Angiotensin Converting Enzyme Inhibitor–Modulated MicroRNAs Targeting Renal Fibrosis

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MicroRNAs (miRNAs) are endogenously produced noncoding RNAs, 20–22 nucleotides in length, that regulate gene expression at the level of translation.[1,2] These short RNAs are involved in cell differentiation, tissue development, apoptosis, and lipid metabolism, as well as the pathophysiology of a growing number of diseases. The latter function is deduced from targeted experiments and exploratory analyses such as genome-wide association studies.

miRNAs are transcribed from their own promoter sites by RNA polymerase II as primary miRNAs, which then undergo two subsequent cleavages by the RNase III enzymes, Drosha, within the nucleus, and Dicer, after translocation into the cytoplasm, result in the formation of double-stranded, mature miRNAs. The guide strand of this mature miRNA is incorporated into the RNA-induced silencing complex (RISC). This RISC-miRNA complex then specifically targets mRNAs and leads to the negative regulation of protein synthesis or mRNA degradation.[1,2]

The human genome contains genes encoding >1000 miRNAs. miRNAs bind to their targets on the basis of nucleotide sequence in the 3′-UTR or other regions of mRNA, as well as genomic DNA, have a one-to-many and many-to-one relationship with their targets, and can potentially influence the expression of nearly all protein-coding genes.[1,2] Several miRNA prediction databases have been developed to aid in sequence-based target identification, although predicted targets on such lists have been shifting through experimental validation and program advancement in each database.

The basic understanding of miRNA biology has led to novel methodologies for manipulating miRNA expression and function. The methodologies use synthetic oligonucleotides that either enhance (miRNA-mimics) or inhibit (anti-miRs) the expression of a mature miRNA of interest.[3,4] These attractive tools are used to study the biological roles of specific miRNAs *in vitro* and *in vivo*.

Renal fibrosis features excessive deposition of extracellular matrix in the interstitium following glomerulosclerosis, tubular atrophy, and interstitial inflammatory infiltration. It is a common hallmark of CKDs such as diabetic nephropathy, hypertensive nephrosclerosis, and chronic GN, the progression of which is parallel to deterioration of kidney function. A number of miRNAs are emerging as common regulators of organ fibrosis and can be divided into two groups: those that regulate the differentiation or proliferation of fibrosis-related cells such as miR-21[5–8] and those that directly govern the translation of extracellular matrix components such as miR-29.[9–11]

miR-21 is widely expressed in the body. However, miRNA-21 is likely dispensable for development, because miR-21 gene-deficient mice grow normally.[5–7] In the obstructed kidney with fibrosis, miR-21 expression in mice significantly upregulates in tubular epithelia in response to the TGF-β/Smad3 pathway, whereas blocking miR-21 with a specific anti-miR attenuates renal fibrosis.[5,6] miR-21 represses Smad7 to promote TGF-β signaling in tubular epithelia and activates the ERK pathway to promote proliferation of fibroblasts, which likely contributes to renal fibrosis.[6,8]

miR-29s are also widely expressed in the kidney, lung, and heart, and their expression is dramatically upregulated in adult mice compared with newborns.[9–11] The miR-29 family consists of three members, and all members target the same gene because they bear an identical seed (complementary) sequence. In obstructed or diabetic murine kidneys with fibrosis, in contrast to miR-21, the TGF-β/Smad3 pathway significantly decreases expression of miR-29, whereas delivery of miR-29-mimics attenuates renal fibrosis. This suggests miR-29 is a downstream inhibitor of TGF-β/Smad3-mediated fibrosis.[9,10] In cultured fibroblasts and tubular epithelial cells, TGF-β1 downregulates miR-29 expression, which then enhances expression of extracellular matrix, because miR-29 targets at least 20 different extracellular matrix–related genes, including types I, III, and IV collagens, to repress their expression.[11] This was pharmacologically confirmed by the administration of Rho-associated kinase inhibitor, fasudil, that restored expression of miR-29 and prevented renal fibrosis in the kidneys of diabetic rats.[10] Additionally, other miRNAs implicated in renal fibrosis thus far include miR-192, miR-200a, and miR-377.[12–14]

In this issue of *JASN*, Macconi *et al.*[15] add miR-324-3p into the interesting mix of miR regulators in renal fibrosis. They found miR-324-3p was significantly upregulated in

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glomeruli from the kidneys of Munich Wistar Fromter (MWF) rats with spontaneous progressive nephropathy. Targets of miR-324-3p, predicted by multiple database searches, contain a serine peptidase, prolyl endopepidase (Prep), which is involved in the formation of the antifibrotic peptide, *N*-acetyl-seryl-aspartyl-lysil-proline (Ac-SDKP), and the antihypertensive peptide, angiotensin1–7, the latter of which was not studied here.

Ac-SDKP is a natural regulator of hematopoiesis that also has anti-inflammatory and antifibrotic effects on kidney, as well as heart, lung, and liver.[16–19] Ac-SDKP is hydrolyzed by angiotensin converting enzyme (ACE) and preserved under the pressure of ACE inhibition.[20] Delivery of a miR-324-3p mimic into cultured tubular epithelial cells resulted in significant downregulation of mRNA encoding Prep and protein expression and suppressed TGF-β1 induction of a profibrotic marker, α-smooth muscle actin, in those cells.[15] *In situ* hybridization and immunohistochemistry also demonstrated that upregulation of miR-324-3p expression associates with reduced expression of Prep in both glomeruli and cortical tubular epithelia in fibrotic areas. Treatment of the rats with an ACE inhibitor, lisinopril, significantly raised urine and plasma concentrations of Ac-SDKP and suppressed interstitial collagen deposition in the kidneys with higher Prep expression and less miR-324-3p expression in the tubular epithelia.[15]

This study strongly suggests that dysregulation of the miR-324-3p/Prep complex contributes to the progression of spontaneous nephropathy in MWF rats. However, its universal validity remains to be clarified, because miR-324-3p has never been identified in any lists of upregulated miRNAs detected in kidney tissues from either diabetic nephropathy or IgA nephropathy,[14,21,22] in which the profibrotic effects of ACE inhibition have been confirmed. In addition, an explanation of how ACE inhibition lowers miR-324-3p expression in the kidney, which was not mentioned in this study, is of great interest.

Although biomedical research concerning physiological and pathophysiological actions of miRNAs is a promising area that has shown rapid progress over the last decade,[1,2] the translation of diagnostic and therapeutic significance of miRNAs into clinical medicine remains to be established. In contrast to the studies described above, some studies provided conflicting results on the roles of profibrotic miR-21 and antifibrotic miR-29 in organ fibrosis. MiR-21 gene-deficient mice were not protected from cardiac fibrosis in response to a variety of cardiac stresses.[7] It has also been reported that there is increased expression of miR-29c in the diabetic kidney of *db/db* mice and that miR-29c knockdown prevents progression of diabetic nephropathy.[23] These divergent results suggest the complex nature of miRNA actions and study methodologies may be context dependent, involving confounding factors such as off-target effects of anti-miRs. Therefore, to explore and provide the specific functions of miRNAs, it is necessary to use genetic and pharmacological approaches. In the case of miR-324-3p, before proceeding to future clinical application and drug development, finding other targets and functions of this miRNA should be explored fully.

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DISCLOSURES

None.

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See related article, "MicroRNA-324-3p Promotes Renal Fibrosis and Is a Target of ACE Inhibition," on pages 1496–1505.

Aquaporin 2: Not Just for Moving Water

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Aquaporin 2 (AQP2) is widely recognized for its role in vasopressin-stimulated water transport across the collecting duct and hence in the production of concentrated urine.[1,2] AQP2 is primarily expressed in the apical plasma membrane and subapical vesicles of the collecting duct, although it has also been detected in the basolateral plasma membrane.[3] In response to vasopressin binding to the V_2-vasopressin receptor, AQP2 is trafficked from the subapical vesicles to the apical plasma membrane; AQP2 is endocytosed and recycled into subapical vesicles when the vasopressin stimulus ends.[1] Water exits collecting duct cells through AQP3 and APQ4, located in the basolateral plasma membrane, resulting in the transcellular reabsorption of water.[4]

In the current issue of *JASN*, Chen *et al.*[5] provide evidence for a novel role for AQP2 in promoting cell migration and epithelial morphogenesis. Their rationale for hypothesizing that AQP2 may do more than transport water merits explanation, as it is quite clever. They noted that the phenotype of AQP2-null and -transgenic mice includes the severe urine concentrating defect that one would anticipate, but also includes renal tubular abnormalities and neonatal mortality from renal failure.[6–8] Mice lacking AQP3 or AQP4 (or AQP1, which is not expressed in the collecting duct) also have a severe urine concentrating defect, but do not have the tubular abnormalities or neonatal mortality.[9] Thus, they reasoned the phenotype of the AQP2-null mouse was not simply the result of polyuria and a severe urine concentrating defect but must result from another, previously unrecognized, function of AQP2.

Insight into what this novel role may be came from Chen *et al.* identifying a potential integrin-binding site in AQP2, which is not present in other aquaporins. In addition, Tamma *et al.*[10] recently showed that integrin signaling modulates AQP2 trafficking. This suggested to Chen *et al.* that an interaction between AQP2 and an integrin at this site may contribute to the tubular abnormalities seen in AQP2-null mice.

What are integrins? Integrins are a large family of cell surface adhesion receptors that transduce signals coordinately with growth factors and the extracellular matrix.[11] Integrins are expressed in the collecting duct and play an important role in kidney development and repair.[12] More specifically, one of the integrins, integrin $\beta1$, plays an important role in collecting duct development and the maintenance of tubular integrity.[13,14] Thus, Chen *et al.* explored whether the potential integrin-binding site in AQP2 could play a role in the abnormalities found in AQP2-null mice.

In their study, Chen *et al.* confirmed that AQP2-null mice have tubular defects that were apparent as early as postnatal 7 days of age. They also found abnormal subcellular distribution of integrin $\beta1$ in the AQP2-null mice, with integrin $\beta1$ being expressed primarily at the basolateral plasma membrane. These *in vivo* studies are important for establishing a potential physiologic role for an AQP2–$\beta1$ integrin interaction.

To further explore the functional significance of an AQP2–$\beta1$ integrin interaction, Chen *et al.* proceeded to study cultured cells. They showed that AQP2 interacts with integrins through the integrin-binding site in AQP2, Arg-Gly-Asp (RGD), which is the same motif identified by Tamma *et al.*[10] They then observed that AQP2 promotes epithelial cell migration, which is an important mechanism of tissue repair following injury, in both MDCK and LLC-PK1 cells, and that promigration effects requires both AQP2 and $\beta1$ integrin. Finally, they proceeded to show that AQP2 promotes epithelial cell migration by facilitating the turnover of $\beta1$ integrin in the focal adhesions.

In the present study, Chen *et al.* elucidate a novel role for AQP2 in modulating $\beta1$ integrin trafficking and surface expression, and turnover at the focal adhesions. This mechanism

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