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# Kidney endothelial cell heterogeneity, angiocrine activity and paracrine regulatory mechanisms

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## Abstract

The blood microvascular endothelium consists of a heterogeneous population of cells with regionally distinct morphologies and transcriptional signatures in different tissues and organs. In addition to providing an anti-thrombogenic surface for blood flow, endothelial cells perform a multitude of additional regulatory tasks involving organogenesis, metabolism, angiogenesis, inflammation, repair and organ homeostasis. To communicate with surrounding cells and accomplish their many functions, endothelial cells secrete angiocrine factors including growth factors, chemokines, cytokines, extracellular matrix components, and proteolytic enzymes. Nonendothelial parenchymal and stromal cells in turn regulate endothelial growth, differentiation and survival during embryonal development and in the adult by paracrine mechanisms. Driven by advances in molecular biology, animal genetics, single cell transcriptomics and microscopic imaging, knowledge of organotypic vasculatures has expanded rapidly in recent years. The kidney vasculature, in particular, has been the focus of intensive investigation and represents a primary example of how endothelial heterogeneity and crosstalk with nonendothelial cells contribute to the development and function of a vital organ. In this paper, we review the morphology, function, and development of the kidney vasculature, with an emphasis on blood microvascular endothelial heterogeneity, and provide examples of endothelial and nonendothelial-derived factors that are critically involved in kidney development, growth, response to injury, and homeostasis.

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Author statement

DR, GL and RFN: conceptualization, writing-original draft, writing-reviewing & editing.

The literature cited in this review includes papers known by the authors from their previous work in vascular biology (DR, GL, RFN) and renal pathology/electron microscopy (RFN) as well papers identified by searching online using keywords such as: angiocrine factors, endothelial heterogeneity, fenestrae, kidney, and transcriptomics. Additional keywords such as PDGFB or VEGF were used to find papers that focused on specific angiocrine/paracrine factors. References of interest were also found in the cited literature of recently published reviews that covered in detail topics discussed in this paper (e. g. Refs. 6, 12, 14). Search engines used for this review include PubMed. Google and Google Scholar. Years covered are 1976–2021.

Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

**Keywords**

Angiocrine factors; Endothelial heterogeneity; Fenestrae; Kidney; Transcriptomics

## 1. Introduction

The blood microvasculature, once considered a passive conduit of oxygen, nutrients, hormones and blood cells, is now recognized as a critical regulator of organ development, maturation, homeostasis, and repair. Of critical importance in the execution of these functions are endothelial cells which line the inner surface of blood vessels throughout the body. Endothelial cells maintain blood fluidity by controlling the equilibrium between hemostasis and fibrinolysis, regulate vascular tone, and play a central role in inflammation, angiogenesis and tissue regeneration. [1,2] Endothelial cells perform the specialized tasks of local microvascular beds by acquiring heterogeneous phenotypes in different organs and within the vascular bed of individual organs [3–6]. Endothelial heterogeneity, or angiodiversity, ensures that microvascular endothelial cells nimbly and effectively adapt to the local structural and functional requirements in various districts of the vascular tree [6,7].

In the embryo, tissue-specific microvascular endothelial cells regulate organogenesis by modulating the growth, differentiation and function of the developing parenchyme and stroma. Parenchymal and stromal cells in turn regulate endothelial growth, differentiation and survival through paracrine mechanisms. Crosstalk between endothelial and parenchyma/stromal cells persists in the adult to maintain organ homeostasis in health and disease. Microvascular endothelial cells accomplish their regulatory function by producing molecules (secreted or membrane-bound), known as angiocrine factors, that control developmental, homeostatic and reparative processes by paracrine or juxtacrine mechanisms [6,8,9]. Endothelial-derived angiocrine factors include inhibitory and stimulatory growth factors, chemokines, cytokines, extracellular matrix components, and proteolytic enzymes. The angiocrine activity of endothelial cells is facilitated by their ubiquitous distribution and proximity to nonendothelial cells [8]. Examples of physiologic, developmental, reparative and pathologic processes modulated by endothelial angiocrine activity are shown in Table I.

The paradigm that blood vessels generate regulatory signals that influence organ development was first proposed in studies of liver and pancreas development, which showed that paracrine factors from endothelial cells control early steps of organogenesis during embryonal development [10,11]. The angiocrine factor concept was later introduced by Rafii and co-workers who proposed that endothelial cells form a vascular niche that can influence pathologic and reparative processes not only by delivering nutrients and oxygen but also by modulation the activity of other cell types through paracrine and juxtacrine mechanisms [12].

Among the most vascularized organs of the body are the kidneys which, combined, account for a fraction of body weight and yet receive up to one fourth of the cardiac blood output. The kidneys provide a remarkable example of how a morphologically and transcriptionally diverse population of microvascular endothelial cells contributes to the development, homeostasis and response to injury of a vital organ [13]. This review

focuses on the heterogeneity and angiocrine properties of the kidney microvascular endothelium and its paracrine regulation by parenchymal and stromal cells during kidney development, postnatally, and in the adult. We also describe how disruption of the paracrine crosstalk between endothelial and nonendothelial cells causes patterns of injury frequently encountered in renal pathology such as glomerulosclerosis, interstitial fibrosis, and thrombotic microangiopathy, resulting in proteinuria and/or renal failure.

## 2. Anatomy and physiology of the kidney vasculature

The kidneys perform a multitude of functions including removal of metabolic waste products and toxins; control of water and electrolyte balance, and blood pressure; regulation of acid-base and calcium metabolism; and endocrine control of hemopoiesis through the production of erythropoietin. The renal vasculature plays a central role in the performance of these functions. In each kidney, oxygenated blood derived from the abdominal aorta flows through the renal artery into gradually smaller branches and finally, arterioles [6,13]. Afferent arterioles carry blood to glomeruli, microscopic filters made of an intricate tuft of capillaries lined by fenestrated endothelium, overlaid by podocytes and supported by mesangial cells and matrix. Blood flowing through glomerular capillaries at high pressure is filtered through the glomerular capillary wall into the Bowman's urinary space and drained by efferent arterioles into peritubular capillaries (PTC). PTC supply the tubular parenchyma with oxygen and nutrients and drain the water and small solutes that have been reabsorbed by tubular epithelial cells. Glomerular blood flow and filtration rate are regulated by afferent and efferent arterioles through their capacity to modulate vascular tone [6,13].

The ultrafiltrate produced in the glomeruli contains low-molecular weight molecules including ions, water, glucose, and nitrogenous waste products which flow into the Bowman's urinary space, proximal tubules, Henle's loop, distal tubules and, finally, collecting ducts that empty into the renal pelvis. As it flows through the tubules, the glomerular filtrate is transported from the renal cortex, where glomeruli reside and most of the reabsorption and secretion of small solutes by tubules occurs, into the medulla. Here a hyperosmolar gradient re-absorbs water, and the glomerular filtrate becomes progressively more concentrated into urine which flows from the collecting ducts into the renal calyceal system and pelvis. The hyperosmolar gradient of the renal medulla is created by a countercurrent mechanism involving the Henle's loop, the descending vasa recta (DVR), which originate from the efferent arterioles of the juxtamedullary glomeruli, and the ascending vasa recta (AVR), which together with the PTC drain into the renal venous system [6,9,13].

The kidneys also contain lymphatic vessels which collect excess fluid and proteins including albumin that have accumulated in the interstitium. Lymphatics are present in the renal cortex but not in the medulla where interstitial fluid is primarily drained by the AVR [9].

## 3. Development of the renal vasculature

Kidney blood vessels develop concurrently with the epithelial components of the nephron from two mesodermal derivatives: the ureteric bud and the metanephric

mesenchyme [14]. Endothelial cells, derived from precursor cells of the ureteric bud / metanephric mesenchyme or extrarenal sources, vascularize the developing kidney through vasculogenesis, angiogenesis and intussusceptive microvascular growth (nonsprouting angiogenesis) [15–18]. Vasculogenesis occurs through *in situ* differentiation of endothelial cells from angioblasts present in the embryonal mesenchyme. Angiogenesis is the formation of new vessels through endothelial sprouting from pre-existing vessels. Intussusceptive microvascular growth is the result of splitting of individual microvessels into two separate vascular tubes through the formation of intraluminal endothelial pillars [2,19,20]. Glomeruli develop in the metanephric mesenchyme from cleft-like structures (S-shaped bodies) lined by cells which differentiate into podocytes. In response to podocyte stimuli, endothelial cells originating from angioblasts migrate into the cleft of the S-shaped bodies forming capillaries primarily by vasculogenesis, though sprouting and nonsprouting angiogenesis have also been implicated in this process [19–21]. PTC arise from vasculogenesis and sprouting angiogenesis [20]. Major renal vessels originate from branches of the aorta and vena cava through an angiogenic sprouting process. [20].

## 4. Morphologic and functional heterogeneity of kidney endothelial cells

Endothelial cell heterogeneity in the kidney is dictated by local functional needs and regulated by a variety of environmental factors including blood flow, oxygen tension, osmolarity, nutrient levels, growth factors/cytokine milieu, and cell–cell interactions (6). Distinct morphologic types characterize the renal endothelium including the fenestrated endothelium of the capillary microvasculature and the continuous endothelium of arterial and venous vessels (Fig. 1).

To eliminate metabolic waste products and toxins from the circulation, renal glomeruli in humans produce approximately 180 l of ultrafiltrate per day, 99% of which is reabsorbed by tubules. This prodigious daily exchange of fluids from and into the renal microcirculation is made possible by the presence of a specialized network of highly permeable and fenestrated endothelial cells in the glomeruli and PTC [6,9,13].

The fenestrated endothelium of the glomeruli is covered by a proteoglycan-rich glycocalyx which contributes negative charges that are critical for the selective nature of the glomerular capillary filtration barrier function. This feature ensures that circulating negatively charged molecules such as albumin are repelled by the glomerular filter and not lost in the ultrafiltrate. During glomerulus development, the endothelial fenestrae ranging in size from 70 to 100 nm in diameter, are subtended by diaphragms, which are gradually lost in post-natal life [9,22] (Fig. 1A–B).

The fenestrae of the PTC endothelium are covered by a thin diaphragm composed of glycoproteins which persists in the postnatal kidney (Fig, 1C–D). The presence of fenestrations in the PTC endothelium facilitates reabsorption of fluids from the tubular lumen into the circulation and exchange of molecules that have been reabsorbed or secreted by tubular epithelial cells. Fenestrated endothelial cells with diaphragms also line the AVR [9,23] (Fig. 1E–F).

Unlike the fenestrated endothelium of the glomerular and peritubular capillaries, the arterial endothelium is continuous and has a well-developed system of tight junctions which limit its permeability and convey mechanical strength in an environment characterized by high rates of pulsatile blood flow (Fig. 1G–H). A continuous endothelium also forms the inner lining of the renal veins. A notable exception to the continuous morphology of the arterial endothelium is the endothelium associated with the juxtaglomerular apparatus of the afferent arteriole, which is fenestrated and without diaphragms, likely due to facilitate exchanges of molecules between circulating blood and the renin-producing cells of the juxtaglomerular apparatus [9].

## 5. Gene expression heterogeneity in kidney endothelial cells

Advances in single-cell transcriptomics have broadened our knowledge of endothelial heterogeneity in different organs, demonstrating the existence of organ-specific signatures of gene expression [6,7,24,25] and revealing up to 24 transcriptionally different endothelial cell populations in the kidney [9]. Distinct transcriptional profiles have been identified for endothelial cells of renal arteries, afferent arterioles, efferent arterioles, glomerular capillaries, peritubular capillaries, descending vasa recta, ascending vasa recta, venules, and veins [9].

The pattern of gene expression in different renal vascular districts reflects the morphological and functional phenotype of the endothelial cells. For example, the fenestrated endothelium of glomerular and peritubular capillary endothelial cells expresses *KDR* (Kinase Insert Domain Receptor)*/Flk1* (Vascular Endothelial Growth Factor Receptor 2, VEGFR2) which transduces VEGF signals from adjacent epithelial cells (podocytes, tubular epithelial cells) regulating endothelial cell migration, proliferation, survival, and differentiation, including formation and maintenance of fenestrae, during embryonal development and in the adult [9].

Unlike the glomerular endothelium, the PTC endothelium expresses *PLVAP*/*Plvap* (Plasmalemma Vesicle Associated Protein) which encodes for a protein associated with the diaphragms that span the endothelial fenestrae [9,24] (Fig. 2). Immature capillaries in the embryonal kidney express *Aplnr* (Apelin receptor) which gets downregulated but is retained in a subset of mature PTC [26,27]. The PTC endothelium also expresses higher levels of *Igfbp3* (insulin-like growth factor binding protein-3 and *Npr3* (natriuretic peptide receptor-3) whereas expression of vWF(von Willebrand factor) is more prominent in the glomerular endothelium [9,24]. Genes specifically expressed in selected renal endothelial cell types also include transcription factors such as *Sox17* (SRY-Box Transcription Factor 17, arterial and arteriolar endothelium), *COUP-TFII* (COUP transcription factor 2, also known as *Nr2f2*, venous endothelium) and *Tbx3* (T-Box Transcription Factor 3) and *Gata5* (GATA Binding Protein 5) (glomerular endothelium) [9,26]. Notably, targeted deletion of *Tbx3* in endothelial cells causes glomerular dysmorphogenesis manifested by focal capillary aneurysms, glomerular hypoplasia and glomerulosclerosis [26]. The endothelium of DVR expresses *Slc14a1* (solute carrier family 14 member 1) which encodes for urea transporter B whereas the AVR endothelium which, like the PTC endothelium, is rich in diaphragmed fenestrae expresses *Plvap* [9,27] (Fig. 2). Intriguingly, the AVR endothelium also expresses the lymphatic cell markers *Prox1* and *Flt4* (VEGFR3). AVR endothelial cells,

however, do not express other lymphatic endothelial cell markers such as *Lyve1* (Lymphatic Vessel Endothelial Hyaluronan Receptor 1) and *Pdpn* (podoplanin). Thus, AVR, uniquely express a dual blood and lymphatic endothelial phenotype and are actively involved in the reabsorption of interstitial fluids, a function typically performed by tissue lymphatics which are not present in the renal medulla. Unlike the glomerular, peritubular and medullary capillaries, which are *Kdr*-positive, both DVR and AVR do not express *Kdr* or express it at low levels [26,27].

Gene expression in kidney cells can also be regulated at the post-transcriptional level by microRNAs (miRNAs) through both translation repression of mRNA and mRNA destabilization. miRNAs modulate kidney development and have been implicated in the pathogenesis of kidney diseases [28]. miRNAs have also been shown to contribute to the heterogeneous response of endothelial cells from different vascular beds to disease stimuli. In mouse models of renal inflammation, glomerular and arteriolar endothelial cells upregulate VCAM-1 mRNA levels to a similar extent, whereas VCAM-1 protein expression is selectively increased only in the arteriolar endothelium [29]. This discrepancy has been linked to miRNA-126 which is highly expressed in the glomerular endothelium where it interferes with VCAM-1 mRNA function [29]. This regulatory mechanism can be blocked through pharmacologic miRNA-126 knockdown which has been shown to unleash VCAM-1 protein expression in the glomerular endothelium in response to inflammatory challenge [29].

In addition to the examples that we have provided here, many more genes characterize the heterogeneous nature of kidney endothelial cells. For a comprehensive list of these genes the reader is referred to recently published single cell-RNA sequencing studies of mouse and human kidney endothelial cells [9,24,26].

## 6. Angiocrine and endothelial stimulatory factors in the embryonal and adult kidney

As endothelial cells differentiate and become established, they form a niche that plays a crucial role in the formation, maintenance and repair of kidney structures during embryonal development and in postnatal life [7,26]. The molecular mechanisms by which the endothelial niche orchestrates these processes in different anatomic regions of the kidney are complex, poorly understood, and strongly influenced by the heterogeneous nature of endothelial cells. We provide here examples of how the renal microvasculature regulates the development and maturation of kidney structures and how in turn the renal parenchymal and stromal cells modulate the growth and differentiation of the renal endothelium.

The importance of the blood microvasculature in kidney embryonal development has been best documented in studies of glomerulogenesis. In the metanephric mesenchyme, the maturation of the S-shaped bodies into fully formed glomerular structures requires blood flow and endothelial angiocrine activity. Strong evidence in support of this finding is provided by the zebrafish mutant *cloche* which, being unable to produce endothelial cells, fails to form a normal pronephric glomerulus [30]. Altered or absent glomerulogenesis also occurs in zebrafish mutants that have deficient blood flow due to mutations causing

cardiac dysfunction, pharmacologic interference of cardiac output, or laser occlusion of blood vessels [31]. Failure of glomerulogenesis in this model has been attributed to marked reduction in the endothelial metalloproteinase-2 (MMP2) production due to insufficient blood flow resulting in defective remodeling of the extracellular matrix, and it can be reproduced by administering the MMP2 inhibitor TIMP2 to zebrafish with normal blood flow [31]. Findings similar to those reported with the Zebrafish model were obtained using inducible pluripotent stem cells (iPSC)-derived kidney organoids, in which maturation of glomeruli and tubules occurs only after the organoids have become vascularized and perfused following transplantation beneath the mouse renal capsule [32]. In the iPSC-derived kidney organoid model, vascular networks that have formed within the transplant by vasculogenesis anastomose with angiogenic neovessels sprouted from preexisting vessels in the host kidney, recapitulating the embryonic development of the kidney vasculature [32].

Development of the embryonal vasculature is critically dependent on the VEGF/ VEGFR system [33]. Angioblasts expressing VEGFRs are identifiable in the pre-vascular embryonic kidney [34]. As S-shaped bodies in the metanephric mesenchyme develop into glomeruli, differentiating podocytes produce VEGF which stimulates vasculogenesis from angioblasts [14]. In response to podocyte-derived VEGF, endothelial cells migrate into the cleft of the S-shaped bodies forming capillary loops and acquiring a fenestrated phenotype [35]. Genetic ablation of *Vegf* (VEGF-A), *Flt1* (VEGFR1) or *Kdr* (VEGFR2) causes embryonal lethality due to severe cardiovascular defects [36]. Podocytes are the primary source of VEGF which is an essential requirement for glomerulogenesis. Podocyte-restricted deletion of VEGF causes hydrops and perinatal death. Glomeruli in these mice are small, with markedly reduced endothelial cells lacking fenestrations and paucity or absence of capillaries [35,38]. Dysregulation of the glomerular endothelium by genetically interfering with VEGF production in podocytes or VEGFR2 expression in mouse kidneys impairs podocyte maturation including formation of foot processes and slit diaphragms, indicating that endothelial cells are essential for maturation of podocytes and the glomerular filtration barrier [39]. Defective differentiation of podocytes evidenced by irregular aggregates of basement membrane and effaced podocyte foot processes also occurs in the *cloche* zebrafish model where endothelial differentiation is blocked at an early stage of embryonal development [30]. Thus, though the development of podocytes is independent of signals from other glomerular cell types, full podocyte differentiation occurs only after endothelial cells have formed capillary loops and provided appropriate angiocrine stimuli.

Podocyte VEGF is essential for endothelial homeostasis also in the postnatal period and in adults. Defective VEGF signaling due to post-natal deletion of *Kdr* causes injury and loss of glomerular endothelial cells [40]. Administration to newborn mice of a soluble VEGFR chimeric protein that blocks VEGF function, leads to renal failure, growth arrest and lethality, associated with a reduction in the number of endothelial fenestrations, accumulation of mesangial matrix, mesangiolysis, and thickening of the basement membranes [41]. Similarly, VEGF inhibitors such as anti-VEGF blocking antibody or soluble VEGFR1 administered postnatally cause glomerular abnormalities characterized by reduced cellularity, reduced capillaries, and loss of endothelial fenestrations [41]. In adult human patients, anti-VEGF medications, used as anti-angiogenic/adjuvant therapy for cancer treatment, can cause endothelial injury characterized by loss of endothelial

fenestrations, endothelial swelling and thrombotic microangiopathy [35]. The same pattern of glomerular endothelial injury and thrombotic microangiopathy can be obtained in adult mice by conditionally ablating *Vegf* in podocytes [35].

Suppression of podocyte VEGF function in humans or experimental animals causes proteinuria and hypertension. Proteinuria is due to disruption of the glomerular filter barrier due to endothelial injury. Hypertension is likely due to defective vascular tone regulation, as VEGF promotes vasodilation by stimulating production of NO which is a potent vasodilator [42]. VEGF also negatively regulates endothelial release of the vasoconstrictor molecule endothelin-1 which controls the tone of afferent and efferent arterioles [43,44].

A proper balance of VEGF production is needed for normal endothelial function, and excessive amounts of VEGF can cause endothelial injury too. Indeed, genetically induced overexpression of VEGF in podocytes leads to glomerular collapse, rapid loss of capillary glomerular endothelial cells and massive proteinuria [37].

VEGF also plays an important role in tubular epithelial cell-endothelial crosstalk and peritubular capillary homeostasis. In mice, tubular-derived VEGF is localized most abundantly in the thick ascending limb of the Henle's loop, but it is also expressed, though to a lesser degree, in the proximal and distal tubules [45]. Embryonic deletion of tubular VEGF causes marked decrease in renal VEGF, formation of smaller kidneys, and a striking reduction in the density of peritubular capillaries resulting in pronounced polycythemia due to increased renal erythropoietin production [45]. On the contrary, tubular overexpression of VEGF results in proliferation of PTC and fibroblasts, fibrosis, epithelial cysts, and increased systemic VEGF levels leading to enlargement of glomeruli and glomerular capillaries and mesangial proliferation [46]. In addition, *in vitro* studies have shown that endothelial cells promote tubulogenesis by producing soluble factors including matrix metal-loproteinases [47].

In the glomeruli, podocytes have the capacity to differentiate in the absence of glomerular capillaries [30], but the vasculature is clearly needed for the formation of the mesangium and the maturation of the glomeruli into fully functional filtering units. Glomerular endothelial cells recruit mesangial cells by secreting PDGFB (Platelet Derived Growth Factor B) which binds to PDGFR (PDGF receptor) β-positive mesenchymal cell. In response to PDGFB stimulation, PDGFRβ-positive cells migrate into the developing glomerulus where they differentiate into mesangial cells, lay down extracellular matrix and form mesangial stalks [48]. Ablation of *Pdgfb* or *Pdgfrb* in genetically modified mice leads to defective glomerulogenesis characterized by failure of mesangial cell recruitment and abnormal glomeruli with single, markedly dilated capillary loops [49,50]. Abnormally dilated glomerular capillaries and marked reduction of mesangial cells was reported in newborn but not adult mice treated with a blocking anti- PDGFR-β antibody [51]. Glomeruli with a single or a few ballooning capillaries and reduced mesangial cells, similar to the abnormal glomeruli of PDGF-B and PDGFR- β knockout mice, were obtained by restricting PDGF-B knockout to endothelial cells [52]. This study shows that endothelial cells are the primary source of the PDGF-B needed for proper glomerulogenesis and glomerular maturation. Collectively, studies with transgenic mice also demonstrate that mesangial cells

are critical for the branching and splitting of capillaries formed in response to podocyte-derived VEGF.

The marked reduction of mesangial cells and abnormal capillary morphogenesis observed in mice with endothelial-restricted *Pdgfb* mutagenesis is largely corrected after birth [52]. Various explanations have been proposed for glomerular recovery in these animals, including the contribution of nonendothelial sources of PDGF-B production, the presence of few PDGF-B-producing endothelial cells, and the involvement of other PDGF ligands such as PDGF-D. As they age, however, these mice develop albuminuria, reduced glomerular cellularity and glomerulosclerosis, suggesting that endothelial-derived PDGF-B in the adult plays an important role in glomerular function, homeostasis and response to injury [52].

ANGPT1 (Angiopoietin 1) and its endothelial receptor TIE2 are required for the development and maturation of the blood vascular systems, and ablation of their encoding genes leads to early embryonal lethality due to cardiovascular defects [53,54]. This abnormal vascular phenotype is due primarily to defective blood flow because it can be reproduced by specifically ablating *Angpt1* in the heart [55]. However, ablation of *Angpt1* between day E10.5 and E12.5 by conditional gene targeting leads to generalized and severe vascular defects [55]. In the kidney, ANGPT1 deficiency leads to glomerular capillary loop dilatation, with in some cases only a single large open capillary loop, basement membrane defects, and reduced mesangial cells [55]. *Angpt1* knockout induced after day E13.5 produces no vascular phenotype and is dispensable for the survival and function of the adult vasculature, but it results in organ damage, accelerated angiogenesis, and accumulation of extracellular matrix when combined with tissue injury [55]. In mice with streptozotocin-induced diabetes, the *Angpt1* ablation causes dramatic mesangial expansion and glomerulosclerosis as seen in advanced diabetic nephropathy, whereas control diabetic mice with normal *Angpt1* expression develop only mild glomerular abnormalities. The same glomerular phenotype was noted in diabetic mice with targeted delection of *Angpt1* in both podocytes and mesangial cells [55]. This suggests that, in the adult glomerulus, podocyte- and mesangial cell-derived ANGPT1 may mitigate the endothelial response to diabetic or other types of injury and limit glomerular sclerosis/scarring [55].

The TIE2 receptor engages an additional ligand, ANGPT2, which antagonizes TIE2 activation, and has context-dependent inhibitory or stimulatory effects on blood endothelial cells depending on expression of TIE2 and ECM integrin receptors [56,57]. ANGPT2 also functions as an endogenous TIE2 agonists in lymphatic endothelial cells and is required for lymphangiogenesis during embryonal development [58]. ANGPT2 deficient mice die within two weeks after birth with chylous ascites and lymphatic dysfunction [58]. In the kidney, deficiency of ANGPT2, which is expressed in renal arteries and at lower levels in tubules, causes dysmorphogenesis of peritubular capillaries with formation of multiple endothelial lumens and aberrant differentiation of periendothelial cells evidenced by accumulation of PDGFRβ+ and αSMA+ pericyte-like cells around the endothelium. No overt glomerular capillary abnormalities were noted in these animals, though the possibility that ANGPT2 could contribute to glomerular maturation cannot be excluded due to the postnatal lethality of the ANGPT2 deficiency [59].

Notably, deletion of *Tek* (TIE2) in late gestation prevents formation of AVR causing interstitial accumulation of fluids in the outer renal medulla associated with formation of fluid-filled cysts and decreased urine concentrating ability [27]. The same phenotype can be obtained by deleting in late gestation both *Angpt1* and *Angpt2* [27]. This intriguing finding points to a critical requirement for the TIE2 / ANGPT1, 2 receptor system in the development and function of the AVR. An additional distinguishing feature of the medullary vasculature has recently emerged from scRNAseq studies that have revealed concentrated expression of IGF1 (Insulin Growth Factor 1) signaling pathway genes at the tip of the medullary pyramids where IGF1 is primarily expressed by endothelial cells of the ascending vasa recta [9].

Semaphorins represent a large group of extracellular signaling molecules involved in the development and maintenance of tissues and organs [60]. Semaphorins signal through different receptor complexes including plexins to regulate cell adhesion, cytoskeleton and morphology. Among them, Sema3a and Sema3c (Semaphorin 3a and 3c) have been shown to regulate kidney vascular development [60]. Sema3a, a podocyte-derived chemorepellent molecule with guidance and vascular functions, modulates vascular patterning and acts as a negative regulator of endothelial cell migration and survival, whereas Sema3c positively regulates endothelial branching morphogenesis [60]. Genetic ablation of *Sema3a* causes poor glomerular capillary lumen development, increased capillary endothelial cells, loss of endothelial fenestrations, widening of podocyte foot processes, and impaired glomerular filtration barrier evidenced by albuminuria [60,61]. Conversely, overexpression of *Sema3a* during embryonal development causes endothelial cell apoptosis and glomerular hypoplasia [60]. Sema3c promotes glomerular endothelial proliferation and survival, enhances cell adhesion to fibronectin and collagen I, and stimulates β1-integrin activity. In addition, Sema3c induces directional cell migration [37] and increases endothelial tube and network formation. These effects are mediated by Sema3c-induced VEGF120 secretion, *via* neuropilin-1 and neuropilin-2 signaling, and are independent of integrin and VEGFR2 signals [62].

As the glomerular capillary wall develops, endothelial cells and podocytes, together, produce a basement membrane which they share [9]. Proper production and assembly of the basement membrane is critical for the maturation and function of the glomerulus. Among the components that are essential for basement membrane assembly is laminin, a heterotrimeric molecule composed of α, β and γ chains. Mature glomerular basement membranes contain laminin 11 (α5β2γ1). Targeted mutation of the *Lama*5 gene which codes for laminin α5 causes failure of basement membrane formation resulting in avascular glomeruli with displaced endothelial and mesangial cells [63].

## 7. Concluding remarks

The renal vasculature contains a highly heterogenous population of endothelial cells with distinct morphologies and transcriptional signatures in different anatomic regions of the kidney. Endothelial cells produce angiocrine factors with the capacity to regulate kidney embryonal development, maturation, homeostasis and response to injury. Parenchymal and stromal cells in turn generate paracrine cues that induce endothelial differentiation, stimulate

endothelial angiogenic activity and regulate endothelial function. Aberrant expression in experimental animals of genes that regulate angiocrine/paracrine activity between endothelial cells and parenchymal/stromal cells can cause glomerulosclerosis, thrombotic microangiopathy and interstitial fibrosis, leading to proteinuria and/or renal failure, as seen in disease processes affecting human kidneys.

Although major advances have been made in dissecting the molecular mechanisms that regulate developmental processes such as glomerulogenesis, our understanding of the angiocrine activity of the endothelium in health and disease is incomplete. Similarly, the transcriptional activity and zonally restricted gene expression that underly renal endothelial cell heterogeneity have only recently been analyzed and more studies are clearly needed to investigate their physiologic significance and role in the pathogenesis of disease processes. Technological innovations such as scRNAseq, spatial transcriptomics, spatial proteomics, cell-specific and conditional knock-in and -out of genes, and multi-photon/intravital microscopy [63,64] are likely to fill these gaps and further elucidate how different types of endothelial cells regulate renal function. As more knowledge is gained in this exciting field of research, new endothelial-related mechanisms of kidney injury are likely to be discovered which could be targeted for therapeutic intervention in disease processes driven by endothelial dysfunction.

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## Data availability

No data was used for the research described in the article.

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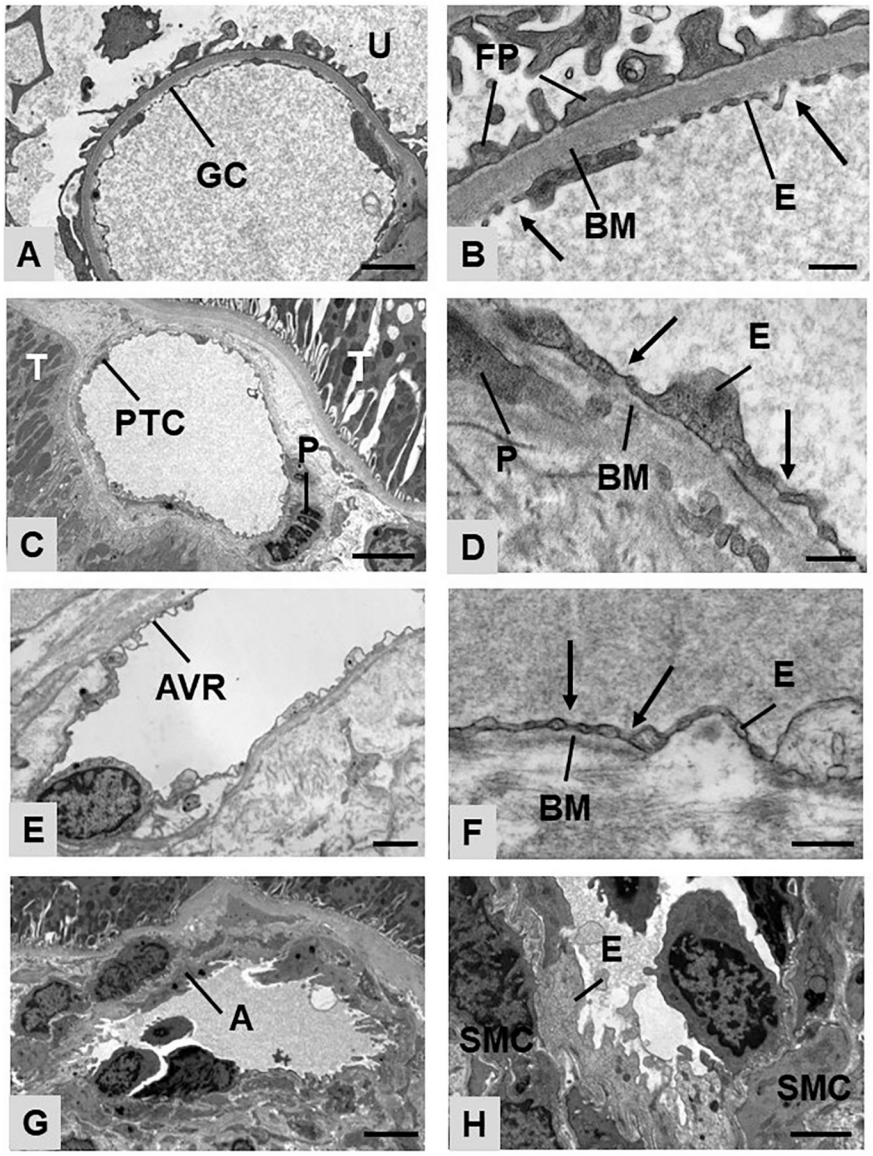


**Fig. 1.**

Morphologic heterogeneity of human kidney microvascular endothelial cells viewed by electron microscopy. A. Glomerular capillary (GC) and adjacent urinary space (U). B. Glomerular capillary wall composed of fenestrated endothelium (E), basement membrane (BM) and podocyte foot processes (FP). Arrows highlight endothelial fenestrae without diaphragms. C. Peritubular capillary (PTC) with associated pericyte (P) and adjacent tubules (T). D. PTC endothelium with diaphragmed fenestrae (arrows) and underlying basement membrane (BM) and pericyte (P) cytoplasmic processes. E. Ascending vas rectum (AVR). F. AVR endothelium (E) with diaphragmed fenestrae (arrows) and underlying basement membrane (BM). G. Cortical arteriole (A). H. Arteriolar endothelium (E) with continuous morphology and underlying medial smooth muscle cells (SMC). Magnification Bars: A, E, H: 2 μm; C 4 μm; G 6 μm; D 200 nm; B, F 400 nm.

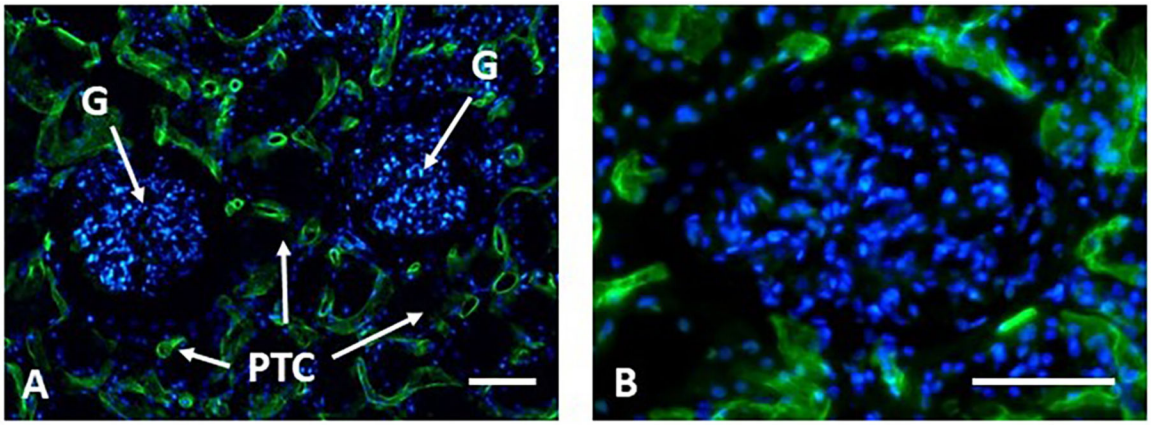


**Fig. 2.**

Heterogeneous PLVAP expression in kidney microvascular endothelial cells. A. Section of human kidney stained with anti-PLVAP antibody (Biolegend, MECA-32) by immunofluorescence shows diffusely positive peritubular capillaries (PTC, arrows) and negative glomeruli (G). Nuclei are counterstained in blue with DAPI. B. Higher magnification view of PLVAP-positive PTC next to next negatively stained glomerulus. Magnification bars: A, B: 50 μm.

**Table I**

Examples of physiologic, reparative and pathologic processes regulated by endothelial angiocrine activity in different organs.

| |
|---|
| 1. Organ development/homeostasis |
| Liver morphogenesis (Matsumoto et al. 2001, doi: https://doi.org/10.1126/science.1063889) |
| Lung morphogenesis (Mammoto et al. 2019, doi: https://doi.org/10.3389/fbioe.2019.00318) |
| Kidney morphogenesis (Lindhal et al., 1998, doi: https://doi.org/10.1242/dev.125.17.3313) |
| Pancreas morphogenesis (Lammert et al. 2001, doi: https://doi.org/10.1126/science.1064344) |
| 2. Self-renewal and differentiation of stem cells |
| Self-renewal of hematopoietic stem cells (Kobayashi et al. 2010, doi: https://doi.org/10.1038/ncb2108) |
| Differentiation of hematopoietic cells (Rafii et al. 2015, doi.org/10.1182/blood.V126.23.SCI-25.SCI-25) |
| Regeneration of neural stem cells (Karakatsani et al. doi: https://doi.org/10.3389/fnmol.2019.00085) |
| Cancer stem cell expansion (Alsina-Sanchis et al. doi: https://doi.org/10.3390/cancers 13,112,610) |
| 3. Organ response to injury/regeneration |
| Regeneration and fibrosis in the liver (Winkler et al. 2021, doi.org/10.1016/j.jhep. 2020.08.033) |
| Regeneration of the pancreas (Lammert et al. 2001, DOI: https://doi.org/10.1126/science.1064344) |
| Regeneration of the lung epithelium (Ding et al. 2011, DOI: https://doi.org/10.1016/j.cell.2011.10.003) |
| 4. Tumor growth |
| Enhanced tumor cells proliferation (Alsina-Sanchis et al., 2021, doi.org/10.3390/cancers 13,112,610) |
| Tumor immune tolerance (Lamplugh and Fan, 2021, doi.org/10.3389/fimmu.2021.811485) |
| Escape from the immune response (Pasquier et al. 2020, doi.org/10.1186/s12967-020-02244-9) |
| Induction of tumor dormancy (Singhal et al. 2020, https://doi.org/10.1158/0008-5472.CAN-19-3351) |