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MicroRNAs and in kidney function and disease

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Abstract

MicroRNAs (miRNA) are short non-coding RNA sequences that regulate gene expression by blocking protein translation or inducing mRNA degradation. miRNA is found in various tissues with variable expression and changes in expression are related to various disease processes. Evidence suggests that changes in miRNA expression are critical for the normal development of kidney tissue. Alternatively, in diseases such as diabetic nephropathy, polycystic kidney disease, and lupus nephritis, specific miRNAs may enhance disease manifestations in a myriad of ways, ranging from activation of fibrotic pathways to anatomical changes that abet proteinuria. The variable expression of miRNA in kidney tissue, whether in the context of normal development or disease processes, makes miRNAs a valuable new tool for understanding, diagnosing, and discovering therapeutic options for pathological processes that affect the kidney.

Introduction

MicroRNAs (miRNAs) are regulatory RNAs that act as posttranscriptional repressors by binding the 3' untranslated region of target genes.(1) The mammalian genome encodes several hundred miRNAs. Interestingly, the miRNA profile in kidney tissue differs greatly from other tissues and indeed, between different segments of tissue within the kidney.(2-5)

As described elsewhere(1,6), miRNA genes, transcribed as long pri-miRNA molecules, have stem-loop structures with imperfect stems. The nuclear RNase III enzyme Drosha and DiGeorge syndrome critical region gene 8 (DGCR8) complex cleave pri-miRNA into pre-miRNA as a hairpin structure with a 3' overhang. Pre-miRNA exported into the cytoplasm via exportin-5 is processed by the RNaseIII enzyme Dicer into mature miRNA, approximating 19-25 nucleotides in length. A strand of mature miRNA enters the RNA-inducing silencing complex (RISC) and usually binds to the 3' untranslated region of target mRNA. This binding reduces expression levels of the target protein via inhibition of translation or elongation, induction of deadenylation, or increased mRNA degradation.(7)

Discrete changes in miRNA can have broad effects in kidney tissue. Deletion of Dicer in renin-secreting cells in murine kidneys leads to altered cell number, renin gene expression, lower plasma renin levels and lower blood pressure levels. Kidneys also develop marked areas of fibrosis and vascular injury.(8)

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Notably, there is also a Dicer-independent pathway involving the Argonaute protein, AGO2, that can convert pre-miRNA to mature miRNA independent of Dicer.(9) This pathway is regulated in cells of kidney origin by Hsp90(10) and AGO2 has been often identified as a link in understanding tubular cell injury during acute kidney injury.(11) Again, this suggests a direct interplay between a miRNA pathway and an organ-level event in the kidney.

miRNA genes are located throughout the genome including introns of protein-coding genes and non-coding transcription units, exons of non-coding transcription units, or intergenic regions.(6,12) Some miRNA genes are located close together in miRNA clusters and may target the same gene or multiple genes in the same pathway, while other miRNA genes may be duplicated on multiple chromosomes and act on the same target gene.(12-14)

miRNAs in kidney physiology

miRNAs that are in greater abundance in kidneys compared to other organs include miR-192, -194, -204, -215, and -216.(4) There are a number of predicted and validated target genes for these miRNAs. Interestingly, there are also a number of miRNAs not present in kidney tissue (Table 1). Their absence may permit protein expression at levels necessary for adequate and constant kidney function. What is clear is the miRNAs are integral for normal kidney development. In animal models involving conditional Dicer knockout mice, miRNAs have critical roles in kidney development. Deletion of Dicer1 in developing ureteric bud epithelium led to hydronephrosis and parenchymal cysts.(15) Podocyte-specific Dicer knockout mice exhibited proteinuria by 3 weeks with segmental foot process effacement, and glomerulosclerosis.(16,17) The foot process effacement was associated, in part, with decreased nephrin and podocin expression in the slit diaphragm.(18)

Studies in Sprague-Dawley rats suggested differential expression of miRNAs between cortex and medulla as well as reciprocal expression of mRNAs in subsections of kidney tissue.(2,19) Proteomic studies have provided additional confirmation of such differential distribution in the kidney. This, in fact, is logical, given the different functions present with cortical and medullary aspects of kidney tissue. One feature of miRNA expression worthy of additional investigation is the possibility that miRNAs in kidney tissue are linked with the protean degree of transport functions along the nephron. There is preliminary data suggesting that miR-192, a miRNA with markedly greater expression in cortical versus medullary kidney tissue, might be associated with sodium transport. Such links mandate further study.(7)

miRNAs in blood pressure regulation

There is circumstantial evidence in several instances of the potential for miRNA to regulate blood pressure. miR-155 appears to suppress expression of the type 1 angiotensin II receptor (AT1R)(20) and as such, would affect blood pressure in several ways. Given that AT1R are encoded on human chromosome 21, it made sense for investigators to take a different view of conditions that might affect human chromosome 21 and examine blood pressure regulation. The most common condition associated with low blood pressure and involving human chromosome 21 is trisomy 21, a condition associated with low blood pressure. In fibroblasts from individuals with trisomy 21, miR-155 levels were higher and AT1R levels lower compared to monozygotic twins.(21) Moreover, miR-155 levels were lower in aortic vessels from 16 week old spontaneously hypertensive rats compared to age-matched Wistar-Kyoto rats.(22)

There are additional associative data examining polymorphisms in the 3' untranslated region of the human L-arginine transporter, SCL7A1.(23) This polymorphism may be linked with a genetic form of hypertension. The long and short variants of this polymorphism can be

distinguished in part by an additional miR-122 binding site, though it remains to be determined whether miR-122 potentiates the development of hypertension for individuals with the long variant.

Finally, data from studies in an experimental model of hypertension, the Dahl salt-sensitive rat, raises the possibility that there might be differentially expressed miRNAs in the medullary region of the kidney compared to salt-insensitive, consomic SS-¹³BN rats.(7)

miRNAs in diabetic and fibrotic kidney disease

Diabetic nephropathy has yielded interesting findings related to miRNA expression. Work from Natarajan and colleagues identified a key role for miR-192 in diabetic nephropathy. miR-192 levels are significantly increased in glomeruli obtained from streptozotocin-induced diabetic animals and diabetic db/db mice.(24) miR-192 in diabetic glomeruli has been shown to be important in mediating transforming growth factor-beta (TGF- β)-induced collagen expression through a mechanism that encompasses TGF- β 1-induced down-regulation of SIP1/ZEB2 via miR-192 and ZEB1/ δ EF1 to increase Col1a2 expression through de-repression of E-box elements.

Some of the same molecules play key roles in epithelial-mesenchymal transition and miRNA has been linked with this process.(25) The miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) prevent expression of ZEB1/ δ EF1 and SIP1/ZEB2, maintaining an epithelial cell phenotype. To date, this process has been studied in only one kidney cell type, Madin-Darby canine kidney (MDCK) cells stably transfected with the protein tyrosine phosphatase, *Pez*.(26) Furthermore, miR-192 seems to have effects that may supersede diabetes alone. Deletion of *Smad7* promoted miR-192 expression in a model of obstructive kidney disease whereas overexpression of *Smad 7* reduced miR-192 expression in kidney tissue.(27)

Finally, it has become apparent over the last decade that diabetic nephropathy is critically dependent on podocyte biology. As noted earlier, miRNAs also have important roles in development and function of podocytes. Knocking out *Dicer* specifically in podocytes in mice leads to proteinuria and even death with evident podocyte and glomeruli abnormalities in these mice.(16-18)

miRNA and other forms of kidney disease

Overexpression of miR-17-92 cluster in mice leads to lymphoproliferative disease with concomitant auto-immune injury in kidneys and likely immune complex deposition in glomeruli.(28) Additional studies have documented differential miRNA expression also in lupus nephritis, with 36 up-regulated miRNAs, including miR-130b, miR-608, miR-124a, and miR-15b_MM1 and 30 down-regulated miRNAs, most notably miR-150 and miR-92b_MM2.(29)

In vitro and *in vivo* studies suggest that miR-15a is down-regulated in liver tissue from patients with autosomal dominant and recessive polycystic kidney disease, congenital hepatic fibrosis and rats with PKD.(30) Other miRNAs have been shown to be differentially expressed in PKD.Mhm(cy/+) rats that develop PKD and control PKD/Mhm (+/+) rats, including the novel mi-RNAs, miR-31 and miR-217. Signaling molecule interactions, signal transduction, immune system regulation, and cell communication represent more than half of over-represented miRNA regulatory pathways in this study.(31) Interestingly, a recent report from Li et al. suggests miR-17 involvement directly targeting PKD2, altering PKD2 expression more so than PKD1.(32) This suggests a differential effect on cystogenesis mediated by this miRNA.

miRNAs also appear to correlate with biopsy-proven rejection in kidney transplant recipients. Anglicheau et al.(33) assayed 33 kidney allograft biopsy samples (12 acute rejection samples and 21 normal samples). The studies demonstrated that miRNA expression associated with human peripheral blood mononuclear cells (PBMCs), miR-142-5p, miR-155, miR-223, were increased in acute rejection. A positive association was evident between tubule-specific NKCC-2 mRNA and miR-30a-3p, miR-10b, and let-7c. Similarly, tubule-associated USAG-1 mRNA was positively associated with these transcripts as well. In vitro experiments using PBMCs and phytohemagglutinin noted that miR-155 increased after mitogen stimulation whereas miR-223 and let-7c decreased. Furthermore, in primary human renal epithelial cell cultures, miR-30a-3p, miR-30a-10b, and let-7c were present and cell stimulation decreased miR-30a-3p expression. The changes in PBMC miRNA expression positively correlating with alterations in tubular miRNA levels raises the possibility that the two could be interrelated expanding our understanding of epithelial cell and PBMC activation as they occur in acute cellular rejection.

Clinical Implications

The variable expression of miRNA in tissues and diseases make them a valuable tool for understanding, diagnosing, and discovering therapeutic options for diseases. While most studies involving miRNA involve tissue specimens, there are other sources including various body fluids. Serum has been shown to be a reliable source of miRNA biomarkers. miRNA circulates in a stable form, resistant to RNase activity(34), and that levels change with physiological changes such as pregnancy.(35) Urine has also shown some promise as a source for biomarkers. Analysis of miRNAs in urine from bladder cancer patients and controls show that high ratios of miR-126:miR-152 and miR-182:miR-152 may be able to differentiate these groups.(36)

Therapeutic options may be focused on manipulating miRNA activity to attenuate disease progression. Inhibitors of miRNA expression include antagomirs which bind directly to miRNA(37), or miRNA sponges which contain tandem repeats of miRNA-binding sites(38). These inhibitors could be used in diseases such as diabetic nephropathy where increased levels of miR-192 have been found.

Summary

These data across various settings demonstrate the burgeoning importance of miRNA in kidney function and disease. The findings to date represent marked strides in identifying unique characteristics about miRNA in kidney tissue and its clinical relevance. Not only are there discrete miRNAs expressed in kidney tissue to a greater degree than other organs but there are noticeably absent miRNAs in kidney tissue as well. This organ-specific expression of miRNA provides insight into their functional roles in development and disease in the kidney. Deletion of Dicer in various parts of the kidney has shown the importance of miRNA in normal ureter development and glomerular function. Kidney specific miRNAs such as miR-192 provide additional insight into the pathogenesis of diabetic nephropathy while the role of miR-155 in AT1R and the miR-17-92 cluster in lymphoproliferative disorders describe indirect methods of injury. Further investigation will provide a more comprehensive understanding of the pathophysiology of kidney disease and may reveal potential therapeutic options.

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Table 1

Differential expression of miRNA in human kidney tissue

High levels of expression in Kidney	
miR-192	
miR-194	
miR-204	
miR-215	
miR-216	
Low levels of expression in Kidney	Highly expressed
miR-133a	Heart/Muscle
miR-133b	Heart/Muscle
miR-1d	Heart/Muscle
miR-296	Heart/Muscle
miR-1a	Heart
miR-122a	Liver
miR-124a	Brain