

for cell polarity, remain a possibility. Relevant to cystogenesis also are studies from the 1980s in which initially the notion of perturbations in cell–matrix interactions were deduced from observations made in a diphenylthiazole-induced murine model of PKD.<sup>9</sup>

Interestingly, as in nephronophthisis, the cystogenesis was associated with TBM thickening and salt wasting into the urine, and it seems that filtered diphenylthiazole targeted the tubular epithelia selectively without affecting the glomerular cells. The TBMs were noted to be deficient in sulfated proteoglycans, although the status of Lam $\alpha$ 5 or the ciliary proteins was not investigated. In line with these studies are the observations made in mice deficient in xylosyltransferase 2 (XylT2), an enzyme that attaches glycosaminoglycan chains onto proteoglycans by O-xylosyl-serine linkage.<sup>10</sup> The XylT2<sup>-/-</sup> mice develop renal cysts with thickened TBMs, suggesting aberrant ECM probably has some role in the pathogenesis of cystogenesis, and this notion is further reinforced by the current elegant Lam $\alpha$ 5 genetic studies.

Finally, this study raises a number of questions that could be the subject of future investigations. Although the authors previously showed that cilia are present in the mutant, do they function properly? Would the cystic kidney disease still occur in the hypomorphs if Lam $\alpha$ 5 were restored in the TBM rather than in the GBM? Can tubular cell injury be documented in the precystic kidney? Would a cilium-relevant mutation exacerbate the cystic phenotype in the Lam $\alpha$ 5 mutant? Addressing these questions might allow the cystic phenotype of these mice to be explained in the context of what is currently known about the pathogenesis of PKD.

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## DISCLOSURES

None.

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See related article, "Maintenance of Glomerular Filtration Barrier Integrity Requires Laminin  $\alpha$ 5," on pages 579–586.

## siRNA Therapy for Glomerulonephritis

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We are well into a new era in pharmacology in which small molecules, often kinase inhibitors, are used to target signal transduction pathways in cancer and other diseases. In addition to small molecules, antibody-based treatments have become a major part of the pharmacologic armamentarium. RNA interference (RNAi)-based treatments promise to be a third pillar in this new frontier. Indeed, several biotech firms have been established in the past decade to develop RNAi-based therapies. In this issue of *JASN*, Shimizu *et al.*<sup>1</sup> bring RNAi-based therapies to bear on kidney disease by demonstrating that small interfering RNAs (siRNAs) to a mitogen-activated protein kinase (MAPK) prevent glomerular disease in a murine model of lupus nephritis.

siRNAs are short, double-stranded RNAi molecules that contain complementary sequences to specific mRNAs encoding proteins. By interacting with mRNAs through these complementary sequences, they act similarly to naturally occurring

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microRNAs to cause degradation of the mRNA or to inhibit translation.<sup>2</sup> Interruption of microRNA processing causes dramatic glomerulotubular injury.<sup>3–6</sup> mRNA degradation is effected by argonaute catalytic proteins that are part of the RNA-induced silencing complex—expressing RNase activity.<sup>5,7</sup> siRNAs have been used for several years in the laboratory setting, primarily in tissue culture experiments to knockdown the expression of desired genes. They can effectively reveal physiologic processes<sup>8–10</sup> or modulate proinflammatory events in renal cells.<sup>11–14</sup> Similar RNAi reagents known as short hairpin RNAs (shRNAs) have been also used in tissue culture or transgenic mice. The distinction between shRNAs and siRNAs is that siRNAs come as commercially available, ready-to-use, double-stranded RNA reagents, whereas shRNAs are typically expressed from a transfected plasmid or transgene and require intracellular processing to remove the hairpin, such that they then resemble siRNAs.<sup>15</sup> Given the great success in using siRNA and shRNA in the laboratory setting, it is no surprise there are many efforts under way to use siRNAs as novel therapies for disease.<sup>15–19</sup> See the July 25, 2009, issue of *Advanced Drug Delivery Reviews* for several recent reviews of this area of research.

There are several hurdles to overcome in bringing RNAi-based therapies to the clinic. First, there is the issue of delivery to a specific location. This is best approached by localized delivery of siRNA molecules or DNA encoding shRNA. A second hurdle is the delivery system itself. Several delivery systems have been used to package and release siRNAs, including lipid-based systems, polycation particles, and others.<sup>16,18,19</sup> For shRNAs, viral vectors and plasmid-based systems are under study.<sup>20</sup> Finally, there is the challenge of identifying a target mRNA whose decreased abundance is likely to have a therapeutic effect while not otherwise inducing unwanted off-target effects that render the treatment unsafe or undesirable.

Shimizu *et al.*<sup>1</sup> approach this problem using siRNAs complexed with polyethylene glycol-poly(L-lysine)-polymers to form nanocarriers in which formation of the complex relies on electrostatic interactions between the positively charged poly(L-lysine) and negatively charged siRNA. First, they establish *in vitro* validation using cultured mesangial cells to knockdown expression of MAPK1, a member of the MAPK family also known as Erk2.<sup>21</sup> The authors also perform essential validation of the localization of these particles to the kidney after intraperitoneal injection along with a demonstration of decreased expression of MAPK1 protein in glomeruli. They claim localization of the particles is in the mesangium, although the stainings shown seem to be broader than typically seen for mesangial cells and may include some endothelial cells as well. The authors then go on to show that suppression of MAPK1 expression decreases glomerular sclerosis in the model of lupus nephritis.<sup>22</sup> TGF- $\beta$  is a widely known mediator that increases expression of connective tissue genes during development and in inflammatory disease<sup>23</sup> through NF- $\kappa$ B activation.<sup>24</sup> TGF- $\beta$  expression was remarkably decreased after the knockdown of MAPK1.<sup>1</sup> Thus, the results from Shimizu *et al.*<sup>1</sup> represent an

important step in bringing RNAi therapy to the treatment of kidney disease.

Many challenges, of course, remain. MAPKs act in a plethora of physiologic and pathophysiologic processes,<sup>21</sup> and suppressing their function in humans over the long term may invite vast off-target effects, unless it is possible to lower specifically hyperexpression at the site of pathology while maintaining relatively normal levels of expression in other, unaffected tissues. It also will probably be necessary to use alternative delivery routes in humans that still allow targeting to the kidney. Even more desirable will be approaches that localize targeting of RNAi molecules to the kidney or even to glomeruli or specific cell types therein. Nevertheless, the work of Shimizu *et al.*<sup>1</sup> represents an important step forward in the use of RNAi-based therapies for kidney disease.

## DISCLOSURES

None.

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See related article, “siRNA-Based Therapy Ameliorates Glomerulonephritis,” on pages 622–633.

## Idiopathic Membranous Nephropathy: Getting Better by Itself

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Understanding the natural history of a disease is crucial in determining whether therapy will be appropriate or useful. Idiopathic membranous nephropathy is a prime example of this

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guiding principle. It has been known for decades, from studies out of France,<sup>1</sup> Italy,<sup>2</sup> and elsewhere,<sup>3,4</sup> that without treatment, membranous nephropathy follows a course punctuated by spontaneous remission and relapse. In this issue of *JASN*, this point is revisited in a large, well-conducted study from Spain. Polanco *et al.*<sup>5</sup> from the Spanish Group for the Study of Glomerular Disease (GLOSEN) managed to gain cooperation from many participating physicians at 14 centers throughout Spain to withhold initial steroid or other immunosuppressive drugs from patients with renal biopsy-proven idiopathic membranous nephropathy, at least until complications ensued or an unsatisfactory evolution of the disease was evident. This approach allowed them to determine the spontaneous remission rate of disease.

The authors wisely limited the study to patients with membranous nephropathy presenting with nephrotic syndrome,<sup>5</sup> because those with initial and persisting non-nephrotic proteinuria would have not required intervention in any case.<sup>6</sup> Although observed remissions were judged to be spontaneous, two thirds of the patients received potentially disease-modifying treatment in the form of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), given both to reduce BP and to diminish proteinuria. Because treatment with these agents was not carried out in a randomized, controlled manner, it was not possible to conclude definitively that nonimmunosuppressive drugs had an impact on the frequency of spontaneous remissions; however, analysis of the observational data does generate a testable hypothesis that ACEIs or ARBs have antiproteinuric actions in membranous nephropathy and may alter the long-term course of the disease, at least in some patients. The strengths of this landmark study are the large number of patients, the strict definitions of remission, and the duration of follow-up, averaging approximately 6 years.

From the perspective of a physician who is seeking guidance as to when to initiate specific immunosuppressive therapy, with its attendant risks for patients with membranous nephropathy and nephrotic syndrome, these data are rather sobering. Almost one third developed a spontaneous complete or partial remission, usually within the first 2 years of observation, but with wide variation in onset; 1 to 66 months for a partial remission and 4 to 120 months for a complete remission. Four features tended to associate with remissions: Female gender, lower proteinuria at baseline, lower serum creatinine at baseline, and treatment with an ACEI or ARB. Histologic predictions of remission were not examined in this study, although previous studies hinted that early morphologic stages of the disease have a greater propensity for spontaneous remission, but this is controversial.<sup>1,7</sup> Remarkably, one in five patients with initial proteinuria >12 g/d underwent spontaneous remission.<sup>5</sup> Most patients exhibited a slow decline in the magnitude of proteinuria over time, and a ≥50% decline in proteinuria during the first year frequently heralded a spontaneous remission.

These humbling observations raise important questions about when to consider immunosuppressive therapy. Patients with declining proteinuria, even if nephrosis remains, are more likely to undergo spontaneous remission and thus not require any specific