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The advance of single cell transcriptome to study kidney immune cells in diabetic kidney disease

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Abstract

Diabetic kidney disease (DKD) is a prevalent microvascular complication of diabetes mellitus and a primary cause of end-stage renal disease (ESRD). Increasing studies suggest that immune cells are involved in regulating renal inflammation, which contributes to the progression of DKD. Compared with conventional methods, single-cell sequencing technology is more developed technique that has advantages in resolving cellular heterogeneity, parallel multi-omics studies, and discovering new cell types. ScRNA-seq helps researchers to analyze specifically gene expressions, signaling pathways, intercellular communication as well as their regulations in various immune cells of kidney biopsy and urine samples. It is still challenging to investigate the function of each cell type in the pathophysiology of kidney due to its complex and heterogeneous structure and function. Here, we discuss the application of single-cell transcriptomics in the field of DKD and highlight several recent studies that explore the important role of immune cells including macrophage, T cells, B cells etc. in DKD through scRNA-seq analyses. Through combing the researches of scRNA-seq on immune cells in DKD, this review provides novel perspectives on the pathogenesis and immune therapeutic strategy for DKD.

Keywords Diabetic kidney disease, Single-cell RNA sequencing, Immune system, Dendritic cells and macrophage, T cells, B cells

Introduction

Diabetic kidney disease (DKD), a microvascular complication associated with type I or II diabetes, poses a significant risk to human health. In most countries, more than 50% of individuals receiving renal replacement therapy (RRT) for ESRD are affected by DKD [1]. Although mortality from DKD has decreased over the past three decades owing to improved diabetes management, the absolute risk of renal and cardiovascular morbidity and mortality remains substantial [2–6]. About 30% of patients with type 1 diabetes mellitus (DM1) and 40% of patients with type 2 diabetes mellitus (DM2) turn up microvascular complications [1, 7]. Several independent familial studies in various populations have indicated a genetic predisposition to DKD [8, 9]. Hence, it is crucial to investigate the pathophysiology and gene expression information of DKD in more depth so that innovative therapeutic methods may be developed to prevent, halt, and reverse DKD. Besides, microalbuminuria, as a

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biomarker for early diabetic nephropathy, has low sensitivity and specificity in predicting DKD [10]. It should be noted that the presence of micro/macroalbuminuria does not always indicate the presence of DKD. Many diabetic patients have declined renal function even without significant proteinuria [11]. Furthermore, renal biopsy is still the method used to diagnose DKD [12]. Yet it is an aggressive procedure related to complications such as infection and hemorrhage [13]. Additionally, it is not feasible to monitor continuously due to the progressive nature of kidney disease and the possibility of sampling errors. Therefore, it is essential to explore noninvasive and highly sensitive immune-associated biomarkers to accurately predict DKD development. The biomarkers provide a promising alternative by offering continuous assessment and early detection of disease progression, addressing the limitations inherent in renal biopsy.

To explain these questions, it is important to explore gene regulatory mechanisms at the cellular level. Fluorescence-activated cell sorting (FACS), as a traditional method for renal cell type studies, characterizes cells based on the expression of surface markers. This approach is crucial for understanding immune responses but imposes stimulus on the cells, potentially altering their expression profiles [14]. The kidney filters and excretes bacterial toxins, circulating cytokines, and inflammatory molecules to maintain the homeostasis of the immune system [15, 16]. The immune system is a complex network of cells, tissues, and organs that work together to defend the body against harmful invaders such as bacteria, viruses, and fungi. Thus, in order to enable detailed characterization of the immune cells, it is necessary to use the sequencing analysis method at the level of single cell. Major progress has been made in exploring the role of immune cells in kidney diseases [15, 16] and applying these findings from laboratory research to clinical practice. However, it is a challenge that determines how these immune cells coordinate kidney immunology in health and disease owing to the small number of these cells and the complex composition of the kidney. So far, we still do not have a panoramic understanding in depth of kidney immunology until now. In this review, we aim to the recent progress made by single-cell studies of DKD. We also discuss recent findings focusing on DKD, an immune-related kidney disease, highlighting the changes in immune cell groups and the potential immune mechanisms revealed by scRNA-seq technology.

Single-cell RNA sequencing technology

Single-cell sequencing technology is a high-throughput transcriptomics technique used to analyze gene expression at the resolution of individual cells [17, 18]. Unlike traditional whole genome sequencing (WGS), which assesses gene expression at the multicellular level, scRNA-seq provides detailed insights into cellular heterogeneity and gene expression variability. This technique allows for precise measurement of gene expression levels and can identify low-abundance transcripts and rare non-coding RNAs. Since 2009, scRNA-seq technology has been rapidly developed during the last decade (Fig. 1)

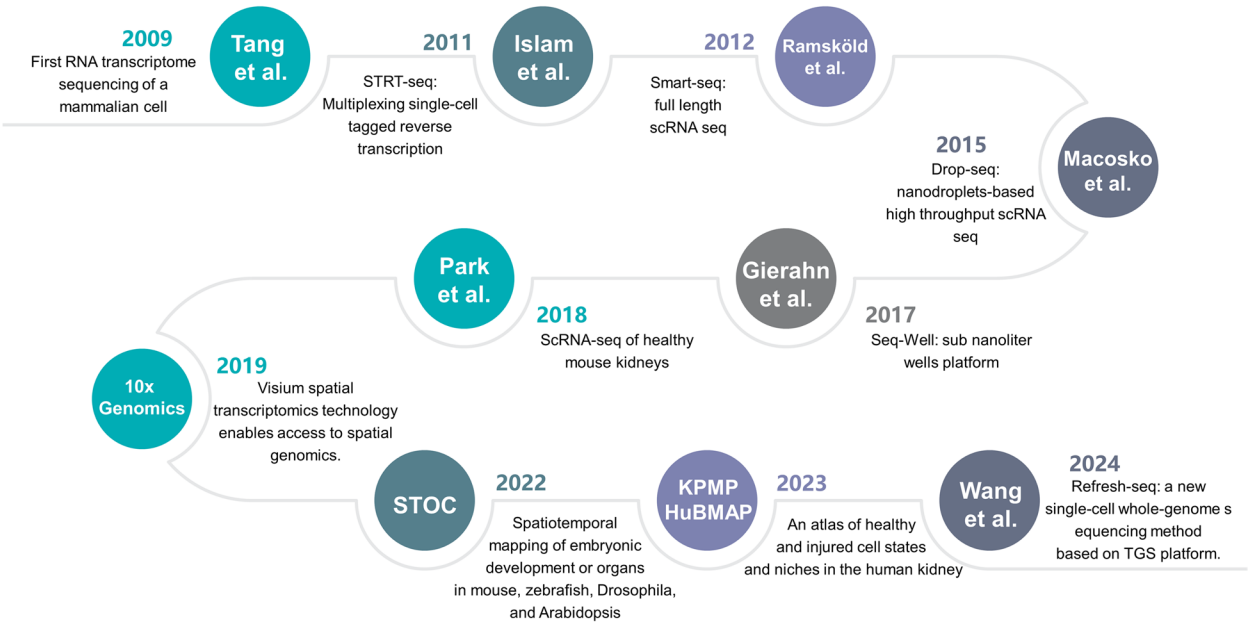

Fig. 1 Development of scRNA-seq and its applications in kidney immunology

[19–28], with significantly reduced costs, increased automation, and rising throughput. It has become prevalent in tackling critical questions in biology and medicine.

The common workflow of scRNA-seq includes single-cell isolation, cell lysis and RNA capture, reverse transcription, complementary DNA (cDNA) amplification and library construction, high-throughput sequencing, and bioinformatic analysis (Fig. 2) [29, 30]. The core strategies consist of separating individual cells, creating sequencing libraries independently, and identifying single cells based on barcode. Zilionis et al. established a method called inDrops, which used droplet microfluidics to encapsulate individual cells in nanoliter droplets and barcoded (indexed) mRNA for genomic or whole transcriptome analysis [31]. This encapsulation facilitates the simultaneous processing of thousands of cells, significantly enhancing throughput. When RNA levels in a cell are insufficient for sequencing, amplification is necessary to enhance the material available for analysis. For genomic DNA amplification in low-biomass samples, whole genome amplification (WGA) techniques, such as multiple displacement amplification (MDA) using phi29 DNA polymerase, are commonly employed [32–34]. Although MDA effectively addresses nucleic acid concentration issues, it can introduce amplification biases that may affect the reliability of quantitative comparisons [34]. Therefore, when applying these methods to metabolomics analysis of environmental samples, careful selection and validation of MDA kits are crucial [35]. For single-cell transcriptome amplification, reverse transcription (RT) of mRNA to cDNA followed by polymerase chain reaction (PCR) is required. This process enables the conversion of RNA into a more stable form, which can then be amplified for sequencing. Picelli et al. dug out smart-seq2 transcriptome libraries that detect precisely, with better coverage, bias and accuracy compared to smart-seq libraries [36]. A critical aspect of scRNA-seq technology is the bioinformatic analysis that follows sequencing, which is essential for interpreting the vast amounts of data generated. This analysis typically involves several key steps: quality control, normalization, dimensionality reduction, clustering, and differential expression analysis. Quality control checks the integrity and purity of the data to eliminate low-quality reads. Normalization adjusts for variations in sequencing depth and RNA composition across cells, enabling meaningful comparisons. Dimensionality reduction techniques, such as PCA (Principal Component Analysis) or t-SNE (t-distributed Stochastic Neighbor Embedding), reduce the complexity of the data, making it easier to visualize and interpret. Clustering algorithms are then applied to identify distinct cell populations based on gene

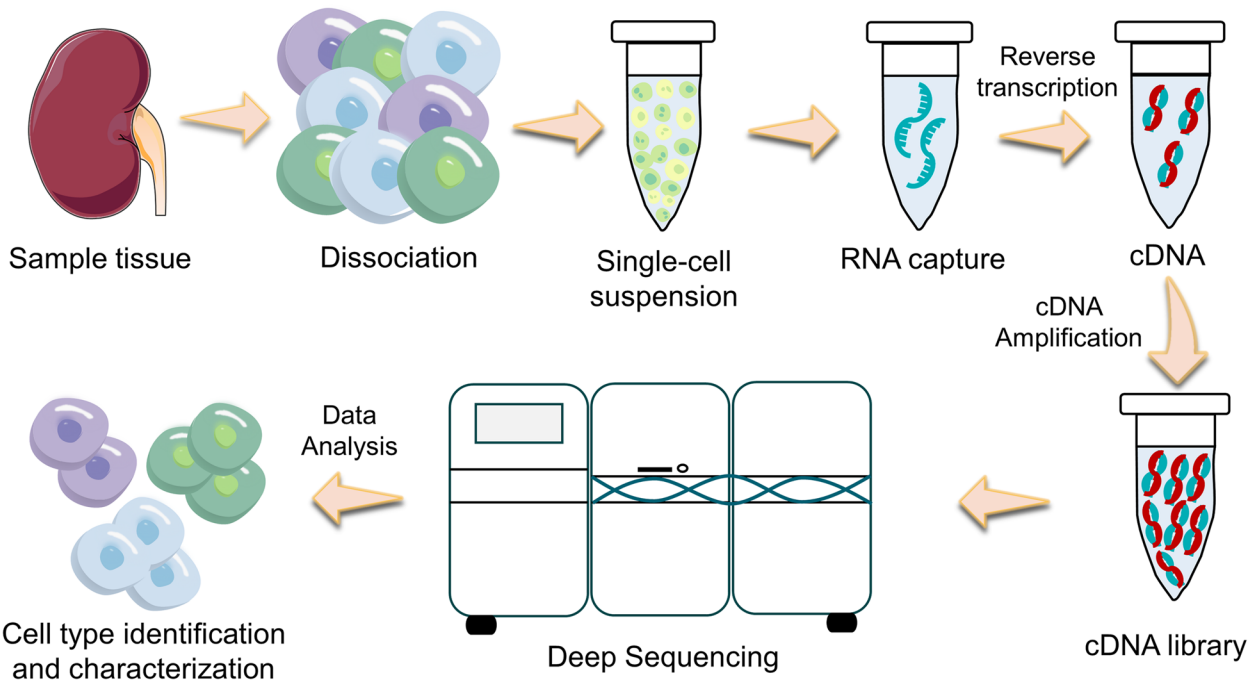

Fig. 2 An overview of the single-cell RNA sequencing procedures. The experimental workflow involves isolating cells from tissue samples and dissociating them into a single-cell suspension; individualized RNA capture; reverse transcription and amplification of complementary DNA (cDNA) and library preparation. After performing high-throughput sequencing and conducting bioinformatic analysis, the specific type of cell would be accurately identified and thoroughly characterized

expression profiles. Finally, differential expression analysis allows researchers to identify genes that are significantly upregulated or downregulated in specific cell types or conditions, providing insights into cellular functions and biological processes.

The advent of scRNA-seq technology has enabled the study of kidney disease at unprecedented resolution. The evergrowing compendium of scRNA-seq-based kidney atlases has catapulted our knowledge of not only the immune cells that comprise the microenvironment but also their cell-state heterogeneity. Spatial transcriptomics (ST), with other technologies such as scRNA-seq, has enabled researchers to dissect the organization and interaction of different cell types within the DKD [37]. Recent advancements in methodologies, such as GeoMx Digital Spatial Profiling (DSP) and LightSeq technology, represent significant strides in the field, offering capabilities that classify these approaches into sequencing-based experimental biological techniques (EBTs) and imaging-based nonexperimental biological techniques (NEBTs). These innovative technologies provide spatially resolved transcriptomic and proteomic data, thereby facilitating a more nuanced understanding of the cellular architecture and functional dynamics in the context of DKD [27].

Single-cell RNA sequencing studies in nephrology

The kidney is a highly complex organ for scRNA-seq studies. To date, mapping kidney gene expression is rapidly becoming an irreplaceable method for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)'s Kidney Precision Medicine Project (KPMP) (https://kpmp.org/), the National Institutes of Health (NIH)'s Human BioMolecular Atlas Program (HuBMAP) (https://hubmapconsortium.org/), Susztak lab's kidney Biobank (KBK) (https://susztaklab.com/), Humphreys lab's Kidney Interactive Transcriptomics (KIT) (http://humphreyslab.com/) and Kuopio University Hospital (KUH)'s Nephrotic Syndrome Study Network (NEPTUNE) (https://neptune-study.org/). The goal of mapping kidney gene expression is to enhance our comprehension of the prevalent types of kidney diseases [38]. Single cell sequencing techniques were initially created to measure gene expression. However, they have now advanced to enable the simultaneous profiling of other features like chromatin accessibility within the nucleus and protein expression at the cell surface [28, 39–44]. Recently, many studies have been published using scRNA-seq technology in kidney atlas, renal tumors, disease mechanisms, therapeutic targets, and so on (Tables 1 and 3).

Researchers have employed scRNA-seq to analyze human and mouse kidney tissues, accurately characterizing the molecular properties and heterogeneity of mesangial cells (MCs). In contrast to the high expression of genes in podocytes and MCs, glomerular endothelial cells (GECs) have only six human-specific genes and no mouse-specific gene [52]. Dumas et al. inventoried how medullary GECs adapt to hyperosmolarity, as well as the molecular and metabolic adaptations to dehydration [47]. While these studies contribute important knowledge about kidney cellular responses, further investigation with more diverse cohorts is necessary to validate findings and enhance their applicability to human disease. A groundbreaking advancement is the creation of a healthy kidney cell atlas by Park et al., which identified a new type of transitional cell by mapping the atlas of adult mouse kidneys, implicating a more complete molecular characterization of the cell types [45]. Sheng et al. constructed expression profiling data from 659 samples to elucidate the relationship between renal cell type abundances and their specific gene expressions, identifying potential therapeutic targets through two analytical strategies [54]. However, limited kidney eQTL datasets have historically constrained GWAS annotations of renal traits. In addressing this issue, researchers doubled the number of identified eQTLs by accounting for tissue heterogeneity using PEER factors and computational deconvolution. Despite these advancements, the eQTL(ci) approach remains dependent on the quality of single-cell expression data. Co-linearity in cell fractions further complicates analysis, particularly for less abundant cell types, highlighting the need for improved methodologies in future studies. Researchers constructed hitherto the most comprehensive tissue atlas of human kidneys, identifying 51 major cell types by analyzing 45 healthy and 48 diseased human kidneys, which contributed to the exploration of new therapeutic strategies for chronic kidney disease (CKD) and acute kidney injury (AKI) [61]. These atlases serve as a solid foundation for further research into kidney physiology and pathology, especially we should expand human genetic data to support the future study.

The studies utilizing scRNA-seq in kidney disease have demonstrated that differentiated gene expressions correlate with various injury states in glomeruli and proximal tubules, ultimately reflecting distinct pathological changes [48, 55]. By integrating snRNA-seq with a murine model of DKD [56], researchers effectively recapitulated key clinical and histological features of the disease. Analysis across both animal and human models revealed a significant proportion of immune cells in diabetic kidneys, highlighting their crucial role in disease progression [46, 57]. Chung et al. sought to improve cell preparation methods and enhance the quality of data from single-cell sequencing, generating a comprehensive and detailed dataset centered on the glomerulus that facilitates in-depth analysis [48]. However, certain approaches identified only a limited number

Table 1 Single-cell transcriptomics studies in nephrology

Article information	Method (s)	Disease/model	Tissue/cell type	Cell number
Park et al. (2018) [45]	ScRNA-seq	Healthy mouse	Kidney	57,979
Fu et al. (2019) [46]	ScRNA-seq	STZ-diabetic eNOS-/- mice	Glomerular cells	829
Dumas et al. (2020) [47]	ScRNA-seq	A hyperosmolarity model in vitro and dehydrated mice in vivo	renal endothelial cell	40,662
Chung et al. (2020) [48]	ScRNA-seq	Healthy mouse	Glomerular cells	75,000
Braun et al. (2021) [49]	ScRNA-seq	ccRCC	Human renal tumors	164,722
Krishna et al. (2021) [50]	ScRNA-seq	ccRCC	ICB-naïve and ICB-treated patients	167,283
Obradovic et al. (2021) [51]	ScRNA-seq	ccRCC	Human renal tumors	200,000
He et al. (2021) [52]	Smart-seq2	Human living donor renal biopsies and mouse	Podocytes, glomerular endothelial cells, MCs and PECs	4,332
Pickering et al. (2021) [53]	ScRNA-seq	CMV	Kidney transplant recipients prior to viremia, acutely after viremia, and long-term post-CMV viremia and propensity-matched nonviremic	\
Sheng et al. (2021) [54]	ScRNA-seq & GWAS	Human kidney	Cell-type-eQTLs	60,661
Li et al. (2022) [55]	ScRNA-seq	Fibrosis mouse	Kidney	309,666
Wu et al. (2022) [56]	SnRNA-seq & Bulk RNA-seq	db/db ($Lepr^{-/-}$)	Kidney	946,660
Lu et al. (2022) [57]	ScRNA-seq	DKD rat model	10 cell types such as immune cells	\
Li et al. (2022) [58]	ScRNA-seq	RCC	Human renal tumors	270,000
Kong et al. (2022) [59]	ScRNA-seq	cABMR after renal transplantation	PBMCs	39,285
Rashmi et al. (2022) [60]	Mux-Seq	Kidney transplants	Human kidney biopsies	50,275
Lake et al. (2023) [61]	ScRNA-seq	Healthy and diseased kidneys	Kidney	400,000
McDaniels et al. (2023) [62]	SnRNA-seq	Kidney transplants	Kidney allograft biopsies	41,893
Wen et al. (2023) [63]	ScRNA-seq	Kidney transplants	Kidney transplantation biopsy cores	81,139
Aidan et al. (2024) [64]	ScRNA-seq	Kidney transplants	Human kidney transplant biopsies	31,203
Lu et al. (2024) [65]	ScRNA-seq	Kidney transplant recipients with COVID-19-induced ARDS	PBMCs	23,980

Abbreviations: *scRNA-seq* single-cell RNA sequencing, *Smart-seq2* Switching mechanism at 5′ end of the RNAtranscript sequencing2, *GWAS* Genome-Wide Association Studies, *snRNA-seq* single-nucleus RNA sequencing, *Bulk RNA-seq* bulk RNA sequencing, *Mux-Seq* Multiplexed droplet single-cell sequencing, *STZ* streptozotocin, *eNOS* endothelial nitric oxide synthase, *ccRCC* clear-cell renal cell carcinoma, *CMV* cytomegalovirus, *DKD* diabetic kidney disease, *RCC* renal cell carcinoma, *cABMR* chronic antibody-mediated rejection, *PBMCs* peripheral blood mononuclear cells

of glomerular cells, which can be attributed not only to their inherent scarcity but also to the inadequacy of tissue-dissociation methods designed for whole kidney preparations, which are not ideal for isolating glomeruli [45, 46]. Analysis of tubular cells from patients with DKD indicated that histological differences are due to the regulation of tubulointerstitial fibrosis and inflammation pathways. McDaniels et al. elucidated the cellular heterogeneity of allograft fibrogenesis in kidney transplant recipients using the single-cell transcriptome, suggesting that inhibiting interactions between immune cells and parenchymal cells may reverse the progression of renal fibrosis, yet the dynamics process of fibrosis is not documented [62]. This insight is particularly relevant in the context of renal tumors, as the understanding of immune cell interactions in kidney transplantation can inform approaches to immunotherapy. In the renal tumor research field, the tumor immune microenvironment is critical in immunotherapy [49–51, 58]. Single-cell RNA (scRNA) and T cell receptor (TCR) sequencing are used to provide a reference value for the treatment of clear cell renal cell carcinoma (ccRCC) [49, 50]. Based on the atlas, multiple approaches have been established to identify potential targets for immunotherapy in ccRCC [50, 51, 58]. Nonetheless, these approaches may be limited by sample sizes and selection biases, potentially impacting the generalizability of the findings. Future studies could focus on enhancing genetic testing rates to mitigate these limitations and improve the robustness of the conclusions drawn from such research.

What's more, numerous studies have explored immune mechanisms in chronic antibody-mediated rejection after kidney transplantation [53, 59, 60, 63, 66]. These multi-omics researches revealed that suppression of immune cell upregulation can significantly improve the survival rate of kidney transplant patients. Especially Aidan et al. inferred from scRNA-seq data the transcriptional profiles of physically interacting cells from human kidney transplant biopsies by the method of sequencing physically interacting cells, which complements

the estimation of cell-cell physical contact from previous scRNA-seq approaches [64]. On the other hand, Lu et al. innovatively studied the peripheral blood mononuclear cells (PBMCs) of kidney transplant recipients (KTRs) with COVID-19-induced ARDS, which showed significant heterogeneity such as elevated antibody levels, impaired T cell differentiation, and dysregulation of innate immunity [65]. While these studies address different aspects of renal pathology, they collectively advance our understanding of renal diseases and potential therapeutic approaches.

Application of single-cell RNA sequencing in diabetic kidney disease

Single-cell RNA sequencing was performed for DKD, which was among the earliest glomerular diseases to undergo this procedure. It is a hot topic to investigate the pathogenesis at the cellular level by using scRNA-seq while exploring the targets for drug intervention in kidney disease research. Recent advancements in scRNA-seq have significantly enhanced our understanding of cell-specific gene expression in DKD. This chapter examines the currently available studies on single-cell transcriptomics related to DKD (Tables 2 and 3).

Experimental DKD

A study on glomerular cells from streptozotocin-induced diabetic eNOS$^{-/-}$ mouse focused on five distinct populations, including glomerular endothelial cells, mesangial cells, podocytes, immune cells, and tubular cells. They revealed increased immune cell infiltration, primarily macrophages, in diabetic glomeruli, alongside dynamic gene expression changes in endothelial and mesangial cells linked to DKD [46]. Chung et al. further demonstrated the varying dynamics of several glomerular cells in ob/ob mice across different ages. The podocyte injury highlighted the activation of the Hippo pathway, suggesting critical therapeutic targets for diabetic nephropathy [48]. Fu et al. performed single-cell RNA sequencing of CD45-enriched immune cells in the kidneys of OVE26 mice with type 1 diabetes, obtaining approximately 17,000 cells and ultimately yielding 11 cell clusters. In the early stages of CKD, macrophages were shown to be involved in the regulation of renal inflammation. In addition, gene expression analysis highlights the dynamically changing macrophage activation in the early stages of DKD and its potential involvement in disease development [66]. Other researchers analyzed the responses of db/db mouse to five therapeutic regimens: control versus angiotensin-converting enzyme inhibitor (ACEi), Rosiglitazone, sodium-glucose cotransporter two inhibitors (SGLT2i), ACEi + Rosiglitazone, and ACEi + SGLT2i. SGLT2i was implied to contribute to regulating alternative splicing in order to activate a protective metabolic switch [56]. Furthermore, a renal cell transcriptome study found that not only SGLT2i affected mitochondrial function in proximal tubules, but also ARBs have the effects against inflammation and fibrosis in much the same way [77]. A latest research of single-cell and bulk RNA sequencing on renal cells from mice with type 2 diabetes (BTBR ob/ob) at early DKD suggested that mechanosensitive transcriptional pathway MRTF-SRF was mainly activated in mesangial cells, providing a potential novel target for diabetic glomerulopathy [67].

Human DKD

The scRNA-seq results from kidney biopsies of both healthy donors and patients with DKD as well as urine samples from COVID-19 patients revealed that ACE2-coregulated proximal tubular epithelial cell expression program in DKD may interact with the SARS-CoV-2 infection processes and ACE2 expression in proximal tubular epithelial cells does not significantly increase with the use of RAAS inhibitors [71]. Furthermore, investigations across 3 independent cohorts have demonstrated no association between RAAS inhibitors and adverse outcomes in COVID-19 patients [71], suggesting a potentially safer profile for these medications in this context. Schaub et al. elucidated the impact of SGLT2 inhibitors on kidney metabolism in young individuals with type 2 diabetes. By demonstrating alterations in transcriptional profiles across nephron segments and modulation of the mTORC1 signaling pathway, the findings suggest that SGLT2i treatment mitigates diabetes-related metabolic disturbances, offering potential renal protection [76]. However, the generalizability of these findings is contingent upon the size and diversity of the studied cohorts, so larger and more representative studies are essential to validate these results and inform treatment strategies for kidney disease patients during the pandemic. In a research concerning human kidney interstitium, Barwinska D et al. mapped renal interstitial marker genes by combining laser micro dissected (LMD) and single nuclear RNA sequencing (snRNA-seq) [72]. Single cell transcriptional analysis was also used to map hyperfiltration-associated gene expression in early diabetic kidney disease, identifying several putative ligand-receptor pairs with downstream intracellular targets linked to cellular crosstalk between endothelial and mesangial cells [73]. A multimodal single cell sequencing research implicates chromatin accessibility and genetic background in diabetic kidney disease progression, which raises the possibility that glucocorticoid receptor inhibition treating DKD with the adverse metabolic effects [74]. A recent study highlights the critical role of MMP7

Table 2 Application of single-cell RNA sequencing in diabetic kidney disease

	Article information	Single-cell technique	Disease/model	Tissue type	Sample number	Cell number
mouse	Fu et al. (2019) [46]	Fluidigm C1 Single-cell Auto Prep System & Illumina NextSeq 500 platform	STZ-induced diabetic eNOS$^{-/-}$ mice	Glomerular cells and immune cells	Control (3) DKD (3)	Total: 829 Control:403 DKD:426
	Chung et al. (2020) [48]	Chromium Single Cell 3′ Library and Gel Bead Kit; Illumina HiSeq 4,000	Nephrotoxic serum nephritis; DKD; Doxorubicin nephropathy; Podocyte-specific genetic disease	Glomerular cells	Control (3) Nephritis (4) DKD (6) Doxorubicin (2) CD2AP (2)	Total: 74,149 Control: 5,488 Nephritis:1,936
	Fu et al. (2022) [66]	10x Genomics Chromium and Illumina Novaseq	OVE26 mice	Immune cells	Control (3) DKD mice (3)	Total:17,000 Control: 10,500 DKD: 6,500
	Wu et al. (2022) [56]	10x Genomics Chromium & Bulk RNA-seq	db/db with unine-phrectomy	Kidney	Mice (70)	Total: 946,660
	Wu et al. (2022) [56]	10×Genomics Chromium and Illumina NovaSeq	db/db	Kidney	db/db (8) db/db + dapa (8) db/db + irbe (8) db/db + dapa + irbe (8)	Total: 83,585
	Liu et al. (2023) [67]	ScRNA-seq & Bulk RNA-seq	BTBR ob/ob	Glomerular cells	Control (8) Ob/ob (8)	Total: 70,944
Animal and Human	Li et al. (2022) [68]	ScRNA-seq & snRNA-seq & Bulk RNA-seq	Wild-type mice, db/db mice	Glomerular, tubules cell	Wild-type mice db/db mice	/
			DKD		Control (3) DKD (3)	
	Tsai et al. (2023) [69]	ScRNA-seq	db/m mice, db/db mice	Blood, urine and kidney	db/m mice + db/db mice	Total: 6,775
			Early DKD		Control (24) T2D (48)	
	Lu et al. (2022) [57]	Illumina NovaSeq 6000 & Illumina HiSeq 4000	DKD rat	Kidney	Control (3) DKD rat (3)	/
			Early DKD		Control (3) DKD (3)	
	Balzer et al. (2023) [70]	10X Genomics, Qiagen, Agilent Technologies, Illumina, Meso Scale Discovery and Olink	ZSF1 rats	Kidney	ZSF1 lean rat (6) ZSF1 obese rat (10)	Total: 4,821
			DKD		Control & DKD (991)	
Human	Menon et al. (2020) [71]	10×Genomics Chromium and Illumina	Early DKD	Kidney biopsy	Control (18) DKD (44)	Total: 111,035
			COVID-19	Urine	DKD (44) SARS-CoV-2 (13)	Total: 25,791
	Barwinska et al. (2021) [72]	LMD and sequenced and sn Drop RNA-seq	DKD	Kidney	Control (9) DKD biopsy (6)	Total: 24,387
	Stefansson et al. (2022) [73]	ScRNA-seq	Early DKD, Hyperfiltration	Kidney	Type 2 diabetes with HF (26) or pre-HF (26)	\
	Wilson et al. (2022) [74]	SnRNA-seq & snATAC-seq	DKD	Kidney cortex	Control (6) DKD (7c)	Total: 107,634
	Hirohama et al. (2023) [75]	ScRNA-seq & Bulk RNA-seq	DKD	Kidney	Control (10) DKD (23)	Total: 64,333
	Schaub et al. (2023) [76]	ScRNA-seq	Type 2 diabetes	Kidney	Control (6) Type 2 diabetes (16)	Total: 40,535

Abbreviations: *scRNA-seq* single-cell RNA sequencing, *snRNA-seq* single-nucleus RNA sequencing, *Bulk RNA-seq* bulk RNA sequencing, *snATAC-seq* single nucleus assay for transposase-accessible chromatin using sequencing, *STZ* streptozotocin, *eNOS* endothelial nitric oxide synthase, *DKD* diabetic kidney disease, *BTBR* black and tan, brachyuric

as a potential biomarker for kidney fibrosis and function decline in diabetic kidney disease. Hirohama et al. implemented histologic analysis and analyzed single cell transcriptome in 23 patients with diabetic kidney disease. The relatively small cohort size may limit the generalizability of the findings, future research should

Table 3 The scale and resolution of single-cell transcriptomics studies in nephrology

Article information	cell number analyzed	Total number of gene	Cell number of DEG[a]	Public databases
Park et al. (2018) [45]	57,979	\	PC: 870 Trans: 110 IC: 1,729	NCBI GEO (GSE107585)
Fu et al. (2019) [46]	644	2,226,308	EC: 369 MC: 144 Pod: 66	NCBI GEO (GSE127235)
Dumas et al. (2020) [47]	40,662	15,977	gRECs: 15,419 cRECs: 11,762 mRECs: 13,481	ArrayExpress (E-MTAB-8145)
Chung et al. (2020) [48]	75,000	1,070	Pod: 1,930 EC: 1,858 MC: 1,197	NCBI GEO (GSE146912)
Braun et al. (2021) [49]	164,722	6,260	Immune Tumor	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8138872/
Krishna et al. (2021) [50]	167,283	16,323	Immune Tumor	https://linkinghub.elsevier.com/retrieve/pii/S1535610821001653
Obradovic et al. (2021) [51]	200,000	30,727	Immune Tumor	https://doi.org/10.17632/nc9bc8dn4m.1
He et al. (2021) [52]	4,332	973(Pod: 424, GECs: 196, MCs: 353)	Pod: 224 GECs: 241 MCs: 17 PECs: 44	NCBI GEO (GSE160048); EGA (EGAD00001006861); https://ega-archive.org/access/dataaccess; https://patrakkalab.se/kidney
Pickering et al. (2021) [53]	34,182	3,000	NK and CD8 + T cell	NCBI GEO (GSE168598, PRJNA745955); ImmPort (SDY1600).
Sheng et al. (2021) [54]	60,661	19,315	PT: 251 Th17: 254 NK: 237 EdnoG: 229	NCBI GEO (GSE173343, GSE115098, GSE172008); http://susztaklab.com/HumanKidneysnATAC/; https://susztaklab.com/VisCello/
Li et al. (2022) [55]	309,666	\	PT: 76,400 TAL: 25,600 DCT: 10,900 CNT: 42,600	http://humphreyslab.com/SingleCell/; NCBI GEO (GSE190887, GSE206084)
Wu et al. (2022) [56]	946,660	8,248	PCs TAL: PECs PT ECs	NCBI GEO (GSE184652, GSE199437); http://humphreyslab.com/SingleCell/
Lu et al. (2022) [57]	\	31,077	10 cell types (tubular cells, endothelium, …)	https://www.frontiersin.org/articles/10.3389/fcell.2022.798316https://doi.org/10.3389/fcell.2022.798316
Li et al. (2022) [58]	270,000	\	Immune PT Fibroblasts	https://doi.org/10.17632/g67bkbnhhg.1 https://www.sanger.ac.uk/project/microenvironment-of-kidney-cancer
Kong et al. (2022) [59]	39,285	53,664,695	T cells B cells	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190329
Rashmi et al. (2022) [60]	50,275	20,150	Immune: 21,038 PT: 11,887 Endo: 13,294	www.kpmp.org
Lake et al. (2023) [61]	400,000	\	PT TAL Immune	https://www.ncbi.nlm.nih.gov/bioproject/PRJNA671343
Wen et al. (2023) [63]	81,139	\	EC, immune, stromal, Endo	\
Liu et al. (2023) [67]	70,944	740	PT Mesan Pod	NCBI GEO (GSE218563, GSE218086, GSE218413)

Table 3 (continued)

Article information	cell number analyzed	Total number of gene	Cell number of DEG[a]	Public databases
Tsai et al. (2023) [69]	6,775	\	Tubular: 5938 Lymphoid: 323 Myeloid: 130 Pod: 19 MC: 21 EC: 202	\
Balzer et al. (2023) [70]	4,821	4,265	PT	NCBI GEO (GSE209821) http://www.susztaklab.com/ZSF1_sGC_snRNA/
Aidan et al. (2024) [64]	31,203	>20	Immune: 3,117 PT: 1,656 GEC: 9,28	NCBI GEO (GSE189536)
Lu et al. (2024) [65]	23,980	\	Immune	National Genomics Data Center (HRA004752, HRA005498)

[a] The numbers listed in this column represent the number of cells expressing the gene. These cell types are representative part of a broader dataset and not an exhaustive representation of all cell types present in our analysis because of space limitations in table

Abbreviations: *DEG* differentially expressed genes, *PT* proximal tubule, *IC* intercalated cell, *Endo* endothelial cells, *PCs* principal cells, *TAL* thick ascending limb, *PECs* parietal epithelial cells, *ECs* endothelial cells, *GEO* Gene Expression Omnibus, *MC* mesangial cells, *Pod* podocytes, *RECs* renal endothelial cells, *GECs* glomerular endothelial cells, *EndoG* glomerular endothelial cells, *Th17* T helper 17 cells, *NK* natural killer cells

incorporate larger, more diverse populations and explore longitudinal designs to strengthen the conclusions better [75].

Combined analyses of experimental and human DKD

Some studies have been conducted that integrate experimental methods utilizing both DKD mouse models and patient samples. This approach aims to enhance the accuracy and translational relevance of experimental results, facilitating a more comprehensive understanding of the disease mechanisms and potential therapeutic interventions. Li et al. integrates bulk and single-cell transcriptome analyses to elucidate the mechanisms underlying DKD, focusing on podocytes. Notably, it identifies dysregulation of spermatogenesis-related genes *TEKT2* and *PIAS2* as key players in DKD progression and they play a role in podocyte cytoskeletal regulation suggests new therapeutic targets [68]. Tsai et al. highlight ferroptosis as a pivotal contributor to DKD progression through immunohistochemistry and other tests, with ceruloplasmin emerging as a key regulator in PT containing AQP4 expression (PTAQP4+). Lower percentages of thick ascending limbs and collecting ducts with impaired metabolism function, as well as *SPP1* and *SEMA3C* causing tubular damage [69]. These findings elucidate essential hub genes that inform the pathophysiological landscape of early DKD and emphasize the need for targeted therapeutic interventions. ScRNA-seq data from human control and diabetic kidney specimens identified immune cells and their marker genes (*EIF4B*, *RICTOR*, and *PRKCB*) as key pathophysiologic factors that might contribute to DKD progression [57]. Another single-cell transcriptomic research on ZSF1 rats suggests pharmacological modulation of soluble guanylate cyclase (*sGC*) as a promising DKD drug target, which aligns with earlier studies employing immunostaining and in situ hybridization techniques [70]. The ZSF1 rat model effectively mirrors human DKD through its phenotypic traits, including obesity, hypertension, and hyperglycemia. However, the interplay of these comorbidities complicates the analysis of renal function. The pronounced metabolic disturbances, while informative, may mask underlying pathophysiological mechanisms specific to DKD progression [78].

In fact, rodent immune systems differ significantly from those of humans. For example, the types and responses of immune cells, cytokine profiles, and the overall architecture of immune responses can vary greatly. These differences can lead to discrepancies in how diseases are modeled and treated in animals compared to humans. Many treatments that show promise in animal models do not translate effectively to human trials. This phenomenon is particularly pronounced in immunology, where the complexity of human immune responses often leads to unexpected outcomes in clinical settings. Two separate phase 2 clinical trials of CCX140-B, a novel CCR2 antagonist, have demonstrated that targeting CCR2 in mouse models does not yield significant results when compared to outcomes observed in patients with DKD [79, 80]. This discrepancy underscores the necessity for improved models that better replicate human immune responses. Researchers are increasingly looking at more

sophisticated models, including genetically engineered mice and humanized models [79], to bridge the gap between preclinical findings and clinical efficacy.

Use of single-cell RNA sequencing to identify immune cell in DKD

ScRNA-seq has significantly advanced our understanding of the immune landscape in DKD, highlighting the heterogeneity and plasticity of immune cell types involved. These techniques enable comprehensive profiling of gene expression across various renal cell populations, revealing not only traditional immune cells such as macrophages, T cells, and dendritic cells, but also less characterized immune cell types and their functional states in diabetes-related kidney injury. Additionally, sequencing technologies allow for the investigation of immune-related pathways and their transcriptional regulation within the kidney microenvironment, identifying key signaling molecules and inflammatory mediators that may drive DKD progression. By integrating bulk and single-cell sequencing data, researchers can better understand the dynamic interactions between immune cells and renal resident cells, enhancing insights into kidney inflammation, fibrosis, and renal dysfunction progression, thus generating potential immune metabolic therapeutic targets (Fig. 3) [81].

Dendritic cells and macrophage

Dendritic cells (DCs) and macrophages are pivotal components of the innate immune system and are widely distributed in kidney tissue. They play critical roles as sentinels and messengers, continuously monitoring the microenvironment for pathogens and other danger signals [82]. Dendritic cells are particularly adept at antigen presentation and activating T cells, thereby bridging innate and adaptive immunity. Macrophages, on the other hand, are phagocytic cells that not only engulf and digest cellular debris and pathogens but also produce a variety of cytokines that modulate the immune response.

Macrophages play a significant role in the pathogenesis of DKD, with two main subtypes exhibiting distinct functions: M1 macrophages, which are pro-inflammatory, and M2 macrophages, which are anti-inflammatory. In the context of DKD, an inflammatory response leads to an increased presence of immune cells, particularly M1 macrophages. This predominance indicates the critical role of M1 macrophages in the immune response and their contribution to renal injury [83]. For instance, You et al. demonstrated that M1 macrophages disrupt podocyte integrity, suggesting that strategies to mitigate the harmful effects of these macrophages on podocytes could be promising for DKD treatment [84]. Macrophages alter their characteristics in response to the local environment, a process called the M1-M2 transition [85]. Fu et al. investigated the transition of macrophage phenotype within the DKD kidney and confirmed the predominance of M1 macrophages over the M2 type in DKD samples, which is consistent with the results of previous studies showing inflammatory macrophages predominate in DKD glomeruli, suggesting the imbalance of M1/M2

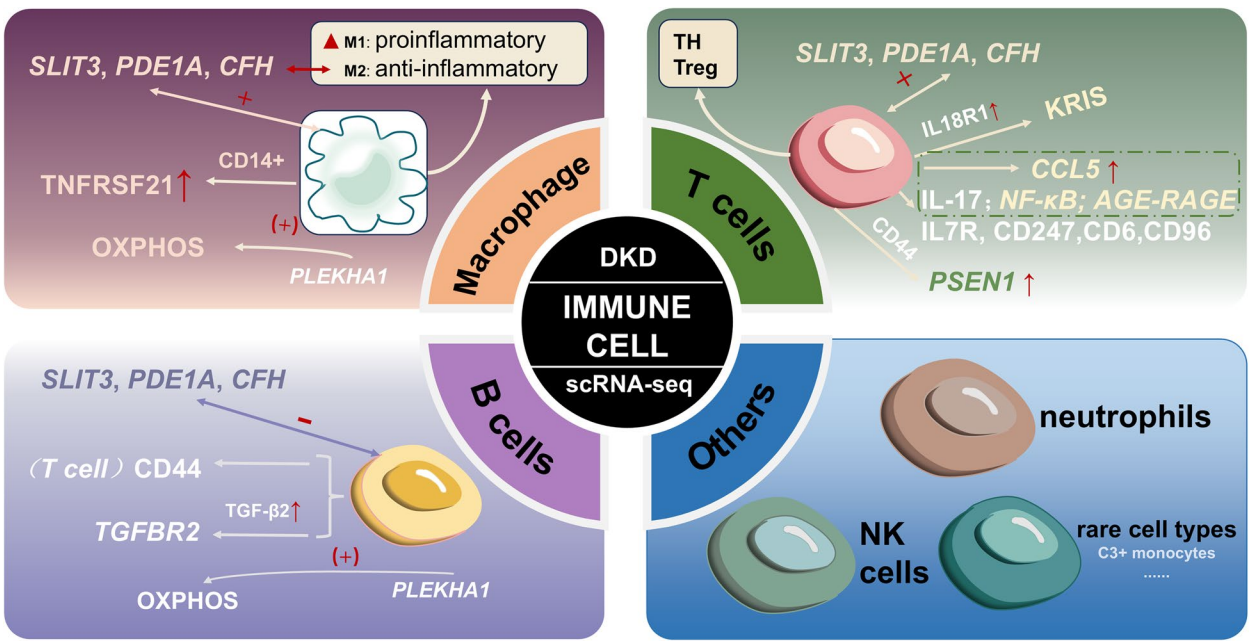

Fig. 3 Use of scRNA-seq to identify immune cell in DKD. Single-cell RNA sequencing has been employed in immune cell (macrophage, T cells, B cells and others) to improve understanding of normal and disease models

polarization results in renal tissue injury in DKD [46].In contrast to the studies above [46], although Wilson et al. found no significant macrophage infiltration in cryopreserved samples of human diabetic kidneys based on the single-cell transcriptomic landscape, a kidney risk inflammatory signature (KRIS) in CD14 + monocyte was upregulated in the diabetic kidney when involving two publicly available PBMC datasets in comparison analysis [86], and a spatial relationship between a C3 + monocyte subset and pro-inflammatory proximal tubules was confirmed [87], which also indicate that macrophages/monocytes have a significant role in the pathogenesis of DKD. On the one hand, the studies referenced have relatively small patient cohorts, which limits the ability to comprehensively characterize inter-individual variability and fully capture the spectrum of disease severity. On the other hand, Wu et al. utilized the dRNA HybISS approach to address the limitations of scRNA-seq, enabling the detection of transcripts in situ without the need for tissue dissociation or nuclear isolation. But it was constrained by a panel of only 200 genes, which limited the ability to identify specific lymphocyte subpopulations. To further investigate DKD, we required a more extensive panel of immune subtype-specific genes. The Xenium platform now provides the capability to utilize up to 480 custom gene probes, facilitating a more comprehensive analysis of immune cell diversity in DKD. Zhang et al. combined transcriptome data and single-cell analysis to increase the sample size, and found that the biomarker *PLEKHA1* has been shown to trigger DKD by activating macrophages, endothelial cells, and other related cells, thereby inducing oxidative phosphorylation (OXPHOS) in renal monocytes [88]. Furthermore, macrophages were regarded as the leading cell in the progression of DKD owing to the correlation between interstitial macrophages and interstitial fibrosis and tubular atrophy, DKD class, and renal function [89]. In flow sorting-assisted scRNA-seq research, macrophages with subtype-specific expression of pro-inflammatory or anti-inflammatory genes contribute to the progression of DKD through immune activation rather than direct mediation [90]. Further analysis showed an increased M1-like inflammatory phenotype, highlighting the dynamic polarization of macrophages in response to the disease environment [66]. Recent high-resolution spatial profiling studies have illustrated the heterogeneity among immune cells in DKD. SnRNA-seq datasets indicate that macrophages and dendritic cells (DCs) are predominant cell types within the renal environment. A reduction in statistical power for identifying differentially expressed genes was noted within immune cell clusters, which may limit the robustness of the findings [56]. Notably, analyzing the relationship between ligands and receptors in the features of single-cell expression profiles revealed that macrophages have a greater potential for interactions with other cell subtypes. Further investigation into the relationship between immune cells and key genes identified three core genes (*SLIT3*, *PDE1A*, and *CFH*), along with macrophage M2 polarization, show a positive correlation with immune cell infiltration [91]. These findings suggest that macrophages and their associated signaling pathways could serve as valuable diagnostic markers and therapeutic targets in the context of DKD.

T cells

T cells exhibit remarkable phenotypic and functional diversity among immune cells. Specialized T helper (TH) subpopulations—including TH1, TH2, TH17, TH9, TH22, and follicular TH cells—and regulatory T (Treg) cells differentiate from CD4 + naïve cells based on the specific cytokine milieu. Similarly, distinct cytotoxic subpopulations—TC1, TC2, TC17, and TC9—as well as CD8 + T regulatory cells arise from the differentiation of CD8 + T cells. These subpopulations respond to various stimuli, including antigens and cytokines, and contribute to the immune response by targeting infected or malignant cells while regulating immune activity to maintain homeostasis.

Recent studies have increasingly highlighted the role of specific genes in modulating T cell behavior and their implications for immune function in DKD. Notably, a comprehensive analysis of five microarray datasets, complemented by advanced techniques such as scRNA-seq, has revealed significant positive interactions between three core genes (*SLIT3*, *PDE1A* and *CFH*) and T cells gamma delta infiltration [91]. This underscores the critical role these genes play in regulating immune cell functions. *SLIT3* has been confirmed to influence DKD pathogenesis by activating extracellular matrix receptor interactions and focal adhesion pathways. *PDE1A* is involved in focal adhesion and lysine degradation pathways, while *CFH* affects peroxisome-generated succinate, contributing to DKD and other metabolic diseases. DKD's PBMC dataset and its samples are less, the anticipated publication of larger datasets will significantly enhance our understanding of the disease and facilitate more robust analyses of the associated genes. In further investigations, Li et al. explored ligand-receptor interactions using differentially expressed genes (DEGs) from T lymphocytes. They identified *PSEN1*, which is upregulated in glomerular cells, as an important player interacting with CD44 on B and T lymphocytes through autocrine and/or paracrine mechanisms [92].

Another research group observed an approximately 7-to 8-fold increase in leukocyte number consisting of T cells, plasma cells, etc., and IL18R1 was increased

in CD4+and CD8+T cells in diabetic patients, which are related to the production of KRIS markers due to immune cells infiltration [86]. The finding indicated a potential connection between immune cell activation and inflammation in diabetes, underscoring KRIS could be used as an immunotherapeutic target. One significant area of research focuses on the relationship between interleukins, T cells, and DKD [93]. A significant area of research focuses on the interplay between interleukins, T cells, and DKD. A series of studies have demonstrated that IL-17, produced by CD8+T cells, is upregulated under pathogenic conditions [94–97]. Elevated levels of IL-17 have been implicated in renal fibrosis and the deposition of complement proteins within the glomeruli [97–99], leading to a cascade of inflammatory responses. This sequence exacerbates leukocyte recruitment, exacerbating proteinuria and albuminuria, while also elevating blood urea nitrogen levels [100]. Several critical signaling pathways, including IL-17, NF-κB, and AGE-RAGE, have been identified in the pathogenesis of DKD. Chen et al. demonstrated that T cells express the chemokine *CCL5*, which recruits immune cells from the bloodstream to sites of infection and inflammation, first utilizing scRNA-seq and ST for analysis [101]. Understanding the roles of innate immunity pathways in DKD could pave the way for identifying novel therapeutic targets. A comprehensive investigation into the integration and regulation of these innate immunity pathways may yield specific targets with reduced toxicity for the treatment of DKD.

B cells

B cells, derived from lymphoid progenitor cells, play a key role in antigen presentation, antibody production, immune memory generation, and immune tolerance promotion. The presence of B cells is rare in healthy kidneys but their numbers increase during inflammatory reactions, and they are associated directly or indirectly with specific renal diseases which involved in immune response and regulatory processes [82].

Before the discovery of a role for B cells in DKD, several studies had confirmed the contribution of CD20 in the pathogenesis of LN, relapsing focal segmental glomerulosclerosis, and membranous nephropathy [102–104]. DKD is characterized by specific immunological changes in peripheral blood, particularly involving B cells. The count of $CD19^+$ $CD38^+$ B cells in peripheral blood was higher in patients with DKD and positively correlated with 24 h proteinuria level that a critical indicator of kidney damage [105]. Two important markers, *TGFBR2* and CD44, have been found to increase in B lymphocytes under diabetic conditions. *TGFBR2* activation, driven by upregulated TGF-β2 in endothelial cells, promotes epithelial-mesenchymal transition and subsequent fibrosis, highlighting the contribution of B cells to DKD pathology [92]. B cells are also known to secrete proinflammatory cytokines such as IL-17, which play a dual role: they enhance the activation of effector T cells and stabilize interactions between B and T cells. This interaction is particularly relevant in the context of autoimmune and autoinflammatory diseases, where sustained T cell activation is detrimental [106].

With the growing recognition of B cells in the pathogenesis of DKD, novel therapeutic strategies targeting B cells have emerged. Based on the snRNA-seq dataset, a high-resolution tool created by Wilson et al. [86], DKD samples with high interstitial fibrosis and tubular atrophy were found having more B cells [107]. According to the results of immune infiltration, Zhang et al. suggested naive B cells have a negative correlation with three key genes (*SLIT3*, *PDE1A* and *CFH*) and DKD patients have high expression of these three genes [91]. The phenomenon raises the prospect of these genes being leveraged as therapeutic targets, especially since targeting the B cell response could provide a dual benefit: modulating the immune landscape while directly influencing pathways associated with fibrosis and inflammation. A biomarker screening research based on single-cell analysis implied *PLEKHA1*, as an important biomarker, also a possible therapeutic target, might initiate DKD by activating B cells, proximal tubular cells, distal tubular cells, macrophages, and endothelial cells, thereby inducing OXPHOS in renal monocytes [88]. Future research should aim to employ single-cell sequencing techniques to elucidate the precise mechanisms through which B cells contribute to diabetic kidney disease DKD pathology and explore the potential synergies of combined immunotherapy approaches.This comprehensive approach may ultimately lead to more effective interventions, such as personalized treatment regimens that mitigate B cell-mediated damage and address the underlying fibrotic processes in the kidneys, resulting in improved outcomes for patients suffering from DKD.

Clinical therapies targeting the immune system for the treatment of DKD

As has been noted, the microenvironments of DKD are infiltrated with the immune cells including DCs, macrophages, T cells, B cells, neutrophils, NK cells, NKT cells and so on. Thus immunotherapy of DKD is naturally explored for potential targets in immune cells [108]. Zhang et al. identified several immunotherapeutic targets, notably the gene *SLIT3*, which has been shown to alleviate abnormalities induced by advanced glycation end products (AGEs) in human renal mesangial cells (HRMCs) associated with DKD. In addition to *SLIT3*, the

genes *PDE1A* and *CFH* were identified through scRNA-seq, DEGs, and weighted correlation network analysis (WGCNA). These findings underscore the potential for targeting these genes in therapeutic interventions for DKD [91]. Moreover, other studies utilizing WGCNA have revealed several gene signatures linked to DKD, including *CD36*, *ITGB2*,*SLC1A3*, *ADI1*, *PTGS2*, *DGKH*, *POLR2B*, *LCK* and *HCK* which are closely associated with immune cells [109–111]. Future studies should investigate how these gene signatures interact within the renal microenvironment and their potential as biomarkers for disease progression or therapeutic targets.

Many researches highlight the complex interplay between immune responses and DKD, emphasizing the potential of targeted therapies to modulate inflammatory mechanisms and improve clinical outcomes. Kaempferol and quercetin were suggested in Ma's research as the potential drugs to improve the immune and inflammatory mechanisms of DKD by affecting ferroptosis [14]. Kong et al. presented the potential therapeutic advantages of targeting the adaptive immune system (T cells) to impede disease progression [112]. Neutrophil extracellular traps (NETs) was also implicated being involved in DKD [113]. NET-induced sterile inflammation promotes diabetes-associated endothelial dysfunction and inhibition of NETs (PAD4 inhibitor) ameliorate endothelial dysfunction and renal injury in DKD [113]. The higher level of the systemic immune-inflammation index (SII), a novel and integrated inflammatory biomarker, is associated with DKD in T2DM patients, implying the SII could be a cost-effective and straightforward approach to detecting DKD [114]. In an experimental diabetic animal research, the interleukin-6 (IL-6), a pleiotropic cytokine was demonstrated to be an effective target, which was potently inhibited in the experiment by the recombinant anti-IL-6R fusion proteins (VHH-0031) in DKD [115]. It has been demonstrated that mesenchymal stem cells (MSCs) could prevent kidney damage in a diabetes model by modulating the immune system, which is achieved by suppressing the responses of CD8 + T cell [116] and the transcription factor EB (TFEB)-dependent M1/M2 macrophages (Mϕ) switch [117]. On the whole, the immune response's role in DKD pathogenesis is intricate and varied. scRNA-seq is going to act as a potent tool for technique application in the future exploration of immunotherapy for DKD.

Limitations of single-cell RNA sequencing in DKD

Single-cell RNA sequencing has emerged as one of the most transforming technologies in the life sciences nowadays. Despite the technology has taken a great leap forward, there are still several limitations. ScRNA-seq captures either entire individual cells or a portion of the molecules physically presenting in the cell, with capture rates varying from 5 to 20% in high-throughput protocols [118] to as high as 30–40% in certain well-based assays [119]. Thus, limited cell sequences accentuate the restrictions and uncertainties of the experiment, something that bulk counterparts do not encounter. Conversely, scRNA-seq is more likely to reflect the differences of gene expression on the cellular level, by examining genomic and transcriptomic changes within individual cells [120]. Although laboratory mice are extensively used in biomedical research, their immune systems differ functionally from those of humans. For instance, laboratory mice are more resistant to certain toxins than humans while differences even exist between species and their respective responses to treatment [121]. In addition, laboratory mice are susceptible to external environmental influences which result in changes in the physiological status of mice due to the breeding environment or inadequate experimental design by researchers [121]. When conducting research on human kidney specimens, the primary challenges are the difficulty in obtaining them and the ethical issues that they pose [74, 91]. The storing technique for human kidney tissue need to be improved while the availability of such tissue is limited. The renal stroma of adults is relatively dense, which makes the isolation of cells and capturing of RNA for individualized analysis challenging. Moreover the transcriptional status of an individual cell before dissociation is highly dependent on the quality of single-cell suspensions since enzymatic digestion protocols usually reduce the activity of cells [122].

ScRNA-seq has significantly advanced our comprehension of kidney immunity and has become a highly influential instrument in the investigation of kidney diseases nowadays. Studies on the immunity of DKD are comparatively limited when compared to studies of lupus nephritis, renal tumors, renal transplantation, and other diseases. Immune cells are much less than parenchymal cells, which makes it difficult to characterize immune cell heterogeneity in DKD and cost highly on scRNA-seq studies [56, 86]. The process of single-cell dissociation has been reported to damage cell health, leading to artifactual dissociation-induced transcriptional stress responses and the risk of RNA degradation [123]. Also immune cells in DKD were not comprehensively identified, most likely due to technical limitations in the single-cell transcriptome dataset. To address these challenges, future research should focus on developing improved dissociation protocols that minimize cell stress and enhance RNA quality. Additionally, integrating advanced computational methods to analyze multi-omic data could help characterize immune cell heterogeneity more effectively. The application of scRNA-seq in the immunity of DKD will become more widespread, providing insight into the

role of the immune system in health and disease. Achieving a comprehensive spatiotemporal immune landscape of DKD will necessitate the development of innovative strategies to analyze multiple single-cell profiles and their molecular signatures. Integrating diverse molecular data from various studies is crucial for advancing our understanding of DKD. However, the complexity inherent in synthesizing these diverse findings into a coherent framework poses significant challenges, particularly in this rapidly evolving field. Addressing these challenges will be essential for unlocking new insights and therapeutic approaches in DKD research.

Conclusions

ScRNA-seq is a revolutionary technology. At present, our understanding of kidney immunology is being rapidly advanced by scRNA-seq. Transcriptomic profiling of individual cells from DKD kidneys has substantially improved the resolution with which we understand the immune cell infiltrates in DKD, overviewing numerous macrophages, T cell, and B cell subsets that accumulate in DKD. These studies applied single-cell sequencing to investigate the immunology of DKD by examining renal biopsy samples, urine cells, and/or blood samples, leading to the identification of novel immune cell populations, gene regulation, and signaling pathways. Although interpreting scRNA-seq data comes with important limitations, utilizing these methods on informative cohorts of patients with DKD and other kidney diseases might provide clinically effective evaluation for prognosis and treatment response.

In this review, we summarize the study and application of single-cell transcriptomics in DKD immunology. Although there are still some limitations and challenges, scRNA-seq has a wide range of potential applications in the fields of kidney immunology, disease mechanism research, and drug therapeutic targets.

Abbreviations

scRNA-seq	single cell RNA sequencing
DKD	Diabetic kidney disease
ESRD	End-stage renal disease
RRT	Renal replacement therapy
DM1	Type 1 diabetes mellitus
DM2	Type 2 diabetes mellitus
FACS	Fluorescence-activated cell sorting
WGS	Whole genome sequencing
cDNA	Complementary DNA
WGA	Whole genome amplification
MDA	Multiple displacement amplification
PCR	Polymerase chain reaction
MCs	Mesangial cells
GECs	Glomerular endothelial cells
CKD	Chronic kidney disease
AKI	Acute kidney injury
TCR	T cell receptor
ccRCC	Clear cell renal cell carcinoma
PBMCs	Peripheral blood mononuclear cells
KTRs	Kidney transplant recipients
ACEi	Angiotensin-converting enzyme inhibitor
SGLT2i	Sodium-glucose cotransporter two inhibitors
LMD	Laser micro dissected
snRNA-seq	single nuclear RNA sequencing
MMP7	Matrix metalloprotease 7
PT	Proximal tubule
PTAQP4$^+$	PT containing AQP4 expression
DCs	Dendritic cells
KRIS	Kidney risk inflammatory signature
OXPHOS	Oxidative phosphorylation
TH	T helper
Treg	Regulatory T
DEGs	Differentially expressed genes
TGF-β2	Transforming growth factor-β2
AGEs	Advanced glycation end products
HRMCs	Human renal mesangial cells
WGCNA	Weighted correlation network analysis
NETs	Neutrophil extracellular traps
SII	systemic immune-inflammation index
IL-6	Interleukin-6
MSCs	Mesenchymal stem cells
TFEB	Transcription factor EB
eQTL	Expression quantitative trait locus
ST	Spatial transcriptomics

Authors' contributions

All literatures were reviewed by F.Y and C.D., Materials were collected by M.W, N.C, L.D, S.Z, T.W, J.Y., The final manuscript was drafted by C.D and F.Y., reviewed by C.D and approved by all authors.

Conflict of interest

The authors declare no conflicts of interest.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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