A Tale of Rescue:
Autosomal Dominant Polycystic Kidney Disease and the C-Terminal Tail of Polycystin-1

David C. Cantong[1], Raj Pandya[1], Laura Onuchic[1], MD, Valeria Padovano[1], PhD, Nikolay P. Gresko[1], MD/PhD, Michael J. Caplan[1], MD/PhD

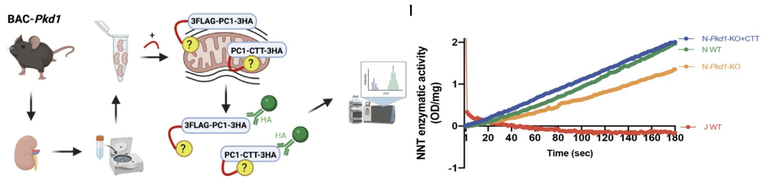
[1]Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT

Introduction

1 in 1,000 people suffer from Autosomal Dominant Polycystic Kidney disease (ADPKD). ADPKD is a painful and debilitating disease that causes cyst formations and abnormal enlargement of the kidneys from fist-size to football-size. Roughly three quarters of ADPKD is caused by a mutation in the *PKD1* gene. *PKD1* encodes the protein Polycystin-1 (PC1). The C-terminal tail (the last 200 amino acids) of PC1 has shown to significantly suppress both cyst formation and growth of the kidneys when it interacts with the mitochondrial enzyme Nicotinamide Nucleotide Transhydrogenase (NNT) (Onuchic et al). These discoveries could provide the potential for future therapeutic use of the C-terminal tail of PC1.

Background

- Onuchic et al. found that in “Knock Out” mouse models (that lack PC1 protein expression), reinsertion of the last 200 amino acids from the C-terminal tail resulted in disease suppression. (**Figure 1b, Onuchic et al.**)
- Suppression is defined as slowing the cystic growth and kidney growth.
- These finding were discovered *in vivo.* (**Figure 1a, Onuchic et al.**)



Methods

- Cell Lines: Mouse Pkd (mPkd) cells.
- Transfection: Plasmid vectors to enter the cell and determine if there was change in NNT enzymatic activity due to the C-terminal tail expression.
- Kinetic Assay: Spectrophotometer to measure NNT enzymatic activity in the separate constructs and cell lines.
- Bradford Assay: Determined protein concentration in the mitochondrial extracts post transfection.
- Western Blot: Separate protein by size and charge and to identify them.

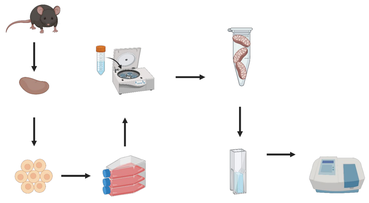


Figure 2: In order to run the enzymatic assay, immortalized mouse kidney cells are maintained, transfected, and then differentially centrifugated to isolate the mitochondria.

Results

Question: Can we develop an *in vitro* system that allows us to reproduce the PC1 C-terminal tail modulation of NNT function observed in animal models? If so, can we employ this system to identify the smallest physiologically relevant portion of the PC1 C-terminal tail?

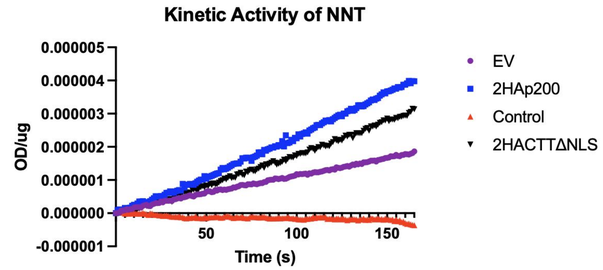


Figure 3: In this figure, 2HAp200 (C-terminal tail insertion) demonstrates more enzymatic activity when compared to the Empty Vector and control.

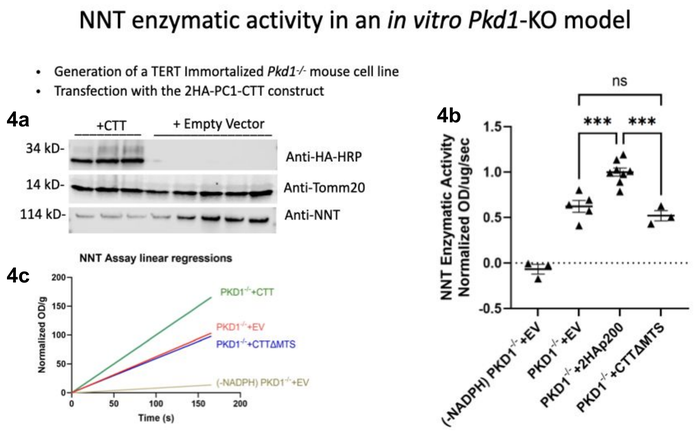


Figure 4a, 4b, 4c: 4a shows that PC1 was successfully transfected in 2HAp200 and not in the Empty Vector. 4b shows the increased NNT enzymatic activity of 2HAp200 compared to the control and Empty Vector. 4c shows the linear regression of NNT in the different constructs.

Conclusions

- We were able to see relatively similar results utilizing both cuvettes and micro-cuvettes, streamlining the amount of protein needed per assay.
- During our experiments, we found that we could indeed replicate the enzymatic activity of previous in vivo derived assays: 2HA-PC1-CTT expression significantly increased NNT enzymatic activity. Furthermore, deletion of the mitochondrial targeting sequence (MTS) contained within the C-terminal tail completely abrogated this effect.
- This opens the door for continuing to run experiments in a time saving and efficient manner.

Future Questions

- Which direction is the enzymatic activity moving toward?
- How can we use the experiment data to make the in vivo model more precise for future experiments?
- What are the long term downstream effects of manipulation of the NNT enzyme?

References

1. The C-terminal tail of polycystin-1 suppresses cystic disease in a mitochondrial enzyme-dependent fashion: Laura Onuchic, Valeria Padovano, Giorgia Schena, Vanathy Rajendran, Ke Dong, Nikolay P. Gresko, Xiaojian Shi, Hongying Shen, Stefan Somlo, Michael J. Caplan bioRxiv 2021.12.21.473680; doi: https://doi.org/10.1101/2021.12.21.473680
2. Dong K, Zhang C, Tian X, Coman D, Hyder F, Ma M, Somlo S. Renal plasticity revealed through reversal of polycystic kidney disease in mice. Nat Genet. 2021 Dec;53(12):1649-1663. doi: 10.1038/s41588-021-00946-4. Epub 2021 Oct 11. PMID: 34635846; PMCID: PMC9278957.
3. Ma M, Tian X, Igarashi P, Pazour GJ, Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. Nat Genet. 2013 Sep;45(9):1004-12. doi: 10.1038/ng.2715. Epub 2013 Jul 28. PMID: 23892607; PMCID: PMC3758452.
4. Images created with www.Biorender.com

Acknowledgements

This project would not have been possible without the support or mentorship from the following wonderful people: The Caplan Lab, Michael Caplan, Laura Onuchic, Raj Pandya, Nikolay Gresko, Vanathy Rajendran, Giorgia Schena, Andrea Reyna-Neyra, Victoria Rai, Nick Iftimia, Forest Mahoney, The Somlo Lab, The Howard Hughes Medical Institute, and the REVU Program. Marla Geha, Jeremy Bradford and the 2022 REVU Fellows.