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Identification of a Multipotent Self-Renewing Stromal Progenitor Population during Mammalian Kidney Organogenesis

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SUMMARY

The mammalian kidney is a complex organ consisting of multiple cell types. We previously showed that the *Six2*-expressing cap mesenchyme is a multipotent self-renewing progenitor population for the main body of the nephron, the basic functional unit of the kidney. However, the cellular mechanisms establishing stromal tissues are less clear. We demonstrate that the *Foxd1*-expressing cortical stroma represents a distinct multipotent self-renewing progenitor population that gives rise to stromal tissues of the interstitium, mesangium, and pericytes throughout kidney organogenesis. Fate map analysis of *Foxd1*-expressing cells demonstrates that a small subset of these cells contributes to *Six2*-expressing cells at the early stage of kidney outgrowth. Thereafter, there appears to be a strict nephron and stromal lineage boundary derived from *Six2*-expressing and *Foxd1*-expressing cell types, respectively. Taken together, our observations suggest that distinct multipotent self-renewing progenitor populations coordinate cellular differentiation of the nephron epithelium and renal stroma during mammalian kidney organogenesis.

INTRODUCTION

The kidney contains multiple specialized cell types that have district physiological functions. During embryogenesis, the urogenital system, including the kidney, is derived from the intermediate mesoderm of the developing embryo (Herzlinger, 1995; Saxen, 1987). Formation of the definitive kidney or metanephros in mammals is initiated by reciprocal interactions between two tissues types, the ureteric bud and metanephric mesenchyme, starting around 10.5 days postcoitum (dpc) in the mouse (Costantini and Kopan, 2010; Dressler, 2009; Little and McMahon, 2012; Schedl, 2007). At this time, analysis of transcription factor expression distinguishes two morphologically distinct populations: a core of *Six2*-expressing (*Six2*+) mesenchyme, and an outer layer of *Foxd1*+ cells that likely emerge from a common *Osr1*+ mesenchymal progenitor (Kobayashi et al., 2008; Levinson and Mendelsohn, 2003; Mugford et al., 2008). As the outgrowing ureteric bud enters this mesenchyme and branches, *Six2*+ cells condense around the bud tip forming the cap mesenchyme, whereas *Foxd1*+ cortical stromal cells make up the renal capsule and nephrogenic interstitium between the *Six2*+ population and the outermost connective tissue (the presumptive renal fascia or Gerota's fascia) that surrounds the kidney and adrenal gland (Costantini, 2006; Kobayashi et al., 2008; Levinson et al., 2005).

The *Six2*+ cap mesenchyme is a multipotent self-renewing nephron progenitor population (Kobayashi et al., 2008). In conjunction with ureteric branching, a ureteric epithelium-derived WNT9B signal induces a pathway of nephrogenesis within a subset of *Six2*+ progenitors (Carroll et al., 2005; Karner et al., 2011; Park et al., 2007, 2012). Induced cells undergo an initial aggregation to form the pretubular aggregate. Subsequently, through a mesenchymal-to-epithelial transition, the pretubular aggregate transitions to the renal vesicle that undergoes a series of morphological transformations and patterning processes generating the main body of the nephron from the proximal glomerulus to the distal connecting segment.

The mature nephron, and its accompanying vascular network, is embedded within the cortical and medullary interstitium (Little et al., 2007). This comprises pericytes and mesangial cell types that are intimately associated with the general kidney vasculature and the specialized vasculature of the glomerular capillary loops, respectively (Quaggin and Kreidberg, 2008; Wiggins, 2007), and interstitial fibroblast-like cells that are most prevalent within medullary regions of the mature kidney. Currently, the origins and interrelationships among these cell types are unclear, and the precise role of these stromal components in development, normal kidney function, and disease is poorly understood.

In this study, we have determined the fate map of the *Foxd1*+ cortical stromal cells during kidney development

in vivo in the mouse. These studies demonstrate that the *Foxd1*+ cortical stroma is a multipotent self-renewing progenitor population for stromal cells in the kidney, giving rise to cortical and medullary interstitial cells, mesangial cells, and pericytes of the kidney. Interestingly, *Foxd1*+ stromal progenitors and *Six2*+ nephron progenitors form two mutually exclusive progenitor compartments shortly after the onset of ureteric branching. Prior to this stage, we observed a small but significant contribution of *Foxd1*+ cells to the *Six2*+ progenitor population. Our observations also suggest that the *Foxd1*+ stromal progenitor and *Six2*+ nephron progenitor populations temporally and spatially coordinate cellular differentiation. These data highlight the roles of distinct progenitor compartments in the assembly of the mammalian kidney.

RESULTS

Generation of *Foxd1-Cre* Knockin Mouse Alleles

During early stages of kidney development, *Foxd1* is specifically expressed in the cortical stroma of the nephrogenic zone (Das et al., 2013; Hatini et al., 1996; Levinson et al., 2005). To determine the fate map of this *Foxd1*-expressing (*Foxd1*+) population, we generated three *Foxd1-Cre* knockin alleles in the mouse, where e*GFPCre* ($Foxd1^{GC/+}$), *eGFPCreER*^{T2} ($Foxd1^{GCE/+}$), and *CreER*^{T2} ($Foxd1^{CE/+}$) transgenes were introduced into the 5′ UTR of the endogenous *Foxd1* locus (Figure S1 available online). These *Foxd1*-Cre knockin alleles ablate *Foxd1* function; however, mice heterozygous for these and previously described null alleles are phenotypically normal and fertile (Hatini et al., 1996; Levinson et al., 2005) (data not shown). The *GCE* and *CE* alleles allow tamoxifen-dependent regulation of Cre recombinase activity (Indra et al., 1999; Kobayashi et al., 2008).

To validate transgene expression patterns of the *Foxd1-Cre* knockin alleles, we examined GFP expression in the developing kidney of $Foxd1^{GC/+}$ and $Foxd1^{GCE/+}$ embryos. In both lines, GFP expression was observed in the cortical stroma during kidney development (Figure S2; data not shown). The nuclear FOXD1 protein colocalized with nuclear GFP in $Foxd1^{GC/+}$ kidneys (Figure S2I), whereas FOXD1 was surrounded by cytoplasmic GFP in $Foxd1^{GCE/+}$ kidneys (Figure S2J). These observations confirmed GFP expression in FOXD1+ cortical stromal cells in the *Foxd1-GC* and *Foxd1-GCE* alleles.

Genome-wide gene expression projects (GenePaint and GUDMAP) have documented *Foxd1* expression in the glomerulus at a low level at 14.5 dpc and at a higher level at 19.5 dpc (Figures S3A and S3B) (Harding et al., 2011; Visel et al., 2004), and microarray analysis suggests podocytes as the likely cell source (Brunskill et al., 2011). Although *Foxd1* mRNA appears to be expressed in most podocytes of maturing-stage glomeruli (Figures S3A and S3B), a recent study showed that Cre recombination was observed only in a subset of podocytes in *Foxd1-eGFPCre* mice during kidney development (Boyle et al., 2014), indicating posttranscriptional regulation for *Foxd1* expression or different sensitivity of detection methods. Consistent with these findings, we detected expression of GFP and FOXD1 in a subset of both podocytes and parietal epithelial cells of maturing-stage glomeruli, but not in less-differentiated capillary loop-stage glomeruli, in the $Foxd1^{GC/+}$ kidney at 15.5 and 18.5 dpc (Figure S3B and S3C; data not shown). We observed *Foxd1-GFP* expression only in the cortical stroma, the visceral (podocytes), and the parietal epithelial cells of the glomerulus. No *Foxd1-GFP* expression was observed in any other tissues of the developing kidney. Thus, the *Foxd1-Cre* knockin alleles faithfully document endogenous FOXD1 expression.

Foxd1+ Cells within the Cortical Stroma Show a Distinct Fate Map to that of *Six2*+ Nephron Progenitors in the Cap Mesenchyme

The fate map of the *Foxd1*+ cortical stroma was compared to that of the *Six2*+ cap mesenchyme. $Foxd1^{GC/+}$ and *Six2-tetoff-eGFPCre* ($Six2^{TGC/+}$) (Kobayashi et al., 2008) mice were intercrossed with mice carrying a *R26R-lacZ* reporter allele ($R26R^{lacZ/+}$) (Soriano, 1999) to permanently label descendant cells from the *Foxd1*+ cortical stromal and *Six2*+ cap mesenchymal cells by β-galactosidase (β-gal) expression.

As expected from our previous study (Kobayashi et al., 2008), analysis of $Six2^{TGC/+}$; $R26R^{lacZ/+}$ kidneys at 14.5 dpc showed β-gal activity confined to the cap mesenchyme and all nephron epithelia including the renal vesicle, S-shaped body, nephron tubule, and visceral and parietal epithelia of the glomerulus (Figures 1A, 1C, and 1E). In striking contrast, $Foxd1^{GC/+}$; $R26R^{lacZ/+}$ displayed a reciprocal pattern of β-gal activity restricted to the cortical stroma, cortical and medullary interstitium, and the glomerular mesangium region (Figures 1B, 1D, and 1F). At later stages, we also observed β-gal activity within podocytes of glomeruli at the maturing stage consistent with *Foxd1* expression within this population (Figures S4A and S4C). A low-variable incomplete β-gal activity was observed in a subset of nephrons (Figures 1D and S4E). Taken together, our observations suggest that the fate maps of *Six2*+ cap mesenchyme and *Foxd1*+ cortical stroma are largely distinct: the former contributing to nephron epithelial cells, and the latter to renal stromal cell types.

The low contribution of *Foxd1*+ descendant cells to regions of the nephron epithelium, normally populated only by cells originating from the *Six2*+ cap mesenchyme, outside of the *Foxd1*+ visceral and parietal epithelia