Mini-review: emerging roles of microRNAs in the pathophysiology of renal diseases

Kirti Bhatt, Mitsuo Kato, and Rama Natarajan

Department of Diabetes Complications and Metabolism, Diabetes and Metabolic Research Institute, Beckman Research Institute of the City of Hope, Duarte, California

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MICRORNAS (miRNAs/MIRNS) ARE a unique class of short (~22 nucleotides) single-stranded endogenous noncoding RNAs (7, 18). miRNAs contain nucleotide sequences complementary to binding sites on the 3′-untranslated regions (3′-UTRs) of protein-coding messenger RNA (mRNA) transcripts. Through direct binding to the mRNA 3′-UTRs, miRNAs can induce repression of target gene and protein expression (56). Individual miRNAs can often engage numerous target mRNAs, thereby influencing several components of a single or multiple cellular pathways (3). By regulating and modulating gene expression via posttranscriptional mechanisms, miRNAs are now recognized as critical players in a variety of developmental, physiological, and pathological conditions.

miRNA biogenesis, processing, and actions occur by discrete mechanisms (44, 56, 66). miRNA genes are located within various genomic contexts, including introns of noncoding or coding transcripts as well as exonic regions. miRNAs are transcribed by RNA polymerase II as long precursor RNAs, called primary miRNAs (pri-miRNAs), which are sequentially processed by key nucleases in the nucleus and cytoplasm to generate mature miRNAs (44). The initial pri-miRNA transcript contains a local stem-loop structure harboring the mature miRNA sequence. Several extensive maturation steps are required to further process the pri-miRNA transcript, leading to the formation of mature miRNA. Initially, within the nucleus, the microprocessor complex, which includes RNase III Drosha and DiGeorge syndrome chromosomal region 8 (DGCR8) crops the stem-loop in the pri-miRNA, which releases a small hairpin-shaped RNA of ~65 nucleotides in length, called pre-miRNA. This pre-miRNA is then exported into the cytoplasm by a multiprotein transport complex, which includes exportin 5 and GTP-binding nuclear protein RAN-GTP. In the cytoplasm, pre-miRNA is cleaved by the RNase III-type endonuclease Dicer, which liberates a small RNA duplex. The resultant RNA duplex is subsequently loaded onto argonaute proteins (AGO1-4) in the RNA-induced silencing complex (RISC) machinery, forming a site where the “passenger” strand is degraded while the mature microRNA (miRNA) remains in complex with the AGO proteins. Besides this canonical biogenesis pathway, various alternative mechanisms can also generate miRNAs (44). These biogenesis pathways have been described in depth in several recent review articles (44, 81, 161). Once processed and loaded into the silencing complex, the mature miRNAs pair with target mRNAs, leading to direct translational repression, mRNA destabilization, or a combination of the two.

More than 2,000 miRNA genes have been identified in humans, which are thought to regulate a high percentage of protein-coding transcripts (3). Small variations in the levels of miRNAs can have major cellular effects, and alterations in miRNA expression are observed during progression of various human diseases (98). Notably, functional studies have established miRNAs as critical modifiers of several disease conditions (98). As a result, therapeutic targeting of miRNA function through systemic or local delivery of miRNA inhibi-
tors or mimics is being extensively pursued (80). The recent successful clinical trial (53) utilizing a miRNA therapeutic for suppression of hepatitis C virus replication have raised the possibility for developing miRNA-based therapeutics for other diseases as well. Moreover, miRNAs have emerged as prognostic and predictive biomarkers for human diseases owing to their high stability and presence in blood, urine, and other body fluids (29). Along with cancer, cardiovascular, and neurological diseases (98), recent work has revealed the key roles played by miRNAs in the development and progression of kidney diseases (64, 141). These studies have raised the possibility of development of novel diagnostic and therapeutic strategies that can be clinically translated for the prevention and treatment of various renal diseases.

**Diabetic Nephropathy**

Diabetic nephropathy (DN) is one of the most common and debilitating complications of diabetes (40, 57) that can quite often lead to proteinuria and end-stage renal disease (ESRD) (57). Patients with DN also have a higher risk of macrovascular complications (40, 57, 126). Glomerular mesangial hypertrophy, mesangial expansion, and accumulation of extracellular matrix (ECM) proteins in the glomerular and tubular compartments, as well as podocyte effacement and apoptosis, are major features of DN (23, 60, 100, 107, 120). Although improved glycemic control reduces the rates of microvascular complications including DN (27, 28, 105, 111), the global incidence of diabetes (64, 66, 141). Notably, severe glomerular and tubular injury, proteinuria, and renal failure were observed in mice with podocyte-specific deletion of Dicer, an essential and important nuclease involved in miRNA biogenesis (production), suggesting the involvement of miRNAs in kidney dysfunction (48, 52, 130). However, because this strategy inhibits several miRNAs, the functions of specific miRNAs in renal failure were not clear from these studies. Early comprehensive studies showed that specific miRNAs are enriched in the kidney compared with other organs (135), and furthermore different miRNA expression patterns were observed in the renal cortex vs. medulla, suggesting their cell type- and tissue-specific functions (138).

High-glucose (HG) conditions seen during diabetes can adversely affect several renal cells involved in DN and also increase the formation of advanced glycation end products (AGEs) and production of growth factors such as transforming growth factor-β1 (TGF-β1), angiotensin II, and platelet-derived growth factor-β1 (PDGF-β1), which can cooperate to further augment fibrotic gene expression in DN (61, 67). Although both miR-216a and miR-217 were shown to target PTEN, one of the key ECM and fibrotic gene collagen, type I, other miRNAs such as miR-216a/miR-217 and miR-200b/c have been shown to play critical roles in DN because they can target key transcription factors, fibrotic, inflammatory, cell cycle, or other genes associated with DN. In the first demonstration of a functional role for a kidney miRNA in DN, miR-192 was found to be upregulated by TGF-β1 and in the cortex of diabetic mice. miR-192 could upregulate the key ECM and fibrotic gene collagen, type I, α2 (Col1a2) in mesangial cells (MC) by targeting the E-box repressors Zeb1 and Zeb2 (which control the expression of TGF-β1-induced collagen) (70). Interestingly, miR-192 was found to upregulate other miRNAs such as miR-216a/miR-217 and miR-200b/c through targeting Zeb1 and Zeb2, initiating amplifying circuits to further augment fibrotic gene expression in DN (61, 67). Both miR-216a and miR-217 were shown to target PTEN, leading to the activation of Akt kinase in mouse MC treated with TGF-β1, which in turn could enhance cellular hypertrophy (67). miR-200b/c in turn were shown to upregulate collagen expression and initiate the autoregulation of TGF-β1 in diabetic conditions (23, 60, 129, 165).

TGF-β1 and its effects on fibroblasts and mesangial cells have been extensively studied because they can target key transcription factors, fibrotic, inflammatory, cell cycle, or other genes associated with DN. In the first demonstration of a functional role for a kidney miRNA in DN, miR-192 was found to be upregulated by TGF-β1 and in the cortex of diabetic mice. miR-192 could upregulate the key ECM and fibrotic gene collagen, type I, α2 (Col1a2) in mesangial cells (MC) by targeting the E-box repressors Zeb1 and Zeb2 (which control the expression of TGF-β1-induced collagen) (70). Interestingly, miR-192 was found to upregulate other miRNAs such as miR-216a/miR-217 and miR-200b/c through targeting Zeb1 and Zeb2, initiating amplifying circuits to further augment fibrotic gene expression in DN (61, 67). Both miR-216a and miR-217 were shown to target PTEN, leading to the activation of Akt kinase in mouse MC treated with TGF-β1, which in turn could enhance cellular hypertrophy (67). miR-200b/c in turn were shown to upregulate collagen expression and initiate the autoregulation of TGF-β1 in diabetic conditions (23, 60, 129, 165).

### Table 1. Concise list of miRNAs involved in the pathogenesis of diabetic nephropathy and acute kidney injury

<table>
<thead>
<tr>
<th>Disease</th>
<th>miRNA</th>
<th>Targets</th>
<th>Pathways</th>
<th>Function</th>
<th>Selected Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DN</td>
<td>miR-192</td>
<td>αEF1, SIP1, ZEB1/2</td>
<td>TGF-β, AKT</td>
<td>Pathogenic</td>
<td>61, 67, 70</td>
</tr>
<tr>
<td>DN</td>
<td>miR-216a/217</td>
<td>PTEN, YBX1</td>
<td>TGF-β, AKT</td>
<td>Pathogenic</td>
<td>67</td>
</tr>
<tr>
<td>DN</td>
<td>miR-200b/c</td>
<td>ZEB1, FOG2</td>
<td>TGF-β, AKT</td>
<td>Pathogenic</td>
<td>61, 110</td>
</tr>
<tr>
<td>DN</td>
<td>miR-21</td>
<td>PTEN, PRAS40, SMAD7, TIMP1/3</td>
<td>TGF-β, AKT</td>
<td>Pathogenic</td>
<td>32, 153, 169, 170</td>
</tr>
<tr>
<td>DN</td>
<td>miR-25</td>
<td>NOX4</td>
<td>Oxidative stress</td>
<td>Protective</td>
<td>41</td>
</tr>
<tr>
<td>DN</td>
<td>miR-377</td>
<td>MnSOD, PAK</td>
<td>Extracellular matrix</td>
<td>Pathogenic</td>
<td>156</td>
</tr>
<tr>
<td>DN</td>
<td>miR-29</td>
<td>Collagens</td>
<td>Extracellular matrix</td>
<td>Protective</td>
<td>22, 34, 148</td>
</tr>
<tr>
<td>DN</td>
<td>miR-29c</td>
<td>SPRY1</td>
<td>Rho kinase</td>
<td>Pathogenic</td>
<td>88</td>
</tr>
<tr>
<td>DN</td>
<td>Let-7b</td>
<td>TGFBR1 Collagens</td>
<td>TGF-β Extracellular matrix</td>
<td>Protective</td>
<td>15, 109, 146</td>
</tr>
<tr>
<td>DN</td>
<td>miR-130</td>
<td>TGFBR1</td>
<td>TGF-β</td>
<td>Protective</td>
<td>17</td>
</tr>
<tr>
<td>DN</td>
<td>miR-93</td>
<td>VEGFA</td>
<td>VEGF</td>
<td>Protective</td>
<td>87</td>
</tr>
<tr>
<td>DN</td>
<td>miR-26a</td>
<td>CTGF</td>
<td>TGF-β/CCTGF</td>
<td>Protective</td>
<td>31, 71</td>
</tr>
<tr>
<td>AKI</td>
<td>miR-24</td>
<td>H2AX, HO-1</td>
<td>Oxidative stress</td>
<td>Pathogenic</td>
<td>89</td>
</tr>
<tr>
<td>AKI</td>
<td>miR-494</td>
<td>ATF3</td>
<td>ER stress</td>
<td>Pathogenic</td>
<td>78</td>
</tr>
<tr>
<td>AKI</td>
<td>miR-687</td>
<td>PTEN</td>
<td>Hypoxia, cell cycle</td>
<td>Pathogenic</td>
<td>11</td>
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<tr>
<td>AKI</td>
<td>miR-150</td>
<td>IGF1R</td>
<td>IGF</td>
<td>Pathogenic</td>
<td>121</td>
</tr>
<tr>
<td>AKI</td>
<td>miR-126</td>
<td>PI3K/PI4</td>
<td>PI3K/PI4akt</td>
<td>Protective</td>
<td>14</td>
</tr>
<tr>
<td>AKI</td>
<td>miR-127</td>
<td>KIF3B</td>
<td>Cell trafficking</td>
<td>Protective</td>
<td>2</td>
</tr>
</tbody>
</table>

miRNA, microRNA; DN, diabetic nephropathy; AKI, acute kidney injury; TGF, transforming growth factor. See the text for additional definitions.
MMC by targeting Zeb1 (61). Furthermore, other studies showed that miR-200b/c also activate Akt by targeting FOG2, an inhibitor of phosphoinositide 3-kinase (PI3K) (110). It is interesting to determine the mechanisms by which specific miRNAs are transcribed and regulated under diabetic conditions. Interestingly, TGF-β1-induced regulation of the miR-192 promoter in MMC involved not only Smads, but also Ets-1 and p53 transcription factors, and chromatin remodeling through histone acetylation by the histone acetyltransferase p300, which is activated by Akt kinase (30, 63, 66). miR-192 promoter was also regulated by hepatocyte nuclear factor-1 (HNF-1) in other cell types (54, 66). Multiple other studies have reported these miRNAs (miR-192, -200 family, 216a, -217) to be induced in renal cells treated with HG or TGF-β1, or in animal models of kidney injury (26, 50, 101, 134, 157). Moreover, diabetic miR-192 knockout mice or diabetic mice treated with a oligonucleotide miR-192 inhibitor were protected from key features of DN (30, 119), suggesting miR-192 can serve as a major upstream miRNA regulator of genes and factors involved in the early stage of DN (26, 30, 61, 63, 66–68, 70, 110).

Another miRNA that has been widely studied and implicated in several cell and animal models of DN and kidney injury is miR-21 because its targets (which include PTEN) are related to fibrosis, Akt activation, hypertrophy, and apoptosis (32, 153, 170). Recently miR-21 was shown to promote fibrosis by targeting metabolic pathways. Relative to wild-type or untreated mice, miR-21 knockout mice or mice treated with anti-miR-21 oligonucleotides showed less severe interstitial fibrosis in response to renal injury (20). On the other hand, miR-21 was downregulated in db/db mice, and its overexpression inhibited MC proliferation (169). More severe renal phenotypes were detected in miR-21 knockout mice crossed with TGF-β1 transgenic mice relative to TGF-β1 transgenic mice (77). Interestingly some miRNAs like the miR-29 family members can directly regulate ECM accumulation because these miRNAs target multiple collagens and hence a decrease in their levels can augment collagen deposition in the kidney or in renal cells treated with TGF-β1 or HG (22, 34, 73, 148). Importantly, miR-29b overexpression was found to significantly alleviate DN in db/db mice (22). Interestingly, an anti-diabetic drug, linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, was shown to upregulate miR-29 and prevent fibrosis in a mouse model of DN (59). On the other hand, in another study miR-29c was found to be upregulated under diabetic conditions and activated Rho kinase by targeting Sry-1, leading to ECM accumulation and podocyte apoptosis (88). Other miRNAs have also been studied in DN. MiR-377 upregulated by HG or TGF-β1 in MC induced fibronectin expression and oxidant stress by targeting manganese superoxide dismutase and p21-activated kinase (156). Let-7 family members known as tumor suppressors (55, 97, 125) were also downregulated by TGF-β1 in renal cells, and this could promote fibrosis through upregulation of the let-7 targets TGF-β1 receptor type-1 (TGF-β1R1) and collagens (15, 109, 146). Mechanistically, it was interesting that let-7 family members downregulated by TGF-β1 induced upregulation of lin28b (negative regulator of let-7 processing) through Smad2/3 (109). The let-7-TGF-β1R1 connection illustrates another signal-amplifying loop involving miRNAs that can accelerate DN (65, 66). Interestingly, the downregulation of let-7a-3 in DN was associated with promoter DNA hypermethylation, suggesting an epigenetic regulatory mechanism (114). TGF-β1-induced decrease of miR-130b was shown to upregulate its target, TGF-β1R1, in MC through mechanisms involving a decrease in Ybx1/NFYC targeted by miR-216a, thus revealing another miRNA-mediated amplifying cascade (17). miR-135a, which promotes ECM accumulation by targeting a transient receptor potential cation channel, subfamily C, member 1 (TRPC1), was enriched in serum and renal tissue from patients with DN and db/db mice (50). miR-30 family members were decreased in accelerated DN, and this was related to the upregulation of their target, connective tissue growth factor (CTGF) (162). miR-22 regulates BMP-7 and BMP-6 and further increases TGF-β1 signaling in mouse models of kidney fibrosis (86). miR-93 was identified as a key miRNA downregulated in podocytes and renal microvascular endothelial cells treated with HG as well as in glomeruli of diabetic db/db mice, and this enhanced angiogenesis via an increase in its target, VEGF-A (87). miR-26a was downregulated in DN patients and also by TGF-β1 in cultured podocytes, leading to an increase in its target, CTGF (71). Interestingly, miR-26a was also upregulated by HG in MC and could activate mTORC1, enhance hypertrophy, and ECM accumulation (31).

Oxidant stress is another important factor in the pathogenesis of DN, and several miRNAs that modulate oxidant stress have been identified (6, 39, 41, 102, 127, 171). The balance of pro-oxidant miRNAs and antioxidant miRNAs may be a key to control renal oxidant stress and DN (62).

Notably, distinct effects of key miRNAs in renal cells have been reported. For example, in some other models of more severe or late stages of DN and in some studies of cultured proximal tubular epithelial cells treated with HG or TGF-β1, decreases in miR-192 were reported (74, 145), although as noted earlier miR-192 was upregulated in renal cells treated with TGF-β1 or HG and many animal models of earlier DN in several studies (26, 30, 61, 63, 66–68, 70, 110). miR-26a was downregulated by TGF-β1 in cultured podocytes (71) although it was upregulated by HG in cultured MC (31). Cell type-specific effects of miRNAs and differences in the animal models studied may be the reason. The cell type-specific response of miR-192 to TGF-β1 can also be explained by the cellular status of transcription factors such as p53, Ets-1, or Smads, since TGF-β1-induced miR-192 expression was abolished in MC from p53−/−, Ets-1−/−, or Smad3−/− mice (26, 30, 63). miR-200 family members were increased related to ECM accumulation and Akt activation in primary MMC (61, 110), although miR-200a was downregulated in TGF-β1-treated proximal tubular epithelial cells also related to fibrosis through TGF-β2 (147). Importantly, the effects of miRNAs such as miR-200 can vary in primary epithelial cells vs. cancer cells (66). Furthermore, it is likely that miRNA levels are lower in renal tissues obtained from patients in late stages of DN relative to early (74), due to poor quality of RNA arising from tissue necrosis and apoptotic cells. Thus it is worthwhile to profile miRNAs in models of early DN (including animal models), rather than late stage, as such miRNAs can be good targets to prevent progression to overt renal failure (30, 65, 66, 119). This is illustrated by data showing that inhibition of miR-192, which is highly expressed in mouse models of early DN (63, 64, 70) but decreased in biopsies from patients in late
stages of DN (74), prevented key features of DN in animal models (30, 119).

An area that is showing great interest and promise is in harnessing the potential of miRNAs as novel biomarkers. Early diagnosis of DN is of tremendous value to prevent progression to renal failure and dialysis. Since there have been significant advances in sensitive and precise detection of miRNAs in human biofluids such as urine and plasma, miRNAs are emerging as promising candidate noninvasive biomarkers for human diseases (36, 38, 139, 154). Comprehensive profiles of miRNAs in urine, urinary sediment, and serum from patients in specific stages of DN, fibrosis, renal function decline (GFR), albuminuria, or rapid progression to ESRD have been reported (5, 8, 13, 16, 33, 50, 51, 92, 93, 106, 115, 136, 151, 152, 166). Because molecular mechanisms underlying DN may vary from patient to patient, individual miRNA profiles could be used as a key factor for personalized medicine and treatment (65).

Another area of active research is miRNA-based therapy, and several delivery approaches in animal models of DN have been attempted to control miRNA expression in vivo (33, 64, 66). Chemically modified stable nuclease-resistant oligonucleotides have been developed that might be used in future for patients. Antisense miRNAs modified with Locked nucleic acid (LNA) are relatively popular due to their superior targeting efficiency (67, 75, 119) and also being evaluated in some clinical trials (82, 144). Targeting several miRNAs by LNA-modified or 2’-O-methyl antisense oligonucleotides has been successful to reduce specific miRNAs and inhibit fibrosis and hypertrophy in mouse models of DN and fibrosis (20, 30, 50, 61, 67, 88, 119, 170). It is anticipated that more sensitive and precise methods to monitor miRNAs in human biofluids will be established, while success in preventing DN in animal models with chemically-modified oligonucleotide inhibitors of specific miRNAs provides hope for clinical translation to human DN in the future.

Acute Kidney Injury

Acute kidney injury (AKI) is characterized by a rapid decline in renal function due to ischemic or toxic damage in renal tubular cells (10). Mortality rates for AKI are extremely high in part due to the lack of sensitive diagnostic markers and an absence of therapeutic options (10). Importantly, it has been recognized that AKI is a key contributing factor in the development of chronic kidney disease (CKD) (21). Even mild AKI episodes can lead to CKD and an increased long-term risk of developing ESRD (21).

Recent studies have revealed the critical role played by miRNAs in modulating multiple cellular and molecular pathways that contribute to AKI (159). In one of the initial studies (158) examining the functional role of miRNAs in AKI, it was found that global miRNA depletion by conditional Dicer ablation in renal tubular cells provides significant protection from ischemic AKI. This study established the pathogenic role of Dicer and associated miRNAs in ischemic AKI. Subsequent studies (159) have now unveiled both protective and pathogenic roles of specific miRNAs in the development of AKI. Based on these studies, several miRNAs (Table 1) have been postulated to have diagnostic utility, while miRNA-based therapeutic strategies have also been suggested for AKI.

Renal tubular cell death is the major contributing factor in the development of AKI (83). Multiple miRNAs have been shown to contribute to renal tubular cell apoptosis. The apoptosis-associated miR-24 is upregulated in the murine kidneys after ischemia-reperfusion (I/R) injury and in patients after kidney transplantation (89). Importantly, silencing of miR-24 in mouse models of I/R injury resulted in significant renoprotection. The apoptotic role of miR-24 was found to be mediated through direct regulation of two target genes, namely, H2A histone family member X and heme oxygenase 1 (89). These results indicate that miR-24 inhibition may be a promising therapeutic option in the treatment of patients with ischemic AKI. miR-494 is another cell death-associated miRNA, which plays a pathogenic role during AKI (78). Initial studies found that urinary miR-494 levels were many-fold higher in patients with AKI than normal controls. In mouse models, renal overexpression of miR-494 led to increased renal damage, which was attributed to reduced levels of its target gene, activating transcription factor 3 (ATF3) (78). Similar to miR-24 and miR-494, miR-687 has recently (11) been proposed to contribute to I/R-mediated AKI. This study (11) identified a novel hypoxia-inducible factor HIF-1/miR-687/PTEN signaling pathway, where miR-687 antagonism preserved PTEN expression, blocking cell cycle activation and preventing renal apoptosis, resulting in significant protection in mouse models of AKI. In a novel myocardial infarction-induced AKI mouse model, miR-150 deficiency also suppressed AKI, which was associated with suppression of inflammation and interstitial cell apoptosis (121). Interestingly, preischemic conditioning protected mice from AKI, and these effects were not observed in miR-21 knockout mice, suggesting that miR-21 plays a protective role during AKI (164).

Along with miRNAs that contribute to AKI, renoprotective miRNAs have also been identified. Bijkerk et al. (14) showed that overexpression of miR-126 in the hematopoietic compartment can protect the kidney via preservation of microvascular integrity during ischemic renal injury and supports recovery by promoting vasculogenic progenitor cell mobilization. In another study (2), miR-127 was identified as a renoprotective miRNA during ischemic renal injury. Ischemic induction of miR-127 was found to be mediated by HIF-1α stabilization, and functional studies showed that miR-127 is involved in cell matrix and cell-cell adhesion maintenance. Similarly, the p53-inducible miR-34a may play a cytoprotective role during cisplatin-induced renal tubular cell death (12).

Besides their roles in pathogenesis or protection from AKI, studies (9, 76, 113, 160) on miRNA expression profiles have illustrated the usefulness of miRNAs as biomarkers for diagnosis of AKI. Although, extensive human studies are still required, initial steps have been taken to demonstrate the potential utility of miRNAs, especially in the urine, for assessing AKI. Multiple studies (90, 96, 104, 113, 124, 155, 159, 160) in humans and animal models have identified specific miRNAs as potential diagnostic markers for the early detection of AKI. Such approaches would be of tremendous clinical value to prevent progression from AKI to renal failure.

IgA Nephropathy

IgA nephropathy is a common form of glomerulonephritis that can lead to ESRD (163). The major hallmark of IgA
nephropathy is glomerular deposition of IgA immune complexes, causing activation of mesangial and inflammatory cells, resulting in renal injury (99). miRNAs have been implicated in the pathogenesis of IgA nephropathy and have received significant attention as potential diagnostic and prognostic markers (137, 167). Interestingly, aberrant O-glycosylation in the hinge region of IgA1 is a common feature of IgA nephropathy (137). In an important study (128), it was found that this aberrant O-glycosylation of IgA1 is due to increased expression of miR-148b, which targets the glycosylation enzyme, β1,3-galactosyltransferase 1 (C1GALT1). This study (128) provides a direct link between the overexpression of miR-148 and the aberrant glycosylation of IgA1, which plays a pathogenic role in the early phase of IgA nephropathy. The miR-29 family which targets several extracellular matrix genes has also been implicated in the pathogenesis of IgA nephropathy (137). In particular the HIF-1α-dependent miR-29c has been suggested to play an anti-fibrotic role by down-regulating collagen type II α1 (COL2A1) protein, and tropomyosin 1α (TPM1) proteins (37). Global analysis of renal miRNA expression has also identified several miRNAs related to immunological and pathological changes that might contribute to the development or progression of IgA nephropathy (137). Additionally, urinary levels of several miRNAs are significantly altered in IgA nephropathy patients and have the potential to be used as biomarkers for diagnostic purposes (58, 104, 137).

**Polycystic Kidney Disease**

Polycystic kidney disease (PKD) is an inherited disorder characterized by development of clusters of cysts within the kidneys (47). The inherited mutations are frequently observed within specific genes, including PKD1, PKD2, and PKHD1, which contribute to abnormal proliferation resulting in cyst formation (19). Recent studies with global miRNA profiling have shown that the miRNA expression pattern is significantly altered in PKD models, suggesting that miRNAs may act as disease modulators downstream of PKD mutations (25, 108). In an interesting study (112), it was shown that the miR-17–92 cluster is upregulated in mouse models of PKD. Moreover, transgenic overexpression of miR-17–92 in kidneys produced cysts in normal mice, while inactivation of miR-17–92 in mouse models of PKD retarded kidney cyst growth and provided significant survival benefits. Mechanistic studies showed that the miR-17–92 cluster promotes aberrant renal cell proliferation through repression of several PKD genes (112).

Other studies have also shown that the PKD genes are miRNA targets, providing a direct link between PKD gene expression, miRNAs, and cystogenesis (116, 141). For example, miR-17 has been shown to target PKD2 and overexpression of miR-17 can promote aberrant cellular proliferation in cell culture models (133). Moreover, transgenic mice expressing miRNAs targeting PKD1 develop several pathophysiological features of PKD (149). Interestingly, knockout mice of bicaudal C (Bicc1), an evolutionarily conserved RNA-binding protein, develop a PKD phenotype (140). Mechanistic studies showed that Bicc1 antagonizes the function of miR-17 and stabilizes PKD2 mRNA. This was substantiated in *Xenopus* models, where the renal defects of Bicc1 knockdown were rescued by reducing miR-17 activity (140). These studies (112, 117, 133, 140) have provided solid proof-of-principle evidence and a strong rationale for targeting miR-17 as a therapeutic strategy in the treatment of PKD.

Autosomal recessive PKD (ARPKD), a subtype of PKD, is generally caused by mutation in the polycystic kidney and hepatic disease 1 (PKHD1) gene (46). PKHD1 was found to be a direct target of miR-365-1 (35). Polycystic liver disease, a frequent feature of PKD, is associated with a reduction in miR-15a expression (79). Loss or reduction in miR-15a expression could directly contribute to aberrant cell cycle progression through increased expression of cell cycle-regulatory genes like cdc25a (79).

**Other Renal Diseases and Conditions**

Along with the above-mentioned renal diseases, miRNAs have also been studied in lupus nephritis, acute allograft rejection, hypertensive nephropathy, and other disorders (141, 159). Altered expression of several miRNAs has been demonstrated in lupus nephritis (131, 132, 141). These differentially expressed miRNAs include miR-638, miR-663, miR-198, miR-155, and miR-146a (91, 141). Similarly, acute allograft rejection, a frequent complication of renal transplantation, is associated with alterations in miRNA expression (4, 84, 95, 143, 160). Global miRNA profiling of human hypertensive nephrosclerosis biopsies also revealed a number of differentially expressed miRNAs, including miR-200a, miR-200b, miR-141, miR-429, and miR-192 (150). Similar profiling has been performed in Dahl salt-sensitive rats (103). miR-29b is upregulated in renal medullary tissues in Dahl salt-sensitive rats fed a high-salt diet, and miR-29b overexpression in cultured cells can suppress collagen expression (85). These findings demonstrate a negative regulatory role of miR-29b in collagen and extracellular matrix accumulation in hypertensive renal injury. Interestingly, recent work has implicated miR-217 in mediating the protective effects of the dopamine D2 receptor on fibrosis in renal tubular cells (45). Additionally, miR-192 and mir-324-3p may play a role in hypertensive nephropathy (150). In another important study, miR-382 was found to target kallikrein 5 and contributes to the development of renal inner medullary interstitial fibrosis (72).

miRNAs have also been implicated in focal segmental glomerulosclerosis (FSGS). Higher expression of miR-193a was observed in isolated glomeruli from individuals with FSGS compared with normal subjects, and transgenic overexpression of miR-193a in mice induced FSGS and podocyte foot process effacement. This was mechanistically attributed to miR-193a-mediated targeting of Wilm’s tumor protein (WT1), a critical regulator of podocyte differentiation and homeostasis, and was associated with downregulation of key WT1 target genes, including podocalyxin and nephrin (42). Increased expression of miR-21 was also observed in Alport nephropathy, a genetic disorder characterized by chronic kidney disease, such as glomerulonephritis. Notably, in this study it was demonstrated that inhibition of miR-21 by antisense oligonucleotides in a mouse model prevented typical features of this disease, including glomerulosclerosis, interstitial fibrosis, tubular injury, and inflammation (43). It is important to note that despite the evidence of differential expression of various miRNAs and their potential functions in renal physiology or pathology, the functional significance in many cases remains unknown and is expected to remain an area of active investigation.
Conclusions and Future Perspectives

Significant progress has been made in our understanding of how miRNAs regulate normal and abnormal kidney function. Strong experimental data in animal models have emerged showing the role of specific miRNAs in the development and progression of renal diseases. Moreover, it has become possible to target miRNAs in vivo (to inhibit or restore miRNA expression), which provides hope for the future development of renal miRNA therapeutics. Furthermore, miRNAs have significant potential as novel diagnostic and prognostic biomarkers for kidney diseases. However, several challenges still remain for translating animal studies to the clinic. Some of these challenges include targeting of miRNA-based therapeutics to specific renal cells and the possibility that more than one miRNA may have to be targeted to have significant clinical benefits. Furthermore, as discussed earlier, miRNAs can contribute to the biological processes affecting kidney functions via direct as well as indirect effects. Added to this complexity of miRNA-mediated actions, one miRNA can target multiple genes and, furthermore, since miRNAs can have cell-specific effects, this poses significant challenges especially in a heterogeneous organ like the kidney. In terms of their clinical diagnostic potential, there is a need for intensive clinical studies in appropriate cohorts with patients from various stages of the disease to identify which miRNA(s) could be used as biomarkers of disease progression. Several studies have identified differentially expressed miRNAs in the blood, urine, and kidney, raising the possibility of developing miRNAs as diagnostic markers. However, these studies have one or more limitations, including insufficient sample size, single time points, lack of appropriate controls, insufficient information on the effect of other comorbidities, and lack of secondary validations. On the other hand, the uniqueness of the miRNAs differentially expressed in the diseases of interest and the cell type- or tissue-specific expression may render them as valuable and specific biomarkers. Notwithstanding these challenges, pitfalls, and promises, understanding the role of miRNAs in renal diseases has a significant potential to lead to the development of a novel class of diagnostic and therapeutic tools for the early detection and treatment of renal diseases.

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