Vascular Endothelial Growth Factor A Signaling in the Podocyte-Endothelial Compartment Is Required for Mesangial Cell Migration and Survival

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The glomerular filtration barrier separates the blood from the urinary space and consists of two major cell types: podocytes and fenestrated endothelial cells. Mesangial cells sit between the capillary loops and provide structural support. Proliferation and loss of mesangial cells both are central findings in a number of renal diseases, including diabetic nephropathy and mesangiolysis, respectively. Using cell-specific gene targeting, it was shown previously that vascular endothelial growth factor A (VEGF-A) production by podocytes is required for glomerular endothelial cell migration, differentiation, and survival. For further investigation of the effect of gene dose and VEGF-A knockdown within the glomerulus, mice that carry one hypomorphic VEGF-A allele and one podocyte-specific null VEGF-A allele (VEGFhypo/loxP,Cre+/−) were generated; in these mice, the “allelic dose” of VEGF-A is intermediate between glomerular-specific heterozygous and null states. VEGFhypo/loxP,Cre+−/+ mice die at 3 wk of age from renal failure. Although endothelial cell defects are observed, striking loss of mesangial cells occurs postnatally. In addition, differentiated mesangial cells cannot be found in glomeruli of podocyte-specific null VEGF-A mice (VEGFhypo/loxP,Cre+/−). Together, these results demonstrate a key role for VEGF-A production in the podocyte for mesangial cell survival and differentiation.


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showed elegantly that elevations in soluble VEGFR-1 correlate with the severity and incidence of preeclampsia in patients and that injection of soluble VEGFR-1 leads to proteinuria and endotheliosis in rats (15). Neutralization of circulating VEGF-A by anti-VEGF-A antibodies and soluble VEGFR-1 induces proteinuria (16). By contrast, 15- to 20-fold upregulation of VEGF-A mRNA in podocytes leads to a collapsing glomerulopathy that resembles the lesion seen in patients with HIV (14). These results demonstrate that VEGF-A production by the podocyte is critical for endothelial cell migration, survival, proliferation, and differentiation within the glomerulus and suggest that changes in VEGF-A production, delivery, or storage may be involved in a wide number of glomerular pathologies.

The endothelial defects were not unexpected as VEGF-A was shown previously to be a critical mediator of endothelial health in other tissues (17–20). However, the exquisite dosage sensitivity and the range of glomerular injuries that were observed on the basis of tweaking the VEGF-A gene dose were not predicted. To dissect further the role of VEGF-A in glomerular biology, we generated mice that carry one hypomorphic VEGF-A allele (21) and one podocyte-specific VEGF-A null allele (14). These mice have in their podocytes an intermediate VEGF-A “gene dose” that lies between null and haploinsufficient mice (14). Although mesangial cells can be found in normal number in glomeruli from newborn VEGFhypo/loxP,Neph-Cre+/− mice, they undergo rapid mesangiolysis by 10 d of age. In VEGFhypo/loxP,Neph-Cre+/−, where podocytes produce no VEGF-A, differentiated mesangial cells are absent from developing and mature glomeruli. Together, these results demonstrate multiple roles for VEGF-A in mesangial cell biology, recruitment, and/or differentiation and survival.

Materials and Methods

Generation of Transgenic Mice

Generation of null VEGF-A mice was described previously (14). To generate a glomerular-specific VEGFhypo/loxP allele, we bred podocyte-specific VEGFhypo/loxP,Neph-Cre+−/− mice with heterozygous hypomorphic mice that express a chimeric VEGF-A protein fused to lacZ and has a reduced function (21). Heterozygous hypomorphic mice have no phenotype. VEGFR-2-GFP (Flk-1-GFP) and VEGFR-1lacZki/− mice were supplied by J. Rossant laboratory (Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada) (22,23).

Genotyping

Genomic DNA isolation from mouse tails and generation and genotyping of the nephrin-Cre transgenic mice have been described previously (14). Presence of the floxed VEGF-A gene was detected by PCR (14). The VEGFhypo+/− allele was identified by PCR using three primers published elsewhere (21) as well as PCR for the β-galactosidase gene (24).

Phenotypic Analysis

Collected urine was probed on a urine dipstick (Chemstrip 5L; Roche Diagnostics Corp., Indianapolis, IN) for the presence of protein and blood in samples. The standard colorimetric assay was performed according to the manufacturer’s instructions. For detection of the presence of protein in urine samples, 2 µl of urine was run on an SDS-PAGE gel. Blood samples were collected by the puncture of femoral vein, and blood chemistry and hematology analyses were performed as described previously (14).

Histologic Analysis

For histologic analysis, freshly dissected kidneys were fixed in 10% formalin/PBS, embedded in paraffin, and sectioned at 4 µm. Sections were stained with hematoxylin and eosin and Masson trichrome, examined, and photographed with a DC200 Leica camera and Leica DMLB microscope (Leica Microsystems Inc., Deerfield, IL). Tissue for electron microscopy was fixed in 1.5% glutaraldehyde, embedded in Spurr (Canemco Inc., Saint-Laurent, Quebec, Canada), and sectioned. In the figures, glomeruli are shown at two time points. The “early time point” is defined as mature-stage glomeruli from postnatal days 1 to 3, and the “late time point” is defined as mature-stage glomeruli from postnatal days 10 to 12. These two time points were chosen because of the dramatic and consistent pathologic differences between them. One hundred percent of early time point glomeruli showed relatively preserved structure with defects in the endothelial compartment alone. By contrast, 100% of late time point glomeruli showed major defects throughout the capillary tuft. Within the first 2 postnatal wk, all stages of glomerular development may be observed in rodent kidneys. Glomeruli from mutant and wild-type littermates were matched for developmental stage and age.

In Situ Hybridization

Kidneys were dissected from mice on postnatal days P1, P3, P5, P10, and P12. Staining was performed as described previously (14). Digoxigenin-labeled probes were prepared according to the Roche Molecular Biochemicals protocol (Roche Molecular Biochemicals, Mannheim, Germany). Probes used for in situ analysis were nephrin (NPHS1), Wilms tumor suppressor gene (WT-1), and vascular α-smooth muscle actin (V SMA-α) (14). Details of the in situ hybridization analysis protocol may be obtained upon request.

Immunohistochemistry

Deparaffinized and rehydrated 5-µm paraffin sections of formalin-fixed kidneys were placed in citrate-buffered solution (pH 6.0) and then boiled using a microwave oven for 20 min for antigen retrieval. Endogenous peroxidase was blocked with 3% hydrogen peroxide, and nonspecific binding was blocked with blocking solution (DAKO North America, Carpinteria, CA). Samples were incubated in a diluted primary antibody at 4°C overnight, washed for 1 h in three changes of PBS, then incubated with the secondary antibody for 1 h. Primary antibodies that were used for this study were monoclonal mouse anti-human smooth muscle actin (clone 1A4; DAKO) at 1:50 dilution and rabbit polyclonal anti-GFP (Molecular Probes, Eugene, OR) at 1:2000. M.O.M kit (BMK-2202; Vector Laboratories, Burlingame, CA) was used for detecting mouse V S M A - α antibody. Immunohistochemical staining was performed with Vectastain ABC kit (Vector Laboratories). Diaminobenzidine (Vector Laboratories) was used for the color reaction. After counterstaining with hematoxylin, slides were dehydrated, mounted, and photographed. Secondary antibody alone was consistently negative on all sections.

Immunofluorescent Staining

PFA-fixed kidneys (4%) were sectioned at 5 µm (detailed protocol provided upon request). We used rat anti-mouse CD31 (platelet endothelial cell adhesion molecule 1 [PECAM-1]) mAb (cat. no. 557335; BD Biosciences, San Jose, CA) with Cy3-conjugated donkey anti-mouse IgG at 1:300 dilution as secondary antibody; rabbit polyclonal anti-GFP
(Molecular Probes) at 1:2000 dilution with secondary goat anti-rat fluorescein (FITC) antibody at 1:300 (Jackson ImmunoResearch Laboratories Inc., West Grove, PA); and monoclonal mouse anti-human smooth muscle actin (clone 1A4; DAKO) at 1:50 dilution with secondary Cy3-conjugated donkey anti-mouse IgG antibody at 1:300 dilution. The detailed procedure has been described previously (25).

Antibodies used for dual immunofluorescent staining on VEGF$^{hypo/loxP,Neph-Cre^{-/-}}$ kidneys are anti-GFP (rabbit polyclonal; Molecular Probes), VSM-A-α (monoclonal mouse anti-human, clone 1A4; DAKO), WT-1 (Santa Cruz Biotechnology, Santa Cruz, CA), Laminin-α5 (rabbit anti-mouse) (26), desmin (monoclonal mouse anti-human, clone D33; DAKO), PECAM (rat anti-mouse; Pharmingen), PDGF receptor β (PDGFR-β; anti-mouse, ebioscience), and β-galactosidase (anti-rabbit, Biogenesis); species-specific fluorescein (FITC) and Cy3-conjugated AffiniPure were used as secondary antibodies. The detailed protocol was described previously (26).

β-Galactosidase Staining

Kidneys from VEGFR-1lacZki/+ mice (23) were dissected and fixed in 4% paraformaldehyde and 1% glutaraldehyde and stained for β-galactosidase activity as described previously (27).

Statistical Analyses

All data are expressed as the mean ± SD. Paired evaluations were made for VEGF$^{hypo/loxP,Neph-Cre^{-/-}}$ and control groups, and statistical significance between means was determined with t test. Statistical analysis was carried out using SigmaStat version 2.01 (Jandel Corp., San Rafael, CA) with the level of significance for comparison set at P < 0.05.

Results

VEGF$^{hypo/loxP,Neph-Cre^{+/-}}$ Mice Hypomorphic for VEGF-A in the Podocytes Develop Glomerular Mesangiolysis

The hypomorphic VEGF-A allele was generated through the insertion of a lacZ cassette into the 3′-UTR of the VEGF-A gene. This allele produces a C-terminus modified VEGF-A protein that is only partially active (21). Homozygous VEGF-A hypomorphic (VEGF$^{hypo/hypo}$) mice die at 9.0 d post coitum because that is only partially active (21). Homozygous VEGF-A hypomorphic (VEGF$^{hypo/hypo}$) mice die at 9.0 d post coitum because of severe abnormalities in the yolk sac vasculature and deficiencies in the development of the dorsal aortas (21), whereas heterozygotes survive to adulthood with no apparent defects (24). To knockdown the level of VEGF-A produced by podocytes, we bred mice that are heterozygous for the VEGF-A hypomorphic allele to mice with a podocyte-selective deletion of one VEGF-A allele (VEGF$^{+/loxP,Neph-Cre^{+/-}}$) (Figure 1). VEGF$^{hypo/loxP,Neph-Cre^{+/-}}$ mice were born in the expected Mendelian ratio. However, they developed macroalbuminuria and renal insufficiency by 5 d of age (Figure 2). One hundred percent of VEGF$^{hypo/loxP,Neph-Cre^{+/-}}$ mice were dead by 3 wk of age as a result of end-stage kidney failure.

Loss of Mesangial Cells in Postnatal Glomeruli

At birth, the number and the structure of glomeruli were grossly normal by light microscopy. However, by day 3, the glomeruli from mutant mice were hypopcellular with “ballooned” capillary loops. Masson trichrome staining showed massive mesangiolysis (Figure 3A). The area between glomerular capillary loops where mesangial cells are normally situated was replaced by a foamy substance devoid of cell nuclei. Immunostaining for VSMA-α (Figure 3B) and in situ hybridization analysis for the PDGFR-β (Figure 4B) that are two markers of the mesangial cell lineage showed that mesangial cells are present in capillary loop stage and mature glomeruli from VEGF$^{hypo/loxP,Neph-Cre^{+/-}}$ mice at P3. However, this staining is lost and completely absent in glomeruli from VEGF$^{hypo/loxP,Neph-Cre^{+/-}}$ mice at 10 to 12 d (Figures 3B and 4B). In contrast, VSMA-positive (Figure 3B) and PDGFR-β-positive (Figure 4B) mesangial cells are present at this stage in control glomeruli. Immunofluorescent staining for the mesangial cell marker desmin and the endothelial cell marker PECAM confirmed that both endothelial and mesangial cell lineages are present at birth (Figure 5). Ultrastructural analysis of the glomeruli showed that mesangial cells are present at birth and are lost postnatally (Figure 6).

To determine the effect of the VEGF-A allelic dose on mesangial cells, we examined glomeruli from mice that produce no VEGF-A in their podocytes (VEGF$^{loxP/loxP,Neph-Cre^{+/-}}$). In con-
Serum creatinine measurements were markedly increased in mice (VEGF<sup>loxP+/−</sup>,Neph-Cre<sup>+/−</sup>). PDGFR-β and VSMA-α stains were negative in VEGF<sup>loxP+/−</sup>,Neph-Cre<sup>+/−</sup> mice but clearly present in VEGF<sup>loxP+/−</sup>,Neph-Cre<sup>+/−</sup> mice (14; data not shown). These genotype-phenotype differences are observed easily on light micrographs of histologic specimens (Figure 7).

**Endothelial Cell Defects.** Although endothelial cells are present at birth (Figures 4C and 6), electron micrographs showed rapid loss of glomerular endothelial cells by days 0 to 3 that preceded the loss of mesangial cells (Figure 6). By day 3, only capillary loop “ghosts” remained as the endothelial cell layer had completely disappeared (Figure 6), whereas mesangial cells were still present. However, by day 5, neither endothelial nor mesangial cells could be identified ultrastructurally. Double immunostaining for the endothelial-expressed marker PECAM and the mesangial-expressed markers desmin and VSMA-α confirmed that the postnatal loss of endothelial cells in VEGF<sup>hyp</sup>/Neph-Cre<sup>+/−</sup> mice (Figure 5) precedes the loss of mesangial cells as shown by both desmin and VSMA-α staining. This sequential loss is observed from differentiated glomeruli.

**Podocyte Differentiation.** In contrast to the major defects observed in the endothelial and mesangial cells, markers of podocyte differentiation, including WT-1 and NPHS1, were well preserved in VEGF<sup>hyp</sup>/Neph-Cre<sup>+/−</sup> mice until 2 wk of age (Figure 4A). Transmission electron micrographs showed that podocyte foot processes were normal, although “coarsening” was observed after the onset of proteinuria at approximately P5 (Figure 6).

**Glomerular Expression of VEGF Receptors.** It is well established that endothelial cells express both VEGFR-1 and VEGFR-2 in vivo (4–8). Expression of VEGFR-1 and VEGFR-2 has been reported in mesangial cell lines but not in vivo. VEGFR-2 is the major receptor that transduces VEGF-A signaling (5,28), whereas VEGFR-1 is thought to modify receptor signaling, perhaps by binding and reducing the amount of VEGF-A that is available for VEGFR-2–mediated signaling. To determine whether mesangial cells express the VEGFR-2 and/or VEGFR-1 in vivo, we examined kidneys from a VEGFR-2-GFP (Flk-1-GFP) reporter gene knock-in murine line (gift of J. Rossant) (22). Robust expression of VEGFR-2 was observed in glomerular endothelial cells, but no expression was seen in other glomerular cells types (Figure 8A). During glomerulo genesis, VEGFR-2–positive endothelial cells are seen migrating into the vascular cleft of S-shape stage glomeruli and in capillary loop and mature stage glomeruli. Double immunostaining was performed with antibodies to VSMA-α and GFP to detect mesangial and VEGFR-2–positive cells. No cells that co-expressed both VEGFR-2 and VSMA-α (Figure 8B) were identified. VEGFR-1 expression was determined using a VEGF<sup>−1αlacZki</sup> allele (Figure 8C) (23) and confirmed that VEGFR-1 expression is restricted to the endothelial compartment. Double immunostaining with antibodies that recognize β-galactosidase and the mesangial marker PDGFR-β (Figure 8D) showed that no cells in the glomeruli of VEGF<sup>−1αlacZki</sup> mice express both markers. Together, these data demonstrate that healthy mesangial cells do not express detectable levels of the major VEGF receptors in

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**Figure 2.** Clinical course of VEGF<sup>hyp</sup>/Neph-Cre<sup>−/−</sup> mice. (A) Representative SDS-PAGE blot shows frank nephrotic-range proteinuria (>5 g/L) in VEGF<sup>hyp</sup>/Neph-Cre<sup>−/−</sup> mice by 5 d of age. Molecular weight (MW) markers: lane 1, urine from a control (VEGF<sup>hyp</sup>/P<sup>−/−</sup>) mouse; lane 2, urine from a mutant (VEGF<sup>hyp</sup>/Neph-Cre<sup>−/−</sup>); and lanes 3 and 4, positive controls from mice with known albuminuria. The arrow points at the large amount of albumin (66.2 kD) present in a sick mouse. A total of 2 μL of urine was loaded in each lane. (B) The bar graph shows reduced hemoglobin (Hb) levels in VEGF<sup>hyp</sup>/Neph-Cre<sup>−/−</sup> mice compared with controls that carry the VEGF<sup>−/−</sup> and/or VEGFR-1 allele (Figure 8C). (C) Analysis of serum creatinine levels. Serum creatinine measurements were markedly increased in mutant mice by 10 d of age. Values represent mean ± SD (n = 3 in each group); **P < 0.01. 100% of VEGF<sup>hyp</sup>/Neph-Cre<sup>−/−</sup> mice (n = 18) were dead by 3 wk of age.
Discussion

In the mouse, glomerular development begins at 12.5 d post coitum, when mesenchymal condensates that have been induced by adjacent ureteric bud tips begin to epithelialize (29). Podocyte precursors first appear at the S-shape stage of glomerular development as columnar shaped cells. At this point, presumptive podocytes begin to express VEGF-A and a vascular cleft forms. VEGFR-2-positive endothelial cells migrate into this vascular cleft to form the glomerular capillary network (29). By the capillary loop stage of development, fenestrations can be seen in the endothelium and mesangial cells that express desmin, and, later, VSMA-α migrate into the center of the glomerulus (30,31).

Gene-targeting studies have demonstrated a critical role for PDGF-B and its receptor PDGFR-β in mesangial cell migration. Loss of either the ligand, which is expressed by endothelial cells, or its receptor, expressed by incoming mesangial cells, leads to a single “ballooned” capillary loop and absence of mesangial cells (32,33). These results clearly demonstrate that mesangial cells, which normally contact the fenestrated endothelium directly, are required to form the glomerular capillary network. More recently, Betsholtz and co-workers (34,35) elegantly showed that endothelial production of PDGF-B is critical for mesangial cell recruitment as endothelial-specific deletion of PDGF-B results in a similar glomerular phenotype to the

**Figure 3.** Mesangiolysis in VEGF<sup>hypo/loxP,Neph-Cre<sup>+/−</sub> mice. (A) hematoxylin and eosin (H&E) and Masson trichrome staining (MAS) demonstrate loss of mesangial cells within differentiated glomeruli as early as 1 to 3 d of age compared with a day 3 (early time point) control. The mesangial cell compartment was replaced by material that has a foamy appearance. (B) Vascular α-smooth muscle actin (VSMA-α) immunostaining confirms that some mesangial cells (brown) are still present in glomeruli at 3 d (early time point) but that they are completely lost by 10 d (late time point). Control glomeruli at P10 to P12 are stained positive for VSMA-α. Early time point includes days 1 to 3 of age (P1 to P3); late time point includes days 10 to 12 of age (P10 to P12).
Figure 4. Molecular marker analysis of cell compartments within the glomerulus of VEGF\textsuperscript{hypo/loxP,Neph-Cre\textsuperscript{+/--}} mice at birth and days 10 to 12 (late time point). (A) In situ hybridization: Podocyte-specific markers: Wilms tumor suppressor gene (WT-1) and nephrin are expressed by podocytes at levels similar to controls at day 1 (early time point). (B) PDGFR-\(\beta\) receptor marks mesangial cells in developing and mature glomeruli. At birth, PDGFR-\(\beta\) receptor staining is similar to controls but completely disappears by 10 to 12 d of age (late time point) compared with control glomeruli of the same stage. (C) Immunostaining for the podocyte cell marker WT-1 and the endothelial cell marker platelet endothelial cell adhesion molecule 1 (PECAM-1) versus WT-1 and the early mesangial cell marker desmin. At day 1 (early time point), dual immunofluorescent staining demonstrated the presence of both endothelial (green in the top row) and mesangial (green in the bottom row) cells. WT-1–positive cells are podocytes (red).
PDGF-B or PDGF-β receptor knockout mice. It is interesting that rescue of the mesangial cell migration defect seems to occur postnatally, suggesting that other signaling pathways may compensate.

The fetal origin of mesangial cells is still not known, but mesangial precursors (35) or more mature “reserve” mesangial cells (36) remain in the adult kidney and sit just outside the glomerular tuft. It has been suggested that mesangial and endothelial cells may have a common origin (37). In the $VEGF^{hyp/loxP, Neph-Cre^{+/−}}$ transgenic mice, numerous PDGFR-
β-positive cells are present outside the glomerular tuft at day 5, whereas mesangial PDGFR-β expression in the glomerulus is dramatically reduced (data not shown). Mesangial cells are often described as glomerular pericytes because of their intimate association with endothelial cells and that they express many of the same markers as pericytes, including the PDGF-β receptor (35). Mesangial cells also exhibit characteristics of smooth muscle cells; they can contract in vitro and express smooth muscle cell markers during development including VSMA-α. In the healthy adult glomerulus, mesangial cells no longer express VSMA-α, but in glomerular injury models and diseases in which mesangial cell activation occurs, this marker is re-expressed. Mesangial cell proliferation and production of extracellular matrix by mesangial cells in diabetic nephropathy and other glomerular diseases is believed to play an important role in the progression of glomerular injury (38,39).

By contrast, mesangiolysis describes a process whereby mesangial cells degenerate and the mesangial matrix dissolves or is attenuated. This form of injury is seen in a variety of glomerular diseases, including thrombotic microangiopathies (e.g., preeclampsia, hemolytic uremic syndrome) and toxic glomerulopathy, and in renal transplantation (40–42), in which the primary injury is believed to occur in the endothelial cells. In animal models, mesangiolysis can be induced in one of two ways: direct damage to mesangial cells, as occurs with Habu snake venom (43) and anti-Thy-1 antibody (44), or direct damage to the endothelial compartment through injection of anti-endothelial antibodies (45) or monocrotaline, an alkaloid from Crotalaria spectabilis (46). Both pathways lead to mesangiolysis within minutes after injection of the toxin. Intravenous administration of aluminum lactate to rabbits causes deposition of aluminum in the mesangial cells that also results in mesangiolysis (47).

In this article, we demonstrate that reduced production of VEGF-A by podocytes also leads to mesangiolysis. In glomeruli from podocyte-specific null VEGF-A mice, markers of differentiated mesangial cell, such as VSMA-α, are completely absent. These results suggested that migration and/or differentiation...
of this cell lineage is dependent on VEGF-A signaling from the podocyte. In glomeruli from hypomorphic VEGF-A mice, mesangial cells initially migrate into the glomerulus in normal number and differentiate as shown by the expression of VSMA-α and PDGFR-β (Figures 3 and 4), but they undergo rapid mesangiolysis by 10 to 12 d of age.

Why do the mesangial cells degenerate? We were unable to detect an increase in apoptosis in the mesangial cell lineage of our transgenic mice by transferase-mediated dUTP nick-end labeling analysis (data not shown), suggesting that the mesangial cells are undergoing necrosis. Previous studies have reported that VEGFR-2 is expressed in mesangial cell lines in vitro and that VEGF-A leads to proliferation of this lineage (12,48). However, we were not able to demonstrate expression of VEGFR-2 or VEGFR-1 in mesangial cells in vivo (Figure 8). Taken together, these results are most consistent with a model in which the mesangial cell defects are due to disruption of the endothelial cell compartment (Figure 9).

Several lines of evidence support this model. First, mesangiolysis occurs in clinical conditions in which the primary defect occurs in endothelial cells (40–42) and injection of recombinant human VEGF (165) seems to enhance glomerular capillary repair in a rat model of induced glomerulonephritis with severe mesangiolysis (48). Second, endothelial cell loss occurred before mesangiolysis in our model. We showed previously that VEGF-A is produced by the podocytes and is required for endothelial cell migration, survival, and differentiation. Absolute loss of VEGF-A leads to the most dramatic endothelial phenotype in which no endothelial or mesangial cells can be identified in mature glomeruli (14). Production of a small amount of glomerular VEGF-A as seen in the VEGF<sup>hypo/lox<sub>P,Neph-Cre</sub></sup> mice permits ingrowth and formation of glomerular capillaries, but these endothelial cells rapidly disappear. During glomerular development, PDGF-B that is secreted by the endothelium recruits mesangial cells from the adjacent metanephric mesenchyme. On the basis of the requirement for PDGF-B production by the endothelial cells for mesangial cell migration, it follows that mesangial cell migration should be disrupted in VEGF<sup>hypo/lox<sub>P,Neph-Cre</sub></sup> mice. Our results suggest that ongoing production of PDGF-B and/or other factors by the endothelium is also required for mesangial cell survival (Figure 9).

In addition to the observation that VEGF-A is required for mesangial cell development and differentiation, our results emphasize the exquisite dose sensitivity that exists for VEGF-A in the developing glomerulus. For each of four individual VEGF-A gene doses within the podocyte (14), distinct clinical courses and glomerular lesions are observed. Clinically, disrup-
tion of VEGF-A production, delivery, and/or storage as a result of primary injury of the podocyte, glomerular basement membrane, or elevations of soluble receptors that reduce local levels of VEGF-A within the glomerulus all may contribute to renal injury.

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followed rapidly by mesangiolysis. VEGF-A production in podocytes leads to endothelial cell loss for glomerular development and mesangial cell recruitment and survival. VEGF-A production by podocytes is required for mesangial cell recruitment and in the mature glomerulus for mesangial cell survival. Reduction of VEGF-A production in podocytes leads to endothelial cell loss followed rapidly by mesangiolysis.

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