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Size doesn't matter; in the stroma, little things make all the difference

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In recent years there has been an increase in the number of studies into the role of stromal cells and microRNAs (miRNAs) in kidney development. Nakagawa *et al.* combine the two in a study of a stromal cell-specific knockout of *Dicer1*. The work identifies many important roles for miRNAs in these cells and kidney development in general, partially through their modification of the β-catenin signaling cascade.

Kidney International (2015) **87,** 1087–1089. doi:10.1038/ki.2015.77

For a long time, the study of early kidney development has focused on two cell types. These are the mesenchymal cells of the metanephric mesenchyme, which via a mesenchymal-to-epithelial transition form the nephrons, and the epithelial cells of the ureteric bud, which form the collecting duct system. Increasingly, however, more attention is given to the third cell type found in the early embryonic kidney, the stromal cells. Similarly, the vast majority of work was, and still is, focused on the protein-coding part of the genome, with the understanding of the role of non-coding transcripts of different sorts and sizes still having a lot of catching up to do. Naoki Nakagawa, Jeremy Duffield, and colleagues[1] (this issue) combine both hiatuses in one study by analyzing the phenotype of a stromal-specific *Dicer1* knockout mouse.

As with many developmental systems, lineage tracing has been essential to get a better understanding of the origins and further fates of the stromal cells. It is now clear that Osr1$^+$ cells from the intermediate mesoderm give rise to all cell types in the kidney, including the stroma. *Foxd1* expression labels the stromal progenitor cells, and lineage tracing of these cells has shown that the stroma gives rise to the endothelial cells, mesangial cells, pericytes, vascular smooth muscle cells, and fibroblasts in the kidney.[2] *Foxd1*, previously called BF-2, is not only a marker for renal stromal progenitor cells, it is an essential gene; constitutive knockout of the gene results in disturbed development of the kidney capsule as well as direct and indirect phenotypes in the nephrogenic and ureteric bud compartments.[3] Nakagawa *et al.*[1] now use this knowledge to delete *Dicer1*, the RNase III enzyme that performs a central role in the processing of pre-microRNAs into biologically active microRNAs (miRNAs), from the renal stromal lineage to study the role of miRNAs in these cells and the developing kidney.

Previous work from different laboratories had already highlighted the importance of miRNAs in the other two cell lineages in the developing kidney. Deletion of *Dicer1* using a *Hoxb7*-Cre driver was shown to result in severe phenotypes in the ureteric bud, including increased cell proliferation and death, renal cysts, and disturbed ciliogenesis and branching.[4] In the nephrogenic lineage, deletion of *Dicer1* in Six2$^+$ cells results in depletion of the nephron progenitors at mid-gestation through increased apoptosis and a large decrease in renal vesicles and comma- and S-shaped bodies.[4] An even earlier mesenchymal loss of *Dicer1* using a *Pax3*-Cre allele led to an apoptotic response shortly after the first nephron induction.[5] *Dicer1* loss in tubules using Ksp-cadherin (*Cdh16*) regulatory elements results in tubular and focal glomerular cysts, with or without hydroureter and hydronephrosis,[6] though the latter could result from *Dicer1* loss in the ureter cells where this Cre driver is also active. Finally, podocin (*NPHS2*)-Cre-driven loss of *Dicer1* in podocytes results in rapid proteinuria and end-stage kidney disease through dedifferentiation and disruption of the cytoskeleton.[7] To this list of renal-specific *Dicer1* mouse models, Nakagawa *et al.*[1] now add the *Foxd1*-eGFPCre-driven mutant in the stromal progenitor cells.

The resulting phenotype observed was severe (Figure 1); the mice died within 2 days after birth, and mutants exhibited cyanosis and lung defects in addition to severe kidney failure. The kidneys were smaller with tubular defects and had a reduced nephrogenic zone, abnormal podocytes, and fewer glomeruli, which were mostly cystic. Furthermore, the inner medulla was absent, and the outer medulla was shortened. Several stages of tubulogenesis were disturbed, such as differentiation of distal tubules and loop of Henle, as well as the polarization of proximal tubules. The results also implicated a role for stromal miRNAs in vasculogenesis with abnormal vacuolation of pericytes and reduced mesangial cellularity in glomeruli. Genome-wide expression analysis of mRNAs revealed that genes involved in stromal-cell migration and activation were suppressed, and the authors identified and confirmed specific miRNAs that affect the canonical Wnt signaling pathway and are involved in some of these phenotypes.

The data presented by Nakagawa *et al.*[1] reinforce the notion that the

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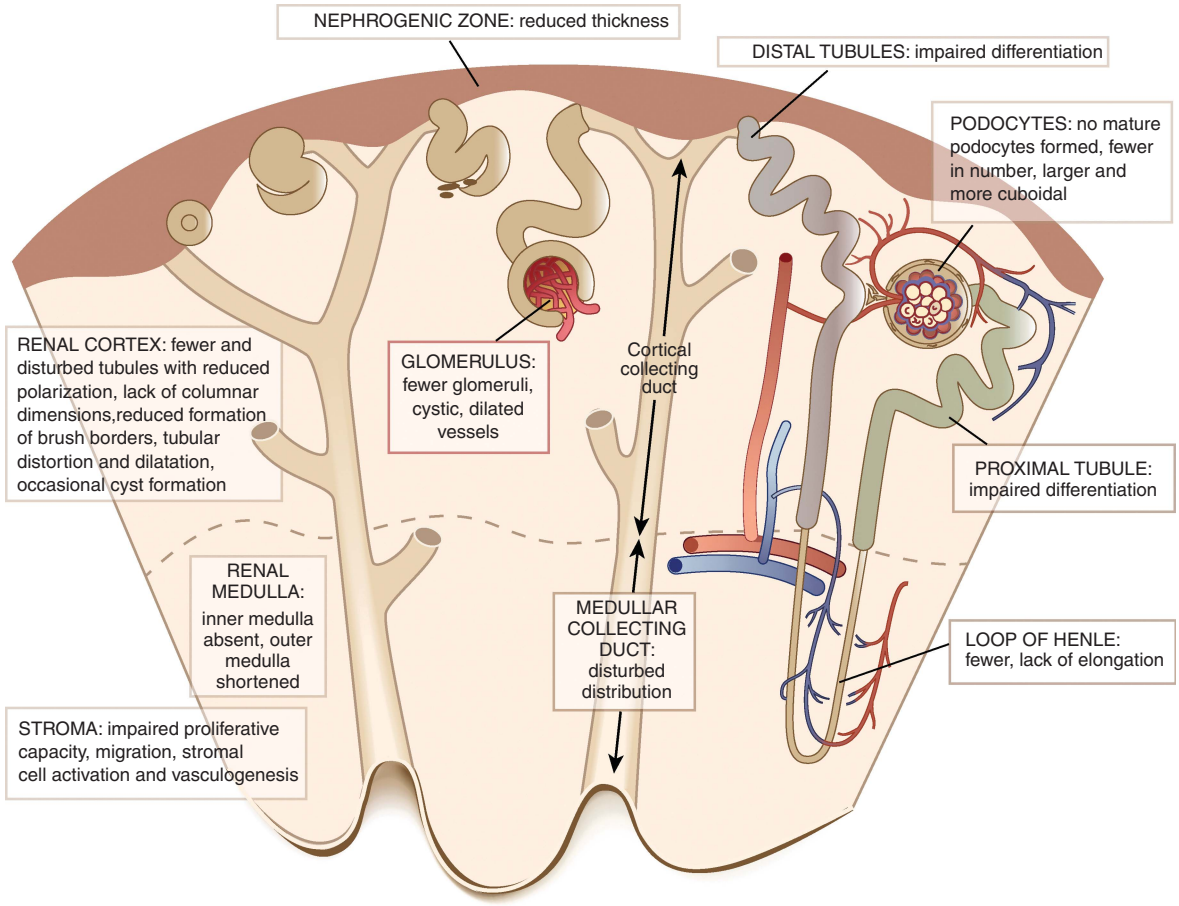

Figure 1 | Summary of the phenotypes found in the stromal-specific *Dicer1* knockout mouse. Adapted from original designed by Kylie Georgas, University of Queensland, for the GUDMAP database (http://www.gudmap.org).

stromal cells have many essential roles for the overall structure and function of the kidney. The *Foxd1* knockout mouse had previously shown direct and indirect roles for this gene in multiple kidney compartments and cell types,[3] and the recently described ablation of stromal cells using *Foxd1*-Cre-induced expression of diphtheria toxin[8] has shown this is a function of the stromal cells in general. The stromal cell-specific *Dicer1* knockout now shows that miRNAs are important in many of these functions. This essential role for miRNAs in kidney development is further supported by the recent realization that many miRNA processing genes, such as *DROSHA*, *DGCR8*, *TARBP2*, *XPO5*, and *DICER1* itself, are commonly mutated in Wilms' tumors, pediatric kidney tumors that are the result of disturbed kidney development.[9]

The data presented by Nakagawa *et al.*[1] suggest a strong effect of miRNA loss on the β-catenin-mediated canonical Wnt signaling pathway. They present a model that could explain the attenuated elongation of the loop of Henle found in mice lacking *Wnt7b* or with *Foxd1*-Cre-mediated deletion of β-catenin, suggesting a role for epithelial–stromal cross-talk.[10] Other data on the role of stromal cells in β-catenin signaling are available. In a seminal study, Das *et al.* showed that stromal cells play an essential role in the control of nephron progenitor cells.[8] Ablation of the Foxd1$^+$ stromal progenitors resulted in a massive expansion of the Six2$^+$ nephron progenitor population at the cost of highly reduced numbers of pretubular aggregates and renal vesicles. The authors identified a Fat4-Yap/Taz-mediated pathway that modulates the outcome of the Wnt9b–β-catenin nephron induction switch and determines which target cells will self-renew and which will differentiate into nephrons. In contrast, in the stromal *Dicer1* mutants the nephrogenic zone is thinner and the number of Six2$^+$ cells are reduced. Nephrons are forming, are found in reduced numbers. This could suggest that stromal miRNAs are involved in the control of the Fat4 signal, for instance by targeting negative controllers of this signal.

Although Nakagawa *et al.*[1] explain that not all phenotypes can be described as effects on the β-catenin pathway, the effect of stromal cells on the nephron progenitor cells through modulation of the β-catenin self-renewal/differentiation switch could be exemplary of the role of stromal cells in the developing kidney in general and explain the broad

effects that stromal mutations, including *Dicer1* loss, have on kidney development. It is striking that the phenotypes in *Dicer1* knockouts in the ureteric bud and nephrogenic lineages are by and large restricted to the mutant lineage or cells, whereas the stromal *Dicer1* knockout seems to affect all lineages. Just as the stromal cells above the cap mesenchyme control the nephron progenitors that lie beneath them, at other sites in the kidney stromal cells could influence other cell types through close or direct contact and modulating signals. The epithelial–stromal cross-talk proposed to be responsible for the Wnt7b phenotype[10] would be another example of this. There is no reason why miRNAs would not be involved in the control of these modulating signals. In fact, through their very nature miRNAs could target multiple proteins or even multiple signals in parallel, making them ideal for such a task. As such, miRNAs might be an ideal genetic entry point to identify these signals.

There is clearly scope for more detailed analyses of specific miRNAs in the developing kidney. In some of the renal *Dicer1* knockouts, specific miRNAs have been identified as important players in the observed phenotypes. In the case of the stromal *Dicer1* knockout, miR-214, miR-199a-3p, and miR-199a-5p are clearly implicated in different aspects of the phenotype in these mice; other miRNAs were identified in other renal *Dicer1* mutants. Transgenic mouse lines with reporter and conditional knockout construct alleles for miRNAs and the availability of Cre drivers for most stages of kidney development will undoubtedly lead to an increased appreciation of the role of specific miRNAs in kidney development and disease.

DISCLOSURE
The authors declared no competing interests.

ACKNOWLEDGMENTS
The Roslin Institute receives Institute Strategic Program Grant funding from the Biotechnology and Biological Sciences Research Council.

REFERENCES

1. Nakagawa N, Xin C, Roach AM *et al.* Dicer1 activity in the stromal compartment regulates nephron differentiation and vascular patterning during mammalian kidney organogenesis. *Kidney Int* 2015; **87**: 1125–1140.
2. Kobayashi A, Mugford JW, Krautzberger AM *et al.* Identification of a multipotent self-renewing stromal progenitor population during mammalian kidney organogenesis. *Stem Cell Reports* 2014; **3**: 650–662.
3. Levinson RS, Batourina E, Choi C *et al.* Foxd1-dependent signals control cellularity in the renal capsule, a structure required for normal renal development. *Development* 2005; **132**: 529–539.
4. Nagalakshmi VK, Ren Q, Pugh MM *et al.* Dicer regulates the development of nephrogenic and ureteric compartments in the mammalian kidney. *Kidney Int* 2011; **79**: 317–330.
5. Chu JY, Sims-Lucas S, Bushnell DS *et al.* Dicer function is required in the metanephric mesenchyme for early kidney development. *Am J Physiol Renal Physiol* 2014; **306**: F764–F772.
6. Patel V, Hajarnis S, Williams D *et al.* MicroRNAs regulate renal tubule maturation through modulation of Pkd1. *J Am Soc Nephrol* 2012; **23**: 1941–1948.
7. Harvey SJ, Jarad G, Cunningham J *et al.* Podocyte-specific deletion of dicer alters cytoskeletal dynamics and causes glomerular disease. *J Am Soc Nephrol* 2008; **19**: 2150–2158.
8. Das A, Tanigawa S, Karner CM *et al.* Stromal-epithelial crosstalk regulates kidney progenitor cell differentiation. *Nat Cell Biol* 2013; **15**: 1035–1044.
9. Hohenstein P, Pritchard-Jones K, Charlton J. The yin and yang of kidney development and Wilm's tumors. *Genes Dev* 2015; **29**: 467–482.
10. Yu J, Carroll TJ, Rajagopal J *et al.* A Wnt7b-dependent pathway regulates the orientation of epithelial cell division and establishes the cortico-medullary axis of the mammalian kidney. *Development* 2009; **136**: 161–171.

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The vascular secret of Klotho

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Klotho is an evolutionarily highly conserved protein related to longevity. Increasing evidence of a vascular protecting effect of the Klotho protein has emerged and might be important for future treatments of uremic vascular calcification. It is still disputed whether Klotho is locally expressed in the vasculature or whether its vascular effects arise uniquely from its presence in the circulation.

Kidney International (2015) **87,** 1089–1091. doi:10.1038/ki.2015.80

α-Klotho (Klotho) gene and protein were discovered in 1997 by Kuro-o and co-workers. The name Klotho refers to Greek mythology, where Klotho is the one of the Fates, who is spinning the thread of life. Klotho-deficient mice manifest a phenotype resembling accelerated human ageing. Klotho-deficient mice have a short lifespan, and overexpression of Klotho in mice extends lifespan significantly in comparison with normal mice, which is taken as proof of the concept that Klotho is associated with longevity. In a human population study, *Klotho* gene variations were found to be associated with life extension. Of particular interest was the finding that Klotho deficiency in mice was associated with a severe vascular phenotype of arteriosclerosis, impaired endothelial function, and impaired angiogenesis.

Klotho protein in mammals is present in different isoforms, as a membrane-bound protein and as a soluble form. Soluble Klotho can be generated by shedding of the extracellular domain of membrane Klotho, containing two internal repeats, KL1 and KL2. Membrane

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