Published in final edited form as:

Semin Cell Dev Biol. 2019 July ; 91: 132–146. doi:10.1016/j.semcdb.2018.06.001.

Development of the renal vasculature

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Abstract

The kidney vasculature has a unique and complex architecture that is central for the kidney to exert its multiple and essential physiological functions with the ultimate goal of maintaining homeostasis. An appropriate development and coordinated assembly of the different vascular cell types and their association with the corresponding nephrons is crucial for the generation of a functioning kidney. In this review we provide an overview of the renal vascular anatomy, histology, and current knowledge of the embryological origin and molecular pathways involved in its development. Understanding the cellular and molecular mechanisms involved in renal vascular development is the first step to advance the field of regenerative medicine.

Keywords

Vasculogenesis; angiogenesis; endothelial cells; mural cells; lineage; fate tracing

1. Introduction

The mammalian kidneys are highly vascularized organs. In humans, the kidneys receive approximately 20% of the cardiac output, even though they constitute less than 1% of total body mass. From this enormous blood flow (1.0 to 1.2L/minute), the glomeruli filter 90 to 120 mL/min which gets mostly reabsorbed resulting in the formation of a small quantity of urine (1 mL/minute). Nephron number varies widely among individuals, but in average each human kidney has approximately one million nephrons.

Over the last two decades major advances have been made on elucidating the mechanisms involved in the development of the kidney vasculature. These include the identification of vascular precursors, their differentiation pathways, the assembly and maintenance of vascular structures and the molecular pathways involved. In this review we will discuss mostly studies performed in mice and humans.

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2. Anatomy and Histology of the Kidney Vasculature

In mammals, the kidney vasculature consists of arteries, arterioles, capillaries, veins, and lymphatics. Arteries and veins are composed of three layers: 1) the endothelium, 2) the muscular media, and 3) the adventitia that fuses with the interstitial tissue. The muscular media, composed of vascular smooth muscle cells is more prominent in arteries whereas the adventitia (including connective tissue, autonomic nerves and vasa vasorum) is more prominent in veins. Capillaries consist of endothelial cells covered by pericytes (smooth muscle like cells). The architecture and composition of the kidney vasculature is rather complex and includes unique microvascular networks exemplified by the glomerular circulation attended to by two arterioles (afferent and efferent) with an intervening capillary tuft and the postglomerular circulation which possesses an extensive net of peritubular capillaries adapted to perform distinctive functions in the cortex and medulla [1]. Furthermore, the afferent arterioles, the main resistance vessels in the kidney that regulate blood flow by contraction or relaxation of the smooth muscle layers contain a specialized group of cells that produce, store and release renin, a hormone crucial for the control of blood pressure and fluid and electrolyte homeostasis. Blood supply for each kidney is provided by a renal artery that branches directly from the abdominal aorta. The right renal artery is a little longer and lies at a higher level than the left. In humans the renal artery usually divides into an anterior and a posterior branch at a point just before entry into the renal parenchyma, and then into multiple segmental vessels, four from the anterior branch and one from the posterior branch. Given the segmental nature of the renal blood supply and the lack of significant collateral circulation between the segments, complete obstruction of an arterial segmental vessel usually results in ischemia and infarction of the tissue in the vessel's area of distribution. Ligation of individual segmental arteries has frequently been used to create and study a model of chronic renal failure in the rodents, such as the 5/6 nephrectomy model, obtained by uninephrectomy and ligation of two or three segmental arteries of the remaining kidney. Morphologic studies in this model described the presence of ischemic zones surrounding the totally infarcted areas. Those studies showed that some regions have at least partial dual supply as demonstrated by viable glomeruli [1,2]. The segmental arteries in turn branch into interlobar arteries which give rise to the arcuate arteries. Each arcuate artery supplies several interlobular (or corticoradial) arteries that divide into multiple afferent arterioles that connect with individual glomeruli. After entering each glomerulus, where blood filtration occurs, the afferent arterioles are continued by the glomerular capillaries which in turn drain into a single efferent arteriole that divides into a second set of capillaries (peritubular capillaries and vasa recta) which surround the renal tubules allowing reabsorption and excretion from the tubular compartment to occur. Next, blood is carried by the venous circulation (interlobular veins, arcuate veins, and interlobar veins) to the renal veins which drain to the inferior vena cava. Of note, morphological studies of the kidney vasculature performed in humans and several other species, showed that it is not uncommon to have more than one renal artery supplying each kidney. A recent study showed that 51% of kidney donors had renal artery variations, of those 38% had an accessory renal artery and 13% had an earlier division of the renal artery. Hence, awareness of variations by evaluating the donors is a must before renal transplantation, urological procedures and angiographic interventions are performed [3]. Some species such as the rat,

pig, and rabbit, contain only one renal artery per kidney [4–6]. Variations on the renal venous have also been reported in humans [7].

In our laboratories, studies performed in rodents including microdissection and histological analysis during embryonic and postnatal life revealed that the first renal arterioles are evident around E15 in the mouse and E16–17 in the rat. The basic layout of arterial and arteriolar development is present by 18–19 days of gestation, followed in the subsequent days by a burst of branching and elongation of new arterioles that repeat the basic process for about a week postnatally, resulting in a remarkable increase in the complexity and surface area peculiar to the renal vasculature (Figure 1). Lymphatics are structurally and functionally specialized vessels responsible for maintaining interstitial fluid homeostasis, absorption of dietary lipids and immune cell trafficking. The blind-ended lymphatic capillaries uptake excrescent interstitial fluid, proteins and white blood cells and transport them through the larger collecting lymphatic vessels to the veins. In a healthy adult human, the lymphatics drain daily about 8L of interstitial fluid containing 20–30g/L of protein back to the blood circulation [8]. Unlike the blood vessels, lymphatic capillaries are composed of a thin and highly permeable layer of lymphatic endothelium (LEC) that shows distinct morphology and gene expression [8]. In the adult kidney, lymphatic vessels are mainly distributed around the larger arteries (arcuate arteries and interlobular arteries) in the cortex, whereas the medulla seems to be devoid of lymphatic vessels. A subset of lymphatic vessels is present within the renal capsule and sub-capsular cortex area. They all converge in the renal hilum surrounding the renal artery.

Despite early reports about renal lymphatics in the mid-1900s, the normal distribution and development of renal lymphatic vessels in humans and other species has not been well described until two or three decades ago owing to the lack of a specific and a reliable marker for the lymphatic endothelium. The first intrarenal lymphatic vessels in mice are observed at embryonic (E) day 13 (E13), whereas the extra-renal lymphatic vessels are well formed already at E12 [9]. The best characterized factors that regulate lymphangiogenesis include vascular endothelial growth factors C and D [9]. The renal lymphatic system has implications for cancer spread. For instance, renal cell carcinoma can spread via lymphatic drainage from the kidney to the paraaortic lymphatics, and systemic malignancies such as lung and stomach carcinomas can find their way to the kidney via the lymphatic vessels. Hence, evaluation of the perinephric lymphatics by images such as MRI is of clinical importance when evaluating those conditions [10]. The lymphatics also play a critical role after kidney transplantation since lymphoceles and lymphorrhea can develop especially with the common use of steroids and mTOR inhibitors for immunosuppression following transplantation increasing the risk of renal allograft rejection [11].

3. Lineage of the Kidney Vascular Cells

3.1 Mouse Models for Cell Lineage Tracing. Cre-Lox system

Significant advances in the study of cell lineage and fate have been made possible with the advent of transgenic approaches combined with refined imaging techniques. Cell lineage analysis has been crucial to advance our understanding of the normal differentiation of diverse cell populations from unique precursors as well as for the identification of different

subtypes of progenitor cells. Cell fate tracing has been invaluable to identify, *in vivo*, the mechanisms involved in adjustments in cell identity that occur during regeneration and repair as well as during pathological processes. The utilization of genetically engineered mice, particularly mice carrying Cre recombinase and/or reporter genes (e.g. LacZ or fluorescent proteins), has been a powerful tool to study cell fate. The Cre-lox system consists of two components derived from the bacteriophage P1: the *Cre* (cyclization recombination) gene that encodes a site specific DNA recombinase, and the loxP recognition site, a specific DNA sequence of 34 bp consisting of two palindromic sequences, 13 bp each and separated by an 8 bp spacer. Cre recombinase recognizes DNA sequences flanked by two loxP sites and excises or inverts the floxed sequences depending on the similar or opposing orientation of the loxP sites respectively [12,13]. Another recombination system available, the Flp-FRT, is conceptually similar to the Cre-lox system but involves a flippase (FLP) recombinase (derived from the *Saccharomyces cerevisiae*) that recognizes a pair of FLP recombinase target (FRP) sequences that flank a DNA region of interest [14]. Cre reporter mouse lines have a loxP-flanked stop sequence followed by the reporter gene usually inserted into the ubiquitously-expressed endogenous ROSA26 locus in chromosome 6 [15]. A variety of fluorescent reporter mice are currently available [16]. These include single, double or multiple fluorescent reporters which can be expressed in the cytoplasm, nucleus or plasma membrane. Double fluorescent reporters such as the membrane Tomato/membrane GFP (mT/mG) in which the mT is expressed in all the cells prior to Cre excision while the mG is expressed only in cells that undergo recombination allows visualization and identification of recombined and non-recombined cells [17]. Because during the *Cre*-induced transition from mT to mG expression the onset of mG labelling starts within the first day of the transcriptional switch while the mT protein is still detectable for 1–2 days after [17,18], this system allows during this timeframe, the identification of newly differentiated double positive (mT+mG+) cells. This reporter mouse has been of use to study transdifferentiation of insulin-producing cells in the pancreas and renin cells in the kidney [17–19]. A multicolor reporter mouse (Confetti) generated by insertion of the Brainbow construct into the ROSA26 locus can also be used for lineage tracing [13,20]. Inducible Cre mouse models (i.e. Tetracycline/Doxycycline, Tamoxifen and RU486) allow Cre recombinase activation *in vivo* at different time points both prenatally and postnatally. Furthermore, mice with a flox-stop-flox diphtheria toxin subunit alpha (DTA) cassette inserted in the ROSA26 locus allow the controlled injury or death of a specific cell population when crossed with mice expressing Cre recombinase in the desired/target cell type [21].

Multiple Cre expressing mouse lines have been generated either by standard transgenesis by DNA pronuclear injection (including the use of bacterial artificial chromosomes), homologous recombination or clustered regulatory interspaced short palindromic repeats (CRISPR) approaches. Whereas their availability has resulted in major advances in the study of cell lineage, there are still several limitations in their use. It is crucial to validate and carefully evaluate where the Cre-driving promoter is expressed. With standard Cre transgenic mice the random insertion of the construct and the uncontrolled copy number of the transgene can result in off target effects such as ectopic expression or silencing of the transgenes. Furthermore, cells from diverse lineages might express shared markers at

different developmental stages potentially leading to wrong conclusions. Effects related to the genetic background are not uncommon. The efficiency of Cre recombinase may be altered by ligands such as Tamoxifen and Doxycycline, therefore the dose of these compounds should be determined based on the overall purpose of the experiment, for instance small doses are preferably used to establish clonality, while larger doses can be used to identify the entire progenitor population. Another important consideration is that tamoxifen induced Cre-lox recombination may continue for weeks after administration.

3.2 Vascular precursors

As shown in Figure 2 the metanephric kidney develops basically from two derivatives of the *Osr1*⁺ intermediate mesoderm: the ureteric bud and the metanephric mesenchyme. The ureteric bud gives rise to the ureteral and collecting duct epithelium. The metanephric mesenchyme can be divided in two morphologically distinct and dynamic compartments, the condensing mesenchyme, which gives rise to all the epithelial nephron from podocytes to the distal tubules and the loose mesenchyme which harbors the precursors for the whole kidney vasculature and interstitium.

Lineage tracing studies using multiple techniques determined that ureteric bud precursors express the homeodomain protein *Hoxb7* [22,23] and the condensing mesenchyme precursors the homeodomain transcriptional regulator *Six2* [24–26]. The cellular composition of the renal vasculature include endothelial cells, vascular smooth muscle cells, renin cells, pericytes, fibroblasts, mesangial cells and the nerve fibers that innervate the vessels. As described below, renal vascular progenitor cells are already present in the metanephric kidney very early, well before formed vessels can be discerned. Within the loose mesenchyme mentioned above, there are two separate progenitors: *Foxd1* positive cells which are stromal cells that express the forkhead transcription factor *d1* and *SCL* positive cells which are endothelial/hematopoietic precursors that express the proto-oncogene *c-Kit* and the stem cell leukemia/T-cell acute lymphoblastic leukemia protein 1 (*SCL*) [27–29]. *Foxd1* positive cells are the precursors of all the renal vascular smooth muscle cells, renin-expressing cells, mesangial cells, and renal interstitial pericytes [27,30,31]. Renin precursors *per se* give rise to a subset of renal arterial vascular smooth muscle cells, which are capable to de-differentiate and re-express renin when homeostasis is threatened [32]. *Tbx18* positive cells in the developing kidney also correspond to a subset of *Foxd1* derived cells localized mostly in the developing medulla surrounding the ureteric bud and which differentiate into vascular smooth muscle cells, mesangial cells and pericytes [33]. Whether there is an overlap between the *renin*⁺ and *Tbx18*⁺ cells during renovascular development is not known.

Whereas the hierarchy of the precursors for all the mural cells of the kidney vessels is fairly well established, the intermediate precursors for the renal endothelial cells remain more elusive. Previous studies showed that a subgroup of *cKit* positive cells which also expresses *Flk1* or *Tie1* appears to give rise to all of the renal vascular endothelial cells [34–36]. Recently our group showed that the renal endothelium has a distinct origin from the renal mural cells. Using inducible transgenic mice that specifically label endothelial and hematopoietic precursors for fate tracing studies in combination with cross-transplantation

and hematopoietic colony forming assays we identified a common progenitor expressing the helix-loop-helix transcription factor SCL that gives rise not only to the renal endothelium but also to blood precursors [37]. Another recent study, using *ex vivo* re-aggregation assay combined with fate mapping and *in vivo* and *in vitro* depletion of specific populations of EC precursors identified heterogeneous sub populations of endothelial precursors, one of them expressing the melanoma cell adhesion molecule (MCAM/MUC18/CD146) and highlighted an important role of CD146+ cells during renal vascular development [38]. In fact, removal of the CD146+ cells from the embryonic metanephric mesenchyme prevents the differentiation of endothelial cells [38].

Interestingly, *Flk1* is also expressed in a subset of *Foxd1* positive renal stromal cells that are integrated within a subpopulation of peritubular capillaries [39]. Deletion of *Flk1* using a *Foxd1-Cre* mouse resulted in dilation of peritubular capillaries, paucity of mature collecting ducts and urine concentrating defects [39].

The potential role of the endothelial progenitors and hemangioblasts derived from the bone marrow is yet to be defined. The autonomic nerve fibers that supply the renal blood vessels follow renin cells during development [40] and appear to develop from progenitors that express Wnt-1 proto-oncogene (unpublished data from our lab).

4. Morphogenesis of the Kidney Vasculature

4.1 Embryonic Origin of the Kidney Vasculature

The embryonic origin of the epithelial nephron from intrinsic precursors within the metanephric mesenchyme has been established long ago based on *in vitro* studies that allow the growth, branching and differentiation of the ureteric bud as well as the mesenchymal to epithelial transformation of the condensing mesenchyme [41–43]. However, the embryonic origin of the renal vasculature has been more controversial due to the lack of *in vitro* models that allow the differentiation of mature vascular structures. Previous interspecies kidney transplant studies involving quail, chick and mouse suggested that the renal endothelium originates from the recipient, implying an extra-renal origin [44,45]. With the advent of transgenic mice, several labeling and lineage tracing studies (with *Flk1*, *Flt1*, *Tie1*, or all the cells genetically labeled with β -galactosidase) showed that the early metanephric kidney, at a time when the vessels are still not developed, possesses precursors that can differentiate into mature arterial and arteriolar vessels containing renin and smooth muscle cells in the appropriate location as well as glomerular and interstitial capillaries [34,46–48]. This conclusion was made possible by transplanting the early nonvascular embryonic kidney under the renal capsule -or into the anterior chamber of the eye- of adult mice and showing that all the vascular structures developed from the embryonic kidney [46–49]. Furthermore, experiments where the endothelial precursors of the embryonic kidney were ablated (with endogenous expression of diphtheria toxin subunit A) after transplantation, resulted in lack of differentiation of intrinsic precursors and no compensation with endothelial cells from the host [37]. Those studies underscored the importance of the endothelial precursors present within the early embryonic kidney. In addition, as further described below, the embryonic kidney possesses hemogenic precursors that can differentiate into all the hematopoietic lineages [37,50]. However, if the embryonic kidney is transplanted under the kidney capsule

of a newborn mouse, a time when nephrogenesis in rodents is still ongoing, the vascularization of the glomeruli within the transplanted kidney occurs as a dual chimeric origin of endothelial cells originating from both the embryonic kidney and from the newborn host [34]. In this case there is also contribution of endothelial cells from the embryonic kidney to the capillaries within the still developing renal cortex of the host. This indicates that during development, endothelial precursors could potentially be attracted from and to any surrounding tissue by angiogenic factors that allow their differentiation into endothelial cells. In fact, the first endothelial cells are already present in the mouse at E10.5 surrounding the invading ureteric bud and scattered (not connected to vessels) around the metanephric mesenchyme [47,49]. A recent static anatomical spatiotemporal analysis of the renal vascularization of the embryonic mouse kidney suggests that the kidney vascularization begins at E11 with the formation of a vascular plexus surrounding the ureteric bud [51]. From then on, the authors observed endothelial plexus following the dividing ureteric branches which stay always connected to the renal artery [51]. Whereas this study using confocal images immunostained for the pan-endothelial marker CD31 increased our understanding of the tri-dimensional distribution of the developing vasculature it still fails to determine whether all the renal endothelial cells originate from the original peri ureteric bud plexus or from precursors within the metanephric mesenchyme that are recruited to differentiate and form the subsequent plexus. In this regard, the *in vitro* generation of kidney organoids from human embryonic stem cells or inducible pluripotent stem cells which are not connected to any circulation, still show development of an endothelial network with lumen formation and with endothelial cells incorporated into the glomerulus [52]. This suggests that there are intrinsic endothelial progenitors within a developing kidney or organoid. Interestingly, when kidney organoids are subjected to shear stress the number of endothelial cells increases dramatically. Regarding the embryonic origin of the renal lymphatic vessels there is still little information. Within the mouse embryonic kidney the first lymphatic vessels start to develop in the renal hilum at E13 [53]. It has been suggested that they may develop by branching from extra-renal lymphatics. However, it remains unclear whether the kidney possesses intrinsic renal lymphatic precursors that contribute to the development of the renal lymphatic vessels.

On the other hand, there are no controversies regarding the origin of the precursors for all the mural cells of the kidney vasculature (renin cells, vascular smooth muscle cells, interstitial pericytes and mesangial cells), which derive from the Foxd1-positive intrinsic metanephric mesenchymal cells [27].

4.2 Mechanisms for renal vascular development

Two major distinct but also overlapping processes are involved in renal vascular formation: vasculogenesis and angiogenesis [31,54]. Additional important processes include hemovasculogenesis [50] and lymphangiogenesis.

Vasculogenesis occurs by *de novo* differentiation and assembly of endothelial tubes followed by recruitment of vascular smooth muscle cells that differentiate from the surrounding mesenchymal precursors (in the case of the kidney, the smooth muscle cells derive from the Foxd1 positive stromal cells mentioned above).

Angiogenesis is the generation of new vessels from pre-existing ones and involves proliferation, migration and sprouting of differentiated endothelial cells and recruitment of vascular smooth muscle cells as the vessels are forming.

Studies using chimeric mice and inter-species surgical grafts of embryonic kidneys showed that the renal vasculature was generated by a combination of vasculogenesis and angiogenesis [31,54]. Major renal vessels seem to originate from vascular plexus branches of the aorta and vena cava whereas the smaller vessels may differentiate and connect to the bigger vessels. Interestingly, whereas the endothelial tubes are predominantly monoclonal, the mural cells of the renal arterial tree are polyclonal [31,54]. A recent study mentioned above using advanced tissue engineering techniques coupled with *in vivo* and *in vitro* re-depletion of specific populations of endothelial cell precursors, plus an *ex vivo* re-aggregation assay gathered new evidence in support of a vasculogenic mechanism for renal vascular development [38]. Another important process involved during vascular formation is hemovasculogenesis, the concomitant formation of blood precursors and vessels [50]. Hemovasculogenesis can also occur in pathological conditions such as myeloproliferative disorders and hemolytic anemias in the form of extramedullary hematopoiesis [50,55]. More recent studies suggest that hemovasculogenesis occurs in the kidney mostly from hemogenic endothelial cells [28].

Lymphangiogenesis is the process whereby lymphatics are formed. Until the last decade, due to lack of specific markers, little was known about the development of the lymphatic system. Using cell culture and microarray analysis, a set of functional marker genes were identified, such as lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), vascular growth factor receptor 3 (VEGF-3), podoplanin and prospero-related homeobox gene 1 (Prox-1) [8,53]. However, none of these markers is exclusively expressed in LECs. For example, LYVE-1 is also expressed in endothelial cells of blood vessels during early development [56]. To date, the cellular origin of the lymphatic endothelial cells is still controversial. A hundred years ago, Florence Sabin proposed that the lymphatic system originated from the veins, which has been confirmed recently by lineage tracing studies using lymphatic endothelial cell markers [8,57]. More recently, identification of lymphatic precursors in both embryonic and postnatal life suggests that lymphatic endothelial cells originate independently from mesenchymal derived lymphangioblasts, which then connect to the veins [58]. Whereas in mice capillary tubes are first seen surrounding the ureteric bud at E11, the first arterioles are formed at E14–15 of gestation. For about 10 days after birth, the branching and elongation of arterioles is remarkable and significantly increases the complexity and surface area of the renal arterial tree [31]. Each arteriolar tip needs to connect to a glomerulus to form a functional nephron. Therefore, spatial and temporal control of the differentiation, migration and assembly of the vascular cells are required for the synchronized connection of arterioles with their associated nephrons.

Pericytes communicate with endothelial cells not only via direct physical contact but also by means of secreted molecules such as VEGF. For appropriate proliferation, migration and remodeling of EC during angiogenesis, pericytes must detach from the growing vessels. Recently, endothelial-mural cell dynamics have been studied *in vitro* to characterize the

physical versus the molecular relationships between the two cell types [59]. There are still no written reports regarding the embryonic formation of the intrarenal venous system.

4.3 Mural cells and vascular development

Foxd1 positive cells are stromal progenitors that express the forkhead transcription factor 1 and reside in the outer layer of the developing kidney and are maintained by self-replication [60]. As mentioned above *Foxd1* positive cells are the precursors of all the renal vascular smooth muscle cells, renin-expressing cells, mesangial cells, and renal interstitial pericytes [27,30,31]. Two mouse models have been used to study the role of *Foxd1* cells in kidney development, the *Foxd1* gene knockout and the ablation of *Foxd1* cells by expression of Diphtheria toxin subunit A (*Foxd1*-DTA) resulting in cell death directed to *Foxd1* precursors [27,61–64]. Similar structural changes were noted in both models including abnormal capsular morphogenesis, renal hypoplasia due to reduced nephron number and abnormal branching of the ureteric bud [61–63]. The renal vasculature is markedly abnormal with absence of the main renal artery and replacement with multiple branches from the aorta that enter the kidney through the renal capsule in a centripetal way and not through the hilum [27]. Renin expression in the afferent arterioles of mutant *Foxd1* mice is significantly reduced [27]. The fact that either the ablation of the *Foxd1* precursor cells (*Foxd1*-DTA) or deletion of the *Foxd1* gene results in a similar phenotype suggests that the transient and early actions of the transcription factor *Foxd1* are crucial for the normal differentiation of the kidney vasculature. Furthermore, a recent study showed that *Foxd1* knockout mice developed reduced branching of the ureteric bud accompanied by downregulation of all of the components of the renin angiotensin system [65]. The underlying molecular mechanisms involved are yet to be identified.

Tbx18 is a Tbox transcription factor crucial for the development of multiple tissues and organs. In the kidney, it is expressed in a subpopulation of the metanephric mesenchyme surrounding the ureteric stalk and in all the mesenchyme around the developing ureter. *Tbx18* has been shown to be essential for ureteral and kidney development [33,66]. Genetic deletion of *Tbx18* in mice results in hydroureter and hydronephrosis due to abnormal development of the smooth muscle cells of the ureter [66] and nephrovascular alterations [33]. The kidneys are smaller, with abnormal formation and arrangement of the vasculature and glomerular aneurisms due to reduced or incomplete differentiation/proliferation of vascular smooth muscle cells and mesangial cells [33]. Expression of *Foxd1* in the developing renal stroma is an earlier event than *Tbx18* and is not affected by global deletion of *Tbx18* at E12.5 suggesting that *Foxd1*⁺ cells are an earlier precursor of *Tbx18*⁺ cells [33,66]. Further mechanisms involved in the differentiation of the renal stroma are reviewed in [67].

Renin is an enzyme traditionally associated with regulation of blood pressure and maintenance of homeostasis. Renin producing cells, in the adult mammal, are classically located in the juxtaglomerular (JG) area in the walls of the afferent arterioles at the entrance to the renal glomeruli where they sense changes in perfusion pressure and release renin accordingly. Renin, the key regulatory enzyme of the renin angiotensin system cleaves its only known substrate, angiotensinogen, and ultimately leads to formation of angiotensin II, a

potent vasoconstrictor and regulator of fluid electrolyte homeostasis. Renin cells themselves are crucial for the normal development of the kidney and its vasculature [68–73].

Renin cell precursors are derived from *Foxd1* positive cells and appear in the kidney around E12.5–E14.5 in mice, well before the arteriolar development has been initiated. Next, renin cells are found at the tip of the newly branching arterioles and widely distributed along the large intrarenal arteries [74]. This wide distribution of renin cells in the embryonic kidney is very distinct from the restricted localization in the juxtaglomerular area in the adult. As the intrarenal arteries and arterioles mature, renin cells progressively differentiate into vascular smooth muscle cells.

In utero exposure to medications that interfere with the renin angiotensin system such as Angiotensin Converting Enzyme inhibitors or Angiotensin Receptor Blockers can lead to severe renal parenchymal and vascular malformations. Moreover, genetic mutations of the renin angiotensin genes in humans leads to vascular abnormalities and renal tubular dysgenesis, a severe disorder characterized by oligohydramnios and lung hypoplasia which can be incompatible with life [75–77].

Several mouse models were used to study the role of renin cells in kidney development *in vivo* by deleting any of the genes that are associated with the renin angiotensin system [31,69,71,78–80]. All result in a severe renal vascular pathology characterized by arterial and arteriolar hypertrophy, similar to the one characteristic of hypertensive disease, but in these cases the animals are hypotensive. Interestingly, this hypertrophy is not observed when renin cells undergo ablation by expression of diphtheria toxin *in vivo*, indicating that renin cells *per se* underlie the etiology of the hypertrophy [73]. A recent study using *Ren1^c* knockout mice crossed with mice expressing YFP driven by the renin promoter confirmed that in the *Ren1^c* knockout model, renin cells not only survived but also extended beyond the classic juxtaglomerular area [71]. This study also showed that renin cells contributed physically to the vascular hypertrophy [71], but whether they also contribute by secreting growth factors remains to be determined. Cells of the renin lineage (renal arteriolar smooth muscle cells) have the plasticity to reactivate the renin program when homeostasis is threatened to produce more renin and restore homeostasis. Once that is accomplished those cells retransform back to smooth muscle cells by a mechanism that is yet to be defined [72].

Wnt7b is a secreted Wnt ligand that regulates the development of multiple tissues acting via canonical [81,82], non-canonical [83], and G-protein coupled pathways [84]. In the kidney, by activation of the canonical Wnt signaling, *Wnt7b* regulates elongation of the renal medulla (via oriented cell division), and elongation of the loop of Henle (through proliferation) [85]. Recently, it has been shown that via *Wnt7b*, the developing ureteric bud regulates proliferation of both mural and endothelial cells of the medullary peritubular capillaries [86].

4.4 Development of the Glomerular vasculature

The glomerular vessels consist of a specialized fenestrated capillary tuft formed by endothelial cells and their associated perivascular cells, the mesangial cells. The glomerular capillary wall is fundamental for filtration; it is composed of three layers including the

capillary endothelium, the glomerular basement membrane and the podocytes. Defects in any of these components lead to disruption of the glomerular filtration barrier and ultimately proteinuria. Progenitors of the glomerular endothelium as well as of the podocytes and mesangial cells are all present in the embryonic kidney prior to development of the blood vessels [46–49]. The first step in glomerular development is the formation of an epithelial vesicle which then invaginates to create a vascular cleft (the so called S-shape body), where endothelial and mesangial progenitors enter the developing glomerulus (Figure 3). In response to vascular endothelial growth factor A (VEGFA) secreted by the developing podocytes, the endothelial precursors, which in turn express the VEGF receptors 1 (Flt1), 2 (Flk1) and the co receptor neuropilin-1 [87], differentiate and proliferate to form first a single primordial capillary loop. The latter undergo maturation by a process of intussusception and branching remodeling [88,89] that requires apoptosis of mesangial and endothelial cells for the successful formation of the mature capillary loops [90].

Expression of VEGFA by the podocytes as well as its receptors in the endothelial precursors is a crucial event for the normal development of the glomerular capillaries and formation of a functional glomerular filtration barrier [91,92].

The glycoprotein Angiopoietin1 (expressed by podocytes and mesangial cells) and its tyrosine receptor Tie2 (expressed by endothelial cells) are also key players for the development and function of the glomerular vasculature but their role varies depending on the developmental time point [93,94]. Early deletion of Ang1 results in embryonic lethality with abnormal glomerular capillary development. The capillary tufts show dilatations and an abnormal glomerular basal membrane. Deletion of Ang1 later in fetal life is not lethal, it does not affect glomerular morphology but it increases susceptibility to kidney damage when exposed to stressors and or injury [95,96].

Appropriate development of the glomerular mesangium is fundamental for the development and maintenance of the glomerular vasculature and glomerular function. Mesangial cells are specialized contractile pericytes which give structural and functional support to the glomerular capillaries. The platelet-derived growth factor B (PDGFB)/Pdgf-receptor β (PDGFR β) pathway is crucial for the development of mesangial cells [97]. Glomerular endothelial cells produce PDGFB which acts on PDGFR β expressed by mesangial cells. Deletion of PDGFB and/or PDGFR β in mice results in absence of mesangial cells and formation of glomerular aneurisms [97]. The transcription factor *Tbx18* is necessary for PDGFR β expression. *Tbx18* knockout mice show lack of ureteral smooth muscle development, vascular dilatation and glomerular aneurisms due to reduced proliferation of glomerular endothelial cells and increased apoptosis of mesangial cells [33,66]. Deletion of the transcription factor *Rbp-J* in mesangial cells results in a similar glomerular phenotype highlighting the crucial role of the Notch signaling pathway in the normal vascularization of the glomerulus [30,98]. Further, the Sphingosine 1-Phosphate (S1P) signaling pathway is essential for the normal development of the glomerular endothelium. Deletion of the tyrosine kinase S1P receptor 1 in endothelial cells results in dilated vessels with abnormal smooth muscle coating and clumping of mesangial cells with the formation of a single, dilated capillary shunt within each glomerulus [28].

As further expanded below, for the proper development of the glomerulus several signaling molecules exerting attraction and repulsion guidance are required. These include SDF1, ephrins and semaphorins. The chemokine SDF1 expressed mostly by podocytes acts on its receptors CXCR4 and CXCR7, expressed in glomerular endothelial cells. Deletion of SDF1 or its receptors results in partial recruitment/clumping of mesangial cells and dilated glomerular capillaries [99]. The transmembrane protein EphrinB2 and its receptor EphB4 are equally essential for vascular remodeling in the early embryo [100]. EphrinB2 is differentially expressed throughout glomerular maturation whereas EphB4 is restricted to the post-glomerular peritubular capillaries and veins [101]. Deletion of ephrinB2 in progenitors of mural cells including mesangial cells results in glomerular aneurisms [102]. The pleiotropic chemorepellant glycoprotein Semaphorin 3a (Sema3a) is produced by podocytes and is crucial for the normal development of the glomerular capillaries. Deletion of Sema3a results in an increase in the number of glomerular endothelial cells with poor glomerular capillary lumen development [103].

5. Major Molecular Mechanisms in Kidney Vascular development (Figure 4)

5.1. Vascular Endothelial Growth Factor (VEGF)

The VEGF family and its receptors are essential regulators for vascular development. There are five related growth factors (VEGFA-D and Placental GF) that bind to three transmembrane tyrosine kinase receptors (VEGFR1–3) and two co receptors, neuropilin-1 and neuropilin-2. VEGF is produced by various cell types including platelets, macrophages, renal podocytes and mesangial cells, keratinocytes and tumor cells. VEGF can function both in an autocrine and paracrine fashion. Vascular endothelial cells express VEGFR1 (Flt1) and VEGFR2 (Flk1/Kdr) and bind to VEGFA, a potent angiogenic factor essential for migration and proliferation of endothelial cells as well as their coating by pericytes and vascular smooth muscle cells. In fact, loss of a single allele of VEGFA in the embryo is incompatible with life [104]. Homozygous deletion of each of its receptors (VEGFR1 and VEGFR2) is also embryonic lethal due to vascular defects [105,106]. Alternative mRNA splicing of VEGFA results in multiple isoforms with different heparin-binding affinity and/or antagonistic effects. VEGFR3 is expressed in lymphatic endothelial cells but also in vascular endothelial cells during embryonic development. Through its binding to VEGFC and VEGFD, VEGFR3 plays an essential role in lymphangiogenesis. The NOTCH pathway regulates expression of all the VEGFRs; Notch activation increases the levels of VEGFR1 but decreases the levels of VEGFR2 and VEGFR3.[107] On the other hand, VEGFA directly stimulates the NOTCH pathway during vascular development [108].

Upon binding to VEGFA, the VEGFR2 is internalized, a process that requires the presence of Ephrin-B2 [107]. VEGFR2 forms complexes with other transmembrane proteins such as VEGFR3, Neuropilin-1, thrombospondin receptor CD47, VE-cadherin and some integrins [109] that can also affect its endocytosis and signaling. Runt domain transcription factors (RUNX1, 2, and 3) regulate expression of VEGF by direct binding to the promoter. The VEGF promoter comprises a total of three RUNX1, one RUNX2 and three RUNX3 binding sites. VEGF is negatively regulated by RUNX1 and RUNX3 whereas RUNX2 increases the expression of VEGF. (reviewed in [110])

VEGFA and its receptors are expressed in the early developing metanephric kidney before vessels are formed [35,48,111,112]. As nephrogenesis ensues, VEGFA is expressed in the developing epithelial structures including podocytes whereas its receptors are located predominantly in endothelial cells [113].

As mentioned above, in the developing nephron, expression of VEGFA by the podocytes as well as its receptors in the endothelial precursors is a key event for the normal vascularization of the glomerulus and establishment of a functional glomerular filtration barrier [91,92]. In fact, conditional deletion of VEGFA in podocytes (or the VEGFR2 in glomerular endothelial cells) results in perinatal mortality due to hydrops fetalis and kidney failure with abnormal glomeruli showing fewer, poorly differentiated and degenerating endothelial cells [91,114]. Interestingly, heterozygous deletion of VEGFA in podocytes leads to glomerular endotheliosis, hypertrophy of endothelial cells with loss of fenestrations [91]. On the other hand, overexpression of VEGF in the podocytes also results in renal failure due to a glomerulopathy with collapsed capillary tufts [91]. Furthermore, postnatal administration of a neutralizing antibody against VEGF in mice showed abnormal vessel formation in the superficial renal cortex, a reduced nephrogenic zone with marked decrease in the number of developing nephrons many of which lack capillary tufts [115].

It has been shown that Crim1, a transmembrane cysteine-rich repeat-containing protein related to chordin, is involved in the delivery of VEGFA to the glomerular endothelial cells. Mice with a homozygous mutation of Crim1 displayed an increased activation of the VEGFA signaling pathway resulting in glomerular capillary dilatation and congestion, overgrowth of endothelial cells, mesangiolysis, and podocyte effacement [116].

In the developing kidney, VEGF actions are not limited to the development of the vasculature, it also plays a role in branching morphogenesis of the ureteric bud [117].

5.2. Transforming Growth Factor β (TGF β)

First identified in platelets, TGF β 1 is a polypeptide cytokine of the TGF β superfamily that regulates multiple cellular processes including cell growth, proliferation, differentiation and apoptosis both in an autocrine and paracrine fashion. TGF β and its receptors are expressed in endothelial and vascular smooth muscle cells and are involved in physiological and pathological processes.

TGF β is required for the development of endothelial and vascular smooth muscle cells. It directly regulates the components of the endothelial basement membrane [118,119] and extracellular matrix deposition [120]. Mice with conditional deletion of TGF β receptors in endothelial or vascular smooth muscle cells die in utero at E10.5–12.5 due to vascular abnormalities [121]. TGF β requires VEGF to induce apoptosis for the removal of redundant endothelial cells, required for capillary lumen formation and angiogenesis [122,123]. In fact, administration of an anti-TGF β 1 neutralizing antibody results in an accumulation of undifferentiated glomerular endothelial cells without a patent lumen formation. Within the glomerulus this process is also co regulated by Sema3a [103,124]. Furthermore, via PDGF- β , TGF β induces proliferation, recruitment and differentiation of pericytes and vascular smooth muscle cells [125].

5.3 Platelet-Derived Growth Factor (PDGF)

The PDGF family includes four isoforms (PDGF-A–D). PDGF receptors are dimers of α and/or β chains; whereas PDGF-A binds to the α chain only, PDGF-B binds to all receptor types [126]. These growth factors have mitogenic and chemotactic roles and are expressed in the renal mesangium, interstitium, vasculature and tubules [127]. PDGF-B produced by the endothelial cells is essential for the recruitment, proliferation and maturation of pericytes and smooth muscle cells required for normal vascular development. Consequently, absence of PDGF-B or PDGFR β in mice results in severe vascular malformations with decreased vascular smooth muscle differentiation and subsequent dilated and leaky vessels that lead to embryonic hemorrhages and death [128,129]. PDGF is one of the most characterized growth factors in renal disease and is a strong activator of mesangial proliferation. As mentioned above, PDGF-B is produced by glomerular endothelial cells and acting on the PDGFR β expressed in mesangial precursors stimulates the development of the glomerular mesangium. In fact deletion of PDGF-B or its receptor in the kidney results in major glomerular defects including failure of development of the mesangial cells and formation of aneurysmatic glomeruli [128,130], while overexpression of PDGF-B results in mesangioproliferative disorder and renal fibrosis [126]. The TALE transcription factor Pbx1 suppresses the transcription of *Pdgfr β* by binding to a cis-regulatory element within the *Pdgfr β* locus [131]. Conditional deletion of *Pbx1* in renal mural cell precursors (Foxd1+ cells) results in marked renal vascular abnormalities due to premature and excessive differentiation of mural cell progenitors into PDGFR β + vascular smooth muscle cells, leading to increased and abnormal arterial branching [131]. The transcription factor *Tbx18* may also be involved in the regulation of PDGFR β . *Tbx18* knockout mice showed reduced proliferation of PDGFR β + cells and therefore fewer cells in the mesangium and developing stroma resulting in development of vascular dilatation and glomerular aneurisms in addition to lack of ureteral smooth muscle development [33].

5.4. Angiopoietins

The angiopoietin (Ang)-Tie pathway is essential for angiogenesis, vascular remodeling and maintenance of vascular function during embryonic and postnatal life and also involved in angiogenesis of pathological processes [132,133]. Angiopoietins include Ang1–3, (in the mouse), and Ang4, which are glycoproteins produced by various cell types that bind to the tyrosine kinase receptor Tie2 in endothelial cells [133]. Ang1 is a required Tie2 ligand and is expressed in the mesenchymal cells surrounding the developing vasculature and in the differentiated mural cells (pericytes, vascular smooth muscle cells), fibroblasts, and monocytes. Ang1 functions in a paracrine fashion to promote vessel stability and inhibit fibrosis [133]. Ang2 is expressed almost exclusively by the endothelial cells and its autocrine functions are context dependent: 1) on Tie2- expressing endothelium it antagonizes Ang1 effects and 2) on invading and migrating tip cells (with low Tie2 expression) Ang2 acts as a proangiogenic factor through binding to integrins [134]. Mice knockout for either Ang1 or its Tie2 receptor show embryonic lethality around E10.5–12.5 with similar vascular abnormalities [95,135–137]. Ang1 knockout embryos develop endothelial cells with poor attachment to the underlying basement membrane [137]. Timed-inducible deletion studies showed that Ang1 actions vary depending on the developmental time point [95]. Ang1 exerts crucial vascular morphogenetic actions during early

development and it has a potential functional role later in life in preventing kidney damage upon exposure to injury or microvascular stress (i.e. during diabetic nephropathy) [95].

The orphan receptor Tie1 is also crucial for embryonic vascular development [138]. Tie1 is almost exclusively expressed in endothelial cells and is highly homologous with Tie2. Lack of Tie1 in the mouse embryo leads to hemorrhages, lymphatic malformations and fetal demise by E13.5 [138]. Tie1 also exerts context dependent functions. It acts as a negative regulator of Tie2 in sprouting tip/angiogenic endothelial cells, and as a positive regulator of Tie 2 in remodeling stalk cells [139]. Tie 1 has been proposed to act as a mechanoreceptor because its expression is regulated by sheer stress [140–142].

5.5. Cell Guidance Molecules

The stromal cell-derived factor 1 [SDF1, also known as C-X-C motif chemokine 12 (CXCL12)], is a chemoattractant cytokine that acting on its receptors CXCR4 and CXCR7 plays an important role in organogenesis, regeneration and tumorigenesis [143]. In the embryonic kidney SDF1 is expressed in stromal cells (surrounding developing nephrons and blood vessels) and in podocytes whereas its receptors are expressed mostly in endothelial cells (Cxcr4) or tubular cells (CXCR7) [99,144]. Deletion of Sdf1 or Cxcr4 in mice results in an identical kidney phenotype with glomerular aneurisms and decrease in the number of mesangial cells.[99] Deletion of Cxcr7 also leads to glomerular abnormalities with capillary dilatation and collapsed mesangial cells [99]. The precise molecular mechanisms underlying the Sdf1/Cxcr4-Cxcr7 axis in the regulation of renal vascular formation are still unknown.

Semaphorins are a large and diverse family of membrane-bound or secreted guidance molecules. Semaphorin-1a (SEMA-1A) was first identified as a guiding molecule involved in for axonal growth. However, the role of semaphorins as repulsive and attractive signals extends to multiple tissues during developmental and pathological processes. Semaphorins act on multimeric receptor complexes that include neuropilins and plexin receptors [145]. Class 3 semaphorins bind to neuropilin receptors with the exception of SEMA-3E (the most studied one) which binds to plexin-D1 in endothelial cells. Deletion of plexin-D1 in mice leads to neonatal death due to vascular, cardiac and skeletal malformations [146]. During development SEMA-3A regulates the number of glomerular endothelial cells, as overexpression of it leads to endothelial cell apoptosis, while deletion results in formation of glomeruli with excessive endothelial cells.[103] SEMA-3C has a similar function to VEGF-A and an opposite one to SEMA-3A. *In vitro* SEMA-3C enhances glomerular endothelial proliferation and tube formation via integrin phosphorylation and VEGF120 secretion [147].

Ephrins and its receptors are membrane- bound proteins that require direct cell to cell interaction for signaling. They regulate various biological processes during development, including arterial and venous specification and maintain angiogenesis and stem cell differentiation during postnatal life. Eight ephrin ligands (ephrinA1–5 and ephrinB1–3) bind to nine ephrinA receptors (EphA1–8 and EphA10) and five EphBs (EphB1–4 and EphB6) respectively. Of the three ephrin-B ligands, ephrin-B2 has been found to play significant roles in angiogenesis and lymphangiogenesis in zebrafish and mice by regulating the internalization of VEGFR3 required for downstream signal transduction of the VEGF signaling pathway [148]. Ephrin- B2 in mural cells is essential for the normal orientation of

the pericytes and smooth muscle cells and coverage during blood vessel assembly through cell-cell dependent and independent functions [102]. In the kidney, lack of ephrinB2 in smooth muscle cells and pericytes leads to dilatation of poorly organized glomerular capillaries [102]. It has also been postulated that ephrins are implicated in the pathogenesis of congenital anomalies of the kidney and urinary tract [149].

5.6. Sphingosine 1-phosphate pathway

Sphingosine 1-phosphate (S1P) is a phospholipid that is released to the circulation by different types of cells including erythrocytes, platelets, neutrophils and mast cells. It functions as a signaling molecule to promote vascular stability and it is degraded by the S1P lyase 1 [150]. S1P is necessary for regulation of EC permeability through modulation of endothelial tight and adherens junctions [151]. It has been shown to inhibit angiogenesis in the retinal vessels during vascular development by regulating the interaction of VEGFR2 and vascular endothelial cadherin [152,153]. S1P acts on a family of five G-protein coupled receptors (S1PR1–5). Global deletion of the S1PR1 results in embryonic lethality by E14.5 [154]. The knockout embryos display severe edema and hemorrhages due to lack of appropriate vascular smooth muscle cell coating of the vessels suggesting an important role for S1PR1 in vascular maturation [154].

S1P via its S1PR1 is also crucial for the appropriate morphogenesis of the kidney vasculature including glomerular capillary development, arterial mural cell coating and lymphatic vessel development [28]. Embryos with inducible S1PR1 deficiency (to overcome mortality before renal vascular maturation) showed endothelial cell hyperplasia with increased proliferation in arteries, veins and peritubular capillaries, together with dilatation of the renal arteries and veins and abnormal glomeruli with capillary shunts and collapsed mesangial cells, suggesting a distinct role of S1PR1 in the development of glomerular capillaries in comparison with other renal blood vessels. In addition, the development of lymphatic vessels in the kidney was stunted [28]. S1P is necessary not only during embryonic development but it also prevents vascular leakage in postnatal life [150]. Deletion of endothelial S1PR1 before renal ischemia reperfusion injury results in exacerbation of kidney injury and inflammation and eventually fibrosis via activation of leucocyte adhesion molecules, suggesting that S1P is reno-protective and necessary for recovery from acute kidney injury [155]. In humans, mutations in S1P lyase leads to congenital forms of steroid resistant nephrotic syndrome, characterized by focal segmental glomerulosclerosis, mesangial sclerosis and rapid progression to end stage renal disease [156].

5.7. Notch Signaling

The Notch signaling pathway, a highly conserved cell to cell communication system, is required for numerous cellular processes during development and disease. In mammals, members of the Delta-like (DLL1, DLL3, DLL4) and the Jagged (JAG1, JAG2) families bind to four Notch receptors (NOTCH1–4), all act via a common effector, the transcriptional regulator Recombination signal Binding Protein for immunoglobulin kappa J region (RBP-J). Notch signaling is a limiting factor for angiogenesis and for endothelial cell activation via regulation of VEGF receptor expression [157,158]. Congenital diseases related to mutations in Notch signaling were first described in 1996 with Notch 3 mutation causing CADASIL

(Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) [159]. Another condition, the Adams-Oliver syndrome is due to mutations in Notch 1 pathway, characterized by skeletal, vascular and cutaneous malformations, suggested to be secondary to the impaired circulation [160].

In the kidney, the Notch signaling pathway plays important roles in the development of the epithelial nephron, the mesangium and the renal vasculature [30,98,161,162]. Conditional deletion of *Rbpj* in renal mural cell precursors results in a marked decrease in arterial and arteriolar development (Figure 5), with reduced endowment of smooth muscle and renin cells, and lack of mesangial cell differentiation resulting in gigantic glomerular aneurysms [30,98]. Individual deletion of Notch1, Notch2 or Notch3 does not result in lack of mesangial cell differentiation suggesting a redundant function for Notch receptors in the renal mesangium [98].

This pathway is also essential for the identity of renin cells as conditional deletion of RBP-J in cells of the renin lineage results in the loss of their typical endocrine contractile phenotype and their ability to respond to homeostatic threats that normally elicit an increase in the production of renin [163,164]. The cells lose the expression of renin as well as smooth muscle markers and increase the expression of genes involved in hematopoietic and immune processes suggesting that in renin cells RBP-J not only acts as a direct activator of the myo-endocrine program but also by inhibiting the expression of unwanted ectopic genes whose expression could have devastating consequences in the control of homeostasis.

5.8. MicroRNAs

MicroRNAs (miRs) are highly conserved, noncoding single-stranded RNA molecules (~22 nucleotides) that act as epigenetic posttranscriptional regulators of various biological processes during developmental, physiological and pathological states. Primary miRs, transcribed in the nucleus by RNA polymerase II, are processed by the enzyme Drosha to form a smaller ~ 70 nucleotide precursor miRNA which is then transported into the cytoplasm and undergoes cleavage by the ribonuclease Dicer to generate the ~22 nucleotide mature miR. Dicer and Drosha play important roles in development and integrity of the vasculature: lack or downregulation of any of them results in abnormal angiogenesis both in vitro and in vivo [165]. Endothelial cell specific deletion of miRNA-126 results in abnormal vascular development with delayed angiogenic sprouting and disrupted vascular integrity leading to a leaky endothelium and hemorrhages [166,167]. Several miRs are involved in angiogenesis by modulating downstream effectors of VEGF signaling. For example, miR-15a, -16, -93, -200b, and -424 affect the levels of Vegf ligand, miR-16 and -424 regulate Vegf receptors and miR-23, -27, -126, -132, -218, and -221 target positive and negative regulators of the Vegf signal transduction cascade (Reviewed in [168]).

MiRs and their processing enzymes have also been shown to be essential for nephron development, renal tubule maturation and podocyte homeostasis and function [169]. Furthermore, they are crucial for the normal development of the renal vasculature. Mice lacking the ribonuclease Dicer in renin expressing cells exhibit vascular abnormalities, renal fibrosis resembling cyclosporine toxicity and decreased renin expression [170]. Deletion of Dicer in the upstream Foxd1+ precursors results in parenchymal and vascular abnormalities

that include reduced smooth muscle in afferent arterioles, decreased renin-expressing cells, and progressive mesangial loss with the consequent development of glomerular microaneurysms [171]. Those studies highlight an intrinsic requirement for miRNAs in the renal stroma and renin cell derivatives. The role of endothelial specific miRNAs in kidney vascular development remains to be studied.

5.9. Mechanical Forces and Shear Stress

The endothelium at its luminal surface is constantly exposed and reacts to hemodynamic shear forces. Physiologically shear stress regulates acute changes in the vessel diameter. Shear forces are also essential for vessel maturation which requires recruitment of mural cells such as pericytes and smooth muscle cells [172].

Furthermore, time-lapse confocal microscopy studies showed that adequate blood flow is necessary for induction of vascular remodeling in the yolk sac [173]. The mechanisms by which shear stress regulates vessel maturation are still poorly understood. Mechanotransduction is the cell signaling cascades triggered by flow mediated endothelial responses which occur at different subcellular levels [172]. It has been suggested that shear stress-regulated miR-27b stimulates the interaction between endothelial cells and pericytes via SEMA-6A and SEMA-6D repression [174]. The role of shear forces in kidney vascular development remains to be studied.

6. Conclusions/Perspectives

Over the last few decades great progress has been made in understanding some of the key signaling events in renal vascular development. Nevertheless, important challenges remain. Understanding how the three-dimensional architecture of the kidney and its vasculature is attained and how vessels connect in a precise timely and spatial manner to their corresponding nephrons will be a significant achievement. Further, it is necessary that we develop the means to understand the role of physical forces, including blood flow, and the vasculature itself in the development and organization of the highly functional architecture of the kidney.

Finally, development of kidney organoids from human iPS cells holds a significant promise for our patients in need of a kidney transplant. However, those organoids currently do not possess a properly formed kidney vasculature and therefore have limited function. Conquering the vascularization of renal and non-renal organoids, will be a major scientific and medical breakthrough. We envision that the next few years will bring about important progress in those areas, advancing the field of regeneration and ultimately benefitting our patients in need of properly functioning organs.

Acknowledgments

We thank Dr. Ariel Gomez for critical reading of the manuscript and discussions. Work in MLSSL's laboratory is supported by NIH DK096373. TM is a pediatric nephrology fellow.

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Highlights

- The early embryonic kidney possesses all the precursors for renal vascular development.
- Early renal vascular precursors differentiate and assemble with the proper spatial organization when the embryonic kidney is transplanted into an adult host.
- Renin cells are a key component of the kidney vasculature with functions during development as well as in adult life in health and disease.
- Vascular disease is a major initiating or contributing factor to most renal diseases.
- The proper vascularization of renal organoids derived from induced pluripotent stem cells will be a major scientific and medical achievement.

Figure 1.
Microdissected renal arterial trees throughout development. Modified from [31].

Figure 2. Developmental origin and lineage relationships of the precursors for the metanephric kidney. Arrows indicate current knowledge and question marks possibilities that require further investigation.

Figure 3.

Development of glomerular capillaries in S-shaped glomerulus. [A-C] 50 nm embryonic mouse kidney sections under transmission electron microscopy showing “tip cell” like endothelial cell (EC) (A, squared) with filopodia (B) and red blood cells (RBCs) in the vessel lumen formed by the invading ECs (C) in S-shaped glomeruli. [D] Schematic diagram demonstrating that ECs (in yellow) form vessel lumens with RBCs (in red) in the vascular cleft of an S-shaped glomerulus (in blue). M, mesangial cell. PD, podocyte. PT, proximal tubule. Scale bar: A-C, 5μm. From [175]

Figure 4.

Major molecular mechanisms in kidney vascular development. Representation of interactions between the endothelial and mural cells (vascular smooth muscle and renin cells). Within the glomerulus a podocyte and a mesangial cell are represented whereas endothelial cells (which express the same receptors and ligands as the one on the left side) are not depicted for clarity.

Figure 5.

Rbpj is required for the proper pattern and formation of the renal arterial tree.

Microdissected renal arterial trees from control and *Foxd1^{Rbpj-/-}* mice showing a marked decrease in arterial and arteriolar branches and duplication of the renal artery (arrowheads) when *Rbpj* is missing in the progenitors of the renal mural cells.