Next on our to-do list, and coming soon, is a remake of our website.

DISCLOSURES

None.

Repairing the GBM Step by Step

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The glomerular basement membrane (GBM) lies between the glomerular endothelium and the podocyte foot processes. In conjunction with the slit diaphragm, GBM constitutes the glomerular filtration barrier that prevents proteinuria.1 The GBM’s major components in the mature kidney are laminin trimers (α5, β2, and γ1; called LM521), collagen IV trimers (α3, α4, and α5), agrin, and nidogen.2 Collagen IV is synthesized exclusively by podocytes, whereas LM521 is synthesized by both endothelial cells and podocytes.3 High-resolution imaging has identified how these molecules are distributed and interact within the GBM.4 LM521 resides next to endothelial cells and podocytes, oriented head to head, along with agrin, whereas collagen IV networks localize to the center of the GBM, next to nidogen and the short arms of LM521 that mediate polymerization, linking collagen IV and laminin networks.4

Laminin 521 and collagen IV mutations cause GBM defects due to truncation or absence of individual chains, impaired secretion, defective polymerization, or network formation, all of which lead to severe proteinuria, nephrotic syndrome, and ESRD as well as associated abnormalities that relate to other organs where the mutant protein is also expressed.5 Alport syndrome is caused by collagen IV mutations (X-linked COL4A5 or autosomal COL4A3 or COL4A4) that disrupt collagen IV network formation, resulting in GBM thinning, thickening, and splitting, which lead to progressive CKD and ESRD associated with sensorineural deafness and lens defects.6 Pierson syndrome, characterized by congenital nephrotic syndrome and microcoria, is caused by homozygous or compound heterozygote truncating mutations in LAMB2, whereas missense LAMB2 mutations manifest as childhood nephrotic syndrome without associated eye or neurologic abnormalities.7 Recent work from the laboratory of Miner and coworkers8 showed that LAMB2 variants can worsen Alport syndrome phenotype in mice, functioning as a modifier gene that may explain the highly variable phenotype of Alport syndrome within families.

In their exciting work published in this issue of the Journal of the American Society of Nephrology, Lin et al.9 test the potential for parenteral treatment of GBM defects using human LM521 in their mouse model of Pierson syndrome. They document that hLM-521 reaches the glomeruli and accumulates on the endothelial aspect of the GBM, leading to modest improvement of foot process effacement and podocyte injury as well as some delay in the development of nephrotic syndrome, which provide proof of principle that systemic treatment can alter the GBM composition.

These thoughtfully designed experiments also provide relevant mechanistic information and rule out confounding factors (e.g., the role of anti-hLM521 antibodies and unspecific trapping). High-resolution imaging and chase studies documented that incorporation of the hLM-521 into the GBM lasts about 3 weeks. Assessment of the longer-term changes in proteinuria and ultrastructure were confirmed in LAMB2-/--; Rag1-/- mice, which are unable to develop antibodies against hLM521.

Although Lin et al.9 show that changing the composition of the GBM by parenteral hLM521 administration delays the development of proteinuria and temporarily improves foot process effacement (FPE), these effects do not fully prevent the development of nephrotic syndrome in the Pierson mouse. The fact that success is partial and transient can be attributed to incorporation of hLM521, a large 800-kD protein limited to the endothelial side of the GBM (super-resolution immunofluorescence showed that hLM521 did not cross the lamina densa). Alternatively, the transient effect of hLM521 could have been due to development of anti-hLM521 antibodies, which the authors showed experimentally. However, mice unable to mount an immunologic response to the heterologous laminin also had a partial transient response to hLM521 administration, suggesting that incomplete incorporation is the main limiting factor.

Recent studies examining permeation of fluorescent and gold-tagged nanoparticles in vivo1 provide insight into the observed incomplete incorporation of hLM521 into the GBM, showing that IgG dimer–sized nanoparticles (approximately 300 kD) do not cross the normal GBM lamina densa, whereas IgG monomer–sized nanoparticles (approximately 150 kD) partially do. In contrast, nanoparticles of both sizes permeate across the thickened abnormal GBM of Pierson syndrome mice and Alport syndrome mice.1 These findings are consistent with the results of the work by Lin et al.,9 supporting the notion that size matters for permeation, even through abnormal GBMs. Taken together, these studies predict that smaller fragments of hLM521 or chimeric laminins (150–300 kD) may achieve an appropriate incorporation into abnormal GBMs.

Previous work from the laboratory of Miner and coworkers10 showed, using a genetic approach, that podocyte LAMBI over-expression can successfully replace deleted LAMB2, thus minimizing proteinuria and preventing the development of FPE and
nephrotic syndrome, although the normal mature GBM contains LM521 rather than LM511. Similarly, transgenic expression of chimeric laminin/nidogen proved to successfully improve congenital muscular dystrophy in mice.\textsuperscript{11,12} Collectively, these studies strongly support the notion that GBM defects can be repaired with chimeric protein replacement.

Further studies are warranted to test hLM521 fragments or chimeras, determine how long hLM521 fragments or chimeras stay in the GBM, and assess their effect on proteinuria and FPE. Should such studies succeed at improving proteinuria, FPE, and survival in Alport syndrome, Pierson syndrome, and other genetic nephrotic syndrome animal models, it would be tempting to speculate that intermittent laminin replacement could potentially be tested as alternative therapy in human clinical trials. Given that GBM thickening (with attenuation of the lamina densa and altered laminin/collagen composition) and microalbuminuria precede foot podocyte effacement in diabetic nephropathy, the potential translational implications of this work extend beyond genetic GBM diseases.

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**DISCLOSURES**

None.

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**WNKs on the Fly**

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A central role of the With No Lysine (K) (WNK) kinases in renal electrolyte balance and BP control was illuminated when mutations in WNK1 and WNK4 were identified as a cause of a genetic intolerance to sodium and potassium\textsuperscript{1} (pseudohypaldosteronism type 2; also known as familial hyperkalemic hypertension or Gordon syndrome). In this issue of the Journal of the American Society of Nephrology (JASN), Sun et al.\textsuperscript{2} report on a new player in the WNK pathway and a surprising intricacy of the signaling mechanism.

According to current understanding, the WNK kinases orchestrate a switch response that toggles the activities of two distal nephron segments (distal convoluted tubule and aldosterone sensitive distal nephron) to maintain sodium and potassium balance over widely varied potassium intake.\textsuperscript{3} WNK kinases in the distal convoluted tubule together with a downstream kinase, Ste20-related proline/alanine-rich kinase (SPAK), form a potassium-sensitive signaling cascade that controls the activity of the thiazide-sensitive sodium–chloride cotransporter (NCC) on demand. WNK signaling is activated in response to low plasma potassium in dietary potassium deficiency, and this stimulates NCC to limit potassium loss from the aldosterone sensitive distal nephron at the expense of retaining sodium.\textsuperscript{4}–\textsuperscript{6} Conversely, when dietary potassium is plentiful, the WNK cascade is inhibited, and this suppresses NaCl absorption and enhances potassium excretion.\textsuperscript{7} Understanding why the WNK-SPAK signaling is so exquisitely sensitive to plasma potassium has been the subject of great interest.

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