TRANSPLANTATION

Single-cell analysis of a biopsy sample

Despite advances in technology for tissue characterization, the techniques that are used to analyse kidney biopsy samples have not changed substantially in the past 25 years. Now, Haojia Wu, Andrew Malone and colleagues have used single-cell RNA sequencing (scRNA-seq) to profile a kidney allograft biopsy sample from a patient with mixed T cell-mediated and antibody-mediated rejection (ABMR).

"We used a massively parallel microfluidic droplet technology called InDrops to perform scRNA-seq of the biopsy sample," says Wu. "This approach allowed us to measure the expression of thousands of genes from thousands of individual cells." In total, the researchers sequenced 4,487 cells and detected a mean of 1,481 transcripts from 827 genes per cell. They identified 16 different cell types in the allograft sample, including 4 types of tubular cells, various lymphocyte and leukocyte populations, 3 types of stromal cells, endothelial cells and a population of actively proliferating cells. A comparison of the epithelial transcriptome of the allograft with that of healthy adult kidney tissue showed downregulation of terminal differentiation markers and upregulation of pro-inflammatory genes in the allograft.

"We have defined the heterogeneity of cell types and cell states in an allograft biopsy at single-cell resolution," says Malone. "Our study provides a proof-of-principle for what we believe may be a transformative advance in the way biopsies are read. For example, bulk-tissue resolution profiling has established that endothelial cells have critical roles in ABMR; our single-cell resolution profiling revealed that there are three different endothelial states in mixed rejection: a resting state, an angiogenic state and an Ig phagocytosis state."

The researchers are now scaling up their scRNA-seq technique to enable the detection of rare cell types and finer distinctions among different cell subtypes. "The goal is to generate clinically useful data sets to improve diagnostic and prognostic accuracy, enable disease subphenotyping and accelerate adoption of molecular biopsy interpretation," says Wu.

Ellen F. Carney

ORIGINAL ARTICLE Wu, H. et al. Single-cell transcriptomics of a human kidney allograft biopsy specimen defines a diverse inflammatory response. *J. Am. Soc. Nephrol.* https://doi.org/10.1681/ASN.2018020125 (2018)

PODOCYTOPATHIES

Pivotal role of CaMK4 in podocyte injury

Disruption of podocyte structure and function ultimately leads to chronic kidney disease. A new study by George Tsokos and colleagues reports a key role of calcium/calmodulin-dependent protein kinase type IV (CaMK4) signalling in podocyte dysfunction.

The researchers found that podocyte CaMK4 expression is upregulated in patients with lupus nephritis (LN) or focal segmental glomerulosclerosis (FSGS) and in mouse models of podocyte injury. To specifically inhibit podocyte CaMK4 in mice, they used nanolipogels loaded with a CaMK4 inhibitor and tagged with either anti-podocin or anti-nephrin antibodies. Treatment with these nanolipogels protected mice against non-immune podocyte injury induced by polysaccharide or adriamycin (a model of FSGS) and prevented podocyte foot process effacement and the development of LN in lupus-prone mice.

"Our most exciting finding was that when we inhibited podocyte CaMK4 in lupus-prone mice, we did not see any dense deposits in the glomeruli despite the fact that autoimmunity was rampant in the periphery," says Tsokos. "My interpretation of this observation is that if podocytes keep their structural and functional integrity, deposition of immune complexes does not occur. This concept upsets the classic dogma that immune complexes deposit indiscriminately and cause inflammation."

Further investigations indicated that CaMK4 suppresses nephrin expression and increases podocyte motility and actin cytoskeleton remodelling via a mechanism involving increased expression of activated Rac1 and synaptopodin degradation. By contrast, podocyte-specific inhibition or silencing of CaMK4 preserves synaptopodin expression and protects against damage to actin fibres and the cytoskeleton.

"CaMK4 is a molecule of pathogenic importance for immune and non-immune kidney diseases," concludes Tsokos. "Our findings could have repercussions for the treatment of patients with LN and FSGS."

Ellen F. Carney

ORIGINAL ARTICLE Maeda, K. et al. CaMK4 compromises podocyte function in autoimmune and nonautoimmune kidney disease. *J. Clin. Invest.* https://doi.org/10.1172/JCI99507 (2018)

DEVELOPMENT

Hedgehog–TGFβ signalling controls nephrogenesis

Stromal cells are crucial for nephron development through their interaction with nephron progenitor cells (NPCs), but little is known about the pathways involved. Now, Rowan et al. describe a Hedgehog (HH)–GLI–TGFβ axis that controls nephrogenesis.

"In our previous work, we noted a zone of HH activity in the cortical stroma of the embryonic kidney," explains lead investigator Norman Rosenblum. To investigate this further, the researchers generated mice with stromal-specific deficiency of the HH effector Smoothened (SMO). In embryonic day 15.5 (E15.5) SMO-deficient mice, the renal stromal capsule was disrupted and the number of NPCs was increased. Surprisingly, however, kidney size and nephron number were reduced in SMO-deficient newborn and E18.5 mice, respectively. Thus, stromal HH signalling cell-autonomously controls renal capsule formation and non-cell-autonomously controls NPC number.

Further analysis suggested that defective mesenchymal–epithelial transition in NPCs was responsible for the reduced nephron number in SMO-deficient mice. Moreover, HH signalling exerts these effects on stromal cells and NPCs by controlling the levels of the transcription repressor GLI3R. RNA sequencing of E12.5 kidneys of wild-type and SMO-deficient mice revealed that TGFβ2 was differentially expressed. Inhibition of TGFβ2 in wild-type embryonic kidney explants reduced nephron number, and deletion of TGFβ2 receptor 2 (*Tgfbr2*) in both stromal cells and NPCs mostly recapitulated the renal defects in SMO deficiency, suggesting that TGFβ2-dependent crosstalk between stromal and cells and NPCs is important for mouse kidney development.

"Our work provides a foundation to study the molecular basis of nephron deficiency, an important cause of childhood and adult-onset kidney disease," says Rosenblum.

Grant Otto

ORIGINAL ARTICLE Rowan, C. J. et al. Hedgehog-GLI signaling in *Foxd1*-positive stromal cells promotes nephrogenesis via TGFβ signaling. *Development* https://doi.org/10.1242/dev.159947 (2018)