The Long and Short of MicroRNAs in the Kidney

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ABSTRACT

MicroRNAs (miRNAs) are a group of small, noncoding RNAs that act as novel regulators of gene expression through the post-transcriptional repression of their target mRNAs. miRNAs have been implicated in diverse biologic processes, and it is estimated that up to half of all transcripts are regulated by miRNAs. Recent studies also demonstrate a critical role for miRNAs in renal development, physiology, and pathophysiology. Understanding the function of miRNAs in the kidney may lead to innovative approaches to renal disease.

In 1993, the first microRNA (miRNA), lin-4, was described in Caenorhabditis elegans.1 The phenomenon of a small RNA that repressed gene expression was initially thought of as a unique means of controlling developmental timing in the nematode. Over the past two decades, it has become clear that miRNA-mediated regulation of gene expression also occurs in multiple biologic processes and is conserved from plants to animals; over 19,000 miRNAs have been reported in 153 species (miRBase, version 17).2 Currently, it is estimated that at least half of all transcripts are regulated by miRNAs.3 There are several current, comprehensive reviews of miRNAs and their roles in the kidney.4–10 This article highlights recent novel insights into miRNA function and the implications for miRNAs in renal disease.

miRNAs are small, endogenous, noncoding RNAs that bind to their respective target mRNAs and recruit the RNA-induced silencing complex (RISC) shown in Figure 1. After recruitment of RISC, miRNAs usually decrease expression of their mRNA targets through translational repression, deadenylation, or enhanced mRNA decay.11 Bioinformatic analysis of conserved miRNA target sites suggests that mammalian miRNAs have on average approximately 300 mRNA targets per miRNA family.12 With few exceptions, the key feature of miRNA target recognition is mRNA sequence complementarity to an eight-nucleotide (nt) seed miRNA sequence found in the 3′-untranslated region.13 The sequence context in which the miRNA target site resides confers additional specificity to miRNA-mRNA interactions, and conservation of specific miRNA target sites in mRNAs across species is predictive of biologically relevant miRNA-mRNA interactions.14

Gene regulation mediated by miRNA has distinct features when compared with transcription factor regulatory networks.15 Experimental approaches to identify miRNA targets show that the degree of miRNA repression is relatively modest for individual proteins and that individual miRNAs modulate the expression of hundreds to thousands of proteins.16,17 Another distinguishing feature is the speed and potential reversibility of miRNA repression because miRNAs act at the site of protein production, the ribosome.18 Furthermore, miRNAs distribute to different subcellular compartments according to their association with the site of protein translation.19,20 Broadly speaking, miRNAs fine-tune existing transcriptional programs in magnitude, time, and space.

An additional layer of complexity is conferred in the regulation of miRNA production and function. Much like other genes, transcription of miRNA genes is largely dependent on transcription factors. Recently, candidate miRNA promoters have been systematically identified using chromatin immunoprecipitation–sequencing data for chromatin marks specific to transcriptional initiation sites, and linked to the binding of embryonic stem cell–specific transcription factors.21 This type of information reveals how miRNAs and transcription factors are integrated into gene regulatory networks.15

A large number of miRNAs are also subject to post-transcriptional regulation.22,23 Generally, RNA polymerase II generates the primary miRNA transcript, which is processed by the microprocessor complex in the nucleus to produce stem-loop precursor miRNAs (Figure 1). The
 precursor miRNA is subsequently cleaved by Dicer to form mature functional miRNA. There is evidence that the processing of primary miRNA transcripts and precursor miRNAs is regulated in different cell types, resulting in differential expression of mature miRNAs.22,23 In addition, the primary transcripts of some miRNA genes undergo RNA editing by adenosine deaminases acting on RNAs that convert adenosine to inosine, resulting in changes in the target specificity of the mature miRNA.24,25 Finally, although most miRNAs repress their respective target mRNAs, some miRNAs also activate targets depending on the cellular context.26

The discovery of miRNAs as critical regulators of fundamental biologic processes led to the emergence of a new field of study: the role of miRNAs in normal renal development, physiology, and pathophysiology. Several recent studies demonstrated that miRNAs are essential in specific tissue lineages using a conditional approach to knock down Dicer, which is required for the production of functional miRNAs (Figure 1). In the kidney, conditional Dicer models have been reported for nephron progenitors, ureteric epithelium, podocytes, proximal tubules, and juxtaglomerular cells.27–33 During kidney development, the global loss of miRNAs in nephron progenitors results in a premature depletion of this population and, as a consequence, a marked decrease in nephron number.29,30 This is mediated by increased apoptosis and upregulation of the pro-apoptotic protein Bim in the absence of miRNAs.29 In contrast, removal of Dicer function from the ureteric lineage results in cystic kidney disease in association with aberrant proliferation, apoptosis, and branching morphogenesis.30

Podocyte-specific loss of Dicer activity causes proteinuria, foot process effacement, and glomerulosclerosis with rapid progression to renal failure.27,28,32 Although the initial specification of podocytes occurs normally in these mice, the maintenance of podocyte structure and function requires miRNA function. The inducible deletion of another miRNA processing enzyme, Drosha, in podocytes in 2- to 3-month-old mice also results in a similar phenotype, demonstrating an ongoing need for miRNA activity in mature podocytes.34 Mice with a Dicer deletion in renin-secreting cells in the juxtaglomerular apparatus demonstrate loss of juxtaglomerular cells, striped fibrosis, and vascular abnormalities.31 Interestingly, the loss of miRNAs in the proximal tubule after 3 weeks of age confers resistance to ischemia-reperfusion injury in mice.33

These initial studies provide crucial insights into a functional requirement for miRNAs in multiple cell lineages in the kidney. However, defining biologically relevant miRNA-mRNA target interactions remains a challenge. Experiments using microarray or proteomic analysis after alterations in miRNA expression have been used to develop and validate bioinformatic algorithms that predict target interactions.16,17 These algorithms are hampered by a high false-positive prediction rate and can fail to predict the most biologically important miRNA targets.35 Nevertheless, they remain a powerful tool and are the basis for the identification of several miRNA-mRNA target interactions that have been verified experimentally.

A more recent approach involves high-throughput RNA sequencing of RISC-bound RNA fragments, which allows for the identification of RISC-associated miRNAs and their target mRNAs.36–38 The experimental and bioinformatic approaches to miRNA target identification are becoming more robust as the determinants of miRNA-mRNA interactions are better defined. A further consideration is the experimental observation that miRNAs regulate many hundreds of proteins, often in related signaling pathways. Thus, rather than conceptualizing miRNA function as repression of a critical single transcript, it may be more informative to describe miRNA activity in the modulation of signaling pathways at multiple levels as part of larger regulatory networks.39

The evidence that miRNAs are crucial in normal kidney development and physiology leads naturally to the question of what role miRNAs play in kidney disease. Research over the past several years has focused on the analysis of differential miRNA expression in renal disease and in the study of specific miRNAs that regulate pathologic processes (see recent reviews for a systematic description).4,5,8,10). These studies implicate TGF β regulation of...
miRNA expression in diabetic nephropathy,\textsuperscript{40–43} p53 induction of miR-34a in ischemic acute kidney injury,\textsuperscript{44} and miR-15a regulation of the cell cycle regulator Cdc25A.\textsuperscript{45} The best studied of these is diabetic nephropathy. Natarajan and colleagues observe upregulation of miR-192, miR-216a, and miR-217 in response to TGFβ signaling in a rodent diabetic mouse model and in glomerular mesangial cells.\textsuperscript{41–43} Furthermore, they identified targets, such as SIP1, PTEN, and Ybx1, that may play critical roles in collagen expression and diabetic nephropathy.\textsuperscript{41–43} However, renal biopsies of patients with diabetic nephropathy show significantly lower miR-192 expression; resolving this apparent discrepancy will require further study.\textsuperscript{46} In other studies, miR-335 and miR-43a promote renal cell senescence and aging by suppressing mitochondrial antioxidant enzymes,\textsuperscript{47} and miR-192 mediates WNK1-regulated sodium and potassium balance\textsuperscript{48} and TGFβ-driven fibrosis.\textsuperscript{49} Differential miRNA profiling as a systems biology approach is also being used in a variety of settings in rodent disease models and renal biopsy samples, including acute kidney injury,\textsuperscript{33} polycystic kidney disease,\textsuperscript{50,51} acute rejection,\textsuperscript{52,53} renal cell carcinoma,\textsuperscript{54,55} lupus nephritis,\textsuperscript{56} and IgA nephropathy.\textsuperscript{57} Thus, investigators are only beginning to elucidate the pathologic mechanisms that are regulated by miRNAs during kidney disease.

What does all this mean for patients with kidney disease? With the emerging data associating miRNA expression patterns to different stages of renal disease, one application is the use of miRNAs as novel biomarkers for diagnostic and prognostic purposes. One of the distinguishing features of miRNAs is their stability—indeed, one recent study suggests their average half-life may be approximately 5 days.\textsuperscript{58} Furthermore, miRNAs are transported in the plasma\textsuperscript{59} and can be isolated from urine,\textsuperscript{60,64} increasing their potential utility as biomarkers.

miRNAs also have significant promise as novel drug targets, particularly given the rapidly increasing knowledge about miRNA regulatory networks in renal pathophysiology. Several experimental approaches to modulate miRNA activity \textit{in vivo} are in development. Chemically engineered oligonucleotides, termed antagonirs, successfully target endogenous miRNAs in mammals and can target miRNAs in the kidney.\textsuperscript{10,62} Alternatively, locked nucleic acid–modified oligonucleotides have been used in a nonhuman primate model with chronic hepatitis C virus infection to repress miR-122.\textsuperscript{63} Other approaches include the development of miRNA sponges, in which miRNA-binding sites are stably introduced into the genome to sequester endogenous miRNAs, or the introduction of oligonucleotide target maskers that protect miRNA targets against miRNA-mediated repression.\textsuperscript{10,64}

There has been an incredible explosion of information regarding miRNAs since their initial discovery less than two decades ago, and there remains much to learn about miRNA-mediated regulation of normal and abnormal kidney function. Although many challenges remain, understanding the role of miRNAs in renal pathophysiology offers the hope of innovative approaches to novel therapies for renal diseases.

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DISCLOSURES

None.

REFERENCES

SCIENCE IN RENAL MEDICINE


