MicroRNAs in Pathogenesis of Acute Kidney Injury

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Key Words

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Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression mainly by repressing their target gene translation. A large spectrum of human diseases is associated with significant changes in miRNAs. Many miRNAs are induced in diseases, whereas some others are downregulated. The significance of miRNAs has been demonstrated in renal development and physiology, and in major kidney diseases such as acute kidney injury (AKI). Recent studies have further implicated specific miRNAs in the pathogenesis of AKI. miRNAs also have the potential to become new diagnostic biomarkers of AKI. Further investigation will identify the key pathogenic miRNAs in various types of AKI and test miRNA-based therapeutics and diagnosis.

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Introduction

MicroRNAs (miRNAs) are non-coding RNA molecules of 21–25 nucleotides that regulate gene expression through the post-transcriptional repression of their target mRNAs [1]. They can induce mRNA degradation or more frequently result in repression of protein translation by binding to the 3′-untranslated region (UTR) of the target mRNAs [2]. Accumulating evidence suggests that a majority of genes are subjected to miRNA regulation. Interestingly, one miRNA may regulate different genes and one gene may be regulated by multiple miRNAs. In the kidney, miRNAs play critical roles in a variety of cellular and physiological activities. Emerging evidence suggests that miRNAs play essential roles in the pathogenesis of acute kidney injury (AKI) [3]. Further investigation of miRNAs in AKI may lead to breakthroughs in the development of novel diagnostic tools and therapeutic interventions.

Acute Kidney Injury

AKI, characterized by a rapid decline of renal function, is a common renal disease with high morbidity and mortality. Clinically, the causes of AKI mainly include sepsis, ischemia/reperfusion (I/R), and various endog-

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Table 1. Major miRNAs studied in AKI

miRNAs	Targets	Renal cell functions	Reference
miR-687	PTEN	Hypoxia/HIF, cell cycle, pathogenic	[8]
miR-489	PARP1	Hypoxia/HIF, protective	[9]
miR-494	ATF3	Inflammation, pathogenic	[10]
miR-24	S1PR1, H2AX, HO-1	Stress, apoptosis, fibrosis, pathogenic	[11]
miR-21	PDCD4, PTEN, HIF-1	Apoptosis, protective	[12, 15, 22]
miR-126	PI3K	Cell survival, protective	[14]
miR-34a	Foxo3	Cell death, cell cycle, protective	[17–19]
miR-155	c-Fos	Cell death, protective	[20]
miR-125b	AhRR	Cell growth, protective	[19]
miR-181a	Bcl-2, Bax	Cell death, pathogenic	[21]

enous as well as exogenous nephrotoxins, such as cisplatin [4]. The pathogenesis of AKI is multifactorial, involving tubular injury, vascular dysfunction, and inflammation. Multiple cell types and different cellular processes and molecular mediators/regulators are responsible for the initiation and progress of the disease [5]. Despite notable progresses made in recent years, the molecular and cellular mechanisms of AKI are still poorly understood.

miRNAs in I/R

Renal I/R injury is a major cause of AKI. Pathologically, ischemic AKI is characterized by sublethal and lethal damages in renal tubules, especially the proximal tubules. Both cell death and repair occur not only in experimental models but also in patients with I/R [6]. To study the possible involvement of miRNAs in ischemic AKI, we established a conditional knockout mouse model in which Dicer (a key enzyme for miRNA biogenesis) was ablated specifically from kidney proximal tubules (PT-Dicer^{-/-}) [7]. In PT-Dicer^{-/-} mice, >80% miRNAs were depleted from renal cortex. These mice did not show renal development defects probably due to the late (2–3 weeks after birth) Dicer deletion driven by PEPCK-Cre. However, upon renal I/R these mice demonstrated significantly better renal function and less tubular cell injury/death, and consequently, these mice survived much better than their wild-type littermates. As a result, this study provided the first evidence for a crucial role of Dicer or associated miRNAs in the pathogenesis of AKI [7]. Subsequent studies have examined the involvement of several specific miRNAs, such as miR-687, miR-489, miR-494, miR-24, miR-21, and miR-126 (table 1).

Microarray analysis of miRNA expression suggested that 13 specific miRNAs were increased, while a dozen of miRNAs were decreased during ischemic AKI in mice [7]. Among them, miR-687 showed >1,700 fold increase in renal cortical tissues after 30 min of ischemia and 12 h of reperfusion. miR-687 induction during renal I/R was recently verified by Taqman real-time PCR, Northern blotting, and in situ hybridization, which further localized the induction specifically in proximal tubules. Interestingly, miR-687 induction was transient, peaking at 12–24 h of reperfusion and decreasing to basal levels after 48 h of reperfusion. Similarly, miR-687 was induced by hypoxia (1% oxygen) in cultured renal cells at 24 h. miR-687 induction is mediated by hypoxia-inducible factor-1 (HIF-1), because the induction was diminished in HIF-1^{-/-} cells and proximal tubule-specific HIF-1 knockout mice. To understand the pathogenic role of miR-687, we tested the effect of anti-miR-687-LNA, which protected against hypoxic injury in vitro and ischemic AKI in vivo. Thus, miR-687 is an injurious miRNA that plays a pathogenic role in ischemic AKI. To determine the mechanism by which miR-687 participates in AKI, bioinformatics analysis was conducted to predict the likely downstream target gene(s). The search of several databases suggested phosphatase and tensin homolog (PTEN) as a target of miR-687. Subsequent experiments verified that PTEN is indeed a target of miR-687 and in ischemic AKI, PTEN may regulate cell cycle and apoptosis. Collectively, these results identify miR-687 as an injury promoter in ischemic AKI and unveil a novel HIF-1/miR-687/PTEN signaling pathway [8].

Another miRNA studied recently is miR-489, which showed a moderate yet constant increase in ischemic AKI [7]. Similar to miR-687, miR-489 induction by hypoxia and renal I/R was HIF-1-dependent [9]. However, in contrast to miR-687, miR-489 was shown to be a protective

miRNA because anti-miR-489-LNA increased apoptosis *in vitro* and worsens ischemic AKI *in vivo*. To identify the target genes of miR-489, we conducted RNA-Sequencing of Ago2 immunoprecipitates. In mammalian cells, Ago2 is a key component of the miRNA-induced silencing complex (miRISC) where an miRNA and its target gene mRNAs associate. We pulled down Ago2 and associated mRNA in the presence or absence of miR-489, and then analyzed the mRNAs by deep RNA sequencing. This analysis identified 417 protein-coding genes whose mRNAs were induced by miR-489 to accumulate in Ago2 immunoprecipitate. In these genes, 127 contained miR-489 targeting seed sequence(s) at 3'-UTR. Interesting, 18 of the 127 genes were shown to be related to cellular stress responses. However, when further analyzed, only PARP1 was proved to be a real target gene of miR-489 during hypoxic and ischemic AKI. PARP1 is known to be a mediator of ischemic AKI and contributor to renal cell death and tissue damage [9]. Thus, upon induction via HIF-1, miR-489 may repress PARP1 to protect tubular cells in AKI.

Recent studies by other investigators have also pinpointed the roles of specific miRNAs in AKI. For example, Lan et al. [10] found that miR-494 was upregulated quickly after renal I/R in kidney tissues and it targeted the activating transcription factor 3. Overexpression of miR-494 not only induced inflammatory mediators, such as IL-6, MCP-1, and P-selectin, but also promoted NF- κ B-dependent inflammatory response, resulting in exacerbated apoptosis and further decrease of renal function. In another study, Lorenzen et al. [11] revealed a specific miR-24 induction in renal endothelial and tubular epithelial cells after I/R. The targets of miR-24 were found to be the sphingosine-1-phosphate receptor 1, H2A histone family member X, and heme oxygenase-1. Anti-miR-24 showed significant beneficial effects on ischemic AKI in mice, supporting its therapeutic potential. Furthermore, anti-miR-24 could delay the process of renal fibrosis [11]. In the model of delayed ischemic preconditioning, Xu et al. [12] showed miR-21 induction in an HIF-1 α -dependent manner. Upregulated miR-21 may target programmed cell death protein 4. Thus, miR-21 may contribute to the protective effect of delayed ischemic preconditioning by repressing programmed cell death protein 4. Likewise, xenon preconditioning could improve the renal function after renal I/R by inducing miR-21. In this model, anti-miR-21 led to a reduction in HIF-1 α , and upregulation of PDCD4 and PTEN, 2 pro-apoptotic target effectors, and resulted in a significant increase in tubular cell apoptosis [13]. Bijkerk et al. [14] verified that the enrichment of miR-126 in the hematopoietic compartment could pro-

tect renal I/R injury by promoting vascular integrity. Kaucsár et al. [15] demonstrated that the miR-21, miR-17-5P, and miR-106a (miR-17-5P and miR-106a belonging to the miR-17 family) were involved in the I/R-induced AKI. Interestingly, their results also indicate the miR-17 and miR-21 could play a critical role in the recovery phase of the disease.

miRNA in Cisplatin Nephrotoxic AKI

Cisplatin is a widely used anti-tumor chemotherapy drug, whereas the renal toxicity limits its application. Cisplatin nephrotoxicity is characterized by cell injury and death in renal tubules, leading to AKI [16]. Generally, oxidative stress and inflammation are responsible for cisplatin nephrotoxic AKI. However, accumulating evidence suggests that DNA damage and the associated DNA damage response are the indispensable pathogenic mechanism. p53, a well-known tumor suppressor protein, is considered closely related to DNA damage induced by cisplatin [17]. In 2010, we reported the first evidence of miRNA regulation in cisplatin nephrotoxicity [18]. miR-34a was upregulated during cisplatin treatment both *in vivo* and *in vitro* in a p53-dependent manner. As such, miR-34a induction was attenuated by pifithrin- α (p53 inhibitor) or in p53-deficient mice. Moreover, blockade of miR-34a increased apoptosis and decreased the survival of tubular cells during cisplatin treatment, indicating that miR-34a may play a cytoprotective role under such conditions [18]. miR-34a induction during cisplatin nephrotoxicity was verified recently by Lee et al. [19]. Moreover, they showed that miR-34a may repress Sirts, resulting in Foxo3 and p53 activation to promote apoptosis. In addition to miR-34a, they also found that the downregulation of miR-122. miR-122 could directly target Foxo3 and that the downregulation of miR-122 during cisplatin nephrotoxic AKI led to Foxo3 expression followed by p53 activation and apoptosis. These results suggest an miRNA integrative network regulating cisplatin-induced AKI and verified Foxo3 as a bridge molecule to the p53 pathway [19]. Additionally, miR-155 was highly increased following cisplatin treatment and blocking it aggravated kidney toxicity, resulting in increased apoptosis and tissue damage [20]. It was predicted that c-Fos, with 2 miR-155 binding sites in its 3'-UTR, was a most probable target of miR-155 during cisplatin treatment. Interestingly, miR-155 regulation was specifically important for cisplatin-induced damage in the kidneys. In other kidney injury models, such as ischemic AKI and UUO,

miR-155-knockout mice showed no difference in the development of injury compared with wild-type C57BL/6 mice [20]. Furthermore, HK-2 cells treated with cisplatin showed a significant increase of miR-181a, which contributed to cell apoptosis and cell death by directly regulating target genes such as Bcl-2 and Bax. Inhibition of miR-181a promoted the expression of Bcl-2 and suppressed Bax, accompanied by the protection of proximal tubular cells from cisplatin injury [21]. Du et al. [22] further showed that oltipraz treatment increased the miR-125b level, resulting in protecting the kidney from cisplatin toxicity. Mechanistically, miR-125b was shown to be transcriptionally induced by nuclear factor erythroid-2-related factor 2 and target aryl hydrocarbon receptor repressor, leading to the activation of AhR and the consequent induction of mdm2 to suppressed p53, tubular apoptosis and cisplatin nephrotoxicity [22].

miRNAs as Diagnosis Biomarkers of AKI

The existence of miRNAs in urinary and plasma indicates the potential of miRNAs as diagnosis biomarkers. Compared to other biomarkers such as proteins, miRNAs are remarkably stable in biological fluids and can be reliably analyzed and quantified. Perhaps most importantly, the Taqman assay of miRNAs is highly specific because it is a sequence-based analysis [3]. These advantages support the possible use of miRNAs as diagnostic and prognostic biomarkers for AKI in the future. For example, miR-21 in urine and plasma was associated with severe AKI [22]; miR-494 level in urine was elevated earlier than serum creatinine levels after I/R [10]. A group of miRNAs (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 and miR-10a-5) was tested as potential biomarkers of AKI in ICU and CS patients [23]. Probably there are specific miRNAs undiscovered in urinary and plasma that could serve as diagnostic and prognostic biomarkers of AKI.

Important Questions to be Investigated

Despite the progress achieved in recent times, several important questions remain to be addressed in the research of miRNAs in AKI. First, it is generally believed that a specific miRNA may regulate numerous target genes and a gene may be regulated by multiple miRNAs. Nonetheless, this is theoretical and in a specific condition like ischemic AKI, the targets of a specific miRNA appear to be far

fewer than that predicted by the database. In our study of miR-489, although 129 genes showed mRNA accumulation in miRISC and had miR-489-targeting seed sequence, we analyzed over a dozen of these genes and were only able to verify PARP1 as the actual target of miR-489 in ischemic AKI [9]. Thus, although one miRNA may target hundreds of genes, under a specific condition, its actual target(s) is very limited. Future research needs to pinpoint and validate the target(s) of a specific miRNA experimentally. This is very important for the understanding of the mechanism by which an miRNA regulates AKI. Second, there are a number of miRNA species that change their expression in AKI and thus far, only a few have been studied in terms of their regulation and pathological roles in AKI. What are the functions of other miRNAs? How are they up- or downregulated in AKI? What are their key downstream target genes? In addition, miRNA regulation appears to be quite different in different types of AKI. This is very obvious from our work on ischemic and cisplatin nephrotoxic AKI. While the majority of work published so far focuses on ischemic AKI, very limited information is known for miRNA regulation in other types of AKI such as sepsis-associated AKI. Finally, a major objective of miRNA research in AKI is to identify a new therapeutic strategy. Thus, it is extremely important to translate knowledge into clinical application in future. In this regard, miRNA mimics and anti-miRs are being developed and tested in vivo, providing the basis for clinical application. The overall strategy is to inhibit the pathogenic miRNAs by using specific anti-miRs and to enhance the protective miRNAs by administering their mimics to attenuate kidney injury and promote renal repair.

Conclusions

AKI is associated with changes in the expression of specific miRNAs. While some miRNAs contribute to the pathogenesis and progression of AKI, others may serve as protective molecules. The expression of specific miRNAs is controlled by multiple transcription factors, such as HIF and p53. Upon expression, these miRNAs contribute to AKI by repressing downstream target genes. As a result, targeting specific miRNAs may afford renoprotection against AKI. In addition, specific miRNAs in body fluids, especially those in urine and plasma, have the potential to become next-generation disease biomarkers, which may be more sensitive and specific than the traditional protein-based biomarkers.

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Disclosure Statement

The authors do not have competing financial interests.

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