

Managing Microvascular Complications of Diabetes with MicroRNAs

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DNA and proteins have long been viewed as the movers and shakers in genomic studies, with RNA sometimes seen as no more than mere messengers shuttling information between the two. This view, however, dramatically changed when the key roles of microRNAs (miRNAs) in gene expression were gradually disclosed over the last decade.1,2

miRNAs are short ~22 nucleotide noncoding RNAs, which constitute a relatively new class of small RNAs that act as post-transcriptional regulators of gene expression. Most miRNAs in animals share a common biogenesis pathway in which miRNAs are transcribed by RNA polymerase II as precursor RNAs, fold into hairpin structures and are further processed by the endonuclease, Drosha, into pre-miRNAs. The pre-miRNAs are exported from the nucleus to the cytoplasm, where they are cleaved by the endonuclease, Dicer, to yield mature miRNAs. The mature miRNA is then loaded into the RNA induced silencing complex, comprised of the Argonaute family of proteins, where it is able to recognize specific mRNA targets. In general, miRNAs negatively regulate their target mRNAs through Watson-Crick base pairing of nucleotides 2–8 of the miRNA (the seed sequence) with complementary sequences within the target mRNA’s open reading frame and 3’ untranslated region. Dysregulation of a single miRNA can influence an entire signaling network. This is because individual miRNAs have multiple targets and thus can exert robust control over complex biological pathways by targeting multiple interrelated proteins. Indeed, it is becoming increasingly apparent that the aberrant expression of a single miRNA may be causally related to a variety of disease states such as cancer, cardiac diseases, and more recently, kidney diseases. In the kidney, miRNAs play important roles in a variety of pathologic conditions. For example, the consequences of inhibition of miRNAs in the glomerulus was recently examined using conditional deletion of Dicer, the RNAs essential for miRNA biosynthesis, in podocytes by several groups.3–5 Overall, it was reported that podocyte-specific deletion of Dicer leads to increased proteinuria, podocyte effacement, reduced slit diaphragm protein expression, and ultimately renal failure. As well, a podocyte-specific knockout of Drosha leads to collapsing glomerulopathy,6 further establishing the importance of regulated miRNAs in podocytes.

With regard to diabetic nephropathy (DN), Natarajan and colleagues7 were the first to report a role for a specific miRNA. Their group reported that miR-192 was upregulated in mesangial cells in vitro and in glomeruli from streptozotocin (STZ)-induced and db/db mouse models of DN. miR-192 has been shown by others to also modulate Smads and fibrogenesis in DN.8,9 Natarajan and colleagues also convincingly demonstrated that miR-192 targets the E-box repressor Smad-1 interacting protein. More recently, the same group has reported that miR-216a was upregulated by TGF-β in experimental models of DN.10 Our group has identified miR-93 as a signature miRNA in the diabetic milieu.11 Expression of miR-93 is increased in experimental models of diabetes both in vitro and in vivo. We also identified vascular endothelial growth factor-A (VEGF-A) as a putative target of miR-93 in the kidney. Using transgenic mice containing VEGF-LacZ bicistronic transcripts, inhibition of glomerular miR-93 by peptide-conjugated morpholino oligomers elicited increased expression of VEGF.

In this issue of JASN, Wang et al12 provide new insights into the role of the miR-29 family in DN. They report that members of the miR-29 family are downregulated in response to TGF-β stimulation in cultured proximal tubular epithelial cells, podocytes, and mesangial cells. This is accompanied by a concomitant increase in the expression of the validated miR-29 targets, collagens I, III, and IV. The authors report that ectopic overexpression of miR-29 results in increased expression of E-cadherin. Interestingly, a correlative assessment was made between miR-29 expression and treatment of the uninephrectomized STZ-diabetic rats with losartan and fasudil, a Rho kinase inhibitor. The renoprotective effects of fasudil correlate with an increase in
levels of miR-29a and miR-29c expression, whereas treatment with losartan correlates with increased miR-29b expression.

The miR-29 family consists of three members (29a, 29b, and 29c) that are encoded by two different loci that give rise to bicistronic precursor miRNAs (miR-29a/b1 and miR-29b2/c). The findings by Wang et al., are consistent with previous reports in which TGF-β signaling has been shown to regulate miR-29 and extracellular matrix proteins in multiple tissues and cell lines. However, the findings of this study are different from several other studies, including our own, where we showed that miR-29c expression was increased in the glomeruli of db/db mice, an established model of type 2 diabetes, and in vitro in podocytes and endothelial cells under high glucose conditions. Functionally, we identified Sprouty homolog 1 (Spry1), an inhibitor of Rho kinase, as a novel target of miR-29c. Finally, we found that in vivo knockdown of miR-29c using specific antisense oligonucleotides significantly reduces albuminuria and kidney mesangial matrix accumulation in db/db mice. Wang et al. address these distinctly different patterns of miR-29 expression by calling attention to the different animal models of diabetes used and differences in cell types and stimulation (high glucose versus TGFβ).

There are several aspects of the study by Wang et al. that deserve future consideration. First, the critical experiment needed to establish the functional relevance of low miR-29 levels in vivo in their experimental models was not performed; injecting miR-29 mimics or forced overexpression of miR-29 using a genetic approach in diabetic animals could have unraveled the functional role of miR-29 in the pathogenesis and progression of DN. Second, an interesting finding of this study is that different miR-29 family members display distinct patterns of expression in different experimental settings. For example, miR-29a was not significantly downregulated in human podocytes and in tubular cells after 4 days of TGF-β stimulation, whereas only miR-29a and miR-29c were significantly downregulated in STZ-induced apoE knockout mice. Therefore, it appears as if miR-29 family members have distinctive responses to both the diabetic environment and TGF-β stimulation. The reasons for the selective response of miR-29 family members were not explored. Third, it remains unknown whether the pathogenic effects of miR-29 are only based on their effect on matrix proteins or whether the pathogenic effects of miR-29 stem from their pleiotropic effects and independent or in addition to their effects on extracellular matrix proteins.

One of the principle and primary goals of diabetes management is to delay and/or prevent the development of chronic complications. Despite several prominent biochemical theories on how hyperglycemia contributes to microvascular damage, no conclusive genomic pathway is currently thought to contribute to the development of chronic diabetic microvascular complications. The ability of miRNAs to modulate complex biologic processes by regulating multiple targets represents a new strategy for therapeutic intervention. Indeed, the role of miRNAs in DN and other microvascular complications of diabetes is now at a tipping point, where promising therapeutic strategies modulating miRNAs are being considered. While there are clearly many challenges to the development of miRNA-based therapeutics, the central roles of miRNAs in DN and other microvascular complications of diabetes are rapidly emerging.

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DISCLOSURES

None.

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Sphingosine Lipids in the Resolution of Renal Ischemia and Reperfusion Injury

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Despite all research endeavors in the field of AKI, its severity and incidence are still increasing without substantial improvements in its prevention or therapy.1 Thus, AKI is still associated with a dramatic increase in morbidity and mortality in patients; a recent study in hospitalized patients indicated that a rise of serum creatinine of only 0.3 mg/dl is associated with a 70% increase in the risk of death.2 The increase in morbidity and mortality in conjunction with the lack of available therapies and the incredible costs associated with renal failure make it an area of intense investigations.

One of the leading causes of AKI is renal ischemia with attenuated blood supply to the kidneys associated with sepsis.1,3–7 Ischemic tissue damage has multifaceted effects on renal tissues, including renal inflammation, direct tubular damage, and alterations of vascular responses. AKI caused by ischemia can occur in different clinical settings such as surgical procedures, where cross-clamping of the aorta and renal vessels is associated with a renal failure rate of up to 30%.8 Similarly, acute renal failure after cardiac surgery occurs in up to 10% of patients under normal circumstances and is associated with dramatic increases in mortality.9 Therefore, new potential therapeutic targets are of urgent need to prevent renal injury caused by ischemia.10–15 Luckily, mouse models of AKI are very well established, and studies in gene-targeted or conditional mice offer great potential for investigating interesting targets protective of AKI.

Lee and colleagues16–21 have intensively investigated the role of adenosine receptors, which are G-protein–coupled receptors, in the pathophysiology of AKI and have opened novel and promising potential therapeutic pathways in treating AKI caused by ischemia. In this issue of JASN, Park et al.22 investigated the impact of sphingosine 1-phosphate (S1P) and its G-protein–coupled receptor, so called S1P receptor 2 (S1P2R), in renal ischemia reperfusion (IR) injury.

Sphingosine and its receptors belong to the sphingolipid family. One characteristic of lipids compared with other messenger molecules is they can freely diffuse across membranes. Thus, they cannot be stored in vesicles but are biosynthesized on demand. Sphingolipids are a class of lipids containing a backbone of sphingosine bases and aliphatic amino alcohols that includes sphingosine. Sphingosine is generated from N-deacylation of ceramide by ceramidase. It can be phosphorylated by sphingosine kinases (SK1 and SK2) to S1P. Once phosphorylated to S1P, it can activate all of the five known S1P receptors.

Five S1P receptors, discovered in the early 1990s, have been cloned thus far (S1P1R, S1P2R, S1P3R, S1P4R, and S1P5R).23 S1P receptors and their mediators have been recently shown to play an important role as potent bioactive messengers in cell differentiation, proliferation, apoptosis, migration, and angiogenesis.24 Moreover, modulators of S1P receptor attenuated vascular leak during acute lung injury, attenuated ischemia reperfusion injury in the heart and the kidneys,25,26 and improved graft survival27 in animal models. Furthermore, synthetic S1P receptors show therapeutic efficacy in clinical trials in multiple sclerosis.28 The S1P1 receptor is the most extensively studied receptor in immune-modulatory processes, whereas the role of the S1P2 receptor in IR injury is largely unknown. Genetic deletion of the S1P3 receptor in mice causes embryonic lethality due to incomplete vascular maturation,29 whereas genetic deletion of S1P2 leads to deafness.30 In the kidney, S1P receptors are expressed on proximal tubules, endothelial cells, and immune cells.31,32

Thus, the present study of Park et al.22 is of interest, based on recent findings, and develops comprehensive insights into the complex role of the sphingosine receptor, S1P2R, and its mediator, S1P, and sphingosine kinases in renal IR injury. Park et al. observed that pharmacological inhibition of S1P2R provides dose-dependent protection against IR injury. On a genetic level, they could show that gene-targeted mice for the S1P2R or mice treated with small interfering RNA targeting S1P2R were protective of IR injury. To confirm that S1P2R is the most important of the five known S1PRs, the authors determined transcript and protein levels and showed that receptor 2 was the one most upregulated after renal ischemia compared with the other four known receptors.