microRNAs in kidneys: biogenesis, regulation, and pathophysiologcal roles

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Bhatt K, Mi Q, Dong Z. microRNAs in kidneys: biogenesis, regulation, and pathophysiological roles. Am J Physiol Renal Physiol 300: F602–F610, 2011. First published January 12, 2011; doi:10.1152/ajprenal.00727.2010.—MicroRNAs (miRNAs) are endogenously produced, short RNAs that repress and thus regulate the expression of almost half of known protein-coding genes. miRNA-mediated gene repression is an important regulatory mechanism to modulate fundamental cellular processes such as the cell cycle, growth, proliferation, phenotype, and death, which in turn have major influences on pathophysiological outcomes. In kidneys, miRNAs are indispensable for renal development and homeostasis. Emerging evidence has further pinpointed the pathogenic roles played by miRNAs in major renal diseases, including diabetic nephropathy, acute kidney injury, renal carcinoma, polycystic kidney disease, and others. Although the field of renal miRNA research is still in its infancy and important questions remain, future investigation on miRNA regulation in kidneys has the potential to revolutionize both the diagnosis and treatment of major renal diseases.

Dicer; diabetic nephropathy; acute kidney injury; renal cell carcinoma; allograft rejection

MicroRNAs (miRNAs) are endogenously produced, short RNAs of 21–25 nucleotides that are important regulators of gene expression at the posttranscriptional level (4, 12, 52). By binding to the 3'-untranslated region (UTR) of the target gene mRNAs, miRNAs can induce mRNA degradation or, more frequently, result in repression of protein translation (18). miRNAs are produced by gene transcription followed by sequential processing or editing. A few hundred miRNAs have been identified in various organisms, and current estimates indicate that these miRNAs may regulate almost half of protein-coding genes (10). By regulating gene expression, miRNAs play critical roles in a variety of cellular and physiological activities (32). In human diseases, miRNA expression is frequently altered, contributing to pathogenesis (14, 15, 20, 22, 30, 37, 65, 75, 76, 90).

The role of miRNAs in the regulation of renal development, physiology, and pathology has emerged as an important and potentially fruitful area of research (47, 48, 72). The identification and characterization of the miRNAs in various renal diseases may lead to breakthroughs in the development of novel diagnostic tools and therapeutic interventions. The research field of miRNA regulation in kidneys and renal diseases is still in its infancy, and despite its enormous potential, significant roadblocks remain. In this review, we aim to discuss the fundamental aspects of miRNA biogenesis and regulation, analyze the roles of miRNAs in renal pathophysiology, and identify future directions of research in this emerging field.

Biogenesis, Function, and Regulation of miRNAs

Bioinformatic analysis coupled with experimental validation has lead to the estimation that most animals have a few hundred miRNA-coding genes (12, 52). Humans are predicted to have ~800 miRNA genes, although many of them have yet to be validated experimentally (18, 34). The miRNA coding regions are present in the genome as independent genes or are present in the introns of protein-coding genes (12, 52, 66). The miRNA genes are transcribed, leading to the production of large transcripts (pri-miRNAs), which are then processed and edited, resulting in the formation of mature, functional miRNAs of 21–25 nucleotides. miRNAs are finally loaded onto a multiprotein complex that mediates mRNA degradation or translational repression (12, 26, 34, 95).

Transcription of miRNA genes is the first step in the biogenesis of miRNAs (52). RNA polymerase II is usually responsible for the transcription, although a minor group of miRNA genes can also be transcribed via RNA polymerase III (18). The transcription generally results in primary transcripts (called pri-miRNAs) that are several kilobases in length. Notably, these large transcripts have well-defined hairpin structures, which direct the pri-miRNAs to a multiprotein complex called the Microprocessor complex in the nucleus (6, 12, 26, 52). The Microprocessor complex contains two major proteins, Drosha (an RNase III-like protein) and its cofactor DiGeorge syndrome critical region gene 8 (DGC8R8). DGC8R8 recognizes and interacts with the hairpin structure in the pri-miRNAs and recruits Drosha. Drosha then cleaves pri-miRNAs precisely at the stem-loop structure, leading to the production of secondary precursor RNAs of ~70-nucleotides, called pre-miRNAs (52). The pre-miRNAs are then exported to the cytoplasm by ex-
portin 5, a nuclear transport receptor family member that recognizes the stem-loop structure of pre-miRNAs (12, 27, 52).

In the cytoplasm, the pre-miRNAs are further cleaved to release the mature miRNA of 21–25 nucleotides (12, 27). This cleavage involves a multiprotein complex, whose major component is Dicer, an RNase III type enzyme. Dicer is a large (~200 kDa) protein that is remarkably conserved in almost all eukaryotic organisms. Although Dicer is directly involved in the cleavage of pre-miRNA to mature miRNA, its activity is thought to be modulated by other associated proteins such as TAR RNA-binding protein (TRBP) and PACT (52). Of note, in addition to its involvement in mature miRNA generation, the Dicer-containing protein complex also regulates the assembly of mature miRNA into the effector complex (12, 27, 52).

The miRNA effector complex is also called the RNA-induced silencing complex or RISC, which includes argonaute (AGO) proteins, the catalytic enzymes with endonuclease activity that are responsible for target mRNA degradation (52). Most organisms have multiple AGO proteins with sometimes distinct biological functions. However, in humans the four AGO proteins, AGO1–4, have overlapping roles in RISC formation and mRNA silencing (12, 27, 52). As depicted in Fig. 1, mature miRNA is loaded onto RISC. Then, one of the miRNA strands (the passenger strand) is degraded, while the other strand (the guide strand) complexes with AGO to form an active RISC, which is directed to the target mRNA based on the complementary sequences in the miRNA and mRNA. It is noteworthy that the AGO proteins possess robust endonuclease activity to degrade the target mRNA when a perfect complementary miRNA is present; however, the endonuclease activity of AGO is not always essential for RISC formation and translational repression of target mRNAs (10–12, 18, 27, 52).

miRNAs can repress target gene expression by either inducing mRNA degradation or by blocking protein translation; nevertheless, the latter mechanism is much more common in mammals (10, 11, 18, 52). It remains unclear as to how the binding of RISC (AGO/miRNA complex) to the 3′-UTR of target mRNA prevents protein translation. Several possibilities have been postulated: 1) deadenylation of the polyadenylated 3′-end may result in mRNA degradation; 2) the AGO-miRNA complex may compete with translation initiation factors, resulting in reduced translation initiation; 3) binding of the AGO-miRNA complex may induce premature termination and impaired elongation; and 4) the AGO-miRNA complex may recruit peptidases to degrade the growing polypeptide during translation (10, 11, 18).

The amount of a specific miRNA is determined by its biogenesis and decay. miRNA biogenesis is critically regulated at the level of gene transcription (52). Many of the miRNA genes are flanked by promoter regions that are very similar to those of protein-coding genes (12, 18, 52). The promoter activity of miRNA genes is governed by specific transcription factors like MYC, p53, and hypoxia-inducible factor 1 (HIF1α) (21, 25, 80). As a result, under conditions of genotoxic stress where p53 is activated and during hypoxia where HIF1α is activated, miRNAs such as miR-34 and miR-210 are upregulated, respectively, in a p53- or HIF1α-dependent manner (39, 60). These observations suggest that the expression of miRNAs

![Diagram of miRNA biogenesis and function](http://ajprenal.physiology.org/)

Fig. 1. Biogenesis and function of microRNAs (miRNA). Most of miRNA genes are transcribed via RNA polymerase II, leading to the generation of pri-miRNAs that are long transcripts with multiple hairpin loop structures. Pri-miRNAs are processed by the Microprocessor protein complex containing Drosha, resulting in the generation of smaller precursor molecules called pre-miRNAs. Pre-miRNAs are then exported from the nucleus via exportin-5. In the cytoplasm, the pre-miRNAs are further processed via Dicer to generate short, double-stranded miRNAs, which are then converted to mature, single-stranded miRNAs via RISC, a protein complex containing argonaute (AGO). Finally, mature miRNAs direct RISC to target gene mRNA, resulting in mRNA degradation (miRNA finds perfect complementary sequences in mRNA) or translational repression (imperfect complementary sequences in mRNA).
is regulated at the gene transcription level in response to pathophysiological challenges. In addition, global miRNA expression can also be modulated by regulating the proteins or enzymes involved in miRNA processing, e.g., Drosha, Dicer, and AGO (52). miRNAs are also regulated via decay or degradation. It has been recognized that miRNAs can rapidly rise and then decrease under various cellular conditions, although the molecular machinery responsible for miRNA degradation is largely unknown (52).

miRNAs in Renal Pathophysiology

Renal diseases, including progressive kidney disease and acute kidney injury (AKI), are associated with high mortality and morbidity rates with very few effective treatment options. To identify novel treatment modalities, research in recent years has been focused on two major aspects: 1) new and more sensitive biomarkers for early diagnosis, and 2) pathogenic molecular targets or pathways that can be specifically targeted for therapy. The discovery of miRNAs as critical regulators of the fundamental activities of a cell, from proliferation and differentiation to apoptosis, suggests the involvement of miRNAs in the pathogenesis of various diseases including those of the kidneys. Indeed, research during the last couple of years has unveiled an emerging role of miRNAs in both chronic and acute kidney diseases. These studies fall into three general categories: 1) global depletion of miRNAs from specific cell types in kidneys by using conditional Dicer-knockout mouse models; 2) analysis of differential miRNA expression in renal diseases to identify potential pathogenic miRNA species; and 3) study of miRNA regulation of specific genes that play pathogenic roles in renal disease.

Dicer-knockout studies implicating miRNAs in renal pathophysiology. Dicer is the enzyme responsible for the processing of pre-miRNAs into mature, functional miRNAs. As a result, genetic ablation of Dicer leads to global depletion of miRNAs (52). Germline knockout of Dicer results in embryonic lethality in mice, underscoring the critical role played by miRNAs in normal development (12, 52). Recently, tissue-specific deletion of Dicer has been employed as a strategy to study the effect of global miRNA repression in different organ systems (42). In kidneys, conditional knockout of Dicer has thus far been reported in podocytes (38, 41, 77), proximal tubules (92), and juxtaglomerular cells (74). Studies using these conditional knockout models have demonstrated striking phenotypes, providing compelling evidence for the involvement of miRNAs not only in kidney development and maintenance of normal renal function but also in the pathogenesis of renal diseases.

The podocyte-specific Dicer knockout model was reported in 2008 separately by three research groups (38, 41, 77). Notably, their findings are fairly consistent (38, 41, 77). Dicer ablation from podocytes results in proteinuria, tubulointerstitial fibrosis, glomerulosclerosis, and foot process effacement at 2–4 wk after birth. Remarkably, the defects rapidly progress into end-stage renal diseases in about 2 mo, resulting in increased mortality (42). The pathological abnormalities shown in the mouse model clearly suggest that Dicer is critical for the maintenance of podocyte homeostasis and associated renal functions. Mechanistically, it is suggested that the loss of miR-30 family miRNAs from podocytes may be responsible for the observed phenotype in the conditional Dicer-knockout model (38, 41, 42, 77). In line with this possibility, a recent study has demonstrated that the miR-30 family is a critical regulator of pronephric renal development in Xenopus laevis (1). Thus the miR-30 family miRNAs may contribute to the maintenance of the homoeostasis and function of podocytes in kidneys.

Sequeira-Lopez and colleagues (74) have recently established a mouse model with Dicer deletion in the renin-secreting juxtaglomerular cells. Mice in this model show an acute loss of juxtaglomerular cells as evidenced by histological, biochemical, and physiological analyses. As a result, renin expression in kidneys and plasma renin concentration are markedly reduced. Functionally, these changes manifest in the form of a significant decrease in blood pressure. Renal histology of the mice shows prominent striped fibrosis and vascular abnormalities. The latest work by Nagalakshmi et al. (62) further showed that when Dicer is specifically ablated from the progenitors of nephron epithelium, nephrogenesis is abrogated, underlying the critical role played by Dicer and miRNAs in kidney development. These results, along with the podocyte Dicer-knockout studies, suggest that Dicer and associated miRNA production are indispensable for normal structure and proper function of renal cells in kidneys.

We have established a conditional knockout mouse model in which Dicer is specifically ablated from the proximal tubular cells in kidneys (92). In this model, there is a global depletion of miRNAs from renal cortical tissues. Somewhat surprisingly, mice from this model show normal kidney size, histology, and function. The absence of renal phenotypes is most likely due to the late turn-on feature of the PEPCK promoter that drives Cre expression in proximal tubules in this conditional knockout model. In kidneys, the PEPCK promoter is not activated in proximal tubular cells until 3 wk after birth, a time point when kidney development has been mostly completed in mice. While the results do not rule in or out a role of miRNAs in kidney development, it is suggested that global depletion of miRNAs does not markedly affect the normal function and physiology of differentiated proximal tubules in adult mice. Strikingly, although these mice do not show phenotypes under normal physiological conditions, they are remarkably resistant to ischemia-reperfusion-induced kidney injury (see below for further discussion) (92).

These conditional Dicer-knockout studies indicate that miRNAs play critical roles in normal renal function or physiology and, when altered, may lead to renal diseases. Nonetheless, it is important to point out that, while global depletion of miRNAs in kidneys may result in renal diseases, it is unlikely for this to occur in humans. It is more likely that specific miRNAs are differentially regulated in renal diseases and contribute to their pathogenesis. If identified, these miRNAs could be used as either diagnostic tools or molecular targets for the prevention and therapy of the disease.

miRNA regulation in diabetic nephropathy. Diabetic nephropathy leads to chronic renal failure and accounts for almost half the cases of end-stage renal disease (48). The disease is characterized by profound changes in renal vasculature accompanied by increased arterial blood pressure, reduced glomerular filtration rate (GFR), and most notably, proteinuria. Most of the pathological changes observed during diabetic nephropathy are associated with increased extracellular depo-
sitions in the glomerulus. Mesangial expansion as a result of accumulation of extracellular matrix and thickening of the glomerular basement membrane (GBM) is a key pathological feature of diabetic nephropathy (48). Although the exact molecular mechanism responsible for these pathological changes remains unclear, numerous studies have suggested the involvement of transforming growth factor-β (TGF-β), which may induce pathogenic collagen synthesis and cellular hypertrophy (48). Consistently, high levels of TGF-β are detected in the glomeruli of diabetic patients. Interestingly, recent studies have demonstrated compelling evidence for TGF-β regulation of miRNAs and its pathogenic role in diabetic nephropathy. In one of the first studies implicating miRNA regulation in renal diseases, Natarajan et al. (51) have identified miR-192 as a critical regulator of collagen production in diabetic nephropathy. In cultured mesangial cells and diabetic kidneys, miR-192 is induced via TGF-β and following induction, miR-192 targets the E-box repressor protein smad-interacting protein 1 (SIP1). Downregulation of SIP1 by miR-192 leads to the relief of the repression of collagen gene expression, resulting in increased collagen production and deposition in the mesangium (51), a hallmark of diabetic nephropathy. A more recent study by Chung et al. (19) has further suggested a role of miR-192 in renal fibrosis. Interestingly, in renal fibrosis models, miR-192 is also induced in a TGF-β-dependent manner, resulting in collagen production (19). In addition to miR-192, TGF-β also regulates the expression of miR-216a and miR-217 in mesangial cells during diabetic nephropathy (49, 50). Notably, these two miRNAs target phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and due to PTEN repression, Akt, a key mediator of diabetic nephropathy, is activated. The latest work by Natarajan and colleagues (50) has further identified the RNA binding protein Ybx1 as a target of miR-216a during TGF-β-induced collagen expression in kidney cells (50). Together, these studies have established a regulatory role of specific miRNAs in TGF-β-dependent renal pathologies observed during diabetic nephropathy.

Are these miRNAs involved during diabetic nephropathy in humans? To answer this question, a recent study examined human renal biopsy samples from patients with established diabetic nephropathy (55). However, the findings are quite surprising and apparently contradictory to the results from diabetic mouse models. In contrast to the observations in diabetic mice, renal biopsy samples from diabetic patients show significantly lower miR-192 (55). Moreover, the decreased expression of miR-192 seems to directly correlate with tubulointerstitial fibrosis and a low GFR in individual patients. In addition, TGF-β suppresses miR-192 expression in cultured proximal tubular cells in this study. As suggested, TGF-β may regulate miR-192 differently in mesangial vs. proximal tubular cells (55). However, this explanation is challenged by the observations of both up- and downregulation of miR-192 by TGF-β in the same NRK52E renal tubular cell line (19, 85). Downregulation of miR-192 by TGF-β has also been shown in mesangial cells and apoe-knockout diabetic mouse kidneys. The cause of the discrepancies in the results from these studies is currently unclear. A recent study by Kriegel and colleagues (54) has further revealed miR-382 induction by TGF-β in human renal epithelial cells. A direct target of miR-382 is identified to be superoxide dismutase 2 (SOD2). Interestingly, blocking miR-382 or overexpressing SOD2 leads to E-cadherin expression, suggesting that miR-382 is involved in the phenotypic change in the cells during renal fibrosis in diabetic kidneys (54).

In addition to TGF-β induction and diabetic mouse models, miRNA expression has been examined during high-glucose or hyperglycemic treatment of cultured renal cells. While no significant changes in miRNA expression are detected during high-glucose incubation of HK2 human proximal tubular cells (55), high-glucose induces miR-377, -129, and -337 in cultured human and mouse mesangial cells (91). The molecular targets of miR-377 are further identified as p21-activated kinase (PAK1) and manganese superoxide dismutase (mnSOD), which when inhibited resulted in fibronectin expression, a characteristic of mesangial cells during diabetic nephropathy. A very recent study by Long and colleagues (59) has systematically analyzed miRNA expression in high glucose-treated podocytes and kidney microvascular endothelial cells and glomeruli of diabetic db/db mice. While a number of miRNAs show expression changes under these conditions, miR-93 is decreased in all samples. Importantly, follow-up studies demonstrate that miR-93 targets vascular endothelial growth factor (VEGF) (59), a crucial regulator of microvascular complications in diabetes. These results raise the possibility that increased renal VEGF expression in animal models of diabetes could be at least partially attributed to decreased miR-93 expression. Together, these findings have suggested the involvement of miRNAs in diabetic nephropathy, uncovering novel therapeutic targets for this devastating disease.

miRNA regulation in AKI. AKI is a major kidney disease associated with high mortality and morbidity. Notably, it has been recently recognized that AKI is associated with increased risk of chronic kidney disease (CKD) and may be a key contributing factor in CKD (83). Despite decades of investigation, effective therapeutic approaches for AKI are still lacking. Recent studies have unveiled the role and regulation of miRNAs in AKI, raising hopes for novel and effective diagnostic and therapeutic strategies.

The first evidence for a pathogenic role of miRNAs in AKI was demonstrated by using a conditional Dicer-knockout model, in which Dicer was ablated specifically from renal proximal tubular cells in kidneys (92). Mice in this model show normal kidney development, histology, and function. However, when challenged by bilateral renal ischemia-reperfusion, the conditional Dicer-null mice are remarkably resistant to the ensuing AKI compared with their wild-type littermates (92). Better renal function and histology as well as significantly improved animal survival of Dicer-deficient mice provides compelling evidence for a pathogenic role of Dicer and associated miRNAs in ischemic AKI.

Then, which miRNAs may contribute critically to ischemic AKI? To address this question, renal cortical tissues collected from C57BL/6 mice at various time points following bilateral renal ischemia were analyzed by miRNA microarray (92). The microarray analysis has revealed significant expression changes in multiple miRNAs following renal ischemia-reperfusion. Interestingly, while some miRNAs are induced, others are downregulated in injured tissues. In addition, while some miRNAs change only at one time point, other miRNA species (e.g., miRNA-132, -362, -379, -668, and -687) show a continuous change during 12–48 h of reperfusion (92). By miRNA microarray, a recent study by Godwin and colleagues (35) also...
demonstrates miRNA expression changes during renal ischemia-reperfusion in C57BL/6 mice. However, the miRNA species that show significant changes in these two studies (35, 92) do not overlap. Although the exact cause of the discrepancy in the results from these two studies is not entirely clear, several major differences between the experimental models and analysis are noted. First, our study used a bilateral renal ischemia-reperfusion model, whereas Godwin et al. (35) used a unilateral model. There are notable differences between these two models. The bilateral model induces more severe AKI following the same ischemic duration and marked renal functional loss, which is undetectable in the unilateral model. In addition, the unilateral model is complicated by the compensatory response in the contralateral “control” kidney that is not subjected to ischemic injury. Second, we profiled miRNA expression at the acute (12 and 48 h of reperfusion) phase of AKI, while Godwin et al. covered late time points, up to 30 days postischemia. Finally, we analyzed renal cortical tissues, while Godwin et al. used RNA samples extracted from whole kidneys, including papillary, medullary, and cortical tissues.

In another recent study, we have specifically examined miR-34a expression and regulation in the AKI model of cisplatin nephrotoxicity (7). Cisplatin is a widely used cancer therapy drug, which has notable side effects in normal tissues, especially the kidneys. One of the major pathways leading to tubular cell injury and death in cisplatin nephrotoxicity involves a rapid DNA damage response and activation of the tumor suppressor protein p53 (67, 68, 71). We show that miR-34a is induced during cisplatin treatment of renal tubular cells in vitro and mouse kidneys in vivo (7). Interestingly, the inductive response is abrogated by pifithrin-α (a p53 inhibitor) and in p53-deficient mice. Surprisingly, inhibition of miR-34a results in increased apoptosis and reduced cell survival. Together, the results demonstrate a p53-dependent induction of miR-34a, which may play a protective role during cisplatin nephrotoxicity (7). By profiling of miRNA expression and analysis of specific miRNAs, future studies are expected to reveal miRNA changes in different models of AKI, resulting in the identification of potential targets for therapeutic intervention.

miRNA regulation in polycystic kidney disease. Polycystic kidney diseases (PKD) are by far the most common genetically inherited renal diseases (33). The diseases are frequently associated with the mutation of specific genes, including PKD1, PKD2, and PKHD1. It is generally believed that dysregulated expression of these genes leads to abnormal cell division and proliferation, resulting in cyst formation in the kidneys (33). The role of miRNAs in PKD has only been recently implicated, mainly by two important findings. First, the PKD genes are targets of specific miRNAs, indicating a direct regulation of PKD gene expression and cystogenesis by miRNAs. Second, the overall miRNA expression pattern is changed in PKD models, suggesting that miRNAs may also act downstream of PKDs to affect disease progression. Translational repression of PKD2 by miR-17 has been reported by two groups (79, 81). Functionally, ectopic overexpression of miR-17 can promote cell proliferation in HEK cells by targeting PKD2 (79). In line with these findings, transgenic mice expressing artificial miRNAs targeting PKD1 develop PKD (86). In a rat model of PKD, 30 miRNAs are differentially expressed (mostly downregulated) in diseased tissues compared with normal animal tissues (69). Interestingly, bioinformatic analysis suggests that these miRNAs may target genes directly involved in the regulation of cell proliferation and cyst formation (69), a possibility that needs to be verified experimentally. A miRNA that may indeed link cell cycle regulation and PKD is miR-15a. In a rat PKD model and PKD patients, miR-15a is downregulated in cystic liver tissues. Moreover, ectopic expression of miR-15a can reduce cyst formation in an in vitro model. One target of miR-15a may be Cdc25A, a key cell cycle regulator. The results suggest that reduced miR-15a expression in PKD may contribute to hepatic cystogenesis by increasing Cdc25A expression and consequent cell proliferation (56). These studies support a profound role of miRNAs in PKD and related proliferative renal diseases.

miRNAs in renal allograft rejection. Acute rejection and chronic allograft nephropathy are responsible for renal failure during kidney transplantation (9, 61). Using renal biopsies from patients with established acute rejection of transplanted kidneys, two studies have shed some light on the role of miRNAs in this pathological condition. In the first study, 8 miRNAs are upregulated and 12 are downregulated in the renal biopsies from acute rejection patients, compared with those of normal patients (78). Although the functional significance of the changes has not been studied, the differentially expressed miRNAs seem to have potential targets that may affect various processes during renal transplantation. In the second study, 17 miRNAs were found to be differentially expressed in acute rejection biopsies (3). Notably, there is a strong correlation between the expression of a subset of miRNAs (e.g., miR-142–5p and miR-155) and acute rejection, suggesting that these miRNAs can be used as predictive markers for renal allograft function and rejection. In addition, this study raises the interesting possibility that some of the differentially regulated miRNAs could be from an extrarenal source, most probably immunological cells (3). Despite these reports, whether and how the differentially expressed miRNAs contribute to allograft rejection remains to be investigated.

miRNAs in kidney cancer. Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults. Changes in miRNA expression in RCC have been documented extensively, although the reported patterns are not very consistent (16, 17, 36, 40, 43–45, 63, 70, 93, 97). RCC is frequently associated with inactivation of the von Hippel-Lindau (VHL) tumor suppressor, resulting in elevated levels of hypoxia-inducible transcription factors (HIF) (46). Thus some of the miRNA changes observed in RCC may well be HIF dependent. In line with this idea, the hypoxia-dependent miRNA, miR-210, is upregulated in several types of tumors, including RCC (64), and may be a prognostic factor for cancer patients (60). Of note, Neal and colleagues (64) have shown recently that VHL may regulate miRNAs in both HIF-dependent and -independent manners. Compared with adjacent normal tissues, there is a significantly higher expression of miR-210, miR-155, and miR-21 in clear cell RCC tissues. While miR-210 expression correlates with VHL inactivation and HIF induction, several other miRNAs (e.g., miR-21) are not expressed in response to HIF activation, suggesting HIF-independent regulation of these miRNAs. On the other hand, it is also important to recognize that some miRNAs are oncogenic and can act upstream of VHL, HIF, and other key factors in RCC to promote tumorigenesis (94).
In addition to RCC, recent research has also suggested the involvement of miRNAs in Wilms’ tumor, a childhood kidney cancer. Notably, Wilms’ tumor seems to involve the regulation of oncomir-1 (an oncogenic cluster of miRNAs located on chromosome 13) by the transcription factor E2F3 (53). By profiling of both miRNA and miRNA expression, Kort and colleagues (53) detected the highest oncomir-1 family expression in Wilms’ tumors among the kidney tumor types analyzed. Interestingly, in tumor tissues oncomir-1 expression is paralleled by the expression of E2F3 (53), a transcription factor that has been implicated in governing oncomir-1 expression (96). Moreover, recent studies have further detected decreased expression of miR-562 and -185, and increased expression of miR-483–3p in Wilms’ tumors, and suggested their potential involvement in tumorigenesis (29, 84). The roles played by these and other miRNAs in renal cancer development, progression and treatment are being intensively investigated.

miRNAs in other renal diseases. Preliminary evidence has also demonstrated differential miRNA expression in other renal diseases, including lupus nephritis, IgA nephropathy, and hypertensive renal injury. In renal biopsy samples from class II lupus nephritis patients, 66 miRNAs show significant changes in miRNA microarray analysis compared with normal kidney samples (23). A more recent study using peripheral blood mononuclear cells and Epstein-Barr virus-transformed cell lines derived from lupus nephritis patients has further identified five miRNAs (miR-371–5P, -423–5P, -638, -1224–3P, -663) that are differentially expressed in lupus nephritis across different racial groups. In IgA nephropathy, 35 miRNAs (of 132 miRNAs detected) are shown to be differentially expressed in renal biopsy samples. Interestingly, Wang and colleagues (87) have recently detected the expression of miR-200c, -141, -205, and -192 in renal biopsy of IgA nephropathy patients, correlating with the severity and progression of the disease. The same investigators also demonstrated increased expression of several miRNAs (miR-200a, -200b, -141, -205, and miR-192) in hypertensive sclerosis of kidneys (88). It is important to note that despite these reports, the functional significance of the miRNAs in these renal diseases remains to be delineated. In this regard, the latest study by Liang and his laboratory (58) has suggested a protective role of miR-29b in renal injury during high salt-induced hypertension. miR-29b is upregulated in renal medullary tissues in Dahl salt-sensitive rats fed a high-salt diet. Importantly, knockdown of miR-29b results in expression of several collagen genes in the kidneys. In cultured renal medullary epithelial cells, miR-29b can suppress collagen expression. These findings demonstrate a negative regulatory role of miR-29b in collagen and extracellular matrix accumulation in hypertensive renal injury. Obviously, one of the major areas of emphasis and technical challenge in future investigations is identifying the functional significance of individual miRNAs in specific renal diseases.

miRNAs as diagnostic tools in renal diseases. In the last few years, there has been a major thrust to identify novel and reliable biomarkers for renal diseases (8, 28, 82). As discussed above, miRNA studies in various renal diseases have shown not only that miRNA expression is differentially regulated but also that the expression pattern itself could be a useful tool for disease diagnosis. Multiple studies, especially in the cancer diagnostic field, have provided proof of principle that microRNA expression can be used as a useful diagnostic tool (5, 13, 31, 57, 61, 73). The relatively consistent changes of miRNAs in diseases, reliable methods of miRNA analysis, tissue-specific expression patterns, and their presence in blood and urine make miRNAs ideal candidates as renal disease biomarkers.

In 2007, Gottardo and colleagues (36) reported the upregulation of four miRNAs (miR-28, -185, -27, and -let-7f-2) in RCC tissues. However, more recent studies by Huang and colleagues (44) compared the miRNA expression profiles in clear cell RCC and normal kidney tissues and detected changes in a much broader range of miRNAs. Apparently, these miRNA microarray analyses did not show consistent results, and more systematic profiling is required to identify a miRNA expression pattern that is applicable as a useful diagnostic tool. Nevertheless, these studies have suggested the potential use of miRNAs for RCC diagnosis and stage classification. miRNA expression has also been proposed to be a diagnostic marker for IgA nephropathy and lupus nephritis (23, 24). For example, by miRNA microarray analysis Dai and colleagues (24) identified 35 miRNAs that were upregulated in renal biopsy of IgA nephropathy patients. A more recent study further showed specific changes in several miRNAs in the urinary sediment of IgA nephropathy patients (89). Diagnostic use of miRNAs has also been suggested in acute renal allograft rejection in human patients. The Suthanthiran laboratory (3) demonstrated a distinguished intragraft miRNA profile of human allografts from patients with acute rejection. Moreover, the miRNA profile appears predictive of renal allograft function. The strong association between intragraft miRNA expression and renal allograft function or acute rejection indicates that miRNAs may serve as biomarkers of human renal allograft status.

Strategies and Challenges in Renal miRNA Research

A broad strategy to identify and study miRNA regulation in renal physiology and pathology is described in Fig. 2. The general experimental strategy can be roughly divided into three parts: identification and characterization of miRNAs, delineation of miRNA target genes, and determination of the functional significance of miRNA regulation. These experimental strategies have been widely used to identify miRNA regulation in renal and other pathophysiological conditions.

As fascinating as it is, the research of miRNA in renal pathophysiology is highly challenging. In addition to the general research strategy discussed above, technical issues are frequently encountered. In the initial phase of research, miRNA expression changes have to be carefully verified. As discussed earlier, microarray analysis needs to be repeated with samples from separate experiments for better identification of differentially regulated miRNAs. Moreover, the miRNAs identified from the microarray analysis as pertinent to the study need to be subjected to further verification individually by real-time PCR analysis and Northern blotting. It is also noteworthy that the real-time PCR technique used for miRNA detection is not well standardized in general, and as a result inconsistent and sometimes erroneous results (noise) may be generated. It is therefore useful to confirm the real-time PCR results using other techniques like Northern blot analysis. However, probably, the most critical and
A challenging step is to predict and then verify the miRNA target genes. Multiple bioinformatic programs or databases are available that generally predict several potential target genes for a particular miRNA (2). Usually, the target prediction softwares offer broad sensitivity and specificity, generating numerous putative target genes of a specific miRNA (10). Thus biologically significant target gene identification that includes shortlisting and experimental verification of these targets can be a very tedious, subjective, and challenging process.

Kidneys are composed of different types of cells which may be differentially affected in various renal diseases. To understand the pathophysiological role played by a miRNA, it would be necessary to identify the cell type(s) that expresses the miRNA in a particular disease condition. The major tools used to study miRNAs are miRNA mimics and antisense oligonucleotides to overexpress or inhibit the function of a particular miRNA. These oligonucleotides are sometimes difficult to introduce in certain cell lines with low transfection efficiencies and may have some off-target or nonspecific effects when used in the in vivo experimental models. Transgenic animal models with conditional overexpression or knockdown of a particular miRNA might provide the best model to study the function and regulation of miRNA in renal diseases. Once the target protein/s has been experimentally validated, it is very important to identify its functional significance and its contribution to the pathophysiological condition. Identifying the functional significance of a change in miRNA regulation and its target genes under a certain condition can be very difficult because a single miRNA can target several proteins and a single protein can be targeted by several miRNAs.

As a final note, although miRNA regulation of renal diseases is an exciting emerging field of research, we should not be inundated by the information from numerous studies identifying miRNAs differentially regulated in various disease conditions. It is important to generate a coherent picture of the miRNAs, their targets, alterations in renal diseases, and pathophysiological roles, which provides a comprehensive view of the pathogenesis and hopefully leads to the development of novel therapeutics.

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**DISCLOSURES**

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REFERENCES


