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Molecular determinants of nephron vascular specialization in the kidney

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Although kidney parenchymal tissue can be generated in vitro, reconstructing the complex vasculature of the kidney remains a daunting task. The molecular pathways that specify and sustain functional, phenotypic and structural heterogeneity of the kidney vasculature are unknown. Here, we employ high-throughput bulk and single-cell RNA sequencing of the non-lymphatic endothelial cells (ECs) of the kidney to identify the molecular pathways that dictate vascular zonation from embryos to adulthood. We show that the kidney manifests vascular-specific signatures expressing defined transcription factors, ion channels, solute transporters, and angiocrine factors choreographing kidney functions. Notably, the ontology of the glomerulus coincides with induction of unique transcription factors, including Tbx3, Gata5, Prdm1, and Pbx1. Deletion of Tbx3 in ECs results in glomerular hypoplasia, microaneurysms and regressed fenestrations leading to fibrosis in subsets of glomeruli. Deciphering the molecular determinants of kidney vascular signatures lays the foundation for rebuilding nephrons and uncovering the pathogenesis of kidney disorders.
E ndothelial cell (EC) specialization in each organ is essential for executing tissue-specific functions1–3. Kidneys have a unique vasculature to regulate blood pressure (BP), maintain electrolyte homeostasis and pH, and govern red blood cell production1–7. Molecular pathways that determine the structural and functional properties of each blood vessel domain in the kidney, as well as the intrinsic and extrinsic cues that enable adaptation of vessels to these tasks are unknown.

Within each human kidney reside approximately one million nephrons, each consisting of a glomerulus and a system of highly distinct tubules. Blood enters the kidney through the renal artery, branches into the interlobular and arcuate arteries that eventually feed into the glomerular capillaries through the afferent arteriole and exit it through the efferent arterioles8. The glomerulus is a tuft of fenestrated capillaries, podocytes, and mesangial cells that allow low-molecular weight substances including ions, water, glucose, and nitrogenous waste to pass from the blood into the Bowman’s space. The ultrafiltrate of plasma is then transported to multiple segments of the nephron in a series including the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and the collecting duct. The descending vasa recta (DVR) and ascending vasa recta (AVR) blood vessels run parallel with the loop of Henle. The vasa recta slow the rate of blood flow to maintain an osmotic gradient required for water reabsorption. To execute these complex functions, the kidney vasculature has acquired adaptive structural and functional specialization often referred to as vascular zonation9. The intrinsic and extrinsic cues that enable adaptation of the kidney vessels to these tasks are not defined.

The heterogeneity of the kidney vasculature is a major hurdle for the generation of kidney organoids in vitro. Kidney organoids provide avenues for studies of kidney development, disease, and regeneration. Although organoids of the kidney have provided significant insights into kidney physiology and disease, they are mainly limited to the study of developmental processes. Kidney organoids are largely avascular, preventing further maturation and the majority of filtration processes that occur in the kidney. In vivo, the regression of vascular development greatly hinders the organogenesis of the kidney10. Similarly, kidney organoid protocols are hampered from building complete, mature, and functional nephrogenic structures in the absence of proper vascularization of the nephron. Current efforts to vascularize organoids have utilized methods such as microfluidic chips11 or implanting human kidney organoids into the mouse kidney as capsules12. Although several studies have recently mapped morphological mechanisms of vascular development in the kidney13,14, the molecular basis for many of these processes is still largely unknown.

Here, we use bulk and single-cell RNA sequencing (RNA-seq) of the kidney vasculature over a broad range of developmental and adult stages of organogenesis to allow the discovery of distinct transcriptomic signatures associated with different kidney vascular beds. This approach unravels the progression of molecular pathways that specify kidney vascular heterogeneity. We identify several genes—including transcription factors and their putative targets that define the ultrastructural, phenotypic, operational, and functional identity of the glomerular capillaries (GC) and the surrounding vascular network. We additionally perform vascular-specific loss of function experiments on the transcription factor Tbx3, a gene that is expressed robustly in glomerular ECs. Tbx3 regulates a transcriptional network that defines glomerular EC specification and function. In this study, our data uncovers how the transcriptional ontology of the vasculature regulates nutrients and waste in the kidney to sustain chemical and vasomodulatory homeostasis.

Results

Molecular profiling of kidney ECs. To decipher the heterogeneity of the kidney vasculature, we performed comparative transcriptomic analysis of the vasculature of the kidney to that of lungs, liver, and heart at different stages of murine development (Fig. 1a). Each organ was dissected from embryonic stages (E) E13, E14, E15, E16, and E17, postnatal stage (P) P4, and adult mice and dissociated into single cells. We isolated the EC fraction by fluorescence-activated cell sorting (FACS) using fluorescently-conjugated CD31 antibody (Supplementary Fig. 1a, b; Supplementary Data 1).

Affinity propagation clustering (APC)15,16 of the transcriptomic dataset showed the adult and postnatal vascular expression patterns of each organ to be distinct from those of embryonic stages (Fig. 1b). Given the homogeneity of kidney embryonic vascular gene expression, we considered the kidney early embryonic stages (E13-E16) as a single class and sought to determine how vascular expression patterns change from embryonic to adult stages. In embryonic stages, 657 genes were significantly upregulated and were enriched for growth-related pathways (Supplementary Fig. 1c; Supplementary Data 2). APC analyses showed adult stages to cluster separately and away from each other and from embryonic stages from their respective organs. In the adult kidney, 283 genes were significantly upregulated and were enriched in pathways relating to small molecule, water, and amino acid transport (Supplementary Fig. 1d; Supplementary Data 2).

Thus, gene expression programs distinct to the kidney are turned on early in development to promote growth, morphogenesis, and specification. A wave of genes is differentially induced at various stages of development. At the onset of birth, new sets of genes are induced to promote kidney-specific vascular functions, including upregulation of transporters and metabolism programs, setting the stage for the specialization of kidney vasculature function in postnatal stages. Collectively, we show that kidney vascular heterogeneity diversifies perinatally and throughout adulthood.

Kidney ECs are heterogeneous and tightly zonated. To determine how kidney vasculature acquires specialized functions, we dissected kidney EC heterogeneity throughout development. Because kidney-specific vascular genes are induced during late gestation to adult stages (Fig. 1b and Supplementary Fig. 1c, d), we performed single-cell RNA-seq (scRNA-seq) on kidney vascular ECs from fetal E17, perinatal P2, P7 and adult stages (Fig. 1c).

CD31+CD45− “Podoplanin” non-lymphatic ECs were purified by FACS (Supplementary Fig. 1b). Single-cell isolates were then processed for digital droplet scRNA-seq (ddSEQ). We sequenced 5936 cells (Supplementary Data 3), including 922, 1000, 917, and 3097 single cells for E17, P2, P7, and adult kidneys, respectively. Filtering for contaminating epithelial and perivascular cells reduced the dataset to 4552 cells (Supplementary Data 4). Raw data was normalized and the effects of cell cycle and mitotic activity of the renal vein (V), and embryonic capillaries, which we have designated as vascular progenitors (VP) (Fig. 1o).
EC subsets in kidney execute defined vascular functions. For each population, we selected one or two of the specific genes amongst the top differentially expressed genes and validated the presence of protein expression in various EC populations. Most types of ECs identified did not manifest unique markers. Instead, each vessel displayed markers that were unique to two or more types of vessel. Arterial vessels express markers, including Sox17 (Fig. 1f, g), Gja5 (Fig. 1f, h), Cxcl12, low levels of VEGFR2 (Kdr, ...
vascular structures. At the second branchpoint, VPs transition to subtypes, which build up from capillaries at E13 before other Gja5 (but not specifically Fig. 2b) and upregulation of gene signatures found in restriction to capillaries and veins (Fig. 2h, i). Pseudotime from the VPs (early generic capillaries) (Fig. 2a, Supplementary Data 5). RNA sequencing analysis of kidney vascular endothelium throughout development.

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Vascular heterogeneity arises from vascular progenitor cells.

To discern how vascular heterogeneity arises in the kidney, ECs were ordered according to pseudotime (Fig. 2a–c, Supplementary Fig. 2a). The earliest capillary cells correspond to VPs and were labeled by the apelin-receptor gene, Aplnr (Fig. 2d). Quantifications of Aplnr fluorescent staining validated its restriction to capillaries and veins (Fig. 2h, i). Pseudotime trajectory predicts two branch-points from early VPs. At the first branchpoint, pre-glomerular arteries (AA and LA) branch from the VPs (early generic capillaries) (Fig. 2a, Supplementary Fig. 2a). This transition is marked by downregulation of vein and VP signatures, such as Plvap, Aplnr, Nr2f2 (Fig. 2b, d, e, Supplementary Fig. 2b) and upregulation of gene signatures found in (but not specifically) pre-glomerular arteries (AA/LA), such as Gja5, Fbn5, Jag1, Sox17, and Cxcl12 (Fig. 2b, f, Supplementary Fig. 2c, d). Accordingly, arteries and veins are the first vascular subtypes, which build up from capillaries at E13 before other vascular structures. At the second branchpoint, VPs transition into GCs (Lpl4+, Ehd3+, Sema5a+, etc.) (Fig. 2c, g) in association with EAs or the DVR (Scl4a1+, Aqp1+) (Fig. 2c, Supplementary Fig. 2f, g), which is successive to the juxtaglomerular efferent arteriole. Remaining VP capillaries then mature into PTCs in the kidney cortex, downregulating VP markers including Aplnr and Nr2f2, while maintaining partially unique markers Igfbp5 and Plvap (Fig. 2c–e, Supplementary Fig. 2b, e). Large veins may emerge from VP capillaries and maintain expression of venous markers, including Nr2f2+ and Cryab+ ECs (Fig. 2b, Supplementary Fig. 2b).

To unravel the plasticity of kidney EC subtypes, we pursued lineage-tracing clones of VPs. We utilized R26R-Confetti mice expressing four fluorescent reporter genes and crossed them to the vascular-specific and inducible Cdh5(PAC)-CreERT2 strain. Pregnant mice were pulsed with tamoxifen at E11, when ureteric buds emerge. ECs began to express single clones of green (GFP), cyan (CFP), yellow (YFP), and red fluorescent proteins (RFP) at E13 (Supplementary Fig. 2h). By E18, vasa recta, veins, arteries, and glomerular vascular subtypes were labeled with only one fluorescent protein (Fig. 2j, k). Pseudotime analysis predicts the EAs branch off progenitors in tandem with GCs while pre-glomerular arteries and arterioles develop independently. Notably, a single arteriole was found to protrude from developing GCs marked with the same fluorescent protein while pre-glomerular arteries were labeled separately (Fig. 2j). Progenitors of cortical PTC displayed heterogeneous mixture of differentially labeled and diverse ECs. Because progenitor capillaries were the first to be labeled, VP ECs may have migratory potential and do not interact during development, leading to intermingling of confetti colored cells. Collectively, specified ECs appear to remain within their structures after they have developed and do not draw a large degree of plasticity to migrate and become other vascular subtypes. Vasa recta and glomeruli are reported to grow from PTCs in the cortex between E14–E15 stages. Bulk RNA-seq (Fig. 3a) and staining validations of the vasculature at E15 (Fig. 1g, h) show genes specific to vascular subtypes become expressed at E14–E15 stages. Peritubular capillaries (the vascular progenitors) were found to constitute the primary vascular plexus in the cortex of the kidney at E13 stages (Fig. 2i). Following vascular specification, transcripts defining vascular subtypes gradually become upregulated over the course of development, peaking at adult stages. Therefore, vascular heterogeneity in the kidney is specified at E14–E15 stages where they branch off VP capillaries in the cortex. As ECs branch off, they lose and gain several key markers and mostly sustain the phenotype of their designated vascular subtypes.

Changes in kidney expression dynamics during development.

The vasculature in adult kidneys differ from other organs by modulating trafficking of amino acids, water, and ion transport proteins, while embryonic stages are defined by specific induction of cell growth and morphogenic pathways (Supplementary Fig. 1c, d). Hence, we sought to determine how gene expression
associated with transcription factors, solute transporters, and growth factors change within each vascular subpopulation over the course of development. From bulk RNA-seq of the kidney vasculature, we first annotated differentially expressed genes in the kidney vessels at each stage of development. We defined 617 transporter proteins, 1314 transcription factors, and 2032 secreted proteins, from the transporter classification database (http://www.tcdb.org/), the DBD transcription factor prediction database26, and the human protein atlas (https://www.proteinatlas.org/). We find sets of genes expressed
in the early embryo (E13–E16) are highly enriched for transcription factors (hypergeometric test p-values range from 1.1E-4 to 3.1E-5), but not for secreted proteins and transporters. Transcription factors are downregulated at E17 (Fig. 3b), with stabilization of their expression (hypergeometric test p = 0.89) thereafter. Notably, the bulk of gene expression of vascular genes in postnatal and adult stages, are dominated by transporters (adult hypergeometric test p = 5.1.E-18) and secreted proteins (adult hypergeometric test p = 0.027) (Fig. 3b). From the P4 to the adult stage, transporters specific to a wide variety of substrates that are not expressed in other organs are enriched in the kidney vasculature (Supplementary Data 5). We also detected an increase in total numbers of transcripts in these stages compared to embryonic stages (Supplementary Fig. 3a). Notably, membrane transport proteins were transcribed only after birth and their expression are augmented steadily to adulthood. Therefore, transport proteins may be induced in ECs in response to the environmental stimulation and dietary changes.

**Specialized capillaries regulate filtrate reabsorption.** As the glomerular filtrate passes through different segments of the kidney tubule, selective reabsorption of the filtrate is returned to the systemic circulation through the PTC. We investigated whether vasculature zonation accommodates selective filtrate reabsorption and secretion. We compared tubular epithelium and the surrounding stroma through sequencing the mRNA of the non-endothelial fraction (CD31^−VEcadherin^−CD45^−Podoplanin^−) and non-lymphatic endothelial fraction (CD31^+VEcadherin^+CD45^−Podoplanin^−). In kidney ECs, 34 transporters were uniquely expressed, including a variety of calcium and potassium channels as well as transporters for phospholipids, glucose, and amino acids (Supplementary Fig. 3b).

We integrated scRNA-seq data to investigate whether the expression of transporter proteins in kidney vessels is also zonated to guide selective filtrate reabsorption. We find membrane transporters to exhibit both punctate and ubiquitous expression patterns. For instance, Slc25a3 and Slc25a5 that transport mitochondrial phosphate and adenine, respectively, are ubiquitously expressed across the kidney vasculature (Supplementary Fig. 3c). Select transporters were also found to be specific to endothelium, such as Slc9a3r2, a protein involved in sodium absorption (Fig. 3e–g). However, many membrane transport proteins identified were expressed within distinct populations of the kidney vasculature (Fig. 3e–m, Supplementary Data 5) supporting the notion that transporters tailor the function of neighboring nephron segments.

Temporal analysis of transporters specific to vessels reveals that most membrane transport proteins are induced at E14–E15 stages, while being upregulated from E17 to adult stages (Fig. 3d). The GCs specifically express two of the voltage gated channels, Kcnej and Scn7a, which are both linked to hypertension. Slc25a3, which mediates membrane transport of taurine, modulating osmoregulation, membrane stabilization, and secretion of taurine in renal tubules, is highly enriched in venous blood vessels (AVR/V) (Fig. 3e). Thus, this is consistent with the role calcium plays in regulating the contraction and relaxation of smooth muscles surrounding the arterial endothelium. Efferent arterioles present high levels of Slec8a1 and Kcnj4 that pump calcium (Fig. 3e). This is consistent with the role calcium plays in regulating the contraction and relaxation of smooth muscles surrounding the arterial endothelium. Efferent arterioles present high levels of Slec8a1 and Kcnj4 that pump calcium (Fig. 3e).

Kidney EC-epithelial crosstalk evolves over development. Kidney epithelial cells and the surrounding stroma crosstalk to mediate development and homeostasis of the kidney. The vasculature establishes a niche that through the secretion of specific angiocrine signals regulate specific developmental and homeostatic functions. Complete functional vasularization is required for progressing the development of the kidney and kidney organoids. However, the mechanism by which zone-specific vascular niche angiocrine signals sustain nephron homeostasis is unknown.

To address this, we show that multitudes of factors are secreted by different vascular subtypes in different ECs from E17 to adult stages, (Fig. 3c). Notably, GCs supply Fgf1, Vegfa, Notch-ligand Dll4, neuronal guidance cue Semaphorin 5a, and Wnt antagonist Dkk2 (Supplementary Fig. 3d). Contaminating podocyte or mesangial doublets were not detectable in the glomerular EC cluster (Supplementary Fig. 3d). During developmental progression, arterial ECs produce Tgfβ2, Ltb4, and Pdgfβ, while VPs elaborate Igf1 and Igl2 with their expression shifting to veins and arteries, respectively (Supplementary Fig. 3e). Regulatory molecules of IGF signaling zonate throughout the vasculature of the adult nephron.
Thus, a network of angiocrine and autocrine factors manifest restricted transcription among defined kidney vascular zones. In addition, we have developed a database to find what types of vessels in the kidney express specific secreted angiocrine factors that contribute to morphogenesis and patterning of nephrons.
Transcription factors delineate the kidney vasculature. Specific transcription factors are enriched in various stages of developing kidney vasculature (Fig. 3b). The bulk of these genes are Hox family of transcriptional regulators (Supplementary Data 2). However, in postnatal stages, many of these genes are downregulated and other specific sets of transcription factors are induced (Supplementary Data 2). Notably, many different transcription factors are associated with distinct vascular subtypes (Supplementary Fig. 3f, Supplementary Data 6), the majority of which are upregulated during late embryogenesis (E17). Hence, specific sets of transcription factors are induced during later stages of development to promote additional vascular specialization or function.

To identify transcription factors that may be important for the development of the kidney vasculature, we utilized the single-cell regulatory network inference and clustering (SCENIC) method. We identified 216 transcription factor regulons enriched in our dataset, which we then binarized and clustered with a supervised analysis to obtain lists of transcription factors with differential activity amongst each vascular subtype (Fig. 4a, Supplementary Fig. 3f). As expected, arteries and veins manifested an enrichment of Sox17 and Nr2f2 regulons, respectively. The GCs produced the largest and most unique combination of transcription factors. Many components of the AP-1 heterodimer such as Fos, Jun, and Atf were enriched (Fig. 4a). The AP-1 pathway is generally enriched during cellular stress and infection, suggesting that glomerular ECs may be sensitive to cellular stress during processing and tissue digestion. The glomerulus expressed several transcription factors known to be involved in stem cell differentiation and tissue morphogenesis, including Tbx3, Gata5, Prdm1, Irf8, Zbtb7a, Klf4, Maff, and Klf13. These gene transcriptional regulatory networks that can be investigated to further our understanding of kidney physiology and manipulated to enhance the construction of kidney tissues in vitro.

Tbx3 contributes to glomerulus morphogenesis and function. To elucidate mechanisms underlying glomerular development and function, we focused on transcription factors with restricted and abundant expression and reguion activity (Fig. 4a), such as Tbx3 in GCs and EAs (Fig. 4b). Immunofluorescent staining in human kidney tissue confirmed that Tbx3 protein is restricted to ECs in GCs and EAs (Fig. 4c).

To uncover the role of Tbx3 in glomerular specification and function, we conditionally ablated Tbx3 expression in EAs by employing the Cre/LoxP system with Cdh5-Cre (VE-cadherin-Cre) to generate Tbx3ΔEC mice. The Tbx3Δlox allele loses 4.6 kb of genomic DNA encompassing the 5′ UTR promoter, the transcriptional start site and first exon of Tbx3 in the presence of Cre recombinase. Targeted homozygous deletion of the Tbx3 allele in ECs using the vascular-specific VE-cadherin-Cre manifested clearly noticeable morphogenetic defects in subsets of the glomeruli in Tbx3ΔEC mice, but not in the control Tbx3lox/lox or Tbx3lox/fl mice (Fig. 4f, Supplementary Fig. 4a). In 4-month-old mice, most glomeruli appeared normal although 18% developed microaneurysms, 5% of glomeruli exhibited hypoplasia, and 3% of glomeruli became fibrotic (Fig. 4d, e). Affected glomeruli manifested significantly dilated capillaries and arterioles (Fig. 4d–f).

Urinalysis revealed Tbx3ΔEC mice to have higher levels of urea, protein, and salt, suggesting aberrant nephron filtration or reabsorption (Fig. 4g–i). Consistent with most glomeruli appearing normal, serum panel showed few signs of kidney failure (Supplementary Fig. 4b–g). Salt homeostasis, blood urea nitrogen, creatinine, and albumin levels remained normal in Tbx3ΔEC serum, with normal glomerular filtration rate (Supplementary Fig. 4k). Four-month-old Tbx3ΔEC kidneys did not have significantly fewer ECs and did not show signs of kidney inflammation or apoptosis as marked by cleaved Caspase-3 and CD45 staining, respectively (Supplementary Fig. 4h, i). No significant defects were found in major blood vessels in other organs (Supplementary Fig. 4j). Transmission electron microscopy (TEM) revealed a fraction of GCs with microaneurysms, significantly fewer fenestrations, and surrounded by deformed podocyte foot processes (Fig. 4m, Supplementary Fig. 4l). Thus, Tbx3 maintains the structural organization of glomerular capillaries.

To determine whether Tbx3 plays a physiological role in BP homeostasis in adult mice, we measured systolic BP in control and Tbx3ΔEC mice. Systolic BP in mice lacking endothelial Tbx3 was lower compared to that of control mice (Fig. 4n). To assess whether this was due to defects in the renin–angiotensin system, levels of renin, angiotensinogen (Agt), and angiotensin-converting enzyme (Ace) were measured in the kidney, lung, and liver respectively by qPCR. Renin levels were found to be higher in the absence of Tbx3 in the vasculature of the kidney, while Agt and Ace1 transcript levels remained the same (Fig. 4o–q). Therefore, Tbx3 regulates a putative transcriptional program that suppresses or balances blood pressure via regulation of renin in the kidney.

To uncover the mechanism by which Tbx3 mediates glomerular vascular development and function, ECs were isolated from control and Tbx3ΔEC kidneys and processed for RNA sequencing (Supplementary Fig. 4n; Supplementary Data 7). Differential expression analysis of glomerular-specific genes between control and Tbx3ΔEC kidney vasculature revealed an...
array of transcripts that were differentially expressed after Tbx3 loss in mice (Supplementary Fig. 4n). Downregulated genes include the solute transporter Slc44a2, transcription factors Gata5, Klf4, and Smad6, and proteins that mediate adhesion or the cytoskeleton, including Rhob and Itga3. The expression of Ehd3, the most abundant and specific gene in GCs (Fig. 1k, 3a), was also downregulated in the absence of Tbx3. Genes that increased in the absence of Tbx3 include those necessary for
morphogenesis, (Spry4, Rap1b, and Pcdh17), transcription factors (Irf1, Prdm1, Elf1, and Stat3), and the growth factor Fgf1. While glomeruli represent a small fraction of the kidney vasculature, there was a significant overlap between genes that are differentially expressed in the bulk endothelial population of Tbx3ΔEC and genes specifically expressed in the glomerular cluster (hypergeometric test \( p = 6.76E^{-9} \); Supplementary Fig. 4o).

To identify potential targets of Tbx3, either Tbx3 or a control vector was overexpressed in human umbilical vein endothelial cells (HUVECs), representing a generic-like vascular bed, and mRNA was sequenced (Supplementary Data 8). There was a significant overlap between Tbx3-regulated genes in HUVECs and glomerular-specific genes (hypergeometric test \( p = 7.1E^{-10} \), Supplementary Fig. 4o). When we compared glomerular genes that were upregulated in Tbx3ΔEC mouse kidney ECs and Tbx3-regulated genes in HUVECs, we find a high concordance with glomerular genes identified through scRNA-seq (hypergeometric test \( p = 4.8E^{-5} \); Supplementary Fig. 4o). Hence, Tbx3 is a very potent and specific mediator of glomerular gene transcription and identity. **Tbx3, Gata5, Prdm1, and Pbx1 repress and specify GC genes.** Other transcription factors were found to have enriched expression or activity in GCs in addition to Tbx3 (Fig. 4a), prompting the hypothesis that additional factors might cooperate with Tbx3 to establish glomerular function. To determine whether transcription factors cooperate in GCs, we focused on additional factors with differential expression, including Tbx3, Gata5, Prdm1, and Pbx1. Each gene was stably over-expressed in HUVECs and mRNA was sequenced for each condition (Supplementary Data 8). Log, fold-changes were calculated relative to an empty-vector control (EV) to find genes regulated by each transcription factor. We compared the lists of genes that change in response to stable overexpression of each transcription factor and found that a significant proportion of genes that were downregulated correspond to genes found within the GC cluster (Fig. 4r). The overlap, however, was not nearly as prominent for the sets of genes that are upregulated. Thus, the identified zone-specific transcription factors may be performing as repressors and possibly collaborate to regulate a common set of glomerular-specific targets.

We next sought to determine if transcription factor overexpression, either alone or in combination, is enough to approximate glomerular fate in HUVEC cells. As a positive reference control, we used glomeruli isolated from a deceased-donor human kidney that was not transplanted (Supplementary Fig. 4p, q). CD31+VE-cadherin+CD45+ECs were FACS-sorted from glomeruli and processed for RNA sequencing. To enable direct comparison between human glomerular and transcription factor overexpression datasets, all datasets were normalized as a fold-change value relative to the HUVECs expressing an empty lentivirus vector. We selected the top 10 significantly up or downregulated genes in response to each overexpression condition and compared expression patterns to human glomeruli. The top differentially regulated genes in response to each transcription factor overexpression exhibited similar expression patterns and phenocopied expression patterns in human glomeruli (Supplementary Fig. 4r). Expression patterns in response to PRDM1 and PBX1 were highly overlapped with that of Tbx3 and GATA5, despite PRDM1 and PBX1 being more ubiquitously expressed, though significantly higher in GCs (Fig. 4s). We directly compared overlap among the expression of glomerular-specific genes in the human glomerulus and in response to each transcription factor. We find that genes modified in response to overexpression of each transcription factor very highly overlap with genes specifically expressed in the human glomerulus (Fig. 4t). We next overexpressed all 4 transcription factors simultaneously in HUVECs and found the highest overlap with the human glomerular expression patterns, indicating these factors function additively in dictating glomerular specificity. Hence, overexpression of each transcription factor promotes a signature that renders HUVECs more like human glomeruli and opens avenues towards designing systems to conditionally reprogram cultured cells into human glomeruli.

To identify pathways downstream of these transcription factors, we did pathway enrichment analysis on GC-specific genes regulated by these factors (Fig. 4u). Numerous vascular maintenance pathways, including those found to be enriched in the glomerulus were downregulated. These include the CXC4 and VEGF signaling pathways, semaphorin interactions, and integrin signaling. This data prompted the hypothesis that each of these four proteins, controlling a transcriptional hierarchy through which glomerular-specific targets guide vascular functions, are selectively downregulated to execute various glomerular functions. Accordingly, in Tbx3ΔEC mice, we observe subsets of glomeruli manifesting hypoplasia, microaneurysms, and fenestration loss, potentially leading to aberrant BP homeostasis. Notably, BP was decreased and there was no indication of vasculopathy in other organs that could induce secondary hypertensive changes in...
the kidney vasculature (Supplementary Fig. 4j). We conclude that Tbx3, along with other factors turns on the expression of pathways that confer the unique vascular functions of GCs. Dysregulation of these genes could lead to defects associated with developmental patterning, EC rarefaction, EC adhesion and contraction, and solute transport.

Discussion

To decipher the molecular determinants regulating intra-kidney vascular network diversity, we employed dSéq single-cell RNA sequencing.40–42. We identified 6 discrete non-lymphatic vessels in the kidney vasculature. Although several important blood vessels are missing, including lymphatic vessels, we focused primarily on unraveling the signature of glomerular ECs, and broadly the arteries, veins and capillaries. Each vascular domain displays unique membrane transport proteins, regulators of transcription, growth factors, and endocrine hormone binding proteins that serve known and unknown functions in nephron development, filtrate reabsorption, and blood pressure homeostasis.

Kidney vascular specification begins at E14 while waves of organotypic kidney vascular genes are induced at the onset of birth, in perinatal stages, and during maturation into adult vessels. Genes that are unique to the kidney vasculature relative to other organs are robustly expressed after birth. Compared to the early developmental stages, several gene signatures in the adult kidney vasculature are unique. We propose that this unexpected transition of vascular specialization could be due to the physiological stress on the kidney after birth. Notably, membrane transport proteins that are important for the kidney’s vasculature function are not upregulated until after birth, indicating that expression of these transporters may be dependent on stimuli not present during gestation. Alternatively, the transcriptome of the postnatal and adult stages may dramatically differ from the embryonic stages of the same organ through the loss of developmental and mitotic transcripts and the transition to transcripts the kidney needs to function.

How do kidney blood vessels, specifically the capillaries, acquire this complex heterogeneity? One hypothesis suggests extrinsic cues turn on transcription factors driving vascular specialization. To test this, we parsed out genes specific to the kidney and specific to zones of the vasculature. We find that GCs primarily express factors that are suppressors of transcription. Pathways repressed by each of these transcription factors correspond to the same pathways that are enriched in the GCs found using scRNA-seq. This creates a conundrum as to how genes become specifically expressed in the glomerulus, but also downregulated where Tbx3/Gata5/Prdm1/Pbx1 is expressed. It is conceivable that prior to glomerular specification, the ECs activate generic vascular specification programs with broad vascular gene profiles. Tbx3, Prdm1, Gata5, and Pbx1 may then be recruited to prune gene expression and fine-tune the specialization functions of the glomeruli. Each factor may also modulate other suppressors thereby activating GC genes. Alternatively, each gene may solely function to modulate or balance perinatal and adult kidney-specific processes, including BP homeostasis or glomerular filtration dynamics.

As an example of intrinsic transcriptional suppression regulating GC functions, we focused on Tbx3, as it is robustly represented in the GCs and represses transcription of particular genes in a variety of diverse, yet specific, tissues.43 Tbx3ΔEC mice developed phenotypes similar to capillary endotheliosis—glomerular swelling and loss of fenestrations—in subsets of the glomeruli. Differential expression analysis among pooled Tbx3-deleted mouse kidney endothelial cells, TXB3-over-expressing HUVECs, and genes enriched in the murine and human revealed that a network of genes that regulate vasodilation/contraction, adhesion, and solute transport that may contribute to hypoplasia, microaneurysms, and loss of fenestrations in Tbx3ΔEC mice.

A network of developmental genes is also altered in Tbx3-deleted ECs, including Gata5 and Ehd3. Both genes have been shown to be necessary for the integrity of glomerular endothelium. Mice lacking Gata5 and Ehd3 develop glomerular lesions and kidney failure.41,44 Tbx3ΔEC mice also have inversely correlated phenotypes to mice that lack endothelial Gata5, including aberrant BP and renin signaling, suggesting that both transcription factors may balance BP in the kidney and possibly arterial vessels in other organs. Tbx3 in arteries, and glomerular endothelium, may suppress genes that allow crossstalk to juxtaglomerular cells, therefore regulating renin secretion, glomerular filtration rate, and BP homeostasis. Tbx3 may also regulate glomerular filtration dynamics by stimulating crossstalk to podocytes and mesangial cells under certain conditions. Tbx3 was not found to be expressed in blood vessels in other tissues except for the lungs, which may explain why phenotypes were centralized primarily in the kidneys. Tbx3 may also function redundantly with its paralogue Tbx2, possibly masking additional phenotypes. Additional studies are necessary to prove these hypotheses and to gain a better understanding of kidney vascular zonation, development, and function.

Our composite of endothelial-specific signatures bring us closer to engineer zone-specific kidney endothelium. Notably, each vascular bed expresses a combination of secreted factors and transcriptional regulators that define zonated vascular fate. By supplying angiocrine factors, ECs instructively orchestrate tissue healing and regeneration during lung, liver, and bone marrow injury.45–47. Generating kidney-specific endothelium may facilitate approaches to rebuild kidneys. Our dataset compiles an array of genes that could be used to engineer kidney-specific vascular endothelium. Furthermore, this knowledge will lay the foundation for identifying extrinsic environmental cues that confer kidney vessels with their unique region-specific structural and functional activities.

Methods

Cell digestion and FACS sorting. To isolate adult endothelial cells from mouse kidney, liver, heart, and lung, mice were injected intravitally with 25 μg of anti-VE-cadherin-αVE647 antibody (clone BV131, Biologend) retro-orbitally in 6–8-week-old male C57BL/6J mice under anesthesia 10 min before they were killed and the organs harvested. For cell sorting, organs were minced and incubated with Collagenase A (25 mg ml−1) and Dispase II (25 mg ml−1) at 37 °C for 20–30 min to create a single-cell suspension. Cells were filtered through a 40-μm filter immediately before counter staining. The single-cell suspension was first blocked with an Fc-quenching antibody before antibody staining with anti-mouse CD31-Alexa Fluor® 488 (102414, Biologend), anti-mouse CD45-Pacific Blue™ (103126, Biologend), and anti-mouse Podoplanin-PE/Cy7(127412, Biologend). Embryonic tissues were dissected and processed through the same antibodies. Following staining, cells were processed for FACS sorting.

Immunofluorescent staining. Tissues were fixed overnight in 4% paraformaldehyde at 4 °C. The following day, organs were washed in PBS and cryoprotected in 30% sucrose overnight. Tissues were then embedded in Tissue-Tek O.C.T. Compound and sectioned at 10 μm on a cryostat. Frozen sections were washed with PBS to remove O.C.T. Antigen retrieval was performed on select slides using a pressure cooker. During retrieval, slides were soaked in citrate buffer (Espec, buffer B cat. # 62706–11 for cytoplasmic stain and buffer A catalog number 62706–10 for nuclear stain). The tissues were permeabilized in PBS with 0.1% Triton X-100 and blocked for 1 h at room temperature in 5% normal donkey serum. Primary antibody incubations were done at 4 °C. Slides were then washed in PBS, incubated in secondary antibody for 1 h at room temperature. Slides were then washed in PBS and mounted using Prolong Gold Mounting Medium containing DAPI. Images were obtained using an A1R Nikon confocal microscope and a Zeiss LSM 710 CarlZeiss Microscope. The following antibodies were used: chicken anti-GFP (for Flk-GFP; Aves, GFP-1020, 1:500), goat anti-Cx40/Gja5 (Santa Cruz, sc-20466, 1:500), rabbit anti-Collagen IV (Millipore, AB756P, 1:400), goat anti-Nrp1 (R&D Systems). 1:100), rabbit anti-Collagen IV (Millipore, AB756P, 1:400), goat anti-Nrp1 (R&D Systems).
systems, AF566, 1:100), rat anti-F-Pvap (BD Pharmingen, 550563, 1:100), goat anti-Sox17 (R&D Systems, AF1924, 1:100), rat anti-Endomucin (Santa Cruz, sc-65495, 1:100), rabbit anti-Tbx3 (Abcam, ab99302, 1:100), rabbit anti-Aplnr (Protein Tech, 20341–1-AP, 1:100), goat anti-Igfbp5 (R&D Systems, AF578, 1:100), goat anti-Igfbp7 (Abcam, ab129302, 1:100), rabbit anti-Six2 (Protein Tech, 11562–1-AP, 1:100).

In situ hybridization. Fixed E15.5, E18.5, or P5 kidneys were dehydrated to 100% ethanol and embedded in paraffin before sectioning using a microtome. Sections were de-paraffinized in xylene, then rehydrated to PBS before being treated with 15 μg/ml proteinase K for 15 min and fixed in 4% PFA/PBS. Slides were then washed and incubated with a pre-hybridization buffer for 1 h at room temperature before being hybridized with the specific probes at 1 μg/ml overnight at 65 °C. The following day, slides were washed in 0.2x SSC then transferred to MBST, and blocked with 2% blocking solution (Roche) for at least 1 h at room temperature. Slides were then incubated with anti-Dig alkaline phosphatase-conjugated antibody (Roche, 1:4000) overnight at 4 °C. Next day, slides were washed 3x in MBST and NTMT before incubating with BM purple (Roche) for a color reaction. After the color reaction, slides were fixed with 4% PFA and mounted using Permoun mounting solution. Images were taken using a Zeiss Axiostar 200M scope and a DP-70 camera from Olympus.

Fluorescent in situ hybridizations were performed following the same procedure up to SSC washes. Following the washes, slides were transferred to TNT and treated with 0.3% H2O2 for 30 min. Slides were then washed again in TNT and blocked in 1% blocking buffer (Perkin Elmer) for 1 h at room temperature. Slides were then incubated with anti-tyrosinase (Roche, 1:1000), rat anti-Endomucin antibodies overnight at 4 °C. Next day, slides were washed in TNT 3x before incubating with TSA Fluorescein Amplification Reagent (1:50 in Amplification Diluent, Perkin Elmer) for 15 min. Slides were washed in TNT following TSA incubation, incubated with goat anti-rat Alexa Fluor 555 for 2 h at room temperature, and then incubated in DAPI. Slides were washed in TNT and mounted using Prolong Gold Mounting Medium. The slides were imaged using an A1R Nikon confocal microscope.

Masson’s trichrome staining. Masson’s trichrome staining was performed by Histoserv, Inc. To fix the tissues, following euthanasia, mice were perfused with 25 ml PBS then 10 ml of 4% PFA/PBS through the left ventricle of the heart. The right atrium of the heart was severed to accommodate bleeding. Kidneys of the mice were then additionally fixed overnight in 4%PFA/PBS overnight at 4 °C. The following day, the kidneys were washed with PBS then stored in 40% ethanol before being shipped to Histoserv, Inc.

Transmission electron microscopy. TEM was carried out by the Weill Cornell Medicine Electron Microscopy Core Facility per their standard protocols.

Lentivirus protocol. The lentiviral vectors used to overexpress Tri3 (pLV[Exp-Hygro-hPckG > hTbx3][NM_016569.3], GATA5pLV[Exp-Hygro-hPckG > hGATA5[ORF024149].], PRDM1pLV[Exp-Hygro-hPckG > hPRDM1 [NM_001198.3].], and PBX1pLV[Exp-Hygro-hPckG > mPbx1[ORF039780].]) in our study were constructed and packaged by VectorBuilder (Cyagen Biosciences).

Human umbilical vein endothelial cell culture. HUVECs were isolated from umbilical cords at the New York Presbyterian Hospital. The permission and approval for obtaining discarded or left over umbilical cords were obtained from institutional review board (IRB) at Weill Cornell Medicine. The IRB deemed the studies on HUVECs exempt from the requirement of informed consent. The primary HUVECs cultured on plates coated with gelatin in media consisting of M199 (Sigma, M4530), 10% FBS (Omega Scientific), 1% (BioRad, M4530), 10% FBS (Omega Scientific), 1% (BioRad, M4530), rabbit anti-Tbx3 (Abcam, ab99302, 1:100), rabbit anti-Aplnr (Protein Tech, 20341–1-AP, 1:100).

Clinical pathology analysis. Urine and serum analysis and serum panels were carried out by the Memorial Sloan Kettering Cancer Center laboratory of comparative pathology per their standard protocols. Urine was collected using metabolic chambers. Analysis of urine was normalized to the volume that was produced. Serum was collected retro-orbitally via a heparin-coated capillary after mice were anesthetized with isofluorane. Glomerular filtration rate. In all, 100 mg of Inulin-FITC was boiled into 5 ml 0.85% NaCl then filtered into a Bio-Spin gel column. Mice were anesthetized with isofluorane, then retro-orbitally injected with the Inulin-FITC solution (2 μg/l bw). Mouse tails were clipped and blood was collected at 0, 3, 5, 7, 10, 15, 35, 65, and 75 min post injection via a capillary coated with heparin. The blood was spun down then plasma was diluted 1:10 in 0.5 M HEPES. The concentration of FITC was then measured using a Spectra Max photometer (485 excitation and 538 emission). GFR was calculated in GraphPad Prism using a two-phase exponential decay function.

Blood pressure measurement. Between the hours of 8–10 a.m., systolic blood pressure was measured using an ITTC Life Science tail cuff plethysmography blood pressure system.

Animal husbandry. All animal experiments were performed under the approval of Weill Cornell Medicine Institutional Animal Care and Use Committee (IACUC), New York, NY. The breeding and maintenance of animal colonies abided by the guidelines of the IACUC of Weill Cornell Medical College, New York, New York, U.S. Experimental procedures followed the IACUC guidelines. Animal care was carried out in the laboratory or the labs were sent to Transnet (transnet.com). To compare the phenotypes among different mouse genotypes, sex- and weight-matched littermates were used. The study used 4-month-old male mice. Tbx3 mice (Tbx3tm1.1Moon37, provided by Anne Moon (Weis Center for Research), were crossed with Cdh5-Cre (Tg(Cdh5-cdx-cre)386), from Luisa Arispe (UCLA) to produce Tbx3floxed/−, Cdh5CreERT2 mice (referred to as Tbx3flox/−, Cdh5CreERT2). Cdh5CreERT2 mice were kindly provided from Ondine Cleaver at UT Southwestern Medical center. B2R-Confetti mice (Gr (ROSA26)Sor1tm1(CAG-Brainbow2.1)C1a) purchased from fax and crossed to Cdh5 (PAC)-CreERT2 (Tg(Cdh5-cdx-cre)ERT2)R1ha mice from Ralph Adams were kindly donated from Jason Butler at Weill Cornell Medicine.

Human tissue data. Human kidneys used for glomerular endothelial cell isolation or histology were obtained as medical waste from a deceased-donor human kidneys through the Memorial Sloan Kettering Cancer Center laboratory of comparative pathology per the standard NIH definition. Hence no IRB approval of the protocol was required.

Single-cell RNA-seq analysis. Cells were harvested from E17, P2, P7, Adult murine kidneys and were digested into single cells. A single-cell suspension was loaded into the Bio-Rad dSeq Single-Cell Isolator (BioRad, Hercules, CA) on which cells were isolated, lysed and barcoded in droplets. Droplets were then destructed, and cDNA was pooled for second strand synthesis. Libraries were generated with direct tagmentation followed by 3′ enrichment and sample indexing using Illumina BioRad SureCell WTA 3′ Library Prep Kit (Illumina, San Diego, CA). Pooled libraries were sequenced on the Illumina NextSeq500 sequencer at pair-end read (R1 68 cycles, sample index: 8 cycles and R2: 75 cycles). Sequencing data were primarily analyzed using the SureCell RNA Single-Cell App in Illumina BaseSpace Sequence Hub. In particular, sequencing reads were aligned to the mm10 reference genome using STAR aligner49 cell barcodes were used to separate reads from different cells, and unique molecular identifiers (UMIs) were used to remove duplicate reads that were actually derived from the same mRNA molecule. A knee plot was generated based on the number of UMI counts per cell barcode in order to identify quality cells separating from empty beads or noise, and a raw UMI counts table for each gene in each cell was then prepared. The raw counts table was fed into Seurat version 2.0.1 for clustering analysis52. Cells that have between 200 and 8000 and downstream analyses were selected. This step was used to filter for high-quality single cells. Epithelial cells and perivascular cells were filtered based on the expression of Cdh1/Epcam/Cdh16 and Pdgfb, respectively. The resulting data was log-normalized in Seurat. To mitigate the effects of cell cycle heterogeneity in data, we followed a previously published approach by assigning each cell with a score based on its expression of canonical cell cycle markers and then regressing these out using Seurat. We also regressed out effects associated with the number of UMIs, mitochondridal content and ribosomal gene content. Principal component analysis (PCA) was performed on the top variable genes determined in Seurat, where the top 13 principal components were selected by choosing the first 13 eigenvalues in the Scree plot and used for cell clustering and t-SNE projection. t-SNE plots were generated using R ggplot2 package.

Pseudo-time analysis. Data was normalized and cells were filtered using tools available in the Seurat package, as described above. Normalized data was converted to an object useable by Monocle v2 in R. Low quality reads were detected as those with a minimum normalized expression of <0.1 and which were expressed in at least 1% of cells. The dimensional reduction dataset was reduced in dimension to the number of dimensions set at 13, defined according to the procedure listed above. The number of dimensions were selected by choosing the inflection point in the Scree plot. Cells were then ordered in pseudo-time and trajectories were plotted using Monocle 2.

SCENIC. SCENIC was used according to the protocol previously described using the protocol in the SCENIC package in R. Briefly, the transcription factor network was defined based on co-expression and filtering using GENIE3 in R using the GENIE3 and GRNboost packages. Cells were first filtered to those expressed in at least 1% of cells with a count value of at least 3. Targets for transcription factor regulations were then scored with RocTarget. Cells were scored based on the activity of the target gene network with AUCell and cells were clustered according to GRN activity with t-stochastic neighbor embedding using only high confidence regulations. Regulon activity was binned according to the activity above the AUC
threshold. A one-way hierarchical cluster was drawn based on binned regulon activity using the stats package in R. Cells were ordered according to clusters defined in the Seurat package described above.

Isolation of glomeruli from human kidney. Human kidney was decapsulated uniformly and minced into small fragments with scalpels. These small fragments were then digested with 1 mg/ml collagenase III in RPMI medium at 37 °C for 10 min with mild rotation. The specimens were then gently pressed with a flattened pestle and passed through a 100-µm cell strainer. After washing with complete medium (RPMI +10% FBS) for three times, glomeruli were collected and centrifuged at 55 × g for 5 min. The emerging suspension contained uniformly decapsulated human glomeruli with minimal disintegration.

Affinity propagation clustering. APC was performed according to pipelines published by McMillan et al. Replicate values were collapsed by the median expression value. As input to the algorithm, cells were clustered by the top 20% of the most highly variant transcripts, corresponding to 3331 genes.

RNAseq normalization pipeline. As a part of our standard RNA-seq pipeline, Fastq files were quality checked with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and reads were processed to remove adapter sequences with BBtools (https://jgi.doe.gov/data-and-tools/bbtools/). Reads were aligned to the mm10 mouse genome or the hg38 human genome with STAR v2.5.3a.56 Aligned files were sorted and indexed with samtools v1.5.50, and count files were generated with HTseq v0.9.1.51 Counts were imported to R v3.4.0, batch corrected and normalized with the EdgeR package.52 The limma53 package was used to calculate differential expression and assign p and q-values.

Pathway analysis. Genes were associated as being differentially expressed between conditions if the limma derived p-value was <0.001 and the log fold change was >1. Gene sets were curated from Broad MSigDB V3 (Kegg, Reactome, GO) or CORUM55 databases and filtered for gene sets containing between 5 and 200 members. The sets of transcription factors were defined from the transcription factor classification database (http://www.tcfd.org/) and the sets of membrane proteins were defined from the DBD database. Growth factors were curated from the lists of secreted proteins in the human protein atlas. A hypergeometric test was used to calculate enrichment of gene sets in lists of genes and p-values were adjusted for false discovery rate.

Other statistical analyses. Hierarchical clustering was performed using the stats package in R.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All bulk and single-cell expression matrices have been deposited under Gene Expression Omnibus with the accession numbers GSE129005 and GSE137786. Other associated raw data are available in Supplementary Data 1–8. All data are available from the corresponding authors upon reasonable request.

Code availability. Code for processing single-cell RNA-seq is available in the Supplementary Software.

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References

1. Augustin, H. G. & Koh, G. Y. Organotypic vasculature: from descriptive heterogeneity to functional pathophysiology. Science 357, https://doi.org/10.1126/science.aal2357 (2017).
2. Nolan, D. J. et al. Molecular signatures of tissue-specific microvascular engraftment reveal cell-cell heterogeneity in organ maintenance and regeneration. Dev. Cell 26, 204–219 (2013).
3. Vanlandewijck, M. et al. A molecular atlas of cell types and zonation in the brain vasculature. Nature 554, 475–480 (2018).
4. Grabias, B. M. & Konstantopoulos, K. The physical basis of renal fibrosis: effects of altered hydrodynamic forces on kidney homeostasis. Am. J. Physiol. Renal. Physiol. 306, F473–F485 (2014).
5. Danziger, J. & Zeidel, M. L. Osmotic homeostasis. Clin. J. Am. Soc. Nephrol. 10, 852–862 (2015).
6. Hamm, L. L., Nahkouh, N. & Hering-Smith, K. S. Acid-base homeostasis. Clin. J. Am. Soc. Nephrol. 10, 2232–2242 (2015).
7. Julian, B. A. et al. Sources of urinary proteins and their analysis by urinary proteomics for the detection of biomarkers of disease. Proteomics Clin. Appl. 3, 1029–1043 (2009).
8. McMahan, A. P. Development of the mammalian kidney. Curr. Top. Dev. Biol. 117, 31–64 (2016).
9. Molenaar, G. & Aird, W. C. Vascular heterogeneity in the kidney. Semin. Nephrol. 32, 145–155 (2012).
10. Bartlett, C. S., Jeansson, M. & Quaggin, S. E. Vascular growth factors and glomerular disease. Annu. Rev. Physiol. 78, 437–461 (2016).
11. Homan, K. A. et al. Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat. Methods 16, 255–262 (2019).
12. van den Berg, C. W. et al. Renal subcapsular transplantation of PSC-derived kidney organoids induces neo-vasculo genesis and significant glomerular and tubular maturation in vivo. Stem Cell Rep. 10, 751–765 (2018).
13. Daniel, E. et al. Spatiotemporal heterogeneity and patterning of developing renal blood vessels. Angiogenesis 21, 617–634 (2018).
14. Munro, D. A. D., Hohenstein, P. & Davies, J. A. Cycles of vascular plexus formation within the nephrogenic zone of the developing mouse kidney. Sci. Rep. 7, 3273 (2017).
15. McMillan, E. A. et al. Chemistry-first approach for nomination of personalized treatment in lung cancer. Cell 173, 864–878 e829 (2018).
16. Frey, B. J. & Dueck, D. Clustering by passing messages between data points. Science 315, 972–976 (2007).
17. Sattija, R., Farrell, J. A., Gennert, D., Schier, A. F. & Regev, A. Spatial reconstruction of single-cell gene expression data. Nat. Biotechnol. 33, 495–502 (2015).
18. Rafi, S., Butler, J. M. & Ding, B. S. Angiogenic functions of organ-specific endothelial cells. Nature 529, 316–325 (2016).
19. Harvan, F., Sorensen, C. M., Holstein-Rathlou, N. H. & Peti-Peterdi, J. Connexins and the kidney. J. Am. Physiol. Regul. Integr. Comp. Physiol. 298, R1143–R1155 (2010).
20. Aird, W. C. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circ. Res. 100, 174–190 (2007).
21. George, M. et al. Renal thrombotic microangiopathy in mice with combined deletion of endostatic regulators EH3D and EH4D. PLoS ONE 6, e17838 (2011).
22. Trapnell, C. et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. Nat. Biotechnol. 32, 381–386 (2014).
23. Munro, D. A. D. & Davies, J. A. Vascularizing the kidney in the embryo and organoid: questioning assumptions about renal vasculo genesis. J. Am. Soc. Nephrol. 29, 1593–1595 (2018).
24. Snippert, H. J. et al. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell 143, 134–144 (2010).
25. Kemeny-Kozlovsky, Y. et al. Ascending vasa recta are angiopoietin/Tie2-dependent lymphatic-like vessels. J. Am. Soc. Nephrol. 29, 1097–1108 (2018).
26. Kummerfeld, S. K. & Teichmann, S. A. DBD: a transcription factor prediction database. Nucleic Acids Res. 34, D74–D81 (2006).
27. Choi, M. et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Science 331, 768–772 (2011).
28. Zhang, B. et al. The association between the polymorphisms in a sodium channel gene SCN7A and essential hypertension: a case-control study in the Northern Han Chinese. Ann. Hum. Genet. 79, 28–36 (2015).
29. Rembold, C. M. Regulation of contraction and relaxation in arterial smooth muscle. Hypertension 20, 129–137 (1992).
30. De Luca, A., Pierro, S. & Camerino, D. C. Taurine: the appeal of a safe amino acid for skeletal muscle disorders. J. Transl. Med. 13, 243 (2015).
31. Oja, S. S. & Sarasansari, P. Pharmacology of taurine. Proc. West Pharmacol. Soc. 50, 8–15 (2007).
32. Han, X. et al. Knockout of the Taut gene predisposes C57BL/6 mice to streptozotocin-induced diabetic nephropathy. PLoS ONE 10, e0117718 (2015).
33. Roth, M., Obaidat, A. & Hagenbuch, B. OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Br. J. Pharmacol. 165, 1260–1287 (2012).
34. Hagenbuch, B. Cellular entry of thyroid hormones by organic anion transporting polypeptides. Best Pract. Res. Clin. Endocrinol. Metab. 21, 209–221 (2007).
35. Aibar, S. et al. SCENIC: single-cell regulatory network inference and clustering. Nat. Methods 14, 1083–1086 (2017).
36. Schonthaler, H. B., Guinea-Viniegra, J. & Wagner, E. F. Targeting inflammation by modulating the Jun/AP-1 pathway. Ann. Rheum. Dis. 70, 109–112 (2011).
37. Frank, D. U., Emechebe, U., Thomas, K. R. & Moon, A. M. Mouse TBX3 mutants suggest novel molecular mechanisms for Ulnar-mammary syndrome. PLoS ONE 8, e67841 (2013).
38. Alva, J. A. et al. VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. Dev. Dyn. 235, 759–767 (2006).
39. Portis, J. L., Wikel, S. K. & McAtee, F. J. A simplified rapid method for purification of glomeruli. J. Clin. Pathol. 32, 406–409 (1979).
40. Park, J. et al. Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. Science https://doi.org/10.1126/science.aax2131 (2018).
41. Brunskill, E. W. et al. Single cell dissection of early kidney development: multilineage priming. Development 141, 3093–3101 (2014).
42. Lu, Y., Ye, Y., Yang, Q. & Shi, S. Single-cell RNA-sequence analysis of mouse kidney disease. Kidney Int. 92, 504–513 (2017).
43. Washko, A. J., Gavrilov, S., Begum, S. & Papaioannou, V. E. Diverse functional networks of Tbx3 in development and disease. Wiley Interdiscip. Rev. Syst. Biol. Med. 4, 273–283 (2012).
44. Messaoudi, S. et al. Endothelial Gata5 transcription factor regulates blood pressure. Nat. Commun. 6, 8835 (2015).
45. Lee, K. S. et al. Catheter-directed intraportal delivery of endothelial cell therapy for liver regeneration: a feasibility study in a large-animal model of cirrhosis. Radiology 285, 114–123 (2017).
46. Gori, J. L. et al. Endothelial cells promote expansion of long-term engrafting marrow hematopoietic stem and progenitor cells in primates. Stem Cells Transl. Med. 6, 864–876 (2017).
47. Rafii, S., Ginsberg, M., Scandura, J., Butler, J. M. & Ding, B. S. Transplantation of endothelial cells to mitigate acute and chronic radiation injury to vital organs. Radiat. Res. 186, 196–202 (2016).
48. Ema, M., Takahashi, S. & Rossant, J. Deletion of the selection cassette, but not cis-acting elements, in targeted Flk1-lacZ allele reveals Flk1 expression in multipotent mesodermal progenitors. Blood 107, 111–117 (2006).
49. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21 (2013).
50. Li, H. et al. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).
51. Anders, S., Pyl, P. T. & Huber, W. HTSeq – a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169 (2015).
52. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 134–140 (2010).
53. Ritchie, M. E. et al. limma powers differential expression analyses for RNA-sequencing and microarray data. Nucleic Acids Res. 43, e47 (2015).
54. Liberton, A. et al. Molecular signatures database (MSigDB) 3.0. Bioinformatics 27, 1739–1740 (2011).
55. Ruepp, A. et al. CORUM: the comprehensive resource of mammalian protein complexes–2009. Nucleic Acids Res. 38, D497–D501 (2010).

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Author contributions

Conceptualization by D.M.B., Sina R., T.M. and Shahin R.; Methodology by D.M.B., E.A.M., J.X., A.D.L. and R.L.; Software by E.A.M. and T.Z.; Formal analysis by D.M.B., E.A.M., T.Z., B.K. and D.R.; Investigation by D.M.B., B.K., R.L., T.L., E.D., M.Y., J.M.G. and A.S.; Writing by D.M.B and Shahin R. reviewed by all other authors; funding by Shahin R., M.E.C. and O.C.

Competing interests

S.R. is a co-founder and non-paid consultant to Angiocrine BioScience, San Diego CA. The spouse of M.E.C. is a co-founder and shareholder, and serves on the Scientific Advisory Board of Proterris, Inc. All other authors have no conflicts of interest.

Additional information

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