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Mesangial cells organize the glomerular capillaries by adhering to the G domain of laminin α5 in the glomerular basement membrane

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Introduction

Basement membranes are thin, sheetlike structures that abut many cell types, including epithelia, endothelia, muscle, fat, and peripheral nerve. They serve as extracellular barriers and as substrates for cellular interactions. Basement membranes are assembled through complex interactions among the major components: laminins, collagen IV, perlecan, and nidogens (Timpl, 1996). Of these components, it is well-known that components: laminins, collagen IV, perlecan, and nidogens are assembled through complex interactions among the major components: laminins, collagen IV, perlecan, and nidogens (Timpl, 1996). These LG modules are fused to the human laminin α1 globular (G) domain, designated Mr51. Transgene-derived protein accumulated in many basement membranes, including the developing GBM. When bred onto the Lama5/H9251 background, Mr51 supported GBM formation, preventing the breakdown that normally occurs in Lama5/H9251 glomeruli. In addition, podocytes exhibited their typical arrangement in a single cell layer epithelium adjacent to the GBM, but convolution of glomerular capillaries did not occur. Instead, capillaries were distended and exhibited a ballooned appearance, a phenotype similar to that observed in the total absence of mesangial cells. However, here the phenotype could be attributed to the lack of mesangial cell adhesion to the GBM, suggesting that the G domain of laminin α5 is essential for this adhesion. Analysis of an additional chimeric transgene allowed us to narrow the region of the α5 G domain essential for mesangial cell adhesion to α5LG3-5. Finally, in vitro studies showed that integrin α3β1 and the Lutheran glycoprotein mediate adhesion of mesangial cells to laminin α5. Our results elucidate a mechanism whereby mesangial cells organize the glomerular capillaries by adhering to the G domain of laminin α5 in the GBM.

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*Abbreviations used in this paper: G, globular; LG, laminin-type globular; GBM, glomerular basement membrane; Lu, Lutheran blood group glycoprotein; PECAM, platelet endothelial cell adhesion molecule; sol-Lu, soluble Lu.
lead to junctional epidermolysis bullosa, a severe skin blistering disease (Pulkkinen and Uitto, 1999). Targeted deletion of laminin α4 leads to impaired microvessel maturation and aberrant localization of neuromuscular synaptic specializations (Patton et al., 2001; Thyboll et al., 2002). We have shown that mice lacking laminin α5 die during late embryogenesis with several developmental defects, including defects in neural tube closure, digit separation, placentation, and kidney and lung development (Miner et al., 1998; Miner and Li, 2000; Nguyen et al., 2002).

In the kidney, basement membranes serve both as structural barriers for tubular epithelia and as a component of the glomerular filter. The glomerular basement membrane (GBM) contains an atypical assortment of basement membrane protein isoforms, including laminin-11 (α5β2γ1) and collagen α3–α5(IV) (Miner, 1998, 1999). There are transitions in the basement membrane component isoforms that are deposited in the developing GBM (Miner, 1998). During glomerulogenesis, transition of laminin isoforms is especially drastic (Miner et al., 1997; Sorokin et al., 1997a). The nascent GBM initially contains laminin-1 (α1β1γ1) and laminin-8 (α2β1γ1), and laminin-10 (α3β2γ1) joins them at the S-shaped stage. By the capillary loop stage, laminin-1 is eliminated from the GBM, and then laminin-9 (α5β2γ1) and laminin-11 (α5β2γ1) begin to accumulate. At maturity, only components of laminin-11 are detected in the GBM (Miner, 1998, 1999). We have previously shown that mice lacking laminin α5 exhibit avascular glomeruli associated with breakdown of the GBM during glomerulogenesis (Miner and Li, 2000). This defect correlates with failure of the developmental switch in laminin α chain deposition in which α5 replaces α1 in the GBM at the capillary loop stage. However, the specific role of laminin α5 in the glomerulus is still undefined.

To investigate domain-specific functions of laminin α5 in developing glomeruli, we analyzed transgenic mice that express chimeric laminin α chains: Mr51 is composed of laminin α5 domains VI through I fused to the human laminin α1 G domain; and Mr5G2 is composed of α5 domains VI through LG2 fused to human α1LG3-5. These chimeras were expressed on the genetic background of the laminin α5 knockout (Lama5−−). The developing kidney was analyzed by immunohistochemistry and transmission electron microscopy. We found that the adhesion of mesangial cells to the GBM via the G domain of laminin α5 plays a key role in capillary loop formation during glomerular development. In vitro studies suggested that integrin α,β1, and Lu are the receptors that mediate binding of mesangial cells to laminin α5.

**Results**

**The developmental switch from laminin α1 to α5 during glomerular development**

As described in previous papers, transitions in laminin isoform deposition are quite dynamic during kidney development and maturation of the GBM (Miner and Sanes, 1994; Miner et al., 1997; Sorokin et al., 1997a). A crucial developmental switch in laminin α chain deposition occurs in the GBM when the laminin α1 chain, which is predominantly expressed in basement membranes of the S-shape body, is replaced by laminin α5 in the capillary loop stage GBM (Fig. 1, A–D). In Lama5−− mutant glomeruli, where this switch cannot occur, the kidney exhibits avascular glomeruli associated with GBM breakdown (Fig. 1, E and F). The GBM breaks down because laminin α1 is eliminated even in the absence of α5 expression, and without a compensating full-length laminin α chain, basement membrane structure cannot be maintained. As a result of GBM breakdown, the cells that comprise the glomerulus—podocytes, endothelial cells, and mesangial cell—are unable to maintain their proper positions adjacent to the GBM, resulting in failed glomerulogenesis (Miner and Li, 2000). This demonstrates the extreme importance of cell–matrix interactions during glomerulogenesis.

**Expression of the chimeric laminin α chains, Mr51 and Mr5G2, in glomeruli**

To begin to examine domain-specific functions of laminin α5, we produced transgenic mice expressing two different full-length chimeric laminin α chains. These encoded laminin α5 domains VI through I and VI through LG2 fused to the complete human laminin α1 G domain and α1LG3-5, designated Mr51 and Mr5G2, respectively (Fig. 2, B and C). We chose to use the human rather than mouse α1 G domain because of the availability of mouse monoclonal antibodies specific for the human domain (Virtanen et al., 2000); thus, transgene-derived proteins could be specifically localized in transgenic mouse tissues. A transgene encoding the full-length mouse α5 chain, designated Mr5 (Fig. 2 A), served as a control. The widely active regulatory element miw (Suemori et al., 1990) was used to drive transgene ex-
expression. As described in our previous papers, transgene-derived laminin levels were significantly increased in heart and skeletal muscle (Moulson et al., 2001; Kikkawa et al., 2002). Crossing of the Mr5 transgene onto the laminin α5 domains VI through I of mouse laminin α5. (C) Mr5G2 contains laminin α5 domains VI through α5 LG2 fused to the human laminin α1LG3-5 domain. Anti–mouse laminin α5 (*) and anti–human laminin α1 LG1-2 (**) antibody epitopes are indicated.

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The domains present in full-length laminin α5 (A), in the chimeric laminin α chains (B and C), and in full-length human α1 (D) are shown. (B) Mr51 contains human laminin α1 G domain linked to domains VI through I of mouse laminin α5. (C) Mr5G2 contains laminin α5 domains VI through α5 LG2 fused to the human laminin α1LG3-5 domain. Anti–mouse laminin α5 (*) and anti–human laminin α1 LG1-2 (**) antibody epitopes are indicated.

To further investigate the effects of Mr51 and Mr5G2 on glomerulogenesis, we used cell type–specific antibodies to identify the three cell types found in glomeruli. Frozen sections of E17.5 control, Lama5 −/−, Lama5 −/−; Mr51, and Lama5 −/−; Mr5G2 kidneys were stained with antibodies to WT1, platelet endothelial cell adhesion molecule (PECAM), and desmin to label podocytes, endothelial cells, and mesangial cells, respectively (Fig. 4, green), and doubly labeled with an anti–basement membrane antibody (Fig. 4, red). In the control, podocytes were observed in a single cell layer epithelium adjacent to the glomerular capillaries (Fig. 4A), and mesangial cells, which provide tension to maintain the glomerular capillary loop structure, were found associated with endothelial cells in the interior of the glomerulus (Fig. 4I). In the Lama5 −/− mutant, the podocytes were in disarray, and the endothelial cells and mesangial cells were extruded from glomerulus, as we showed previously (Miner and Li, 2000; Fig. 4B, F, and J). In Lama5 −/−; Mr51 glomeruli, the transgene-derived chimeric laminin chain partially rescued the defects observed in the mutant. The podocytes were arranged in a single cell layer (Fig. 4C), and the endothelial cells and mesangial cells were localized in the interior of the glomerulus, similar to the control (Fig. 4G).

Identification of cell types in glomerular structures

Figure 3. Expression of the chimeric α chains, Mr51 and Mr5G2, in transgenic mice. Micrographs show E17.5 glomeruli from Lama5 −/−; Mr51 (E and F), Lama5 −/−; Mr5G2 (J and L), and Lama5 −/−; Mr51, and Lama5 −/−; Mr5G2 embryos exhibited greatly reduced or absent capillary looping (Fig. 3, G, H, and K).
and K). These results suggest that the COOH-terminal portion of laminin α5 is dispensable for the assembly of the GBM and arrangement of podocytes. However, the great reduction in capillary looping (Fig. 4, C and K) is indicative of a mesangial cell defect because a similar phenotype has been observed in the total absence of mesangial cells in mice lacking either PDGF B or PDGF receptor α (Miner and Kovesdy, 1998). Lama5 /–; Mr5G2 glomeruli exhibited a very similar aberrant glomerular phenotype (Fig. 4, D, H, and L).

**Analysis of glomerular ultrastructure and podocyte gene expression**

To further investigate the fine structure of the GBM and adjacent cells, we used transmission electron microscopy to visualize E17.5 control, Lama5 /–; and Lama5 /–; Mr51 glomeruli (Fig. 5). In the control, the GBM was clearly visible between podocytes and endothelial cells (Fig. 5, A and G). Mesangial cells were attached to the GBM at the bases of the capillary loops (Fig. 5 D); this allows them to maintain the capillary loop structure. In the Lama5 /–; glomerulus, the absence of laminin α5, coupled with the programmed disappearance of laminin α1, resulted in breakdown of the GBM by the late capillary loop stage (Fig. 5, B and E). In the Lama5 /–; Mr51 glomerulus, the ultrastructure of the GBM was similar to that observed in control (Fig. 5, C and H), confirming that the transgene-derived chimeric laminin α chain must have been assembled into the GBM, as previously demonstrated by immunofluorescence (Fig. 3). This suggests that the short and long arm regions of laminin α5 are sufficient for directing incorporation of the chimera into the GBM. Although podocytes extended foot processes in both control and Lama5 /–; Mr51 glomeruli (Fig. 5, A, C, G, and H), there appeared to be fewer processes in the latter. This may suggest that the α5 G domain has some role in inducing process extension. However, slit diaphragms, the delicate tight and adherens junctions–related structures that are required for glomerular filtration (Kerjaschki, 2001; Miner, 2002), were observed between many of those foot processes that did form (Fig. 5 J), indicating that this crucial aspect of podocyte differentiation was progressing appropriately. To investigate this in more detail, we used antibodies to slit diaphragm– and foot process–associated proteins. The results were essentially sim-
capillaries inhibit a similar capillary malformation. Thus, the distended capillaries observed upon substitution of the GBM components was altered in \( \text{Lama}5^{-/-} \); Mr51 glomeruli, is cell type–specific. In any event, although laminin \( \alpha 5 \) normally trimerizes with the laminin \( \alpha 5 \) and \( \gamma 1 \) chains to form laminin-11, which is first detected at the capillary loop stage of glomerulogenesis (Miner and Sanes, 1994; Miner et al., 1997). The paucity of laminin \( \alpha 2 \) chain (Fig. 7, E and G), whereas laminin \( \beta 1 \) was present in both control and \( \text{Lama}5^{-/-}; \) Mr51 maturing glomeruli (Fig. 7, A and C).

Laminin \( \beta 2 \) normally trimerizes with the laminin \( \alpha 5 \) and \( \gamma 1 \) chains to form laminin-11, which is first detected at the capillary loop stage of glomerulogenesis (Miner and Sanes, 1994; Miner et al., 1997). The paucity of laminin \( \beta 2 \) in \( \text{Lama}5^{-/-}; \) Mr51 GBM indicates either that \( \beta 2 \) does not efficiently associate with the Mr51 chimera to form a trimer, or that the trimer forms but does not efficiently incorporate into the GBM. On the other hand, \( \beta 2 \) did efficiently incorporate into the GBM in the presence of Mr5G2 (Fig. 7 H).

Although little is known about how specificity of laminin trimerization or incorporation into basement membranes is determined, these results suggest that it may be encoded in the \( \alpha 1 \) G domain. Indeed, laminin \( \alpha 1 \) and \( \beta 2 \) are only rarely found in the same basement membrane (unpublished observations), perhaps because the \( \alpha 1 \) G domain directs preferential assembly with \( \beta 1 \). Nevertheless, the laminin-3 trimer (\( \alpha 1\beta 2\gamma 1 \)) has been isolated from placenta (ChAMPLAUD et al., 2000), suggesting that whatever code exists could be cell type–specific. In any event, although laminin \( \beta 2 \) knockout mice exhibit a congenital nephrotic syndrome

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<th>Control</th>
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Figure 6. Deposition of ECM proteins in control and \( \text{Lama}5^{-/-}; \) Mr51 glomeruli. Sections of E17.5 control and \( \text{Lama}5^{-/-}; \) Mr51 kidneys were stained with specific antibodies to fibulin-1 (A and B), fibulin-2 (C and D), agrin (E and F), nidogen-1/entactin-1 (G and H), nephronectin (I and J), perlecan (K and L), collagen \( \alpha 3(IV) \) (M and N), and collagen \( \alpha 4(IV) \) (O and P). The mutant GBM had a composition very similar to the control. Bar, 50 \( \mu m \).
believe that these in vitro data using laminin trimers justify our conclusions concerning adhesiveness of G domains because in vivo mesangial cells adhere to wild-type α5 but not to Mr51, which contains α5 domains VI through I. Thus, adhesion to laminin-10/11 trimers is likely mediated primarily by the α5 G domain.

Identification of receptors mediating mesangial cell adhesion to laminin-10/11

We next wished to investigate the mechanism of mesangial cell adhesion to the α5 G domain. Cell adhesion to laminin-10/11 is mediated predominantly by the integrin family of adhesion receptors (Kikkawa et al., 1998, 2000). Therefore, we examined the effects of function-blocking monoclonal antibodies against various integrin subunits on human mesangial cell adhesion to laminin-10/11 (Fig. 8 C). Anti-integrin α3 and β1 antibodies significantly inhibited the adhesion of human mesangial cells to laminin-10/11, indicating that integrin α3β1 is a primary receptor. (D) The effect of sol-Lu on adhesion of human mesangial cells to laminin-10/11. Sol-Lu had little inhibitory effect on its own, but cooperated with anti-integrin β1 antibodies to inhibit adhesion by ~90%. This indicates that Lu cooperates with integrin α3β1 to mediate mesangial cell adhesion to laminin α5 in the GBM.

Adhesion of mesangial cells to laminins in vitro

To further investigate interactions between mesangial cells and the laminin α5 G domain, we turned to in vitro adhesion assays that used primary human and rat mesangial cells and purified laminin preparations. We have not yet been able to isolate laminin trimers containing the chimeric α chains, and G domain preparations were not available, so we chose to use commercially available laminins. First, we compared the abilities of laminin-10/11 (α3β1γ1), laminin-1 (α1β1γ1), and laminin-5 (α2β1γ1) to promote adhesion of mesangial cells. Quantitative analysis of both human and rat mesangial cell adhesion to surfaces coated with increasing concentrations of these proteins showed that laminin-10/11 had higher cell adhesion activity than laminin-1, especially at the lower protein concentrations (Fig. 8, A and B). In addition, the cells spread less well on laminin-1 than they did on laminin-10/11 (unpublished data). These data provide an explanation as to why mesangial cells adhere poorly to GBM containing the α1 G domain (Fig. 5, F and J) but adhere well to normal GBM containing the α5 G domain (Fig. 5, D and I).
al., 2001). A splice variant of Lu present in humans that should also bind laminin α5 is known as basal cell adhesion molecule (El Nemer et al., 1998; Zen et al., 1999). In our previous studies, we showed that Lu is expressed on the surface of a subset of muscle and epithelial cells in diverse tissues adjacent to basement membranes containing the laminin α5 chain (Mousson et al., 2001). We have also identified Lu to be a specific receptor for laminin α5 via binding to the α5 G domain by using a recombinant form of soluble Lu (sol-Lu) that contains only the Lu extracellular domain; in addition, the presence of α5LG3 was required for binding (Kikkawa et al., 2002). Lu was coexpressed with integrin α3 on mouse mesangial cells in vivo (unpublished data). To examine whether Lu is also involved in adhesion of mesangial cells to laminin α5, sol-Lu was used in adhesion/inhibition assays (Fig. 8 D). In theory, sol-Lu should bind Lu binding sites on laminin α5 and prevent the Lu present on mesangial cells from interacting with α5. Although sol-Lu alone had no effect on mesangial cell adhesion to laminin-10/11, a significant inhibitory effect was observed when it was combined with the anti-integrin β1 antibody (Fig. 8 D). Thus, Lu may be a secondary receptor for adhesion of mesangial cells to laminin-10/11. In addition, the fact that sol-Lu did not enhance the anti-integrin α3 antibody inhibition suggests that there may be another integrin α subunit that is involved.

Discussion

We and others have previously shown that the laminin α5 chain is widely expressed in mice (Miner et al., 1995, 1997; Sorokin et al., 1997b). Based on knockout studies, laminin α5 has roles in several important developmental processes, including neural tube closure, digit septation, placentation, and lung and kidney development (Miner et al., 1998; Miner and Li, 2000; Nguyen et al., 2002). We found that most of the developmental defects are associated with basement membrane breakdown or discontinuity resulting from the absence of α5. Here, we have begun to investigate the role of laminin α5 using a combined transgenic/knockout approach, which effectively substitutes either all or part of the α5 G domain with analogous segments of α1. The result is expression of full-length chimeric laminin α chains capable of trimerizing with β and γ chains and incorporating into basement membranes. A similar strategy was previously used in vitro to map a synaptic basement membrane localization domain on the laminin β2 chain (Martin et al., 1995). This approach has allowed us to uncover a laminin α5 G domain–specific function in glomerulogenesis that might never have been found through traditional knockouts. It also demonstrates the feasibility of using widely expressed transgenes encoding altered basement membrane proteins to replace existing knocked out genes, effectively generating knockins without the use of further gene targeting in ES cells.

Expression of the Mr51, and presumably Mr5G2, chimeric α chains on the Lama5−/− background was able to rescue the breakdown of the GBM (Fig. 5) that normally occurs in Lama5−/− glomeruli when laminin α1 is eliminated (Miner and Li, 2000). The mechanism of α1 elimination is unknown; but if it is not purely transcriptional, then it must somehow be selective for α1 because α5 is not eliminated. It is likely that primary sequence differences or domain structural differences account for the selective elimination of α1. The G domain of α1, present in Mr51, could have carried a signal for elimination, but our results suggest this not to be the case because Mr51 was not eliminated. We are continuing to investigate this interesting issue using additional α chain transgenes.

The major defect in the Lama5−/−; Mr51 and Lama5−/−; Mr5G2 embryos was ballooning of the glomerular capillaries. This same defect was observed in mice lacking mesangial cells due to absence of PDGFB/PDGF receptor β signaling (Lindahl et al., 1998). However, in our case, mesangial cells were clearly present (Fig. 4, K and L, and Fig. 5 J), so we concluded that they must not be adhering properly to the GBM to maintain capillary looping. As the only known differences between normal and Lama5−/−; Mr51/ Mr5G2 GBMs are the G domain substitutions, and laminin α chain G domains have been shown to harbor recognition sites for numerous cell adhesion receptors (Colognato and Yurchenco, 2000), we hypothesized that mesangial cells normally adhere to the α5 G domain but were unable to adhere tightly to either the complete α1 G domain or to α1 LG3–5. Our in vitro studies confirmed that both human and rat mesangial cells adhere better to α5-containing laminins than to α1-containing laminin (Fig. 8, A and B).

With regard to mechanisms for mesangial adhesion to the α5 G domain, mesangial cells express several β1 integrins, including α1β1, α2β1, α5β1, α6β1, and αβ1 (Gauer et al., 1997; Sterk et al., 1998; unpublished observations). Furthermore, immuno-EM studies have shown that β1-containing integrins are concentrated at the mesangial cell surface adjacent to the GBM and the mesangial matrix (Kerjaschki et al., 1989). Antibody inhibition studies demonstrated that integrin αβ1 plays a major role in mesangial cell adhesion to laminin-10/11 (Fig. 8). In support of this, the glomerular capillaries of Igα3−/− kidneys are dilated (Kreidberg et al., 1996), suggesting a defect in mesangial adhesion to the GBM but the fact that the capillaries are not ballooned suggests that another receptor normally cooperates with αβ1 and is able to partially compensate in Igα3−/− mesangial cells. We found that Lu is expressed on mouse mesangial cells and cooperates with β1 integrins to mediate adhesion in vitro (unpublished data; Fig. 8 D). The fact that Lu was found to be involved is consistent with the fact that Mr5G2 does not support capillary loop formation in vivo, because we have shown that sol-Lu does not bind Mr5G2 (Kikkawa et al., 2002). Lu mutant mice being generated in our laboratory will allow us to more directly address the function of Lu in glomerulogenesis.

An important issue to consider here is the relationship of mesangial cells with laminins in the mesangium, a non–basement membrane ECM, which mesangial cells secrete and in which they are embedded. Several different laminins are found in the mesangium, including substantial amounts of laminins-1 (α1β1γ1), -2 (α2β1γ1), and -10 (α5β1γ1), but others can be detected at lower levels (Miner, 1999). It has not been possible to determine the relative levels of these laminins, but one would suspect that, based on our findings, decreased levels of laminin-10 or increased levels of laminin-1,
as might occur in disease states, could correlate with reduced adhesion of mesangial cells to the mesangial matrix. On the other hand, the fact that mesangial cells are almost totally surrounded by their matrix may make this issue irrelevant, as weaker adhesion may be tolerated, both in disease and in normal states. This would be in contrast to the relationship of mesangial cells to the GBM, with which they make contact only at the bases of the capillary loops. A more robust adhesion to the GBM may be necessary in this setting of limited contact in order to counteract the force of blood pressure. Therefore, interaction with the G domain of α5, normally the only α chain in the GBM, would ensure a tight adhesion.

Two other laminin mutant mice with kidney defects have been described. In mice lacking laminin β2, the β1 chain compensates and allows an ultrastructurally normal basement membrane to form. However, the glomerular filter fails as a barrier to plasma proteins, and the mice die at 3 wk of age with massive proteinuria (Noakes et al., 1995). No defects in mesangial cells (unpublished data), and together with Lu, this may troglycan is also expressed on podocytes but not on mesangial cells to the GBM discontinuities (Willem et al., 2002), and we suggest that these GBM defects prevent mesangial cells from adhering and maintaining the integrity of the capillary loops.

Integrin αβ1, expressed basally on podocytes (Korhonen et al., 1990; Kreidberg et al., 1996), yet detachment of podocytes from the GBM in Lama5−/− Mr51/Mr5G2 glomeruli, as occurred with mesangial cells, was not observed. There are two possibilities to explain this. First, integrin αβ1 on podocytes may serve primarily as a signal transducing receptor rather than as an anchoring one. Dystroglycan is also expressed on podocytes but not on mesangial cells (unpublished data), and together with Lu, this may be sufficient for adhesion of podocytes to the GBM. Second, podocytes and mesangial cells may be adhering only weakly to the chimeric laminins through integrin αβ1. This may be sufficient for long-term adhesion of podocytes to the GBM but not for mesangial cells. Capillary looping was evident in immature Lama5−/− Mr51/Mr5G2 glomeruli, but mesangial cell adhesion to the GBM was apparently too weak to counteract the force of blood pressure, leading to death and capillary ballooning. In support of this is our finding that adhesion activity of laminin-10/11 (αβ/γ1) is present in the GBM (unpublished observations); thus, mesangial cells should still be capable of binding to the GBM and maintaining capillary looping. On the other hand, mice lacking the binding site for nidogen on laminin γ1 exhibit glomerular capillary aneurysms similar to the ballooned capillaries we have reported here. The aneurysms were associated with GBM discontinuities (Willem et al., 2002), and we suggest that these GBM defects prevent mesangial cells from adhering and maintaining the integrity of the capillary loops.

Preparation of the chimeric laminin constructs
Preparation of the chimeric laminin α chains, designated Mr51 and Mr5G2, has been described in Kikkawa et al. (2002). Both chimeric laminin cDNAs were cloned into a modified version of the widely active expression vector mammalian expression vector miw, which contains the RSV LTR inserted into the chicken β-globin promoter (Miner et al., 1997). Rat monoclonal antibodies against human laminin γ3 were produced and characterized as described previously (Miner et al., 1997). Rat monoclonal antibodies against collagen α3(IV) (H31) and α4(IV) (H43) chains have been described previously (Ninomiya et al., 1995). Rabbit anti-tobody against nephronectin (Brandenberger et al., 2001) was a gift from Dr. L. Reichardt (University of California, San Francisco, San Francisco, CA). Rabbit antibody against domain IIIb/IVa of mouse laminin α5 was produced and characterized as described previously (Miner et al., 1997). Rat monoclonal antibody MEC 13.3 to PECA was purchased from BIOMED, Bensheim. Rabbit antisera sc-192 to Wils’m tumor protein (WT1) was purchased from Santa Cruz Biotechnology, Inc. Mouse monoclonal antibody D33 to desmin was purchased from DakoCytomation. Mouse monoclonal antibody to synaptopodin (Mundel et al., 1991) was a gift from Dr. P. Mundel (Albert Einstein College of Medicine, Bronx, NY). Rabbit antibodies to CD2AP, nephrin, and podocin have been described previously and were gifts from A. Shaw (Washington University School of Medicine), L. Holzman (University of Miami Medical School, Ann Arbor, MI), and C. Antignac (Necker Hospital, Paris, France; Dustin et al., 1998; Holzman et al., 1999; Roselli et al., 2002). Monoclonal antibodies against human integrin α1 (FB12), α2 (P1E6), α3 (P1B5), and α6 (GoH3) were purchased from CHEMICON International, Inc.

Generation of knockout and transgenic mice
Production of Lama5 mutant mice and of transgenic mice expressing a full-length laminin α5 transgene or the chimeric laminin transgenes has been described previously (Miner et al., 1998; Moulsdon et al., 2001; Kikkawa et al., 2002). Five independent Mr51 lines, all of which gave similar results, and one Mr5G2 line were produced.

Immunohistochemistry
For immunohistochemistry, mouse embryos from timed matings were frozen whole in ice as OCT compound and quick-freezing in 2-methylbutane cooled in a dry ice ethanol bath. Sections were cut at 7 μm in a cryostat and stained with toluidine blue for light microscopy. For semi-thin and thin sectioning, embryonic kidneys were fixed in 4% formaldehyde in PBS and 1 mg/ml p-phenylenediamine. Sections were examined through a microscope (Eclipse E800; Nikon). Images were captured with a Spot 2 cooled color digital camera (Diagnostic Instruments) using Spot Software Version 2.1. Images were imported into Adobe Photoshop 5.0 and Adobe Illustrator 9.0 for processing and layout.
Thin sections were cut with a diamond knife and stained with lead citrate plus uranyl acetate for transmission electron microscopy. Reagents were obtained from Polysciences Inc.

Cell culture and adhesion assay

Normal human mesangial cells were purchased from Cambrex Life Science Corporation and used within seven passages. Primary rat mesangial cells were provided by Q. Yu and A.R. Morrison (Washington University School of Medicine). Cells were grown in DME supplemented with 20% FBS, 10 μg/ml insulin, 1 mM glutamate, and 1 mM sodium pyruvate (Invitrogen) and used within seven passages. Adhesion assays were performed as described previously (Kikkawa et al., 2000). In brief, 20 μg/ml of recombinant laminin-10/11 was coated onto a 96-well plate at 37°C for 1 h. The wells were blocked with 1% BSA. Mesangial cells were trypsinized and allowed to recover in serum-free medium for ~30 min and 100 μl of mesangial cells at 10^5 cells/ml in DME were added to the wells. After a 1-h incubation, the attached cells were stained with 0.2% crystal violet in 20% methanol for 10 min, 100 μl of 1% SDS was added to dissolve the cells, and absorbance was measured at 570 nm by VERSAmax (Molecular Devices). To identify the receptors for laminin-10/11, 10 μg/ml of monoclonal antibodies against different integrins and the recombinant sol-Lu protein were preincubated individually with mesangial cells in a volume of 50 μl of serum-free DME (5 × 10^5 cells/well) at room temperature for 15 min. The preincubated cells were transferred to plates coated with laminin-10/11 and incubated further at 37°C for 20 min. After incubation, the attached cells were stained with 0.2% crystal violet in 20% methanol for 10 min and counted under the microscope.

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