Post-Transcriptional Gene Regulation Makes Things Clearer in Renal Fibrosis

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The human genome consists of >3 Gb, only 1.5% of which encodes for proteins, and >95% is transcribed into RNAs that function as regulators of gene expression. MicroRNAs (miRNA) approximately 20–22 nucleotide (nt) in length are the best characterized class of noncoding RNAs that act as post-transcriptional repressors. Several hundred genes of the mammalian genome encode for miRNAs that are independent genes or lie into introns of protein-coding genes. The miRNA genes are transcribed as long primary transcripts (called pri-miRNAs), which are trimmed into the nucleus by a microprocessor complex, containing the RNase DROSHA, into secondary 70-nt precursor miRNAs named pre-miRNAs. Short double-stranded pre-miRNAs are exported into the cytosol and further processed by another multiprotein complex whose major component is Dicer enzyme. A strand of mature miRNAs enters the RNA-induced silencing complex that binds the 3' untranslated region of target mRNA, leading to translational repression or mRNA degradation.1

miRNAs are predicted to modulate the expression of almost 60% of protein-coding genes and regulate fundamental cellular processes as differentiation, proliferation, death, and metabolism. Previous studies have anticipated the key role for miRNAs in kidney development as ablation of Dicer function in the embryo results in premature termination of nephrogenesis accompanied by development of renal cysts.2 A cluster of miRNAs, including miR-192, -194, -204, -215, and -216, which are highly expressed in the kidney compared with other organs, help to maintain renal function by regulating water re-absorption, electrolyte homeostasis, and BP.3 Moreover, differentially expressed miRNAs in the kidney contribute to cause disease states associated with renal fibrosis, including polycystic kidney disease, diabetic nephropathy, FSGS, as well as kidney cancer.4,5 Fibrogenesis is preceded by the recruitment the renal interstitium of inflammatory cells that secrete profibrotic cytokines, including TGF-β. Several microRNAs have been found to be modulated by TGF-β/Smads during fibrosis.6,7 The mechanism of TGF-β–mediated fibrosis has been attributed to the cytokine capacity of stimulating Smad3 leading to upregulation of profibrotic miR-21 and miR-192 and downregulation of antifibrotic miR-29 and miR-200.8

Studies have also indicated miRNAs as a novel class of immune regulators that play a key role in controlling immune cell development and function and aberrant expression of miRNAs has been found in autoimmune diseases like SLE.9 Profiling miRNA expression in mononuclear cells and plasma from SLE patients revealed unique miRNA signatures in comparison with healthy controls or other immune-mediated diseases. Altered expression of miRNAs in T lymphocytes from SLE patients accelerated T cell activation-induced cell death contributing to the pathogenesis of the disease.10 Four miRNAs, which target genes involved in TGF-β signaling, were detected in plasma of SLE patients that distinguished SLE from other immune-mediated diseases,11 but the significance of such results is quite uncertain considering the undefined cellular source of circulating miRNAs. Differentially expressed miRNAs were also found in kidney biopsies from lupus nephritis (LN) patients, although available studies did not describe any possible role or targets for the selected miRNAs.12,13

In this issue of JASN, Zhou et al.14 attempted to identify miRNAs as possible biomarkers of renal injury in kidney biopsies from LN patients exploring their potential pathogenic role in renal fibrosis. By performing miRNA microarrays in 18 specimens including baseline and repeated biopsies from the same very LN patients, they found miR-150 as the most differentially upregulated miRNA. The peculiarity of this study lies in the fact that renal miR-150 levels correlated positively with the histologic chronicity index (CI) in baseline biopsies from independent patients and changes of miR-150 expression paralleled changes in CI between baseline and repeated biopsies. Localization of miR-150 by in situ hybridization showed predominant expression in proximal tubular cells as well as in podocytes and parietal epithelial cells (PECs) of the Bowman’s capsule, although to a lesser extent. A similar pattern of expression of miR-324-3p was reported in the kidney from animals with chronic proteinuric nephropathy and fibrosis. Increased expression of miR-324-3p was associated with downregulation of its target prolyl endopeptidase (Prep)—involved in the formation of the antifibrotic peptide Ac-SDKP—in fibrotic areas of the kidneys.
from diseased rats. Overexpression of miR-324-3p increased proximal tubular cell (PTC) susceptibility to developing a fibrogenic phenotype in response to TGF-β. In the study by Zhou et al., renal miR-150 expression correlated with the expression of profibrotic molecules like collagen I (COL1), which were significantly increased in renal tubulointerstitium. Whether the altered expression of miR-150 in LN patients was associated with changes in clinical markers such as proteinuria was not shown, possibly because kidney specimens were retrieved from LN patients enrolled between 1976 and 1999 and no clinical data were available. It would be worthwhile to assess miR-150 expression in another cohort of patients for whom clinical data are available. Furthermore, exploring whether drugs that interfere with the fibrotic process of LN patients could modulate miR-150 might be of interest and may help to find new therapeutic tools to halt the progression of human LN.

The strong miR-150 expression in PECs of the Bowman’s capsule in LN patients with high CI is an interesting observation by Zhou et al. that might warrant further detailed investigation. In human and rat Bowman capsule, PECs are composed by three distinct cell populations, which include progenitor cells, that in disease conditions proliferate abnormally and acquire a migratory phenotype contributing to the formation of hyperplastic lesions and subsequent glomerulosclerosis. Localization of miR-150 in PECs from LN patients was very reminiscent of that of miR-324-3p in Munich Wistar Frontner rats, which develop progressive glomerular injury.

A number of freely accessible miRNA databases are available that can be used to predict miRNAs function and targets. The newly generated miRNA bodymap web tool enables users to prioritize candidate miRNA based on its expression pattern across different tissues predicted by multiple independent databases at the same time (http://www.mirnabodymap.org). Zhou et al. used the European Bioinformatics Institute database (MicroCosm) to predict miR-150 function. With this tool, the suppressor of cytokine signaling-1 (SOCS1), a protein with antifibrotic properties, was identified as one of the possible targets for miR-150. Of interest, a search by the miRNA bodymap tool predicted that SOCS1 is a miR-150 target by three different databases, which strengthens the significance of the present results. SOCS1 was validated as a target of miR-150 in vitro in primary human PTCs. Downregulation of SOCS1 by specific small interfering RNA increased the expression of profibrotic genes both in PTCs and in mesangial cells. In cultured podocytes, TGF-β stimulated miR-150 expression, which correlated with decreased SOCS1 expression and increased COL1 and COL3 expression. All of the in vitro data corroborated the in vivo evaluation performed in renal biopsies from LN patients. SOCS1 mRNA and protein were reduced in biopsies with high miR-150 and high CI, whereas TGF-β staining increased. Crucial to the relevance of SOCS1 in LN are data that knockout mice for SOCS1 spontaneously develop a lupus-like disease, accompanied by hypergammaglobulinemia, autoantibody production and severe GN and renal fibrosis. In experimental diabetes, SOCS proteins were found to control the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling cascade. The JAK/STAT pathway regulates a wide range of genes involved in cell proliferation, inflammation, and fibrosis and SOCS proteins, acting as negative regulators of the JAK/STAT pathway, are able to halt the progression of diabetic nephropathy. Zhou et al. found increased expression of SOCS1 in biopsies of LN patients with low miR-150 and low CI compared with control kidney, suggesting a compensatory protective role for SOCS1 in early stage of renal fibrosis, as previously documented in renal biopsies of patients with diabetes. Zhou et al. found that SOCS1 upregulation was a transient phenomenon that was lost with the progression of LN when miR-150 was upregulated. This is an important observation for future research on novel antifibrotic drugs that explores SOCS1 as a possible novel target. In the meantime, it would be valuable to evaluate whether conventional therapies for SLE actually modulate renal SOCS1 expression considering that SOCS1 mRNA was found increased in peripheral blood mononuclear cells of patients with active SLE under treatment in respect to patients with inactive SLE. Antibodies against double-stranded DNA (dsDNA) are widely used to diagnose SLE and the presence of circulating anti-dsDNA antibody-secreting cells was associated with persistent proteinuria, high SLE disease activity index, and predictable flare up of the disease. In experimental LN, the binding of anti-DNA antibodies to glomerular basement membrane or mesangial matrix is critical to initiate glomerular inflammation. SOCS1 has been shown to mediate DNA double-strand break repair preserving the genomic stability, which highlights another potential protective function of SOCS1 in LN.

As suggested by this and other studies, altered miRNAs can be considered as disease markers and potential therapeutic targets of antifibrotic therapy. Although the clinical utility of antagonomers in CKD still needs to be demonstrated, the pivotal role of miRNAs in renal medicine opens fascinating perspective. A growing list of randomized controlled trials aimed at halting renal disease progression with the available drugs resulted in rather counterintuitive and unexpected negative results; thus, the great hope to treat kidney diseases relies on studies unlocking the genome that pave the way to discover better targeted therapeutic tools.

DISCLOSURES
None.

REFERENCES


