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REVIEW

The origin and role of the renal stroma

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ABSTRACT

The postnatal kidney is predominantly composed of nephron epithelia with the interstitial components representing a small proportion of the final organ, except in the diseased state. This is in stark contrast to the developing organ, which arises from the mesoderm and comprises an expansive stromal population with distinct regional gene expression. In many organs, the identity and ultimate function of an epithelium is tightly regulated by the surrounding stroma during development. However, although the presence of a renal stromal stem cell population has been demonstrated, the focus has been on understanding the process of nephrogenesis whereas the role of distinct stromal components during kidney morphogenesis is less clear. In this Review, we consider what is known about the role of the stroma of the developing kidney in nephrogenesis, where these cells come from as well as their heterogeneity, and reflect on how this information may improve human kidney organoid models.

KEY WORDS: Kidney development, Renal stroma, Lineage, Cortical stroma, Kidney organoid

Introduction

With chronic kidney disease (CKD) responsible for 1.2 million deaths per annum worldwide and rising at >6% each year (GBD Chronic Kidney Disease Collaboration, 2020), there is an immediate need for better models to understand kidney disease aetiology, develop precision therapies and find new approaches to renal replacement. This requires an ability to model the human kidney accurately. Recent years have seen the expansion of research using human pluripotent stem cells (hPSCs) to generate organ-like tissues termed organoids (Lancaster and Knoblich, 2014). The kidney is no exception, with several protocols to generate human kidney tissue from hPSCs published since 2014 (Freedman et al., 2015; Morizane et al., 2015; Taguchi et al., 2014; Takasato et al., 2014, 2015) and a growing body of subsequent work to improve and apply these existing protocols to model development and disease (reviewed by Little and Combes, 2019).

Kidney organoid protocols have been developed based upon the processes underlying kidney development in model organisms, including the mouse, with these conditions mimicked during the *in vitro* culture of hPSCs. As such, this required an understanding of the origins of the cell populations that make up the mammalian kidney *in vivo*. The kidney is regarded as being derived from the intermediate mesoderm (IM), perhaps excepting the neural and immune cells that represent populations that migrate in as development occurs. The kidney is composed of three progenitor lineages: nephron, ureteric and stromal progenitors. Importantly, perturbation to any of these lineages leads to abnormal kidney development, indicating that all are crucial to successful kidney growth (Little and Combes, 2019). Whereas the origin and development of the kidney epithelium, including both the nephrons and the ureteric epithelium, has been well characterised, the origin and role of the stromal populations has not. Within kidney organoids, the stroma is often the most abundant, yet least understood, cell type present. In this Review, we describe what is known about how these three progenitor populations interact during kidney morphogenesis *in vivo* with a focus on the origin, heterogeneity and role of the renal stroma. This framework is then used to discuss the stromal populations present in kidney organoids, their similarity to and differences compared with *in vivo* stroma and why improvements in stromal patterning may be crucial to improve kidney organoid protocols.

Formation of the kidney in mammals

The permanent kidney in mammals, the metanephros, is the third in a progression of paired excretory organs that form during embryogenesis. In the mouse, where kidney development has been extensively studied, the pronephros, mesonephros and metanephros arise in this temporal order from the rostral to caudal end of the embryo. In mammals, only the metanephros persists after birth.

An overview of early kidney development

Kidneys arise from the IM, which lies medio-laterally between the paraxial (PM) and lateral plate (LPM) mesoderm (Fig. 1A). The early, rostral IM, referred to as the anterior IM (AIM), develops by embryonic day (E) 7.5 in the mouse (Fig. 1B, E7.5), initially forming the pronephros by E8.5 at the level of somites 8 and 9 by forming an epithelial tube and a band of mesenchyme adjacent to each other (Redempta Vetter and Gibley, 1966) (Fig. 1B, E8.5). This epithelial tubule, known as the nephric duct (ND), represents the ‘distal tubule’ of the pronephric kidney, as is the case in other organisms in which this is a functional organ. The ND extends caudally as the body elongates and is always adjacent to a nephrogenic mesenchyme. Shifts in the identity and developmental potential of this extending mesenchyme can be tracked through changes in Hox paralogue expression (Hox code) and is guided by anterior-posterior signalling gradients. During extension from E9, this mesenchyme adjacent to the nephric duct undergoes mesenchyme-to-epithelial transition (MET) events to form the mesonephric tubules of the mesonephros, parallel to somites 10-17 (Fig. 1B, E9.5) (Redempta Vetter and Gibley, 1966). The gonadal mesenchyme arises alongside the mesonephros. In mouse, there is a clear distinction between cranial (rostral) and caudal mesonephric tubules, such that the caudal mesonephric tubules do not fuse with the adjacent ND whereas the cranial ones do (Georgas et al., 2011; Redempta Vetter and Gibley, 1966) (Fig. 1B, E9.5). Mesonephric tubule function is transient, with these rudimentary excretory organs regressing as development progresses, contributing to the genital tract by becoming the epididymis and paradidymis (Dudek and Fix, 1998) in males or epoophoron (Russo et al., 2000) in females. The

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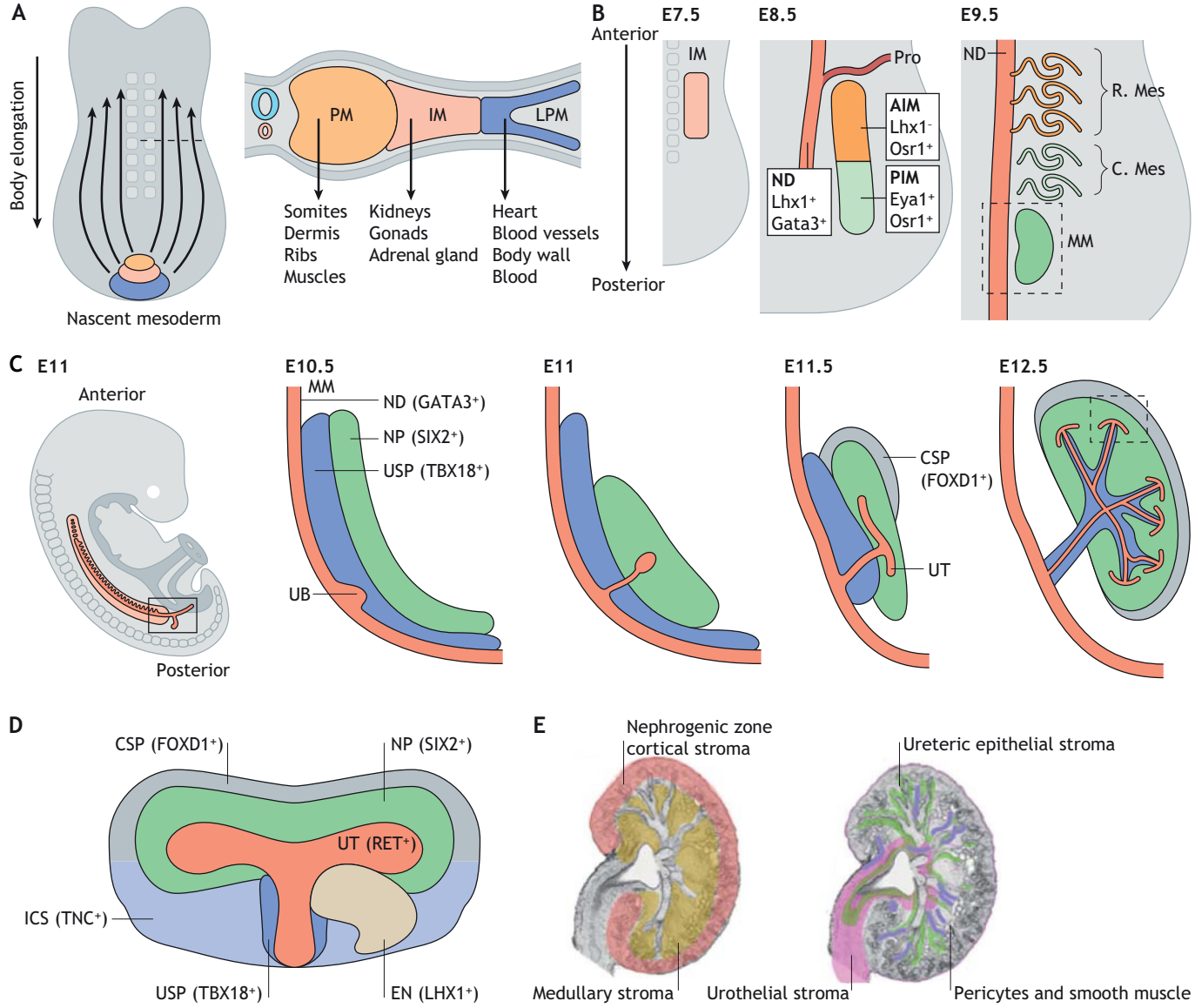

Fig. 1. Organogenesis of the excretory system. (A) Migration of the nascent mesoderm to form the trunk mesoderm segments: the paraxial mesoderm (PM), intermediate mesoderm (IM) and lateral plate mesoderm (LPM). (B) Stages of excretory organ development from the IM between embryonic day (E) 7.5 and E9.5. The nephric duct (ND) and pronephros (Pro) form by E8.5. The rostral (R.Mes) and caudal (C.Mes) mesonephros form by E9.5 from the anterior IM (AIM) and posterior IM (PIM), respectively, whereas the metanephros forms from metanephric mesenchyme (MM) after E.9.5. Box outlines region of interest shown in C. (C) Position of metanephric kidney in an E11 embryo (left; box) with stages of ureteric bud (UB) invasion and initial branching events. E12.5 box outlines the nephrogenic niche region shown in D. (D) Cell populations within the nephrogenic niche. (E) Regional locations of stromal populations in the E18.5 mouse kidney. Modified from Combes et al. (2019b). CSP, cortical stromal progenitors; EN, early nephron (including renal vesicle); ICS, inner cortical stroma; NP, nephron progenitors; USP, ureteric stromal progenitor; UT, ureteric tip.

final excretory organ, the metanephros, arises from the metanephric mesenchyme (MM) at the level of the hindlimbs (Fig. 1B, E9.5); this region displays a Hox11 paralogue gene expression signature that is not present in the more rostral regions of the excretory system (Davis et al., 1995; Patterson et al., 2001). In this region, the ND and MM are adjacent, whereas more anterior regions are separated by an intervening mesenchyme (Wainwright et al., 2015).

Metanephros patterning and the nephrogenic zone

Metanephric patterning commences as the MM interacts with the adjacent ND. Glial cell line-derived neurotrophic factor (GDNF) secretion from the MM binds and activates the RET tyrosine kinase receptor and GDNFRα1 co-receptor expressed along the ND. In response, the ND epithelium undergoes regional proliferation and conformational change in the form of a swelling of the duct (Georgas et al., 2011). This results in the formation of a bud that extends towards and invades the MM (Chi et al., 2009) (Fig. 1C). Following this invasion, the bud balloons and then bifurcates to form two distinct tips (Costantini and Kopan, 2010), representing the first branching event initiating the development of the kidney (Fig. 1C). From this stage, RET expression becomes restricted to the branching tip regions immediately surrounded by the GDNF-expressing MM, forming the first nephrogenic niche. Each niche contains a RET^+ ureteric tip surrounded by $SIX2^+$ nephron progenitor mesenchyme, further surrounded by $FOXD1^+$ cortical stromal progenitors (Fig. 1D). These ureteric tip, nephron and

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cortical stromal populations all represent self-renewing progenitors required for metanephric organogenesis and all are present at the periphery of the developing organ in a region referred to as the nephrogenic zone (Chi et al., 2009; Kobayashi et al., 2008, 2014). Reciprocal signalling within each niche between the ureteric tip and the nephron progenitors drive both nephrogenesis and ureteric branching. In addition, trophic signals from the nephron progenitors drive ongoing ureteric branching and consequential organ expansion. Conversely, the nephron progenitors represent a self-renewing stem cell population (Kobayashi et al., 2008) positioned around the ureteric tip and receiving trophic signals from the ureteric tip that regulate self-renewal, proliferation and migration (Barak et al., 2012; Carroll et al., 2005; Karner et al., 2011; Kopan et al., 2014; Little and McMahon, 2012). Canonical WNT signalling from the ureteric tip also initiates nephron progenitor commitment and MET to form a renal vesicle (Carroll et al., 2005). Each renal vesicle then undergoes elongation and segmentation into the functional filtration units within the kidney: the nephrons.

Human kidney development

Until recently, our understanding of kidney development in human has been limited to anatomical and histological studies with ethical limitations on access to all stages of development and no capacity to apply the transgenic approaches used in mouse to interrogate the molecular basis of morphogenesis. Despite this, it has long been appreciated that very similar morphogenetic events appear to occur (Osathanondh and Potter, 1963a,b). Recent single-cell transcriptional profiling, as well as spatial studies of gene and protein expression have revealed strong congruence between mouse and human metanephric development, particularly with respect to trimester 1 and early trimester 2 (Lindström et al., 2018a,b,c). The transcriptional congruence of nephron progenitors in mouse and human suggests similar cellular identities and a conservation of the accepted signalling pathways, including RET-GDNF regulation of ureteric branching and WNT signalling in nephron formation. This role is reinforced by the phenotypes observed in patients with mutations in these pathways (Nicolaou et al., 2015). Although there is strong similarity between species, there are distinctions, such as the expression of both *SIX1* and *SIX2* in the cap mesenchyme and the expression of *FOXD1* in both cortical stroma and cap mesenchyme in humans, whereas *Foxd1* is completely restricted to the cortical stromal population in mouse (Lindström et al., 2018c). Anatomically, the mouse kidney is unipapillate, whereas the human kidney forms from multiple lobes. The duration and timing of ureteric branching also differs. Branching and nephrogenesis continues in the developing mouse kidney from 11.5 days post-coitum (dpc) to birth; however, in the human, branching ceases during trimester 2 whereas nephron formation persists into trimester 3 (Osathanondh and Potter, 1963a,b; Ryan et al., 2018) generating ∼1 million nephrons per adult kidney (Bertram et al., 2011).

Role of the renal stroma in metanephric morphogenesis

The ratio of stroma to epithelium drastically changes across development, with developing kidneys containing defined regions of stromal cells that are likely to contribute to the differentiation and maturation of the nephrons (Airik et al., 2006; Drake et al., 2020; England et al., 2020; Fetting et al., 2014; Levinson et al., 2005; Yallowitz et al., 2011). The stroma then regresses to form a minor population of interstitial fibroblasts and remaining derivatives, such as the endogenous vasculature and mesangial cells, smooth muscle-like cells that contract to regulate blood flow of the glomeruli (the filtration network of blood vessels within kidney nephrons) (Bohnenpoll et al., 2013; Humphreys et al., 2010; Kobayashi et al., 2014; Mugford et al., 2008). The importance of the renal stroma to kidney development is clear when investigating mutant phenotypes; mutations in many key stromal genes, including *Foxd1* and *Tbx18*, lead to a range of dysmorphologies (Table 1).

Cortical stroma

Cortical stroma development

At the start of metanephric development, *Foxd1*-expressing cortical stromal progenitor cells are located in the nephrogenic zone surrounding the nephron progenitors (Airik et al., 2006; Bohnenpoll et al., 2013; Hatini et al., 1996) (Fig. 1C,D). As the developing kidney expands, the cortical stroma around the nephrogenic niches persists in the periphery of the expanding organ, whereas additional stromal populations are present in the centre of the forming organ, some of which are derived from the Foxd1^{+} stroma and some are not (Kobayashi et al., 2014; Bohnenpoll et al., 2013) (Fig. 1E). For example, *Foxd1* lineage-tracing studies in mouse have identified that Foxd1^{+} cortical stroma gives rise to the pericytes and mesangial cells throughout the final organ (Kobayashi et al., 2014) (Fig. 1E), as well as contributing to postnatal fibrotic lesions (Humphreys et al., 2010). However, there is no evidence that cortical stroma contributes to the epithelial nephron or ureteric structures, although expression of *Foxd1* is later initiated in the podocytes, specialised epithelial cells within the glomeruli (Brunskill et al., 2011). Conversely, nephron progenitors do not give rise to non-epithelial derivatives and the expression of *Pax2* within the nephron progenitors is required to suppress adoption of a stromal identity (Naiman et al., 2017), but this does not clarify whether both populations arise from the same parent population.

Roles of cortical stroma

Loss of *Foxd1* in the kidneys (Fetting et al., 2014; Levinson et al., 2005), or ablation of the Foxd1^{+} population altogether (Das et al., 2013; Hum et al., 2014), results in delayed and disorganised nephrogenesis with an expansion of uncommitted nephron progenitors and aberrant ureteric branching. Impaired TGFβ signalling or Hedgehog signalling upstream of Foxd1 leads to similar phenotypes. For example, *Smo* and *Tgfb2* knockouts (KO), normally expressed by the cortical stroma, also have an expanded nephron progenitor population but fewer nephrons (Rowan et al., 2018). Perturbations to genes expressed in the cortical stroma, such as the Hox10 paralogues *Ecm1*, *Pbx1*, *Pdgfrb* and *Meis1* (Delgado et al., 2021; Hisa et al., 2004; Hurtado et al., 2015; Paroly et al., 2013; Yallowitz et al., 2011), lead to aberrant nephrogenesis and ureteric branching.

Nephron progenitors may be influenced to proliferate through cell-cell interactions with the cortical stroma driven by Fat4 regulated Yap/Taz signalling (Das et al., 2013). Foxd1 itself may regulate nephrogenesis by repressing the medullary stromal gene *Dcn*, which encodes decorin, a BMP-SMAD antagonist. Indeed, BMP is signalling required for nephron progenitor differentiation (Fetting et al., 2014). In addition, loss of stromal *Sall1* expression leads to *Fat4* repression and cortical expression of *Dcn*, phenocopying the *Foxd1*-null kidneys and indicating that that Sall1 is involved in stromal-nephron progenitor interactions, potentially by binding Foxd1 (Ohmori et al., 2015). These perturbations lead to expanded nephron progenitor regions and impaired kidney development.

Ureteric branching is regulated through retinoic acid (RA) signalling and the cortical stroma synthesises RA through Aldh1a2 (also known as Raldh2) (Niederreither et al., 2002a;

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Table 1. Effect of stroma-expressed gene disruptions in mice models

Gene	Phenotype	Stromal expression	Reference
Foxd1	Hypoplastic kidneys, often fused at midline. Delay to nephrogenesis due to dysregulated and expanded nephron progenitors. Loss of cortex-medullary patterning.	CS	Hatini et al., 1996; Levinson et al., 2005
Pdgfrb	Inhibited glomerular maturation. Lack of capillary tuft including mesangials and podocytes.	CS, MS	Alpers et al., 1992; Soriano, 1994
Rara/*Rarb*	Expanded cortical stroma causing dysregulated branching.	CS, MS	Mendelsohn et al., 1999; Batourina et al., 2001
Atp6ap2	Delayed nephrogenesis. Abnormal vascular development.	CS, MS	Yosypiv et al., 2019
Tcf21	Hypoplastic kidneys. Disrupted ureteric branching. Blood vessel abnormalities.	CS, MS, US	Cui et al., 2003; Quaggin et al., 1999
Aldh1a2	Reduction in kidney size by E14.5 with normal kidney architecture. When performing double KO with *Raldh3* (http://www.informatics.jax.org/marker/MGI:1861722*Aldh1a3*) expressed in the ureteric bud, phenotype replicated that of *Rara*/*Rarb* double KO.	CS	Batourina et al., 2005; Niederreither et al., 2002b; Rosselot et al., 2010
Bmp4	Hypoplasia. Dysplasia. Hydroureter. Reduced ureteric branching.	MS, US	Miyazaki et al., 2000
Fgf7	Decreased nephron number. Smaller collecting duct system.	MS, US	Qiao et al., 1999
Agt	Abnormal medulla development. Increase in pericytes around cortical peritubular capillaries.	MS, US, Mes	Nagata et al., 1996; Woolf et al., 2009; Song et al., 2010a
Ace	Abnormal medulla development.	MS	Esther et al., 1996
Sfrp1	Decrease in size of renal papilla.	CS, MS, US	Leimeister et al., 1998; Yoshino et al., 2001; Trevant et al., 2008
Agtr1a	Double KO with *Agt* leads to impaired ureteric branching.	CS	Iosipiv and Schroeder, 2003
Agtr2	Impaired uretic branching.	MS, Mes	Pope et al., 2001; Song et al., 2010b
Pbx1	Hypoplastic kidneys. Unilateral agenesis. Ventrally rotated, mis-positioned caudally. Decreased ureteric branching and ectopic nephrogenesis.	CS, MS	Schnabel et al., 2003
Meis1	Defects in angiogenesis.	CS, MS	Hisa et al., 2004
Sall1	Expansion of nephron progenitors. Kidneys were smaller and heavily cystic.	CS, MS	Nishinakamura et al., 2001; Ohmori et al., 2015
Hox10 paralogues	Reduced and aberrant ureteric branching and decreased nephrogenesis.	CS	Yallowitz et al., 2011
Dicer1	Defects in nephron progenitors, vasculature and glomeruli development.	CS	Nakagawa et al., 2015; Phua et al., 2015
Fat4	Expansion of nephron progenitors and decreased nephrogenesis.	CS, MS	Saburi et al., 2008; Das et al., 2013
Smo	Abnormal capsular morphology. Depletion of cortical stroma and expanded nephron progenitor domain. Decreased nephrogenesis. Hypoplasia.	CS, MS	Fabian et al., 2012; Rowan et al., 2018
Gli3	Phenocopies *Smo* deficiency; double KO with *Smo* rescues phenotype.	MS	Rowan et al., 2018
Tgfb2	Stromal KO causes nephron progenitor expansion but decreased nephrogenesis. KO in both stroma and nephron progenitors leads to hypoplasia.	All mesenchyme	Rowan et al., 2018
Ptch1	Decreased periureteric stroma proliferation and an expanded Foxd1-expressing cortical stromal domain.	MS, US	Cain et al., 2009; Blake et al., 2016

CS, cortical stroma; KO, knockout; Mes, mesangial cells; MS, medullary stroma; US, ureteric stroma.

Rosselot et al., 2010), which binds to the RA receptors *Rara* and *Rarb* expressed throughout the stroma (Batourina et al., 2001; Mendelsohn et al., 1999).

The renin-angiotensin system (RAS) is crucial for kidney development, with many of the genes involved in this system (e.g. *Agt*, *Atp6ap2*, *Ace* and *Agtr1a*) expressed in the stroma. Perturbation to expression of these genes leads to a range of phenotypes from delayed nephrogenesis, impaired ureteric branching and abnormal vascular development (Song et al., 2017a; Yosypiv et al., 2019). *Foxd1* itself plays a role in regulating this system, with loss of *Foxd1* leading to decreased expression of RAS-related genes (Song et al., 2017a). Finally, a depletion of stroma triggers inappropriate vascular patterning, in part owing to a deficit of renin-expressing pericyte populations arising from Foxd1$^+$ stroma (Song et al., 2017a,b; Yosypiv et al., 2019).

Together, these phenotypes demonstrate a crucial role for the stroma in regulating kidney development.

Ureteric stroma

The Foxd1$^+$ stroma does not represent all stromal components of the kidney either during development or in the postnatal organ. In mouse, the renal stroma is derived from at least two distinct populations of cells identified by the expression of either *Tbx18* or *Foxd1*. *Tbx18*-expressing ureteric stroma is located around the invading ureteric epithelium (Fig. 1C,D) and hence contributes to the stroma of the medullary region (Airik et al., 2006) (Fig. 1E). Together, these two populations appear to give rise to the entirety of the renal stroma (Bohnenpoll et al., 2013; Kobayashi et al., 2014) with the Tbx18$^+$ population predominantly generating the smooth muscle surrounding the urothelium and other stromal populations within the medullary region during development (Bohnenpoll et al., 2013) (Fig. 1E).

In *Tbx18*-null mice, ureteric stroma fails to condense appropriately and does not respond to signals from the ureteric epithelium. As a result, *Tbx18*-null mice lack smooth muscle surrounding the urothelium, leading to short hydroureter and hydronephrosis at birth (Airik et al., 2006; Bohnenpoll et al., 2013). Once the invading ureteric tip has branched to form the typical T-shape surrounded by the MM, there is a clear demarcation of these stromal lineages, with Foxd1$^+$ cells present surrounding the nephron progenitors, and the *Tbx18*-expressing cells surrounding the invading bud (Fig. 1C,D).

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Medullary stroma

The medullary stroma of the developing kidney (Fig. 1E) has been shown to play a role in maturation of the collecting ducts and elongation of the developing nephron segments, with a clear role for the Hedgehog and TGFβ signalling pathways in regulating these processes (D'Cruz et al., 2020; Dumbrava et al., 2021). Hedgehog signalling in the cortical stroma initiates TGFβ2 signalling to support nephron formation (Rowan et al., 2018). Conversely, the removal of the Hedgehog effector Smo results in expanded nephron progenitors, reduced nephron commitment and loss of the capsule. This result is mediated by the repressor protein Gli3 and can be phenocopied by driving Gli3 deletion from the *Foxd1* promoter (Rowan et al., 2018). The TGFβ signalling factors Smad2, Smad3 and Smad4 are expressed throughout the cortical and medullary stroma to mediate TGFβ signalling pathways (Banas et al., 2007; Rowan et al., 2018; Vrljicak et al., 2004). *Ptch1* is strongly expressed in the nephron progenitors, and in cortical and medullary stroma, including the periureteric mesenchyme (Blake et al., 2016; Cain et al., 2009). Inhibition or deficiency of *Ptch1* expression leads to decreased periureteric stromal proliferation and differentiation (Cain et al., 2009; Yu et al., 2002). As described above, there are many ligand-receptor pairs expressed between the cortical stroma and nephron progenitors, suggesting that important stroma-epithelial interactions drive kidney development (Das et al., 2013). Indeed, β-catenin has been shown to have a cell-autonomous role in medullary stromal development, which in turn has a non-autonomous effect on adjacent epithelial development (England et al., 2020).

Although KO studies in mice have shown the importance of the stromal populations in regulating kidney development, there remain many gaps in our understanding of the autonomous and non-autonomous functions of the stroma in morphogenesis. Recent transcriptional investigation of genes expressed in adjacent domains of cortical stroma and nephron progenitors have identified both known and putative ligand-receptor pairs that may represent under-investigated signalling pathways from or to the stroma (Combes et al., 2019b). Putative interactions between nephron progenitors and cortical stroma have been proposed involving *Sfrp1*, which has a minor KO phenotype (Leimeister et al., 1998; Trevant et al., 2008; Yoshino et al., 2001), and *Ntn1*, which has conserved expression within the stromal progenitor population (Combes et al., 2019b; England et al., 2020). Combining transcriptional profiling with phenotypic analysis of mice with stromal perturbations will continue to provide insight into the integral role that the stroma plays during kidney development.

Developmental origin of the renal stroma

Origin of cortical stroma

Although the origin of the nephrogenic mesenchyme of the kidney is well characterised, the origin of the component stromal elements is not clear. In mouse, there are several divergent lines of thought regarding the origin of the Foxd1$^+$ stromal progenitors. One theory suggests that the Osr1$^+$ IM precursor population gives rise to both the Foxd1$^+$ stromal progenitors and Six2$^+$ nephron progenitors (Mugford et al., 2008). *Osr1* is an early marker of IM in mouse and lineage tracing using an Osr1-Cre induced between E7.5 to E10.5 labels ureteric epithelium, Six2$^+$ nephron progenitors and Foxd1$^+$ stromal populations (Kobayashi et al., 2008, 2014). However, *Osr1* is not confined to the nascent IM but is expressed more broadly in early nascent mesoderm and later also in LPM (James et al., 2006; Takasato and Little, 2015; Takasato et al., 2015). Taguchi and colleagues have investigated the lineage relationship of between brachyury (T) and Osr1 expression and regional nephrogenic mesenchyme in mouse, concluding that the Osr1-derived ND is distinct from the later more posterior MM that gives rise to the metanephros (Taguchi et al., 2014). Using this distinction between the temporal origins of ND and MM, they have directed the differentiation of hPSCs to create a nephron progenitor population (Taguchi et al., 2014). However, they conclude that this does not include an appropriate stromal population, suggesting a distinct origin for nephron progenitors and cortical stroma.

An alternative view has proposed that the Foxd1$^+$ population arises from a paraxial mesoderm population and migrates into the cortical region of the nascent IM-derived kidney (Guillaume et al., 2009; Levinson et al., 2005). This is strongly supported in non-mammalian models. Guillaume and colleagues have performed *in ovo* experiments in chicken, in which they inject a labelled dye into specific regions of the mesoderm (paraxial or intermediate, and adjacent to different somites) (Guillaume et al., 2009). They found that when dye is injected into the IM, some components of the ND system are labelled; however, there are regions in the kidney lacking labelling, including the interstitium and mesangial cells. When the PM is injected, the somites and the majority of the renal interstitium are labelled, including the mesangial population and the renal capsule, suggesting that the *Foxd1*-expressing interstitial population originates from the PM. In contrast, the Pax2-expressing nephrogenic mesenchyme is not labelled (Guillaume et al., 2009). There is also indirect evidence of *Foxd1*$^+$ cell migration in murine models. Labelled cells in the *Foxd1-lacZ* reporter mouse are not just seen populating the cortical region of the metanephric kidney, but also in PM-derived structures nearest the notochord and the body wall, which appear to be streaming into the cortical region of the kidney at the E13.5 (Levinson et al., 2005).

A third option is a common IM origin, followed by lineage separation prior to the commencement of metanephric development. There is immunohistological evidence from mice that Foxd1$^+$ cells are not present around the forming MM in the earliest stage of ureteric bud invasion at E10.5, but appear to surround the rostral MM at E11.5 and completely surround the cortical MM by E13.5 (Bohnenpoll et al., 2013; Naiman et al., 2017; Yallowitz et al., 2011) (Fig. 1C). A study into the expression of Hox10 paralogue genes has suggested that Foxd1$^+$ cells originate in the nephrogenic mesenchyme immediately rostral to the nascent kidney, where they become concentrated by E12 before migrating posteriorly to the kidney cortex (Yallowitz et al., 2011). *Foxd1 in situ* hybridisation between E10.5 and E11.5 also suggests a field of expression starting rostral to the MM and not yet completely encapsulating the MM (Bohnenpoll et al., 2013) (Fig. 1C). As there is no evidence of a Six2-derived stromal population (Kobayashi et al., 2008), the Foxd1$^+$ stroma cannot derive from a subset of Six2$^+$ MM. As such, this evidence supports the hypothesis that the stromal progenitors originate separately to the nephron precursors before undergoing migration onto the kidney cortex, but does not indicate whether they have a common or distinct mesodermal origin. This controversy is complicated by the observation in human of *FOXD1* expression within the nephron progenitors.

Origin of ureteric stroma

The origin of the *TBX18*$^+$ ureteric stroma is similarly uncertain. *Tbx18* lineage derivatives are present in multiple intermediate mesoderm tissues, including kidney, gonad and adrenal glands (Bohnenpoll et al., 2013). At E9.5, *Tbx18* is expressed in the coelomic epithelium adjacent to the Wolffian duct and co-expressed with *Aldh1a2* and *Osr1* (Bohnenpoll et al., 2013). By E10.5, *Tbx18* expression lies between the nephrogenic mesenchyme and nephric duct. By E11.5,

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the invading ureteric bud of the nephric duct has passed through the *Tbx18* mesenchyme field, at which point the *Tbx18* population condenses around the invading bud (Bohnenpoll et al., 2013) (Fig. 1C). Although the two stromal populations are distinct at E11.5, there is evidence from lineage tracing that some Foxd1$^+$ cells originate from a Tbx18$^+$ precursor (Bohnenpoll et al., 2013). *Tbx18* is expressed in mesoderm prior to *Foxd1*, with some evidence to suggest that at least a subpopulation of *Foxd1* cells arise from a *Tbx18*-expressing precursor, although there is no evidence of co-expression (Bohnenpoll et al., 2013). This may indicate that either a proportion of the Foxd1$^+$ stroma originates from an earlier Tbx18$^+$ population, or that the Tbx18$^+$ mesenchyme can take on a range of stromal fates depending on their environmental signals and are not restricted to forming the ureteric mesenchyme. *Tbx18* is also expressed early in the urogenital region, present in the mouse urogenital precursor region at E9.5 adjacent to the Pax2$^+$ field that forms the kidney. *In situ* hybridisation has identified *Tbx18* expression in a mesenchymal field flanking the MM at E10.5 prior to *Foxd1* expression, which is clearly present by E11.5 surrounding the MM and adjacent to the Tbx18$^+$ field, itself concentrated around the invading ureteric epithelium (Airik et al., 2006; Bohnenpoll et al., 2013) (Fig. 1C).

In summary, the existing evidence points to several possibilities for stromal origins (Fig. 2). The IM or PM may give rise to the cortical and ureteric populations. This may occur as a lineage divergence directly from the IM or PM, or there may be an intermediate precursor population that expresses one or both of *Foxd1* and *Tbx18*. In this scenario, *Tbx18* would need to be expressed first, and any cells that turn on *Foxd1* would be committed to the cortical lineage and downregulate *Tbx18*. This boundary would likely be regional, with a more-anterior population migrating to the cortical nascent kidney while the posterior *Tbx18* population that the ureteric bud migrates through would condense around this epithelium. These lineages would then interact with their respective neighbouring populations and contribute to the mechanisms of normal kidney development. Specific approaches to resolve these options would require careful lineage-tracing analyses, potentially most readily achieved in mouse, or the time-lapse imaging of *ex vivo* urogenital tracts dissected from *Foxd1* and/or *Tbx18* reporter strains. As we discuss below, the pertinence of such studies to human could now be addressed *in vitro* using reporter and lineage-tracing hPSC tools.

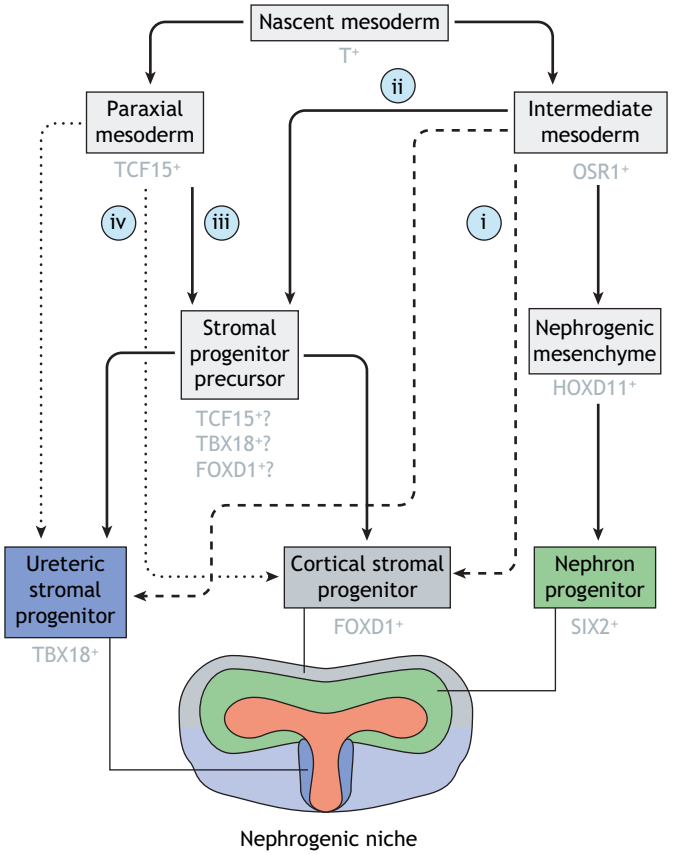

Fig. 2. Potential origins of stromal lineages. The diagram maps out the potential lineages of the mesenchymal populations with a focus on the stroma. The intermediate mesoderm or the paraxial mesoderm may give rise to the cortical and ureteric stromal populations either directly (i or iv) or through a stromal progenitor precursor (ii or iii). A stromal progenitor precursor would likely express either TCF15 or OSR1 depending on the mesoderm of origin. It also may express both FOXD1 and TBX18 prior to lineage specification. The nephron progenitors are known to arise from the nephrogenic mesenchyme of intermediate mesoderm origin.

Transcriptional profiling to understand stromal heterogeneity *in vivo*

Although it is clear that Foxd1$^+$ and Tbx18$^+$ stromal compartments contribute to distinct stromal populations, it has been a particular challenge to pull apart the heterogeneity of the resulting stromal populations present in these tissues (Combes et al., 2019b; England et al., 2020; Lindström et al., 2018c; Little and Combes, 2019). This likely reflects the overall transcriptional similarity between these mesenchymal populations, some of which we know arise from the same progenitors. Detailed single-cell transcriptional profiling in both mouse and human has now described a greater variety of stromal cellular identities, although there remains considerable transcriptional similarity between subclusters of stromal cells with histologically defined stromal subpopulations (Combes et al., 2019b; England et al., 2020; Howden et al., 2021; Lindström et al., 2018c). Stromal regionalisation can be identified most clearly along the cortical-medullary axis of the kidney, which becomes histologically distinct by E15.5 in mice.

As the kidney expands radially, the central portion of the existing stroma expresses distinct transcriptional signatures that define the medullary stroma (Kobayashi et al., 2014). The allocation of stromal subpopulations from cortex to medulla reflects the specific positioning of key epithelial elements within the kidney. The ureteric epithelium is predominantly within the inner medulla, although this epithelium does extend into the cortex connecting to the tips in the nephrogenic zone. Similarly, the nephrons span the radial axis of the organ although the proximal and distal tubular epithelium is concentrated in the cortical region, whereas the loops of Henle descend into the medulla. Conservative histological and transcriptomic analysis has classified distinct stromal subpopulations in the cortex, the medulla, the area surrounding the ureteric epithelium and the area surrounding the ureter (Adam et al., 2017; Combes et al., 2019b; Magella et al., 2018). A more recent, targeted single-cell analysis of the *Foxd1*-derived stroma has identified 17 subpopulations, 12 of which can be identified histologically by markers including *Lox*, *Clca3a1*, *Smoc2*, *Penk* and *Wnt4* (England et al., 2020). The additional clusters can be identified as subpopulations within the cortex or medulla epithelium with histological evidence for these distinct domains. The cortical stroma expressing *Foxd1* also expresses *Ntn1*, *Crabp2*, *Igfbp5*, *Fibin* and *Smoc2*. There are two distinct regions within the Foxd1$^+$ stroma: one region cortical and another lateral to the nephron progenitors. The most cortical Foxd1$^+$ cells can be

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transcriptionally clustered into two populations; one cluster expresses *Nnt1*, *Smoc2* and *Dlk1*, whereas the other cluster expresses *Fibin*. The lateral Foxd1$^+$ population expresses both *Fibin* and tenascin C (*Tnc*) (Fig. 1D). These domains suggest a *Foxd1*-derived population transitioning towards the medulla as the kidney expands, with *Foxd1* expression absent by the inner cortical stroma.

The integration of datasets across species has shown that there is evidence for conservation and similar heterogeneity within the stromal populations in mouse and human (England et al., 2020). However, there are also noticeable differences, such as the expanded expression of FOXD1 and MEIS1 into the nephron progenitor population in human, whereas in mouse there is a strict population boundary (Lindström et al., 2018c). In fact, only 27% of genes identified as markers of mouse stroma are enriched in human stroma (Lindström et al., 2018c). Even though the stromal populations are a major contribution to the captured cells with single-cell foetal-kidney datasets, there has been considerably less investigation into the stromal lineages compared with nephron. Typically, the stroma is clustered conservatively into three or four populations within human foetal kidney data, including progenitors, and cortical, medullary and mesangial populations, which have the most distinct overall transcriptional signatures. However, in some datasets, these populations are given generic stroma or interstitial identities (Howden et al., 2021; Lindström et al., 2018c; Menon et al., 2018; Tran et al., 2019). These data are useful to use as a reference for identification and classification of the stromal cells generated in kidney organoids (Howden et al., 2021; Wilson et al., 2021 preprint).

Stromal populations within kidney organoids

The recent generation of multiple protocols to derive kidney-like tissues (organoids) from hPSCs has opened up possibilities of modelling kidney development and disease in a human context (Freedman et al., 2015; Morizane et al., 2015; Takasato et al., 2015). Organoids have the potential to contribute to and greatly advance personalised medicine by providing a patient-specific tissue for assays, such as drug screens. To fulfil this potential, organoids need to be fully characterised in their cell populations and the tissue itself needs to be as functionally mature as its purpose requires.

Generation of kidney organoids

Organoid protocols are designed to direct differentiation from pluripotent cells through the posterior primitive streak into IM in order to mimic the developmental progression seen in mammalian kidneys. A number of protocols have been described for the directed differentiation of hPSCs to kidney, but all nephron-forming kidney organoid protocols follow a very similar trajectory (reviewed by Little and Combes, 2019). For most protocols described to date, there are both similarities and differences between organoids and foetal kidney (Combes et al., 2019a; Subramanian et al., 2019; Tran et al., 2019; Wu et al., 2018). Nephrons arise and are segmented into proximal and distal regions, a process that includes the formation of glomeruli. Organoid nephrons are derived from *SIX2*-expressing progenitors (Howden et al., 2019), as they are *in vivo* (Kobayashi et al., 2008; Lindström et al., 2018c). Podocytes within the organoid glomeruli have been shown to produce VEGF (van den Berg et al., 2018), and, although vascular networks have been identified, these do not efficiently invade the forming glomerulus, as would occur *in vivo* (Takasato et al., 2015). Although some organoid formats give rise to a complex network of interconnected nephrons (Lawlor et al., 2021; Takasato et al., 2015), transcriptional analyses have subsequently identified these connections represent a GATA3$^+$ distal connecting tubule rather than ureteric epithelium. The absence of bona fide ureteric epithelium, and hence the absence of ureteric tips, prevents the formation of nephrogenic niches in which the nephron progenitors are supported to self-renew. What is also unclear is the appropriateness of the stromal cell types within organoids.

Mesenchymal populations in kidney organoids

Transcriptional analysis of the mesenchymal populations present within organoids from multiple protocols has identified both kidney-like and non-kidney-like cell populations (Table 2). The most extensive analyses of these populations have been performed on organoids derived using protocols described by Takasato et al. (2015) and Morizane et al. (2015). When integrating foetal human kidney and organoid datasets, stromal populations do co-cluster with cells contributing from both foetal and organoid tissue; however, gene expression differences can be identified even within these clusters (Combes et al., 2019a). It has been difficult to relate clustering analysis within organoid single-cell data to the histologically defined regions within mouse or human kidneys. Instead, stromal populations have been classified into the broader – but distinct – populations of cortical, medullary and mesangial stroma (Combes et al., 2019a). Cells that do not fit into these categories are labelled ‘off-target’ populations and are often identified to have transcriptional signatures similar to muscle or neural cell types, suggesting an unwanted path of differentiation in these protocols. As organoids represent an *in vitro* model mimicking development, it is possible that such ‘off target’ populations simply represent adjacent mesodermal populations that arise in embryogenesis for which accurate datasets are not yet available to provide a clear identity. Despite this challenge, we should be able to find the anticipated stromal populations assumed to be required for kidney development. In fact, there is little evidence of *FOXD1* expression in the profiling data from any kidney organoid protocol (Combes et al., 2019a; Harder et al., 2019; Phipson et al., 2019; Subramanian et al., 2019; Wilson et al., 2021 preprint, Wu et al., 2018), despite reports of immunopositivity for FOXD1 and MEIS1 (Takasato et al., 2015). As discussed earlier, *Foxd1* KO mice have perturbed nephrogenic niches and nephrogenesis. As such, a lack of these cells in organoids is likely contributing to the lack of a nephrogenic niche and the loss of ongoing nephron induction (Howden et al., 2019).

Increasing organoid complexity

The concept that the nephric duct/ureteric epithelium and the MM have distinct origins has inspired the derivation of ureteric epithelium *in vitro* (Mae et al., 2020; Taguchi and Nishinakamura, 2017). Recent studies have now reported on recombining ureteric epithelium with nephron organoid protocols (Taguchi and Nishinakamura, 2017; Uchimura et al., 2020). For example, the generation of ‘higher order’ organoids consisting of a connected nephron and ureteric epithelial network has been demonstrated using mouse pluripotent stem cells (Taguchi and Nishinakamura, 2017). Generated via co-culture of separately derived nephron progenitors and ureteric epithelium, these co-cultures also include endogenous stromal progenitors isolated from E11.5 mouse embryos and generate remarkably complex organoids reminiscent of mouse embryonic kidney (Taguchi and Nishinakamura, 2017). However, the same approach has not been successful using hPSC-derived recombinations. The authors have proposed that adding the correct (i.e. cortical) stroma is important for optimal higher order kidney formation, implying that the stromal populations present in human differentiations are not sufficient for

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Table 2. Stromal and off-target populations identified from organoid single-cell RNA-sequencing

Reference	Sample age (days)	Organoid derivation protocol	Stromal/mesenchymal classification	Off targets
Wu et al., 2018	26	Morizane et al., 2015; Takasato et al., 2015	Protocols analysed separately but with very similar outcomes. Three mesenchyme clusters expressing *COL1A1* and *COL3A1* identified, but not characterised further.	Three neuronal clusters expressing *SOX2* and *POU3F2*, one cluster *CRABP1* enriched. Takasato organoids had one melanocyte population and one neuronal progenitor population. Morizane organoids had one muscle population identified.
	34	Takasato et al., 2015	N.A.	Substantial increase in off-target cell types compared with day 26.
	7, 12, 19, 26	Takasato et al., 2015	Time course between days 7 and 26. Mesenchyme progenitor and mesenchyme populations identified with *COL3A1* expression.	Two neuronal populations identified. Pseudotime trajectory revealed that the stroma and neuronal cells have a common precursor.
	26	Takasato et al., 2015 (with BDNF inhibition)	N.A.	Identified from time course, *NTRK2* expression could lead to off-target neuronal cell type. Inhibition of BDNF-NTRK2 signalling led to decreased neuronal off-target population.
Czerniecki et al., 2018	21	Freedman et al., 2015 (with/ without VEGF addition)	Two samples collected with and without addition of VEGF to the media from day 4.5. One stromal cluster expressing *COL3A1*, *COL1A1*, *POSTN* and *ACTA2* resembled a population of myofibroblasts, pericytes and mesangial cells. Subclustering of stromal population led to five clusters. One subcluster expressed the VEGF receptor *FLT1* and the endothelial progenitor marker *MCAM*, and was composed only of VEGF-treated cells.	One very small group of cells identified as endothelial. Small clusters with neural, muscle and undifferentiated off-targets detected.
Howden et al., 2019	18, 25	Takasato et al., 2015	Across the two sample ages, seven stromal populations identified expressing *COL1A1* and *COL3A1*. Three were enriched in the day 25 sample that arose with age, expressing the kidney stromal markers *TCF21*, *DCN* and *CXCL12*. *MEIS1*-expressing stromal cells were lineage traced and found to arise from a *SIX2* precursor.	Two muscle and one neural off-target populations identified.
Combes et al., 2019a	25	Takasato et al., 2015	Organoids from four batches were analysed and directly compared with a cortex-enriched hFK data to analyse congruence. Organoid cells mapped to multiple hFK stromal clusters although differences were observed between organoid and hFK within clusters.	N.A.
Harder et al., 2019	19, 20, 21	Freedman et al., 2015	One mesenchymal cluster identified as kidney stroma. Kidney stroma expressed *COL3A1*, *DLK1*, *CXCL12*, *COL1A1* and *DCN*, among other markers.	Four non-kidney clusters included nonspecific stroma, neural and proliferating cells.
Subramanian, 2019	29	Morizane et al., 2015; Takasato et al., 2015	Compared both protocols with multiple lines, found with mesenchymal clusters. These cells made up more than 70% of captured cells in all samples. Three mesenchyme populations were similar to those of hFK.	One endothelial-like cluster, two muscle-like, two neuron-like and one melanoma-like.
	7, 15, 29, 32	Takasato et al., 2015	Six non-nephron progenitor cell mesenchymal populations and an endothelial progenitor cluster identified.	Distinct off-target cells not identified at day 15 but a *SOX2* population was identified, containing putative neuronal progenitors and enriched in lines leading to more off-target cell types.
Kumar et al., 2019	25	Kumar et al., 2019	Micro-organoid culture led to 50% stromal generation compared with Takasato organoid control. Noticeable *PAX3*-expressing mesenchymal population.	Same contribution of off-target.
Low et al., 2019	10, 12, 14	Low et al., 2019	Organoids analysed at the early nephrogenesis stage. Two stromal populations identified expressing *COL1A1*, *COL3A1*, *MEIS1* and *PDGFRA*.	One endothelial cluster was sub-clustered into three populations; lineage trajectory analysis indicated that these cells arise from a nephron progenitor cell population in this *in vitro* setting.

Continued

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Table 2. Continued

Reference	Sample age (days)	Organoid derivation protocol	Stromal/mesenchymal classification	Off targets
Tran et al., 2019	16, 28	Morizane et al., 2015	Compared with an hFK reference with four interstitial/stromal clusters, one mesangial cluster, and one endothelial cluster. Nine clusters identified as interstitial with various enriched gene profiles, including kidney stromal markers *PBX1*, *ALDH1A2*, *SNAI2*, *DCN*.	Two muscle clusters, one neuronal cluster and one unknown cluster identified.
Lawlor et al., 2021	25	Takasato et al., 2015	Organoids generated with different biophysical properties. Traditional micromass organoids often have a core of undifferentiated mesenchymal cells whereas organoids bioprinted with decreased cellular density do not. Despite this, stromal populations are similar between all organoid types.	N.A.
Uchimura et al., 2020	25	Takasato et al., 2015; Uchimura et al., 2020	Comparison of a Takasato organoid with a novel Uchimura protocol organoid generated from mixed anterior and posterior target populations. Takasato organoids were identified to have 57.2% off-target and 16.2% mesenchyme cells whereas Uchimura organoids had only 9.4% off-target and 44.8% mesenchyme. Mesenchyme expressed *COL3A1* and *COL1A1*. Stromal markers *MEIS1* and *PDGFRB* were shown to be increased by qPCR.	Off-target populations included melanocyte, neuron and muscle.

hFK, human foetal kidney.

appropriate patterning. Targeting a more accurate stromal population from hPSCs *in vitro* could be beneficial for enhanced kidney organoid morphogenesis.

The dilemma of 'off target' versus 'on target' stroma in organoid patterning and maturation

The studies described above do not address the identity of the stroma generated in current protocols. Although it is possible that some of these stromal cell types are 'off target' mesodermal or even non-mesodermal mesenchymal populations, it is also possible that they do not accurately represent any *in vivo* stromal population in the developing urogenital tract. A comparison of congruence between Takasato protocol-derived human kidney organoids and a reference dataset of dissociated foetal kidney cortex cells concluded that the organoids contain stromal populations similar to the cortical stroma present within the reference data (Combes et al., 2019b). Of the five stromal clusters identified within the integrated organoid/foetal dataset, both organoid and kidney contribute to all clusters. However, three clusters with medullary and mesangial identities are dominated by organoid cells, likely due to the foetal dataset being enriched for cortical populations. Within individual stromal clusters, there is differential gene expression between the organoid and foetal kidney cells, highlighting differences even between the most similar cell types (Combes et al., 2019b). However, the original human foetal kidney reference fails to identify a *FOXD1*-enriched population (Lindström et al., 2018c), indicating that these cells are missing from the reference. Regardless, this analysis shows congruence between organoid and foetal kidney stroma.

Using a recently developed cell identity prediction tool trained on a more extensive dataset drawn from multiple human foetal kidneys, a significant percentage of the stromal cells present across many published organoid datasets have not been assigned to any renal identity (Wilson et al., 2021 preprint). These 'unassigned' cells are enriched for genes that indicate an off-target population, such as *MAP2*, *NTRK2* and *MYOG*, and have been interpreted to represent muscle or neural phenotypes (Harder et al., 2019; Howden et al., 2019; Lawlor et al., 2021; Uchimura et al., 2020; Wu et al., 2018). In addition, some stromal cells show a strong *PAX3* (Kumar et al., 2019) expression signature, suggestive of paraxial mesoderm. $FOXD1^{+}$ stroma is absent, and so is $TBX18^{+}$ stroma. Hence, it is possible that a third distinct renal stroma differentiation protocol will be required, with the recombination of nephron, ureteric and stromal populations resulting in a more accurately patterned organ, as predicted by Taguchi and colleagues (Taguchi and Nishinakamura, 2017).

Stromal populations in transplanted organoids

Although improving the stromal composition is crucial for the maturation or successful application of organoids for renal replacement, the transplantation of kidney organoids under the skin or renal capsule of immunocompromised mice has resulted in rapid maturation, including glomerular vascularisation, formation of a right glomerular basement membrane, the development of more mature epithelial ultrastructure and evidence of transcriptional maturation (Bantounas et al., 2018; Kumar Gupta et al., 2020; Low et al., 2019; Nam et al., 2019; Ryan et al., 2021; Sharmin et al., 2016; Subramanian et al., 2019; van den Berg et al., 2018). Although these results are very promising, most studies also report the presence of unwanted stromal expansion, with the formation of off-target tissue types, including cartilage. It is unclear whether this represents misdifferentiation of existing 'off target' stromal populations present in kidney organoids or results from an environmental perturbation of an otherwise appropriate stromal population.

In Wilms' tumour (a paediatric, renal embryonal tumour), the spontaneous formation of cartilage and muscle is regarded as one example of inappropriate development of mesenchymal elements (Bakshi et al., 2003; Cecchetto et al., 2003; Miyagawa et al., 1998). Indeed, mutation of the MM gene *WT1* has been associated with myogenesis in Wilms' tumour (Miyagawa et al., 1998). This abnormal response may be elicited by the local environment, perhaps induced by hypoxia or the innate immune response. Interestingly, inappropriate stromal expansion has also been shown when transplanting embryonic kidney tissue, meaning this morphology may be induced or exacerbated by the transplantation

itself (Kumar Gupta et al., 2020). Conversely, there is also evidence that post-transplantation organoids show an improved transcriptional stromal identity, including a reduction in off-target markers, such as *SOX2* and *PMEL*, and even the formation of an 'on target' population expressing *STMN2*, *CHGA* and *CHGB*, which are otherwise absent in organoids (Subramanian et al., 2019).

Determining which stromal populations in organoids are off target will be crucial, not only in improving organoids as a model of development, but also for disease modelling and therapy. The selective removal of a proposed off-target cell type, an NTRK2-expressing stroma identified in some examples of kidney organoids, has been achieved through the use of NTRK2 inhibitor K252a to inhibit the survival of this population (Wu et al., 2018). This approach represents a 'proof of concept' for removing unwanted component cell types, but does not address the question of which cells need to be removed.

The influence of renal stroma on epithelial patterning

Another key feature absent in kidney organoids is an appropriate corticomedullary anatomy. Nephron formation occurs, but in some protocols these arise as random self-organising events with little evidence of connectivity between nephrons (Freedman et al., 2015; Morizane et al., 2015). This lack of nephron orientation is accompanied by a lack of tubular maturation. Such defects are likely to reflect the lack of appropriate renal stromal cell types, together with a lack of distinct stromal zonation from medulla to cortex. *In vivo*, stromal zonation is arranged around the axis of an invading ureteric bud. Ureteric tree and nephron orientation *in vivo* also involves reciprocal signals between the medullary stroma and the extending and branching ureteric tree, as well as the stroma and the descending loops of Henle (Yu et al., 2002, 2009).

It is clear that the stroma influences epithelial identity, as has been described in a number of other organ systems. Indeed, in seminal studies investigating this stromal-epithelial crosstalk, Taylor and colleagues have shown that co-culture of breast ductal epithelium with prostatic stroma results in the adoption of prostatic epithelial marker expression, including initiation of *Foxa1*, *Nkx3-1* and *Gata3* expression (Taylor et al., 2009). Similarly, co-culture of prostatic mesenchyme with skin, prepubertal mesenchyme with bladder or oesophagus epithelia all support the concept that the stroma can influence epithelial identity, even when the mesenchyme and epithelium derive from different germ layers. This observation has been interpreted as a direct patterning influence by the stromal cells on the adult epithelial stem cells.

A similar role for the stroma in epithelial patterning has recently been demonstrated in the developing mouse (Sallam et al., 2020). In this study, the authors show that hPSC-derived induced ureteric epithelium co-cultured with developing mouse urogenital tract display distinct maturation phenotypes depending on the surrounding stroma. For example, ureteric epithelium placed within the developing metanephric mesenchyme adopts a ureteric tip phenotype, supporting cap mesenchyme and induction of nephrons. Conversely, ureteric epithelium placed within the peri-Wolffian duct stroma (along the nephric duct) induced expression of uroplakins and formed a surrounding αSMA^+ muscular cuff. The plasticity of the ureteric epithelium has previously been appreciated and has recently been manipulated to generate hPSC-derived ureteric epithelium from distal nephron in response to a specific cocktail of ureteric epithelium-supportive growth factors (Howden et al., 2021). Hence, the simple stromal-specific production of ligands and pathway inhibitors could logically guide the identity adopted by adjacent epithelium. In support of this, Uchimura and colleagues have recently combined an AIM differentiation protocol with a more posterior IM differentiation protocol, and showed that the combination of cells from both differentiations enhanced ureteric epithelial gene expression but also the maturation and identity of the nephron epithelia (Uchimura et al., 2020). Such data again suggests the potential power to improve organoid protocols by supplying appropriate instructive stromal populations.

Most existing protocols for directed differentiation of pluripotent stem cells rely upon a stepwise induction of early embryogenic patterning events, first to instruct the appropriate germ layer and then to specify tissue type. Given the success in generating both nephron-forming and ureteric epithelial populations from hPSCs, a similar stepwise differentiation to specific renal stromal populations is logical.

Conclusions

In this Review, we have discussed how the renal stroma forms, its contribution to organogenesis and hence its crucial role in formation of the final adult kidney. Despite the requirement for appropriately patterned renal stroma, there is little evidence for the appropriate generation and localisation of kidney stroma within hPSC-derived kidney organoids. This is likely to be adversely impacting the capacity for these tissues to reach maturation. Given the assumption of a common developmental pathway for both nephron progenitors and cortical stroma, the absence of appropriate stromal patterning may challenge this view. Indeed, the renal cortical stroma may not be derived from the IM, instead migrating from a different population of origin. Hence, as current organoid protocols focus on patterning towards IM, they may fail to generate this population. Cell migration is known to occur in populations such as neural crest and primordial germ cells, giving precedence to the concept of stromal migration. We conclude that generating an appropriate renal stroma for addition to current organoid protocols may improve complexity and maturation in kidney organoids and that this, once again, requires a re-evaluation of normal development.

Competing interests

The authors declare no competing or financial interests.

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