

Single cell RNA sequencing of the human kidney: Identifying new drug targets for kidney fibrosis

Presenter: Christoph Kuppe, Germany

Chairs: Ronald Beatriz Sanchez Alamo, Martin De Borst

Written by: Jasna Trbojevic-Stankovic

Single-cell RNA sequencing (scRNA-seq) is a relatively new, cutting-edge technology in the genomics area. The fundamental objective of this technology is to provide previously unattainable insights into the gene expression patterns in individual cells. With scRNA-seq scientists can examine the molecular profiles of cells and tissues, which can be crucial for identifying new medication targets for conditions such as kidney fibrosis. Although this method has been exploited for some time, it has been revolutionized in recent years by the development of microfluidic chips. These chips have substantially increased the throughput of the procedure, enabling researchers to analyse tens of thousands, to even a million cells, in a single experiment. The gel beads (Fig. 1) that are used in these microfluidic chips are functionalized with unique DNA barcodes, which are used to identify individual cells in the sample. The cells and the necessary reagents for reverse transcription are added to the gel beads, which are then emulsified in an oil solution. Emulsification creates droplets in the oil solution, which contain the cells and the reagents. The gel beads and the oil solution are then loaded onto the microfluidic chip to perform reverse transcription. This process returns DNA molecules, which are then amplified by the polymerase chain reaction (PCR) technique. The PCR amplification allows researchers to quantify the number of transcripts of a particular gene in the sample.

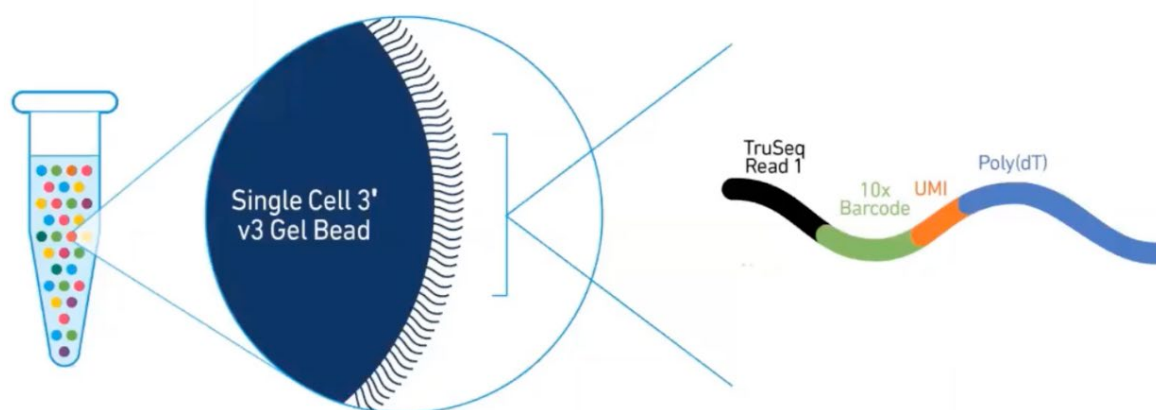


Figure 1. Single cell RNA sequencing: gel bead coated with unique DNA molecules

One of the key innovations in single-cell RNA sequencing is the use of unique molecular identifiers (UMIs) in the DNA barcodes, which allow researchers to distinguish between the transcripts of a single gene in the sample, and to quantify the number of transcripts accurately. In addition to the UMIs, the DNA barcodes also contain a poly deoxythymine primer sequence, which captures the polyA tail of the mRNA. Although this only catches a portion of the transcript, it is enough to count the number of transcripts of a particular gene in the sample. The computational tools and algorithms available for analyzing this data have also improved, including computational pipelines. These biocomputational

tools, such as Scanpy and Seurat, allow for quality control, data normalization, feature selection, dimensional reduction, and cell clustering.

Applying sc-RNA sequencing in nephrology

One of the main goals in nephrology practice is to understand the origin of kidney function decline and disease progression. Fibrosis is a well-described factor that contributes to organ dysfunction and is the final common pathway in many organ failures, including the kidneys. Understanding the heterogeneity of this process and its plasticity is essential to understand the mechanisms that drive the expansion and regression of fibrosis. Additionally, it is essential to understand where myofibroblasts come from, where they differentiate and what drives this process.

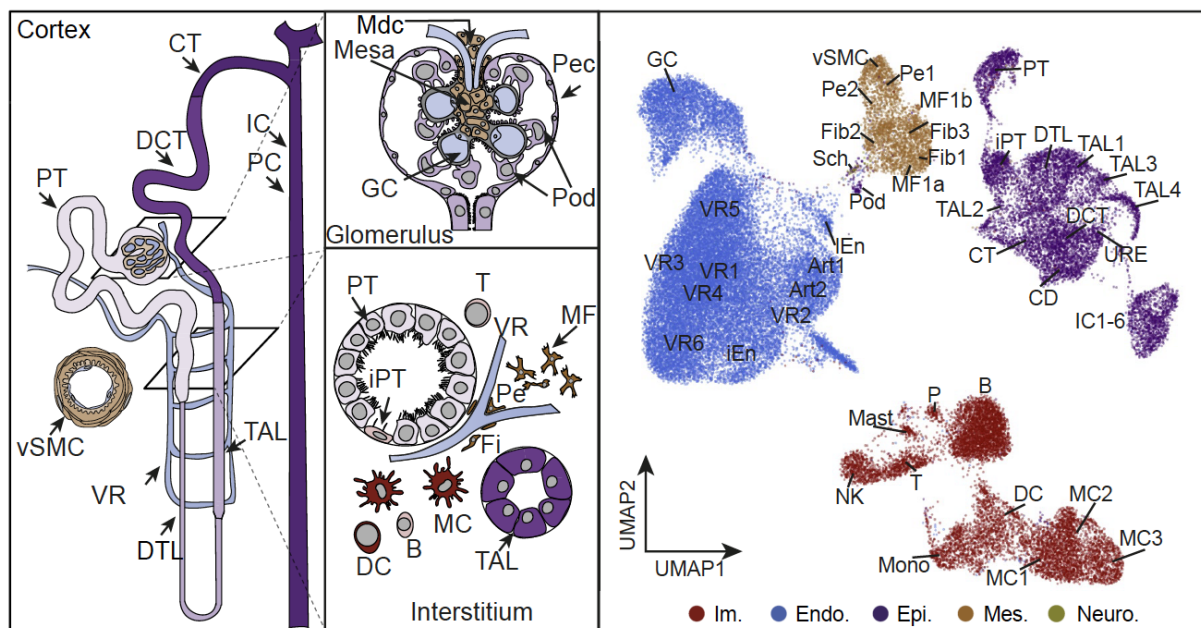


Figure 2. Mapping of the kidney cells

In a recently published study, Kuppe et al. aimed to map all matrix-producing cells in the human kidney to identify the sources of scar-forming myofibroblasts in kidney fibrosis. The research team established a protocol for sampling tumour-distant locations in the tissue during cancer-related resection. Since the kidney is overrepresented by epithelial cells in the cortex, they divided the cell population into an epithelial cell-enriched and a mesenchymal cell-enriched population to understand the development of fibrosis and mesenchymal cells. This required selecting a marker for FACS to optimize how to digest the kidney tissue. Research also focused on developing a protocol to extract nuclei and generate single nuclei RNA sequencing for bio-archived and frozen tissue.

The team characterized the clinical covariates of the patients by looking at the histopathology of the samples. They generated human kidney single-cell transcriptomes from 17 patients and captured different immune cell compartments, endothelial cell compartments, and mesenchymal cells. The authors generated a cell marker catalogue containing unique genes expressed in the kidney, for example only by parasites, glomerular endothelial cells, proximal tubular cells, and Schwann cells. They also captured biology in samples from patients with GFR decline and overexpression of extracellular matrix, and also identified key dysfunctional pathways, such as the fatty acid metabolism, associated with CKD.

The extracellular matrix genes were mostly expressed in the mesenchymal cell cluster, but some cells expressed them less than others. The team developed a score and identified where the single collagen genes were expressed, which enabled them to infer lineages.

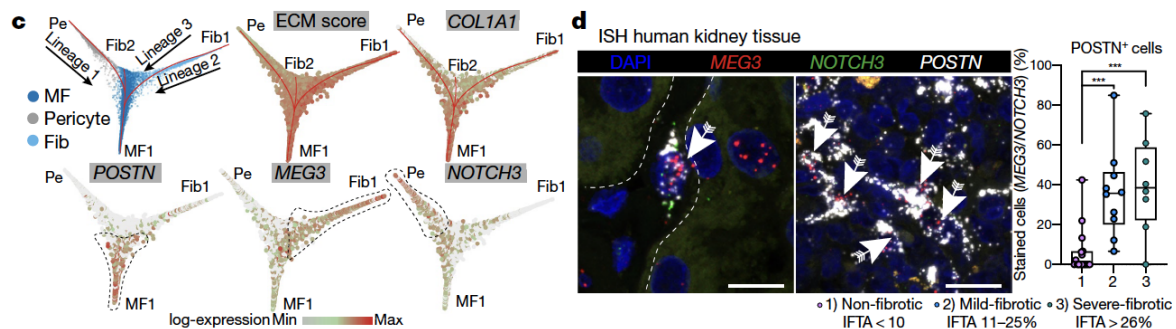


Figure 3. Myofibroblast differentiation lineages

Through diffusion mapping, researchers defined the lineages of differentiation, identified distinct marker genes, and validated the directionality of the diffusion graphs. Additionally, they analysed stromal signalling and identified a strong interaction between myofibroblasts and immune cell compartments. Finally, they used a combination of cell populations and genes associated with high expression of collagen alpha 1 to identify a Wnt pathway regulator, NKD2, as a potential anti-fibrotic target. It was then validated through in situ hybridization and CRISPR-Cas9 experiments.

Understanding the different types of cells involved in fibrosis is essential for developing therapeutic strategies to reduce the progression of kidney disease. These results significantly contribute to the present knowledge and understanding of renal fibrosis as the underlying mechanism of CKD. The work demonstrated the power of single-cell sequencing technology to capture the heterogeneity of cell populations and generate insights into the biology of kidney fibrosis. This research could provide the foundation for the development of therapies to reduce the progression of kidney disease.

Key points

1. Recent research and experiments generated a valuable atlas of human kidney single-cell data of patients with CKD.
2. Distinct pericyte and fibroblast subpopulations are the major sources of kidney myofibroblasts. These can be identified with sc-RNA sequencing.
3. Endothelial and epithelial cells express some extracellular-matrix molecules but do not become true myofibroblasts.
4. NKD2 is a potential anti-fibrotic target.

Further reading

- (1) Kuppe C, Perales-Patón J, Saez-Rodriguez J, Kramann R. Experimental and computational technologies to dissect the kidney at the single-cell level. *Nephrol Dial Transplant*. 2022;37(4):628-637. doi: 10.1093/ndt/gfaa233.
- (2) Kuppe C, Ibrahim MM, Kranz J, et al. Decoding myofibroblast origins in human kidney fibrosis. *Nature*. 2021;589(7841):281-286. doi: 10.1038/s41586-020-2941-1.

