MicroRNAs in kidney physiology and disease

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Abstract | MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression. They have important roles during kidney development, homeostasis and disease. In particular, miRNAs participate in the onset and progression of tubulointerstitial sclerosis and end-stage glomerular lesions that occur in various forms of chronic kidney disease (CKD). Therefore, miRNAs represent potential new therapeutic targets for a debilitating disease that continues to increase in prevalence worldwide and for which fully effective therapies are lacking. Several lines of research aimed at improving common CKD diagnostic tools and avoiding invasive kidney biopsies have also identified circulating miRNAs as possible diagnostic and even prognostic biomarkers of kidney disease. This Review discusses current understanding of the function of miRNAs in CKD, focusing on functions specifically involved in the transforming growth factor β1 pathway, which is activated in CKD. miRNAs that, according to available evidence, seem to be involved in diabetic nephropathy, IgA nephropathy, lupus nephritis, polycystic kidney disease and graft rejection, are also discussed.

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Introduction

Chronic kidney disease (CKD) is an important public health problem that is closely linked to major non-communicable diseases such as diabetes mellitus and hypertension.[1] Less common causes of CKD are hereditary diseases such as polycystic kidney disease (PKD), IgA nephropathy (IgAN) and lupus nephritis, or herbal and environmental toxins. Regardless of the disease aetiology, progression of CKD results in tubulointerstitial and glomerular fibrosis owing to excessive deposition of extracellular matrix (ECM). Other key features of CKD are inflammatory cell infiltration, tubular cell atrophy, mesangial cell hypertrophy and podocyte apoptosis.[2] All of these pathological events are mainly instigated by the cytokine transforming growth factor β1 (TGF-β1).[3]

Current therapies that target the renin–angiotensin–aldosterone system[4] are not always effective in halting progression to end-stage renal disease, a condition that requires renal replacement therapy by dialysis or kidney transplantation. Blockade of TGF-β1 signalling using TGF-β1 neutralizing antibodies or specific inhibitors of TGF-β1 receptors might be a promising therapeutic approach for CKD as these strategies have been shown to attenuate renal fibrosis in various animal models.[5] The success of these approaches in preclinical studies has prompted the search for therapies that interfere with the epigenetic regulation of TGF-β and genes involved in the TGF-β signalling pathway. In this context, non-coding RNA species, including microRNAs (miRNAs), have been widely implicated in the pathogenesis and progression of CKD, particularly in the regulation of TGF-β1-mediated fibrosis. Correction of miRNA expression by *in vivo* delivery of miRNA mimics or inhibitors has therefore emerged as a promising novel therapeutic strategy for the treatment of CKD. In addition, several lines of current research aimed at improving common CKD diagnostic tools and avoiding invasive kidney biopsies have identified circulating miRNAs as possible diagnostic and even prognostic biomarkers.[6,7] This Review focuses on current research investigating the roles of miRNAs in normal kidney physiology and diseases; particular attention is given to the TGF-β1 pathway and its regulation by miRNAs.

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Biogenesis and function of miRNAs

miRNAs are epigenetic regulators of gene expression that are able to modulate several cellular processes, from development to disease conditions. The human miRNAome is composed of 1,881 precursors and 2,588 mature miRNAs, which regulate at least 60% of protein-coding genes.[8] Since 2010, the number of miRNAs included in miRBase has grown by approximately two-thirds owing to the advent of small RNA deep-sequencing techniques.[9]

Here, we briefly mention the key points of miRNA biogenesis and function, as these processes are described thoroughly elsewhere.[10,11] Transcription of miRNAs occurs from individual or clustered genes (that is, the miR-194–192 cluster), although some miRNAs can be encoded from distinct genomic loci.[12] Genes encoding miRNAs are located in non-coding sequences or in introns of either protein-coding genes (miR-trons) or non-coding RNA.[13] Intronic miRNAs are usually coordinately expressed with their host gene and most of the time they both affect the same signalling pathway.[14–16]

RNA polymerase II transcribes miRNAs in the nucleus as long capped and polyadenylated hairpin transcripts, called primary miRNAs (pri-miRNAs).

Key points

- MicroRNAs (miRNAs) are key players in kidney development and physiology
- Transforming growth factor β1 is a major regulator of kidney fibrosis; its signalling is finely regulated by miRNAs
- miRNAs contribute to both the induction and progression of chronic kidney disease (CKD)
- Current translational research on miRNAs in kidney disease is mainly focused on developing reliable biomarkers for diagnosis and prognosis of CKD and renal transplantation
- miRNAs represent novel therapeutic targets for CKD, but delivery and safety issues must be taken into account before translation into clinical practice

These are processed into smaller ~70 nucleotide stem–looped structures, called precursor miRNAs (pre-miRNAs) by the ribonuclease III-like enzyme Drosha together with the microprocessor complex subunit DGCR8. Pre-miRNAs are then exported to the cytoplasm by exportin-5/GTP-binding nuclear protein Ran, where the ribonuclease Dicer yields 22 nucleotide miRNA duplexes consisting of the guide and passenger strands (miRNA:miRNA*). The guide strands are finally assembled into the RNA-induced silencing complex (RISC) and bind through their 'seed sequence' (nucleotides 2–8) to fully or partially complementary sites within the 3' untranslated region of target mRNAs. miRNAs rarely bind to the coding regions of mRNA or genomic DNA, including promoter regions.[17] Target recognition by miRNAs leads to mRNA translational repression and/or mRNA deadenylation and decay,[18,19] although positive regulation has been described in a few cases (Figure 1).[20,21] Beyond the simplistic concept that miRNAs act as repressors of a single transcript, emerging evidence indicates that they are modulators of many hundreds of proteins often involved in related signalling pathways.[22,23]

miRNAs are usually stable with a long half-life, but individual miRNAs can undergo rapid decay in specific cellular contexts in the presence of particular environmental stimuli or cellular factors. Of note, the presence of certain sequences in miRNAs that determine decay rate, together with several ribonucleases that degrade miRNAs, have been identified.[24]

miRNAs also have a key role as regulators of cellular crosstalk.[6] They can be actively secreted into the extracellular microenvironment or into body fluids and captured by other cells, thus altering their transcriptional programme. In addition, circulating miRNAs can be derived from apoptotic cells, with the likely purpose of carrying alarm signals from apoptotic cells to other cells.[6] miRNAs circulate in blood, urine and other body fluids either packaged into microvesicles and/or exosomes, or transported by RNA-binding proteins (Argonaute2 complexes) and lipoproteins, which protect them from degradation by ribonucleases.[6] The consequent stability of miRNAs in biological fluids, together with the fact that miRNAs can be repeatedly collected by non-invasive means and detected with high accuracy and specificity by amplification methods, renders miRNAs potentially better biomarkers than proteins and mRNAs. However, isolation and quantification of miRNAs is time-consuming and expensive, and further optimization of these procedures is required before they can be used in routine clinical practice.

miRNAs in development and physiology

Analyses of miRNA expression profiles have identified a set of miRNAs expressed mainly in the adult human kidney (including miR-215, miR-146a and miR-886); other miRNAs, such as miR-192, miR-194, miR-21, miR-200a, miR-204 and let-7a–g, are enriched in the kidney as well as in other organs.[14,25,26] However, some discordant findings regarding miRNA expression profiles have been reported, probably owing to use of different detection methods and to differences in the intrinsic nature of the analysed samples. The picture is further complicated by the fact that expression levels of selected miRNAs differ between fetal and adult kidneys, suggesting that besides being tissue-specific, miRNA expression is specific to developmental stage.[27]

The generation of mouse models with conditional knockdown of miRNA-processing enzymes or knockdown of specific miRNAs in various cell lineages has enabled the roles of miRNAs during kidney development and homeostasis to be studied. In nephron progenitor cells, conditional deletion of Dicer results in their apoptosis—mediated by the proapoptotic protein Bim—and in the premature termination of nephrogenesis.[28,29] Ablation of the miR-17~92 cluster, composed of miRNAs normally expressed in the developing kidney with well-known roles in development, leads to defective proliferation of progenitor cells and reduced numbers of developing nephrons.[30] Mice deficient in miR-17~92 are characterized by renal hypodysplasia and develop glomerular dysfunction and proteinuria.[30]

In ureteric buds, impaired miRNA processing leads to excessive cell proliferation and apoptosis accompanied by disrupted ciliogenesis within the epithelium of ureteric buds and consequent development of renal cysts.[29,31] In addition, one study suggested that miRNAs modulate later stages of renal tubule maturation as removal of Dicer from maturing renal tubules and collecting ducts leads to the formation of tubular and glomerular cysts in mice through a mechanism that probably involves modulation of *Pkd1* gene dosage by miRNAs.[32]

miRNAs also have a key role in podocyte homeostasis. Indeed, podocyte-specific deletion of Dicer or Drosha in the developing kidney causes disruption of the glomerular filtration barrier, leading to proteinuria and collapsing glomerulopathy with glomerular and tubulointerstitial fibrosis.[33–37] Moreover, miRNA processing is important for maintenance of adult podocyte function and differentiation as selective deletion of Drosha in adult mice results in collapsing glomerulopathy,[37] whereas inactivation of Dicer in postnatal proximal tubules does not affect their histology or function and protects mice from renal ischaemia–reperfusion injury.[38]

miRNAs are also involved in regulating renal physiology, from the control of blood pressure to the maintenance of whole-body fluid and electrolyte homeostasis. The role of miRNAs in blood pressure control was

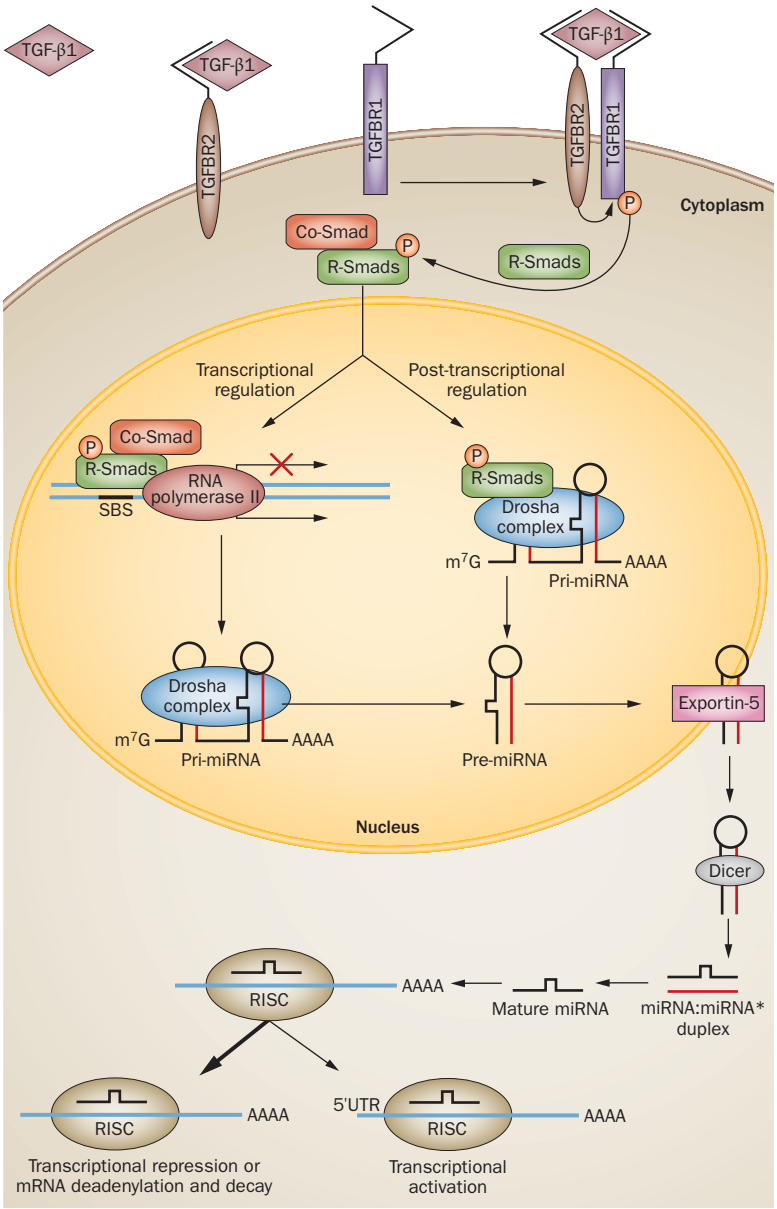

Figure 1 | Interplay between TGF-β1/Smad signalling and the miRNA machinery. TGF-β1/Smad signalling can modulate miRNA expression by regulating transcription and/or facilitating the processing of pri-miRNA into pre-miRNA in the nucleus. Pre-miRNA is exported to the cytoplasm where it is processed into the final mature miRNA, which is loaded into the RISC. Mature miRNA guides the RISC to silence target mRNAs through mRNA cleavage, translational repression or deadenylation. Rarely, transcriptional activation can occur. Abbreviations: Co-Smad, common mediator Smad; miRNA, microRNA; P, phosphorylation; pre-miRNA, precursor miRNA; pri-miRNA, primary miRNA; RISC, RNA-induced silencing complex; R-Smad, receptor-regulated Smad; SBS, Smad binding site; TGF-β1, transforming growth factor β1; TGFBR1, TGF-β receptor type-1; TGFBR2, TGF-β receptor type-2; UTR, untranslated region.

ascertained by generation of mice with specific inactivation of Dicer in renin-expressing juxtaglomerular cells, which leads to loss of these cells accompanied by reduced plasma renin concentration, hypotension and kidney fibrosis.[39,40] Several miRNAs are modulated by hypertonic conditions and have a key role in the control of osmolarity balance by regulating Na^+ and K^+ levels in the different portions of the nephron. miR-192 suppresses the Na^+/K^+-ATPase β1 subunit gene in human renal epithelial cells,[41] and in the distal nephron it inhibits the serine/threonine kinase WNK1, a protein that is essential for the coordinated regulation of electrolyte transport in the kidney.[42] Regulation of Na^+ transport by miRNAs has been ascertained in the mouse cortical collecting duct where aldosterone reduces the expression of a subset of miRNAs that target ankyrin-3. In turn, overexpression of ankyrin-3 increases sodium transport mediated by the epithelial Na channel.[43] Moreover, renal medullary cortical collecting duct cells exposed to high Na^+ concentration are characterized by reduced levels of miR-200 and miR-717 in association with increased levels of their common target NFAT5,[44] which controls the cellular response to osmotic stress. miRNAs are also able to regulate K^+ secretion. Indeed, in the cortical collecting duct, high dietary K^+ intake stimulates the transcription of miR-802 and miR-194, which regulate ROMK channel activity by targeting caveolin-1 (a negative regulator of the ROMK channel)[45] and intersectin-1 (involved in mediating the WNK-induced endocytosis of the ROMK channel),[46] respectively. Finally, in the thick ascending limb of Henle, miR-9 and miR-374 regulate the expression of claudin-14, which is critical for Ca^{2+} reabsorption in the kidney.[47] Interestingly, the transcriptional levels of both miRNAs are directly regulated by the Ca^{2+}-sensing receptor CASR.[48]

miRNAs in TGF-β1-mediated fibrosis

TGF-β1, the most abundant isoform of the TGF-β family, is a pivotal player in CKD progression. Dysregulated TGF-β1 synthesis by distressed resident renal cells and infiltrating inflammatory cells in response to various stimuli, including high glucose,[49] angiotensin II[50] and reactive oxygen species,[51] is an important component of the renal fibrogenic process and is associated with pathological events in the glomerular, tubulointerstitial and vascular compartments.[3,52] TGF-β1 elicits a different response in glomerular cells leading to mesangial cell hypertrophy and proliferation (apoptosis on rare occasions), podocyte apoptosis and detachment from the glomerular basement membrane, and endothelial-to-mesenchymal transition.[3] Similarly, TGF-β1 leads to tubule loss and tubulointerstitial fibrosis[3,53] characterized by scar formation following myofibroblast accumulation. The origin of myofibroblasts in kidney fibrosis has been the subject of heated debate. Previous studies suggested that myofibroblasts originate from endogenous stromal cells (pericytes and interstitial fibroblasts) or resident vascular and tubule cells undergoing endothelial-to-mesenchymal or epithelial-to-mesenchymal transition or from migrating and differentiating extrarenal cells of bone marrow origin.[54] By means of fate-mapping approaches, an elegant study identified that myofibroblasts originate from proliferating local resident fibroblasts or from differentiated bone-marrow-derived mesenchymal stem cells recruited in the diseased kidney, whereas endothelial and proximal tubular cells only account for 5% and 10% of interstitial myofibroblasts, respectively.[55]

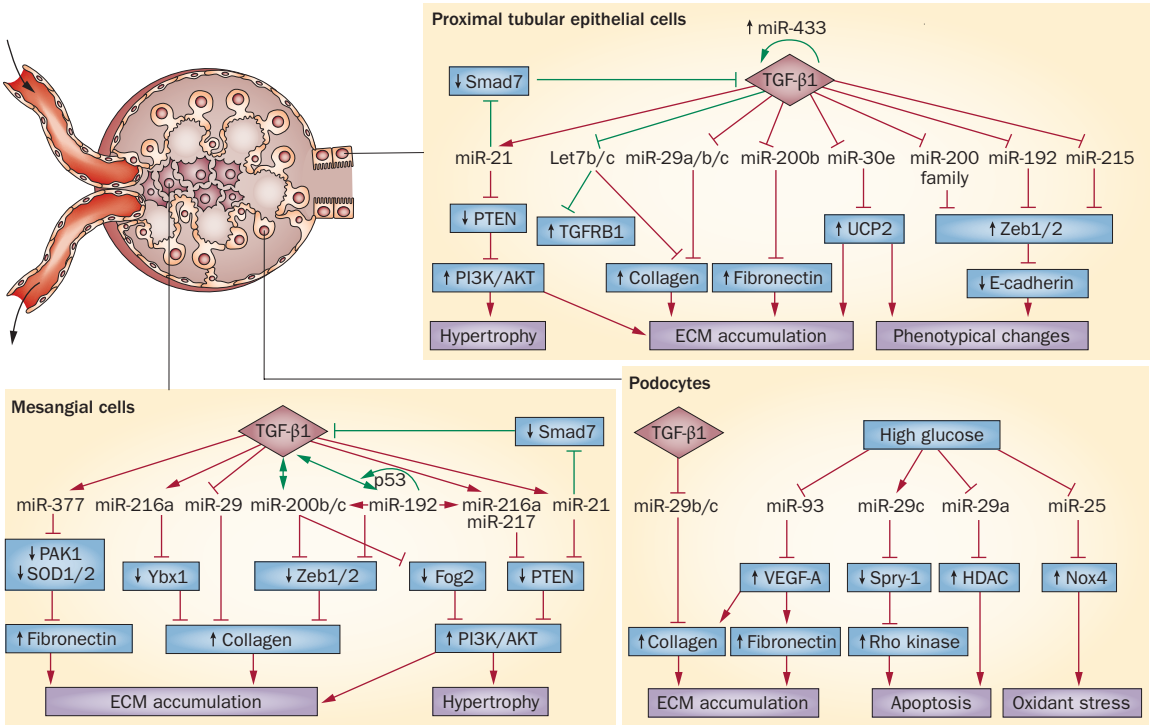

Figure 2 | miRNA-regulatory networks in proximal tubular epithelial cells and mesangial cells in response to TGF-β1, and in podocytes in response to TGF-β1 and high glucose. Up and down black arrows represent upregulated and downregulated, respectively. miRNA-dependent signalling loops through which TGF-β1 amplifies its signal are shown in green. Abbreviations: ECM, extracellular matrix; HDAC, histone deacetylase; miRNA, microRNA; TGF-β1, transforming growth factor β1; TGFBR1, TGF-β receptor type-1.

The major contribution of TGF-β1 to kidney fibrosis is based on its ability to stimulate the synthesis and accumulation of ECM within the glomerulus and the interstitial parenchyma, in part by upregulating plasminogen activator inhibitor, which inhibits ECM degradation.[56,57] The profibrotic effects of TGF-β1 are primarily accomplished by the Smad-dependent pathways,[58] although non-Smad pathways including pp60^{c-src}, epidermal growth factor receptor, MAPK, p53, and PI3K/AKT might also be involved.[59,60] The binding of TGF-β1 to TGF-β receptor type-2 results in recruitment and activation of TGF-β receptor type-1 (TGFBR1). In the Smad-dependent pathway, TGFBR1 activates Smad2 and Smad3 (receptor-regulated [R]-Smads) by phosphorylation, which in turn bind to Smad4 (also called common mediator Smad or co-Smad) forming a complex that translocates into the nucleus and regulates gene transcription, including miRNAs (Figure 1).[61,62] Smad7 negatively modulates the pathway by blocking the phosphorylation of R-Smads and promoting degradation of the receptor complexes, and blocks TGF-β/Smad-dependent fibrosis.[58] Smad-dependent transcriptional regulation of genes in response to TGF-β1 requires binding of the Smad complex to a Smad binding site (SBS) in the promoter regions of target genes and engagement of sequence-specific transcription factors and co-factors that dictate gene induction or repression in a context-dependent manner.[58,60]

Overall, miRNAs are emerging as both downstream effectors of TGF-β-dependent regulation of the fibrogenic process and upstream regulators of TGF-β signalling. Most members of the TGF-β family or TGF-β-dependent pathway are targets of miRNAs, suggesting an autoregulatory feedback loop between TGF-β and miRNAs.[62]

The following sections describe the role of miRNAs in TGF-β1-induced renal fibrosis, which has been investigated using a multifaceted approach combining animal models of CKD and *in vitro* studies. miRNAs modulated by TGF-β1 in proximal tubular cells, mesangial cells and podocytes are represented in Figure 2. Moreover, miRNA-specific targets and their contribution to kidney fibrosis are highlighted.

Regulation of miRNA expression by TGF-β1

TGF-β1 regulates the miRNA transcriptional profile in the kidney in a cell-dependent and context-dependent manner through Smad signalling. R-Smads that have translocated into the nucleus modulate miRNA expression at the transcriptional level by binding to a SBS in DNA or at the post-transcriptional level through direct association with conserved consensus sequences within the stem region of pri-miRNAs. Sequence-specific binding of R-Smads to pri-miRNAs provides a landing site for the Drosha/DGCR8 complex thereby promoting the efficient cleavage of specific pri-miRNAs.[62–64] Smad-activated auxiliary factors such as the RNA helicase p68, a component of the Drosha microprocessor complex, can also facilitate recruitment of Drosha/DGCR8 to specific pri-miRNAs for efficient processing.[63,64]

TGF-β1 upregulates the expression of the profibrotic miR-21 in cultured proximal tubular epithelial cells (pTECs) via Smad3 signalling, both at the transcriptional and post-transcriptional levels.[65] Of note, Smad3 constitutively interacts with SBS1 and SBS2 in the miR-21 promoter, but Smad3 phosphorylation is needed for miRNA transcription. By contrast, binding of Smad3 to a SBS in the promoter region of miR-29, which exerts antifibrotic properties, represses miRNA transcription as documented *in vitro* in cultured pTECs and fibroblasts as well as *in vivo* in the unilateral ureteral obstruction (UUO) model.[66]

A functional link between miR-192 and TGF-β1-driven renal fibrosis has also been documented, although the effect of TGF-β1 on miR-192 expression is not consistent between various studies. *In vitro* studies showed different regulation of miR-192 by TGF-β1 in mesangial cells and pTECs of different origin.[67–70] Similarly, miR-192 was found to be overexpressed in fibrotic kidneys of rodents after UUO[67] or renal mass ablation[67,71] but upregulated or downregulated in models of experimental diabetic nephropathy.[68–70] Conflicting findings may be caused by intrinsic differences in the animal models used, in the disease stage studied and/or in the experimental conditions used for cultured cells. One study described a biphasic induction of miR-192 by TGF-β1 in murine mesangial cells that initially involves the Smad pathway followed by a secondary mechanism that sustains miRNA expression by relaxing the locked chromatin structure of the miR-192 gene through Ets1 and histone H3 acetylation by Akt-activated p300.[72] Similar findings were observed in glomeruli from diabetic db/db mice. By contrast, it has been demonstrated in human pTECs that miR-192 is normally expressed, since hepatocyte nuclear factor (HNF) and p53 constitutive binding sites are found in its promoter region. TGF-β1 represses miR-192 transcription by decreasing HNF binding to the miR-192 gene.[73] In the kidney, HNF expression is restricted to the tubular compartment, whereas it is absent in mesangial cells and podocytes,[74] thus partly explaining the cell-specific regulation of miR-192.

Regulation of ECM and cell adhesion proteins

The gene that encodes collagen, the main constituent of the ECM, is characterized by the presence of E-box regulatory elements located in its far upstream region. In murine mesangial cells exposed to TGF-β1 and in glomeruli from diabetic mice, upregulation of miR-192 and miR-200b/c increases *Col1a2* and *Col4a1* expression by inhibiting the E-box repressors Zeb1 and Zeb2.[68,69,75] Consistent with these findings, miR-192 expression negatively correlates with the expression of ZEB1 and ZEB2 in Southwestern American Indians with type 2 diabetes.[76] In mouse mesangial cells, TGF-β1 promotes increased expression of *Col1a2* through upregulation of miR-216a. This effect is mediated by post-transcriptional upregulation of *Tsc22* by Ybx1, an RNA-binding protein that is a specific target of miR-216a. The interaction of Tsc22 with the transcription factor E3 on the far upstream E-box region increases *Col1a2* expression. In line with this finding, a significant increase of Tsc22 was observed in the glomeruli of diabetic db/db mice.[15]

Several studies have demonstrated that the miR-29 family, which targets different isoforms of collagen, has an antifibrotic role. TGF-β1 counteracts the beneficial role of miR-29 family members by downregulating their expression as demonstrated in pTECs,[77,78] mesangial cells[78] and podocytes.[78] The loss of miR-29b contributes to progressive renal injury in various models of CKD,[66,78,79] whereas overexpression of miR-29b exerts a therapeutic effect in UUO and diabetic db/db mice.[66,80] Of note, treatment of rats with advanced diabetic nephropathy with the angiotensin II-receptor blocker losartan resulted in a marked increase in miR-29b expression, which was associated with decreased expression of collagen, fibronectin and laminin, and with protection from renal fibrosis.[78] Similarly, in Munich Wistar Fromter rats, a model of progressive nephropathy, the angiotensin-converting-enzyme inhibitor lisinopril exerted an antifibrotic effect by preventing miR-324-3p-dependent downregulation of prolyl endopeptidase, a serine peptidase involved in the metabolism of angiotensins and in the synthesis of the antifibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), which has a homeostatic role in maintaining collagen balance.[81]

During renal fibrosis, the ECM is also characterized by increased levels of fibronectin. *In vitro* studies in human and mouse mesangial cells documented that high glucose upregulates miR-377, via TGF-β1, which indirectly increases fibronectin levels by reducing the expression of PAK1, SOD1 and SOD2.[82] Consistent with this *in vitro* finding, miR-377 is also upregulated in a mouse model of streptozotocin-induced diabetes.[82] In human pTECs, expression of fibronectin is affected directly by miR-200b.[83]

TGF-β1-induced downregulation of the miR-200 family,[83,84] miR-192[70,85] and miR-215[70] results in decreased levels of E-cadherin (through direct miRNA targeting of the E-cadherin transcriptional repressors Zeb1 and Zeb2) in pTECs, as well as in models of fibrosis including UUO[73] and diabetes.[70] E-cadherin is an epithelial marker involved in cell–cell adhesion whose dysregulation induces epithelial cell phenotype changes.

Epithelial cell ECM production and phenotype changes are also regulated by the miR-30 family as demonstrated in rat pTECs and in the UUO model, in which TGF-β1 suppresses miR-30e expression, resulting in increased expression of UCP2, an anion carrier protein that regulates mitochondrial ATP production. Dysregulation of the miR-30e/UCP2 axis has been linked to decreased ATP generation that ultimately causes kidney fibrosis.[86]

Regulation of cellular hypertrophy

Glomerular mesangial hypertrophy is a hallmark of diabetic nephropathy. Activation of the PI3K/Akt signalling pathway has an important role in inducing cellular hypertrophy and studies have demonstrated that TGF-β1 activates PI3K/Akt signalling through miRNAs.

Table 1 | miRNAs involved in renal diseases

miRNA	Targets	*In vitro* model	*In vivo* model	Effects	Ref(s)
Diabetic nephropathy					
↓ miR-29a	HDAC	Podocytes	STZ-induced diabetic mice	Podocyte dysfunction	98
↑ miR-29c	Spry-1	Podocytes, RMECs	db/db mice	Podocyte apoptosis	99
↓ miR-25	Nox4	Mesangial cells	STZ-induced diabetic rats	Increased oxidant stress	100
↓ miR-93	VEGF-A	Podocytes, RMECs	db/db mice	Increased angiogenesis and ECM accumulation	101
IgA nephropathy					
↑ miR-148b	C1GALT1	PBMCs	IgAN patients	IgA1 aberrant glycosylation	105
↓ miR-223	Importin α4, importin α5	GEnCs	IgAN patients	Glomerular endothelial proliferation	108
Lupus nephritis					
↑ miR-150	SOCS1	pTECs, mesangial cells	LN patients	ECM accumulation	111
Polycystic kidney disease					
↑ miR-17	PKD1, PKD2	HEK-293	PKD mice	Cyst growth	118, 119, 121
↑ miR-92	HNF-1β	—	PKD mice	Cyst growth	118, 120

Abbreviations: ECM, extracellular matrix; GEnC, glomerular endothelial cell; HDAC, histone deacetylase; IgAN, IgA nephropathy; LN, lupus nephritis; miRNA, microRNA; PBMC, peripheral blood mononuclear cell; PKD, polycystic kidney disease; pTEC, proximal tubular epithelial cell; RMEC, renal microvascular endothelial cell; STZ, streptozotocin.

TGF-β1 activates PI3K/Akt by downregulation of PTEN by miR-21 in pTECs,[87] and by miR-21,[87,88] miR-216a and miR-217 in mesangial cells.[89,90] Increased expression of miR-21 has been detected both in animal models of acute and chronic kidney diseases and in human patients.[22,91,92] PI3K/Akt signalling is also controlled by miR-200b and miR-200c, which repress the PI3K inhibitor FOG2 as shown in TGF-β1-treated mesangial cells and in glomeruli of diabetic db/db mice.[93] Of note, miR-200, miR-216a and miR-217 are all activated by miR-192, which triggers a cascade of miRNA modulation leading to Akt activation, hypertrophy and fibrosis in mesangial cells.

TGF-β1 amplification loop

Not only is TGF-β1 considered to be one of the major driving forces in the induction of signalling pathways leading to the development of renal fibrosis, but it has also been proposed as an inducer of signalling loops that amplify and create a chronic state of profibrotic pathway activation. This scenario has recently been suggested to occur in rat pTECs treated with TGF-β1 and in mouse kidneys after UUO where a positive feedback loop induced by TGF-β1 and mediated by miR-433 was considered to be responsible for the induction of renal fibrosis.[94] In this study, the antizyme inhibitor Azin1, a ubiquitous protein that participates in polyamine metabolism and cell growth, was predicted to be a target of miR-433. High levels of miR-433 correlate with increased levels of antizyme, which induces the degradation of ornithine decarboxylase involved in polyamine biosynthesis. Low levels of polyamines in turn activate and sustain the TGF-β1-induced profibrotic effect. *In vivo* knockdown of miR-433 attenuates the induction and progression of fibrosis. Moreover, it was demonstrated that TGF-β1 is able to reinforce its signalling by suppressing let-7b, which directly targets TGFBR1 in rat pTECs.[95] Consistent with this observation, let-7b expression was reduced in models of both diabetic and non-diabetic renal fibrosis. A study in human pTECs demonstrated a similar role for let-7c, another member of the let7 family that targets TGFBR1 together with COL1A1, COL1A2 and thrombospondin.[96] Lipoxins attenuate renal fibrosis in a rat UUO model by inducing let-7c.[96]

miRNA-regulated circuits that amplify TGF-β1 signalling have also been demonstrated in mouse mesangial cells and in the glomeruli of diabetic mice, with an underlying auto-regulatory loop involving TGF-β1, miR-192 and key miR-200 family members.[75] Along this line, crosstalk between p53 and miR-192 induced by TGF-β1, via the miR-192 target Zeb2, has been described as a circuit loop in mouse mesangial cells and in glomeruli of diabetic mice.[76]

A positive feedback loop between TGF-β1 and miR-21, via the miR-21 target Smad7 in mesangial cells has been shown as a possible mechanism that amplifies TGF-β1 signalling during renal fibrosis in mouse models of type 2 diabetes.[92,97]

miRNAs in diabetic nephropathy

In addition to the miRNAs involved in TGF-β1 signalling, other miRNAs directly modulate the high-glucose-induced perturbation of renal tissue homeostasis that occurs in diabetic nephropathy (Figure 2, Table 1). High glucose levels induce apoptosis of mouse podocytes by downregulating miR-29a,[98] and overexpressing miR-29c.[99] Low levels of miR-29a affect the integrity of podocyte ultrastructure and correlate with increased levels of its target histone deacetylase, which modulates the acetylation status of nephrin.[98] Increased levels of miR-29c promote cell apoptosis and fibronectin synthesis through a mechanism that involves Rho kinase activation by inhibition of Spry-1, a specific gene target of miR-29c.[99] Consistent with these observations, miR-29c is upregulated in glomeruli from diabetic db/db mice. Furthermore, high glucose promotes oxidant stress by downregulating miR-25, which targets the major catalytic subunit of NADPH, Nox4, as demonstrated in rat mesangial cells exposed to high glucose and in diabetic rat kidneys.[100] Moreover, high glucose downregulates miR-93 expression in cultured mouse podocytes, in kidney microvascular endothelial cells and in the glomeruli of diabetic db/db mice. Low levels of miR-93 correlate with an increased expression of vascular endothelial growth factor A together with its targets fibronectin and Col4a3, implicating an antifibrotic and anti-angiogenic role for miR-93.[101]

An analysis of the miRNA expression profile of kidneys from patients with diabetic nephropathy showed that levels of miR-192 decreased with progression of the disease and correlated with estimated glomerular filtration rate and with histopathological evidence of tubulointerstitial fibrosis,[85] whereas miR-200b levels remained unchanged.

Moreover, circulating miRNAs are differentially expressed throughout the progression of diabetic nephropathy, paving the way for the use of miRNAs as predictors of disease course. An analysis of miRNAs in the urine of individuals ranging from those with no renal disease through to those with overt diabetic nephropathy identified a differential signature associated with each stage.[102] Similarly, analysis of miRNA expression in urinary exosomes from patients with type 1 diabetes revealed that patients with microalbuminuria at the early stages of the disease expressed higher levels of miR-130a and miR-145 and lower levels of miR-155 and miR-424 than did patients with normoalbuminuria.[103] Another study of patients with CKD who underwent renal biopsy found that diabetic nephropathy is associated with decreased urinary levels of miR-15.[104]

miRNAs in immune diseases

IgA nephropathy (IgAN) is characterized by aberrant glycosylation of IgA1, a reaction catalysed in part by C1GALT1, which has been proposed to be a target of miR-148b. miR-148b is upregulated in peripheral blood mononuclear cells (PBMCs) isolated from patients with IgAN, and patients carrying the 1365G/G genotype in the miR-148b-binding site of C1GALT1 have increased C1GALT1 binding affinity for miR-148b and consequently lower enzyme expression (Table 1).[105]

Global miRNA expression analysis in kidney biopsy samples from patients with IgAN found that dysregulated levels of miRNAs related to fibrosis (downregulation of miR-200c and upregulation of miR-192, miR-141 and miR-205)[106] and to the immune response (upregulation of miR-155 and miR-146a)[107] correlated with disease severity and progression. Moreover, glomerular endothelial cells of patients with IgAN are characterized by reduced expression of miR-223, which causes glomerular endothelial proliferation, a pathological hallmark of IgAN.[108] Low levels of miR-223 were also found in circulating endothelial cells, providing a possible non-invasive method for evaluating the severity of IgAN (Table 1). Other potential biomarkers of IgAN are miR-155,[109] miR-146a,[109] miR-17[104] and miR-93—which are found at increased levels—and miR-29b and miR-29c—which are found at decreased levels—in the urine of patients with IgAN.[109]

miRNA dysregulation also has a key role in lupus nephritis, an autoimmune disorder with a complex pathophysiology. miRNA expression analysis in kidney biopsy samples of patients with class II lupus nephritis (characterized by pure mesangial involvement), identified 36 upregulated and 30 downregulated miRNAs compared with healthy controls.[110] miR-150 has also been demonstrated to promote renal fibrogenesis in lupus nephritis (Table 1).[111,112] miR-150 targets SOCS1, a negative regulator of the JAK/STAT signalling pathway, which regulates a wide range of genes involved in cell proliferation, inflammation and fibrosis. In human mesangial cells and pTECs, TGF-β1 upregulates miR-150, resulting in decreased expression of SOCS1 and increased production of profibrotic proteins. SOCS1-knockout mice spontaneously develop a lupus-like disease; likewise, the kidneys and in particular the tubular cells of patients with fibrosing lupus nephritis are characterized by increased expression of miR-150 in association with lower levels of SOCS1.[111]

In patients with lupus nephritis of differing severities, a correlation was identified between clinical disease severity and glomerular and tubulointerstitial expression of miR-638, miR-198 and miR-146.[113] Analysis of miRNA expression profiles in plasma,[114] urinary sediment[115] and PBMCs[116] revealed a subset of miRNAs associated with lupus nephritis (miR-342-3p, miR-223 and miR-20a in plasma, miR-221 and miR-222 in urinary sediment, and miR-371-5p, miR-1224-3p and miR-423-5p in PBMCs), which are potential promising disease biomarkers.[117]

miRNAs in PKD

miRNAs can contribute to the onset of PKD by directly regulating the expression of PKD1 and PKD2 (responsible for autosomal-dominant PKD) and PKHD1 (responsible for the less common autosomal-recessive PKD), as well as by affecting numerous target genes involved in cell proliferation (Table 1). The miR-17~92 cluster is upregulated in the kidney of various mouse models of PKD and its overexpression produces cysts in mice.[118] In particular, miR-17 represses PKD1 and PKD2,[118,119] whereas miR-92 inhibits PKHD1 through the transcription factor HNF-1β.[118,120] Tran and colleagues suggested that the binding of miR-17 to PKD2 is antagonized by the RNA-binding protein Bicc1, thus regulating PKD2 gene dosage.[121] Another study demonstrated that Bicc1 also has a key role in the regulation of cAMP signalling—the dysregulation of which is a key aspect of PKD—by inducing the silencing of two key enzymes in this pathway by recruiting miR-125a and miR-27a to their target sites.[122] Consistent with these observations, Bicc1-knockout mice develop a PKD phenotype. Of note, in other experimental settings it has been demonstrated that the miR-17~92 cluster regulates mTOR and TGF-β pathways, both of which are implicated in cyst growth.[118]

In addition to disease induction, miRNAs have a key role in the progression of PKD, affecting several pathways involved in PKD pathogenesis, including TGF-β, MAPK and calcium signalling pathways, as demonstrated in a rat model of autosomal dominant PKD.[123,124] In the same animal model, a parallel analysis of kidney mRNA and miRNA expression using microarrays identified miR-214, miR-31, miR-199a-5p, miR-21, miR-34a, miR-132 and miR-146b as miRNAs that could target major pathways in autosomal dominant PKD.[125] Complete analysis of kidney miRNA expression in human PKD would provide new insights regarding their roles in the development of autosomal dominant PKD.

Table 2 | miRNA-based therapies in models of chronic kidney disease

Targeted miRNA	Approach	miRNA modulation	Gene transfer method	*In vivo* model	Ref(s)
miR-21	Knockdown	miR-21 shRNA plasmid	US-mediated transfer into the tail vein	UUO mice	65
		Inducible miR-21 knockdown plasmid	US-mediated transfer into the tail vein	db/db mice	92
		Anti-miR-21	Intraperitoneal injection	UUO mice	22
miR-29b	Overexpression	Inducible pre-miR-29b plasmid	US-mediated transfer into the tail vein	UUO mice	66,80
miR-29c	Knockdown	miR-29c antisense oligonucleotide	Intraperitoneal injection	db/db mice	99
miR-192	Knockdown	LNA-anti-miR-192	Subcutaneous injection	STZ-induced diabetic mice	69,76
miR-214	Knockdown	Anti-miR-214	Subcutaneous injection	UUO mice	23
miR-433	Knockdown	Inducible miR-433 shRNA plasmid	US-mediated transfer into the tail vein	UUO mice	94

Abbreviations: LNA, locked nucleic acid; miRNA, microRNA; sh, short hairpin; STZ, streptozotocin; US, ultrasound; UUO, unilateral ureteral obstruction.

Based on results of the miRNA profile of urine from patients with autosomal dominant PKD, miR-1 and miR-133b have been proposed as potential biomarkers for monitoring disease progression and response to treatments.[126] These results offer the rationale for further investigation in this field.

miRNAs in kidney transplantation

miRNA profiling could be a promising tool for monitoring the status of transplanted kidneys, which despite advances in immunosuppressive therapy can undergo acute rejection (an event that is becoming increasingly less common) or chronic rejection. Two research groups have identified miRNAs that were differentially expressed in biopsy samples from the kidneys of patients with acute rejection, although no overlapping miRNAs were found.[127,128] In particular, Anglicheau and colleagues demonstrated that increased intragraft levels of miR-142-5p, miR-155 and miR-223, probably derived by graft-invading immune cells, and decreased levels of miR-10b, miR-30a-3p and let-7c, expressed by resident renal cells, were predictive of renal graft function.[128] One study identified miRNA signatures in kidney biopsy samples that were able to distinguish between acute cellular and humoral rejection and delayed graft function.[129] Of note, Lorenzen *et al.*[130] analysed miRNA expression in the urine of patients with acute rejection and proposed miR-210 as a novel biomarker of acute rejection and predictor of long-term graft function.

Interestingly, analysis of a blood-derived miRNA signature of drug-free tolerant patients after kidney transplantation identified eight modulated miRNAs. Among these miRNAs, overexpression of miR-142-3p in PBMCs was correlated with drug-free tolerance, probably owing to the negative regulation of TGF-β signalling by miR-142-3p. Thus, miR-142-3p expression represents a promising predictor of patients who could reduce or even avoid immunosuppressive therapies.[131]

Three reports studied miRNA changes during chronic rejection characterized by interstitial fibrosis and tubular atrophy (IF/TA). miR-142-3p, miR-204 and miR-211 were differentially expressed in kidney biopsy samples and urine of patients with chronic rejection characterized by IF/TA compared with patients with normal histology and a functioning allograft.[132] A subsequent study confirmed miR-142-3p overexpression in biopsy samples with IF/TA, and also reported dysregulation of miR-21, miR-142-5p, the cluster comprising miR-506 on chromosome X, miR-30b and miR-30c in these samples.[133] Investigation of miRNA expression in urinary cell pellets from patients diagnosed with chronic rejection with IF/TA compared with those with normal renal function identified 22 differentially expressed miRNAs, mainly associated with inflammation.[134] More importantly, these researchers identified a subset of miRNAs (miR-200b, miR-375, miR-423-5p, miR-193b and miR-345), which are promising biomarkers for monitoring graft function and anticipating progression to chronic rejection, as they were differentially expressed between the two groups soon after kidney transplantation but before histological injury was evident.[134]

Potential uses of miRNAs for therapy

Mature miRNAs possess distinct features that make them potentially suitable as therapeutic agents, including their short sequence and their high homology across multiple vertebrate species. Manipulation of the activity of specific miRNAs in the kidney can be achieved by *in vivo* delivery of mimics to restore miRNA levels or inhibitors to block miRNA function. miRNA mimics are double-stranded synthetic oligonucleotides that accomplish the endogenous functions of the miRNA of interest, but following chemical modifications possess increased stability and are efficiently taken up by cells. The most widely adopted strategy so far to block miRNA function is with chemically modified oligonucleotides (anti-miRs) designed against the mature miRNA sequence that are stable in circulation and are cell permeable (2'-O-methyl-group-modified oligonucleotides or locked nucleic acid anti-miRs). In addition to anti-miRs, miRNA inhibition can be achieved by expression of miRNA-target sequences able to capture pathogenic miRNAs (miRNA sponge), short hairpin RNA plasmids to abrogate miRNA expression via RNA interference, or using oligonucleotides complementary either to the 3' untranslated region of the target mRNA binding site sequence (masking approach) or to the sequence of the miRNA (erasers).

Many studies in experimental animal models have focused on the therapeutic potential of miRNAs in CKD and promising results in halting renal fibrosis have been obtained by knocking down miR-21,[22,65,92] miR-29c,[99]

miR-214,[23] miR-433,[94] and miR-192[69,76] or overexpressing miR-29b (Table 2).[66,80] Successful kidney transfection was achieved by intraperitoneal, intravenous or subcutaneous injection of either mimics or inhibitors or, more frequently, by intravenous injection of plasmids expressing miRNAs or short-hairpin RNAs.

However, many obstacles must be overcome before miRNA-based therapies for CKD can be translated into clinical practice, including delivery methods and safety concerns. Indeed, the target miRNA should be kidney-specific in order to avoid any potential adverse effects in other tissues and organs, and should affect only one target (or targets acting in the same pathway) to avoid effects on unintended templates, as in the case of miR-21, the knockdown of which induces cell death in addition to halting renal fibrosis.[65] So far, these limitations have been partially overcome either by local administration of miRNA-based drugs or by using vectors containing kidney-specific and inducible promoters.

Conclusions

The importance of miRNAs in the kidney field is increasingly recognized as they enable researchers to understand in-depth the pathways that have a role in kidney physiology and disease. They can also provide an explanation for divergent transcriptomic and proteomic data. Moreover, an miRNA-based therapy that either restores or blocks miRNA expression and activity is very attractive, especially now that the first miRNA-targeted drug (miravirsen for the treatment of hepatitis C) has entered a phase II clinical trial.[135] So far, the potential of miRNAs as an effective antifibrotic therapy has been demonstrated only in experimental models of CKD because many safety concerns must be resolved, from the delivery method to the adverse effects on alternative templates. Instead, a more impending application is the detection and quantification of circulating miRNAs as a novel non-invasive, repeatable method to identify and monitor the degree of disease. However, because most miRNAs are highly pleiotropic and act differently depending on the cell type, a single miRNA is unlikely to be able to diagnose and predict a form of CKD. Instead, a network of correlated miRNAs must be considered.

Review criteria

The PubMed database was searched for scientific papers published up to February 2014 using the following search terms: “microRNA”, “chronic kidney diseases”, “kidney fibrosis”, “TGF-β”, “diabetic nephropathy”, “IgA nephropathy”, “lupus nephritis”, “polycystic kidney disease” and “kidney transplantation”. This Review primarily focused on recent literature published after March 2011, although older papers that are relevant to this topic were also selected.

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Author contributions

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