell- (EPC) derived MVs into mice after surgically inducing ischemia and reperfusion could protect the kidneys from I/R-induced renal damage, reduce apoptosis and inflammatory cell infiltration, and promote epithelial cell proliferation and angiogenesis. However, fibroblast-derived MVs had no such protective effect, and MVs transfected with anti-miR-126 and anti-miR-296 lose this protective effect. These results demonstrate that the renal protection mediated by EPC-derived MVs is primarily achieved by miR-126 and miR-296, indicating that miRNA-containing MVs, as cell-derived packages of information, allow cell–cell communication and might be a potential therapeutic target for AKI.

In addition to miRNAs carried by MVs, exogenous regulation of miRNA expression might also be a potential therapeutic target for AKI. miR-489 was induced by hypoxia-inducible factor 1 (HIF-1) in an ischemia mouse model,[65] and miR-489 inhibition increased both the renal tubular damage and apoptosis. Although not investigated in that study, we can speculate that increasing mir-489 expression would also affect the extent of cell damage and apoptosis. miR-182[81] was identified as the main driver of post-transplantation AKI, and miR-182 inhibition improved the kidney function in an animal model with right nephrectomy and contralateral clamping of the renal pedicle for 40 min. Furthermore, miR-182 inhibition facilitated cell proliferation, metabolism, and angiogenesis. Thus, it is also reasonable to assume that the inhibition of mir-182 is a potential therapeutic target for ischemic AKI. Overall, as miRNAs have achieved good results as therapeutic targets in animal models, they have promise for use in clinical applications. Therefore, the main problem is now heterogeneity among studies, but in the future, this could resolved by more studies being performed with larger sample sizes.

In conclusion, since miRNA was discovered in nematodes, preliminary data and miRNA research methods have accumulated through research in the fields of miRNA isolation, identification, functional analysis, and regulation. Usually, studies are performed to detect miRNA expression profiles in animal and cellular models of disease and to discover the differentially expressed miRNAs by comparison with the expression profile of the control group; then, the miRNA expression is modulated to observe the resulting phenotypic and metabolic changes in the cells or animals and determine the function of the corresponding miRNA. Previous studies have shown that miRNAs are abnormally expressed in acute renal injury; thus, they are expected to play an important role in the development and progression of AKI and to be capable of serving as biomarkers for the early diagnosis and evaluation of AKI, potentially enabling earlier intervention. These reports have greatly encouraged researchers to study the potential of miRNAs in the context of kidney disease. However, much comprehensive and in-depth work remains to explore the molecular mechanisms involved in the specific roles of miRNAs, target genes, and signal transduction pathways, as well as how to use miRNAs as therapeutic targets for AKI. In short, how to apply miRNA research in the field of translational medicine should be considered by the entire scientific community.

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DECLARATION OF CONFLICTING INTERESTS

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