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PERSPECTIVES IN BASIC SCIENCE

Renal microvascular assembly and repair: Power and promise of molecular definition

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Renal microvascular assembly and repair: Power and promise of molecular definition. Developmental assembly of the renal microcirculation is a precise and coordinated process now accessible to experimental scrutiny. Although definition of the cellular and molecular determinants is incomplete, recent findings have reframed concepts and questions about the origins of vascular cells in the glomerulus and the molecules that direct cell recruitment, specialization and morphogenesis. New findings illustrate principles that may be applied to defining critical steps in microvascular repair following glomerular injury. Developmental assembly of endothelial, mesangial and epithelial cells into glomerular capillaries requires that a coordinated, temporally defined series of steps occur in an anatomically ordered sequence. Recent evidence shows that both vasculogenic and angiogenic processes participate. Local signals direct cell migration, proliferation, differentiation, cell-cell recognition, formation of intercellular connections, and morphogenesis. Growth factor receptor tyrosine kinases on vascular cells are important mediators of many of these events. Cultured cell systems have suggested that basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) promote endothelial cell proliferation, migration or morphogenesis, while genetic deletion experiments have defined an important role for PDGF β receptors and platelet-derived growth factor (PDGF) B in glomerular development. Receptor tyrosine kinases that convey non-proliferative signals also contribute in kidney and other sites. The EphB1 receptor, one of a diverse class of Eph receptors implicated in neural cell targeting, directs renal endothelial migration, cell-cell recognition and assembly, and is expressed with its ligand in developing glomeruli. Endothelial TIE2 receptors bind angiopoietins (1 and 2), the products of adjacent supportive cells, to signals direct capillary maturation in a sequence that defines cooperative roles for cells of different lineages. Ultimately, definition of the cellular steps and molecular sequence that direct microvascular cell assembly promises to identify therapeutic targets for repair and adaptive remodeling of injured glomeruli.

VASCULOGENESIS AND ANGIOGENESIS IN THE DEVELOPING KIDNEY

The processes that assemble vascular structures during embryonic life, whether in the kidney or in other sites, are distinguished by the presence or absence of contiguous pre-existing vessels and are termed angiogenesis or vasculogenesis, respectively. Vasculogenesis involves the *de novo* assembly of endothelial progenitor cells into vessels through migration, proliferation, cell-cell aggregation, assembly and morphogenesis [1–3]. During vertebrate embryonic development, vasculogenesis begins at gastrulation (at approximately embryonic day 7, E7, in the mouse). At that time, extraembryonic mesodermal cells from the posterior primitive streak migrate into the yolk sac where they aggregate to form "blood islands." Mesodermally-derived cells in the blood islands differentiate into either endothelial progenitor cells, so called angioblasts, or primitive hematopoietic lineages. The close spatial and temporal association between the development of these two cell lineages and their expression of common markers has suggested they have a common precursor, the "hemangioblast" [3]. By E8.5, endothelial progenitor cells from these blood islands fuse to form a primordial honeycombed vascular network, which subsequently remodels into a highly vascular plexus that feeds the developing embryo. Similar vasculogenic steps lead to development of the dorsal aorta in the embryo proper [4].

In contrast, angiogenesis involves branching, sprouting and anastomosis of existing vessels to create new vessels [5]. Prevailing concepts have asserted that postnatal vascular expansion proceeds through angiogenic processes. Yet notions about strict segregation between vasculogenic and angiogenic processes have become blurred in recent years, particularly in light of better definition glomerular vascular development [6]. It is now clear that vasculogenic processes contribute.

ORIGIN OF GLOMERULAR ENDOTHELIAL CELLS

The metanephric kidney begins to develop around E11.5 in the mouse. When the ureteric bud invades metanephric blastema, it branches and mesenchymal cells are induced to convert to an epithelial phenotype and aggregate in "vesicles" at the tips of the branches (Fig. 1A). Molecular determinants of this bidirectional inductive process include c-*ret* and its ligand, GDNF, a growth factor originally assigned function in neural development [7, 8]. Formation of a cleft, the so-called vascular cleft, in the comma and S-shaped epithelial aggregates provides an entry point to which progenitor endothelial and mesangial cells are recruited (Fig. 1B) [9]. This site ultimately develops into the vascular pole of the glomerular capillary tuft. Although little is currently known about the sequence of events leading glomerular endothelial, mesangial and epithelial elements to assemble the filtering unit, remarkable integration is required and several specialized features of the glomerular microcirculation are noteworthy. Glomerular endothelial cells develop fenestrae typical of endothelial cells exposed to vascular endothelial growth factor (VEGF), share a fused basement membrane with podocytes and are in direct contact with the mesangial cells that function as pericytes [10, 11].

Key words: renal microcirculation, vascular cells, tyrosine kinases, growth factor, vasculogenesis, angiogenesis, metanephric kidney.

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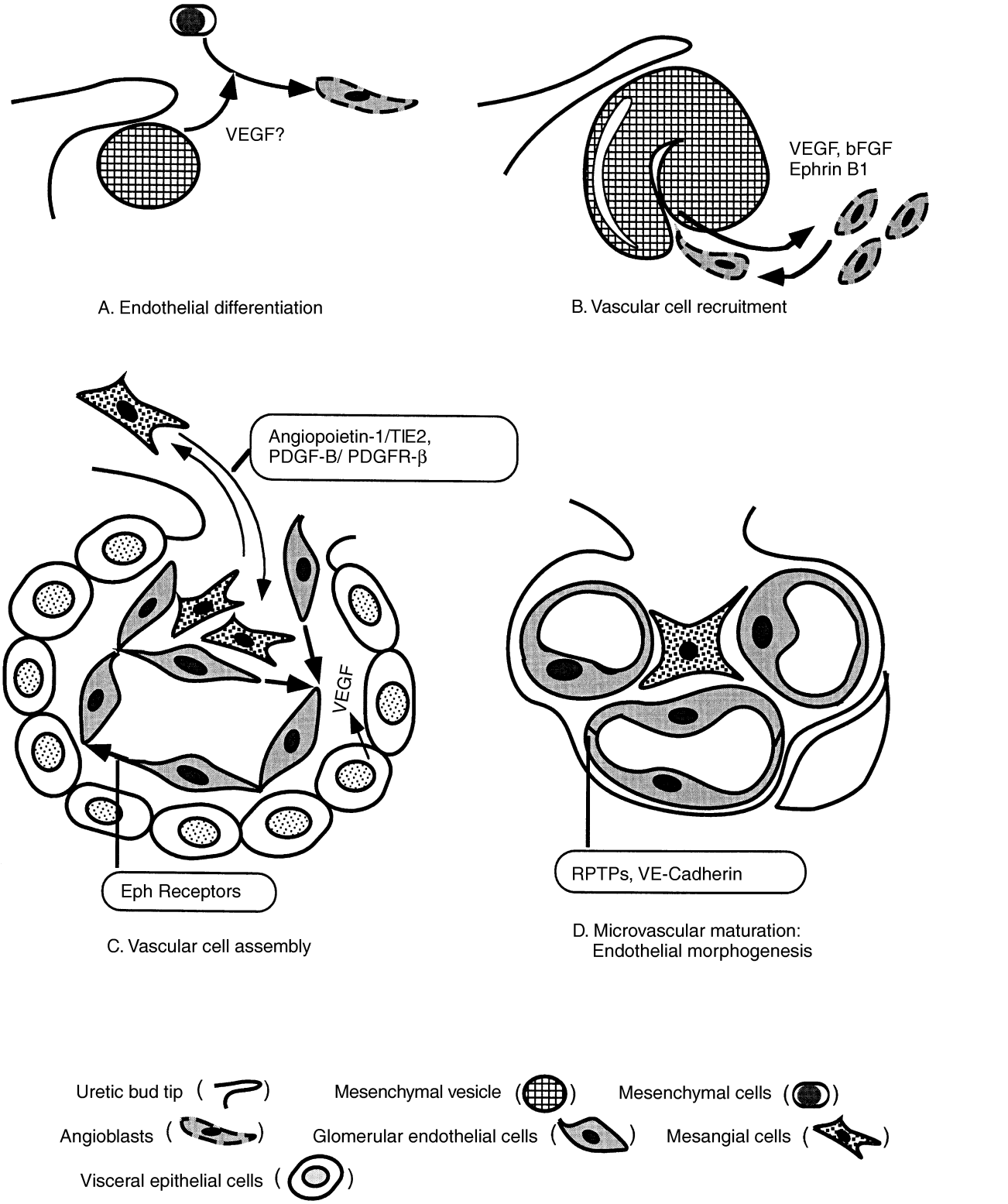

Fig. 1. Glomerular microvascular development. (*A*) Proliferative expansion of this epithelial mass of cells induces vascular endothelial growth factor (VEGF) expression and increases the population of flk-1 expressing "angioblasts" dispersed in adjacent sites. (*B*) VEGF, basic FGF and possibly ephrinB1 direct migration of angioblasts to the developing glomerular vascular cleft. (*C*) Coordinated recruitment and differentiation of mesangial and endothelial cells involves paracrine signaling through receptors for platelet-derived growth factor B (PDGF B) and possibly for angiopoietin-1. EphB1 receptors may direct homologous cell-cell recognition required for coordinated endothelial assembly and capillary network formation. VEGF likely provides ongoing proliferative signals, at least at early steps in the process. (*D*) Late steps in microvascular maturation involve formation of endothelial cell-cell junctions, growth arrest, and remodeling of matrix, where a number of cell to cell signaling systems may be invoked, including receptor protein tyrosine phosphatases (RPTPs) and cadherins.

Although metanephric organ cultures have provided a rich source of information about early glomerular epithelial development, they previously failed to develop glomerular capillaries as *in vitro* tissue culture explants [12, 13]. As a result, considerable efforts have been applied to defining experimental systems where determinants of glomerular capillary assembly could be tested. Early experiments used transplantation of metanephric kidney from avian and mammalian (rodent) sources to *in vivo* sites tractable for experimentation, such as the chick or quail chorioallantoic membrane [14, 15]. Incorporation of vascular cells from the recipient tissue into glomeruli that developed in the transplanted tissue led to the conclusion that glomerular capillary development depended predominantly upon angiogenic processes, in which new vessels are recruited to developing glomeruli from existing vessels in recipient tissue [16]. However, new data have reconstructed the process of glomerular capillary development in the best studied mammals, mice and rats.

Abrahamson and colleagues have defined an important role for recruitment of relatively undifferentiated mesenchymal cells to the developing glomerulus from adjacent sites in a vasculogenic process [6, 17, 18]. When genetically marked embryonic kidneys were transplanted under the capsule of native adult rodent kidneys, the transplanted donor tissue was the predominant source of endothelial cells that populate glomeruli that subsequently develop [18]. This result is consistent with their identification of endothelial progenitor cells, or angioblasts, that express VEGF receptor (flk-1) dispersed throughout the donor tissue [19]. In contrast, recipient-derived endothelial cells incorporated into glomerular capillaries of transplanted tissue when that embryonic tissue was transplanted into neonatal kidneys. The difference, depending upon developmental stage of the recipient tissue, is attributed to the presence of flk-1 (+) angioblasts in the newborn rodent kidneys [19]. These cells provide a tissue source of the angioblasts that are recruited to migrate into the vascular cleft of developing glomeruli and ultimately to assemble into glomerular capillaries (Fig. 1B). Although loose networks of endothelial marker positive cells were evident in the donor tissue [18], substantial evidence supports a role for a vasculogenic process in glomerular capillary development.

SYSTEMS FOR DEFINING MOLECULAR STEPS IN GLOMERULAR MICROVASCULAR ASSEMBLY

Developmental studies of gene "knock out" mice have provided some of the most compelling information about specific roles for individual receptor tyrosine kinases in the stepwise integration of endothelial progenitors into mature functional capillaries. Although some striking findings have been defined explicitly in the glomerular microcirculation, including definition of requirements for PDGF B/c-*sis* [20], other data are necessarily derived from observations of vascular development outside the kidney, since deficits may compromise embryonic viability prior to renal organogenesis. Conditional "knock out" technology promises to solve this problem in the near future, yet each experimental system imposes its own inherent limitations. Cultured cell systems may offer important advantages, particularly for defining later steps of vascular cell assembly and maturation. Due to the remarkable heterogeneity of vascular endothelium derived from different tissue sources, more relevant information may be obtained when the vascular endothelial cells are derived from the kidney and molecules under scrutiny are expressed in a timely manner in developing glomeruli.

GENE TARGETING AND SPECIFIC RECEPTOR TYROSINE KINASES IN VESSEL DEVELOPMENT

At a molecular level, gene "knock out" experiments have assigned critical roles to a number of specific receptor tyrosine kinases expressed on endothelial and mesangial cells, including two of the receptors for VEGF, flt-1 and flk-1 (KDR) [21–23]. Vascular endothelial growth factor (VEGF) is an important regulator of endothelial cell proliferation and migration [24]. High levels of flk-1 and flt-1 expression are detected at early developmental stages in endothelial cells. In fact, among those mesodermal cells that give rise to angioblasts, flk-1 is the first definitive marker of commitment of endothelial progenitors [25, 26]. Mesodermally-derived angioblasts that express flk-1 are contiguous to endodermal cells that coincidentally express VEGF, at E7.5, suggesting that VEGF from this source may support differentiation of flk-1 positive cells to endothelial cells [27].

Definitive evidence that flk-1 signaling has a pivotal role in endothelial differentiation in the mouse embryo comes from targeted gene disruption studies [28, 29]. Flk-1 deficient mice die between E8.5 and E9.5, as a result of an early defect in the development of hematopoietic cells and endothelial cells. Yolk sac blood islands are absent at 7.5 days and organized blood vessels are not observed in the embryo at any time.

Despite the overlapping affinities of flk-1 and flt-1 for VEGF, targeted disruption of flt-1 evokes a different phenotype. In these animals early endothelial differentiation is not blocked in either extra-embryonic or embryonic regions, and animals die at later embryonic stages [30]. The flt-$1^{-/-}$ endothelial cells assemble into abnormal vascular channels containing aggregates of endothelial cells within the lumen, suggesting that flt-1 signals endothelial cell-cell or cell-matrix interactions. Thus, the flt-1 null mice display disordered organization of the early embryonic vasculature, rather than defects in endothelial differentiation.

Similar findings are seen in animals with VEGF gene inactivation [31, 32]. Angiogenesis and blood island formation is impaired. Notably, loss of a single allele is capable of causing embryonic lethality at E11 to E12. In aggregate, these genetic deletion experiments suggest that flk-1 signaling is important in early steps of endothelial differentiation, and that flk-1 may interact with ligands other than VEGF to accomplish these steps. Loss of VEGF or flt-1 function impacts later stages of vessel development, causing failure of organization and integration.

HOW IS RENAL VASCULOGENESIS INDUCED?

The coordinate expression of VEGF and its receptors during renal vasculogenesis suggests pivotal function of this ligand/receptor pair for this process [33, 34]. Two recent reports confirm important roles for VEGF in developing kidney. Culture of metanephric kidneys under hypoxic conditions induces VEGF expression, as well as expression of flt-1, flk-1 on cells that contribute to developing capillaries [35]. Moreover, neutralizing antibodies against VEGF block these responses. These findings suggest that local expansion of cell mass coinciding with cell division induces VEGF expression [35]. *In vivo* administration of VEGF antibodies blocks glomerular and extraglomerular capillary development in newborn mice, consistent with VEGF

function to recruit and promote proliferation of endothelial cells [36]. As depicted in Figure 1B, these findings suggest that hypoxia in the developing glomerular epithelial mass induces VEGF expression, which, in turn, mediates vasculogenesis through recruitment of the flk-1 expressing angioblasts that reside in mesenchymal tissue [19].

ASSEMBLY AND MATURATION OF VASCULAR STRUCTURES: PDGF B AND RECRUITMENT OF PERICYTES

Targeted disruption of genes for other growth factors and their receptors demonstrate that the coordinated recruitment of supportive pericyte-like mesangial cells is critical to the effective development of glomerular capillaries (Fig. 1C). PDGF-B/c-*sis* and PDGFR-β null mice die perinatally from hemorrhage and a general failure of late stage capillary development [20, 37]. These animals have fully developed Bowman's spaces, but the glomerular capillaries are poorly developed, with a complete absence of identifiable mesangial cells. Epithelial and endothelial cell types can be recognized, but their integration into functional capillaries has been aborted by the apparent failure of glomerular mesangial recruitment. Outside the kidney, these animals show a remarkable pattern of intradermal and intracerebral hemorrhage that results from an apparent failure of capillaries to mature and to recruit pericytes [20, 38]. Pericytes function in those microcirculations as mesangial cells do in the glomerulus, to support and contact endothelial cells. Thus failure to coordinate recruitment of pericyte/mesangial cells with endothelial cells has a profound detrimental effect on capillary maturation and integrity.

BASIC FIBROBLAST GROWTH FACTOR, TRANSFORMING GROWTH FACTOR-β AND HEPATOCYTE GROWTH FACTOR IN VASCULAR DEVELOPMENT

A number of other growth factors and growth factor receptor systems have been implicated in angiogenesis and capillary development, although the evidence for their participation in the glomerulus is less direct. The fibroblast growth factors (FGFs) are among the most potent angiogenic factors known. Mice genetically deficient in FGF receptors (FGF-R1) show a lethal phenotype prior to vascular development and are not useful in studying kidney development [39]. FGF-R3 knock-out mice reveal abnormal bone development but show no apparent kidney abnormalities [40].

Recent studies, however, suggest that FGFs and their receptors may participate in renal blood vessel development. Using both reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry, Ford et al found expression of both FGF-1 and FGF-2 in cortex and glomeruli of adult rat kidneys [41]. Although all seven receptor isoforms were expressed in the cortex, only the IIIc splice variants of each receptor (FGF-R1 to FGF-R4) were found in glomeruli. Using a similar RT-PCR strategy, Kee et al identified expression of FGF-R1, FGF-R3 and FGF-R4 in fetal rat kidneys in late gestation (E21) [42]. FGF-R1 and FGF-R3 protein expression was greater in fetal kidneys than in kidneys from 12-week-old rats, while FGF-R4 protein was expressed comparably. Receptor tyrosine phosphorylation patterns leave some doubt as to the developmental roles for FGF in glomerular development, but the differential expression pattern suggests specified function for FGF within glomeruli.

Although it is clear that long-term administration of FGF-2 to rats promoted development of focal segmental sclerosis, with podocyte foot process effacement and detachment from the glomerular basal membrane, angiogenic or endothelial proliferative responses were not identified [43, 44]. Neutralizing antibodies to bFGF did reduce endothelial proliferative responses during the recovery phase of the anti-Thy1 mesangiolytic model [45]. In aggregate, the evidence for FGF and receptor participation in glomerular vascular development and repair is promising, but incomplete.

In addition to VEGF and FGF, transforming growth factor (TGF)-β1 has been assigned important roles in angiogenesis [46]. Exogenously delivered TGF-β1 evokes capillary angiogenesis in the chick chorioallantoic membrane [47]. During embryogenesis, TGF-β1 is expressed in many tissues, including endothelial and hematopoietic precursors [48]. Targeted disruption of the TGF-β1 gene caused fetal demise around E10.5, accompanied by defects in the yolk sac vasculature and hematopoiesis [49]. Although initial endothelial differentiation was apparent, numbers of flk-1 (+) cells were reduced and assembly of capillary-like networks was defective. Contacts between endothelial cells either failed to form or were sufficiently unstable that integrity was compromised, leading to blood cell leakage into the yolk sac cavity. Similar findings have been reported in TGF-β receptor type II deficient mice [50]. *In vitro* endothelial cell/pericyte coculture experiments have suggested that TGF-β mediates important growth inhibitory effects on endothelial cells [51].

Finally, an important role for TGF-β type II receptors in capillary morphogenesis has been demonstrated by Choi and Ballerman, in experiments where endogenous receptor function was abrogated using a dominant negative receptor in transfected endothelial cells [52]. Both apoptosis and capillary formation were eliminated by expression of this dominant negative receptor. At present, it appears that TGF-β may mediate important signals between pericyte (or mesangial cell) and endothelial cells to promote and maintain capillary integrity. Undoubtedly, additional studies will elucidate further roles for TGF-β and its receptors in regulating glomerular capillary development and repair.

A number of findings suggests that hepatocyte growth factor (HGF) may participate in glomerular capillary development. HGF is a potent mitogen and motility factor, acting through its receptor, the receptor tyrosine kinase, MET [53]. HGF is expressed in developing human renal cortex [54]. HGF is a potent angiogenic factor in corneal and in *in vitro* assays [54]. Cultured endothelial cells express MET, and HGF has been suggested to promote angiogenesis through induction of platelet activating factor (PAF) synthesis, *in vivo* [55, 56]. Further definition of the role of HGF and endothelial MET receptors in developing renal glomeruli is needed. Such studies promise to expand our understanding of collaborative interactions between epithelial and endothelial cell types, particularly if communication parallels that between hepatocytes and sinusoidal endothelial cells [57].

NEWLY DEFINED RECEPTOR SYSTEMS AND CAPILLARY ASSEMBLY

TIE2, angiopoietins and capillary maturation

Exciting new information about another family of receptor tyrosine kinases (RTKs), including TIE1 and TIE2 (Tek) amplifies on this developing theme of coordinated vascular recruitment

and maturation, as depicted in Figure 1, panels C and D [58, 59]. The onset of embryonic expression of receptors TIE1 and TIE2 seems to follow that of flk-1 in endothelial progenitor cells [60]. Two ligands for TIE2 have recently been identified and cloned. Angiopoietin-1 is a 70 kDa glycoprotein with fibrinogen-like and a coiled-coil domains [61]. Although angiopoietin-1 binds and induces tyrosine phosphorylation of TIE2, it does not elicit proliferation of endothelial cells or their association into tubules *in vitro*. However, its high expression in the mesenchyme surrounding developing blood vessels suggests it plays a regulatory role in the assembly of non-endothelial vessel wall components [62]. Mice lacking angiopoietin-1 exhibit defects in the remodeling of their vasculature, with fewer branches and homogeneously sized vessels. Ultrastructural analysis shows that endothelial cells are poorly associated with the underlying matrix and do not properly recruit and associate with periendothelial cells. These findings contrast with the earlier defects in vasculogenesis displayed by VEGF- or VEGF receptor-deficient embryos. Additional evidence links human families afflicted with hereditary venous malformations to TIE2 [63]. Two different families show activating mutations in the tyrosine kinase domain of TIE2 that are linked with venous malformations. These lesions are characterized by attenuated smooth muscle layer support in conjunction with the abnormal vascular channels.

A second ligand for TIE2, angiopoietin-2, has been identified [64]. Although highly homologous to angiopoietin-1, it binds TIE2 and blocks its activation. When overexpressed in transgenic mice, angiopoietin-2 disrupts blood vessel formation in the mouse embryo, closely mimicking the phenotype of the angiopoietin-1 knock-out animals [64]. In physiological circumstances of angiogenesis, angiopoietin-2 is expressed following the angiopoietin-1 expression and only at sites of active vessel remodeling, including ovary, placenta and uterus. Thus, endothelial TIE2 appears to communicate critical (yet undetermined) signals for regulating late vascular development. The ligands TIE2 binds, angiopoietin-1 and -2, appear to accelerate, then retard that function, respectively, as they are expressed in a temporal sequence in adjacent supportive cells.

Although explicit roles for TIE2 and angiopoietins in the glomerulus are not yet defined, the data from both patients with vascular malformations and mice provide exciting new information about the collaboration between cellular elements in developing and maturing blood vessels [65]. With the excitement comes a number of new questions about how the paracrine action of angiopoietins from smooth muscle cells or progenitors may participate in endothelial recruitment and integration of those cells. Further studies are needed to evaluate relevance of these findings to the assembly, remodeling and repair of the renal glomerular or renal venous circulations.

Endothelial targeting and EPH receptors in angiogenesis

To this point the data reviewed support the hypothesis that the embryonic glomerular microvasculature develops through sequential and cooperative effects of members of the VEGF, PDGF, TGF-β, FGF, HGF and angiopoietin-1 families. Available information suggests that PDGF and the angiopoietins (1 and 2) participate in the recruitment of pericytes cells to their endothelial partners. Is there an analogous receptor-ligand system that mediates (homologous) cell-cell recognition and targeting between differentiated endothelial cells, permitting them to find the right partner to integrate into capillary networks?

After a primary capillary plexus is formed, endothelial cells extend filopodia and sprout from the plexus in an angiogenic process to develop a mature vascular plexus [66]. The success of this vascular network integration requires that endothelial progenitor cells discriminate the paths they migrate, and the cells they contact in order to develop the highly organized and reproducibly assembled vascular architecture. In no other organ is this architectural assembly more critical than in the kidney, where the filtration apparatus and countercurrent processes require intimate integration between epithelial and vascular structures.

Remarkable similarities exist between the developmental targeting problems confronting axons and those of endothelial cells in the developing vasculature. Axons migrate in highly reproducible patterns toward either neural or muscle cell targets, based on local molecular guidance signals [67]. Despite the great distances axons migrate, they make very few errors of navigation, thanks to remarkably specific guidance cues presented by cells in their environment. Recent evidence suggests similarities in targeting processes are also shared between vascular and neural networks at the molecular level, particularly among receptor tyrosine kinases of the Eph family.

Eph receptors currently comprise a family of at least 13 individual members that are expressed in species from *Xenopus laevis* to *Homo sapiens* in a highly tissue-restricted distribution [68]. Recent changes in the nomenclature have segregated ligands (ephrins) and receptors (Ephs) into two distinct classes based on the structural characteristics of the ephrins [69]. Ligands of the ephrinA series are *g*lycosyl*p*hosphatidyl*i*nositol linked, while those of the ephrinB series are transmembrane proteins. EphA receptors display overlapping affinities for ephrinA series ligands, and the same relationships hold for EphB subclass receptors. Reciprocal compartmentation of ligands with their receptors has been noted during embryonic development, suggesting important roles for these molecules in defining the developmental organizational plan [70].

Interactions between cells displaying ligands and receptors of these families direct cell aggregation and targeting behavior, as well as other organizational responses [68]. The targeting functions of these molecules may be best illustrated in the developing retinotectal system where retinal axons expressing EphA3 receptors migrate through tectal fields of cells displaying a gradient of the ligand ephrinA2. These axonal projections select their final destinations based on local levels of ligand expression [71].

In the vascular system, recent evidence implicates the Eph family receptor, EphA2 (Eck), in angiogenic and chemotactic responses [72]. Endothelial expression of the EphA2 ligand, ephrinA1 (B-61/Lerk-1), is induced by TNFα, and ephrinA1 antibodies block angiogenic responses to TNFα in the rabbit corneal angiogenesis assay. Our lab recently identified expression of a second Eph family receptor, EphB1 (ELK), in human renal microvascular endothelial cells (HRMEC) and in glomerular endothelial cells *in situ* [73]. In collaboration with the Abrahamson lab, we identified high level expression of both EphB1 (receptor) and ephrinB1 (ligand) in developing and mature murine glomeruli. Mesenchymal cells in the subcapsular cortex displayed intense EphB1 and ephrinB1 staining in a pattern similar to that of flk-1. EphB1 staining was seen in developing glomeruli, as was ephrin-B1 immunoreactivity. In contrast to the

Table 1. Neural and microvascular networks share remarkable similarities

		Neural network	Vascular network
Basic mechanisms		1 trillion neurons → growth cones → neurite extension	>1 trillion endothelial cells → filopodial extension → capillary tube formation
Involved processes	—Attachment —Migration —Cell-cell connection —heterologous —homologous	During development only	During development and repair
Key molecules	—Cell adhesion molecules	—Ig-CAM —Cadherin	—PECAM —VE cadherin
	—Receptor tyrosine kinases (RTK)	—FGF receptor —Trk receptor —Eph receptor	—PDGF receptor —VEGF receptor —Eph receptor
	—Receptor protein tyrosine phosphatases (RPTP)	—RPTP B	—DEP-1 (?)
	—Matrix proteins	—Fibronectin —Laminin	—Fibronectin —Laminin
	—Others	—Netrins —Semaphorins	

developing kidney, mesenchymal and interstitial cells no longer expressed EphB1, ephrinB1, or flk-1 in the adult mouse kidney, while EphB1 and ephrin-B1 expression persisted on arteriolar intimal cells and glomerular capillary endothelial cells.

In vitro studies showed ephrinB1 is capable of inducing cultured human renal microvascular endothelial cells to assemble into capillary-like structures, while ephrinA1 did not [73]. Recent evidence supports an important role for juxtacrine (cell to cell) EphB1/ephrin-B1 interactions in signaling recognition steps required for endothelial commitment to capillary-like morphogenesis. Interruption of this interaction blocks capillary-like assembly [92]. Our findings provide early evidence that Eph/ephrin interactions may participate in the cell-cell recognition processes required for glomerular capillary assembly. Indeed, we speculate that targeting functions analogous to retinotectal neural targeting may direct endothelial progenitors to the vascular cleft during glomerular development.

Receptor tyrosine phosphatases and vascular assembly: Predictions

Although sufficient data are not yet in place to assign important functions to yet another class of cell surface receptors, several independent lines of evidence suggest that receptor protein tyrosine phosphatases (RPTPs) may participate importantly in late stages of capillary development (Fig. 1D). If the analogies between guidance systems that direct neuronal network and vascular network assembly hold (Table 1), strong evidence for RPTP function in axonal guidance may be relevant [74]. A particularly critical late step in both developmental and reparative capillary development is the arrest of endothelial cell proliferation (Fig. 1D).

Several studies point to RPTPs as possible molecular transducers of growth arrest signals. Sodium orthovanadate, the potent protein tyrosine phosphatase (PTP) inhibitor, releases density arrest and stimulates capillary morphogenesis in confluent endothelial cells [75–77]. PTP activity is markedly increased in cultured nonendothelial cells at high density where growth arrest is seen [78, 79]. Finally, a Type III RPTP, DEP-1 (or ECRTP/HPTPη) [80–82] is expressed in human renal microvascular endothelial cells and in glomerular endothelial cells, *in situ* [83; T.Takahashi and T.O. Daniel, unpublished results]. The Type III family includes the glomerular epithelial phosphatase, GLEPP1 [84], as well as two Drosophila RPTPs, DPTP10D and DPTP99A [85] that participate in neural targeting [74]. DEP-1 is prominently expressed in vascular sites, including glomerular endothelial cells, and in some renal epithelial cells [83]. Its expression is reduced in migrating and proliferating endothelial cells [83]. Indeed, cultured cell studies of human renal microvascular endothelial cells support a role for DEP-1 to sense and transduce signals that may mediate growth arrest [93]. This and other RPTPs may be required to arrest proliferation and migration of endothelial cells during developmental capillary maturation and upon repair following injury.

Glomerular capillary repair

The findings summarized above have identified molecular determinants that contribute to *developmental* glomerular capillary assembly. A major motivation for this work remains the expectation that, at least at some level, repair recapitulates development. We anticipate that successful repair follows sequential steps represented in the left panel of Figure 2. Derangement of that process may lead to the sclerotic outcome seen in the right panel. Where are the developmental studies applicable to further definition of glomerular and other microvascular renal repair processes?

In several reversible glomerular injury models, many of the endothelial cells display proliferative responses and several morphological features of angiogenesis [45, 86–88]. Johnson and colleagues have shown that brisk endothelial proliferation accompanies glomerular repair following acute injury in the mesangiolytic anti-Thy-1 model [45]. In this experimental system, mesangiolysis leads to ballooning and distention of capillary endothelial cells as they lose their support from adjacent mesangial cells. The number of proliferating endothelial cells in a glomerulus is increased seven- to ninefold during days 2 to 7 following the mesangiolytic injury [45]. Consistent with the increased local

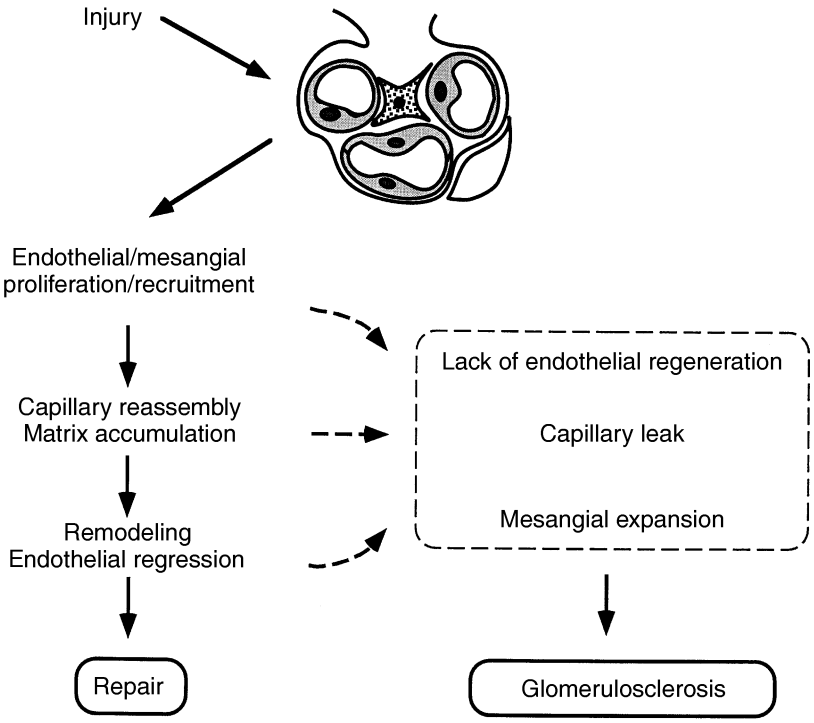

Fig. 2. Alternative post-injury responses. Capillary reassembly into new microvascular networks leads, under optimal circumstances, to remodeling and repair of the glomerulus through processes shown on the left. Alternatively, disordered repair or remodeling may contribute to the destructive processes depicted on the right, ultimately resulting in glomerulosclerosis.

production of VEGF and bFGF following such an injury, neutralizing antibodies against VEGF and bFGF, but not PDGF, reduced endothelial proliferation. However, the outcome of reducing endothelial proliferation upon capillary repair is not yet fully investigated. Other data suggest that repair recapitulates development. In a second model of glomerular injury, the Habu snake venom model, endothelial repair involves migration and elongation of endothelial cell processes from the vascular pole, much as was described above for the developmental steps in glomerulogenesis [87]. It is not yet clear whether this represents recruitment of endothelial replacement cells from a local repository, or from extraglomerular sites.

It is now obvious that glomerular cell proliferation is only one part of the repair process. A number of questions seem important in light of the developmental findings. Are angioblasts recruited to repair sites from repositories either within or outside the glomerulus? Are all glomerular endothelial cells equally competent to proliferate, migrate and integrate into repairing capillaries? How are proliferation of endothelial and mesangial cells coupled to their structural integration into capillaries under repair?

Some provocative results may be pertinent to these questions. We have identified small subpopulations of endothelial cells expressing the PEC-1 antigen in normal human glomeruli [89]. Among cultured human renal microvascular endothelial cells, those expressing this antigen display capacity to assemble into capillary-like structures *in vitro*. We speculate that these rare PEC-1 (+) glomerular cells may serve as a local repository of proliferation competent endothelial progenitors capable of repopulating capillaries following damage.

As is depicted in Figure 2, success (left panels) or failure (right panels) of many of the developmental steps outlined above likely determines whether normal function is repaired following renal injury. Certainly the success of the repair process in any capillary bed, including the glomerulus, requires not only proliferation of endothelial and mesangial cells, but also their reintegration into functional capillary structures.

THE FUTURE

The potential for therapeutic strategies to exploit fundamental properties of the microcirculation is great. The microvasculature, in kidney as in other organs, is a dynamic organ that retains developmental potential throughout life. It is the functional gatekeeper directing flow of inflammatory cells to tissue sites of injury. It actively regulates thrombosis and fibrinolysis. It supports growth and metastasis of solid tumor neoplasms. Its tissue specific heterogeneity fundamentally defines function of organs such as kidney and intestine, and is critical in the central nervous system. It is a major conduit for drug delivery to tissue sites. We anticipate that future gene-based and cell-based therapies are likely to target the microvasculature, expanding considerably on approaches to glomerular disease, cancer, and vascular diseases, as well as chronic inflammatory conditions.

Early experiments have proved conceptually that mesangial cells may be delivered to the glomerular microcirculation and incorporated into viable structures there [90]. Recent evidence shows that endothelial cell precursors can be purified from circulating blood, cultured and returned to the circulation where they incorporate into capillaries that regenerate at sites of skeletal muscle ischemia [91]. These findings suggest the possibility of coupling gene replacement technologies with targeted cell replacement approaches, to deliver genetically modified endothelial cells to tissue sites where they may incorporate into microvascular beds. Once incorporated, such modified endothelial cells could potentially deliver biological products capable of ameliorating or modifying primary disease processes. If cultured "angioblasts" can be isolated from the circulation in sufficient numbers, cultured

and amplified, transfected and selected for exogenous gene production, then finally delivered back into the circulation for integration into selected sites, innumerable therapeutic strategies may develop. Whether or not these approaches ultimately yield tractable therapeutic strategies, their development will provide fertile ground for identifying molecular and cellular processes in experimental systems.

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APPENDIX

Abbreviations used in this article are: bFGF, basic fibroblast growth factor; E number, embryonic day; Eck, Eph family of tyrosine receptors, EphA2; ELK, Eph family receptor, EphB1; Eph, family of tyrosine kinases; ephrins, ligands of Eph receptors; FGF, fibroblast growth factor; flk-1, vascular endothelial growth factor receptor; HGF, hepatocyte growth factor; HRMEC, human renal microvascular endothelial cells; MET, receptor tyrosine kinase of hepatocyte growth factor; PAF, platelet activating factor; PDGF, platelet-derived growth factor; PTP, protein tyrosine phosphatase; RPTP, receptor protein tyrosine phosphatase; RTK, receptor tyrosine kinase; RT-PCR, reverse transcriptase-polymerase chain reaction; TGF-B, transforming growth factor-beta; VEGF, vascular endothelial growth factor.

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