MicroRNAs in Idiopathic Childhood Nephrotic Syndrome

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 Childhood nephrotic syndrome (NS) is associated with an increase in the permeability across the glomerular filtration barrier due to processes that affect the dynamics and permselectivity of the glomerular filtration barrier. This increased permeability leads to an inability to restrict the loss of protein to <100 mg/m² body surface per day (1). The incidence of idiopathic NS has been reported to be 2 to 7 cases per 100,000 children (1), and the reported prevalence is 16 in 100,000 children (1). The disease is classically characterized by distinct clinical abnormalities, such as proteinuria in the nephrotic range (protein excretion >50 mg/kg per day), hypoalbuminemia (<3 g/dL), edema, and hyperlipidemia (1). Severe complications include bacterial infections due to immunoglobulin losses and thromboembolisms related to a relative increase in the hematocrit (1).

Children with NS have been classified into different categories. Patients with primary NS, in which a systemic disease cannot be identified, include children with idiopathic NS (absence of glomerular inflammation on renal biopsy) and children with primary glomerulonephritis. Secondary NS is related to a systemic disease. Less commonly, NS is caused by an inherited renal disease (congenital or infantile NS). This form occurs in children younger than 1 year and is associated with a poor outcome (2).

Primary idiopathic NS may be associated with at least 3 different histologic abnormalities: minimal-change disease (MCD), focal segmental glomerulosclerosis (FSGS), and membranous nephropathy (1). Pathogenetically, MCD and FSGS have long been considered to be caused by a circulating permeability factor (3). Recently, circulating soluble urokinase plasminogen activator receptor has been shown to cause FSGS (4). Congenital or infantile NS is caused by mutations in podocyte or slit diaphragm proteins [e.g., CD2AP (CD2-associated protein), podocin, the Wilms tumor-suppressor gene, and nephrin (1)].

Idiopathic NS has been identified as the most prominent form of childhood NS. In children between the ages of 1 and 10 years, >90% of cases have been described as idiopathic. This number decreases to 50% in children >10 years of age (5). Children with idiopathic NS present with the typical clinical features of NS, as well as histologic signs of MCD (diffuse foot process effacement on electron microscopy and minimal changes on light microscopy), FSGS, or mesangial proliferation. It is debated whether these 3 disorders are separate entities or represent different severities of a single disease process (6).

A majority of children display the characteristic histologic findings of MCD (1). In an attempt to avoid renal biopsy, an initial trial therapy of steroid treatment is started, and 90% of patients respond to this treatment (1). Patients with idiopathic NS are further characterized as “steroid responsive” or “steroid resistant,” depending on their response to steroid treatment. NS in steroid-responsive children is usually related to MCD. The risk of progression to chronic kidney disease is low for these patients. The prognosis for patients with steroid-resistant NS is substantially worse. The histologic variant and the response to steroid treatment is associated with ethnicity (1, 7). In particular, steroid resistance is more likely to be associated with an African or Hispanic ethnicity (7).

Currently, the concentrations of urinary protein, serum lipids, and serum albumin are the only diagnostic biomarkers of NS. Establishing a definitive diagnosis relies on the results of a renal biopsy procedure, which can be associated with severe side effects because of its invasive nature.

Recently, microRNAs (miRNAs) have been drawing attention as their roles as powerful regulators of gene expression have become known. miRNAs are small noncoding RNAs (approximately 22 nucleotides) that repress target genes through the posttranscriptional degradation of mRNA and translational inhibition of protein production (8). miRNAs in different species are highly conserved, and miRNAs in humans are thought to regulate about 50% of the genome. miRNA precursors are formed in a tightly regulated process in the nucleus and then transported into the...
cytosol, where they are further processed to form mature miRNAs (8). Mature miRNAs then bind to response elements in the 3’ untranslated region of their target mRNA molecules, finally leading to the suppression of genetic information. miRNA antagonists (antagomiRs) are available for cleaving specific mature miRNAs, thereby silencing their cellular effects. They thus serve as powerful novel therapeutics for many diseases (8). The overriding importance of these molecules for pathophysiological events through modulation of disease-specific signal-transduction pathways has been demonstrated in numerous studies (9). Until recently, miRNAs were believed to act merely as intracellular modulators of genetic information. In last few years, however, several studies have revealed the remarkable stability of extracellular miRNAs circulating in the blood or excreted in the urine and underscored their key importance as biomarkers of certain diseases (10, 11). Intriguingly, circulating miRNAs may also modulate cellular transcriptional events by transferring genetic information from a donor cell to a recipient cell (12). The stability of circulating miRNAs depends on several critical mechanisms of miRNA transport, without which they are degraded by plasma RNases within minutes (12). Circulating miRNAs are protected from degradation by inclusion into lipid or lipoprotein complexes, such as microvesicles/microparticles or exosomes (12). Additionally, miRNAs may be stabilized by binding to RNA-binding proteins such as Argonaute 2 (12). Endothelial apoptotic bodies released upon cell damage and subsequent apoptosis induction may also transport circulating miRNAs (12). Recently, HDLs also have been demonstrated to carry miRNAs (12).

In the current issue of Clinical Chemistry, Luo et al. describe their investigation of the role of circulating and urinary miRNAs in children and adolescents with idiopathic NS (13). They included 159 patients between the ages of 1 and 14 years in their study. The primary exclusion criteria were secondary NS and congenital NS. Twenty-four patients did not respond to steroid treatment. An initial large analysis of miRNA gene expression was performed with RNA isolated and pooled from serum samples obtained from 33 NS patients and 30 controls. This initial screen revealed deregulation of several miRNAs. Analysis of an independent confirmation cohort of 126 patients and 79 matched controls validated these results for several deregulated miRNAs, including miR-30a-5p, miR-151–3p, miR-150, miR-191, and miR-19b. Moreover, these miRNAs were shown to have prognostic importance for this patient cohort. To assess the response to treatment, Luo et al. studied 50 paired serum samples obtained from patients before and after steroid therapy (steroid-responsive patients). After 4 to 8 weeks of steroid therapy, the serum concentrations of all 5 selected miRNAs had declined significantly. Finally, the investigators evaluated the excretion characteristics of these selected miRNAs in NS patients. The urinary concentration of miR-30a-5p was increased in NS patients, whereas the concentrations of urinary miRNAs miR-150 and miR-191 were unchanged. miR-151–3p and miR-19b were undetectable. Furthermore, the urinary concentration of miR-30a-5p was decreased in NS patients who responded to steroid treatment. The authors did not detect a difference between steroid-responsive patients and steroid-resistant NS patients with respect to circulating miRNAs.

This study is the first to investigate the role of circulating and urinary miRNAs in NS patients. This non-invasively acquired molecular fingerprint of NS patients might aid in the diagnosis, elucidation of potential therapeutic response, and risk stratification of NS patients, thereby precluding the need for a renal biopsy and its associated potential for complications.

Intriguingly, the investigated miRNAs might provide insight into the pathophysiological disturbance in NS patients. Dysregulation of the immune system, particularly cellular immunity, has been shown to be involved in idiopathic NS (14). miR-150, which is produced in lymph nodes, is associated with the maturation of functional T cells and B cells (15). miR-19b has been shown to have a critical role in promoting Th1 cell responses and in suppressing inducible regulatory T-cell differentiation via targeting phosphatase and tensin homolog (PTEN) (16).

The critical role of the miR-30 family for podocyte homeostasis was shown by targeted deletion of the enzyme dicer, which is indispensable for miRNA biogenesis, in mature podocytes in vivo (17).

Unfortunately, Luo et al. were unable to demonstrate a difference in circulating miRNAs between patients with steroid-resistant NS and those with steroid-sensitive NS. Of particular interest to the clinician is to have the ability to identify patients likely to respond to steroid treatment at the onset of disease. That would prevent unnecessary steroid treatment in patients who are unlikely to respond.

The authors do not provide a clear proposal regarding the origin of circulating and urinary miRNAs. One can only speculate regarding whether the serum miRNAs investigated in this study are released by immune cells or by a cell type yet to be determined and play a causal role in the induction of NS, or whether they are released by damaged kidney cells (most likely the podocyte). In addition, it remains to be determined whether urinary miRNAs are shed from tubular epithelial cells or are bound to protein in blood and subsequently lost through a leaky glomerular filter. In that context, it is interesting to consider whether the ob-
served decrease in miR-30a-5p in NS patients responding to steroid treatment is just a consequence of a restored barrier function of the glomerular filter.

In conclusion, Luo et al. present a detailed and innovative analysis of circulating and urinary miRNAs in NS patients. Although there are still unresolved questions to be addressed, the results of investigations of miRNAs with this patient cohort may have great future diagnostic potential, and these markers could represent a novel and noninvasive means of monitoring and stratifying patients with this serious complication.

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