Understanding Cyst Formation in Polycystic Kidney Disease: GSK3β's Effect on Anks3

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a genetic condition affecting up to 1 in 400 individuals. It is predominantly caused by mutations in the *PKD1* gene, which codes for the protein Polycystin-1 (PC1). The absence of PC1 causes cysts to grow on both kidneys. Nearly 50% of cases lead to kidney failure, and there is currently no cure and few treatment options.

Many pathways to cyst formation have been discovered, but developing an effective treatment requires an understanding of how these pathways function and interact with one another. We examined the interaction between pathways involving the protein kinase GSK3β, which is inhibited by PC1, and the cilia dependent cyst activation (CDCA) pathway.

Using qPCR and a GSK3β inhibitor, we assessed whether inhibition reduces the expression of a downstream CDCA pathway target GLIS2. These findings suggest GSK3β does not reduce the expression of GLIS2. A better understanding of these pathways will be required to support the development of targeted treatments that suppress cyst growth without affecting normal cell function.

Introduction

Normal Kidney

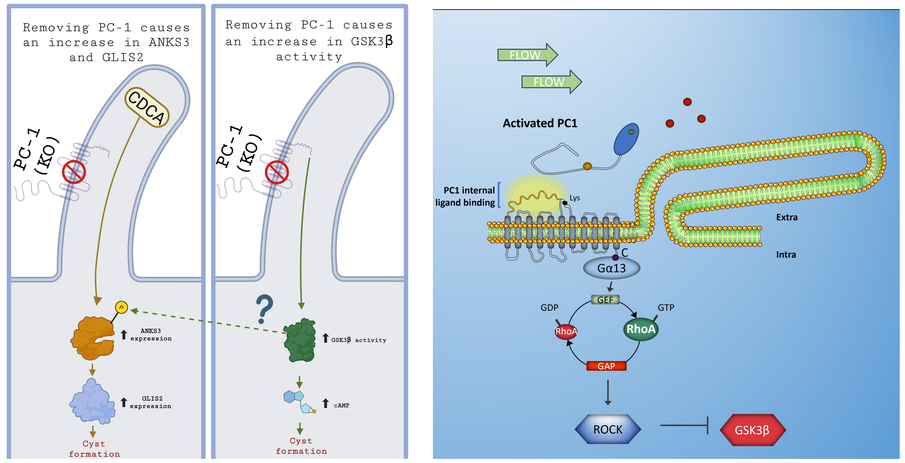
End Stage Cystic Kidney

- Polycystic Kidney Disease (PKD) is a genetic condition predominantly caused by mutations in the gene *PKD1* which causes cysts to grow on both kidneys.[1]
- By age 50, kidneys often expand to five times their normal size, roughly the size of a football.[1]
- Around 50% of cases lead to kidney failure; there is currently no cure and few treatment options.[1]

Citations

1. Boletta, A., & Caplan, M. J. (2025). Physiologic mechanisms underlying polycystic kidney disease. *Physiological Reviews, 105*(3), 1553–1607. https://doi.org/10.1152/physrev.00018.2024
2. Tao, S., Kakade, V. R., Woodgett, J. R., Pandey, P., Suderman, E. D., Rajagopal, M., & Rao, R. (2015). Glycogen synthase kinase-3β promotes cyst expansion in polycystic kidney disease. *Kidney International, 87*(6), 1164–1175. https://doi.org/10.1038/ki.2014.427
3. Walker, R., Maranto, A., Palicharla, V., Hwang, S.-H., Mukhopadhyay, S., & Qian, F. (2022). Cilia-localized counterregulatory signals as drivers of renal cystogenesis. *Frontiers in Molecular Biosciences, 9*, Article 936070. https://doi.org/10.3389/fmolb.2022.936070
4. Wei, Z., Rehman, M., Gu, J., Lerner, G., Dong, K., Roy, K., Cordido, A., Cai, Y., Tian, X., Tham, M. S., Kanyo, J., Lam, T. T., Zhao, H., & Somlo, S. (2025). Anks3 mediates cilia dependent polycystin signaling and is essential for adult kidney homeostasis. *bioRxiv*. https://doi.org/10.1101/2025.04.22.649832
5. Created in BioRender.com

Hypothesized CDCA and GSK3β Interaction



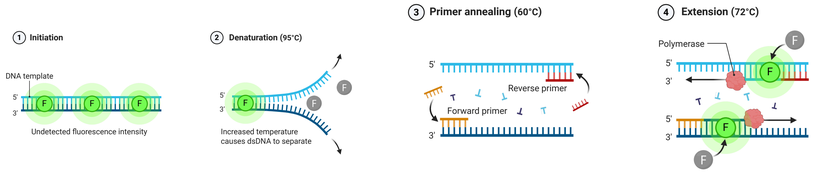
- The presence of cilia has been shown to be involved in the mechanisms that govern cyst formation.[2]
- In the absence of PC1, cilia produce a signal (CDCA) that drives cyst formation.[2]
- The proteins ANKS3 and GLIS2 have been shown to participate in cyst formation as effectors of the CDCA pathway.[3]
- ANKS3 acts upstream of GLIS2: ANKS3 is expressed at higher levels and more heavily phosphorylated *Pkd1* knockout (KO) cells.[3]
- PC1 inhibits GSK3β under normal conditions, reducing its ability to phosphorylate proteins.[4]
- In *Pkd1* KO cells, GSK3β is not inhibited, leading to more kinase activity (phosphorylation).
- **Does GSK3β phosphorylate ANKS3 in *Pkd1* KO cells?**

Hypothesis

- **GSK3β phosphorylates ANKS3, which leads to upregulation of GLIS2, causing cyst formation.**

Methods

Fluorescent Dye-Based Real Time PCR



- A GSK3β inhibitor (SB) was used on Wild-type (WT) and KO IMCD3 (inner medullary collecting duct) epithelial cells.
- qPCR was used to measure mRNA levels of ANKS3 and GLIS2.
- **If GSK3β phosphorylates ANKS3, and GLIS2 is regulated by the phosphorylation of ANKS3, there should be less GLIS2 mRNA when GSK3β is inhibited.**

Results

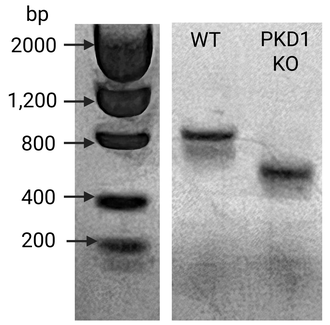


Figure 1: Verification of WT and KO cells via DNA gel genotyping.

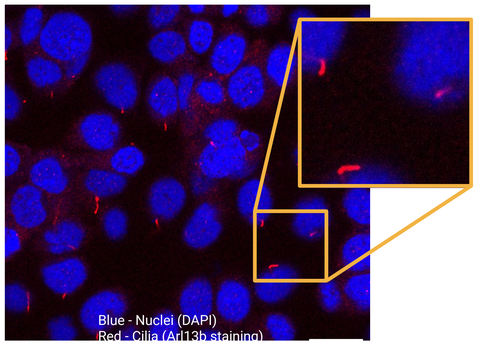
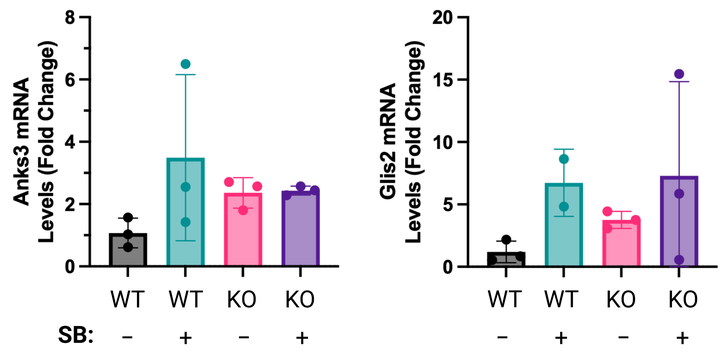


Figure 2: Verification of cilia through ARL13B staining

- The IMCD3 cells were first tested to verify wildtype and *Pkd1* knockout.
- The IMCD3 cells were tested to verify cilia were present.



- ANKS3 levels were not affected in KO cells treated with SB.
- GLIS2 levels did not decrease in the KO cells treated with SB, and may even have increased.

Conclusion

- Previously published results of GLIS2 and ANKS3 upregulation in *pkd1* KO cells were replicated.
- These data indicate that inhibiting GSK3β does not reduce GLIS2 and ANKS3 mRNA transcripts. It may have even increased GLIS2.
- **These data suggest that the initial hypothesis was incorrect, and GSK3β does not play an obligate role in CDCA signal transduction.**

Future Directions

- It may be that GSK3β phosphorylates ANKS3, but if this is the case, these data show that it is unlikely this phosphorylation affects GLIS2 expression.
- Perform a western blot to determine if inhibiting GSK3β affects ANKS3 phosphorylation at the protein level.

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