Dicer regulates the development of nephrogenic and ureteric compartments in the mammalian kidney

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MicroRNAs (miRNAs) are a large and growing class of small, non-coding, regulatory RNAs that control gene expression predominantly at the post-transcriptional level. The production of most functional miRNAs depends on the enzymatic activity of *Dicer*, an RNase III class enzyme. To address the potential action of *Dicer*-dependent miRNAs in mammalian kidney development, we conditionally ablated *Dicer* function within cells of nephron lineage and the ureteric bud-derived collecting duct system. *Six2Cre*-mediated removal of *Dicer* activity from the progenitors of the nephron epithelium led to elevated apoptosis and premature termination of nephrogenesis. Thus, *Dicer* action is important for maintaining the viability of this critical self-renewing progenitor pool and, consequently, development of a normal nephron complement. *HoxB7Cre*-mediated removal of *Dicer* function from the ureteric bud epithelium led to the development of renal cysts. This was preceded by excessive cell proliferation and apoptosis, and accompanied by disrupted ciliogenesis within the ureteric bud epithelium. *Dicer* removal also disrupted branching morphogenesis with the phenotype correlating with downregulation of *Wnt11* and *c-Ret* expression at ureteric tips. Thus *Dicer*, and by inference *Dicer*-dependent miRNA activity, have distinct regulatory roles within different components of the developing mouse kidney. Furthermore, an understanding of miRNA action may provide new insights into the etiology and pathogenesis of renal cyst-based kidney disease.

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MicroRNAs (miRNAs) are a large and growing class of small, non-coding, regulatory RNAs that control gene expression predominantly at the post-transcriptional level through direct binding to target mRNAs. In general, miRNAs function by inhibiting protein translation and/or degrading target mRNAs (for recent reviews, see Carthew and Sontheimer[1]; Behm-Ansmant *et al.*[2]; Pillai *et al.*[3]), although recent reports indicate that miRNAs may occasionally enhance expression from their target mRNAs in a tissue context- or cell cycle state-dependent fashion.[4,5] A single miRNA may regulate hundreds of target genes, and a given gene can be regulated by multiple miRNAs. In this way, miRNA action underpins many fundamental biological processes, including developmental timing, apoptosis, cell proliferation, cell-fate choice and morphogenesis, as well as pathogenic cellular activities, notably oncogenesis.[6]

miRNAs are initially transcribed as long pri-miRNAs from miRNA-coding genes and need to be processed to function. *Dicer*, an RNase III class enzyme, is required for the processing of most miRNAs. pri-miRNAs are first processed in the nucleus by another RNase III, Drosha, in a complex with DGCR8, or the splicing machinery, to ~70 nt stem-loop pre-miRNAs. *Dicer* then processes pre-miRNAs in the cytoplasm to mature miRNAs, which recognize cognate mRNAs through an Argonaute-dependent process, modulating the stability or translation of mRNA targets (see Carthew and Sontheimer[1] for a recent review). Genetic studies with conditionally removing *Dicer* function have demonstrated critical roles for *Dicer*-mediated miRNA regulation in the development and function of a variety of mammalian tissues and organs.[7,8]

Kidney development is driven by reciprocal interactions between the ureteric bud (UB) epithelium and the overlying metanephric mesenchyme (for a recent review, see Dressler[9]), with additional inputs from the renal interstitium and vascular tissues.[10–16] The UB epithelium originates as an evagination of the Wolffian duct epithelium at the hind-limb level.[17] UB-derived signals regulate maintenance and nephron commitment within a *Six2*+ mesenchymal progenitor compartment that caps the branching ureteric tips (nephron progenitors).[18–20] Inductive UB signals induce a

mesenchymal-to-epithelial transition within overlying progenitor pools, resulting in the emergence of the renal vesicle beneath the UB tip.[19] The renal vesicle is the nephron precursor, and its morphogenesis, patterning, and differentiation establishes the mature renal tubular, visceral, and parietal epithelia of the nephron. Metanephric mesenchyme-secreted Glial cell line-derived neurotrophic factor (GDNF) stimulates branching morphogenesis of the subjacent UB tips, establishing the highly branched network of the renal collecting duct system (recently reviewed in Costantini[17]). Epithelial growth within both nephron and collecting duct epithelia is tightly controlled; deregulation leads to cystic dilation and renal cystic diseases. Through these intricate interactions, the UB branching morphogenesis regulates nephron numbers, a predisposing factor for renal diseases and hypertension.[21,22]

Evidence for the involvement of *Dicer* and miRNAs in kidney development and homeostasis is accumulating.[23–25] miRNAs have been detected in embryonic and adult kidney tissues and changes in miRNA expression have been observed in various kidney diseases, including polycystic kidney disease, diabetic nephropathy, kidney and bladder cancer, and in autoimmune disease like lupus nephritis.[23–25] Podocyte-specific removal of *Dicer* causes multiple abnormalities in postnatal renal corpuscle homeostasis and function, leading to proteinuria and a rapid progression to end-stage kidney diseases.[26–28] Maintenance of Juxtaglomerular cells also requires *Dicer* input.[29] Removal of *Dicer* within the collecting duct epithelium results in postnatal hydronephrosis and collecting duct cysts,[30] demonstrating the functional importance of *Dicer* and miRNAs in kidney homeostasis and function. In addition, miRNAs regulate *Xenopus* pronephros patterning and differentiation.[31]

In this paper, we use nephron progenitor-specific *Dicer* removal to demonstrate the importance of *Dicer* in the maintenance of nephron progenitors and nephron epithelia. Conditional removal of *Dicer* within the adjacent ureteric bud epithelium extends a previous report[30] of *Dicer*/miRNA-associated cystic dilation by identifying a requirement for *Dicer* in controlling cell proliferation and apoptosis in the collecting duct epithelium. Furthermore, we demonstrate that *Dicer* action is required for normal branching growth of the ureteric network.

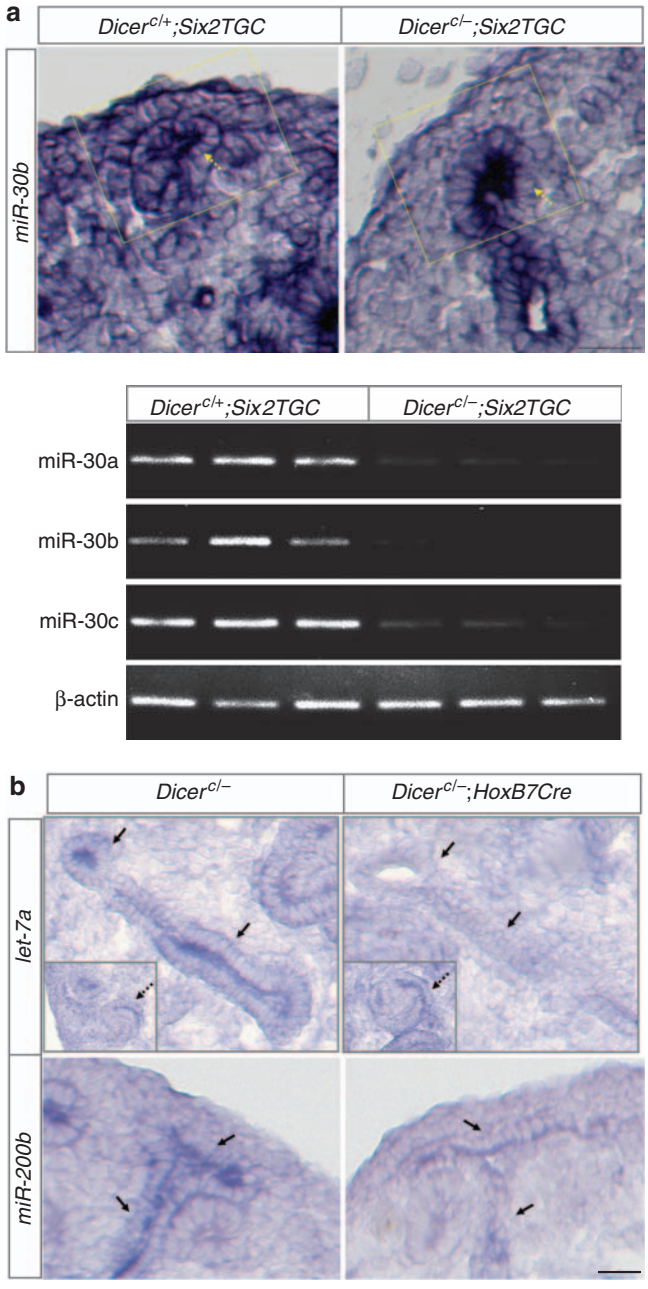

Figure 1 | MicroRNA (miRNA) biogenesis is disrupted from *Dicer* depletion. *In situ* hybridization for miRNAs with locked nucleic acid (LNA) probes on E14.5 kidney sections and semiquantitative miRNA reverse transcription-PCR (RT-PCR) analysis on the cap mesenchyme. (**a**) miR-30b expression in the nephron progenitors (the cap mesenchyme cells overlying the ureteric tip in the boxed area) is markedly reduced to background levels in Dicer nephron mutants. Its expression in the ureteric bud epithelium (dashed arrows) is unaffected. Semiquantitative RT-PCR for miR-30a, miR-30b, miR-30c, and β-actin (control) in the fluorescence-activated cell sorting (FACS)-sorted green fluorescent protein (GFP)-positive cap mesenchyme (nephron progenitors) from three controls and three mutants showing the reduction of mature miRNAs in the E13.5 Dicer nephron mutant cap mesenchyme. The trace amount of mature miRNAs in the mutants may be due to incomplete degradation of mature miRNAs previously produced in a subset of cap mesenchyme cells because of more mosaic expression of *Six2TGC* at earlier developmental stages (Kobayashi *et al.*[18] and unpublished observations, JY). (**b**) let-7a and miR-200b expression is greatly reduced in the ureteric bud (UB) epithelium (solid arrows) in Dicer UB mutants but unaffected in non-UB cell types (S-shaped bodies, insets and dashed arrows). Scale bars = 20 μm.

RESULTS

Removal of *Dicer* activity from the nephron lineage leads to premature depletion of nephron progenitors

To examine the overall involvement of nephron lineage-expressed *Dicer* and miRNAs in kidney development, we ablated *Dicer* function from the entire nephron lineage using