Revisiting Basement Membrane Pathology in Renal Cystic Disease

Yashpal S. Kanwar
Departments of Pathology and Medicine, Northwestern University Medical School, Chicago, Illinois

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Efforts to delineate the mechanisms of polycystic kidney disease (PKD) during the past decade have focused on the primary cilium of renal tubular epithelia.\textsuperscript{1–3} The current consensus is that genes mutated in PKD code for proteins that somehow modulate the ciliary pathobiology. Similarly, the same issue arises in patients with nephronophthisis (NPH), a distinct cystic disease whereby mutations in NPHP1, NPHP2, and NPHP3 associate with tubular disruption and basement membrane thickening at the corticomedullary junction, leading consequentially to polyuria and salt wasting.\textsuperscript{4} These NPHP genes are expressed in the cilia located on the apical surface of the tubular epithelial cells. Thus, the prevailing hypothesis is that cilia act as mechanosensors responding to the flow of glomerular filtrate along the tubular lumen of the nephron. This sensor function results in signal transduction from the flow of Ca\textsuperscript{2+} as a result of the opening of cation channels formed in part by polycystins-1 and -2 transmembrane proteins that are affected by mutations in the autosomal dominant form of PKD (ADPKD).\textsuperscript{1–4} The net result being that abnormalities in ciliary function cause changes in epithelial cell signaling, polarity, proliferation, and fluid accumulation, ultimately resulting in cystogenesis.

Despite this highly attractive notion, not all forms of cystic kidney disease have a cause that is unswervingly related to defects in cilia. In 2006, Miner and colleagues\textsuperscript{5} reported in a landmark article that mice with a hypomorph mutation in the laminin α5 gene (Lama5\textsuperscript{neo} allele) develop PKD-like renal disease along with thickened tubular basement membranes (TBM) in addition to proteinuria and hematuria, the latter being characteristic of glomerular dysfunction. Incidentally, the homozygous mutants (Lama5\textsuperscript{neo/neo}) die of apparent renal failure at approximately 4 weeks of age.

Lama5 is an extracellular matrix (ECM) protein, and it is not a constituent of the apical ciliary protein complex. It is an integral component of both glomerular basement membrane (GBM) and TBM.\textsuperscript{6} In the design of the hypomorphic mutant, there is a deliberate insertion of an FLP recombinase-recognition target (FRT) site flanking the neo gene in a Lama5 intron that facilitates the generation of a conditional null allele, which seems to cause frequent abnormal splicing of the primary Lama5 transcript, reducing synthesis of full-length Lama5 protein.\textsuperscript{8} Reduced Lama5 levels were observed in many nephron segments. From these seminal studies, the authors concluded that reduced Lama5 in the GBM causes proteinuria and hematuria, whereas reduced Lama5 in the TBM induces PKD by altering tubular epithelial cell behavior and perhaps by impairing cilium-mediated signaling events.

In a follow-up study that appears in this issue of \textit{JASN}, Goldberg \textit{et al.}\textsuperscript{7} investigate the role of Lama5 in the GBM and its relationship to the pathogenesis of PKD. Mutating Lama5 selectively in glomerular podocytes using a fully functional but conditionally null allele and a podocyte-specific Cre causes proteinuria that has a variable onset but invariably progresses to nephrotic range. Intriguingly, there were no tubular abnormalities and no evidence of PKD. This is consistent with the fact that in these mutant mice, the TBMs are unaffected. Moreover, these results reemphasize the point that GBM components are vital for the integrity of the filtration barrier, a concept advanced by Farquhar and Kanwar\textsuperscript{8} decades ago.

In a complementary set of studies, Goldberg \textit{et al.}\textsuperscript{7} rescue by two different methods the Lama5 expression defect in the hypomorphic Lama5\textsuperscript{neo} mutants specifically targeting podocytes. In the first approach, they removed the FRT site flanking the neo insertion using a novel FLP recombinase transgene expressed only in podocytes using the nephrin promoter. Although the authors did not document the timing of recombination, it seems to be early enough to ensure that podocytes can make the required amount of Lama5. In the second approach, they expressed a human Lama5 transgene only in podocytes using a doxycycline-inducible system. Both approaches rescue the glomerular proteinuria and hematuria without affecting the abnormal TBM composition. In addition, in both instances, cystogenesis is inhibited. This means that the glomerular filtration barrier defects somehow initiate cystogenesis in the hypomorphic mutants.

In these experiments, it seems difficult to invoke the involvement of cilia in the pathogenesis of PKD in this unique murine model. Moreover, there are no precedents for congenital proteinuria causing cystogenesis. The authors thus suggest that in the precystic kidneys, some of the excessively filtered proteins in the glomerular ultrafiltrate damage the tubular epithelial cells, whose abnormal basement membrane and consequent abnormal cell–matrix interactions might synergize with this injury to promote cystogenesis.

Long before the cilium became a focus of PKD research, earlier studies of polycystin-1 suggested that its large extracellular domain is involved in interactions with ECM proteins.\textsuperscript{1–3} Although such interactions have not yet proved relevant to disease pathogenesis, the notion that ciliary function in epithelial cells might be influenced by some aspect of cell–matrix interactions, which are clearly important.
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.9

Interestingly, as in nephronophthisis, the cystogenesis was associated with TBM thickening and salt wastage into the urine, and it seems that filtered diphenylthiazole targeted the tubular epithelia selectively without affecting the glomerular cells. The TBM were noted to be deficient in sulfated proteoglycans, although the status of Lamα5 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.10 The XylT2−/− 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 Lamα5 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 Lamα5 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 Lamα5 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.

REFERENCES


See related article, “Maintenance of Glomerular Filtration Barrier Integrity Requires Laminin a5,” on pages 579–586.

siRNA Therapy for Glomerulonephritis

Jordan A. Kreidberg

Department of Medicine, Children’s Hospital Boston, and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts


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.1 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 miRNAs through these complementary sequences, they act similarly to naturally occurring...