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Single cell RNA sequencing of the adult mouse kidney – from molecular cataloging of cell types to disease-associated predictions

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Single-cell transcriptomic analyses have emerged as a powerful tool in dissecting cell diversity and function within complex biological systems[1]. As high-throughput approaches have evolved[2–4], the field has moved towards large datasets comprising thousands of cells or nuclei [5–8]. In a recent report in Science from the Susztak laboratory, *Park et al.* describe single cell RNA sequencing (scRNA-seq) analyses of 57,979 cells from the kidneys of seven adult male mice. Analysis of these data by computational approaches, and follow up studies *in vivo*, led to the authors to three key conclusions: (1) they have developed a comprehensive cell atlas for the mouse kidney and identified novel cell-types, (2) kidney disease-associated genes highlight specific cell-types, thus identifying the cellular origin of each disease, (3) in there is a rare cell type in the collecting system, a rare cell-type which shares features of both intercalated and principal cells, and can transition into either cell-type depending on Notch pathway activity.

What does this study show?

In an attempt to describe the cellular diversity in the adult male mouse kidney, *Park et al.* used scRNA-seq to identify 16 cell-clusters, 13 with profiles expected of known cell-types (nephron, interstitium, immune, and vascular cells) and 3 reported as novel. Secondary clustering showed endothelial, proximal tubule and intercalated cell populations of 3, 3 and 2 distinct cell-types, respectively. Since cell clusters do not necessarily represent individual cell-types, the reported 21 cell-types may not represent the full cellular diversity of the mouse kidney. Consistent with this view, at least 14 cell-types have previously been identified in the nephron and collecting epithelium alone[9] and no signatures are reported for the connecting tubule, parietal epithelium of the renal corpuscle, macula densa cells, and distinct interstitial cell-types of the mesangium and juxtaglomerular apparatus. Of the three novel cell-types, two are highlighted by expression of *Mki67*, a well-known marker of cell proliferation suggesting these populations are most likely an amalgamation of proliferating

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cell types, not novel mature cell types. The third shares both principal cell (PC) and intercalated cell (IC) signatures, and confirms prior observations from the Knepper[11] and Barasch[12] laboratories.

There are some surprising results amongst the clustering and sub clustering. For example, the vasculature endothelial grouping on secondary analysis breaks down into three distinct clusters assigned as vascular endothelium, pericytes and the descending loop of Henle. As these derive from three distinctly different lineages, one might expect each to have separable expression-profiles and not to group together into one related-cluster in the primary analysis. Of these, the descending loop of Henle cluster is identified on the basis of expression of *Aquaporin 1,* which does indeed demarcate the descending limb of the loop of Henle. However, *Aquaporin 1* also labels a subset of renal vasculature[10]; hence, these cells may actually be a distinct vascular component.

In summary, the current level of analysis largely confirms known cell diversity in the mammalian kidney but provides a new depth to the transcriptional profiles within those cell types. Sexual heterogeneity was not addressed in this study of only male mice with, some sex-related differences expected[13]. This should be a focus for future studies. Further, the kidney is organized along a cortical-medullary axis of structure and function, information that is lost on whole kidney dissociation. Ultimately, a complete understanding of cell diversity within the mammalian kidney would also incorporate axial positional information to map cell types to specific regions of the kidney.

scRNA-seq can provide critical information identifying cells expressing disease-related genes. To explore whether monogenic kidney disease-causing genes could be linked to specific cell-types, the authors analyzed expression of 29 genes associated with nephrotic syndrome and proteinuria. Most showed enriched expression in podocytes and the authors concluded podocyte dysfunction is unequivocally the principal reason for proteinuria in this monogenic disease cohort. Similar arguments were applied to other genes and diseases (acidosis, nephrolithiasis, and chronic kidney disease). These are powerful approaches but there are reasons to be cautious in going beyond hypothesis to draw hard conclusions. In the absence of knowing when disease states are established, it may be difficult to draw cell assignment inferences based on gene-to-cell correlations in the adult kidney. Further, if a cell cluster is composed of a single cell-type such as the podocyte, specific genes may be readily detected by scRNA-seq in those cells, but poorly in other cells and consequently overlooked in comparative cell-averaging approaches. Although additional work is necessary to confirm that the disease target genes indicated in *Park et al.* are really as uniquely expressed as the current level of analysis suggests, this interesting study adds to the ongoing discourse in pinpointing disease to its cellular roots.

ICs and PCs are collecting duct cells that regulate acid-base balance and sodium, potassium, and water levels, respectively. *Park et al.* describe a transitory cell-type displaying gene expression characteristic of both ICs and PCs. A similar cell-type has been reported in the developing and adult mouse kidney through immuno-staining approaches and in scRNA-seq analyses focused on the collecting system[11,12]. Using genetic lineage tracing approaches to extend published studies[12], the authors activated a reporter gene within either the PC or the

IC lineage. Both crosses labelled PC and IC cells, consistent with previous studies arguing for an intermediate progenitor, with shared PC and IC gene signatures. A key question on the functional significance of this population in the adult remains unanswered as the genetic labelling in this strategy starts in the fetal kidney. Temporally-refined genetic tools that enable adult specific cell labelling can provide a definitive insight.

Several studies have linked Notch signaling to promoting the PC fate in the developing kidney[14]. In the adult, IC cells produce the Notch ligand Jagged1 while PC cells show ongoing Notch signaling[12]. *Park et al.* confirmed *Jagged1* expression by ICs in the scRNA-seq dataset and explored the potential for Notch signaling to modulate IC and PC ratios in the adult kidney by ectopic activation of the Notch signaling pathway. Consistent with plasticity amongst mature cell-types, or directed differentiation of PC/IC dual signature cell-types, the authors observed a modest increase in the PC to IC ratio. Up-regulated Notch signaling has been reported in patients and animal model with kidney disease[15,16]. *Park et al.* examined a folic acid model of chronic kidney disease and observed a loss of intercalated cell-types and acidosis consistent with a gain-of-Notch function. However, no direct link was established with the Notch pathway. Further, through a deconvolution of kidney biopsies from patients with diabetic kidney disease, *Park et al.* suggest an elevated ratio of PC to ICs, an interesting proposition that warrants scrutiny by more direct approaches.

How does this study compare with prior studies?

From the perspective of scRNA-seq analysis of cell complexity in the mammalian kidney, prior studies have largely focused on the developing mouse[8,17] and human kidney[6,18], or a specific lineage within the adult mouse kidney[11]. However, a recent large scale scRNA-seq analysis of adult organs in the mouse, included 6665 single cells from the kidney[19]. It will be interesting to compare and contrast these datasets with those of *Park et al.* to provide insights into protocol and sex-specific biases, given protocol variations and the inclusion of male and female kidneys in other studies[19].

What are the implications for nephrologists?

Single-cell transcriptomics tools are enabling a new wave of analyses of biological systems and disease. These resources “democratize science” empowering anyone to download and scrutinize data with a specific hypothesis in mind. In this new era, published datasets will be thoroughly mined with robust algorithms and the strengths and weaknesses of datasets will be found, and the technical and analytical strategies refined. *Park et al.* report a large dataset of adult mouse cells and the data will be a valuable resource to understand cell-types in the mouse. The value of these datasets in linking disease genes to a given cell type is well-illustrated by the Park et al study of podocyte transcriptional signatures. With potential gene/cell origin disease association, researchers are in a strong position to generate accurate disease models to understand disease states, and identify novel therapeutic strategies. Parallel efforts to obtain large datasets for normal and diseased human organs, including the kidney, are progressing (Human Cell Atlas: https://www.chanzuckerberg.com/human-cell-atlas; Kidney Precision Medicine Project: https://www.niddk.nih.gov/research-funding/research-programs/kidney-precision-medicine-project-kpmp). These will become important

resources for the nephrologists identifying and tracking disease. scRNA-seq datasets that fully incorporate normal cell diversity are also an essential resource for programs aiming to reconstruct normal kidneys (ReBuilding a Kidney: Oxburgh et al., 2017, https://www.rebuildingakidney.org/).

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