Can Biomarkers of Disease Activity Guide Treatment in FSGS?

Kirk N. Campbell and John C. He


FSGS is a heterogeneous disorder that is an important cause of nephrotic syndrome (1). It is not a disease but rather a histologic pattern of injury characterized on kidney biopsy by segmental areas of mesangial sclerosis in some but not all glomeruli (2). Podocyte foot process effacement is invariably detected on electron microscopy, and no specific immune deposits are usually seen with immunofluorescence microscopy (3). Secondary causes of FSGS include obesity, hypertension, and viral nephritides (2). Therapy is directed to individuals with primary FSGS, particularly those with nephrotic-range proteinuria, who are deemed high risk for progression to ESRD. In addition to angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, these patients are typically treated with high-dose steroids for at least 8–12 weeks (4). If they do not achieve a clinical remission, agents such as calcineurin inhibitors or other immune modulators are administered (5). Patients who do not respond to steroids have a poor prognosis, being much more likely to experience a progressive decline in renal function (6).

Advances in cell and molecular biology have identified the podocyte as the key target cell of injury in FSGS. Positional cloning has identified several important constituents of podocytes and their interposed slit diaphragm, such as Nphs1 (nephrin), Nphs2 (podocin), and Actn4 (actinin-4) (7–9). Mutations in these and many other recently identified genes result in podocyte injury, FSGS, and a clinical course often characterized by steroid resistance (10). Further evidence establishing the role of the podocyte in the progression of FSGS has been provided by experimental models of isolated podocyte depletion, wherein a critical threshold of a 40% reduction in podocyte number closely correlates with irreversible, high-grade proteinuria and a progressive decline in renal function (11). Despite these advances, the pathogenesis of podocyte injury remains unclear and options for targeted therapy are lacking. Efforts to identify biomarkers to predict steroid responsiveness, determine risk of disease recurrence after transplant, and guide therapeutic decision making in patients with FSGS are at an early stage and not widely available.

MicroRNAs (miRNAs) are small noncoding RNA molecules that regulate gene expression at the transcriptional or post-transcriptional level (12,13). They are ubiquitously expressed with variable cell and tissue specificity and, unsurprisingly, are essential for normal functioning of eukaryotic cells (14). Aberrant miRNA expression was first associated with chronic lymphocytic leukemia and has since been detected in such diverse clinical conditions as other hematologic and solid malignancies, cardiovascular disease, and some psychiatric conditions (15,16). Relevance to kidney disease has been illustrated by the podocyte-specific deletion of the pre-miRNA processing enzyme Dicer, which results in proteinuria and progressive glomerulosclerosis (17,18). Additionally, miR-192, miR-200b, miR-200c, miR-216a, and miR-217 levels are increased in glomeruli isolated from mouse models of streptozotocin-injected type 1 and db/db type 2 diabetic mice (19). Overexpression of miR-377 in mesangial cells in vitro induces fibronectin protein production, a potential contributing factor to the progression of diabetic nephropathy (20). In polycystic kidney disease, miR-17 directly targets the 3′-untranslated region of the PDK2 gene, thereby repressing its expression (21,22). miR-21, which is involved in apoptosis and fibrotic signaling pathways, has been postulated as a biomarker for AKI (23).

A recent study showed that transgenic expression of miR-193a causes extensive podocyte foot process effacement and progressive FSGS (24). miR-193a levels were upregulated in isolated glomeruli from patients with FSGS (24). Mechanistically, miR-193a exerts its deleterious effect by downregulating WT1, a master regulator of podocyte homeostasis (24). Separately, in a cohort of patients with idiopathic nephrotic syndrome, miR-192 and miR-205 levels were higher in the serum of patients with FSGS than in those with minimal-change disease (25). miR-192 levels also correlated significantly with the degree of interstitial fibrosis in patients with FSGS (25).

miRNAs have also been detected in the urine, with miR-126:miR-152 and miR-182:miR-152 ratios elevated in patients with urothelial bladder cancer (26). This study raised the possibility of miRNAs being used as biomarkers for diagnostic and prognostic purposes. miR-27b and miR-192 levels are increased in the urinary exosomes of patients with lupus who have nephritis, again raising the possibility that miRNAs can be used to determine renal involvement in this patient population (27). Differential expression of miR-10a, miR-10b, and miR-210 has been detected in urine samples of kidney transplant recipients with acute T cell–mediated rejection (28). miR-210 could also be a potential guide to treatment...
response because levels are different before and after acute rejection episodes (28).

In this issue of CJASN, Zhang et al. sought to identify urinary biomarkers for disease activity in patients with FSGS (29). The authors performed a meticulous stepwise validation protocol in which 196 miRNAs were initially noted to be altered in the urine of patients with active FSGS with nephrotic-range proteinuria (FSGS-A) compared with FSGS in complete remission (FSGS-CR) and normal controls. On the basis of differential expression levels, relevance to kidney disease, and the immune response, 54 candidate miRNAs were then subjected to confirmation and validation by real-time quantitative PCR. The levels of four miRNAs—miR-196a, miR-30a-5p, miR-490, and miR-155—were significantly higher in the urine of patients with FSGS-A patients than in those with FSGS-CR and in controls. Although the urinary levels of these miRNAs were not differentially expressed on the basis of the histologic variant in FSGS, within each subtype the miRNA levels were higher in patients with FSGS-A compared with those who had FSGS-CR.

The authors sought to determine whether the four identified miRNAs were specific biomarkers for FSGS and measured their urinary expression in patients with membranous nephropathy (MN) and diabetic nephropathy (DN). They found specificity for FSGS, with miRNA levels not distinguishing between active MN and MN in remission or active and incipient (early stage, microalbuminuria) DN. Interestingly, compared with normal controls, miR-196a, miR-30a-5p, and miR-490 levels were significantly higher in both active MN and active DN. However, unlike in FSGS, the miRNAs were also elevated in MN in remission as well as incipient DN. The urinary expression of these miRNAs therefore do not appear to correlate with the degree of proteinuria in MN and DN, thereby seemingly limiting their utility to distinguish active versus remission states to FSGS. Caution should be applied to this interpretation because the sample sizes (active MN: 29 patients; MN in complete remission: 26 patients; active DN: 23 patients; and incipient DN, 27 patients) were relatively small.

Zhang et al. then measured urinary miR-196a, miR-30a-5p, and miR-490 levels prospectively in 55 patients with FSGS-A, including 33 who had a complete remission (defined as urinary protein < 0.4 g/24 hours) after 8 weeks of 1 mg/kg corticosteroids (maximum, 80 mg/d) and 22 who continued to have nephrotic-range proteinuria (29). They found that miR-196a, miR-30a-5p and miR-490 levels decreased significantly in patients who responded to steroids but not those who were unresponsive. Receiver-operating characteristic curve analysis showed area under the curve values > 0.80 for each miRNA with a composite 3-miRNA signature area under the curve of 0.94. These values suggest the utility of the miRNAs as possible biomarkers to distinguish FSGS-A from FSGS-CR. In a clinical setting, such an approach would appear to be redundant when proteinuria data are readily available. More useful would be a biomarker to predict the likelihood of a remission prior to or early in the course of treatment. Zhang and colleagues partially addressed this need by prospectively demonstrating that miR-30a-5p levels could predict the response to steroid therapy after 4 weeks of treatment. At this time point miR-30a-5p levels were decreased in complete remission versus non–complete remission patients while levels of proteinuria were not. This raises the prospect of miRNA levels being used as a clinical decision tool to determine the duration of therapy with steroid versus consideration of an alternative agent. The data for miR-30a-5p were limited in this setting because values were reported for a retrospective cohort of patients with FSGS-A treated with steroids. The findings will require validation in a prospective cohort.

The use of noninvasive biomarkers to guide treatment in FSGS could greatly help simplify therapeutic approaches for this heterogeneous disorder, for which cell-specific therapy is unavailable. The prospect of using urinary miRNAs has been enhanced by the current study, although important gaps remain. The molecular mechanisms underlying the urinary expression of the identified miRNAs remain unclear. It is also unknown what their role in disease pathogenesis could be. This same group recently reported that a reduction in miR-30 family miRNAs promotes podocyte apoptosis, depletion, and glomerular disease progression (30). It remains to be determined how these observations correlate with their current findings on urinary miRNAs. Further studies will also be needed to test whether the utility of these biomarkers is generalizable considering the present study was carried out exclusively in a Chinese population. Nonetheless, this study was well designed and adds much to the clinical and scientific discourse in raising awareness for an unmet clinical need.

Disclosures
None

References


Published online ahead of print. Publication date available at www.cjasn.org.

See related article, “Evaluation of MicroRNAs miR-196a, miR-30a-5P, and miR-490 as Biomarkers of Disease Activity among Patients with FSGS,” on pages 1545–1552.