Single-Cell RNA Sequencing Identifies Cell-Type Specific Cellular Responses To Renal Ischemia-Reperfusion Injury

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Background: Therapeutic options for treating acute kidney injury (AKI) and subsequent development of chronic kidney disease (CKD) are limited, leaving affected patients at high risk of cardiovascular events, end-stage kidney disease, and mortality. The lack of clear molecular understanding of its pathogenesis and renal reparative pathways contributes to this scarcity of targeted therapeutics. Recent technological advancements in single-cell RNA sequencing have revolutionized our understanding of complex and dynamic tissues such as the kidney. However, optimization is still required to successfully apply this technology to rodent AKI models.

Hypothesis: Understanding cellular events in AKI at single-cell resolution will guide us to develop new therapeutic strategies.

Methods: We have optimized the kidney digestion protocol to achieve high viability (>95%) and very few doublet formations to avoid flow-cytometry-based cell isolation. We used our unilateral ischemia-reperfusion injury (IRI) mouse model, which causes severe renal atrophy at 21 days after IRI. Droplet-based single-cell RNA-seq libraries were created and sequenced from a total of 10,000 cells from both injured (IRI) and contralateral kidneys (CLK) using a Drop-Seq platform. Single-cell transcriptome profiles were clustered and annotated based on the expression patterns of known marker genes.

Results: Our t-distributed Stochastic Neighbor Embedding (tSNE) analyses identified at least 25 clusters in our combined dataset of IRI and CLK. We captured podocytes in 1.97% of total cells, which is close to the published single-nucleus RNA-seq dataset (2.4%; Wu et al., JASN 2019). There was clear separation among epithelial cells between IRI and CLK kidneys. We successfully mapped the known expression pattern of epithelial injury marker genes such as *Havcr1* (encoding kidney injury molecule1, KIM1), *Lcn2* (encoding neutrophil gelatinase-associated lipocalin, NGAL), and cytokeratins. Gene ontology analyses identified unique cell-type-specific signaling in each cellular cluster, such as oxidative stress responses in the KIM1-expressing proximal tubular segment.

Conclusions: We have developed an optimized platform for generating and analyzing the single-cell transcriptome of mouse kidneys that underwent IRI. Future studies using this platform will inform us as to how each cell responses/adapts to ischemic stress during the course of the disease and guide us to identify novel therapeutic approaches for AKI and its transition to CKD.