Defining cellular complexity in human autosomal dominant polycystic kidney disease by multimodal single cell analysis

Yoshiharu Muto, MD, PhD;[1] Eryn E. Dixon, PhD;[1] Yasuhiro Yoshimura, MD, PhD;[1] Haojia Wu, PhD;[1] Chidambaram Ramachandran, PhD;[2] Andrew J. King, PhD;[2] Eric Olson, PhD;[2] Marvin Gunawan, MSc;[2] Stephen L. Seliger, MD;[3] Owen M. Woodward, PhD;[3] Paul A. Welling, MD;[4] Terry J. Watnick, MD;[3] Benjamin D. Humphreys, MD, PhD;[1,5]

[1]Division of Nephrology, Department of Medicine, Washington University in St. Louis, St. Louis, MO, USA; [2]Chinook Therapeutics, Inc., Seattle, WA and Vancouver, BC, Canada; [3]University of Maryland School of Medicine, Baltimore, MD, USA; [4]Johns Hopkins School of Medicine, Baltimore, MD, United States; [5]Department of Developmental Biology, Washington University in St. Louis, St. Louis, MO, USA

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is the leading genetic cause of end stage kidney disease and is characterized by formation and progressive expansion of kidney cysts. Most ADPKD cases arise from mutations in either the *PKD1* or *PKD2* gene, but the precise downstream signaling pathways driving cyst growth are not well understood.

Methods: We performed paired single nucleus RNA-seq and single nucleus ATAC-seq on human kidney tissue samples from 8 ADPKD subjects with end stage kidney disease and 5 control subjects. Nuclear preparations were processed using 10X Genomics Chromium 3' v3 chemistry for snRNA-seq, and Chromium ATAC v1 for snATAC-seq. The libraries were sequenced by NovaSeq and reads were counted with CellRanger to generate data matrices.

Results: We generated ~100,000 single nucleus transcriptomic and ~50,000 single nucleus epigenetic data for ADPKD and control kidneys. The integrated datasets identified 11 primary cell clusters, including most epithelial cell types as well as large fibroblast and leukocyte cell types. Proximal tubular cells from ADPKD kidneys expressed a failed repair transcriptomic signature characterized by profibrotic and proinflammatory mRNAs. We identified distinct principal cell subclusters in ADPKD, one of which was distinguished by expression of GPRC5A+ in cyst lining cells. These cells co-expressed ROR1, a non-canonical Wnt pathway coreceptor that regulates both NF-kB and the Hippo pathway. Consistent with this finding, inflammatory gene expression was enriched in this subpopulation. Additionally, transcription factor DNA-binding motif enrichment analyses of snATAC-seq suggested activation of Hippo pathway effector TEAD3. A cis-coaccessibility network constructed on snATAC-seq data elucidated the enhancer landscape around GPRC5A locus and potential regulatory mechanisms that included cAMP and retinoic acid pathways

Conclusion: We performed single cell multimodal analysis on human ADPKD kidneys, revealing previously unappreciated cellular heterogeneity and potential therapeutic targets in ADPKD.

Funding Sources: NIDDK Support and Commercial Support (Chinook Therapeutics)