RENAL INJURY RESPONSE IN AN ADULT *Pkd2* MOUSE MODEL

UAB Graduate Biomedical Science

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ABSTRACT

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is caused by mutations in either the PKD1 or PKD2 gene, resulting in progressive renal cyst formation. Previous studies have shown that renal injury accelerates cyst formation in mouse models of PKD, suggesting *Pkd1* and *Pkd2* (encoding PC1 and PC2, respectively) may be involved in regulating injury and repair responses. We are evaluating the presence of malrepaired cells, defined as the persistent expression of injury markers following injury, such as SOX9, in *Pkd2* mutant mice and how the loss of *Pkd2* may affect this process following injury.

Cisplatin, a chemotherapeutic drug that has a nephrotoxic side effect, was used to give an injury to the kidney. The percentage of SOX9-expressing renal epithelial cells in *Pkd2* mutant and control kidneys were compared using fluorescent microscopy imaging. The number of cells expressing SOX9 peaked 3 days post-injury in *Pkd2* mutants and 7 days post-injury in controls and decreased through 28 days post-injury. At day 28, *Pkd2* mutants showed an increased number of persistent SOX9-expressing cells compared to controls. This finding shows a defect in repair processes suggesting that *Pkd2* may be involved in the repair response pathway. An increase in SOX9+ cells were observed from D28 to D35 post-injury in *Pkd2* mutants, indicating that the cells that failed to repair are proliferating or additional cells are being further injured during cyst formation. This evidence suggests that *Pkd2* mutants are more sensitive to injury and there is a fail to repair mechanism possibly playing a role in ADPKD progression.

INTRODUCTION

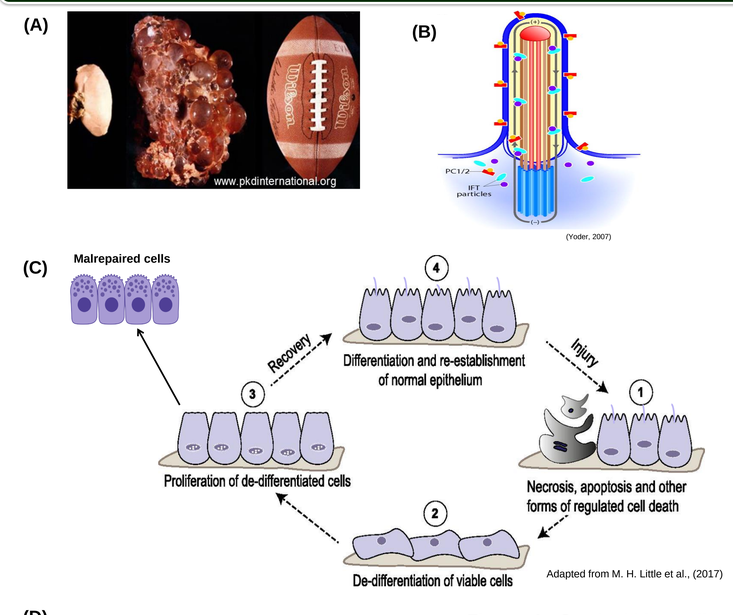

Figure 1. Cystic kidney disease and renal injury. (A) Comparison between a normal kidney and an ADPKD kidney. **(B)** Diagram of primary cilia, a long, thin organelle protruding from the apical surface of almost all cell types, most commonly from epithelial cells **(C)** Response of renal epithelium to injury. **(D)** Renal injury, acting as a disease progression factor, accelerates cyst formation in cystic kidney animal models.

RESULTS

❖ Cisplatin treatment accelerates cyst formation in *Pkd2* mutants

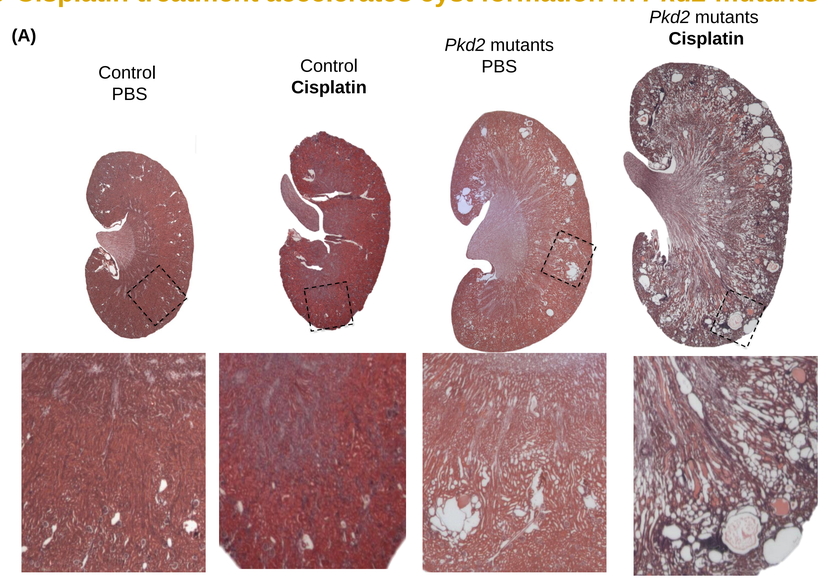

Figure 2. (A) H/E staining of 8 weeks posts cisplatin and PBS -treated kidney sections of control and *Pkd2* mutants. The cysts form throughout the kidney following injury but develop in focal regions in non-injured *Pkd2* mutants.

❖ PKD2 regulates injury response after cisplatin treatment

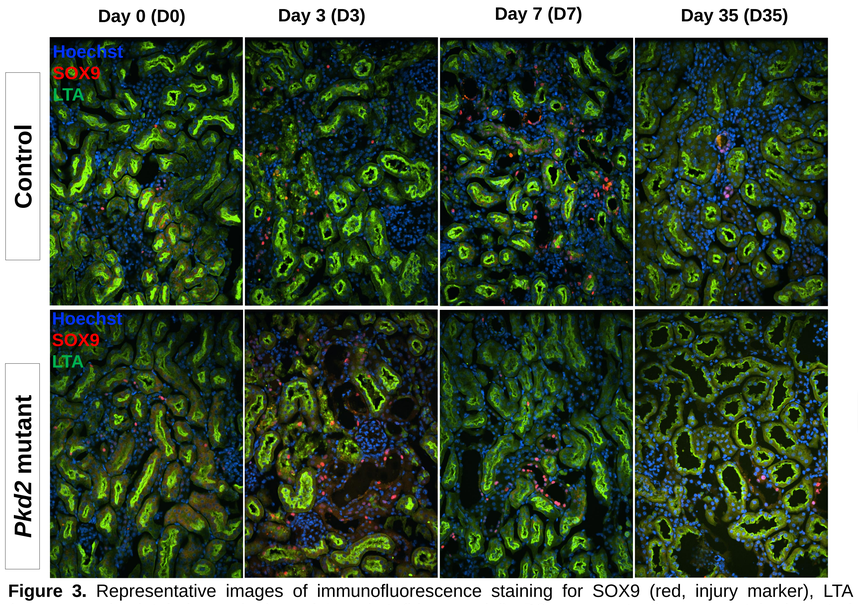

Figure 3. Representative images of immunofluorescence staining for SOX9 (red, injury marker), LTA (green, proximal tubule marker), and Hoechst (blue, nuclei). D0 represents the age-matched control kidney without cisplatin and expresses very few SOX9+ cells(intrinsic injury). Upon cisplatin treatment, the number of injured cells expressing SOX9 increases and then gradually decreases through D28. However, an increase in SOX9+ cells were observed from D28 to D35 post-injury in *Pkd2* mutants may indicate that the malrepaired cells are proliferating or additional cells are being further injured in *Pkd2* mutants.

RESULTS

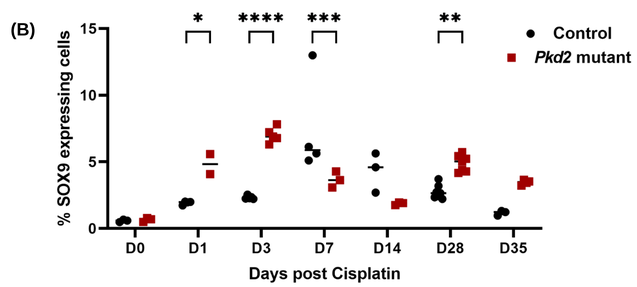

Figure 4. The scatter plot depicts mean percentage of SOX9 expressing cells in the kidney as compared to the total number of nuclei. To assess renal injury, the expression of a renal injury marker, SOX9, at several time points were quantified post cisplatin-induced injury in *Pkd2* mutants and controls. The percentage of SOX9+ cells peaked at D3 in *Pkd2* mutants and D7 in controls indicating the higher sensitivity to injury in the mutants. Furthermore, the percentage of SOX9+ cells did not return to baseline levels in both controls and mutants, and continued to express SOX9 through D28, where renal repair is expected to be fully resolved. The increase in the number of malrepaired cells in *Pkd2* mutants as compared to controls at D28 shows a defect in repair processes suggesting that PKD2 may be involved in the repair response pathway.

FUTURE DIRECTIONS

- ❖ Test the hypothesis that malrepaired cells contribute to cyst formation
- ❖ Lineage tracing of SOX9 expressing cells using SOX9CreERT2 and Brainbow fluorescence reporter mouse
- ❖ Study the clonal expansion of labeled cells around the cyst

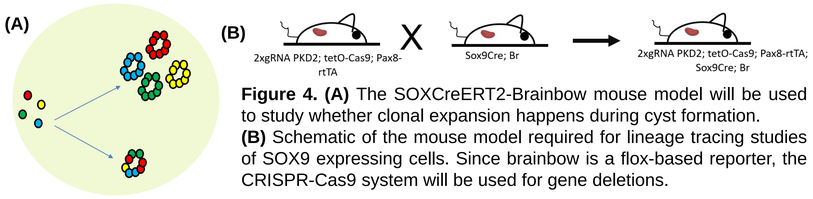

Figure 4. (A) The SOXCreERT2-Brainbow mouse model will be used to study whether clonal expansion happens during cyst formation. **(B)** Schematic of the mouse model required for lineage tracing studies of SOX9 expressing cells. Since brainbow is a flox-based reporter, the CRISPR-Cas9 system will be used for gene deletions.

CONCLUSIONS

- ❖ *Pkd2* mutants are more sensitive to injury as compared to controls.
- ❖ The increase in number of malrepaired cells in *Pkd2* mutants relative to controls at D28 shows a defect in repair processes in *Pkd2* mutant kidney.
- ❖ This suggests that PKD2 may be involved in the repair response pathway.
- ❖ An increase in SOX9+ cells from D28 to D35 may indicate that the malrepaired cells are proliferating and involved in cyst formation in the *Pkd2* mutants.

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NIH National Institute of Diabetes and Digestive and Kidney Diseases

PKD FOUNDATION Polycystic Kidney Disease