CHANGES IN TUBULE FLOW AND CELL DIFFERENTIATION DURING RENAL CYST DEVELOPMENT

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Background

Polycystic kidney disease is an inherited disorder in which clusters of cysts develop within the kidney causing them to enlarge and lose function. Cyst development results from mutations in ciliary localized proteins, PC1 and PC2 (encoded by the *Pkd1* and *Pkd2* genes, respectively), or due to loss of the cilium (e.g., in *Ift88* mutants). This leads to changes in the nephron associated with de-differentiation of cells, cyst formation, abnormal injury responses, and alterations in tubule flow. It is currently unknown whether flow is disrupted prior to or after cyst initiation, or whether tubule flow is important in maintaining the cells' differentiation state. While the function of the cilium in the kidney remains enigmatic, one proposed role is as a mechanosensor that detects changes in flow through the tubule lumen.

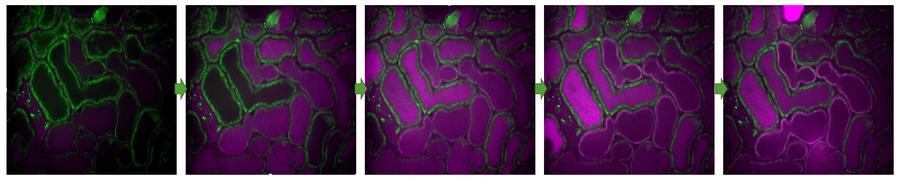


Figure 1: Intravital imaging of flow through the renal tubules using LMW dextran. Dextran (10kDa) was delivered via retro-orbital injection (20ug/mouse) and imaged immediately. Tubule flow is indicated by the presence of dextran (purple) within the tubule lumen. Flow is seen instantly, and we visualize tubules clearing the dextran. In WT samples, dextran can be seen in all tubules within the field of view. In future studies we will visualize flow through tubules of cystic *Pkd2* mutant mice to determine if there are differences compared to WT.

Methods

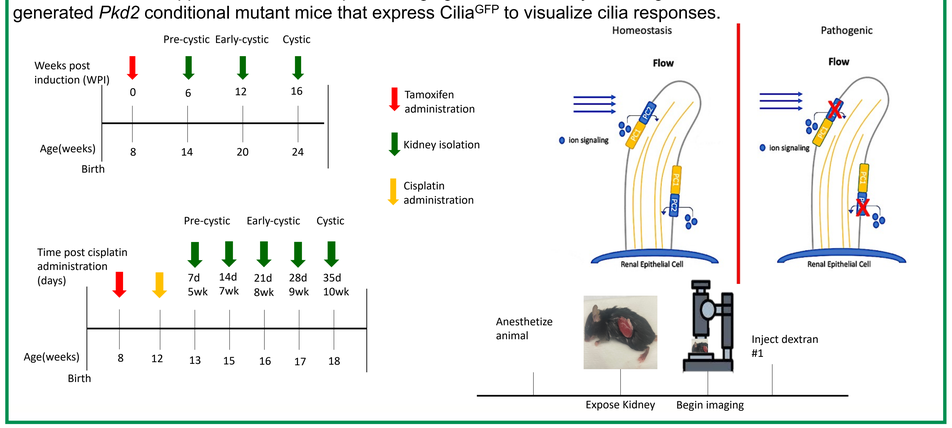
Conditional *Pkd2;CAGG-creER* mice were injected with tamoxifen (3 doses @ 6 mg/40 g BW) at 8 weeks of age. Samples were isolated at three time points after tamoxifen administration: 6 weeks (pre-cystic), 12 weeks (early cystic) and 16 weeks (late cystic). For injured groups, the mice follow the same induction timeline and were given cisplatin (1 dose @ 9 mg/kg BW) at 12 weeks of age and isolated at 7, 14, 21, 28, and 35-days post cisplatin.

Mice were analyzed by intravital imaging (IVI) of a low molecular weight (LMW) fluorescent dextran at all time points after tamoxifen and cisplatin administration to determine whether flow was present or absent within the tubules. Dextran was injected one day prior to isolation of the samples.

Using Fluorescence Activated Cell Sorting (FACS) we quantified the percentage of proximal tubule cells (LTA+) that receive flow (dextran +) or do not receive flow (dextran -).

Immunofluorescence (IF) microscopy on frozen sections were analyzed to visualize dextran absorption, cyst formation, proximal tubule differentiation (LTA, Aqp1, Hnf4a, Megalin, CD13, etc.), and injury response (Sox9, Kim1).

We will use IVI approaches with an optical imaging window to analyze changes in tubule flow *in vivo*. We also generated *Pkd2* conditional mutant mice that express Cilia^{GFP} to visualize cilia responses.



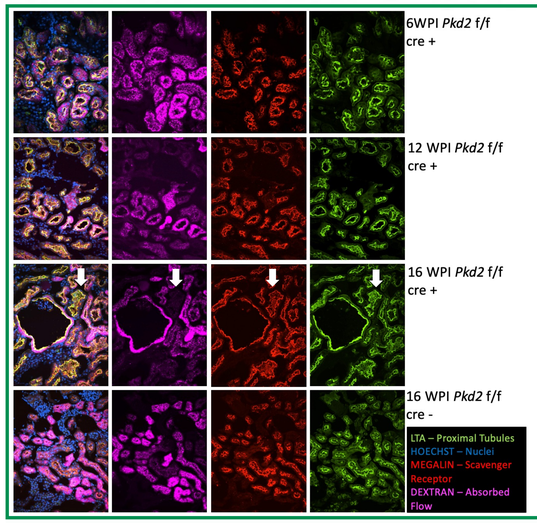


Figure 2: Analysis of tubule flow in adult induced Pkd2 conditional mutants. Mutants isolated at three time points showing the changes in tubule dilations as cysts expand. We note flow is present in progressive stages of cystogenesis using 10kd dextran, which is taken up in the proximal tubules by a scavenger receptor megalin. Some proximal tubules show no flow (no dextran) indicating a disruption to flow at 16WPI post induction (white arrow; proximal tubule with no flow(no dextran).

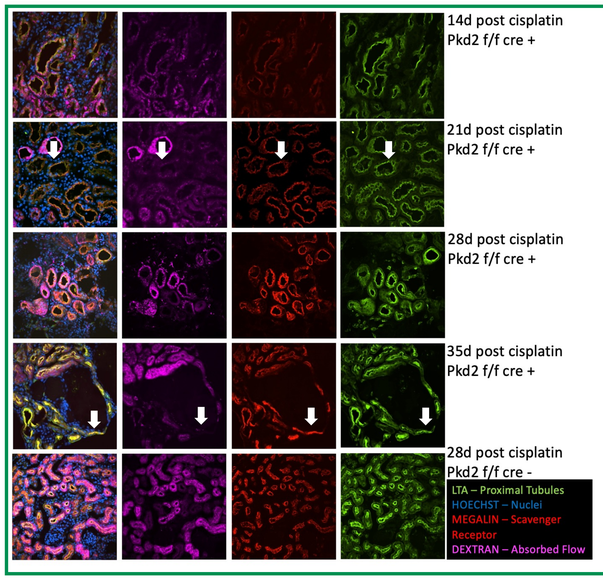


Figure 3: Analysis of tubule flow in adult induced, injured Pkd2 mutants. Mutants isolated at 4 time points showing tubule dilations and flow as cysts expand. We note flow is present at each isolation point during injury induced cyst formation. This data suggests that there is no loss of flow leading to accelerated cytogenesis. With similar data from both non-injured and injured cohorts we report that a loss of flow does not precede cyst formation and both models follow similar initiation methods. Arrows indicate proximal tubules that are megalin and LTA positive that are dextran negative.

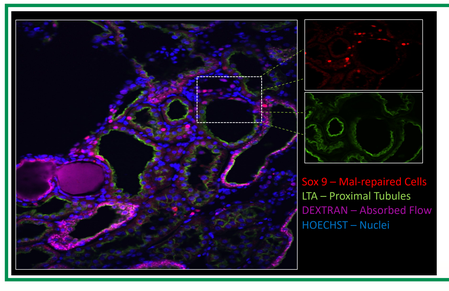


Figure 4: Dextran uptake and PT cell markers in late cystogenesis. Despite advanced proximal tubule cyst formation in *Pkd2* mutant mice (16-weeks post tamoxifen), dextran uptake (purple) is present indicating that tubule flow is still present in cystic tubules. Sox9 (red), an indicator of PT cell injury, is observed in advanced cysts.

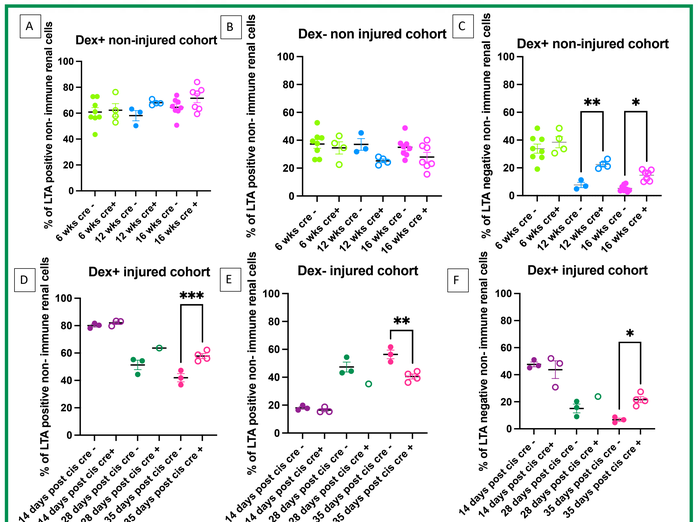
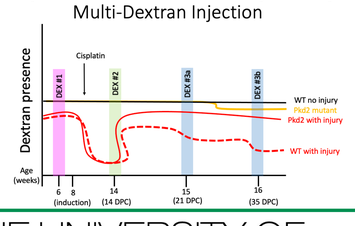


Figure 5: Flow cytometry quantification of proximal tubule cells receiving flow. Graphs A&B show no overall decline in proximal tubule cells receiving flow (Dex+) in non-injured cohorts during cystogenesis. Graph C shows an increase in LTA-/Dex+ cells as cyst progress which correlates to immunofluorescent images showing loss of LTA in some cells in a proximal tubule cyst. Graphs D&E agree with non-injured data where overall flow is not lost and Dex+ cells show a statistical increase in the 35d (cystic) injured cohort. Graph F also agrees with non-injured data and shows an increase in LTA-/Dex+ cells. This data is indicative of the loss of LTA in proximal tubule cells that are still receiving flow.

Conclusions and Future Directions

- Sox 9, an indicator of cell injury is observed in advanced cysts but is not associated with overt changes in tubule flow.
- Cystic tubules continue to receive flow as indicated by a lack of change in dextran uptake.
- Accelerated cystogenesis caused by injury follows a similar initiation mechanism.
- Next, we will examine proximal tubule cell cycle dysregulation and determine whether the response to tubule flow is lost, contributing to cyst expansion.
- Use intravital imaging to study the response of cilia to the presence of flow during cystogenesis.
- Study proximal tubule cell de-differentiation to verify a relationship to cystogenesis.
- Determine if there are ciliary changes during cyst formation and whether these changes are the same following renal injury.
- Utilize bulk RNAseq to identify transcriptional changes during cyst progression in non-injured and injured *Pkd2* mutants
- Use multiple dextran injections in a continuous study to verify tubule flow during cyst progression.



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