Myeloid heterogeneity in kidney disease as revealed through single cell RNA sequencing

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Key Points:
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Kidney disease represents a global health burden of increasing prevalence and is an independent risk factor for cardiovascular disease. Myeloid cells are a major cellular compartment of the immune system; they are found in the healthy kidney and in increased numbers in the damaged and/or diseased kidney, where they act as key players in the progression of injury, inflammation and fibrosis. They possess enormous plasticity and heterogeneity, adopting different phenotypic and functional characteristics in response to stimuli in the local milieu. Though this inherent complexity remains to be fully understood in the kidney, advances in single-cell genomics promises to change this. Specifically, single-cell RNA sequencing (scRNA-seq) has had a transformative effect on kidney research, enabling the profiling and analysis of the transcriptomes of single cells at unprecedented resolution and throughput, and subsequent generation of cell atlases. Moving forward, combining scRNA- and single-nuclear RNA-seq with greater resolution spatial transcriptomics will allow spatial mapping of kidney disease of varying aetiology to further reveal the patterning of immune cells and non-immune renal cells.

This review summarises the roles of myeloid cells in kidney health and disease, the experimental workflow in currently available scRNA-seq technologies and published findings using scRNA-seq in the context of myeloid cells and the kidney.

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Myeloid heterogeneity in kidney disease as revealed through single-cell RNA sequencing

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Abstract

Kidney disease represents a global health burden of increasing prevalence and is an independent risk factor for cardiovascular disease. Myeloid cells are a major cellular compartment of the immune system; they are found in the healthy kidney and in increased numbers in the damaged and/or diseased kidney, where they act as key players in the progression of injury, inflammation and fibrosis. They possess enormous plasticity and heterogeneity, adopting different phenotypic and functional characteristics in response to stimuli in the local milieu. Though this inherent complexity remains to be fully understood in the kidney, advances in single-cell genomics promises to change this. Specifically, single-cell RNA sequencing (scRNA-seq) has had a transformative effect on kidney research, enabling the profiling and analysis of the transcriptomes of single cells at unprecedented resolution and throughput, and subsequent generation of cell atlases. Moving forward, combining scRNA- and single-nuclear RNA-seq with greater resolution spatial transcriptomics will allow spatial mapping of kidney disease of varying aetiology to further reveal the patterning of immune cells and non-immune renal cells.

This review summarises the roles of myeloid cells in kidney health and disease, the experimental workflow in currently available scRNA-seq technologies and published findings using scRNA-seq in the context of myeloid cells and the kidney.

Introduction

The kidney is a complex organ that performs diverse functions essential for physiological homeostasis and consequently, patients with kidney disease often present with significant complications and comorbidities[1-2]. The manifestations of kidney disease are wide-ranging, encompassing acute kidney injury (AKI), autoimmune disorders and rejection of kidney transplants, all of which are directly or indirectly immune-mediated[3]. Due to an increased prevalence rate, kidney disease is having a major effect on global health, both as a direct cause of morbidity and mortality, and as a major risk factor for cardiovascular disease[1].

Myeloid cells are the most abundant nucleated haematopoietic cells in the body, encompassing monocytes, macrophages, dendritic cells (DCs) and granulocytes. They make up a critical arm of the immune system and have diverse functions[4]. In the kidney, macrophages and DCs have most notably been shown to influence organ homeostasis, injury and reparative processes following diverse insults. Their function, phenotypic spectrum and interplay with other immune and non-immune cells is complex and incompletely understood[3,5].

Our understanding of the immune system has advanced through the application of technologies that allow single cell resolution, primarily microscopy and flow cytometry. The number of parameters that can be measured simultaneously with these is limited and reliant on prior knowledge of which antigens are present. Over recent years, progress in single-cell RNA sequencing (scRNA-seq), which aims to measure the expression levels of genes in cells in a comprehensive way, has transformed our ability to profile immune cells[2,6-7].

Roles of myeloid cells in kidney health and disease

Myeloid cells have an adaptive nature, demonstrating transient responses, controlled differentiation, and (re)location into tissues as well as plasticity in response to various environmental stimuli, whether they are homeostatic or inflammatory[4]. This represents a complication regarding their study within the kidney, as this major haemo-filtration organ offers a challenging milieu that is not spatially uniform, and differences in immune cell types in the renal cortex and medulla have been reported[8,9].

Macrophages are the most abundant myeloid cell type in the kidney and, together with DCs, coordinate inflammatory responses to freely filtered antigenic material and safeguard the

kidney from infection[8]. Importantly, they are also involved in initiation and progression of renal disease, as well as the subsequent tissue regeneration. Due to this diversity, it is generally suggested that macrophages are divided into proinflammatory (M1 or 'classically activated') and tissue reparative (M2 or 'alternatively activated') phenotypes. However, this characterisation is oversimplified with not only macrophages, but also DCs possessing inherent plasticity. Indeed, there is robust evidence for disease-associated myeloid cells sharing signature marker genes of both pro- and anti-inflammatory characteristics[4,10].

Immune cells are typically defined by different cell surface markers, though different myeloid cell types have many markers in common, therefore, several will often be required to identify the cell type of interest. In addition, markers can differ between species (*Table. 1*). Immunohistochemistry and flow cytometry are often used to identify both intra- and extracellular markers[6]. In mice, kidney resident macrophages ($CD11b^{lo}F4/80^{hi}$) are of dual haematopoietic origin arising from the yolk-sac and from definitive haematopoiesis, with $Ly6C^{lo}$ 'patrolling' monocytes ($CD14^{lo}CD16^{hi}$ in humans)[11-13]. Under inflammatory conditions, $Ly6C^{hi}$ monocytes ($CD14^{hi}CD16^{-}$ in humans) infiltrate the kidney, differentiating into ($CD11b^{hi}F4/80^{lo}$) macrophages[11-13]. Despite being limited to around 16 parameters and problems that arise from spectral overlap, flow cytometry in particular has been very useful and represents the most basic single cell technology, but it is unlikely to provide the full phenotypic spectrum of myeloid cells[6-7, 10].

Overview of single-cell sequencing technologies

High throughput gene expression techniques, such as microarray and bulk RNA-sequencing, have provided a better understanding of complex organ transcriptomes, however, their RNA input requirements have limited studies to pooled populations. As the kidney contains more than 20 distinct cell types including: epithelial, endothelial, mesenchymal, and immune populations, the resulting gene expression profile of renal tissue reflects only an average for these heterogeneous constituents[14]. Within the kidney the proximal tubule cells dominate in number, thus can obscure the expression profile of rarer populations. scRNA-seq technologies have addressed these limitations by enabling the objective investigation of transcriptomes of individual cells in a given sample, with generation of cell atlases of mammalian renal components. Notably, the kidney research community has been invested in open access and has made these data freely available via websites (*Table. 2*). This permits the

unbiased assessment of cellular heterogeneity, identification of new cell states and subtypes, and dynamic cellular transitions at very high resolution and accuracy[6,14-15].

To date, a number of scRNA-seq techniques have been proposed, and the development of new protocols is a very active area of research (as reviewed by [16]). Generally, all scRNA-seq experiments share a common workflow: sample preparation, single-cell capture, reverse transcription and transcriptome amplification, library preparation, sequencing, and analysis (*Figure. 1*).

Single-nuclear sequencing (snRNASeq) is an increasingly popular alternative to scRNAseq. Here, nuclei are isolated from cells and used for droplet-based sequencing[14]. snRNA-seq has been reported to have comparable gene detection to scRNA-seq in the adult kidney and to eliminate dissociation-induced transcriptional stress responses in the inflamed fibrotic kidney. Moreover, it is compatible with frozen samples, but captures immune cells with lower efficiency[19]. The reason for this remains unclear. As such, it might be necessary to isolate $CD45^{+}$ cells, which only account for 2-17% of the total kidney cell population[20], in order to obtain the greatest possible insight into renal leukocytes using this method.

Numerous other single-cell techniques are under development to allow the direct measurement of microRNA, proteomic or metabolic data, which scRNA-seq and snRNA-seq approaches do not provide. Most notably, simultaneous expression profiling of transcripts and cell surface proteins, such as RNA expression and protein (REAP)-seq[21] and cellular indexing of transcriptomes and epitopes (CITE)-seq[22] is now possible, permitting the correlation of protein expression with transcriptomic data. In addition, Assay for Transposase-Accessible Chromatin (ATAC)-seq allows profiling of chromatic accessibility enabling investigation of the epigenetic heterogeneity[23]. It has recently been used in combination with snRNA-seq to identify unique cell states within the proximal tubule and thick ascending limb in the human kidney[24], as well as key chromatin remodelling events and gene expression dynamics associated with kidney development in mice[25] (*Figure. 2*).

Due to the kidney having distinct regional differences, anatomic localisation and intercellular interactions of cells is important, yet it is largely lost when analysing dissociated single cells. Interactions may be somewhat gleaned from receptor-ligand pair analyses for chemokines and cytokines, however, direct visualisation of the spatial relationships among cells is needed

to fully answer such questions. This further means that it is not possible to discriminate leukocyte populations that infiltrate or reside in the kidney from those that may be circulating through the kidney vasculature[6,15]. Combining scRNA-seq and snRNA-seq with spatial transcriptomics may improve this aspect. Indeed, Ferriera and colleagues recently utilised these complementary technologies to spatially map the transcriptomic signature in mouse AKI models and demonstrated how this may be applied to human samples[26]. They identified patterns of immune and epithelial cell co-localisation and although this it was limited by resolution, it is likely to improve with future work.

scRNA-seq studies in kidney health

A comprehensive cellular anatomy of the normal kidney is crucial to completely understand the development and resolution of kidney disease yet to date, attempts to fully map immune populations in the kidney in health are limited. In 2018, Park and colleagues provided the first and largest scale murine kidney cell scRNA-seq so far from healthy mouse kidney[2]. They identified major cell subtypes that comprise the nephron and previously known renal immune cell types, including resident macrophages and neutrophils. However, it is possible that some myeloid subtypes were undetected, perhaps due to immune cell types contributing a small proportion in the uninjured kidney and/or due to sample preparation as approximately 25% of cells did not pass quality control in this study.

A recent key study used scRNA-seq with flow and mass cytometry to define the global immune landscape in healthy human adult and foetal kidneys, profiling cells across the lifespan[9]. Mononuclear phagocytes (MNPs), natural killer (NK) cells and T cells were most prevalent, and within the mature kidney myeloid compartment four subsets of MNPs, neutrophils, mast cells, and plasmacytoid DCs (pDCs) were identified. The MNP cluster was dominated by two monocyte-derived macrophage populations; one was transcriptionally similar to $CD14^{+}$ classical monocytes, and the other to $CD16^{+}$ non-classical monocytes. It also contained a conventional DC (cDC) population and tissue-macrophage population that was skewed toward an anti-inflammatory M2 transcriptome expressing CD206. In addition, researchers demonstrated anatomical localisation of immune cells by referencing their work to publicly available bulk RNA-seq data generated from known kidney regions, revealing differential distribution of immune subsets in the medulla and pelvis compared to the cortex. Analysis of ligand-receptor interactions, indicating epithelial and immune cell crosstalk, was predicted to localise antibacterial macrophages and neutrophils to regions of the kidney most

susceptible to ascending infection from the urinary tract. This is in line with previous work that showed high interstitial sodium concentration of the medulla stimulates the production of chemokines by tubular epithelial cells, which helps position macrophages to counter the immunological threat posed by ascending bacterial infection[8]. The capability to orchestrate localisation of immune cells to specific regions was absent in foetal kidneys, with postnatal acquisition of transcriptional signatures indicative of roles in inflammation and immune defence[9]. Another recent study by Menon and colleagues identified resident immune cells in non-diseased human samples from tumour-nephrectomy, surveillance, and pre-perfusion biopsies; these included two myeloid clusters, although they were not defined into subtypes[27].

Dynamic changes in the immune system are a hallmark of ageing[28]. Recently, scRNA-seq was performed on >350,000 cells from several organs and tissues taken from C57BL/6J mice belonging to age groups ranging from 1 to 30 months[29]. Following computation of overall diversity score, two clusters of renal macrophages were identified for which composition changed significantly with age. One cluster was mostly composed of cells from 1- and 3-month-old mice enriched with an anti-inflammatory signature (e.g., *Cq1a, Cd74, Cd81*). Conversely, the other cluster was primarily composed of cells from 18-, 21-, 24- and 30-month-old mice that resembled a proinflammatory macrophage state (e.g., *Intgal, Msrb1*). At present, such comprehensive ageing studies in humans are missing but these will be important to undertake in future due to the prevalence of kidney disease increasing with age[1].

Though studies have been carried out on both human and mouse tissue, there are shortcomings in acquiring enough fresh human samples, highlighting the importance of overcoming translational issues. Studies have identified well defined markers of myeloid cell types across murine tissues, but they lack conservation across species. A well-known example is F4/80 (encoded by *adgre1*) which is used to identify tissue resident macrophages in mice but is not expressed by macrophage populations in humans. Furthermore, markers that were historically used to differentiate DCs from macrophages, such as CD11c and MHCII, are now known to be commonly expressed by tissue macrophages[28,30]. Zimmerman and colleagues used scRNA-seq to identify a cross-species kidney macrophage-specific marker[31]. $CD45^+$ cells with the lymphocyte population excluded were isolated from one mouse, rat, pig and human kidney, revealing a cluster of cells across all which express *C1q.* Other scRNA-seq analyses of whole kidney tissue from mice[2,10] and humans[32] have also identified this *C1q*-expressing cluster. In the *C1q*-expressing cluster, novel surface markers

Cd74 and *Cd81* were identified as potential candidate markers of kidney resident macrophages across species[31]. These were validated by flow cytometry showing $CD74^{+}CD81^{+}$ cells were more abundant in resident- ($CD11b^{lo}F4/80^{hi}$) compared to infiltrating ($CD11b^{hi}F4/80^{lo}$) macrophages, and using the parabiotic mouse model they confirmed minimal exchange with bone marrow derived cells. It remains unknown if these candidates vary throughout the time course of inflammation. Indeed, *C1qa* expression varied over twelve myeloid subsets in our reversible-unilateral ureteral obstruction (R-UUO) study[10]. In future it will be important to further investigate the effect of mouse strain in order to contextualise findings. Indeed, inbred laboratory mouse strains can be highly divergent in their immune response patterns for example, after unilateral ischaemic reperfusion injury (IRI) 129/Sv mice have been shown to have fewer infiltrating leukocytes than C57BL6/J mice[33].

scRNA-seq studies in kidney injury and disease

A phase-dependent influx of myeloid cells and changes in their phenotype occur during the time course of kidney diseases[34]. To define the cellular landscape in the progression of AKI to chronic kidney disease (CKD), do Valle Duraes and colleagues employed sc-RNAseq in a unilateral IRI model with or without immediate contralateral nephrectomy to study kidney regeneration or fibrosis, respectively[35]. Seven main clusters were identified including: resident and inflammatory macrophages, neutrophils/monocytes, DC/monocytes, NK cells, T and B cells. The healthy kidney was dominated by resident macrophages, whereas following injury inflammatory macrophages significantly expanded and resident macrophages disappeared, regardless of the model used or post-injury timepoint. The mixed populations of neutrophils/monocytes and DC/monocytes also expanded after injury. In another study, snRNA-seq was used with a bilateral IRI model of AKI[36]. Though proximal tubules were the focus here, six leukocyte clusters were identified: three macrophage subtypes, DCs, T and B cells. Ligand-receptor analysis performed on the combined leukocyte cluster suggested changes in Ccl2 (tubulointerstitium) to Ccr2 (leukocytes) signalling over time; fibroblasts and endothelial cells were the first cell types to signal leukocytes, followed by leukocyte-leukocyte signalling and increasing Ccl2-Ccr2 signalling from proximal tubules that failed to repair at 2 days and 6 weeks post-injury. The first and second macrophage cluster are likely resident and inflammatory macrophages, based on markers such as *F13a1* and *Vcan*. The third cluster expressed high levels of *Mmp12*, a macrophage-specific metalloproteinase, and

Gpnmb, a negative regulator of inflammation proposed to promote M2 polarisation. This and the DC cluster were most abundant at 2- and 6 weeks post-injury, in which the kidney is in the reparative phase. Our group recently used scRNA-seq in the mouse R-UUO model, in which regression of established tubulointerstitial fibrosis, a hallmark of CKD, occurs following reversal of obstruction[10]. Interestingly, we also reported a macrophage cluster that was solely in kidneys that had undergone UUO reversal; it was also characterised by expression of *Mmp12* and scavenger receptors, including *Gpnmb* and *Mrc1*. We have previously reported a similar *Mmp12*$^+$ macrophage in liver during the resolution of liver disease[37]. Whilst these *Mmp12*$^+$ cells mapped to macrophages on the ImmGen database, they morphologically resembled monocytes and expressed high levels of *Ccr2* but low levels of F4/80 by immunofluorescence. Furthermore, their transcriptome most closely aligned to monocytes infiltrating the kidney during recovery from IRI. We found that bone marrow derived monocytes that were fed FITC labelled collagen increased their expression of *Mmp12* and *Gpnmb*, suggesting that phagocytosis of collagen may induce expression of these markers.

In our characterisation of myeloid cells in renal injury and repair, we identified a further eleven myeloid cell subsets in the R-UUO model, including some with a novel phenotype. This was uniquely done by integrating droplet and plate based scRNA-seq with index linkage to map the subsets onto monocyte and macrophage gates on flow cytometry[10]. Three monocyte clusters were identified: patrolling and inflammatory subtypes, and a unique 'pro-fibrotic' subtype expressing *Arg1* that was exclusively present at UUO day 2 (acute injury phase). These *Arg1*$^+$ cells expressed markers of Ly6C$^+$ inflammatory monocytes, including *Ccr2* and *F13a1*, though not *Ly6c2*. Moreover, they were shown to express hypoxia, pro-fibrotic and pro-inflammatory genes, as well as genes encoding ECM components and ECM cross-linkers. Together with a number of ligand-receptor pairs revealed between *Arg1*$^+$ monocytes and mesenchymal cells, these data suggest *Arg1*$^+$ cells could be derived from recruited Ly6C$^+$ monocytes that become activated acutely toward a profibrotic phenotype in the hypoxic and inflammatory environment of the injured kidney. Indeed, there are waves of monocyte ingress during chronic progressive kidney injury[35]. Interestingly, in a model of renal injury due to sepsis, a subcluster of macrophages showed increased *Arg1* expression in the later post-injury timepoints, albeit with markers of alternative macrophage activation, such as *Mrc1*[38]. We further tracked the fate of circulating immune cells recruited to the kidney using paired blood exchange (PBE), which holds advantages over parabiosis, with the

donor cells persisting at large numbers in the circulation for a relatively short time, and, thus, permits the tracking of cells at multiple time points after injury or during resolution of disease. Combining PBE, flow cytometry and pseudotime analyses we showed early recruitment of donor monocytes to the obstructed kidney at UUO day 2; where at UUO day 7 they transitioned to a $CCR2^{hi}$ macrophages with a transcriptome almost identical to resident macrophages. Other work is indicative of $CCR2^{+}$ cells being detrimental; in an IRI model with CCR2 inactivation there was a reduction in the expansion of kidney F4/80 macrophages and severity of renal fibrosis[5]. It remains to be determined if these novel populations are present at all stages in the context of CKD; for example, are the *Mmp12*$^{+}$ macrophages present at early points of injury where endogenous kidney repair mechanisms are active and attempting repair, or only when a scarred matrix is present with no ongoing injury stimulus?

Dhillon and colleagues performed scRNA-seq analysis on healthy and fibrotic mouse kidneys from a folic acid nephropathy model[39]. Fourteen immune clusters were identified, compared to only five in their previous study[2] including: macrophages, subclusters of DCs (CD11b+/-, pDC), granulocytes and lymphocytes. Another study compared scRNA-seq and snRNA-seq on obstructed (fibrotic) mouse kidney at UUO day 14. Both methods had comparable gene detection; however, compared with scRNA-seq, snRNA-seq had a reduced dissociation bias and dissociation-induced transcriptional stress responses[19]. One macrophage cluster was identified here, though researchers identified a novel "de-differentiated" proximal tubule cluster that expressed numerous secreted proinflammatory cytokines, including Ccl2, macrophage proliferative cytokine Il-34, and neutrophil chemo-attractants Cxcl1 and Cxcl2. This study, again, indicates that snRNA-seq is not as capable as scRNA-seq at detecting immune populations in the damaged and inflamed kidney. Indeed, the same group performed sn-RNAseq on cryopreserved human diabetic kidney samples[40]. Diabetic kidneys were found to have increased leukocyte numbers compared to control kidneys, as expected. Monocytes, plasma cells, T and B cells were detected, however, a macrophage population was not, despite the role of macrophages in type 2 diabetes being well-documented[41]. Researchers further compared their diabetic samples to publicly available peripheral blood mononuclear cell datasets as the leukocyte numbers in control samples was small. Increased inflammatory markers were observed in both infiltrating diabetic $CD14^{+}$ monocytes and $CD16^{+}$ monocytes.

Recently, Kuppe and colleagues generated a single cell map of the human kidney, with a focus on the tubulointerstitium and fibrosis development in CKD[42]. However, in the $CD10^{-}$

(non-proximal tubular) sorted cells, three macrophage subtypes, monocytes, DCs and mast cells were identified, as well as NK, T and B cells. Researchers proposed a working model of the pathways involved in renal fibrosis, but immune cells, which play important roles in pro-fibrotic signalling, were absent from this despite being captured. Further extending our understanding of immune cells in different pathological states, a study described the immune landscape of the human kidney in patients with lupus nephritis, a frequent complication of systemic lupus erythematosus[43]. Kidney samples from patients with lupus nephritis and from healthy controls were analysed using scRNA-seq. This revealed twenty-one immune clusters including numerous subsets of myeloid cells in both pro-inflammatory responses and inflammation-resolving responses: inflammatory $CD16^+$ macrophages, phagocytic $CD16^+$ macrophages, tissue resident macrophages, M2-like $CD16^+$ macrophages, cDCs and pDCs. Tissue resident macrophages were the dominant cluster in healthy kidneys.

Myeloid cells are also increasingly recognised as major players in transplant rejection. Dangi and colleagues used scRNA-seq to dissect the contribution of myeloid cell subsets to kidney transplant rejection in a mouse model[18]. Thirteen clusters were identified as immune cell types, including two macrophage subsets, monocytes, pDC, cDC and a transitioning monocyte/macrophage population. As expected, the number of cells in all immune cell clusters was the highest in rejecting kidneys followed by tolerised kidneys, and it was markedly lower and often negligible in naïve kidneys. A macrophage cluster was the second most abundant behind T cells. Interestingly, among the top cluster-defining genes, *Axl* was exclusively expressed only by the graft-infiltrating myeloid cell clusters macrophage cluster 1 and 2, and the macrophage/monocyte cluster but not by any other cell types. $Axl1^+$ macrophages were found to be significantly higher in rejected versus tolerised kidneys, and Axl promoted intragraft differentiation of inflammatory macrophages. In another scRNA-seq study, Wu and colleagues analysed a kidney allograft biopsy from a recipient undergoing acute rejection[32]. They identified two distinct monocyte populations, likely a proinflammatory and classic or intermediate, further supporting a role of myeloid cells in kidney rejection in humans.

Conclusions

Over the past few years single-cell genomics, with scRNA-seq leading the way, has provided researchers with a new experimental toolbox to dissect the roles and interactions of individual cell types in health and disease. It is allowing the re-evaluation of our understanding of

myeloid cell biology in complex organs, including aspects of cellular ontogeny, differentiation, homeostasis, and range of activation states. This is especially important in the kidney where heterogeneity and plasticity of cells within the myeloid compartment is reflected under both homeostatic and disease conditions. Integration of data sets from separate experiments using human samples will further be important; humans are genetically heterogeneous and have variable environmental exposures, and procurement of enough samples can be a challenge. This also highlights the need to continue to determine the translatability of our findings. The application of scRNA-seq in both basic and translational research is expected to grow exponentially with the continued advancements and standardisation of experimental and analytical methods.

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Author Contributions

Rachel Bell: Visualization; Writing - original draft; Writing - review and editing

Laura Denby: Conceptualization; Funding acquisition; Resources; Supervision; Writing - original draft; Writing - review and editing

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Detection of myeloid ($CD45^+$) cell types by flow cytometry		
Markers	**Mouse**	**Human**
Monocyte	CD11b, Ly6C, CD192 (CCR2), CX3CR1	CD11b, CD14, CD16
Macrophage	CD11b, F4/80, CD68, CD64	CD11b, CD68, CD163
Pro-inflammatory	CD86, MHCII	CD86, HLA-DR
Anti-inflammatory	CD206 (MRC1), IL-4R	CD206 (MRC1), IL-4R
Tissue resident	CD192 (CCR2), CD206 (MRC1), CD68, CD74, CD81	CD192 (CCR2), CD68, CD74, CD81
Dendritic cell	CD11c, CD11b, MHCII	CD11c, CD11b, HLA-DR
Conventional	CX3CR1, CD103	CD1c (BDCA1), HLA-DR
Plasmacytoid	Ly-49Q, CD317 (Bst2)	CD123 (IL-3Rα), CD303 (BDCA2)
Neutrophil	CD11b, Ly6G, CD18	CD11b, CD16, CD32, CD66b
Eosinophil	CD170 (Siglec-F), CD193 (CCR3)	CD329 (Siglec-8), CD193 (CCR3)
Basophil	CD123 (IL-3Rα), FcεRIα	CD123 (IL-3Rα), FcεRIα
Mast cell	CD117 (c-Kit), FcεRIα	CD32, CD117 (c-Kit), FcεRIα

Table. 1 Examples of markers commonly used to identify populations of myeloid cell types in mice and humans. Combinations and levels of expression of markers can vary with subtype/level of activation.

Species (genotype)	Disease/Model	Method(s)	Website link
Mouse (C57BL6/J)	Fibrosis (R-UUO)	scRNA-seq,	Gene Atlas of Reversible Unilateral Uretic Obstruction Model (rUUO) http://www.ruuo-kidney-gene-atlas.com
Mouse (C57BL6/J)	Healthy (Ageing, 1 to 30 months)	Bulk RNAseq, scRNA-seq,	Tubula Muris Senis https://tabula-muris-senis.ds.czbiohub.org
Mouse (C57BL6/J)	Acute injury (LPS-induced endotoxemia)	scRNA-seq	Mouse Kidney Single-cell Expression https://connect.rstudio.iu.edu/content/18/
Mouse (CFW)	Acute injury (IRI)	scRNA-seq	Mouse IRI scRNA https://research.cchmc.org/PotterLab/scIRI/
Mouse (C57BL6/J) Human	Healthy, Fibrosis (FAN) Healthy	scRNA-seq, snATAC-seq	Susztaklab Kidney Biobank https://susztaklab.com
Mouse (C57BL6/J) Human	Healthy, Fibrosis (UUO), Acute injury (IRI) Allograft rejection, Diabetes	scRNA-seq, snRNA-seq	Kidney Interactive Transcriptomics (KIT) http://humphreyslab.com/SingleCell/
Human	Healthy (including foetal)	scRNA-seq	Kidney Cell Atlas https://www.kidneycellatlas.org
Human	Healthy (including foetal)	scRNA-seq	Nephrocell http://nephrocell.miktmc.org
Human	Healthy (foetal)	scRNA-seq	The Human Nephrogenesis Atlas https://sckidney.flatironinstitute.org

Table. 2 Single-cell atlases. Single-cell expression atlases from studies discussed in this review and of interest. R-UUO, reversible unilateral uretic obstruction; FAN, folic acid nephropathy; IRI, ischaemic reperfusion injury.

Figure Legends:

Figure 1. Mapping myeloid cells in kidney injury. For dense tissues like the kidney cell dissociation, typically achieved using both physical disaggregation and enzymatic digestion, is the most important step; it directly affects the molecular profiles of cells and can introduce stress-induced transcriptional artifacts[9,17]. This is particularly limiting when looking at kidney disease samples where tissue is subject to inflammatory stress, though it may be overcome by excluding low quality cells for example, on the basis of high mitochondrial RNA content[18]. Cell isolation and capture is the most unique hurdle to overcome for scRNA-seq. The use of FACS allows for the selection of cells based on surface markers and is therefore useful when isolating a specific subset of cells for sequencing, including CD45 for immune cells. Adapted with permission from [10].

Figure 2. Combining technologies to fully characterise renal immune populations. Using sc/sn-RNA-seq together with other historical and novel technologies has enabled the identification of new myeloid cell markers. Immune cells may be mapped onto flow cytometry plots, for example.

Figure 1

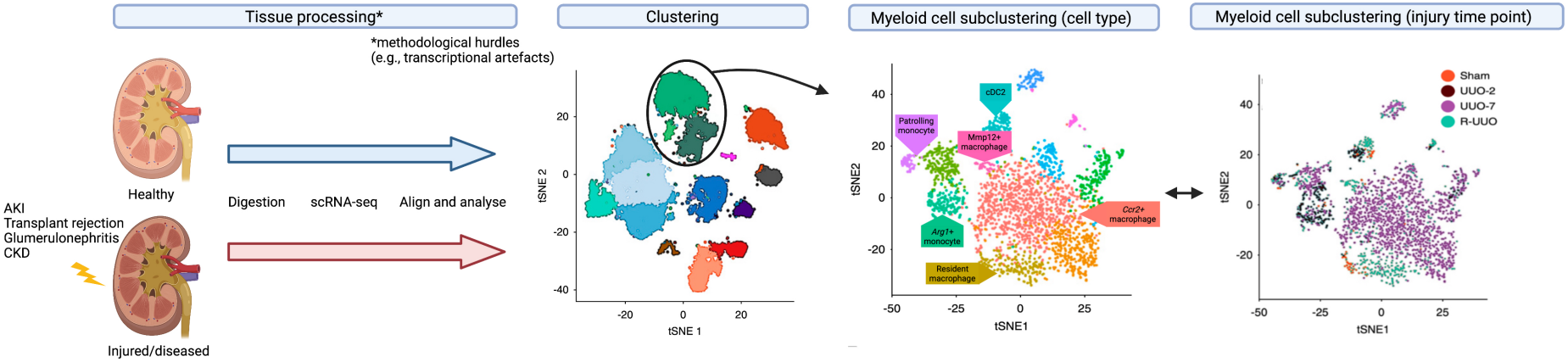
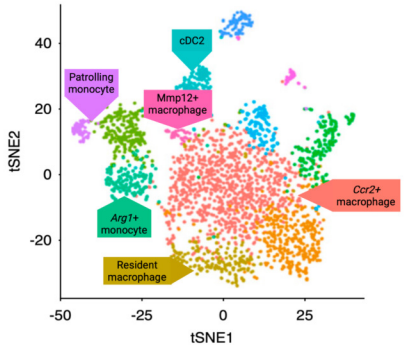
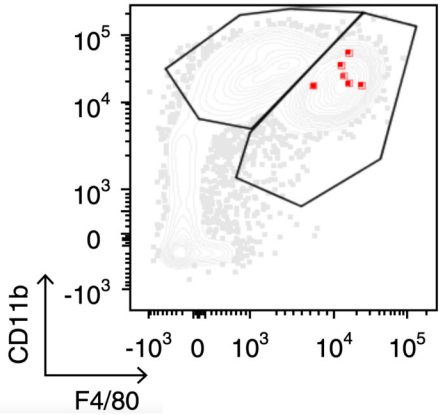

Tissue processing*
*methodological hurdles
(e.g., transcriptional artefacts)
Healthy
AKI
Transplant rejection
Glumerulonephritis
CKD
Injured/diseased
Digestion
scRNA-seq
Align and analyse
Clustering
tSNE 1
tSNE 2
Myeloid cell subclustering (cell type)
Patrolling monocyte
cDC2
Mmp12+ macrophage
Arg1+ monocyte
Ccr2+ macrophage
Resident macrophage
tSNE1
tSNE2
Myeloid cell subclustering (injury time point)
Sham
UUO-2
UUO-7
R-UUO

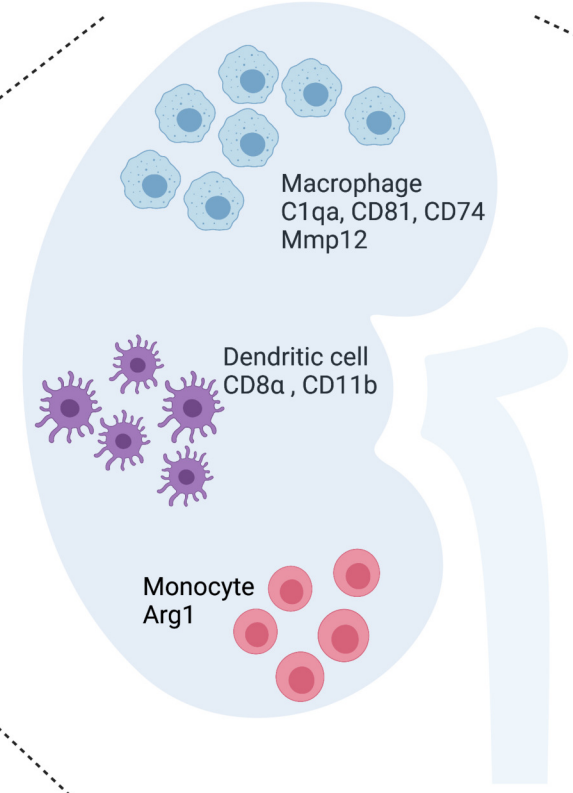
Figure 2



sc/snRNA-seq in combination with

- multi-omics (e.g., ATAC-seq, CITE-seq, REAP-seq)
- immunohistochemsity
- flow cytometry/FACS





Lineage
- Predict cellular ontogeny

Cell state
- Probe cellular heterogeneity in kidney
- Uncover tissue resident signatures
- Spatial organisation of tissue immunity

Pseudotime
- Putative cell-cell signalling networks