

Unique Gene Expression in Developing Ascending Vasa Recta: A Tale of Tie

David P. Basile¹ and Mervin C. Yoder²

¹Department of Cellular and Integrative Physiology and ²Department of Pediatrics and the Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana

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To facilitate its role in maintaining plasma homeostasis, the kidney relies on a complex vascular architecture to carry out multiple functions. Given that the plasma volume is filtered approximately 60 times per day and that approximately 99% of the filtrate is reabsorbed and returned to the plasma volume, the renal vasculature is noteworthy in the amount of blood perfusion and hydraulic conductivity. The unique role of the renal vasculature is further highlighted by its countercurrent exchange function, allowing for the maintenance of medullary osmotic gradients. Understanding the molecular basis for the development of specific renal vascular components is surely required if regenerative strategies are ultimately pursued to affect patient care.

Studies on kidney development have suggested that the renal vasculature requires vascular endothelial growth factor (VEGF) signaling in the glomerulus to recruit fetal liver kinase 1+ angioblasts into the vascular crest, some of which reside in the metanephric mesenchyme.^{1–3} By day E13 in mice, capillaries expressing endothelial cell markers, such as CD31 (platelet endothelial cell adhesion molecule 1) and fetal liver kinase 1+, form networks around kidney epithelial cells. On E14, arteriogenesis begins with early arterial patterning.^{1,4} Presumably, this complex network is mediated by paracrine cues emanating from the interstitium. Pharmacologic and genetic approaches have identified the importance of various factors, such as VEGF-A,⁵ EphrinB2-EphB4,⁶ and TGF- β ,⁷ as well as other molecules in modulating vascular pattern formation in the developing kidney.

The angiopoietin/Tie-2 system plays an important role in both vascular and lymphatic development. Angiopoietin 1 and

2 both interact with the Tie-2 receptor and mediate distinct effects. Although it was originally considered that Angpt 2 may act as an inhibitor of Angpt1, Angpt2 null mice were shown to display impaired lymphatic development, suggesting that its activity is cell-type specific.⁸ Not surprisingly, the angiopoietin/Tie-2 system has also been investigated in the setting of renal development. *Angpt1* null mice manifest defects in vascular development and die during embryonic development, although exogenous Angpt1 enhanced vascular growth in metanephric organ culture.⁹ Complete deletion of *Angpt2* disrupts the formation of postglomerular peritubular capillaries, and these mice die shortly after birth.⁹ Unfortunately, the early lethality observed in these mice makes it difficult to provide a more detailed understanding of the Tie-2 system in renal vascular development.

In this issue of the *Journal of the American Society of Nephrology*, Kenig-Kozlovsky *et al.*¹⁰ further explored the angiopoietin/Tie-2 system in renal development. Using a Tie-2-Cre reporter system, the investigators showed that all glomerular, peritubular capillary, and vasa recta beds derive from Tie-2-expressing cells. They also used a system of inducible mutant mice, in which *Angpt1*, *Angpt2*, *Tie-2*, or a combination of genes could be deleted at day E16.5 (denoted as A1 ^{Δ 16.5}, A2 ^{Δ 16.5}, Tie-2 ^{Δ 16.5}, or double A1/A2 ^{Δ 16.5}, respectively) to circumvent problems associated with the early embryonic lethality previously encountered. Under these conditions, mice were not embryonic lethal but did manifest specific defects in renal vascular structure and function. Reductions in vascular density of both cortical peritubular capillary and medullary vasa recta was observed in Tie-2 ^{Δ 16.5} and A1/A2 ^{Δ 16.5} mice. Large outer medullary cysts developed in both Tie-2 ^{Δ 16.5} and A1/A2 ^{Δ 16.5} mice, but mice lacking Angpt1 or Angpt2 individually (*i.e.*, A1 ^{Δ 16.5} or A2 ^{Δ 16.5}) did not develop cysts. These cysts did not express markers of epithelial cells or endothelial cells but did express mesenchymal markers.

Follow-up studies revealed the most interesting aspects of this report. Using elegant immunofluorescence staining, the authors showed that the primary defect was the loss of plasmalemmal vesicle-associated protein-positive structures indicative of the ascending vasa recta (AVR), whereas urea transporter B-expressing vessels of the descending vasa recta (DVR) were largely unaffected.¹⁰ Thus, whereas lineage tracing reveals that all cells of the medullary vascular bundles derive from Tie-2-expressing cells, there is a time-sensitive component of AVR development that requires Tie-2 signaling after day E16.5. The AVR is a highly fenestrated structure thought to be important in fluid uptake, and this study provides the first functional evidence showing a specific alteration in AVR development that alters urine concentrating capacity, presumably by participating in countercurrent exchange of solute in the medulla.

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Correspondence: Dr. David P. Basile, Department of Cellular and Integrative Physiology, Indiana University School of Medicine, 635 Barnhill Drive, MS 334, Indianapolis, IN 46202. Email: dpbasile@iupui.edu

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The second significant observation of the study relates to the fact that selective mutation of both *Angpt1* and *Angpt2* together but not separately at E16.5 specifically attenuated AVR development. The result was similar to that of a previous report by this group that showed disrupted development of Schlemm's canal in the eye,¹¹ a structure with both lymphatic and vascular characteristics. The investigators hypothesized that AVR similarly represents a hybrid vessel displaying both vascular and lymphatic markers. Cells of the AVR expressed common vascular markers, such as CD31, as do the cells of the DVR.¹⁰ However, AVR, but not DVR, expressed some markers of lymphatic vessels (Prox-1, and VEGFR3), whereas AVR did not express other markers of lymphatics, such as LYVE and podoplanin. These cells were specifically lost when Tie-2 signaling was disrupted. Moreover, classic renal lymphatics expressing the above classic lymphatic markers were not affected when Tie-2 signaling was disrupted at day E16.5.

The renal medulla is typically thought to be devoid of a classic lymphatic system, where the highly fenestrated AVR is thought to conduct fluid reabsorption to the circulation.¹² In a highly vascularized organ, such as the kidney, serious complications, like the development of interstitial edema and increased interstitial pressure, may result in a reduction in filtration. Thus, it is interesting to note that, in this study, animals lacking the AVR showed a significant reduction in GFR. However, at this time, the underlying basis for the reduction in GFR has not yet been elucidated. In addition, the presence of cystic development in mice lacking the AVR may also be indicative of an elevation in interstitial pressure, which will need further study.

Therefore, this interesting study makes a substantial contribution to our understanding of renal vasculature development but also opens up a variety of new questions worth considering. For example, does the AVR derive from a different set of vascular progenitors, or is there a differentiating step that occurs during development to form AVR and DVR from a common vascular progenitor cell? Does the AVR have more in common with the development of lymphatics, which are thought to be derivatives of venous circulation and also highly influenced by Tie-2 activity?¹³

Also, could one speculate on the possible contribution of AVR in lymphangiogenesis, which occurs in a variety of inflammatory renal states? Indeed, lymphatic vessels appear in the renal interstitium in disease models associated with renal fibrosis, whereas vascular capillaries simultaneously decrease.¹² Neolymphangiogenesis is thought to be mediated by the activity of VEGF-C, and inhibition of lymphangiogenesis has been associated with an attenuation of renal inflammation and fibrosis.¹² The origin of these new lymphatic vessels in disease states and after transplant is not yet clear, and it has been suggested that it derives from circulating progenitor cells.¹² However, the presence of a hybrid phenotype provides for the possibility of plasticity, whereby inflammatory conditions transition renal capillaries to a hybrid

phenotype, or that new lymphatics might derive from differentiation or expansion of existing AVR cells. Of course, such possibilities have yet to be addressed specifically. However, given the recent uptick of interest in lymphangiogenesis in the setting of inflammatory CKD, this study may help to shape interpretation of many studies using various markers for lymphatic vessel appearance. At the least, the utilization of a panel of markers for both vascular and lymphatic vessels should be used to discern modulation of a hybrid phenotype under these conditions.

DISCLOSURES

None.

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See related article, "Ascending Vasa Recta Are Angiopoietin/Tie2-Dependent Lymphatic-Like Vessels," on pages 1097–1107.