The Transcription Factor *Sry-Related HMG Box-4 (Sox4)* is Critical for Nephron Endowment and Renal Development in Vivo.

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Introduction

Defects in nephrogenesis and ureteric branching result in Congenital Anomalies of the Kidney and Urinary Tract (CAKUT). Nephron deficiency is a hallmark feature of CAKUT. Low nephron endowment - although asymptomatic early in life - is associated with adult-onset hypertension, a leading cause of coronary heart disease, stroke, and renal failure in North America. We have previously identified the *Sox4* transcription factor as essential for normal renal development *in vivo*. Conditional ablation of *Sox4* in nephron progenitor cells and their derivatives results in early-onset glomerular injury, which progresses to end-stage renal failure in mice.

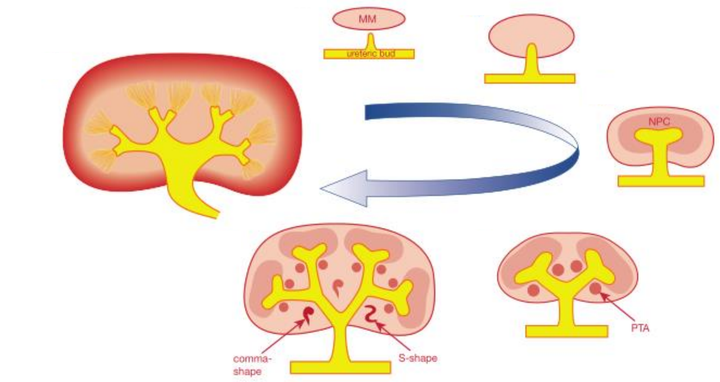


Figure 1. Kidney development results from reciprocal signaling between the metanephric mesenchyme (MM) and the ureteric bud. NPC – nephron progenitor cells, PTA – pretubular aggregates.

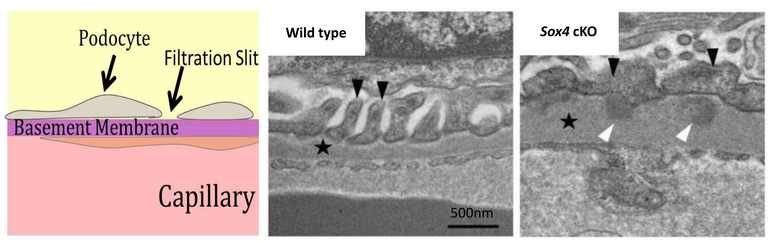


Figure 2. Conditional ablation of *Sox4* in nephron progenitor cells (*Sox4* cKO) results in renal injury. Ultrastructural glomerular damage is characterized by thickening of the basement membrane (star), podocyte foot process effacement (black arrowheads), and aggregates of electron-dense material in the basement membrane (white arrowheads).

Methods

- Conditional Knockout (cKO) of *Sox4* was targeted to nephron progenitor cells using *Six2-Cre*.
- Counts and approximations of glomerular number were performed on paraffin embedded samples using the gold standard physical disector/fractionator combination and TrakEM2 plugin for Fiji.
- Explant experiments were carried out using the following approach:

Embryonic Kidneys (E12.5) → Culture for 72hrs → Immunofluorescence: PTA – Red; Ureteric bud - Green

Results

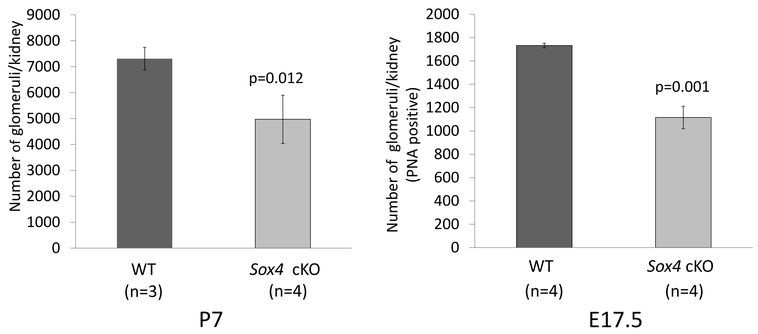


Figure 3. The absence of *Sox4* in nephron progenitor cells (*Sox4* cKO) leads to a >30% reduction in glomerular number at both postnatal day (P)7 and embryonic day (E)17.5.

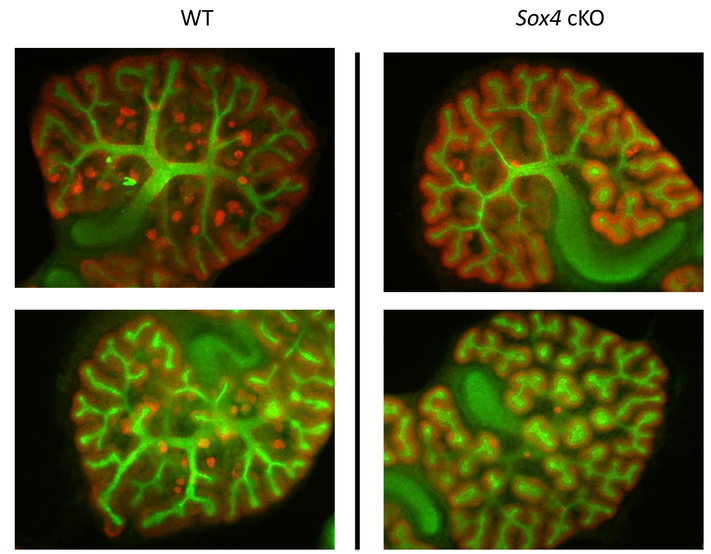


Figure 4. Embryonic kidney explants exhibit a 47% reduction in pretubular aggregate (PTA) formation in the absence of Sox4. Representative explants after 72 hours in culture (top), and normalized PTA ratios (bottom) are shown. Red: nephron progenitor cells and pretubular aggregates (WT1). Green: ureteric bud (cytokeratin).

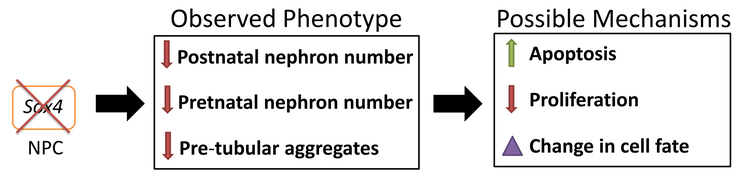
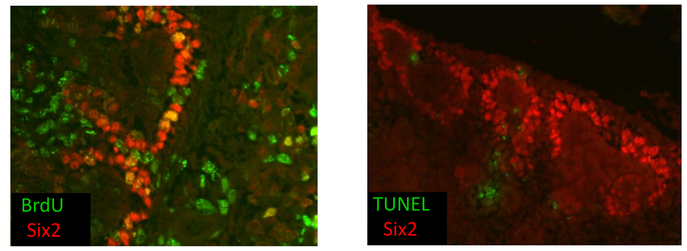


Figure 5. The absence of *Sox4* in nephron progenitor cells (NPC) results in a significant decrease in the number of nephrons and pretubular aggregates. Three possible mechanisms are: 1) increased apoptosis of NPC, 2) decreased apoptosis of NPC, and 3) a change in cell fate of NPC.



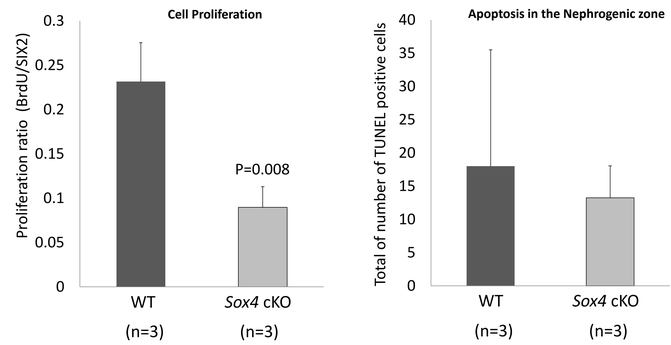


Figure 5. Preliminary data indicate that ablation of *Sox4* in Six2 positive nephron progenitor cells leads to a significant reduction in cell proliferation with no affect on apoptosis at embryonic day (E)15.5.

Summary

Here we report that low nephron endowment is a primary developmental defect in *Sox4*-deficient mice. Cultured *Sox4*-deficient kidney explants exhibit a 47% reduction in pretubular aggregate formation at embryonic day (E) 12.5 and a 36% reduction in glomerular number is observed at E17.5. Current experiments are underway to investigate whether reduced nephron endowment in *Sox4*-deficient kidneys may be due to 1) increased apoptosis, 2) decreased proliferation, or 3) a change in cell fate of nephron progenitor cells during nephrogenesis *in vivo*.

Acknowledgements

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