

Chimerism of the Renal Glomerulus Revisited

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Since the early descriptions of Alport syndrome, when the glomerular basement membrane (GBM) was found split into multiple laminar strands, investigators have wondered about the contribution of various cell types in the differential synthesis of these strands¹; podocytes and endothelia flanking the GBM were always potential candidates. To address this issue, investigators began to examine various developmental events during glomerulogenesis using interspecies grafting, transplantation, and the generation of mutant mice with the ultimate goal of producing hybrid glomeruli showing chimerism among the laminin and collagen chains of the GBM. The article by Abrahamson *et al.*² in this issue highlights the value of generating hybrid glomeruli during mouse embryonic development to tease out the cells synthesizing various laminin isoforms.

Morphogenesis in mammals is governed by various homotypic and heterotypic cell interactions leading to differentiation of epithelia or endothelia followed by the directional migration of endothelial cells to vascularize various local compartments.³ Nephrogenesis ensues through similar cell interactions during embryonic life, when the glomerulus passes through a series of developmental stages: Vesicle, comma- and S-shaped body, precapillary, and maturing capillary stages.⁴ Accompanying these stages are changes in the basal laminae lining epithelial and endothelial surfaces. Initially the two basal laminae form a loosely organized matrix in S-shaped cleft and precapillary stages that ultimately assembles into a compact GBM with maturity. This process suggests dual cellular origin of the GBM.⁵

The GBM is an amorphous scaffold of matrix stratified into a central compact lamina densa that is flanked by relatively loose lamina rara interna and externa. These regions

are composed of high molecular weight proteins, including type IV collagen, laminins, entactin/nidogen, and sulfated proteoglycans.⁶ The last are known modulators of morphogenesis with a wide variety of potential functional domains.⁶ Their glycosaminoglycan chains are made up of either heparan or chondroitin sulfate, which is attached to its respective core peptides. In the early 1980s, Farquhar and colleagues⁵ suggested the occurrence of a switch in the expression of these glycosaminoglycan chains during development. In this switch, chondroitin sulfate, distributed randomly in the matrix but relatively closer to the endothelia, is substituted by heparan sulfate in the mature GBM and equally distributed in the lamina rarae, thus raising the possibility of the dual cellular source for their synthesis by cells endowed with differential capabilities for posttranslational modification. Similarly, a dual origin of various peptide chains of laminin and collagen, albeit at the translational level or by alternative splicing, and their substitution during metanephric development would be possible.

Both laminin and collagen have a number of peptide chains most likely synthesized by different cells but assembled in various combinations in the matrix to yield differential properties while retaining the characteristic functional domains of each specific isoform.^{4,6} The cellular source of individual isoforms would dictate function, and the ideal model for delineating such functional effects would be the generation of hybrid glomeruli producing a chimeric GBM—an idea of Lauri Saxen that needs revisiting.

In the mid-1980s, Saxen and colleagues^{7,8} designed a series of interspecies (mouse/quail or quail/chick) grafting experiments to generate hybrid glomeruli to study the origin of endothelium. Eleven-day mouse, avascular kidney explants were transplanted onto quail chorioallantoic membranes. The grafts were vascularized by invading avian-derived endothelial cells, as assessed by the presence of deeply stained nucleoli, whereas podocytes lacking prominent nucleoli were of murine origin.⁷ Similar results were observed in quail/chick transplantation experiments. No vascularization was seen when uninduced mouse metanephric mesenchyme was transplanted onto chorioallantoic membrane, suggesting that early progenitor epithelial cells in the induced kidney produce a chemoattractant for migrating endothelial cells that guide them to vascularize these hybrid glomeruli.⁸

The GBM that formed in these hybrid glomeruli demonstrated co-reactivity with species-specific antibodies to both type IV collagen and laminin, suggesting dual origin.⁹ The other basement membranes in these interspecies metanephric explants were exclusively of either avian or murine origin. Co-reactivity of the GBM with species-specific antibodies led to studies of the cellular source of GBM proteins, and having an incomplete fusion of the GBM in these interspecies hy-

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brids yielded this opportunity¹⁰; murine-specific antibodies that stained the GBM strands in apposition with the visceral epithelium exhibited podocyte reactivity and similarly was the case with strands in proximity to the endothelium, confirming dual cellular synthesis of the GBM.¹⁰

The dual cellular source of GBM was further supported by observations of Abrahamson in neonatal rats, in which intracellular localization of intravenously administered anti-laminin IgG localized both in glomerular podocytes and endothelia and in respective strands of the incompletely fused immature GBM.¹¹ In the 1990s, the chimerism of GBM synthesized by glomerular podocytes and endothelia was elucidated by new intraspecies experiments.^{12,13} Avascular embryonic kidney explants, with genetic backgrounds different than the host, were transplanted into the anterior chamber of the eye or underneath the renal capsule of neonatal mice.¹³ Surprisingly, the explants vascularized and formed hybrid glomeruli with well-developed capillaries.

The feasibility of these intraspecies experiments led Abrahamson and colleagues to initiate new experiments to dissect out the cellular source, podocyte *versus* endothelium, for the synthesis of individual collagen or laminin peptide chains. Such experiments led to the notion that for glomerulogenesis to proceed properly, peptide chains present in comma- or S-shaped bodies are substituted by other isoforms as glomeruli mature; that is, type IV collagen $\alpha 1$ and $\alpha 2 \rightarrow \alpha 3, \alpha 4$, and $\alpha 5$; and laminin $\alpha 1\beta 1\gamma 1 \rightarrow \alpha 5\beta 2\gamma 1$.^{6,14,15} Genetic mutations or deficiencies in collagen $\alpha 3(\text{IV})$ and $\lambda 2$ results in GBM abnormalities and proteinuria, as observed in Alport and congenital nephrotic syndrome, respectively.^{16,17}

In the studies of Abrahamson *et al.*², intraspecies experiments were carried out in mice lacking specific laminin isoforms. Hybrid glomeruli were generated by transplanting 12-d embryonic kidney explants from laminin $\alpha 5$ null mice underneath the renal capsule of neonatal mice expressing the reporter transgene *LacZ*. Normally, *Lama5*^{-/-} null mice have avascular glomeruli, defective glomerulogenesis, disarray among podocytes, and extrusion of endothelial and mesangial cells with absent GBM.¹⁸ Upon transplantation, the *Lama5*^{-/-} null grafts vascularized with glomerular capillaries and GBM, but the podocytic abnormalities were only partially corrected. The GBM in these hybrid glomeruli formed with equal contribution of laminin $\alpha 1$ chains from podocytes and laminin $\alpha 5$ chains from endothelial cells. This suggests the laminin $\alpha 5$ isoform is essential for guiding host endothelial cells into the graft to complete GBM assemblage. Conceivably, a structural change, deficiency, or lack of accessibility for a given component of the matrix likely induces a conformational change in other components of the capillary wall. In support of this hypothesis are studies in which disruption of the α -dystroglycan/matrix transmembrane integrin complex leads to disordered organization of the GBM with foot process effacement and proteinuria.¹⁹

The hybrid glomeruli studies of Abrahamson *et al.*² set the stage for future transgenic investigations in which intraspecies

experiments will be able to delineate the function of each of the isoforms of various GBM proteins. Such studies should accelerate further analysis of chain-specific domains in matrix proteins (*e.g.*, the laminin G domain)²⁰ in terms of their interaction with specific integrin receptors and their role in the pathobiology of glomeruli relevant to various renal diseases.

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DISCLOSURES

None.

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See the related article, "Partial Rescue of Glomerular Laminin α 5 Mutation by Wild-Type Endothelial Cells Produce Hybrid Glomeruli," on pages 2285–2293.

Angiotensin-2 and Glomerular Proteinuria

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The close spatial apposition between podocytes, fenestrated endothelia, and mesangial cells within the glomerulus has led to speculation that paracrine growth factors secreted by these cells are required to maintain structural and functional integrity of glomerular permselectivity in adults. This line of thinking has advanced the concept that disruption in the normal balance of these paracrine growth factors might give rise to proteinuric renal disease.

Angiotensin-2 is an antiangiogenic growth factor that is secreted by endothelial cells during periods of active vascular remodeling and opposes the proangiogenic effects of angiotensin-1 mediated through activation of the endothelial tyrosine kinase receptor Tie-2.^{1,2} Previous studies have shown that angiotensin-2 is expressed in developing glomeruli, where it is normally downregulated after birth³ but is upregulated in a variety of

experimental models of glomerular disease, including diabetes.^{4–6} As such, angiotensin-2 is a candidate growth factor that might play a role in destabilizing glomerular endothelia, causing a breakdown of glomerular permselectivity in proteinuric renal diseases.

In this issue of *JASN*, Davis *et al.*⁷ address this question using an inducible transgenic strategy to promote prolonged (5 to 10 wk) ectopic expression of angiotensin-2 in adult mouse podocytes. These mice develop low levels of nonselective proteinuria, indicating that angiotensin-2 has the capacity to modify glomerular permselectivity. These observations raise two important questions that warrant further discussion: How does angiotensin-2 cause proteinuria, and what is the significance of these findings for pathogenesis of glomerular disease?

Electron microscopic studies in these angiotensin-2–overexpressing mice demonstrate glomerular endothelial apoptosis, but there is no evidence of glomerular capillary collapse or foot process effacement. These findings are consistent with the role of angiotensin-2 in destabilizing endothelial cell integrity⁸ but raise questions about the mechanism of proteinuria.

The authors provide evidence that the slit diaphragm protein nephrin, an essential component of the glomerular permselectivity barrier,⁹ is downregulated in angiotensin-2–overexpressing mice. On the basis of the observation that proteinuria has been described in the absence of foot process effacement, the authors argue that these changes in the expression of nephrin may give rise to a defect in slit diaphragm function without inducing a structural abnormality in podocytes. This is certainly a possibility that might be confirmed by more detailed ultrastructural analysis of the slit diaphragm. However, an alternative possibility is that the primary defect in these mice results from loss of glomerular endothelial integrity.

This speculation is consistent with Davis's observations of endothelial cell apoptosis and that expression of the angiotensin-1/2 receptor Tie-2 is generally restricted to endothelial cells.¹⁰ Furthermore, despite the important focus on podocyte biology in the pathogenesis of proteinuric renal disease,⁹ there is emerging evidence that the specialized, fenestrated endothelia along the glomerular capillary also play a significant role in maintaining the charge selective barrier to proteinuria.^{11,12} In addition, it could be argued that the low levels of proteinuria observed in the angiotensin-2–overexpressing mice are more consistent with human microalbuminuria that is thought to reflect a primary defect in endothelial as opposed to glomerular epithelial function.¹³

The functional significance of these changes in angiotensin-2–overexpressing mice for glomerular pathology is even less clear cut. For example, it is uncertain whether mild proteinuria without evidence of structural abnormalities in glomerular architecture will give rise to progressive renal disease. Long-term studies using this mouse model would establish whether this is the case. More important, however, is that it is unclear whether persistent, isolated expression of angiotensin-2 in podocytes reflects the more complex environment of

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