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LABORATORY STUDY

Comprehensive profiling of cell type-specific expression and distribution of complement genes in mouse and human kidneys: insights into normal physiology and response to kidney transplantations

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ABSTRACT

Background: Recent studies innovatively revealed the localized expression of complement genes in kidneys and shed light on the vital roles of the intracellular complement system in the physiologic function and pathological conditions. However, a comprehensive analysis of the expression of complement genes in the context of the evolving cellular landscape of the kidney is not available.

Methods: We analyzed single-cell RNA sequencing data from healthy human subjects, C57BL/6 mice, and kidney transplant-rejected mice. The data were sourced from the NCBI Gene Expression Omnibus and processed using quality control measures and unsupervised clustering. Differential gene analyses were based on expression levels.

Results: In total, 50 complement genes were categorized into pattern recognition molecules, proteases, complement components, receptors, and regulators. In normal mice kidneys, complement genes were expressed at relatively low levels. Among different complement gene categories, receptor genes were most widely expressed in kidney cells. Comparatively, macrophages and mesangial cells are the most abundant immune and nonimmune cell types for complement gene expression. A comparison of human and mouse data showed similar expression patterns, but human kidney complement gene expression was more abundant. Comparative analysis between mouse transplant-rejected and normal kidneys demonstrated stronger complement gene expression in transplant-rejected kidneys.

Conclusions: This study illustrated significant similarities in complement gene expression between murine and human kidneys and highlighted the responsive nature of complement genes to kidney injury, underscoring the dynamic nature of local complement regulation. These findings enhance our understanding of the complex regulation of the complement system within the kidney, offering insights into its role in renal disease pathogenesis.

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Introduction

The kidney is responsible for filtering metabolites from human blood and reabsorbing nutrients to maintain the body's internal environment [1]. The complexity and diversity of renal function stem from the remarkable and complex cellular heterogeneity [2]. Innovations in omics-based technologies have facilitated the identification of different renal cell types [3]. A recent study successfully established the first multidimensional single-cell reference atlas of the human kidney, encompassing various cell types in the human kidney [4]. These cell types include various nonimmune cell populations such as proximal renal tubular cells, distal tubular epithelial cells, collecting duct cells, endothelial cells, podocytes, mesangial cells, and vascular smooth muscle cells, as well as some immune cell populations including mononuclear phagocytes and lymphocytes. Each of these cell populations exhibits functional plasticity and is involved in multiple regulatory pathways [4].

The kidney is particularly vulnerable to dysregulated complement activity, and numerous studies have shown that the

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complement system is activated in renal diseases of different etiologies, including various forms of glomerulonephritis, tubulointerstitial diseases, thrombotic microangiopathy, and transplant rejection [5]. There are three pathways of activation of the complement system: the classical pathway (CP), the alternative pathway (AP), and the lectin pathway (LP) (Supplementary Figure 1). Activation of CP is initiated by IgG or IgM that binds specifically to the target antigen, forming an immune complex that further activates the C1 protein complex. LP is activated by pattern-recognition molecules, such as mannan-binding lectin proteins (MBL), which recognize and bind specific sugar groups on the surface of microorganisms [6]. AP is primarily triggered by nonspecific interactions of the organism with biological surfaces, independent of antibody involvement, and possesses the property of continuous low-level activation of plasma C3 to form C3b in a process called tickover. In addition, it can also be activated as an amplification loop for other pathways. Activation through each pathway produces C3 convertase, which cleaves circulating C3 into C3a (allergenic toxin) and C3b (opsonin) fragments, followed by the formation of C5 convertase capable of cleaving C5 into C5a and C5b. C5b binds to C6, C7, C8, and multiple C9 molecules to form the membrane attack complex (MAC, C5b-9) [7]. The complement activation pathway described above involves regulatory interactions between a network of about 50 complement proteins, which can be divided into five major categories: pattern recognition molecules, proteases, complement components, receptors, and regulators.

Despite the traditional view favoring hepatic synthesis of inactive complement precursors, the local production of complement proteins in other cells have attracted increasing interest [8,9]. Several recent studies have innovatively revealed the localized expression of complement genes in the kidney [10,11] and shed light on the intracellular complement system-complosome-serves vital roles in regulating multiple functions of kidney cells, such as regulating mitochondrial respiration, remodeling the cytoskeleton, altering the cell cycle, and mediates inflammatory responses [12].

Numerous studies have demonstrated that defining molecular diversity at the single-cell level is crucial for understanding kidney health and injury, as well as for identifying specific therapeutic targets for kidney diseases [13,14]. Recent advancements in single-cell and multi-omics technologies have revolutionized kidney research, enabled high-resolution mapping of cellular states and identified biomarkers for kidney injury and repair [4,15,16]. Landmark studies have defined nephron and stromal alterations during injury [4] and uncovered species-specific kidney disease traits [17]. Despite these advances, a comprehensive analysis of the expression of complement genes in the context of the evolving cellular landscape of the kidney is not available.

In this study, we initially performed a bioinformatics analysis of single-cell RNA sequencing data (scRNA-Seq) obtained from healthy mice and human kidney tissues. We aimed to elucidate the expression patterns of complement genes across different kidney cell types under normal physiological conditions. Considering the widespread complement activation throughout the kidney, including the glomeruli, tubules, vasculature, and interstitial tissues, differed from mainly glomerular complement activation in lupus nephritis and IgA nephropathy, we subsequently also examined the complement transcriptional profiles of diverse kidney cell populations in mouse transplant-rejected kidneys.

Methods

Data retrieval

Raw counts and associated metadata for the scRNA-Seq mouse kidney atlas were procured from the NCBI Gene Expression Omnibus database (accession number GSE193649, GSE140023, GSE157292) [18–20]. In total, the mouse dataset comprised 10599 normal kidney cells from six healthy C57BL/6 mice and 20570 cells from transplant-rejected kidneys of two BALB/c mice (Supplementary Table 1).

For human kidney atlas, data generated by Zhang Y et al. [21] and Liao J et al. [22]was analyzed (accession number GSE159115, GSE131685), which comprised 36418 cells from healthy tissue of 9 human kidneys (Supplementary Table 1).

In total, 50 complement proteins genes, including nine pattern recognition molecules, eight proteases, 10 complement components, 12 receptors, and 11 regulators were selected according to previous report [23] and pathways of complement in public databases (Reactome: R-MMU-166658 and KEGG: map06410).

Data analysis

The mouse and human datasets were analyzed using R (version 4.3.2, https://www.r-project.org/) and Seurat R package (version4.3, https://satijalab.org/seurat/).

The design of this study is illustrated in the Supplementary Figure 2. We first merged the mouse and human kidney datasets separately using the MergeSeurat function [24]. Based on quality control assessment, the dataset was filtered to exclude low-quality cells. Only cells that exhibited the expression of more than 200 genes and greater than 200 unique molecular identifiers (UMIs) per cell while maintaining mitochondrial gene expression below 20% were deemed eligible for subsequent analysis. The relationship between the percentage of mitochondrial genes and mRNA readings, as well as the relationship between the number of mRNAs and the reads of mRNA, were detected and visualized. To exclude data variations due to the cell cycle, we normalized the data using the NormalizeData function of Seurat (v. 4.3.0.1) and calculated and examined cell cycle phase scores based on the expression of canonical markers [25]. After re-normalization the data using Seurat's NormalizeData function, we identified all highly variable genes in single cells, controlling for the relationship between average expression and dispersion, and used them for downstream principal component analysis (PCA). Given that these data originate from distinct samples, we employed a

strategy known as 'Harmony' to mitigate potential batch effects and ensure the integrity of downstream analyses [26]. To reveal the underlying structure of the dataset, non-linear dimensional reduction was executed using UMAP, relying on the top 50 principal components with the highest variance. Nearest neighbors were identified based on the K-nearest neighbor graph using FindNeighbors function and the cells were grouped based on the Louvain algorithm using the FindClusters function, resulting in different clusters and classified into different cell types in mice and human datasets, respectively. Cell type assignment was based on canonical markers from literature, and marker genes for cell clustering was included in Supplementary Table 2. Next, the FindMarkers function was used to find differentially expressed genes between different groups of each cell type. All dot plots, violin plots and heatmaps, as well as differential expression analyses relied on RNA assays.

Cell culture

The human proximal tubular epithelial cell line HK-2 (ATCC, USA) was cultured in MEM medium supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Beyotime, China). Primary human mesangial cells (ScienCell, USA) were cultured in mesangial cell medium (ScienCell, USA) supplemented with mesangial cell growth supplement (ScienCell, USA) and 2% FBS (Gibco, USA). Bone marrow-derived macrophages (BMDMs) were generated from C57BL/6 mice. Bone marrow cells were flushed from femurs and tibias, filtered, and cultured in 1640 medium with 10% FBS Gibco, USA), 1% penicillin-streptomycin, and 20 ng/ml M-CSF (PeproTech).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cultured cells using the RNAex Pro reagent (Agbio, China) following the manufacturer's instructions. Reverse transcription was performed using the Evo M-MLV kit (Agbio, China). The mRNA levels were quantified by PCR (Supplementary Table 3) using the SYBR Green Pro Taq HS kit (Agbio, China). GAPDH and β-actin were used as internal controls. The PCR products were subsequently analyzed by agarose gel electrophoresis.

Immunofluorescence

HK-2 cells and BMDMs on slides were fixed with 4% paraformaldehyde and permeabilized with Triton X-100. Then, primary antibodies including rabbit anti-C3 antibody (Proteintech) and Mouse anti-C3a antibody (Abcam), followed by secondary antibodies including Alexa Fluor 647 AffiniPure Goat Anti-Rabbit IgG (Jackson ImmunoResearch) and Alexa Fluor 488 AffiniPure Fab Fragment Goat Anti-Mouse IgG (Jackson ImmunoResearch) were added for detection. Nuclei were counterstained with DAPI.

Statistical analysis

Differential gene expression between different cell types and groups was examined using the two-sided Wilcoxon Rank Sum Test, implemented *via* Seurat's FindMarkers function. Parameters included a log2 fold-change (log2FC) threshold of ≧0.75 and a minimum percentage (min.pct) of 0.1, which ensured that only genes expressed in at least 10% of the cells were tested. All differential analyses were based on expression levels. Significance was set at a threshold of 0.05, and adjusted p-values < 0.05 were deemed statistically significant.

Results

Expression profiles of complement genes across diverse mice kidney cell types

To elucidate the cell type-specific expression of complement genes in normal kidney cells, we obtained single-cell sequencing datasets of kidney tissues from NCBI's Gene Expression Omnibus database for computational analysis, which were derived from the studies of Gaedcke S et al. [18] and Conway BR et al. [19].

After quality control, preprocessing, and unsupervised clustering, we categorized the dataset into 14 different cell types, including a variety of immune cells (e.g., macrophages, dendritic cells, T cells, and B cells) and nonimmune cells (e.g., renal tubular epithelial cells, podocytes, mesangial cells, and endothelial cells) **(**Figure 1(A)**)**.

We subsequently analyzed the cell type-specific expression and distribution of 50 complement genes, which were classified into five groups: pattern recognition molecules, proteases, complement component genes, receptors, and regulatory genes (Supplementary Table 4). Overall, complement genes were expressed at low levels in normal mice kidney cells. Comparatively, macrophages and mesangial cells are the most abundant immune and nonimmune cell types for complement gene expression.

Pattern recognition molecules

Pattern recognition molecules (PRMs) identify and bind to specific molecular patterns on pathogens or damaged/infected cells, activating complement and triggering an inflammatory response to remove these threats. We examined the expression of 8 PRMs in mouse kidney cells, finding high expression of C1q in macrophages and Cfp in neutrophils. C1q, a cyclic protein complex of six identical trimers, primarily binds to antigen-antibody complexes, initiating the classical pathway of the complement cascade. In normal kidney cells from C57BL/6 mice, all three subunits of C1q were expressed in 75% of macrophages **(**Figure 1(B)**)**. Properdin (Cfp) is a positive regulator of the complement alternative pathway that enhances the effects of the alternative pathway by stabilizing the C3bBb complex, and it was highly expressed in 25% neutrophils in mouse kidney **(**Figure 1(B)**)**.

Ficolin-1 (Fcna) and Ficolin-2 (Fcnb), as well as mannose-binding lectin 1 (Mbl1) and 2 (Mbl2) can recognize and bind

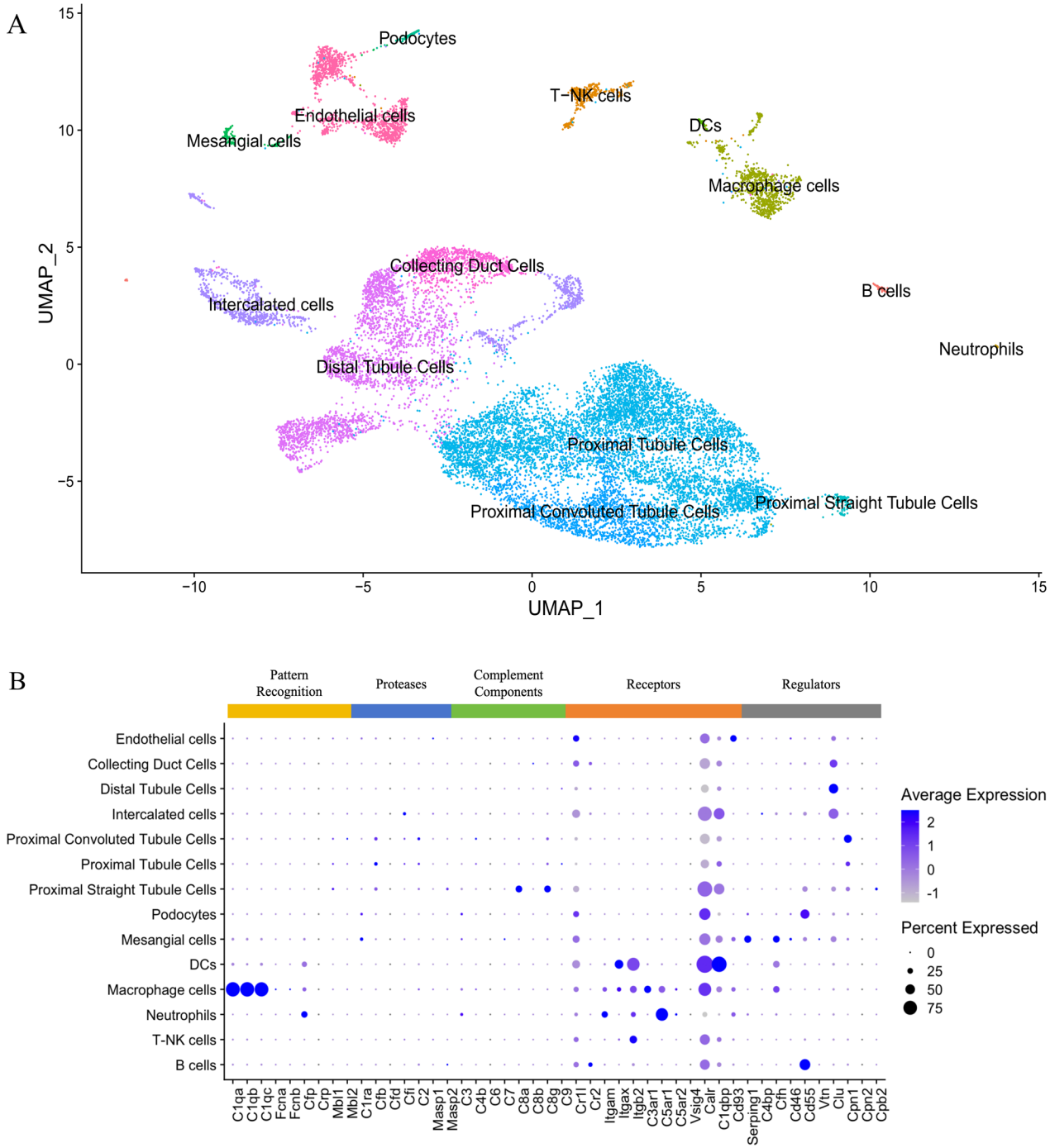

Figure 1. Complement gene expression in mouse normal kidney tissues.
A. Visualization of different cell types identified in the mouse kidney transcriptome. Unified manifold approximation and projection (UMAP) plot of the kidney single-cell transcriptome from healthy C57BL/6 mice (from 6 mice, 8-12 weeks old). B. Dot plot illustrating the expression pattern of all complement genes in various cell types identified in the single cell transcriptome data for normal mouse kidney (n = 6). Size of the dots represents the percentage of cells expressing a gene and color intensity represents the average expression level. Missing complement components indicated that their expression was not detected in the datasets. Data from healthy C57BL/6 mice (8–12 weeks old).

to specific glycosyl group on the surface of pathogens, causing complement activation of the lectin pathway. However, only small amounts of expression of them were found in a variety of micemic kidney cells (Figure 1(B)).

Proteases

Binding of pattern recognition molecules or complement components to zymogen proteases activates the complement system. Serine proteases act synergistically with each other to ensure proper recognition, labeling, and clearance of pathogens in the immune response while avoiding undue damage to host tissues.

In our study, we found that in normal kidney tissues of mice, complement component 1 subcomponent r-A (C1ra) was expressed in approximately 10% of mesangial cells and 5% of podocytes, complement factor B (Cfb) and complement

component 2 (C2) were expressed in 10% of proximal tubular cells, and mannan-binding lectin serine peptidase 2 (Masp2) and complement factor I (Cfi) were expressed in 5% of proximal tubular cells and 10% of intercalated cells, respectively (Figure 1(B)). These proteases function in CP (C1ra, C2), AP (Cfb, Cfi), and LP (MASP-2, C2), respectively.

Interestingly, the above protease genes are mainly expressed in non-immunized cells, especially in proximal tubular cells and mesangial cells. In addition, other serine proteases, including Complement component 1 subcomponent r-B (C1rb), Complement component 1 subcomponent s (C1s), Mannose-binding lectin serine peptidase 1 (Masp1), and Complement factor d (Cfd) were not detected in any of the cell types in the mouse kidney dataset (Figure 1(B)).

Complement components

Complement components C3, C4, C5, C6, C7, C8, and C9 are key functional proteins in the complement cascade reaction and are essential for the immune response and terminal effects of complement activation.

The analysis showed low expression of C3 in a variety of cells (e.g., mesangial cells, proximal tubule cells, podocytes, macrophages, and neutrophils) in mouse normal kidney (Figure 1(B)). Expression of C7 was only detected in small amounts in mesangial cells. C8 is a complex composed of three subunits, C8a, C8b, and C8g. The results showed that C8a and C8g were highly expressed in 30% of proximal straight tubule cells and that C4b, C8a, and C8g were also lowly expressed in other tubule cells, predominantly proximal convoluted tubule cells and distal convoluted tubule cells (Figure 1(B)). Expression of other complement components (C8b and C9) was relatively low in the mouse dataset. C4a, C5, and C6 expression were completely undetectable in these mice.

Overall, complement component genes were expressed at low levels in normal mouse kidney tissues, suggesting that complement component genes are produced locally less in the kidney under normal conditions.

Receptors

Complement receptors are proteins present on the cell surface that mediate the cellular response to the complement system by binding to complement molecules. The activity of the complement system is not only controlled by the activation and regulation of complement components, but also by a variety of complement receptors.

The Cr1l gene for complement receptors showed moderate expression in almost all non-immune cells. Cr2 gene was expressed only in a small number of B cells (Figure 1(B)). Itgam and Itgax were highly expressed in 25% of neutrophils and 50% of dendritic cells, respectively, whereas Itgb2 was expressed at high levels in 25%-50% of macrophages, dendritic cells, and T-NK cells. Concerning allergy toxin receptors, C3ar1 was highly expressed in approximately 25% of macrophages, and C5ar1 was highly expressed in about 25% of macrophages and 50% of neutrophils (Figure 1(B)). Notably, C3ar1 and C5ar1, two inflammation-associated receptors, were co-expressed in macrophages, a well-documented infiltrating inflammatory cell type in kidney diseases, and there was a positive, albeit weak, correlation between their expression in macrophages ($R = 0.37$) (Supplementary Figure 3A, B).

Calr was involved in protein folding, quality control, and transport in the endoplasmic reticulum, as well as in apoptosis and immune system regulation, and was universally highly expressed in almost all renal cells. The CD93 gene was expressed in only about 25% of endothelial cells (Figure 1(B)). Overall, the expression of complement receptors favored immune cells compared to non-immune cells.

Regulators

Complement regulators are found both on cell surfaces and in body fluid. They regulate the activity of complement proteins by a variety of mechanisms, including inducing their inactivation, preventing their binding to target structures, and facilitating their degradation, to ensure that the complement system is activated when it is appropriate and inhibited when it is not necessary.

Among the different regulators of the complement system investigated, Complement Factor H (Cfh), Membrane Cofactor Protein (MCP/CD55), and Clusterin (Clu) were expressed at high levels in mouse normal kidney. Expression of the C1 inhibitor (C1-INH/Serping1), Cfh and Clu was detected in approximately 30% of the mesangial cells. Of these, Cfh was also highly expressed in 30% of macrophages and dendritic cells, whereas Clu was mainly expressed in nonimmune cells (Figure 1(B)). Membrane attack complex inhibitory factor (Cd59b) was also predominantly expressed in non-immune cells. At least 40% of B cells and podocytes highly expressed the Decay-Accelerating Factor (Cd55/DAF). Carboxypeptidase-N1 (Cpn1) gene is highly expressed mainly in 40% of proximal renal tubule cells. Complement Component 4 Binding Protein (C4bp), Carboxypeptidase-B2 (Cpb2), Vitronectin (Vtn), and Membrane Cofactor Protein (Cd46/MCP) were expressed at low levels (Figure 1(B)), while Carboxypeptidase-N2 (Cpn2) did not have any related mRNAs detected in the normal mouse kidney dataset.

Complement expression in human kidney

To understand the similarities and differences in local complement expression within the kidney between mice and humans, we also analyzed human kidney datasets, which consisted of 15 distinct cell types (Figure 2(A)). There were many similarities and some differences in the expression patterns of complement genes between human and mouse kidneys. The most striking similarity was the high expression of several complement genes in macrophages and mesangial cells, as observed in mice.

In human kidney, C1QA-C and CFP were mainly expressed in macrophages and dendritic cells, and CFP expression was not detected in neutrophils. Interestingly, we observed FCN2 and FCN3 expression in human endothelial cells (Figure 2(B)), whereas they were barely detectable in mouse kidney (Figure 1(B)).

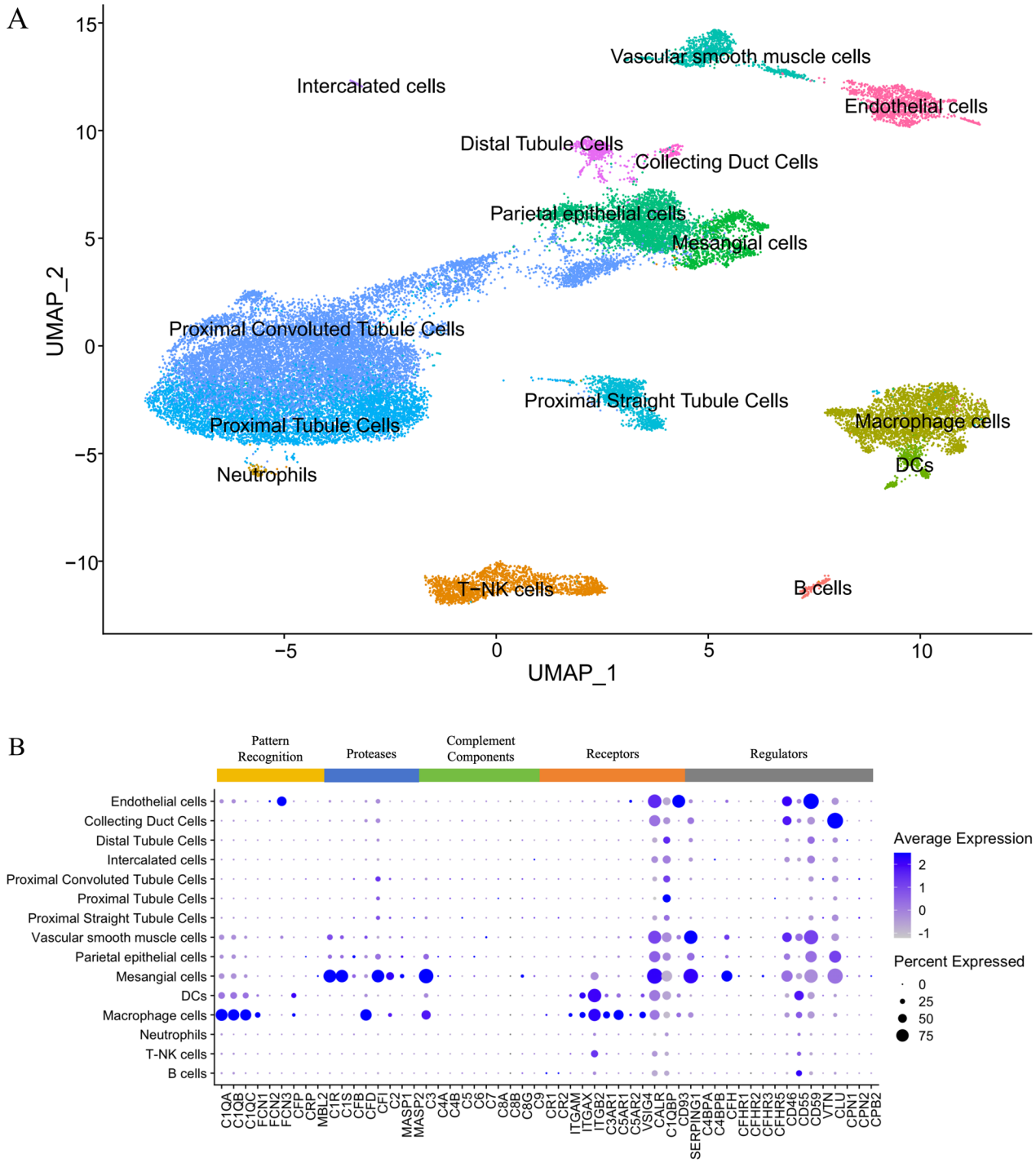

Figure 2. Complement gene expression in healthy human kidney tissues.
A. Visualization of various cell types identified in the human kidney transcriptome. Unified manifold approximation and projection (UMAP) plot of the kidney single-cell transcriptome from healthy human kidney samples. B. Dot plot illustrating the expression pattern of all complement genes in various cell types annotated in the single cell transcriptome data of healthy kidney tissue of human patients ($n = 9$). Size of the dots indicated the percentage of cells expressing the gene, and the color intensity represented the average level of expression. The human data were based on single-cell RNA sequencing of healthy, uninvolved renal regions from patients undergoing partial nephrectomy for renal tumors ($n = 6$), as well as single-cell RNA sequencing of renal tissue from radical nephrectomy ($n = 3$). Normal kidney tissues were obtained at least 2 cm away from tumor tissue.

Regarding the expression of complement proteases, unlike in mice, high expression of C1R, C1S, CFI, C2, and MASP1 was observed in human mesangial cells and a small number of parietal epithelial cells, as well as high expression of CFD and moderate expression of C2 in human macrophages (Figure 2(B)).

Complement component protein C3 was highly expressed in 25-50% of human mesangial cells and macrophages and moderately expressed in parietal epithelial cells and dendritic cells, whereas the expression pattern of complement components C4-C9 was essentially the same in both human and mouse kidneys (Figures 1(B), 2(B)).

The complement receptors ITGAM, ITGAX, ITGB2, C3AR1, C5AR1, C5AR2, and VSIG4 were mainly expressed in myeloid cells, such as macrophages and dendritic cells (Figure 2(B)). ITGB2 was also detected in T-NK cell populations and human mesangial cells, which was not found in mouse mesangial cells (Figure 1(B)). Expression of CALR and C1QBP was ubiquitous, with the highest levels observed in endothelial cells, vascular smooth muscle cells, mesangial cells, collecting duct cells, macrophages, and dendritic cells. CD93 was highly expressed in endothelial cells, similar to mouse kidney (<25% of cells), dendritic cells and macrophages. However, CD93 was more abundant in human kidneys (<50% of cells) (Figure 2(B)).

The complement regulator SERPING1 was abundantly expressed in 75% of mesangial cells and vascular smooth muscle cells. CFH was highly expressed in 50% human mesangial cells, similar to mouse kidney, but was less expressed in macrophage cells (Figure 2(B)). Expression of CD46, CD55, and CD59 appeared to be more prevalent and abundant in human kidney compared to mouse kidney, with high levels of expression observed in clusters of human endothelial cells, vascular smooth muscle cells, and mesangial cells (Figure 2(B)). CLU was highly expressed in human collecting duct cells, mesangial cells and parietal epithelial cells. C4BP, VTN, CPN1, CPN2 and CPB2 were expressed at low levels in most cell clusters of the human kidney, whereas the CPN1 gene was moderately expressed in mouse proximal tubular cells **(**Figures 1(B),2(B)**)**. In addition, co-expression of C3AR1 and C5AR1 was also found in the human kidney dataset, similarly, there was a positive correlation between them (Supplementary Figure 3C, D).

In summary, the kidney dataset from nine human patients recapitulated the major findings in terms of the expression of several complement proteins in kidney cells from six wild-type mice, although differences in some cell types and complement genes were also noted.

Complement expression in mouse transplant-rejected kidney

Comparative analysis of complement genes expression trends in mouse transplant-rejected kidney tissues and normal kidney tissues revealed stronger complement gene expression in transplant-rejected kidney.

The pattern recognition molecule C1q was the most commonly differential expression complement gene in transplant-rejected and normal kidneys, with elevated expression in most cells in transplant-rejected kidney. In addition, the expression of Cfp was increased in macrophages and monocytes of transplant-rejected kidney. Regarding proteases, C2 gene in loop of henle cells was the only differentially expressed gene between transplant-rejected and normal kidneys (Tables 1,2).

Expression of complement component gene C3 was significantly increased in monocytes, macrophages, mesangial cells and proximal renal tubule cells of transplant-rejected kidney compared with normal kidney. Similarly, C4b expression was elevated in macrophages, proximal tubule cells and loop of henle cells (Tables 1, 2) (Supplementary Figure 4).

Complement receptor Calr was more abundant in almost all renal tubular cells of transplant-rejected kidneys, and C5ar1 expression was reduced in macrophages of transplant-rejected kidneys compared to normal kidney (Tables 1, 2).

Expression of complement regulators Cd59a in mesangial cells and Cfh in macrophages was reduced in transplant-rejected kidneys compared with normal kidneys. Meanwhile, in transplant-rejected kidneys, Serping1 expression level was elevated in podocytes and endothelial cells, whereas the expression level of Cpn1 was decreased in proximal tubule cells (Tables 1,2).

Complement gene expression in mouse BMDMs, human mesangial cells and HK-2 cells

To validate the complement gene expression in the kidney, we then detected the mRNA and protein expression of some complement genes in mouse BMDMs, human mesangial cells and HK-2 cells, which showed relatively higher expression of complement genes. We confirmed the mRNA expression of C3, C5, C3ar1, C5ar1, Cfp, and Cfb in BMDMs, as well as C3 and C3AR1 in both human mesangial cells and HK-2 cells (Figure 3(A)). Additionally, using immunofluorescence staining we confirmed the expression of C3 protein in both BMDMs and HK-2 cells (Figure 3(B)).

Discussion

In our study, we provided a comprehensive overview of the localized expression of complement genes through bioinformatics analysis of open-access mouse and human scRNA-Seq profiles. Our results highlighted the expression of complement pattern recognition molecules, proteases, complement components, receptors, and regulators across different kidney cell types. These findings suggest that tissue-specific complement proteins in the kidney may play roles in both physiological and pathological conditions.

Previous studies have demonstrated that although complement produced in the kidneys is in small amounts, it is significant [27]. For example, deficient local synthesis of C3 in the kidney leads to defective T-cell priming and reduces renal allograft rejection, with local extravascular concentrations of C3 being more crucial than circulating C3 in determining acute rejection [28]. The glomerular basement membrane, primarily composed of extracellular matrix, is particularly prone to complement activation due to its lack of intrinsic complement regulators and reliance on soluble complement regulators [29]. In certain glomerular diseases, the expression of complement regulators increases, suggesting a protective response [30]. These studies indicate that locally expressed complement proteins contribute to complement activation and regulation in the kidney, though the functions of many complement proteins remain unknown. Our analyses identified the expression levels of 50 complement genes across 14 mouse kidney cell types and 15 human kidney cell types. We found that pattern recognition molecules were expressed at higher levels in immune cells, while proteases

Table 1. Differential complement genes in non-immune cells: Transplantation vs. Control.

Cell type	Gene	p_val	avg_log2FC	pct.1	pct.2	adjusted_p_val
Mesangial cells						
	C3	4.86E-12	2.23	0.40	0.01	1.57E-07
	C4b	1.23E-06	1.09	0.18	0	4.01E-02
	Cd59a	1.83E-07	−1.98	0.15	0.62	5.93E-03
Podocytes						
	Cd93	4.87E-30	1.35	0.56	0.06	1.58E-25
	Serping1	2.73E-39	1.69	0.49	0.01	8.87E-35
Proximal Straight Tubule Cells						
	C1qb	1.81E-31	1.03	0.35	0.03	5.89E-27
	Cpn1	1.09E-29	−1.027	0.18	0.44	3.53E-25
	C4b	8.87E-124	1.95	0.82	0.02	2.88E-119
	Calr	1.40E-83	1.11	0.95	0.74	4.55E-79
Proximal Tubule Cells						
	C1qa	5.33E-62	1.67	0.27	0.01	1.73E-57
	C1qb	1.26E-72	1.76	0.33	0.02	4.09E-68
	C1qc	2.67E-28	0.99	0.14	0.01	8.68E-24
	Cpn1	9.23E-52	−1.356	0.04	0.28	2.99E-47
	C3	1.03E-26	0.85	0.14	0.01	3.35E-22
	C4b	8.94E-105	1.77	0.39	0	2.90E-100
	Calr	8.02E-115	1.66	0.67	0.18	2.60E-110
Proximal Convoluted Tubule Cells						
	C1qa	1.26E-11	0.90	0.25	0.02	4.09E-07
	C1qb	1.40E-18	1.24	0.35	0.01	4.55E-14
	C4b	1.53E-37	1.86	0.60	0.01	4.96E-33
	Calr	3.06E-30	1.12	0.93	0.63	9.94E-26
Intercalated cells						
	C1qa	1.71E-11	2.12	0.31	0.01	5.56E-07
	C1qb	4.59E-14	1.60	0.38	0.01	1.49E-09
Distal Tubule Cells						
	C1qa	1.82E-22	2.21	0.31	0.01	5.89E-18
	C1qb	2.24E-28	2.24	0.38	0.01	7.25E-24
	C1qc	4.26E-10	1.11	0.15	0.01	1.38E-05
	Calr	1.73E-33	1.61	0.60	0.14	5.60E-29
Collecting Duct Cells						
	Calr	2.44E-27	1.50	0.98	0.61	7.91E-23
	Clu	6.58E-13	2.14	0.69	0.24	2.14E-08
Endothelial cells						
	C1qa	2.47E-38	1.51	0.31	0.01	8.01E-34
	C1qb	3.94E-35	1.58	0.31	0.01	1.27E-30
	C1qc	8.11E-16	0.76	0.14	0.01	2.63E-11
	Serping1	1.76E-31	1.17	0.31	0.02	5.70E-27
Loop of Henle cells						
	C1qb	7.05E-09	1.49	0.32	0	2.29E-04
	C2	1.83E-10	0.89	0.42	0.03	5.94E-06
	C4b	3.49E-14	1.50	0.49	0	1.13E-09
	Calr	1.11E-13	1.17	0.92	0.65	3.59E-09

p_val: The original unadjusted p-value, indicating the significance level of differential expression.
avg_log2FC: The average log2 fold change, representing the mean logarithmic fold change in gene expression levels between the two groups. A positive value indicates upregulation of the gene in the transplantation group relative to the control group, while a negative value indicates downregulation.
pct.1: The percentage of cells expressing the gene in the transplantation group.
pct.2: The percentage of cells expressing the gene in the control group.
p_val_adj: The p-value adjusted for multiple hypothesis testing.

and regulators were more expressed in non-immune cells. This study lays a foundation for investigating the complement regulatory functions of renal local complement components.

Another important finding was that several complement proteins are expressed at mid-to-high levels in mouse and human mesangial cells compared to other non-immune cells, suggesting significant roles in renal physiology or pathological states. Mesangial cells, with their irregular morphology and well-developed organelles, perform functions such as synthesis, secretion, phagocytosis, degradation, and contraction [31,32]. In pathological conditions like glomerulonephritis, mesangial cell proliferation and functional activities are enhanced [33]. Complement activation in mesangial cells may exacerbate renal pathological injury and fibrosis [34]. The non-canonical roles of complement and the intracellular complement system (complosome) are gaining attention, potentially playing critical roles in cellular functions that are not yet fully understood [35,36]. Further exploration is needed to determine whether complement proteins in mesangial cells have non-canonical functions involved in renal disease pathogenesis.

Consistent with previous studies [37,38], our research identified the expression of various complement genes in immune cells, such as C1Q, CFH, and C3 in macrophages, ITGB2 in T-NK cells, and CD55 in B cells. Beyond their role in

Table 2. Differential complement genes in non-immune cells: Transplantation vs. Control.

Cell type	Gene	p_val	avg_log2FC	pct.1	pct.2	adjusted_p_val
T-NK cells						
	C1qa	1.82E-16	1.39	0.23	0.01	5.90E-12
	C1qb	1.41E-19	0.89	0.29	0.02	4.56E-15
Neutrophils						
	C1qa	6.53E-07	1.72	0.25	0	2.12E-02
	C1qb	6.47E-09	1.77	0.33	0.01	2.10E-04
Macrophage cells						
	Cfp	7.40E-103	1.47	0.61	0.11	2.40E-98
	C3	7.99E-191	2.57	0.77	0.05	2.59E-186
	C4b	6.35E-41	0.94	0.26	0.01	2.06E-36
	C5ar1	4.27E-17	−0.93	0.25	0.35	1.38E-12
	Cfh	7.95E-20	−0.79	0.19	0.32	2.58E-15
DCs						
	C1qb	2.24E-07	0.86	0.31	0.07	7.27E-03
	C3	3.78E-07	0.84	0.27	0.05	1.23E-02
Monocytes						
	Cfp	2.01E-08	1.28	0.89	0.46	6.52E-04
	C3	2.23E-09	2.10	0.70	0	7.24E-05

p_val: The original unadjusted p-value, indicating the significance level of differential expression.
avg_log2FC: The average log2 fold change, representing the mean logarithmic fold change in gene expression levels between the two groups. A positive value indicates upregulation of the gene in the transplantation group relative to the control group, while a negative value indicates downregulation.
pct.1: The percentage of cells expressing the gene in the transplantation group.
pct.2: The percentage of cells expressing the gene in the control group.
p_val_adj: The p-value adjusted for multiple hypothesis testing.

inflammatory responses, recent studies have shown that complement proteins in immune cells perform non-canonical functions in metabolism and immune regulation [39,40]. In transplant-rejected mouse kidneys, we found the expression of several complement proteins in immune cells was altered, with C1Q showing elevated levels in nearly all immune cells. C1q has been identified as a central modulator of immune cell activity [41], linked to mitochondria-driven ROS production and oxidative stress, as well as AKT activation, which triggers downstream mTOR activation and cell proliferation [42]. Growing evidence suggests that various intracellular complement components serve as central regulators of fundamental cellular processes and immune modulation [43,44], and similar roles may be present in kidney immune cells.

In this study, we analyzed both mouse and human kidney datasets independently in order to ensure that the observed complement genes expression patterns are not confined to a single species. Similar gene expression trends were identified in both mouse and human, providing strong evidence of a conserved complement activation and regulation process in the kidney. And the identified concordance between mouse and human kidney datasets allowing researchers, who focused on complement in the kidney, to better interpret the relevance of mouse findings to human kidney biology and disease.

Given the kidney's unique susceptibility to complement attack, extensive studies are underway to evaluate the potential efficacy of complement inhibitors in various renal pathological conditions, including PNH, lupus nephritis, kidney transplantation, IgA nephropathy, aHUS, and C3 glomerulopathy [45,46]. The traditional complement-targeted therapies primarily target serum-active or extracellular complement [47]. However, our findings underscore the importance of considering locally produced renal complement proteins. The kidney cell-specific complement gene expression profile may provide insights for more inclusive design strategies, potentially leading to the development of novel targeted therapies with enhanced efficacy and fewer side effects.

Increasing evidences indicated the important roles of complement activation in both acute and chronic rejection following transplantation [5,48,49]. In patients receiving kidney transplantation, elevated perioperative sC5b-9 levels in organ donors correlate with the risk of transplantation rejection and delayed graft function [50,51]. Furthermore, transplant biopsies have revealed deposits of MAC and C3d in the transplant-rejected kidney [52]. Moreover, C5aR inhibition or C3aR/C5aR defects reduce immune responses, diminish inflammation and prolong graft survival, highlighting complement's dual innate-adaptive role [53–55]. Our findings regarding changes in complement gene expression during kidney transplantation support the involvement of complement activation in transplantation and also indicated the induction of local complement expression and regulation in this context. Animal models have shown that the local synthesis of C3 is critical for an effective immune response. Lack of local C3 synthesis results in impaired T cell activation and subsequently weakened immune responses to donor antigens, leading to prolonged graft survival [28]. In addition to C3, many other complement proteins that changed during kidney transplantation require further exploration.

Given the close relationship between complement system activation and transplant rejection, the expression patterns of complement proteins in kidney cells could potentially serve as important biomarkers for predicting kidney transplant outcomes. Such biomarkers would help clinicians to detect transplant rejection early, enabling timely interventions and treatments. Furthermore, these biomarkers could assist in optimizing transplant matching during the early stages, allowing for the avoidance of high-risk matches that are likely to result in rejection. Additionally, future single-cell RNA sequencing data from kidney tissue could provide a valuable tool for personalized immunosuppressive therapy in kidney transplant patients. By identifying specific complement gene expression profiles in individual patients, clinicians could tailor immunosuppressive treatments more precisely, thereby optimizing therapeutic efficacy while minimizing side effects. We believe that further exploration of complement gene expression in the kidney will greatly enhance our understanding of the immune mechanisms underlying kidney transplant rejection. This approach to precision medicine could pave the way for better management of kidney transplant patients and improve long-term transplant outcomes.

Our study has several limitations that warrant consideration. Firstly, although single-cell RNA sequencing is a powerful analytical tool, it is susceptible to certain technical biases. These include variations in cell capture efficiency, RNA

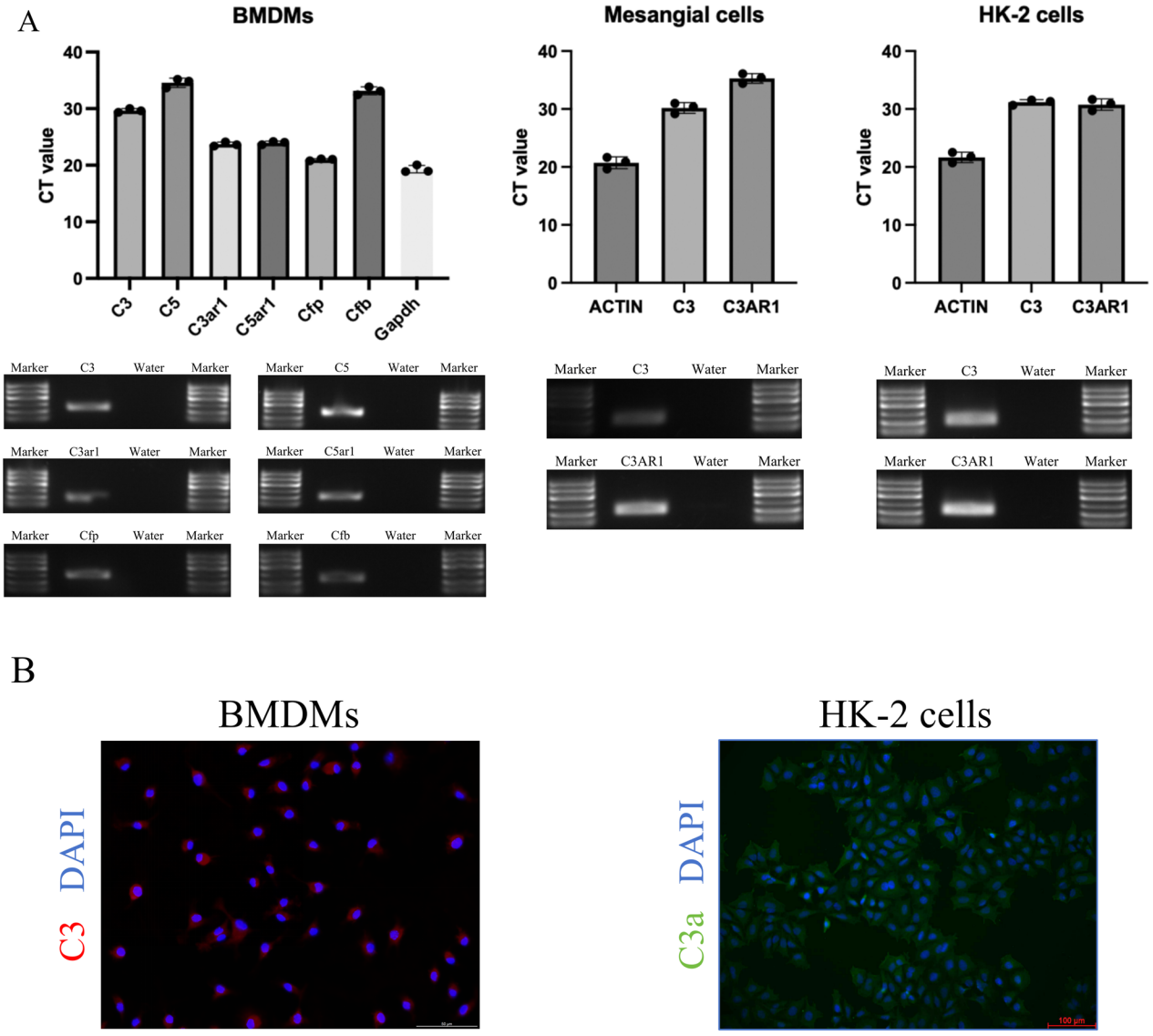

Figure 3. Complement gene expression in mouse BMDMs, human mesangial cells and HK-2 cells.
A. qRT-PCR and agarose gel electrophoresis results demonstrated the expression of C3, C5, C3ar1, C5ar1, Cfp, and Cfb in BMDMs, C3 and C3AR1 mRNA were expressed in both human mesangial cells and HK-2 cells. B. immunofluorescence staining confirmed the expression of C3 protein in both BMDMs and HK-2 cells.

degradation rates, and differences in library preparation protocols, all of which can affect data quality, potentially leading to underrepresentation of specific cell populations or gene transcripts. Secondly, our study primarily employed bioinformatic analysis. While our findings provide a preliminary atlas of complement gene expression in the kidney, they should be viewed as a foundation for further investigation. The complement system is an exceptionally complex and finely regulated network that maintains dynamic homeostasis, enabling the body to respond to sudden infections, injuries, and other stressors. Although the classical functions of the complement system have been widely studied, much remains unknown and uncertain regarding the full scope of its activities. The emerging field of intracellular complement function has revealed the high variability and complexity of complement gene expression in renal cells during both physiological and pathological processes. This variability further underscores the critical role of complement in kidney health and disease. Additional studies are necessary to elucidate the precise roles of complement genes in both normal kidney physiology and pathological states. Such research will be crucial in unveiling the exact functions of the complement system in kidney diseases and identifying potential therapeutic targets.

In conclusion, our findings provide a preliminary atlas of complement genes in human and mouse kidneys, offering a unique and valuable perspective on cell type-specific dysregulation of the complement system in the pathogenesis of some kidney diseases, especially those involving complement activation.

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Author contributions

Li Zhu conceived and designed the study. Xianzhi Li performed the experiments. Xianzhi Li analyzed the data. Xianzhi Li and Li Zhu prepared the manuscript. All authors have read and approved the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data sharing statement

All single-cell RNA sequencing data reported herein can be found in the Gene Expression Repository (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE193649, GSE140023, GSE157292, GSE159115, GSE131685.

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