MicroRNA: a new frontier in kidney and blood pressure research

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Liang M, Liu Y, Mladinov D, Cowley AW Jr, Trivedi H, Fang Y, Xu X, Ding X, Tian Z. MicroRNA: a new frontier in kidney and blood pressure research. Am J Physiol Renal Physiol 297: F553–F558, 2009. First published April 1, 2009; doi:10.1152/ajprenal.00045.2009.—MicroRNA (miRNA) has emerged rapidly as a major new direction in many fields of research including kidney and blood pressure research. A mammalian genome encodes several hundred miRNAs. These miRNAs potentially regulate the expression of thousands of proteins. miRNA expression profiles differ substantially between the kidney and other organs as well as between kidney regions. miRNAs may be functionally important in models of diabetic nephropathy, podocyte development, and polycystic disease. miRNAs may be involved in the regulation of arterial blood pressure, including possible involvement in genetic elements of hypertension. Studies of miRNAs could generate diagnostic biomarkers for kidney disease and new mechanistic insights into the complex regulatory networks underlying kidney disease and hypertension. Further progress in the understanding of miRNA biogenesis and action and technical improvements for target identification and miRNA manipulation will be important for studying miRNAs in renal function and blood pressure regulation.

hypertension; acute kidney injury; chronic kidney disease; gene

Generally, genes are transcribed into RNAs that are either translated into proteins (mRNA) or make up the machinery for protein translation (rRNA and tRNA). However, some RNA molecules play a regulatory role, affecting the expression of other genes. MicroRNA (miRNA) is a recently discovered class of regulatory RNA molecules that mainly suppress the expression of protein-coding genes. Several hundred miRNA genes have been identified in human, mouse, and rat genomes (http://microrna.sanger.ac.uk) (25). The biogenesis and action of miRNA have been extensively reviewed elsewhere (18, 20, 21, 53). Briefly, a miRNA gene is transcribed as a long pri-miRNA molecule that contains one or more stem-loop structures with imperfect stems. A microprocessor complex in the nucleus involving the RNase III Drosha trims pri-miRNA into pre-miRNA that is basically a hairpin structure with a 3′ overhang. Pre-miRNA, facilitated by exportin 5, is transported into the cytosol, where it is further processed by another RNase, Dicer, into mature, ~22-nucleotide-long miRNA. One strand of the mature miRNA enters the RNA-induced silencing complex (RISC) and binds to the 3′-untranslated region (UTR) of target mRNA through imperfect base pairing. The binding reduces the expression level of the target protein. The reduction of target abundance may be mediated by a number of mechanisms including inhibition of translational initiation (55), inhibition of elongation, and induction of deadenylation that decreases mRNA stability and increases the rate of mRNA degradation (21).

Some, but not all, miRNA genes are located in introns of protein-coding genes or form transcriptional units with adjacent protein-coding genes (60). Some miRNA genes are located close to each other in the genome and form miRNA clusters. In addition, some miRNAs, such as miR-194 and miR-29b, are encoded by multiple copies of genes. One strand of a mature miRNA often exhibits a much higher level of abundance than the other strand. The minor strand is denoted with an asterisk (*) at the end of the name. In some cases, both strands can be detected at significant levels and are named with 5p and 3p to indicate their sequence orientations.

The size of miRNA and its suppression of protein expression are reminiscent of small interfering RNA (siRNA), which has become a popular experimental tool in the last few years. However, miRNAs are encoded by the genome and produced endogenously, while siRNA or its precursor, with some exceptions, is introduced experimentally from outside of animal cells. Animal miRNAs usually have only partial homology with their target genes, while siRNA is often designed to have perfect homology with target sequence. Animal miRNAs usually bind to the 3′-UTR of their target miRNAs and may suppress the translational activity while leaving the target mRNA intact in some cases. siRNA usually induces degradation of the target mRNA.

The fact that animal miRNAs do not require perfect homology with their target sequences makes it difficult to identify specific target genes for a miRNA. Experimentally proven miRNA-target pairs indicate several characteristics of target
miRNA IN THE KIDNEY

miRNA in Renal Physiology

miRNA expression profiles have been reported in the kidneys of humans and mice (4, 42, 49, 64, 66, 69). Examples of miRNAs that are enriched in the kidneys compared with other organs include miR-192, miR-194, miR-204, miR-215 and miR-216. Predicted and validated target genes for these miRNAs can be retrieved from the databases described above. Equally interesting are miRNAs that are not expressed in the kidney or are expressed at lower levels in the kidney compared with other organs. The absence of these miRNAs may contribute to permitting the expression of target proteins that are important for the kidney function.

Tian et al. (70) compared miRNA expression profiles in the renal cortex and the renal medulla of Sprague-Dawley rats. It was found that 6 and 11 miRNAs were preferentially expressed in the renal cortex and the renal medulla, respectively (see Fig. 1 in Ref. 70). Importantly, Tian et al. showed that several predicted miRNA-target pairs were reciprocally expressed in the kidney regions (see Table 1 in Ref. 70). The target genes were examined at the protein level with proteomic techniques (70). The two kidney regions differ in several general aspects including filtration and specific transport activities, blood and oxygen supply, and interstitial osmolarity. miRNAs preferentially expressed in one kidney region compared with the other may contribute to these differences that are important for normal renal physiology.

The nephron consists of distinct segments that are characterized by a mosaic of transport activities. A particularly exciting possibility is that miRNA may regulate certain transporters or channels and contribute to maintaining cellular identities in various nephron segments. For example, we have found that miR-192, which is expressed at levels at least 20-fold higher in the renal cortex than the medulla (70), might be involved in the regulation of the molecular machinery used for sodium transport in renal epithelial cells (Liu and Liang, unpublished data).

miRNA in Renal Disease

miRNA might be functionally important for the development of diabetic nephropathy. miR-192 levels have been found to increase significantly in glomeruli isolated from streptozotocin-treated diabetic mice and diabetic db/db mice (36). miR-192 targets Smad-interacting protein 1 and appears to contribute to the upregulation of collagen type I α2 in mouse mesangial cells treated with transforming growth factor (TGF)-β1. miR-377 has also been reported to be upregulated in models of diabetic nephropathy in vivo and in vitro (79). miR-377 may reduce the expression of p21-activated kinase and superoxide dismutase, indirectly contributing to enhanced fibronectin protein production characteristic of mesangial cells in diabetic nephropathy.

An important role for miRNA in the development and function of podocytes and glomeruli has been demonstrated by several studies in mice (28, 29, 63). Dicer mediates the generation of mature miRNAs as discussed in the introduction. Loss of Dicer specifically in podocytes in mice led to the development of proteinuria and eventually death within a few weeks after birth. Podocytes and glomeruli from these mice exhibited a variety of abnormalities. The specific miRNAs and their target genes that are responsible for the observed damage remain to be identified.

Transgenic overexpression of the miR-17-92 cluster in mice led to the development of lymphoproliferative disease (80). These mice also showed signs of autoimmune injuries in the kidney, including possible immune complex deposition in glomeruli. Proteinuria in these mice was sporadic (80).

Lee et al. (45) showed that miR-15a was downregulated in cholangiocytes from models of polycystic kidney disease and might contribute to in vitro cystogenesis by targeting Cdc25A, a cell cycle regulator. In situ hybridization suggested that miR-15a was downregulated in the liver tissue of patients with autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, or congenital hepatic fibrosis, as well as rats with polycystic kidney disease (45). Several miRNAs in the kidney have been reported to be differentially expressed between PKD/Mhm(+/+) rats that develop polycystic kidney disease and control PKD/Mhm(+/-) rats (58).

Expression changes of miRNA have been reported in renal tumors (24, 52, 56) and in the kidneys from a small number of sequence including homology with the first 7 or 8 bases of the miRNA (the seed region) and conservation across species. These characteristics have been extrapolated to computationally predict target genes for miRNAs. The predictions can be accessed at http://www.targetscan.org/ (23, 26, 46), http://pictar.mdc-berlin.de/ (6, 38), http://www.microrna.org/ (5, 33), and several other Web sites. Some in silico analyses indicate that >60% of all protein-coding genes in humans, or thousands of protein-coding genes, are potential targets for the few hundred known miRNAs (23). The predicted targets varied between studies depending on the details of the informatic model used. The apparent promiscuity and widespread nature of miRNA effects are reminiscent of transcriptional factors (30).

Experimental validation of a miRNA-target pair usually includes evidence of reciprocal expression between the miRNA and the target, the latter being the native target miRNA and/or protein as well as a reporter gene linked to the 3′-UTR of the target miRNA (39). Validated miRNA-target pairs have been curated in several online databases such as TarBase (http://diana.cslab.ece.ntua.gr/tarbase/).

It is important to note that the understanding of miRNA biology is still in its early stage and is evolving rapidly. For example, targeting of 3′-UTR and conservation across species have been shown to be true for many miRNAs. However, some miRNAs could target coding regions, might lack the typical seed region, and might not be well conserved (22, 67).

While it is experimentally challenging to validate a miRNA-target pair in mammalian species, intensive research over the last few years has resulted in a rapid expansion of our knowledge about the function of specific mammalian miRNAs. A wide range of biological functions has been shown to be influenced by miRNAs. These include many key biological processes and are as diverse as oncogenesis, apoptosis, cardiac development, adipocyte differentiation, insulin secretion, and angiotensin II signaling. Numerous review articles are available to provide additional details of the function of miRNA including reviews in specific areas of particular interest to renal physiologists such as the cardiovascular system (14, 43, 44, 74, 75, 84).

miRNA in Renal Disease
miRNA in Hypertension

miR-155 was reported to suppress the expression of angiotensin II type 1 receptor (54), a protein intimately involved in the regulation of arterial blood pressure. miR-155 is located on human chromosome 21. Trisomy 21 is associated with lower blood pressure. Analysis of fibroblasts from monozygotic twins showed that miR-155 levels were higher, while angiotensin II type 1 receptor protein levels were lower, in trisomy 21 (62). The miR-155 target site in the 3′-UTR of human angiotensin II type 1 receptor contains single nucleotide polymorphism rs5186. The 1166C allele of rs5186 has been associated with hypertension in some subpopulations. Interestingly, miR-155 was shown to downregulate the expression of only the 1166A, but not the 1166C, allele of rs5186 (62). In addition, miR-155 levels were found to be lower in aorta of 16-wk-old spontaneously hypertensive rats than in age-matched Wistar-Kyoto rats (81). mRNA levels of angiotensin II type 1 receptor were not significantly different, and protein levels were not analyzed. It is unclear whether miR-155 could regulate rodent angiotensin II type 1 receptor since the regulation in humans appears to be dependent on a specific allele (62). These reports suggest that miR-155 might contribute to lowering arterial blood pressure in part by targeting angiotensin II type 1 receptor.

The polymorphism ss52051869 in the 3′-UTR of human L-arginine transporter SLC7A1 has been shown to potentially contribute to a genetic predisposition to essential hypertension (83). The minor allele of the polymorphism is associated with a long variant of the 3′-UTR of SLC7A1. The long variant contains one more predicted miR-122 binding site than the short variant and reduced the expression level of reporter genes, although the polymorphism per se did not seem to be directly involved in the regulation of the reporter gene expression (82). It remains to be determined whether miR-122 is involved in the reduced expression of SLC7A1 that is associated with the minor allele of the 3′-UTR polymorphism.

An inversion region has been reported in the linkage interval on chromosome 12p in several families of patients with autosomal-dominant hypertension and brachydactyly (2). No protein-coding sequences were identified in the region. However, an exon in the region that was exclusively expressed in non-affected individuals appeared to contain a miRNA-like sequence (2).

The Dahl salt-sensitive rat is a widely used genetic model of human salt-sensitive forms of hypertension and renal injury (9, 59, 72). Naraba and Iwai (57) analyzed expression levels of 118 miRNAs in the kidneys and cardiac ventricles in Dahl salt-sensitive rats and Lewis rats fed a 0.2% or 8% NaCl diet for 9 wk. A single pool of samples was analyzed for each group, and a few miRNAs were further analyzed with individual samples. No significant difference in miRNA expression was identified (57). Our laboratory has found that several miRNAs in the renal medulla were differentially expressed between Dahl salt-sensitive rats and salt-insensitive consomic SS-13th rats. Some of these miRNAs appeared to be functionally important in Dahl salt-sensitive rats (Liu and Liang, unpublished data).

Opportunities

Recent progress in the understanding of miRNA provides a wide range of opportunities for kidney and blood pressure research, including identification of new diagnostic biomarkers and therapeutic targets and generation of novel insights into disease mechanisms.

The value of miRNAs as disease biomarkers has been demonstrated in previous studies (52). Early diagnostic markers would be highly valuable for kidney diseases including acute kidney injury (AKI) and chronic kidney disease (CKD). AKI is consistently associated with a high mortality rate. The prevalence of CKD has increased by ~30% between the periods of 1988–1994 and 1999–2004, and it is estimated that 13.1% of the US population aged 20 yr or more have CKD stages 1 through 4 (8). Current diagnosis of AKI and CKD relies primarily on serum creatinine in combination with urine output for AKI and urinary findings, such as proteinuria, for CKD. These indexes, however, change substantially only in established AKI or CKD and do not allow very early detection that is critical for effective preventative management of these conditions.

Significant efforts, including proteomic analyses, are being made to identify biomarkers to improve early diagnosis of kidney disease such as AKI (7, 73). Several hypoxia-responsive miRNAs have been reported (19, 41). Hypoxia is an important part of ischemic injury, which is the major cause of AKI. It would be interesting to determine whether hypoxia-responsive miRNAs or other miRNAs could serve as early biomarkers, or part of a set of early biomarkers, for AKI.

The causes of advanced kidney failure requiring renal replacement therapy include diabetes mellitus, hypertensive and large vessel disease, glomerulonephritis, and several others. A significant role for miRNA in several models of CKD, such as models of diabetic nephropathy, has been supported by recent studies as discussed in miRNA in Renal Disease above. Further studies are needed to examine the value of miRNA in the early detection of CKD.

Hypertension, CKD, and AKI are complex diseases that are likely mediated not by a single gene or pathway but by treelike networks of molecular, cellular, and physiological mechanisms (47, 48, 51, 71). The treelike regulatory networks may involve numerous susceptibility and modifier genes, resembling leaves on a tree. The genes interact with environmental factors and specific genomic contexts, leading to changes in a variety of molecular and cellular pathways that gradually converge into physiological pathways, resembling branches of a tree that eventually leads to the disease. A single miRNA could regulate multiple pathways or multiple components of a pathway. For example, it was reported recently that miR-29 could contribute to tumor metastasis and cardiac fibrosis by simultaneously regulating several extracellular matrix genes (61, 77). The potential ability to mediate coordinated changes makes miRNAs particularly suitable as key elements of the regulatory trees underlying complex diseases.

One of the most clearly established roles for miRNA is its contributions to organism development and cell differentiation (3, 20, 31). It is being increasingly recognized that cell differentiation and dedifferentiation are importantly involved in CKD, AKI, and possibly hypertension. Examples of related mechanisms include epithelial-mesenchymal tran-
sition, inflammation, and activation of renal stem cells (1, 32, 34, 35, 50). It would be very interesting to determine whether miRNA is involved in these important disease mechanisms.

Challenges

Studies of miRNA in kidney and blood pressure research face several challenges, some of which are related to the fundamental biology of miRNA while others are inherent to kidney and blood pressure research. Three major questions regarding the fundamental biology of miRNA remain to be fully addressed. First, it is not completely clear how miRNA is produced and processed. Many miRNA genes are located within introns of host genes. The expression of these miRNAs tends to be correlated with that of the host genes. However, the correlation is not perfect (4, 42), suggesting the presence of posttranscriptional regulation (68). The major and minor strands of a miRNA may be coexpressed but in most cases would target different sets of genes, the implications of which might be more significant than currently recognized.

Second, it is not fully understood how mature miRNA interacts with target molecules in mammalian cells. Binding of miRNA to a 3′-UTR may suppress translational initiation by interfering with the binding of essential translational initiation factors (55). Other translational repression mechanisms and even translational activation and transcriptional effects may also be involved (18, 37, 78).

Finally, and perhaps most importantly, specific target genes for most miRNAs remain unclear. In silico sequence analyses predict the presence of hundreds of thousands of miRNA-target pairs, only a small number of which have been experimentally validated. Proteomics is considered a promising new approach that may help to identify miRNA targets (27, 70). Improvements of target prediction and validation methods are also desirable.

In addition to target identification, techniques for efficiently manipulating miRNA in vivo are critical for studying the role of miRNA in renal function and blood pressure regulation as well as developing miRNA-related therapeutics. The kidney is a highly compartmentalized organ containing numerous cell types. Some aspects of renal physiology, AKI, and CKD may be studied with informative in vitro models or ex vivo preparations, but the significance of in vitro findings must be validated in vivo. The regulation of arterial blood pressure involves complex interactions between the kidney and other organ systems, which is difficult to directly examine in any in vitro settings.

Part of the technical challenge in manipulating miRNA in vivo is similar to well-known challenges in manipulating protein-coding genes and could benefit from progress in general gene manipulation techniques. Several studies have demonstrated the efficiency of modified oligonucleotides, gene knockout, and transgenesis in altering specific miRNAs in vivo (15–17, 40, 76, 77, 85). Techniques for delivering expression constructs directly to the kidney including the renal medulla (51), a region importantly involved in the long-term regulation of arterial blood pressure (10, 11), have also been reported.

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