MicroRNAs in kidney diseases: new promising biomarkers for diagnosis and monitoring

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ABSTRACT

A series of microRNAs (miRNAs) have a critical role in many cellular and physiological activities such as cell cycle, growth, proliferation, apoptosis and metabolism. miRNAs are also important in the maintenance of renal homeostasis and kidney diseases. In vitro and in vivo animal models have shown a critical role of miRNAs in the development of diabetic nephropathy (DN) and in the progression of renal fibrosis. Specific miRNAs in renal tissue and peripheral blood mononuclear cells (PBMCs) are up and downregulated in different kidney diseases. They represent new potential biomarkers for diagnosis and targeted therapy. In addition, urinary miRNAs may be considered non-invasive biomarkers for monitoring the progression of renal damage. The activity of miRNAs can be modified by different approaches such as the use of antisense oligonucleotide inhibitors (antagomirs), tandem miRNA-binding site repeats manufactured by Decoy or Sponge technologies and miRNA mimics. The use of miRNA blockers or antagonists as therapeutic agents is very attractive but new information will be necessary considering their role in other systems.

Keywords: biomarkers, epigenomics, microRNAs, renal disease, therapy
strands are likely degraded. The main constituents of the RISC are members of the Argonaute (AGO) family that have robust endonuclease activity to degrade the target mRNAs or to block protein translation. The mechanism of how miRNA induces the inhibition of translation is currently unknown. In animal cells, single-stranded miRNAs associated with RISC usually bind to target mRNAs through partial base-pairing, and result in translational repression. In plants, most miRNAs trigger mRNA cleavage through perfect base-pairing between miRNAs and target mRNAs [2, 3]. Each miRNA can potentially regulate the translation of a large number of different mRNAs, and each mRNA can possess multiple binding sites for a single or for many different miRNAs because the specificity of miRNAs is mainly determined by Watson–Crick base pairing at the 5′-region of the miRNA [4].

During these last few years, the number of miRNAs in the miRBase registry has increased. Different methods have been developed for miRNA expression profiling in order to better understand gene regulation under normal and pathological conditions. Microarray technologies are particularly used for miRNA profiling as they are able to screen large numbers of miRNAs simultaneously. However, miRNA expression obtained from high-throughput arrays has to be validated with alternative technologies. A quantitative real-time PCR has become the gold standard of miRNA quantification because it offers the highest sensitivity from small amounts of starting material and it is able to detect as less as 1-nt difference between miRNAs [5]. Moreover, an miRNA real-time PCR array can be used for the screening of miRNAs obtained from small amounts of blood samples, cells and renal biopsies (frozen or formaline-fixed paraffin-embedded material).

FIGURE 1: The multistep process of miRNA biogenesis in the cell. Length of miRNA: primary-miRNA (pri-miRNA) (several thousand bases long); precursor-miRNA (pre-miRNA) (~70 nucleotides); mature miRNA (21–25 nucleotides).

MODULATION OF PHYSIOLOGICAL RENAL ACTIVITIES

By regulating gene expression, miRNAs have critical roles in innate immunity and in a variety of cellular and physiological activities such as cell cycle, growth, proliferation, apoptosis and metabolism. miRNAs are also important in the maintenance of renal homeostasis.

As illustrated in the previous section, Dicer is an enzyme responsible for the processing of pre-miRNAs into mature miRNAs. The genetic ablation of Dicer leads to global depletion of miRNAs. Knockout conditions of Dicer have been created in cultured cells and in mice for kidneys. Thus, knockout models of Dicer have been obtained in podocytes, proximal tubular cells and juxtaglomerular cells. Interestingly, the podocyte-specific Dicer knockout model showed foot process effacement at 2–4 weeks after birth, massive proteinuria, tubulointerstitial fibrosis and glomerular sclerosis [6–8]. These findings clearly demonstrate that Dicer is critical for the maintenance of podocyte homeostasis and associated renal functions. In this case, the Dicer deletion was responsible for the loss of miR-30 family which modulates the podocyte functions.

Mice lacking Dicer in kidney tubules and collecting ducts show hydronephrosis, hydroureter and cyst formation [9]. Patel et al. [9] used the Dicer ablation of epithelial tubules for demonstrating that the miR-200 family targets polycystin-1 and interferes with tubule formation in IMCD3 cells. Thus, the regulation of polycystin-1 is important for the cystogenesis in polycystic kidney disease (PKD). Another miRNA family, miR-17, is implicated in PKD because it targets polycystin-2, involved in cyst formation [10]. These data demonstrate that
perturbation of miRNAs in renal epithelial cells contributes to PKD development in the organogenesis.

Wei et al. [11] created a knockout mouse model with ablated Dicer in the proximal tubular cells. After a bilateral renal ischaemia-reperfusion was induced, this mouse was resistant to acute kidney injury (AKI). Microarray analysis demonstrated some change in miRNAs (miR-132, miR-362, miR-379, miR-668, miR-687) during 12–48 h of reperfusion. In another model of acute kidney injury induced by cisplatin nephrotoxicity, an upregulation of miR-34a was observed [12]. The administration of pithrin-α (a miR-34a inhibitor) showed an improvement in the renal function. These findings suggest that future studies may prove different profiling of miRNAs in different forms of AKI, and they may be potential targets for therapeutic indications.

Sequeira-Lopez et al. [13] induced Dicer deletion in the renin-secreting juxtaglomerular cells. In this mouse model, an acute loss of juxtaglomerular cells associated with reduced expression of renin in kidneys and reduced plasma renin concentration was observed. The mouse model developed decreased blood pressure, vascular abnormalities and renal striated fibrosis. In another model, Nagalakshmi et al. [14] demonstrated that Dicer ablation played a critical role in the development of nephrogenic and ureteric compartments in the mammalian kidney.

These different animal models of Dicer deletion clearly show that miRNAs play an important role in the physiological functions of kidneys.

**IN VITRO AND IN VIVO ANIMAL MODEL STUDIES**

Fibronectin is a key matrix protein highly accumulated in diabetic nephropathy (DN). Wang et al. [15] demonstrated that in cultured human and mouse mesangial cells exposed to high glucose and TGF-β, which simulate the diabetic milieu, miR-377 was consistently upregulated. This miRNA was associated with increased expression of the matrix protein, fibronectin. Computational analysis indicated that some target genes such as PAK 1 and SOD1/2 were reduced in their activity by miR-377. This leads to enhanced susceptibility to oxidant stress and accumulation of extracellular matrix fibronectin. Thus, this miRNA could have a critical role in the mesangial cell response to the diabetic milieu and could be considered a target miRNA for therapy.

The profibrotic synthesis and accumulation of the extracellular matrix induced by TGF-β1 are also modulated by another miRNA, miR-29, as shown by Bo Wang et al. [16] after exposition of proximal tubular cells, primary mesangial cells and podocytes to TGF-β1. Suppression of miRNA-29 by TGF-β1 promoted collagen deposition and renal fibrosis.

Kato et al. [17] identified SIP1 (Smad-interacting protein 1) as a target of miR-192 in mouse mesangial cells. SIP1 is an E-box repressor belonging to the same family as δEF1, a key inhibitor of E-cadherin. Smad transcription factors have been shown to be the major effectors of TGF-β signalling. Collagen 1-α 1 and -2 (Col1a1 and -2) and other extracellular matrix (ECM) genes are regulated in mesangial cells by TGF-β via Smads. The collagen gene has E-box elements in the far upstream enhancer region. They discovered a mechanism for TGF-β-mediated collagen regulation that involves a cross-talk between E-box repressors (δEF1 and SIP1) and miR-192 [17]. Thus, TGF-β-induced downregulation of SIP1 (via miR-192) and δEF1 (via as-yet-unknown mechanisms) and can cooperate to enhance Col1a2 expression via derepression at E-box elements. They also confirmed these results in a mouse model of diabetes showing that TGF-β inducing miR-192 is responsible, at least in part, for increased Col1a2 expression not only in vitro in mesangial cells but also in vivo [17].

Furthermore, Putta et al. [18] in a mouse model of streptozotocin-induced DN evaluated the efficacy of a locked nucleic acid (LNA)-modified inhibitor of miR-192 designated LNA-anti-miR-192. This inhibitor significantly reduced the expression levels of miR-192, increased the activity of the E-box repressors (SIP1 and δEF1) and downregulated the expression of collagen, TGF-β and fibronectin. These findings support the possibility that anti-miR may contribute to the treatment of renal fibrosis in DN.

Zhang et al. [19] investigated the potential role of miR-451 in mesangial hypertrophy during early DN in vitro and db/db mice, which is a model of type 2 diabetes. From bioinformatic analysis, they discovered five potential target genes for miR-451 which downregulated the expression of Ywhaz (tyrosine3-monooxigenase, tryptophan 5 monooxigenase activation protein, zeta) gene in the 3’ UTR. The overexpression of miR-451 caused the reduction activities of two kinases (p38 MAPK and MKK3) and the suppression of Ywhaz. This miR-451 targeting Ywhaz prevented mesangial hypertrophy and glomerular mesangial cell proliferation in vitro and in vivo. Therefore, this miRNA may be a potential target for intervention and prevention of DN in an early stage.

Fu et al. [20] demonstrated in diabetic rats induced by streptozotocin a significant reduction of miR-25 in kidneys and in high-glucose-treated mesangial cells, accompanied by an increase of NOX 4 expression levels. These results indicate that miR-25 is an endogenous silencing gene which contributes to the regulation of NOX 4 expression and NADPH oxidase derived superoxide function in DN in which hyperglycaemia induces oxidative stress. This silencing action of miR-25 on NOX 4 gene may represent a novel molecular mechanism in the regulation of redox signalling molecules. Targeting miR-25 could be an ideal approach for therapeutic intervention in chronic kidney disease (CKD) related to DN.

Renal fibrosis is characterized by increased synthesis and deposition of extracellular matrix within glomeruli and tubointerstitium in CKD. TGF-β regulates several miRNAs to control epithelial-to-mesenchymal transition and fibrosis. It upregulates the expression of miR-21 which is present in kidney and other organs. Recently, Zhong et al. demonstrated that miR-21 expression was upregulated in tubular epithelial cells in response to TGF-β1 and in the mouse fibrotic kidneys of the unilateral ureteral obstruction [21]. This upregulation was dependent on the activation of TGF-β/Smad3 signalling. Interestingly, when Zhong et al. suppressed miR-21 by a short hairpin RNA (shRNA) plasmid, they ameliorated the renal...
fibrosis in the mouse model of obstructive nephropathy and halted the progression of renal fibrosis downregulating the expression of collagen I, fibronectin and α-SMA. These findings clearly show that miR-21 is a promoter of renal fibrosis and its inhibition may be a therapeutic approach to suppress renal fibrosis. Increased miR-21 is also reported in hypertensive nephropathy, anti-Thy1,1 glomerulonephritis and DN. Thus, miR-21 is one of the most extensively investigated miRNAs.

A piece of recent information on the role of miR-21 in promoting renal fibrosis was given by Chau et al. [22] who demonstrated that this upregulated miRNA inhibits several major metabolic pathways, in particular the lipid metabolism and the oxygen radical production. In these pathways, Pparγ and Mpv17L are the miR-21 targets. Silencing of miR-21 may be effective in limiting renal fibrosis in chronic glomerulonephritis and chronic allograft dysfunction.

TGF-β also downregulates the expression of miR-29a/b/c family, which targets collagen gene expression and increases the expression of ECM proteins. Wang et al. [16] demonstrated in three models of renal fibrosis, representing early and advanced stages of DN, low levels of miR-29. The administration of the Rho-associated kinase inhibitor fasudil or losartan prevented renal fibrosis and restored expression of miR-29. These findings suggest that pharmacological modulation of these miRNAs may have a potential benefit for progression of renal fibrosis.

Ichii et al. [23] created an animal model of CKD in B6. MRLc1 mice which spontaneously developed chronic kidney inflammation, membranoproliferative glomerulonephritis and interstitial fibrosis with mononuclear cell infiltration, similar to human CKD. This model showed mild glomerular lesions in the 6-to-8-month-old mice, whereas interstitial lesions and severe glomerular lesions were observed in the 10-to-12-month-old mice. They found high expressions of miR-146a/b in the kidneys which increased with the ageing and the development of CKD. In situ hybridization demonstrated high expression of miR-146a in interstitial lesions containing inflammatory cells. Bioinformatic analyses evidenced that target genes RELA, IRAK 1, IL 1 B, IL 10 and CXCLs, were present in monocytes. The miR-146a renal expression and its urinary excretion were associated with the development of interstitial lesions and correlated with infiltrating inflammatory cells, especially those of the monocytic lineage. Furthermore, the in vitro study demonstrated that the expression of miR-146a increased in monocytes stimulated with lipopolysaccharides and cytokines (IL-1β and TNF-α). Thus, macrophages expressing miR-146a in inflammatory lesions may participate in the progression of renal damage through NF-κB-dependent or independent signalling pathways. This data suggests the important role of miR-146a in the local regulation of inflammatory mediators and provide new information on the molecular pathogenesis of CKD. Recently, Maccioni et al. [24] investigated the role of miRNAs in the fibrotic process of Munich-Wistar rats which develop spontaneous progressive nephropathy. They found an upregulation of miR-324-3p in microdissected glomeruli which was associated with markedly reduced expression of prolyl endopeptidase (Prep), a serine peptidase involved in the metabolism of angiotensin, which is the predicted target of miR-324-3p. ACE inhibition downregulated miR-324-3p at glomerular and tubular levels and promoted renal Prep expression. These data suggest that the renoprotective effect of ACE inhibitors may be due, in part, to the modulation of the miR-324-3p/Prep pathway which is another potential target. In conclusion, the results of these in vitro and in vivo studies support the possibility that anti-miR and drug molecules may be considered as a future translational approach for the treatment of DN and CKD.

**INTRARENAL EXPRESSION OF miRNAs**

A greater abundance of miRNAs compared with other organs is present in the kidneys. Their expression makes them a valuable tool for understanding, diagnosing and discovering therapeutic options for renal diseases. miR-192, miR-194, miR-204, miR-215 and miR-216 are highly expressed, whereas miR-133a, miR-133b, miR-1d, miR-296, miR-1a, miR-122 and miR-124a are poorly expressed [25]. Differential expression of miRNAs between the cortex and medulla is present; in fact, miR-192 is 20-fold higher expressed in cortical than in medullary kidney tissue, where it is involved in the regulation of sodium transport [26]. miR-155 appears to suppress expression of the type 1 angiotensin II receptor and would affect blood pressure [27].

Wang et al. [28] studied the intrarenal expression of miRNAs in IgA nephropathy (IgAN) patients which was characterized by downregulated miR-200c and upregulated miR-141, miR-192 and miR-205. These miRNAs diversely correlated with disease severity and progression of renal damage. These findings suggest that some miRNAs may have an important role in the progression of renal damage. In this process, the deregulated expression of miR-200c and miR-205 is very interesting for their link with epithelial-to-mesenchymal transition.

Dai et al. [29] studied the microRNA expression profile in a small number of renal biopsy samples from patients with class II lupus nephritis and healthy controls. Sixty-six miRNAs were found differentially expressed (36 upregulated and 30 downregulated) in lupus nephritis [28]. High intrarenal expression of miRNAs (miR-141; miR-192; miR-200a; miR-200b; miR-205; miR-429) was also found in renal biopsies of patients with hypertensive glomerulosclerosis and the degree of upregulation correlated with disease severity [30].

Expression profiling of miRNAs from formalin-fixed, paraffin-embedded tissue of renal biopsies of patients with DN showed higher miR-192 in progressors and non-progressors compared with late presenters, suggesting loss of this miRNA expression in advanced DN [31]. An in situ hybridization study localized miR-192 in both glomerular and tubular cells. In addition, miR-192 expression correlated inversely with loss of renal function and with the presence of tubulointerstitial fibrosis. In vitro experiments on proximal tubular epithelial cell lines demonstrated a decrease of miR-192 expression in response to TGF-β which post-transcriptionally regulated this miRNA. These results are different from those of Kato et al.
found an increase of miR-192 after stimulation of mesangial cells with TGF-β. This suggests that mesangial cell and proximal tubular epithelial cell miRNA expression profiles may exhibit divergent responses to profibrotic stimuli such as TGF-β.

Suthanthiran’s group, recently, studied the miRNA expression profile of human kidney allograft tissue with interstitial fibrosis/tubular atrophy and individualized miR-21 overexpression in resident renal cells involved in the fibrotic process in association with other miRNAs (miR-142-3p, miR-223, miR-150, miR-155) which express localized intrarenal circulating lymphomonocytes [32]. Thus, these expressed miRNAs and their predicted targets are candidates for the mechanism of interstitial fibrosis and tubular atrophy in kidney transplantation.

Scian et al. [33] studied miRNA expression in renal biopsies of transplant patients with chronic allograft dysfunction. They found the differential expression profile of miR-142-3p, miR-204 and miR-211 in the kidney and in paired urine samples. Because of the high correlation between miRNAs and mRNA pairs related to immune cellular process, the urinary pattern may be considered an attractive, non-invasive tool for early diagnosis of chronic allograft dysfunction.

The different expression of miRNAs in renal biopsy samples of patients with different kidney diseases is very interesting; however, additional studies could explain whether these miRNA alterations contribute to development of the renal disease or are disease specific.

**RENAL CELL CARCINOMA**

A number of miRNA expression studies have been carried out in renal cell carcinoma (RCC). A decreased miR-199a expression was found in fresh surgical renal specimens, which correlated with high expression of nuclear GSK-3β and higher tumour stage. Re-expression of miR-199a downregulated GSK-3β and suppressed cancer cell growth [34]. These findings demonstrate low miR-199a expression in advanced RCC, and its re-expression may be considered a new potential target for the treatment of RCC.

The expression of miR-205 was significantly suppressed in renal cell cancer lines and RCC samples [35]. This miRNA suppresses some potential gene targets encoding Src, Lyn, Yes and Lck which are involved in cell migration invasion and cell proliferation. Therefore, its downregulation may facilitate RCC proliferation and diffusion. This study demonstrates that local administration of miR-205 in established tumours may induce a dramatic regression of tumour growth.

Expression of miR-584 was found downregulated in RCC tissue samples [36]. It was correlated with high expression of R0CK-1 protein which modulates the cell motility. This condition promotes tumour cell diffusion; therefore, the upregulation of miR-584 may improve progression of RCC and patient survival.

Aberrant expression of miR-1285 may inhibit cancer cell proliferation, invasion and migration [37]. Downregulation of this miRNA, which targets oncogenic genes, might contribute to RCC development. This novel molecular target may provide new insights into the potential mechanisms of RCC oncogenesis.

**CIRCULATING miRNAs**

The majority of miRNAs are present in the cytoplasm of cells, but many miRNAs are outside the cells and in the body fluids [38, 39]. Circulating miRNAs are stable and have distinct expression profiles among different fluids. While miRNAs are stable in the extracellular compartment, miRNAs maintain their stability which is probably due to the presence of ribonucleases in serum and other body fluids [40, 41].

The function of circulating miRNAs is poorly understood; however, one of the most intriguing hypotheses is their role as mediators in the cell–cell communication [42]. In fact, miRNAs and their corresponding RNAs are present in microvesicles (ranging from 10 to 1 μ) and exosomes (ranging from 30 to 100 nm). These vesicles are released from bone marrow stem cells [43], resident adult renal stem cells [44], endothelial cells [45] and dendritic cells [46].

miRNAs are also transported by the RNA-binding protein Argonaute 2, which is part of the RNA-induced silencing complex, or by nucleophosmin 1. Other processes carrying miRNAs are represented by cell damage culminating in apoptosis [47] and high-density lipoprotein in subjects with familial hypercholesterolaemia [48]. For instance, miRNAs in peripheral circulation may serve as a measure of disease stage or for monitoring therapeutic response.

Many studies have shown that circulating miRNAs are remarkably stable in body fluids [49]. However, a recent study demonstrated that circulating levels of some miRNAs are reduced in plasma samples of patients with severe chronic renal failure when compared with subjects with mild renal impairment or minimal renal function [50]. These findings have important implications for the use of circulating miRNAs as biomarkers in individuals with severe renal damage.

In a global analysis of circulating plasma miRNAs, Lorenzen et al. [51] revealed 13 of them with different regulation between patients with AKI and healthy controls. The authors quantified three miRNAs (miR-16 and miR-320 as downregulated; miR-210 as upregulated), and concluded that miR-210 might be considered a strong independent predictor of survival in critically ill patients with AKI. Circulating miRNAs were analysed in serum samples of patients with RCC. Among 36 upregulated miRNAs in RCC tissue and serum, Wulfken et al. [52] identified the circulating miR-1233 as a potential biomarker for RCC patients.

Low levels of miR-508-3p and miR-509-3p have been found in plasma and renal cancer tissue samples of patients with RCC [53]. The over-expression of these miRNAs in vitro suppressed the proliferation of RCC cells, induced cell apoptosis and inhibited cell migration. These findings suggest that miR-508-3p and 509-3p have an important role as modulators in tumour formation, and they may be considered as novel diagnostic biomarkers for RCC.
miRNAs IN PERIPHERAL BLOOD MONONUCLEAR CELLS

The miRNA profile has been investigated in peripheral blood mononuclear cells (PBMCs) of some renal diseases. Our group recently studied the miRNA profile in PBMCs of IgAN patients [54]. Six miRNAs were found significantly upregulated in IgAN patients compared with healthy subjects. Among them, the upregulated miR-148b targeted the glycosyltransferase C1GALT1, which is typically downregulated in IgAN. Based on our results, we demonstrated that in IgAN, lower levels of C1GALT1, due to inhibiting effect of over-expressed miR-148b, lead to an increased production of circulating deglycosylated IgA1.

Dai et al. [55] studied the microRNA expression in PBMCs of 23 Chinese patients with systemic lupus erythematosus and 10 healthy controls. They found a differential expression of 16 miRNAs (7 downregulated and 9 upregulated).

Te et al. [56] analysed miRNA expression in PBMCs in Epstein-Barr virus transformed cell lines of Afro-American and Euro-American patients affected by lupus nephritis. Five miRNAs (miR-371-5p; miR-423-5p; miR-638; miR-663; miR-1224-3p) were differently expressed across different ancestries and all tested specimens. miR-371-5p, miR-1224-3p; miR-423-5p were associated with lupus nephritis. All these miRNAs may serve as SLE-specific signature and could be used as biomarkers for the diagnosis of lupus nephritis.

Anglicheau et al. [57] investigated the miRNA profile of PBMCs as well as of kidney tissue biopsies in patients with acute renal allograft rejection who showed an miRNA pattern represented by overexpressed miR-142-5p, miR-155 and miR-223. This pattern was confirmed in renal biopsies of same patients, indicating that the PBMC pattern of miRNAs may be used as a non-invasive diagnostic test in patients with acute allograft rejection.

URINARY miRNAs

Urinary miRNAs are filtered or excreted from the kidney and/or urinary tract.

Clinical data from a recent paper by Lan et al. [58] demonstrated that high urinary levels of miR-494 preceded the increase of serum creatinine in patients with kidney injury. These data imply that urinary miR-494 may be used as an early and non-invasive indicator of acute renal damage.

Zhou et al. [59] showed that a combination of miR-192 and miR-27b from urinary exosome could differentiate lupus patients with or without nephritis. Ichii et al. [23] observed high levels of miR-146a in the urinary sediment of B6.MRL-1 mice developing CKD. The level of this miR represents the pathological activity of the renal damage and thus, may be used as a non-invasive biomarker for monitoring the progression of renal damage in CKD. Hanke et al. [60] demonstrated that some miRNA ratios (miR-126/miR-152 and miR-182/miR-152) were significantly increased in urine of patients with urothelial bladder cancer. Lorenzen et al. [61] analysed the miRNA expression profile in urine of patients with acute T cell-mediated renal allograft rejection who showed reduced levels of miR-210 that improved after response to corticosteroid pulse therapy. Thus, urinary miRNAs might represent an attractive, non-invasive tool for the early diagnosis of many diseases.

THERAPEUTIC SPECULATIONS

The possibility of silencing a specific gene target is of considerable interest for therapeutic applications. RNA interference may be obtained by small interfering RNAs (siRNAs) or by miRNAs. An interesting review focuses on siRNAs [62]. Shimizu et al. [63] ameliorates renal damage by siRNA nanocarrier complex-based therapy in a mouse model of lupus nephritis, thus improving the kidney function, reducing proteinuria and ameliorating glomerular sclerosis. They silenced some intraglomerular genes, mainly the mitogen-activated protein kinase 1 (MAPK1), which influences the TGF-β1 mRNA expression.

miRNAs represent potentially novel therapeutic targets. It would clearly be desirable to specifically modulate the levels of individual miRNAs in vivo. Several methods can be used for overexpressing and ablating miRNAs for therapeutic applications. First, the use of antisense oligonucleotide inhibitors (antagomirs) which can be produced chemically [64].

Antagomirs target mature miRNAs. They are synthetically produced and contain a cholesterol group, which promotes their entrance into the cells. Alternative RNA chemistries, such as LNAs, may bind and inhibit miRNA function in vivo [65]. These alternative chemistries are more resistant to degradation.

Second, miRNA activity can also be inhibited by the transgenic introduction of tandem miRNA-binding site repeats (Decoy or Sponge technologies). miRNA sponges are genetically engineered competitive miRNA inhibitors designed by insertion of multiple binding sites into the 3’ UTR of targets of analogous miRNAs. The sponges are introduced into the cell by subcloning the miRNA binding site region into a vector containing a U6 small nuclear RNA promoter with 5’ and 3’ stem-loop elements [66].

Third, the introduction of oligonucleotides with perfect complementarity in the 3’ UTR of the target miRNA-binding site sequence may interfere with the binding of the miRNA. This approach is referred to as ‘masking’ or ‘target occupation’ [67].

Fourth, a further approach to inhibit miRNA function can be achieved by so-called ‘erasers’. Here, the expression of a tandem repeat of a perfect complementary sequence of the target miRNA inhibits endogenous miRNA.

Several targets of miRNAs in renal diseases have been identified (Table 1). These target genes modulate cystogenesis, growth of cysts and cell proliferation in PKD [68]. Others modulate mesangial cell proliferation and mesangial expansion [15, 17, 19].

In kidney diseases, in which miRNAs are downregulated, the administration of miRNAs mimics may restore their functions. This approach generates non-natural double-stranded miRNA-like RNA fragments. Such a RNA fragment is
designed to have its 5′-end bearing a partially complementary motif to the selected sequence in the 3′ UTR unique to the target gene. Once introduced into cells, this RNA fragment, mimicking an endogenous miRNA, can bind specifically to its target gene and produce post-transcriptional repression, more specifically translational inhibition, of the gene. Another approach is the introduction of viral vectors encoding shRNAs into the cells which block the mature miRNAs. In this way, it is possible to achieve a specific and continuous level of expression [69].

These strategies have been used with great success in animal models [16, 18]; however, it is necessary to take into consideration that manipulation of miRNAs may affect many components of a pathway in a disease. The potential effect of a therapeutic miRNA may depend on some important factors such as the circulating system, the cell membrane and finally the escape from the endosomal vesicles into the cytoplasm. In addition, the small size of these molecules (7–20 KDa), which are filtered by the kidneys and excreted, reduces the transfer of these therapeutic molecules from the blood to the target tissue. Therefore, the local introduction of these molecules by injection into the tissue increases the bioavailability in the target tissue [70]. In other cases, it is possible to administer PEGylated liposomes, lipiodoids and biodegradable polymers. In addition, to avoid the kidney filtration, these therapeutic molecules can be encapsulated in liposomes forming vesicles >50 KDa that do not pass the glomerular filter. Liposomes contain cationic lipids that drive the interaction between the lipid bilayer of the cell and the negative charge of the nucleic acids. The balance between the anionic nucleic acids and the cationic lipids is in favour of a net positive charge, thus favouring the binding of liposomes to the anionic cell surface molecules [71].

These great efforts achieved in the translational research need further information on the safety and reliability of these therapeutic molecules.

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CONCLUSIONS

Translational research on miRNAs is today mainly oriented to develop reliable biomarkers for diagnosis and prognosis of renal diseases, renal cancer and kidney transplantation. The use of miRNAs as therapeutic agents is very attractive but new information is necessary considering their specificity and influence on other systems. In these cases, bioinformatic analyses and systems biology could drive the research for knowing new informative miRNA targets. Meanwhile, the administration of antagomirs as miRNA inhibitors could modulate the effect of miRNAs, but we do not have sufficient information on the safety and toxicity of these antisense oligonucleotide inhibitors.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

Kidney Disease: Improving Global Outcomes—an update

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ABSTRACT

Kidney Disease: Improving Global Outcomes (KDIGO) was founded in 2003 to fulfill a need for international cooperation and consolidation in the development and implementation of clinical practice guidelines. KDIGO has experienced a rapid growth in the development of guidelines, producing three guidelines in its first 6 years and another six in the last 3 years. In addition, it has held 12 global conferences on important issues in kidney disease and its treatment. A major effort is under way to support the dissemination and implementation of KDIGO guidelines through various channels, including an Implementation Task Force with official representatives in 86 countries. KDIGO is now under its own management and remains committed to the development of evidence-based guidelines. Future challenges include finding adequate sources of funding and building stronger links with other organizations involved in guideline development and implementation.

Keywords: controversies conferences, guidelines, implementation, KDIGO, methodology

INTRODUCTION

Clinical practice guidelines are ‘Systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances’ [1]. The need for guidelines has arisen from the growing volume and

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